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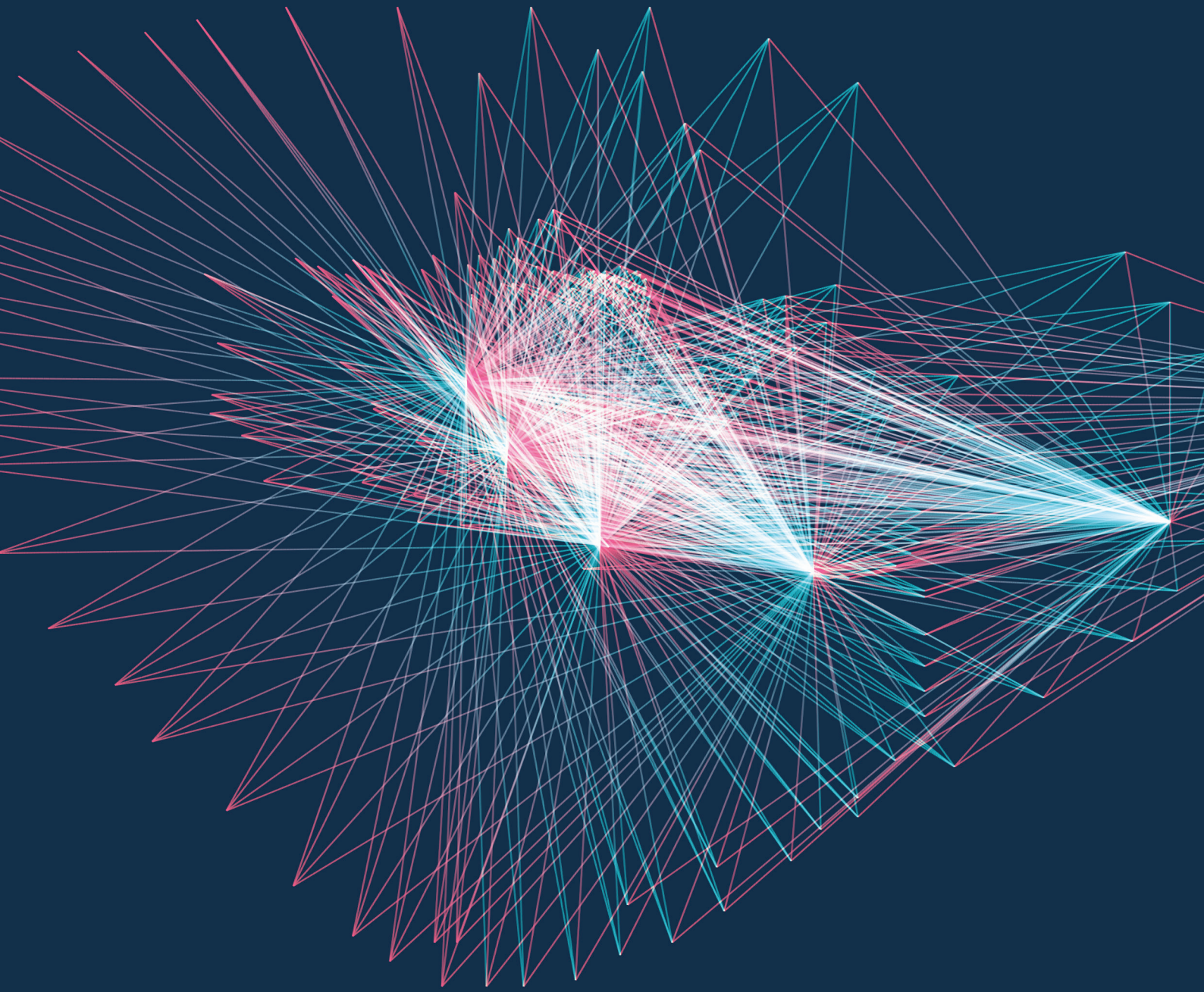

Chiang Mai University's Faculty of Dentistry publishes academic research articles in the newly titled - **Oral Sciences Reports,** which was previously known as *Chiang Mai Dental Journal (CMDJ)*. The journal was originally established for the purposes of publishing academic research articles by the Faculty of Dentistry at Chiang Mai University in 1977. In the current report, editors and experts in their respective fields review articles received from authors prior to being published to ensure that the content of all articles is up-to-date, universal, logical, and in accordace with academic principles so the reader can apply knowledge and cite works in the development of dentistry for the purposes of advancing future research while being beneficial to patients and society.

At present, Oral Sciences Reports openly receives all submissions through an online journal review process system. The new online system also allows reviewers and researchers an ability to read 3 issues each year.

Aim and Scope of the journal

To compile research and content that is up to date and usable to all branches of dentistry and related fields. The articles in Oral Sciences Reports are fundamental research work, including original articles, review articles, case reports/ series, short communications, and letters to the editor.

Policy

Accepted articles will be fairly reviewed by the editors and experts with full transparency through the following process.

1. The articles must be correct according to academic principles and not duplicate works that have been previously published.
2. The articles will be considered and reviewed through a non-bias process by concealing the names of authors and related persons in the considered documents while also concealing the names of the experts and reviewers who review the articles (double-blind review).
3. The review process can be tracked online. The article authors can review the status of their article and are able to follow up on the article evaluation through the online process. The duration of each step is closely monitored so that the articles can be published on time.
4. Authors of articles are responsible to review and verify the accuracy of the text, images, tables in the articles before publication.
5. Articles published in Oral Sciences Reports are the copyright of Oral Sciences Reports, which forbids anyone from duplicating published articles for any purpose without explicit permission from Oral Sciences Reports.

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Thai Journal Citation Index Centre (TCI) Tier 1
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Google Scholar

Editorial Board

Types of Submission

Oral Sciences Reports invites the following submissions:

1.	Original Articles	Original contributions of research reports or unpublished recent academic research to the development and applications in dentistry and related fields. The original article must not exceed 4000 words in length and must contain no more than 10 figures and tables in total.
2.	Review Articles	Comprehensive reviews of special areas of focus in dentistry and related fields. Articles that contain important collected data from numerous books or journals and from the writer's experience. Information should be described, reviewed, compared, and analyzed. The review article must not exceed 4000 words in length and must contain no more than 10 figures and tables in total.
3.	Systematic Reviews	Clearly formulated reviews that uses systematic and reproducible methods to identify, select and critically appraise all relevant research, and to collect and analyze data from the studies that are included in the review.
4.	Case Reports/Series	Original findings that highlight novel technical and/or clinical aspects in dentistry and related fields which include clinical symptoms, diagnosis, patient care, treatment, follow-up, and evaluation. The report must not exceed 2500 words in length and must contain no more than 5 figures.
5.	Letters to the Editor	Commentaries on published papers in the journal and other relevant matters that must not exceed 1000 words in length
6.	Short Communications	Original contributions describing new developments of high impact that justify expedited review. The report must not exceed 2000 words in length and must contain no more than 3 figures.

Submission Checklist

Authors should ensure to prepare the following items for submission. Failure to complete the required items may contribute to the delay of publication process. Please check the relevant section in this guideline for more details.

1. Title page — Must include title of the article, author names and affiliations. One author has been designated as the corresponding author with contact details (e-mail address and full postal address) (see 'Title page' section for more information and an example)
2. CRediT Contribution — Author will be asked to provide CRediT Contributions as well as their degree of contribution at the time of the original submission. CRediT Contribution is a high-level classification of the diverse roles performed in the work leading to a published research output in the sciences. Its purpose to provide transparency in contributions to scholarly published work, to enable improved systems of attribution, credit, and accountability.
3. Abstract — Must not exceed 250 words. Relevant keywords (up to five keywords) must be included at the end of the abstract. (see the 'Abstract' section for more details)
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7. Supplementary data (if applicable)

Additional considerations the author should confirm before submission:

1. Manuscript must be 'spell-checked', 'grammar-checked', and 'plagiarism-checked'.
2. All figures, tables, and references mentioned in the text should match the files provided.
3. Permission must be obtained for use of copyrighted material from other sources (including the internet).
4. Authors must provide conflicts of interest statement, even if there is no conflict of interests to declare.

Ethical Guidelines

Authors must acknowledge to the following ethical guidelines for publication and research.

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All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee's approval for each study. Experimentation involving human subjects will only be published if such research has been conducted in full accordance with the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements or with ethical principles of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above-mentioned principles.

Experimentation involving animal subjects should be carried out in accordance with the guidelines laid down by the National Institute of Health (NIH) in the USA or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

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All clinical trials must register in any of the following public clinical trials registries:

- Thai Clinical Trials Registry (TCTR)
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- EU Clinical Trials Register
- ISRCTN Registry

The clinical trial registration number and name of the trial register should be included in Materials and Methods of the manuscript. For epidemiological observational trials, authors of epidemiological human observations studies are required to review and submit a 'strengthening the reporting of observational studies in Epidemiology' (STROBE) checklist and statement. Compliance with this must be detailed in Materials and Methods.

D. Systematic Review

The abstract and main body of the systematic review should be reported using the PRISMA for Abstract and PRISMA guidelines respectively. Authors submitting a systematic review should register the protocol in one of the readily-accessible sources/databases at the time of project inception and not retrospectively (e.g. PROSPERO database, OSF registries). The protocol registration number, name of the database or journal reference should be provided at the submission stage in Materials and Methods. A PRISMA checklist and flow diagram (as a Figure) should also be included in the submission material.

E. Conflicts of Interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Potential sources of conflict of interest include (but are not limited to) patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If there are no interests to declare, please state 'The authors declare no conflict of interest'. Authors must disclose any interests in the section after acknowledgments.

F. Submission Declaration and Verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright- holder. The conference proceedings are allowed to be part of the article if the contents do not exceed 70% of the article.

G. Copyright

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All texts in the submitted manuscript are required to be inclusive language throughout that acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (for instance by using 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess'). Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, religion, culture, or any other characteristic.

A. Title page

The title page will remain separate from the manuscript throughout the peer review process and will not be sent to the reviewers. It should include these following details:

- Title should be concise, information-retrieval, and not exceed 30 words. Please avoid abbreviations and formulae where possible.
- Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript number immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and the e-mail address of each author.
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B. Abstract

Abstract must not exceed 250 words with concise and informative explanations about the article. Authors must prepare an abstract separately from the main manuscript using Microsoft Word processing software (.doc or .docx). Please avoid references and uncommon abbreviations, but if essential, abbreviations must be defined at their first mention in the abstract itself. Abstract structure of the original articles must consist of ‘Objectives, Methods, Results, and Conclusions’.

Abstract of other types of submitted articles should be summarized in one paragraph. Up to five keywords relevant to the articles must be provided and arranged in alphabetical order.

C. Manuscript

Oral Sciences Reports adheres to a double-blinded review. The main body of the paper (including the references, figures, tables and any acknowledgements) must not include any identifying information, such as the authors' names. The layout of the manuscript must be as simple as possible with double-spaced, single column format with Sans Serif font and uploaded as an editable Microsoft Word processing file (.doc or .docx). Complex codes or hyphenate options must be avoided, but the emphatic options such as bold face, italics, subscripts, and superscripts, etc. are encouraged.

1. Original article

• *Introduction* should include literature reviews of previous studies, research questions, and the rationale for conducting the study. The Introduction should not be too long and should be easy to read and understand while avoiding a detailed literature survey or a summary of the results.

• *Methods* should provide sufficient details in a logical sequence to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized and indicated by a reference. If quoting directly from a previously published method, use quotation marks and cite the source. Any modifications to existing methods should also be described.

• *Results* should show the data gained from the study's design in text, tables and/or illustrations, as appropriate, and be clear and concise.

• *Discussion* is criticism, explanation, and defense of the results from the standpoint of the author, and comparison with other peoples' reports. The discussion can include criticism of materials, methods and study results, problems, and difficulties, pointing out the benefits of adoption and providing feedback where appropriate. Discussions should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature.

• *Conclusions* refers to a summary of the study or research results.

• *Acknowledgments:* Please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study.

Formatting of funding source:

This work was supported by the 1st organization name [grant numbers xxxx]; the 2nd organization name [grant number yyyy]; and the 3rd organization name [grant number zzzz].

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant or funding from funding agencies in the public, commercial, or not-for-profit sectors.

• *References* should be confined to documents relating to the author's article or study. The number should not exceed 80, placed in order and using numbers which are superscripted and put in parentheses, starting with number 1 in the article and in reference document's name. (see 'References' section for more information regarding reference formatting)

2. Review articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described, and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

3. Systematic review

• Introduction should be focused to place the subject matter in context and to justify the need for the review.

• Methods should be divided into logical sub-sections in order to improve readability and enhance understanding (e.g. details of protocol registration, literature search process, inclusion/exclusion criteria, data extraction, quality assessment, outcome(s) of interest, data synthesis and statistical analysis, quality of evidence).

• Results should present in structured fashion (e.g. results of the search process, characteristics of the included studies, results of primary meta-analysis, additional analysis, publication bias, quality of evidence).

• Discussion should summarize the results, highlighting completeness and applicability of evidence, quality of evidence, agreements and disagreements with other studies or reviews, strength and limitations, implications for practice and research.

• Conclusion(s) should reach clear conclusions and/or recommendations on the basis of the evidence presented.

4. Case reports/series should be divided into Introduction, Case report, Discussion and Conclusions. They should be well illustrated with clinical images, radiographs and histologic figures and supporting tables where appropriate. However, all illustrations must be of the highest quality.

There are some necessary considerations which should be comprehended and consistent throughout the article:

1. Abbreviations: define abbreviations at their first occurrence in the article: in the abstract and in the main text after it. Please ensure consistency of abbreviations throughout the article.

2. Mathematical expressions: the numbers identifying mathematical expressions should be placed in parentheses after the equation, flush to the right margin; when referring to equations within text, use the following style: Eq. (5), Eqs. (3-10), [see Eq. (4)], etc.

3. Nomenclature: abbreviations and acronyms should be spelled out the first time they are used in the manuscript or spelled out in tables and figures (if necessary). Units of measure and time require no explanation. Dental nomenclature in the manuscript should be complete words, such as maxillary right central incisor. Numbering of teeth from pictures or tables should follow the FDI two-digit system.

4. Units: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

5. Product identification: all products mentioned in the text should be identified with the name of the manufacturer, city, state, and country in parentheses after the first mention of the product, for example, The ceramic crown was cemented on dentin surface with resin cement (RelyX^{TM} U200, 3M ESPE, St. Paul, MN, USA)…

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Figures should be prepared and submitted separately from the main manuscript. Color artworks are encouraged at no additional charge. Regardless of the application used other than Microsoft Office, when the electronic artwork is finalized, please ‘save as’ or ‘export’ or convert the images to **EPS, TIFF, or JPEG format with the minimum resolution of 300 dpi.** Keep the artwork in uniform lettering, sizing, and similar fonts. Please do not submit graphics that are too low in resolution or disproportionately large for the content. Authors must submit each illustration as a separate file.

Please ensure that each illustration has a caption according to their sequence in the text and supply captions separately in editable Microsoft Word processing file (.doc or .docx), not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

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Please submit tables as editable Microsoft Word processing files (.doc or .docx), not as images, and avoid using vertical rules and shading in table cells. Each table should be placed on a separate page, not next to the relevant text

in the article. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body while ensuring that the data presented in them does not duplicate results described elsewhere in the article.

F. References

Citation in text

Any citations in the text should be placed in order and using numbers which are superscripted and put in parentheses. Please ensure that all citations are also present in the reference list consecutively in accordance with their appearance in the text.

Reference style

All references should be brought together at the end of the paper consecutively in accordance with their appearance in the text and should be in the Vancouver reference format. Please follow these examples of correct reference format below:

1. *Journal article*

1.1. One to six authors

Author(s) – Family name and initials. Title of article. Abbreviated journal title. Publication year;volume (issue):pages.

Example:

Parvez GM. Pharmacological activities of mango (Mangifera Indica): A review. J Pharmacognosy Phytother. 2016;5(3): 1-7.

Or

Choi YS, Cho IH. An effect of immediate dentin sealing on the shear bond strength of resin cement to porcelain restoration. J Adv Prosthodont. 2010;2(2):39-45.

Or

Firmino RT, Ferreira FM, Martins CC, Granville-Garcia AF, Fraiz FC, Paiva SM. Is parental oral health literacy a predictor of children's oral health outcomes? Systematic review of the literature. Int J Paediatr Dent. 2018;28(5):459-71.

1.2. More than six authors

Author(s) – Family name and initials of the first six authors, et al. Title of article. Abbreviated journal title. Publication year;volume(issue):pages.

Example:

Vera J, Siqueira Jr JF, Ricucci D, Loghin S, Fernández N, Flores B, et al. One-versus two-visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study. J Endod. 2012;38(8):1040-52.

1.3. Article in press

Authors separated by commas – Family name and initials. Title of article. Abbreviated journal title in italics. Forthcoming - year of expected publication.

Example:

Cho HJ, Shin MS, Song Y, Park SK, Park SM, Kim HD. Severe periodontal disease increases acute myocardial infarction and stroke: a 10-year retrospective follow-up study. J Dent Res. Forthcoming 2021.

2. *Books*

2.1. Book with author (s)

Author(s) – Family name and initials (no more than 2 initials with no spaces between initials)– Multiple authors separated by a comma. After the 6th author add - "et al". Title of book. Edition of book if later than 1st ed. Place of publication: Publisher name; Year of publication.

Example:

Sherwood IA. Essentials of operative dentistry. Suffolk: Boydell & Brewer Ltd; 2010.

Or

Abrahams PH, Boon JM, Spratt JD. McMinn's clinical atlas of human anatomy. 6th edition. Amsterdam: Elsevier Health Sciences; 2008.

2.2. Book with no author

Title of book. Edition of book if later than 1st ed. Place of publication: Publisher name; Year of publication.

Note: Do not use anonymous. Please begin a reference with the title of the book if there is no person or organization identified as the author and no editors or translators are given.

Example:

A guide for women with early breast cancer. Sydney: National Breast Cancer; 2003.

2.3. Chapter in a book

Author(s) of chapter - Family name and initials, Title of chapter. In: Editor(s) of book - Family name and initials, editors. Title of book. edition (if not first). Place of publication: Publisher name; Year of publication. p. [page numbers of chapter].

Example:

Rowlands TE, Haine LS. Acute limb ischaemia. In: Donnelly R, London NJM, editors. ABC of arterial and venous disease. 2nd ed. West Sussex: Blackwell Publishing; 2009. p. 123-140.

3. Thesis/dissertation

3.1. Thesis in print

Author - family name followed by initials. Thesis title [type of thesis]. Place of publication: Publisher; Year.

Example:

Kay JG. Intracellular cytokine trafficking and phagocytosis in macrophages [dissertation]. St Lucia, Qld: University of Queensland; 2007.

3.2. Thesis retrieved from full text database or internet

Author - family named followed by initials. Thesis title [type of thesis/dissertation on the Internet]. Place of publication: Publisher; Year [cited date – year month day]. Available from: URL

Example:

Pahl KM. Preventing anxiety and promoting social and emotional strength in early childhood: an investigation of risk factors [dissertation on the Internet]. St Lucia, Qld: University of Queensland; 2009 [cited 2017 Nov 22]. Available from: https://espace.library.uq.edu.au/view/UQ:178027

4. Webpage

4.1. Webpage with author

Author/organization's name. Title of the page [Internet]. Place of publication: Publisher's name; Publication date or year [updated date - year month day; cited date - year month day]. Available from: URL

Example:

American Dental Association. COVID-19 and Oral Health Conditions [Internet]. Chicago: American Dental Association; 2021 Feb 12 [updated 2021 Feb 12; cited 2021 Jun 24]. Available from: https://www.ada.org/en/press-room/news-releases/2021-archives/february/covid-19-and-oral-health-conditions

4.2. Webpage with no authors

Title [Internet]. Place of publication (if available): Publisher's name (if available); Publication date or year [updated date (if available); cited date]. Available from: URL

Example:

Dentistry and ADHD [Internet]. 2019 Jan 15 [updated 2019 Jan 15; cited 2020 Apr 8]. Available from: https://snoozedentistry.net/blog/dentistry-and-adhd/

4.3. Image on a webpage

Author/organization. Title [image on the Internet]. Place of publication: Publisher's name; Publication date or year [updated date; cited date]. Available from: URL

Note: If the image does not have a title - give the image a meaningful title in square brackets.

Example:

Poticny DJ. An Implant-Supported Denture Offers a Number of Advantages [image on the Internet]. Texas: Office of Dan Poticny; 2018 Nov 21 [updated 2018 Nov 21; cited 2019 Aug 30]. Available from: https://www.dfwsmiledoc.com/blog/post/an-implant-supported-denture-offers-a-number-of-advantages.html

5. Government publications/reports

5.1. Reports and other government publications

Author(s). Title of report. Place of publication: Publisher; Date of publication – year month (if applicable). Total number of pages (if applicable eg. 24 p.) Report No.: (if applicable)

Example:

Australian Institute of Health and Welfare. Oral health and dental care in Australia: key facts and figures trends 2014. Canberra: AIWH; 2014.

5.2. Government reports available online

Author(s). Title of report. Report No.: (if applicable). [Internet]. Place of publication: Publisher or Institution; Publication date or year [updated date - year month day; cited date - year month day]. Available from: URL

Example:

World Health Organization. WHO mortality database [Internet]. Geneva: World Health Organization; 2019 Dec 31 [updated 2019 Dec 31; cited 2021 Mar 29]. Available from: https://www.who.int/data/mortality/country-profile

6. Tables/Figures/Appendices

Follow the format of book, journal or website in which you found the table/figure/appendix followed by: table/figure/image/appendix number of original source, Title of table/figure/appendix from original source; p. Page number of table/figure/appendix from original source.

Note: each reference to a different table/figure within the same document requires a separate entry in the Reference list. Please provide permission documents from the original sources.

Example:

Smith J, Lipsitch M, Almond JW. Vaccine production, distribution, access, and uptake. Lancet 2011;378(9789):428-438. Table 1, Examples of vaccine classes and associated industrial challenges; p. 429.

7. Journal abbreviation source

Journal names should be abbreviated according to the Web of Science - Journal Title Abbreviations.

Peer-review Process

Oral Sciences Reports follows a double anonymized review process. Each manuscript will be assigned to at least three expertises for consideration. The identities of both reviewers and authors are concealed from each other throughout the review to limit reviewer bias. To facilitate this, please ensure that the manuscript keeps anonymity before submission such as affiliation, author's gender, country or city of origin, academic status, or previous publication history. Our peer review process is confidential and identities of reviewers are not released. Letters and technical comments are sent to the authors of the manuscript on which they comment for response or refutation, but otherwise are treated in the same way as other contributions with respect to confidentiality.

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A manuscript must be submitted electronically on the OSR ScholarOne submission site. When entering the submission page for the first time, you will be asked to create an account with your e-mail and password followed by your personal data.

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. Please follow the submission process carefully. The system converts your article files to a single PDF file used in the peer-review process. Editable Microsoft word processing files are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent to your registered e-mail.

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Prognostic Significance of C-Reactive Protein in Oral Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the sixth most prevalent type of cancer worldwide. One of the most investigated potential biomarkers in this context is C-reactive protein (CRP), a constant biomarker and an acute segment reactant related to inflammation and tissue injury.

Aims and Objectives: This systematic review and meta-analysis aim to investigate the role of CRP as a potential biomarker for predicting prognosis in OSCC, specifically in terms of overall survival (OS) and disease-free survival (DFS).

Methodology: This systematic review (ID: CRD42022344744) has been registered with the International Prospective Register of Systematic Reviews (PROSPERO). We conducted a systematic literature search using PubMed, Scopus, and Google Scholar. From this search, 638 studies were initially identified. The PRISMA flow diagram was then developed to guide study selection, and Version 5.3 of the Review Manager (RevMan) software was used to do the meta-analysis.

Results: Our systematic review and meta-analysis included articles that completely satisfied our inclusion and exclusion criteria. In our meta-analysis, the forest plot model calculates a combined Hazard ratio of more than 1.5 (1.85 for DFS and 1.97 for OS), suggesting a poor prognosis for OSCC patients regarding DFS and OS, particularly in those with high CRP.

Conclusions: A high CRP level was found to be a significant indicator of poor prognosis compared to OSCC cases with low CRP levels, in terms of patients' overall survival and disease-free survival. Thus, these findings suggest that CRP may be a potential marker for predicting prognosis.

Keywords: biomarker, carcinogenesis, C-reactive protein, diagnosis, oral cancer, prognosis

Introduction

Globally, oral squamous cell carcinoma (OSCC) is the sixth most prevalent type of cancer. Crucial and main risk factors for developing OSCC are heavy alcohol, smoking, betel nut chewing, infection, as well as human papillomavirus (HPV).[1-3] The most vital predictors for prognosis in patients include lymph node metastasis and tumor size. Potential biomarkers are required to predict prognosis and improve patients' quality of life through therapeutic approaches.[4-6] One of the most investigated potential biomarkers in this context is C-reactive protein (CRP), a chronic biomarker and an acute segment reactant related to inflammation and tissue injury. Raised CRP has been linked to poor prognosis in patients with a variety of solid tumours, including lung, breast, and renal cell carcinoma. Numerous evaluations and investigations have been conducted to determine the prognostic association between CRP and oral cancer.[7-10]

CRP levels fluctuate daily and progressively rise with age, blood pressure, coffee, alcohol, and smoking.[11-13] The production of CRP in hepatocytes is regulated by pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). TNF-α levels and CRP expression are positively correlated.[14-17] CRP has been traditionally used as a crucial biomarker for infection. Previous studies on cardiovascular disease have provided evidence of its role in the inflammatory process, including the host response and infection, which involves apoptosis, primarily through the production of IL-6 and cytokines such as tumour necrosis factor, and facilitates phagocytosis by releasing nitric oxide.[18-20] It was very early appreciated that CRP can function as an opsonin.[7,21-24] CRP plays a crucial role in host defence against infection, in which Opsonisation is one of the mechanisms.[25]

CRP plays a vital role in the body's immune defense, particularly by initiating the complement cascade. It also eliminates autoantigens, which are widespread throughout the body. CRP promotes this clearance by binding to Fc receptors and activating the complement pathway on the surface of phagocytic immune cells. Various theories have been proposed; systemic inflammatory reactions mediated by CRP and different white blood cell types are essential in conditions such as tumor cell death, low-oxygen environments (hypoxia), and localized tissue damage. Consequently, both CRP and leukocyte subtypes are widely recognized as key biomarkers for evaluating systemic inflammation.[26-33]

The liver produces CRP, an acute-phase protein, when inflammatory cytokines, particularly IL-6, increase significantly during infection. CRP is a non-specific biomarker widely used to indicate systemic inflammation, including infections. Significantly increased levels of CRP are often detected in inflammatory conditions such as osteoarthritis. Additionally, CRP serves as an essential clinical indicator for cardiovascular events, including unstable angina, thrombosis-related complications, and systemic inflammatory activity. In contrast, modest elevations in CRP are more frequently associated with autoimmune diseases like systemic lupus erythematosus, scleroderma, Sjögren's syndrome, polymyositis, and other long-term inflammatory disorders. Multiple diagnostic approaches are used to measure CRP concentrations in clinical settings. These techniques include visual agglutination, rapid immunodiffusion, and immune-turbidimetry. Among the earliest and most commonly employed methods is the latex agglutination assay. This technique involves latex beads coated with goat-derived IgG antibodies specific to human CRP. When exposed to a CRP-positive sample, these particles aggregate, producing visible clumping within two minutes. This allows for quick and semi-quantitative assessment of CRP levels.[30-33] In certain primary malignancies like oesophageal cancers, hepatocellular cancers, colorectal cancers, cervical cancers, bladder cancers, as well as in advanced stages of any cancers or any microbial infections, can lead to systemic inflammation, and the serum level of CRP plays a vital role as both a direct and indirect indicator of this inflammatory response.[11,20,33-36]

Numerous investigations have examined the relationship between pro-inflammatory cytokines and blood levels of acute-phase proteins, specifically CRP, in patients with oral squamous cell carcinoma.[37,38] These investigations have consistently shown that CRP concentrations rise significantly during the active phase of the disease. Based on this observation, CRP has been proposed as a potential biomarker for OSCC and may also assist in identifying cases of local recurrence in head and neck malignancies.[18,37-39]

Furthermore, IL-6 enhances the interaction between CRP and tumor cells, possibly contributing to the lysis of malignant cells. As such, increased CRP levels could

reflect tumor-associated tissue damage and the broader inflammatory response mounted by the host against the tumor microenvironment.[16,40] Therefore, by doing a systematic review and meta-analysis, we aimed to determine the elevated CRP as a potential biomarker for prognostic prediction in OSCC in terms of overall survival (OS) and disease-free survival (DFS).

Systematic review

This systematic review (ID: CRD42022344744) has been registered with the International Prospective Register of Systematic Reviews (PROSPERO). The most recent Meta-Analyses and Preferred Reporting Items for Systematic Reviews (PRISMA) criteria were used in reporting this systematic study.[41]

• The focus question was–Can CRP play a prognostic biomarker role in OSCC?

• PECO Criteria–This sub-heading includes

• Population (P)–OSCC patients for whom CRP has been evaluated.

• Exposure (E)–States the cut-off value of C-reactive protein.

• Comparison (C)–of <5 and ≥5 of CRP value

• Outcome (O)–Value of HR (hazard ratio) with a 95% confidence interval (CI) as an effect size measure for the variables' overall survival and disease-free survival.

• We conducted a systematic literature search using PubMed, Scopus, and Google Scholar. The systematic review search topic was registered in 2022. Search was performed by December 2022-2024, using the following keywords: “CRP” OR “C-REACTIVE PROTEIN” AND ‘BIOMARKERS' AND “PREDICTION” AND “PROGNOSIS” AND “ORAL CANCER” OR “OROPHARYNGEAL SQUAMOUS CELL CARCINOMA” OR “ORAL SQUAMOUS CELL CARCINOMA” OR “HEAD AND NECK SQUAMOUS CELL CARCINOMA”.

• After searching and screening, all potentially associated studies were evaluated for their contents, followed by exclusion and inclusion criteria, and data were extracted.

Inclusion criteria

I. Cohort studies that reported the prognostic value of CRP for survival (OS, DFS) and clinic-pathological factors in OSCC, such as tumour size, histological grade, clinical stage, or N status.

II. Research articles written in English.

III. Research in which pre-operative levels of CRP have been evaluated by univariate analysis.

IV. Studies in which the cut-off value of CRP was taken as ≥5.

Exclusion criteria

I. Studies in which HR of DFS and OS was not directly mentioned or could not be calculated using estimation methods.

II. Review articles of CRP.

III. Secondary or metastatic cases of OSCC.

Data were extracted from all the eligible studies for systematic review and Meta-analysis and retrieved under the following headings

I. Demographic data concerning population, survival rates, and ethnicity during the follow-up.

II. Information on tumours (sample size, lymph node invasion, TNM staging, and histological grading).

III. Experiments involving materials, study design, and meeting CRP cut-off requirements.

IV. Data on survival and clinic-pathological factors that include univariate analysis of CRP and HR data for OS and DFS with matching 95% CIs, respectively.

V. Publication information, such as the author's name, the year of publication, and journal titles.

The Newcastle-Ottawa scale (NOS) was used in the included studies to assess the risk of bias. Selection, outcome, and comparability were all employed to measure study qualities.[42] NOS score ≥7 is commonly accepted as “good quality” in meta-analyses. Calibration ensures consistent and reproducible scoring. RevMan 5.3 software version was used to perform the meta-analysis of the accumulated studies from selected articles. A forest plot was generated using the OSCC patient survival data (HR and 95% CI) to illustrate the association between CRP and its prognostic role in OSCC, expressed in terms of OS and DFS. The inverse-variance approach was used to meta-analyze pooled estimates. This random-effects approach considers the potential for distinct underlying findings between research subgroups (i.e., variations in oral subsides associated with geographical regions or the intrinsic variability of the experimental procedures). Heterogeneity was assessed using RevMan 5.3 software, where a p-value less than 0.10 and an I-squared value greater than 50% indicate the presence of considerable heterogeneity across the studies. An observed HR>1 suggests that the direction of the effect tends to favour poor prognosis in the research

group exposed to raised or elevated CRP levels.

Results

PRISMA chart description: Records identified from various databases, PubMed (n=21), Scopus (n=606), and Google Scholar (n=11). A total of 638 articles were identified. Five hundred ninety-two articles were removed before screening, as they did not align with the topic's specificity and were duplicates. The remaining 46 articles were then screened, and five were excluded as review articles. So, 41 articles reports sought for retrieval. Among these 25 studies were removed as they did not mention the prognostic outcome of CRP. The eligibility of the remaining sixteen items was then evaluated. However, the following factors led to the exclusion of 12 studies. Reasons for exclusion: 1) Missing HR data in 5 studies. 2) The CRP cut-off value did not meet the inclusion criteria (≥5 mg/L) in 6 studies. 3) HR reported for recurrence only, not overall survival, in 1 study. Although it has been observed that these 12 studies reviewed were supportive of the increased value of CRP in predicting a poor prognosis for OSCC cases, the details provided in the manuscript did not meet the inclusion criteria. Therefore, these studies were excluded. Lastly, findings of the remaining four studies were included to assess the contribution of CRP to OSCC prognosis in terms of OS and DFS, and a systematic review and meta-analysis were conducted. Which entirely satisfy our inclusion and exclusion criteria, as shown in Figure 1. The complete clinical data, including histological grading, TNM staging, lymph node status, and HR values, have been summarized in Table 1. All four studies you listed, from Taiwan and Austria, performed univariate analysis to examine the predictive role of C-reactive protein (CRP) in OSCC. A total of three out of four studies were conducted in Taiwan, while the other was conducted in Austria. All four articles reported the prognostic outcome associated with CRP and included the key characteristics of HR and 95% CI values–an overview of all the selected articles and prognostic outcome-giving studies related to CRP and OSCC. All studies had NOS scores between 7 and 8 (Table 2). Three papers received a NOS score of "7," while one study received an overall score of eight. The quality of the included retrospective cohort studies was evaluated using the Newcastle-Ottawa Scale in three domains: outcome (0-3 points), comparability (0-2 points), and selection (0-4 points). Nine is the highest possible score. Studies with a score of 6-9 are often regarded as high-quality. Therefore, all studies included in our systematic review and meta-analysis had overall good NOS scores.

The risk of selection bias is low in relation to the individual domains. Regarding all the included studies, all have successfully reported at least six months of follow-up with the Hazard ratio value.

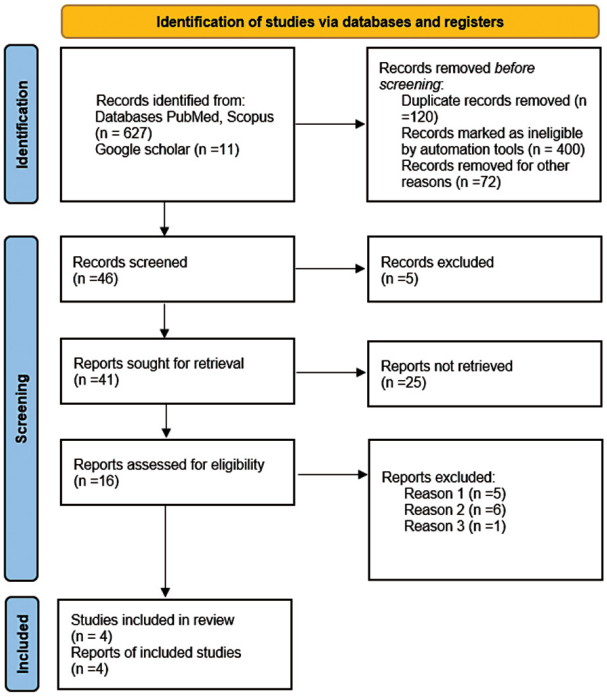

Figure 1: PRISMA 2020 flow diagram for systematic review. (Showing literature search and study selection for the present systematic review)

Meta-analysis outcome

The HR and 95% CI observed in our included studies were combined to investigate the relationship between CRP and OSCC. If the 95% Confidence Interval (CI) for the HR>1, the result is statistically significant. In cancer prognosis of OSCC and its relation to CRP, an HR>1 means the event is more likely to occur in the exposed group (OSCC cases). While HR>1 indicates a higher probability of recurrence in disease-free survival and a higher risk of mortality (overall survival).

In our meta-analysis, the forest plot model calculates a combined Hazard ratio of more than 1.5 (1.85 for DFS and 1.97 for OS), suggesting a poor prognosis for OSCC patients regarding DFS and OS (Figures 2 and 3). Figure 2 shows in DFS (Meta-analysis), very low heterogeneity

Table 1: Description of study articles included finally in the systematic review and meta-analysis.

SN	Year	Author	Nation	Sample size	M: F	Median Age	Predominant Site	Follow up	TNM staging	Histological staging	Number of cases analyzed: CRP	CRP vs. T				CRP Vs. N				CRP vs. HR (95%CI)	
												<5		≥5		<5		≥5		DFS	OS
												E	A	E	A	E	A	E	A		
1	2012	Huang SF	Taiwan	142	133:9	52.06	Buccal Mucosa 67 (47.2%)	3 Years	I-37 II-28 III-12 IV-65	Well/ Moderate-117 Poor-25	109*	50	36	9	14	70	16	13	10	2.888 (1.625-5.135)	2.526 (1.245-5.136)
2	2017	Tai SF	Taiwan	343	318:25	52.21	Tongue 132 (38.5%)	6 Months -6years	I-76 II-66 III-43 IV_a-133 IV_b-25	Well-107 Moderate-192 Poor-44	343	181	68	29	65	192	56	57	38	1.902 (1.302-2.778)	2.235 (1.393-3.585)
3	2018	Graupp M	Austria	197	146:1	58.9	Tongue 197 (100%)	Long-term cohort study (Up to 13 Years	I-22 II-29 III-28 IV-118	N.A.	150**	31	35	36	48	23	43	52	59	1.454 (0.949-2.227)	1.616 (1.026-2.546)
4	2019	Dante D.P.	Taiwan	246	225:21	53	Tongue 106 (43%)	6-72 Months	I-63 II-51 III-35 IV-97	Well-66 Moderate-150 Poor-29 Undifferen-tiated-1	246	134	51	20	41	152	33	41	20	1.714 (1.029-2.854)	1.879 (0.981-3.599)

Abbreviations'*- M: F- Male: Female Ratio, CRP: C- reactive protein, OS: Overall-survival, DFS: Disease-free survival, SZ: Sample-Size, Tumor Stage, N-Lymph Node, CI: Confidence Interval, HR: Hazard Ratio, NA: Not Available, *(33 patients excluded due to not available data on CRP), **(47 patients excluded due to not available data on CRP). N_0, N_1 – Early (E). N_2, N3- Advance (A) and T_0, T_1- E (Early), T_2, T_3, T_4, T_{4a}, T_{4b}, T_{4c}- Advance (A).

Table 2: Studies of quality assessment by the Newcastle-Ottawa scale.

Study	Selection	Comparability	Outcome	NOS Score
SF.Huang *et al* (2012), Taiwan	● ● ● ●	● ●	● ○ ○	7
S.F.Tai *et al* (2017), Taiwan	● ● ● ●	● ●	● ○ ○	7
M.Graupp *et al* (2018), Austria	● ● ● ●	● ●	● ○ ●	8
Dante De Paz *et al* (2019), Taiwan	● ● ● ●	● ●	● ○ ○	7

● Point rewarded; ○ no point rewarded

was observed with Tau^2=0.01, Chi^2=3.63, p<0.00001. I^2=17%. The overall effect is obtained as Z=4.78. The combined HR value in this figure is calculated as 1.85 (1.44-2.39) with a 95% CI. The findings revealed a strong correlation and an increase in the HR value of DFS, indicating a poor prognosis. HR=1.8 DFS, which means that patients with high CRP have an 80% higher risk of recurrence or progression of OSCC than those with lower CRP.

Figure 3 shows the overall survival (meta-analysis), which shows zero heterogeneity (Tau^2=0.00) with a combined value of HR obtained as 1.97(1.50-2.58), with a confidence interval. Chi^2=1.49 and p-value (p<0.00001) with an overall effect; Z=4.91, I^2=0.00. The result also states that an increase in HR value (OS) is associated with the worst prognosis in OSCC patients. The statistical analysis was performed using RevMan software version 5.3 for meta-analysis.

Discussion

The studies included in our review support the prognostic value of elevated CRP (≥5 mg/L) in OSCC, linking it to advanced tumor stage, nodal involvement, and reduced survival outcomes. Despite some heterogeneity in methodology and cut-off values in the broader literature, a consistent trend suggests that CRP is a low-cost, easily measurable, and clinically relevant biomarker for risk stratification and treatment planning in OSCC.

S.F. Huang *et al.,*[7] conducted a retrospective study involving 142 patients diagnosed with OSCC. The cut-off value for CRP was set at ≥5 mg/L. The average age of the patients was 52.06 years, with the majority being men. The tongue (35.2%) and buccal mucosa (47.2%) were the most often occurring primary tumour sites. Early pathological tumor status was noted in 92 patients, while 50 presented with advanced disease. CRP levels were measured using an auto-analyzer. The study reported HR of DFS and

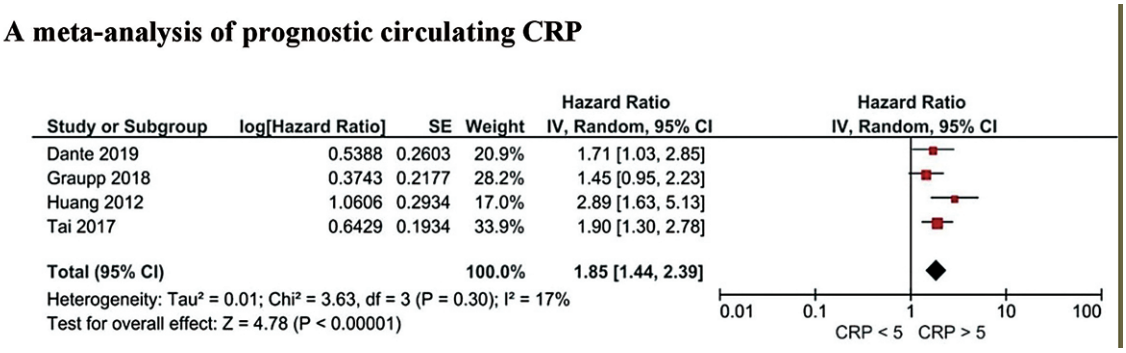

Figure 2: Forest plot for survival outcome of up-regulated CRP in OSCC patients. (Disease-Free Survival).

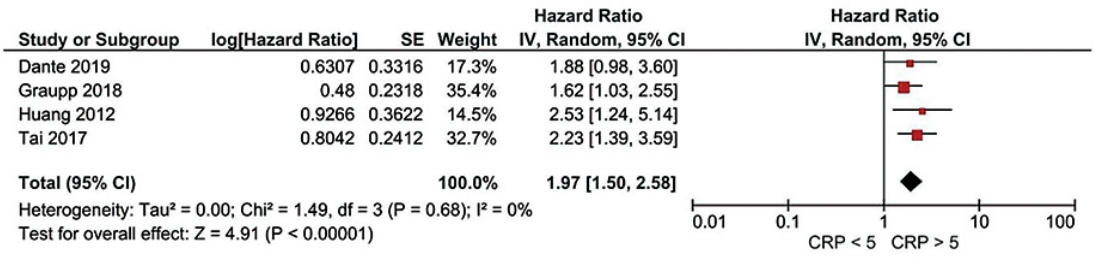

Figure 3: Forest plot of survival outcome associated with CRP &OSCC patients. (Overall Survival).

OS and suggested that elevated preoperative CRP levels serve as a significant prognostic biomarker in OSCC, particularly in relation to lymph node metastasis, tumor recurrence, and advanced tumor stage.

Graupp *et al.,*[12] analyzed 197 patients with tongue OSCC, focusing on OS and DFS as the primary outcome measures. Males comprised the majority (n=146), with a mean age of 58.9 years. Of the individuals in the group, 112 had advanced disease and 85 had early-stage tumours. Patients with CRP ≥5 mg/L included 36 with early-stage and 48 with advanced-stage tumors. Positive lymph node involvement was seen in 59 of these patients. The study found a significant association between elevated CRP and poor survival, with an HR of 1.616 (95% CI: 1.026-2.456) and an HR of 1.45 for disease-free survival DFS, establishing CRP as a cost-effective, clinically relevant prognostic biomarker in tongue SCC.

Tai *et al.,*[43] included 343 OSCC patients with CRP ≥5 mg/L as the cutoff. We used an auto-analyzer (Hitachi, Tokyo) to measure CRP levels. The most frequently affected areas were the buccal mucosa (n=126) and the tongue (n=132). The majority of patients (n=318) were male. The distribution of tumour stages was as follows: Stage I (n=76), Stage II (n=66), Stage III (n=43), and Stage IV (n=158). There was a strong correlation between advanced pathogenic nodal status (p=0.006) and lymphatic invasion (p=0.068), as well as elevated CRP levels (≥5 mg/L). The HR was 2.235 (95% CI: 1.393-3.585) for OS and 1.902 (95% CI: 1.302-2.778) for DFS. The authors concluded that CRP is an essential biomarker for prognosis, particularly effective in buccal cancer, potentially linked to areca nut and tobacco use.

Dante De Paz *et al.,*[13] assessed 246 OSCC patients (225 males and 21 females) with CRP levels ≥5 mg/L, which was defined as the threshold. The mean age was 53 years. Tumors were most commonly located in the tongue (n=106), followed by buccal mucosa (n=83). Among patients with CRP levels ≥5 mg/L, 20 were in the early stages and 41 were in the advanced stages. The median follow-up period was 24 months (6-72 months). The HR for DFS was 1.714 (95% CI: 1.029-2.854), and for OS, it was 1.879 (95% CI: 0.981-3.599). The study concluded that CRP significantly correlates with poor prognosis in OSCC and may inform treatment modifications and adjuvant therapy strategies.

Several additional studies have demonstrated a correlation between elevated CRP and poor prognosis in OSCC. Still, they were excluded from our systematic analysis because they did not meet the inclusion criteria, primarily due to the absence of hazard ratio (HR) reporting or inconsistent CRP cut-off values. For instance, Khandavilli *et al.,*[1] Kruse *et al.,*[2] Anderson *et al.,*[3] Kawasaki *et al.,*[4] and Blatt *et al.,*[5] found a significant association between elevated CRP and OSCC. Still, they were excluded because they did not report HR for OS or DFS, which is essential for meta-analysis. Other studies were excluded because they used CRP cut-off values inconsistent with the ≥5 mg/L threshold defined in our inclusion criteria. Farhan *et al.,*[10] used a cut-off of 10 mg/L. The readings reported by Chen *et al.,*[6] had a cut-off of ≤5 mg/L, and Peter *et al.,*[9] applied a threshold of <2 mg/L. Similarly, Grimm *et al.,*[8] employed a CRP cut-off of ≥1.1 mg/dL, Matsuki *et al.,*[14] did not adhere to the ≥5 mg/L standard, and Aarstad *et al.,*[15] used a threshold of 1 mg/L. Chen *et al.,*[44] focused exclusively on recurrent OSCC cases and provided HR values only for recurrence, not OS or DFS, and were therefore excluded. These exclusions were necessary to ensure methodological consistency and improve the reliability of the prognostic implications derived from CRP levels in OSCC. Some systematic reviews also supported the significance of CRP in OSCC, as seen in Rivera *et al.,*[45] who identified CRP among 41 significant prognostic biomarkers associated with early mortality in OSCC patients. Machado *et al.,*[46] suggested that systemic inflammation in patients can be measured by CRP. Stec-Martyna *et al.,*[47] suggested that CRP serves as a sensitive indicator of inflammation and, to some extent, cellular damage; its kinetics differ significantly depending on the underlying condition.

However, it is essential to note that many studies have used different cut-off values for CRP, which limits its overall predictive capacity in relation to OSCC. Our systematic review included only studies that used a CRP cut-off value of ≥5 to ensure more consistent and reliable results. Despite the variation in cut-off values across the literature, the evidence suggests that elevated CRP levels are associated with a poor prognosis in OSCC.

Limitations & future perspectives

As with any retrospective study, our analysis has certain limitations. Notably, the follow-up period was

relatively short, which may affect the strength of long-term prognostic conclusions. To address this, future prospective studies with a minimum follow-up duration of five years are warranted. Another critical limitation lies in the inconsistency of CRP cut-off values used across different studies. For improved standardization and comparability, adopting the internationally recognized CRP cut-off value of ≥5 mg/L is essential. Furthermore, to validate and generalize the prognostic utility of CRP in OSCC, well-designed, multi-institutional studies conducted across diverse populations and geographic regions are highly recommended.

Conclusions

After a thorough and careful review of various studies involving CRP and OSCC, this meta-analysis and comprehensive systematic review found that an increase in CRP levels of ≥5 is associated with poor outcomes in OSCC patients. The elevation in the Hazard ratio of OS and DFS suggests a poor prognosis in these patients.In studies conducted in Taiwan and Austria, most OSCC cases were of tongue carcinoma, followed by buccal mucosa carcinoma, as observed in the present review. The CRP test has advantages over other biomarkers, as it is inexpensive, rapid, and repeatable. However, further studies with a 5-year follow-up are required to confirm the prognostic capacity of CRP as a biomarker for predicting the prognosis of OSCC.

Conflict of Interest

The authors declare no conflict of interest.

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Social Messaging Apps as Oral Health Promotion Tools: A Literature Review

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Abstract

Social messaging apps are now integral to daily life, transforming communication and information sharing. These platforms show potential as tools for health promotion. However, existing studies vary in scope, outcomes, and platforms, leading to inconsistencies in assessing the impact and effectiveness of oral health (OH) promotion. This review aims to examine the type of social messaging apps used for OH promotion and their impact on OH knowledge, attitude, behavior, and OH status. The search was conducted across four electronic databases using relevant keywords to identify articles published between 2004 and 2024. This review includes experimental and quasi-experimental study designs, published in the English language. Data such as the study design and the characteristics of social messaging apps for OH promotion and their effect on knowledge, attitude, behavior, and OH status were extracted. The systematic search identified 601 articles, with 15 studies being included in the analysis. Four studies reported on OH knowledge and behavior outcomes, six reported on OH status outcomes, and five reported a combination of both outcomes. WhatsApp was the most utilized social media platform reported in these studies. OH promotion interventions leveraging these platforms demonstrated significant improvements in OH knowledge and status, particularly when using customizable, engaging content supported by continuous information delivery. However, more robust primary studies investigating specific tools, engagement strategies, user preferences, and patterns of patient interaction can offer substantial benefits to patient health, in line with technological adoption.

Keywords: messaging apps, mobile application, oral health, oral health promotion

Introduction

Oral diseases persist as a significant public health burden globally, impacting populations across nations despite advancements in technology. The Global Burden of Disease Study identified oral pathologies as the most widespread and impactful condition worldwide from a public health perspective.[1] Dental caries and periodontal disease remain prevalent among the adult population globally and are frequently associated with pain, discomfort, and early tooth loss.[1,2]

Historically, oral health (OH) promotion relied on traditional methods, including brochures, posters, OH talks, exhibition booths and community outreach programs.[3] The digital revolution, however, has led to a more innovative and dynamic approach through the use of social media and messaging apps, which have become ubiquitous in the lives of the younger generation, thus offering opportunities to leverage these platforms for health promotion.[4] Social media consumers also perceived the benefits of using social media as being easily available, in addition to increasing their awareness, providing online support, and helping them conform to current informational norms.[5,6]

Although several studies have investigated social media and mobile applications for OH promotion, there is considerable variations in terms of the scope, outcome, and platform involved.[7-9] A previous systematic review examined broad social media platforms for OH promotion but had limited focus on messaging apps and outcomes related to OH attitudes and behaviors.[7] Furthermore, considering the contemporary and evolving nature of existing social and messaging apps platforms, each with unique features, there is a need to synthesize the evidence related to specific platforms. Synthesizing this evidence would enhance understanding of OH promotion initiatives that employ these specific messaging apps technologies. Therefore, this literature review aims to synthesize existing evidence on the use of social messaging apps for OH promotion by identifying the types of apps used and examining their reported impact on improving OH knowledge, attitudes, behaviors, and OH status.

Materials and Methods

Search strategy

A systematic search was conducted across four databases (PubMed, Scopus, Web of Science, and Medline) using a combination of search terms for social messaging apps, oral health promotion, and oral health impacts to address the research objectives. Boolean search methods such as “AND” and “OR” were used to find relevant articles. Example of search strategies include ("social networking" OR “instant messaging” OR “messenger” OR “chat” OR Snapchat OR WeChat OR WhatsApp OR Telegram) AND ("oral health education" OR "oral health promotion" OR "oral health information") AND ("oral health literacy" OR "oral health knowledge" OR "oral health attitude" OR "oral health behaviours" OR "oral health practice" OR "oral hygiene") AND ("young adult" OR adolescent OR adulthood OR youth OR "college student"). Database-controlled vocabulary was used to search subject headings, with relevant synonyms being utilized for searching titles, abstracts, and keywords. The search was limited to English publications from 2004 to Dec 2023 and an updated search was conducted until March 2024. The search results were then exported into the Rayyan web application (http://rayyan.qcri.org) to further assist in the screening process and removal of duplicates.

Selection process

Two reviewers screened and analyzed the titles and abstracts from the list of electronic searches using a screening form based on the inclusion and exclusion criteria. The inclusion criteria are as follows: (1) studies involving social messaging apps for OH promotion; (2) studies with OH knowledge, attitude, behavior, and OH status based on clinical indicators; (3) experimental and quasi-experimental study designs; (4) peer-reviewed journal articles. The exclusion criteria are as follows: (1) studies of social messaging apps for surveillance/mediators; (2) editorial, case report, book chapter, literature review, and consensus; (3) studies of training or personal development using social messaging apps; (4) studies of social messaging apps as recruitment tools; (5) studies involving specific mobile health application.

Data extraction

Data were extracted by the first reviewer and verified by the second reviewer using a standardized data extraction form. The extracted information included general study characteristics (author, country, year, study design, and target group), study duration, types of social

messaging apps, OH promotion methods, and KAB or OH status outcomes (Table 1). Any discrepancies were resolved through discussion with a third reviewer.

Results

Literature identified from the search

A total of 601 articles were identified through electronic database searches. Of the total, 106 duplicate articles were removed, and 495 articles underwent screening based on their titles and abstracts. Among these, 431 articles were considered irrelevant and consequently removed, resulting in 64 articles retrieved for their full-text review. Subsequently, another 49 articles were excluded, resulting in 15 articles eligible for data extraction and analysis.

Characteristics of included articles

A total of fifteen studies were included for analysis. These studies originated from twelve countries spanning from Asia, the Middle East, Europe, and South America. The most common studies are from Saudi Arabia (n=4), India (n=3), Malaysia (n=2), and China (n=2). Single studies were conducted in Italy, Thailand, Brazil, and Iran. In terms of the study design, most of the studies utilized randomized controlled trials (n=12). Specifically, there were six randomized controlled trials[10-15], three single-blinded randomized controlled trials[16-18], two non-blinded randomized control trials[19-20], and one double-blinded randomized controlled trial.[21] The remaining three studies were quasi-experimental designs.[22-24]

Four studies evaluated outcomes solely in terms of self-reported improvements in OH knowledge, attitudes, and behaviors.[12,22,23] Meanwhile, six studies reported quantifiable OH status using clinical parameters (i.e, gingival status, dental plaque, caries free) as primary outcomes.[11,14,15,17,19,21] The remaining five studies assessed a combination of variables on OH knowledge, attitudes, and behaviors as well as OH status as study outcomes.[13,18,20,23,24] The characteristics of included studies are summarized in Table 1.

Characteristics of social messaging apps used

The most popular social messaging intervention was delivered using WhatsApp (n=9). On the contrary, Telegram was used as an intervention in two studies, similar to the WeChat application.[10,17] A single study utilized Snapchat[16] and Chatbot Messenger.[24]

The included studies identified several OH promotion activities using social messaging applications. Most of the studies utilized social messaging apps to deliver educational and motivational content (n=8). Three studies employed these apps to send reminders to practice oral hygiene, while one study provided support to encourage self-monitoring of OH behavior. The remaining three studies combined multiple OH promotion activities delivered through social messaging apps (Table 2).

These various activities share common characteristics: 1) providing tailored and personalized educational materials, 2) offering interactive and engaging content, and 3) providing continuous information and support to participants. Firstly, tailored OH promotion delivered via social messaging apps effective on specific user groups such as parents/caregivers[23], patients[15], students[13], and pregnant women[16] to improve their oral hygiene and gingival health.

Secondly, social messaging apps enable experts to disseminate visually interactive content like videos and infographics to educate audiences and promote OH behaviors.[18,23] For example, delivering infographic-based information through WhatsApp to orthodontic patients as opposed to providing the same information through plain text messages.[11] Moreover, social messaging enables audio narration to complement the information delivered for better comprehension.[18] Additionally, social messaging platforms permit discussions between patients and OH providers in order to exchange condition-specific information, advice, and treatment recommendations.[12]

Finally, this platform also allows ongoing informational and social support delivery for the intended audiences. For instance, regular reminders and OH notifications delivered to patients over social messaging apps provide a consistent flow of information to address the needs and demands of the target populations.[13,15,17,18]

Impact of social messaging app interventions on knowledge, attitude, and behaviors

Two studies of college students demonstrated that social messaging apps have significantly improved oral cancer knowledge and promoted further online discussions compared to conventional audio-visual aids.[12,13]

Table 1: Summary of the characteristics of included studies (n=15).

Study	Country	Study Design	Sample Size	Outcome Measure
Aboalshamat *et al*., 2019	Saudi Arabia	Single-blinded parallel group RCT	68	OH Knowledge • Pregnancy-related OH knowledge
Al Gunaid *et al*., 2021	Saudi Arabia	Quasi-experimental	70	OH Knowledge • Orthodontic topics
Al-ak'hali *et al*., 2020	Saudi Arabia	Randomised controlled trial	43	OH Status • PI • GI
Borujeni *et al*., 2021	Iran	Single-blinded parallel group RCT	60	OH Status • PI • Gingiva colour & consistency
Deghatipour *et al*., 2021	Iran	Randomised controlled trial	439	OH Behaviour • Childern's OH care behaviour • Children's sweet consumption OH Status • % caries-free children
Li *et al*., 2016	China	Non-blinded, two-arm randomised controlled trial	224	OH Behaviour • No failed attendance • Duration of treatment OH Status • PI • MGI
Lotto *et al*., 2020	Brazil	Single-blinded, 2-parallel arm, randomised controlled trial	104	OH Knowledge • Parental eHealth literacy • Children's sugar-free sweets consumption OH Status • % caries free lesion
Malik *et al*., 2019	India	Randomised controlled trial	34	OH Status • PI • MGI • BI
Mustafa *et al*., 2022	Malaysia	Quasi-experimental	123	OH Behaviour • Children's oral care
Nayak P *et al*., 2018	India	Cluster randomised controlled trial	182	OH Knowledge • Tobacco • Oral cancer
Pithpornchaiyakul *et al*., 2022	Thailand	Quasi-experimental	71	OH Knowledge • Toothbrushing information OH Behaviour • Toothbrushing frequency
Saxena & Gunja, 2022	Malaysia	Double-blinded parallel group RCT	54	OH Status • Mean plaque scores
Subburaman *et al*., 2021	India	Randomised interventional study	140	OH Knowledge • KAB score OH Status • PI • OHI • MGI
Wu *et al*., 2022	China	Non-blinded randomised controlled trial	44	OH Status • PI • BOMP
Zotti *et al*, 2019	Italy	Randomised controlled trial	60	OH Status • Changes in intercanine width OH Behaviour • Compliance with orthodontic treatment

BI: bleeding index; BOP: bleeding on probing; BOMP: bleeding on marginal probing index; GI: gingival index; KAB: knowledge, attitudes, behaviour; MGI: modified gingival index; OH: oral health; OHE: oral health education; OHI: oral hygiene instruction; PI: plaque index.

Table 2: Oral health promotion activities identified on social messaging apps.

Oral health promotion activities	References	Number of articles
Deliver educational and motivational content	Aboalshamat *et al*, 2023[16]; Al-Ak'hali *et al*, 2020[15]; Al-Gunaid *et al*, 2021[22]; Borujeni *et al*, 2021[17]; Deghatipour *et al*, 2021[10]; Lotto *et al*, 2020[18]; Mustafa *et al*, 2022[23]; Subburaman *et al*, 2021[13]	8
Deliver oral hygiene reminders	Li *et al*, 2016[20]; Malik *et al*, 2019[14]; Saxena and Gunjal, 2021[21]	3
Support self-monitoring	Zotti *et al*, 2019[14]	1
Multiple activities	Nayak *et al*, 2018[12]; Pithpornchaiyakul *et al*, 2022[24]; Wu *et al*, 202[2][19]	3

Parental intervention via WhatsApp increased OH knowledge and shifted attitudes and behaviors around children's oral health.[23] Additionally, orthodontic patients receiving WhatsApp images, videos, and texts showed substantial gains in OH knowledge.[22] Table 3 shows a summary of the reported OH knowledge, attitude, and behavior (KAB) outcomes.

Impact of social messaging apps intervention on OH status outcomes

WhatsApp reminders and motivation increased oral hygiene compliance among participants.[11,13] Another study found gradual gains in oral hygiene and gingival health over time. Additionally, WhatsApp reminders with added graphic image on toothbrushing, reduced plaque, gingival bleeding, and inflammation as opposed to plain text messages reminders.[11] Tracking active engagement through intraoral photos through WeChat intervention has also strengthened intentions and behaviors beyond passive information consumption among orthodontic patients.[14,19] The impact of social messaging apps intervention on OH status outcomes is summarized in Table 4.

Impact of social messaging apps on the combination of OH knowledge, attitude, behavior and OH status

Studies reporting a combination of KAB and OH outcomes showed that social messaging interventions boosted parental eHealth literacy and reduced child sugar intake.[18,23] Meanwhile, a Chatbot messenger intervention targeting parents and caregivers, significantly increased overall OH knowledge and reduced plaque accumulation.[24] However, when comparing social messaging app interventions to a more comprehensive mixed approach, a study[10] found that social messaging apps alone resulted in the lowest percentage of caries-free children compared to groups receiving a blended intervention including in-person education and dialogue (Table 5).

Discussion

Since social messaging apps have achieved widespread use and acceptance, they present potential and economical tools for impactful and equitable OH promotion across populations. Compared to conventional brochures, public service announcements, or mass media, social messaging platforms provide a personalized and engaging avenue for healthcare communication.[25] For instance, social messaging apps permit consumer-centric content sharing, thus allowing some degree of anonymity or desired personal connection.[26,27] Furthermore, transforming OH promotion intervention into a more accessible, interactive, and engaging process offers diverse tools for healthcare experts to creatively connect with both patients and the public.[27]

Recent evidence highlights that messaging apps are increasingly preferred channels for delivering OH information, particularly among adolescents and young adults.[7] Unlike traditional social media platforms, where lengthy posts are often overlooked, users of messaging apps tend to engage more with concise, visually supported content.[23,26,27] Kite *et al*.,[28] noted that young individuals generally avoid reading comprehensive health materials, suggesting that within messaging environments, health messages must be brief, visually appealing, and supported by images or short videos to sustain attention and encourage information sharing. Photographs, in particular, have been shown to be highly effective for conveying OH information due to their ability to communicate messages quickly and clearly.[22,23]

In addition, messaging apps allow users to receive content directly and privately, making it easier to blend succinct text with multimedia elements such as images,

Table 3: Social messaging applications and oral health knowledge, attitude, and behavior outcomes.

Reference (Country)	Study design	Study population and sample size	Study duration	Social messaging apps	OH promotion methods	Findings
Aboalshamat *et al*, 2019[16] (Saudi Arabia)	Single-blinded parallel group RCT	68 pregnant women in Saudi Arabia	2 weeks	Intervention: Snapchat	Snapchat stories (5-10 minutes video sent twice a week)	No significant differences were found in the scores between groups at all times (p>0.050)
				Comparison: WhatsApp	Written flyers	
Al-Gunaid *et al*, 2021[22] (Saudi Arabia)	Quasi-experimental	70 members of the public	14 days	Intervention: WhatsApp	Orthodontic-related information being sent to the groups every day	Drastic improvement in patient knowledge from early stage to end-stage, with a significance level of p<0.01. All types of media formats (image, video, text) were significantly effective in improving the patients' knowledge (p<0.001).
Nayak *et al*, 2018[12] (India)	Cluster RCT	182 first- and second-year Bachelor of Commerce students	4 weeks	Intervention: WhatsApp	WhatsApp messages (pictures and video) were sent daily with a group discussion on the WhatsApp chat room every Saturday	The intervention group showed a statistically significant increase in all knowledge core topics except the etiology of oral cancer (p=0.280). The control group showed a significant increase in knowledge scores found in only two core topics; signs of oral cancer (p<0.001) and epidemiology of oral cancer (p<0.001). A significant difference between knowledge scores post-intervention (t=−15.05, p<0.001).
				Comparison: Power-Point presentation	A PowerPoint presentation was held twice every week, and at the end of the sessions, queries were answered.	
Mustafa *et al*, 2022 (Malaysia)[23]	Quasi-experimental	123 parents and care-givers of preschool children	5 months	WhatsApp	20 infographics of oral health (based on 10 dental topics) sent 2 weeks	Significant differences in oral and dental care of children before and after the infographics were sent to the respondents (p<0.001)

OH: oral health; RCT: randomized controlled trial

Table 4: Social messaging applications and oral health status.

Reference (Country)	Study design	Study population and sample size	Study duration	Social messaging apps	OH promotion methods	Findings
Al-ak'hali *et al*, 2020 (Saudi Arabia)[15]	RCT	43 male dental patients with gingivitis	3 months	Intervention: WhatsApp	Weekly Dental care WhatsApp messages	Statistically significant improvement in oral health status (PI and GI score) in both groups.
				Comparison: Conventional oral health education	Comprehensive OHE given at baseline	The averages of PI and GI were not significantly different between both groups at any time point of measurement (baseline, after one, and three months; $p>0.05$).
						Indicative no additional effects for using WhatsApp messages for study purposes.
Borujeni *et al*, 2021[17] (Iran)	Single-blinded RCT	60 fixed orthodontic patients	16 weeks	Intervention: Telegram	OHE videos of 5-7 minutes via Telegram	There was a statistically significant difference in PI and BOP between the two groups at the 3rd and 4th appointments ($p\leq0.01$).
				Comparison: Conventional OHE	Conventional comprehensive OHE after the 1st session	Significant differences in gingiva consistency between the intervention and control groups at the 3rd and 4th appointments ($p\leq0.05$).
Malik *et al*, 2019[11] (India)	RCT	34 orthodontic patients in active treatment with fixed appliances in both arches	5 weeks	Intervention group: WhatsApp	WhatsApp reminders on OHI with added graphics sent twice a week	Significantly lower BI ($p<0.001$), MGI ($p<0.001$), and PI ($p<0.001$) scores were observed at follow up (after 8 weeks) and improved over time in the intervention group.
				Comparison: Practo Software (text messaging)	Text messages on OHI without added graphics sent twice a week	WhatsApp messages with graphic content were more effective in reminding orthodontic patients of oral hygiene practices.
Saxena and Gunjal, 2022[21] (Malaysia)	Double-blinded parallel-group RCT	54 orthodontic patients with fixed orthodontic mandibular and maxillary appliances	4 weeks	Intervention: WhatsApp	WhatsApp reminders (text and images) to maintain oral hygiene sent once a week	No statistically significant difference in the mean plaque scores intergroup regardless of time ($p=0.360$). At eight weeks, the WhatsApp group showed a greater reduction in the plaque score with a mean difference of 8.12, followed by the email group with a mean difference of 6.32 and the least reduction in the control group with a mean difference of 3.90.
Wu *et al*, 2022[19] (China)	Non-blinded RCT	44 fixed orthodontic patients	12 weeks	Intervention: WeChat	WeChat mini program: - Notification for toothbrushing - Periodic use of a disclosing agent with photography - Toothbrushing, clocking with videos of toothbrushing	Significant differences in dental plaque between the intervention and control at 6 weeks ($p<0.0001$) and 12 weeks ($p<0.005$). The intervention group recorded a gradual declining trend of gingival bleeding than the control group.
				Comparison: Conventional routine oral health education	Received care via conventional methods	
Zotti *et al*, 2019[14] (Italy)	RCT	60 patients undergoing post-orthodontic treatment	12 months	Intervention: WhatsApp	Intraoral selfies shared in the WhatsApp group to earn points for the "Relapse Game" Interaction with another member of the group using text and emoticons.	A significant difference in intercanine width between the groups was noted ($p\leq0.05$). Higher compliance with wearing post-orthodontic retainers among adolescent patients in social media activity with a lower relapse rate.

BI: bleeding index; BOP: bleeding on probing; GI: gingival index; MGI: modified gingival index; OH: oral health; OHE: oral health education; OHI: oral hygiene instruction; PI: plaque index; RCT: randomized controlled trial

Table 5: Social messaging applications, KAB, and oral health status.

Reference (Country)	Study design	Study population and sample size	Study duration	Social messaging apps	OH promotion methods	Findings
Deghatipour *et al*, 2021[10] (Iran)	RCT	439 pregnant women in the second/third trimester of pregnancy	Beginning of pregnancy until 18 months post-delivery	Intervention: Telegram	Telegram messages on behavioral content (n=41) and nutritional content (n=43) every week	A higher number of caries-free children in the intervention group (78.8%) than in the control group (56%). All the four intervention groups except social networks resulted in more chance of being caries-free compared to the control group. Higher frequency of using finger tooth brushing (30%) at 24 months in the intervention groups compared to the control group, but the lowest difference was in the social network group. Higher no-sweet intake in the intervention group than in the control group, but the lowest difference was in the social network group. Intervention of social networks alone has not been effective in the study compared to other methods.
				Comparison: Comprehensive	Comprehensive intervention combining all methods	
				Group discussion	Group discussions performed every three months	
				Face-to-face intervention	Face-to-face by PHCP implemented (at a 6-month interval) during routine baby checkups	
Li *et al*, 2015[20] (China)	Non-blinded, two-arm randomized controlled trial	224 adolescent and adult orthodontic patients	33 months	Intervention: WeChat	Reminder (brief text) and educational message (rich texts and pictures) sent via WeChat	Shorter duration of treatment in the intervention group than in the control group (range 66-93 weeks vs 75-103 weeks; p=0.007) A smaller percentage of patients with failed attendance in the intervention group than in the control group (RR = 0.42; 95% CI: 0.31- 0.57) There was no significant difference between the two groups in the OPI or MGI in either baseline or endpoint evaluation.
				Comparison group: Conventional management	Not mentioned	
Lotto *et al*, 2020[18] (Brazil)	Single-blinded, 2-parallel arm, randomized controlled trial	104 parents and children aged 36-60 months	6 months	Intervention: WhatsApp	Parents or caregivers received educational text messages and audio narration of ECC via WhatsApp messages every 2 weeks	Similar percentages of caries-free lesions were reported from both groups after 6 months. Participants with the increment of maximum ICDAS did not increase significantly in the intervention group (15.4-23.1%; p=0.125) as opposed to the control group (21.2-36.5%; p=0.008) between 3- and 6-month follow-ups. No significant difference in the parental eHealth literacy eHEALS score between groups at all periods. Parents of the intervention group reported significantly higher consumption of sugar-free sweets but lower intake of sugar-free foods by their children.
				Comparison: Not mentioned	Did not receive any other educational material or information	
Pithpornchaiyakul *et al*, 2022[24] (Thailand)	Quasi-experimental design (pre-test and post-test)	71 pairs of caregivers and children aged 6 months to 36 months	Study I: 21-day FunDee Study II: 30 days FunDee	2 chatbots, 21-day FunDee (Study I) and 30-day FunDee (Study II), based on the protection motivation theory	The chatbots operating on Facebook Messenger were designed to engage and motivate the users through texts, videos, clips, or infographic exchanges from 3-5 minutes each.	Both chatbots recorded statistically significantly increased overall knowledge of children's toothbrushing information. Study I showed a significant difference in the improvement of frequency of daily toothbrushing (p=0.02), along with a significantly greater understanding of child oral healthcare and significant plaque reduction.
Subburaman *et al*, 2021[13] (India)	Randomized controlled trial	140 college students	3 months	Intervention: WhatsApp	Received oral health education through WhatsApp (pictures, videos, and text messages)	Higher mean difference in KAB scores in the intervention group (170.73%, 73.38%, 60.19%) than in the control group (46.19%, 36.45%, 35.67%). Gradual reduction of mean OHI-S and MGI scores recorded in the intervention group. In the control group, the OHI-S score was reduced in the first month but increased during the third and sixth months.
				Comparison: None	Restrained from any form of oral health education after initial exposure at baseline	

CI: confidence interval; ECC: early childhood caries; eHEALS: health literacy scales; ICDAS: international caries detection and assessment system; KAB: knowledge, attitude, behavior; MGI: modified gingival index; OH: oral health; OHI-S: simplified oral health index; OPI: ortho-plaque index; PHCP: primary health care providers; RCT: randomized controlled trial; RR: relative risk.

voice notes, and short video clips. Several studies have demonstrated that supplementing text messages with multimedia components, such as images and audio narration can improve comprehension of OH messages.[11,17,20] For instance, one study enhanced OH information for young parents and caregivers by incorporating voice-read passages within mobile messages, which increased accessibility and supported better understanding.[18] This aligns with U.S. findings showing that multimedia-rich messages are more engaging, reduce language barriers, and provide clearer modelling of preventive behaviors.[29]

In terms of improving OH knowledge among young adults, messaging platforms have been shown to facilitate stronger engagement and retention of key information when motivational and educational content is delivered directly to users' mobile devices.[18,22,23] Additionally, several studies have reported positive changes in oral hygiene behaviors, such as increased tooth brushing frequency, healthier dietary choices, and improved compliance with orthodontic retainer use, following interventions delivered through social messaging apps.[14,24]

A study by Li *et al*.,[20] reported that orthodontic patients showed better attendance and shorter treatment duration. However, the use of messaging app teaching and reminders did not lead to improvements in oral hygiene. In contrast, Wu *et al*.,[19] demonstrated significant oral hygiene improvements among young adults with fixed orthodontic appliances using a WeChat intervention. The main distinction is that the Wu and colleagues study incorporated additional behavior change strategies into the social messaging app, including goal setting, self-monitoring, discussions on coping strategies, and motivational messaging during the volitional phase. These examples suggest that active social communication and accountability via messaging platforms are more effective than simple reminders or information alone.

Studies have shown that physicians and health professionals were the first sources of health information among participants.[30,31] However, clinical consultations between patients and providers are typically brief due to time constraints, limiting tailored interventions and ongoing support. Social messaging enables ongoing convenient OH education and knowledge transfer beyond dental clinic visits. It allows for an ongoing, tailored key OH education exchange between dental appointments.[13,22] Social messaging apps also create opportunities for dialogues for patients to ask questions and clarify misunderstandings and doubts about their condition[12], enabling healthcare providers to give real-time feedback on techniques like brushing beyond clinical settings and correcting of improper methods to reduce disease risk.[19] Similarly, an alcohol reduction study among students stresses the importance of having timely psychosocial support from healthcare providers. Collaboration by setting appropriate goals with students through messaging apps can improve the outcomes of drinking habits.[32]

Despite evidence of improved KAB and OH status, several studies did not show significant differences in either KAB or OH status.[15,16,21] A study from Al-ak'hali *et al*.,[15] reported that WhatsApp usage in health education delivery did not yield any added benefit to the conventional method of oral hygiene delivery, which is likely due to the intensive baseline instructions provided by an OH professional to both groups on proper brushing techniques, flossing, and oral hygiene aids. Such intensive initial education may have minimized any additional benefits of WhatsApp messaging.[15] Moreover, interventions that combine social messaging app components with other modalities, such as in-person instruction and dental visits, seem to achieve better outcomes than social messaging apps alone.[10] Thus, it is arguable that comprehensive in-person intervention has probably managed to provide better skill demonstrations and support that may be limited to digital-only interventions. Similarly, according to a systematic review, digital interventions such as using smartphone apps provide mixed evidence of effectiveness against non-digital interventions on health behaviors among adults.[33] Therefore, in light of evidence that has been explored in the literature, a blended model that integrates social messaging apps as a complement to non-digital delivery methods such as in-person interventions and peer support should be considered to provide optimal OH promotion efforts.

This review is subject to several limitations. Firstly, it restricts inclusion to English publications, excluding non-English publications and grey literature. The scope is confined to studies employing intervention and control or pre-post analysis, with a specific focus on the impact of OH knowledge, attitude, behavior and selected OH status using clinical indicators. The review also does not account for potential influences arising from varying follow-up periods or differences in patient participation. Thus, care-

ful interpretation of the review's findings is warranted.

Conclusions

It can be concluded that social messaging apps demonstrate potential to promote positive OH knowledge, attitudes, and behaviors, and to some extent improve OH status (i.e, gingival status, dental plaque, caries free). Given the platform's contemporary form and continuous evolution, more in-depth research into optimal use for OH promotion is required. Investigating user preferences, engagement tactics, and the most beneficial social messaging approach can inform patient-centric solutions that enhance OH status. Furthermore, examining consumer privacy and functionality needs will support the development of adaptive, ethical interventions that enhance patient health alongside technological adoption.

Conflict of Interest

The authors declare no conflict of interest.

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Dental Stem Cells: A Gateway to Regenerative Dentistry and Medicine

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Abstract

Dental stem cells (DSCs) have emerged as a pivotal resource in the evolving fields of regenerative medicine and dentistry, owing to their accessibility, ethical acceptability, and multipotent to pluripotent capabilities. Sourced primarily from dental pulp, exfoliated deciduous teeth, and periodontal tissues, DSCs demonstrate robust potential to differentiate into various cell lineages, including osteoblasts, neurons, hepatocytes, insulin-producing cells, and cardiomyocytes. This review explores the origin, classification, and biological properties of embryonic, fetal, and adult stem cells, with a dedicated focus on DSCs. It discusses the current and potential therapeutic applications of DSCs in treating neurological disorders, cardiovascular diseases, diabetes, liver conditions, ocular defects, and bone regeneration. The manuscript also emphasizes the significance of DSC banking, technological advances in their isolation and application, and the molecular mechanisms underpinning their regenerative capacity. By integrating recent findings and clinical insights, the study underscores DSCs as a promising, minimally invasive, and patient-specific tool for future personalized regenerative therapies.

Keywords: dental stem cells, regenerative dentistry, regenerative medicine, stem cell banking, stem cell therapy

Introduction

Stem cells are a unique group of undifferentiated cells possessing the ability to self-renew and differentiate into specialized cell types, playing a vital role in tissue homeostasis and repair. These cells form the biological foundation for regenerative medicine due to their capacity to restore or replace damaged tissues. The concept of stem cells was first introduced in the late 19th century by German biologist Ernst Haeckel, and further established when Alexander Maximow, in 1909, proposed the existence of a common precursor for blood cells–paving the way for modern stem cell biology.[1]

Stem cells are broadly classified based on origin (Embryonic Stem Cells (ESCs), Fetal and Extra-Embryonic Stem Cells, Adult stem cells) and potency (Totipotent, Pluripotent, Multipotent and Unipotent) (Figure 1). By origin, they include ESCs, derived from the inner cell mass of the blastocyst during early embryonic development, are pluripotent cells capable of differentiating into all three germ layers, ectoderm, mesoderm, and endoderm except for placenta and umbilical cord tissues. They possess unique properties such as indefinite self-renewal, high proliferative capacity due to a shortened G1 phase, and the potential to form diverse cell types, making them valuable in regenerative medicine, tissue replacement, and research on development and diseases. However, their use is limited by ethical concerns surrounding embryo destruction, the risk of immune rejection, limited functional integration in tissues like the heart, and the potential formation of tumor such as teratocarcinomas, raising significant safety and ethical considerations for clinical application.[2]

Fetal stem cells, the third major stem cell class, originate from the fetus proper and extra-embryonic structures such as the placenta, amniotic fluid, amniotic membrane, and Wharton's jelly. These perinatal tissues, often discarded after birth, are rich sources of various stem cells, including hematopoietic stem cells (HSCs) and mesenchymal stromal cells (MSCs). Fetal stem cells are characterized by high plasticity, survival in low oxygen environments, and secretion of angiogenic and trophic factors that promote tissue regeneration. Extraembryonic stem cells, in particular, offer ethical and practical advantages due to their non-invasive sourcing and lower immunogenicity, making them attractive for therapeutic use. However, their limited differentiation potential, lower proliferative capacity, and the need for further research on their applications restrict their current clinical utility.[3] (Table 1)

Adult stem cells, derived from adult tissue. Also known as somatic stem cells or resident stem cells. The adult stem cells have the ability to self-renew themselves, and can be differentiated into a limited number of mature cell types.[4] Adult stem cells include: hematopoietic stem cells, epidermal stem cells, adipose stem cells, neural stem cells, limbal stem cells and hepatic stem cells and mesenchymal stem cells, including dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), and stem cells from apical papilla (SCAPs) and are widely used in therapeutic applications due to lower ethical concerns and immune compatibility.[5]

Since adult stem cells are multipotent cells they are reprogrammed to acquire pluripotent state, the stem cells formed are called Induced Pluripotent Stem Cells (iPSCs). these cells were similar to human ESCs and they could differentiate into cell types of the 3 germ layers *in vitro*.[7] and are a powerful tool in personalized regenerative therapies, albeit with ongoing concerns about genomic instability and tumor risk.[6]

In terms of potency, stem cells may be Totipotent, can differentiate into both embryonic and extraembryonic tissues (e.g., zygote). Pluripotent, can form all cells of the three germ layers (e.g., ESCs, iPSCs). Multipotent, limited to cell types within a lineage (e.g., MSCs, HSCs) and Unipotent, which can generate one cell type (e.g., skin stem cells), with self-renewal ability.[7]

The advent of regenerative medicine has ushered in a transformative era for tissue repair and organ replacement, with stem cells at its core. Among the various sources of stem cells, DSC have garnered increasing attention due to their non-invasive harvesting, immunomodulatory functions, and multilineage differentiation potential, especially for craniofacial, neurological, and orthopaedic regeneration.[8] Compared to other MSC sources such as bone marrow or adipose tissue, dental stem cells are younger in origin, exhibit higher proliferative rates, and retain epigenetic plasticity that enhances their regenerative utility.[9]

Emerging research also highlights the feasibility of using DSC in neurodegenerative diseases, such as Parkinson's and Alzheimer's, due to their neurotrophic

factor secretion and differentiation into functional neurons *in vitro* and *in vivo*.[5] Moreover, ongoing developments in biomaterials, scaffolding, and 3D bioprinting are accelerating the clinical translation of DSC based therapies.[10] As DSC bridge the interface between dental practice and advanced cell-based therapies, they represent a strategic, ethical, and scalable solution for personalized regenerative applications. This review presents DSCs as a promising avenue for future personalized regenerative medicine.

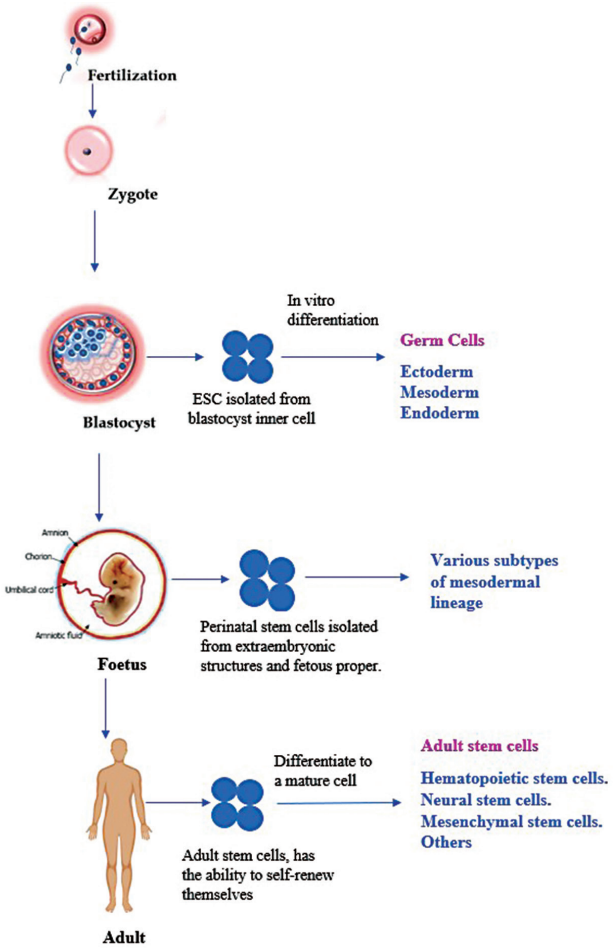

Figure 1: Origin of stem cells.

Dental stem cells

Unlike bones, human teeth have very limited ability to heal or regenerate after injury or disease. Enamel, the outermost layer of the tooth, is acellular and cannot regenerate. However, other dental tissues like dentin, pulp, cementum, and periodontal ligament have some regenerative capacity, depending on conditions.[11]

DSC are a type of adult stem cell found in various parts of the tooth. They can be easily collected from extracted teeth or naturally shedding baby teeth, making them a convenient and minimally invasive source. They are less likely to cause immune reactions or tumors compared to ESCs.[12] Interestingly, stem cells can also be isolated from inflamed or diseased teeth, and these still retain similar regenerative abilities as those from healthy teeth. This suggests that even damaged teeth can be a valuable source of stem cells.[13-15]

DSCs are derived from ectomesenchyme (a mix of ectoderm and mesoderm origin), giving them the ability to form both epithelial (like enamel-producing ameloblasts) and mesenchymal cells (like dentin-producing odontoblasts, cementoblasts, and bone-forming osteoblasts). This dual potential makes them especially useful for full tooth regeneration or repair in the future.[16]

Although dental tissues such as exfoliated deciduous teeth, impacted third molars, and orthodontically extracted premolars are widely recognized as rich sources of MSCs, it must be emphasized that the harvested cellular populations from these tissues are inherently heterogeneous and do not exclusively comprise stem cells.[17] These tissues contain a mixture of various cell types including fibroblasts, endothelial cells, immune cells, and progenitor cells, necessitating rigorous methodologies to isolate and characterize the true stem cell fraction.[18]

Table 1: Stem cells isolated from extra embryonic structures.

Extra embryonic structures	Stem cells
Amniotic membrane	Amniotic epithelial stem cells Amniotic mesenchymal stem cells Amnion-derived stem cells
Amniotic fluid	Amniotic fluid mesenchymal stem cells Amniotic fluid derived - stem cells
Wharton's jelly	Umbilical cord matrix mesenchymal stem cells
Placenta	Placenta derived stem cells

To accurately identify MSCs within these mixed populations, standard protocols typically begin with enzymatic digestion, such as collagenase and dispase treatment, followed by *in vitro* culture under MSC-supportive media.[19] Following isolation, surface marker profiling remains a critical step, with cells required to express positive markers like CD73, CD90, and CD105 while lacking hematopoietic markers such as CD34 and CD45, in accordance with the International Society for Cellular Therapy (ISCT) criteria.[20] Additional validation involves functional assays including colony-forming unit fibroblast (CFU-F) assays, trilineage differentiation into osteogenic, adipogenic, and chondrogenic lineages, and assessments of proliferation and immunomodulatory capacity.[21,22] Only cell populations meeting these phenotypic and functional benchmarks should be classified as dental MSCs or dental stem cells.[23]

Classification of dental stem cells

To date, seven distinct types of human dental stem or progenitor cells have been isolated and characterized, including DPSCs[11], SHED[24], PDLSCs[25], dental follicle progenitor cells (DFPCs)[26], and SCAP.[27] Among these, PDLSCs and DFPCs are classified as periodontium-related stem cells[28], while DPSCs, SHED, and SCAP are considered dental pulp-related stem cells. In addition, subsequent research has identified other DSCs, such as human natal dental pulp stem cells (NDP-SCs) [29] and gingival mesenchymal stem cells (GMSCs).[30] (Figure 2)

Dental pulp stem cells

DPSCs, first identified in 2000 by Gronthos *et al*., are a type of adult MSC found in the pulp tissue of permanent teeth.[11] They possess high self-renewal capacity and can differentiate into multiple cell types including odontoblasts, osteoblasts, neurons, adipocytes, chondrocytes, myocytes, and even melanocytes. DPSCs are easily accessible from extracted teeth and even from inflamed pulp tissues, making them a convenient and promising source for regenerative therapies.[31] They can be isolated using either enzymatic digestion or a simple outgrowth method, and they retain their regenerative potential even after long-term preservation. To understand and identify these cells, researchers look for specific surface and intracellular markers.

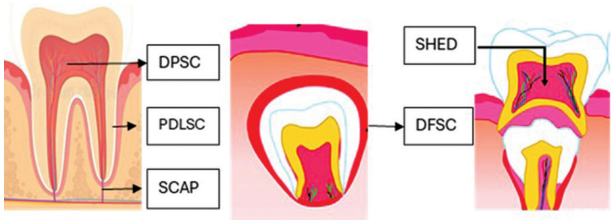

Figure 2: Different DSC types and where they are found in related tissues DPSCs-dental pulp stem cells, PDLSc- periodontital ligament stem cells, SCAP-stem cells from apical papilla, DFSc- dental follicular stem cells, SHED- stem cells from human exfoliated deciduous teeth, are used.

They express classical MSC markers like CD73, CD90, and CD105[32], while lacking hematopoietic markers such as CD34 and CD45.[32] Intracellular pluripotency markers like OCT-4, NANOG, and SOX2 suggest their stemness and multipotent nature.[33] Recent research highlights a regenerative subpopulation marked by CD24a and Sp7 with enhanced dentin-forming ability.[34] Additionally, perivascular markers such as CD146 and NG2 support their role in vascular and neural repair.[35] Upon differentiation, they express markers like ALP, DMP-1, BSP, and OPN, indicating odontogenic and osteogenic potential. These insights affirm DPSCs as a versatile tool for tissue engineering and regenerative dentistry.[36]

Stem cells from human exfoliated deciduous teeth

SHED are a population of highly proliferative, multipotent stem cells derived from the pulp of naturally shed deciduous teeth, first described by Miura *et al*., in 2003.[17] These cells possess the capacity to differentiate into various lineages including odontoblasts, osteoblasts, adipocytes, chondrocytes, neural cells, and hepatocyte-like cells, making them a valuable source for regenerative therapies.[37] SHED is easily accessible during physiological tooth exfoliation, providing a non-invasive and ethically acceptable route for stem cell procurement.[37] Compared to other DSCs, SHED show higher proliferation rates and enhanced telomerase activity, contributing to their superior regenerative potential.[38]

SHED express a typical MSC profile, including surface markers such as CD29, CD44, CD73, CD90, CD105, CD166, and STRO-1, while lacking hematopoietic markers like CD14, CD34, CD45, and HLA-DR.[39,40] They also show expression of pluripotency-associated markers including OCT-4, NANOG, and SSEA-4.[41] Their strong

neurogenic potential is reflected by the spontaneous expression of neural markers such as nestin, βIII-tubulin, SOX1, SOX2, CD271, MAP2, GFAP, CD56, and doublecortin (DCX), even before neurogenic induction.[41,35] Flow cytometric studies indicate consistently high expression rates (70-95%) for CD73, CD90, and CD105, supporting their MSC identity.[41] These findings further validate SHED as a potent source for tissue engineering, particularly in neural and craniofacial applications.

Periodontal ligament stem cells

PDLSCs were first identified by Seo *et al*., in 2004 from the periodontal ligament tissue of extracted human third molars.[18] They are mesenchymal progenitors located in the ligament that connects the tooth root to the alveolar bone. PDLSCs exhibit high clonogenicity, proliferation, and multipotent differentiation into osteoblasts, cementoblasts, chondrocytes, and adipocytes, making them excellent candidates for periodontal and alveolar bone regeneration.[42] They can be non-invasively harvested, cultured, and preserved, offering great potential in regenerative dentistry.

PDLSCs express a standard MSC surface marker profile including CD29, CD44, CD73, CD90, and CD105, along with the perivascular marker CD146, while lacking hematopoietic markers CD34 and CD45.[43] Uniquely, they also express periodontal-specific extracellular matrix proteins like asporin, osteopontin (OPN), and osteocalcin, which are not typically seen in bone marrow or adipose tissue-derived MSCs.[42] These markers support their specialized role in osteogenic and periodontal tissue regeneration.

Dental follicle progenitor cells

DFPCs were first isolated and characterized by Morsczeck *et al*., in 2005 from the dental follicle tissue that surrounds unerupted tooth germs.[19] These progenitor cells are essential precursors for periodontal tissues such as cementum, alveolar bone, and periodontal ligament. DFPCs exhibit fibroblast-like morphology, high migratory capacity, and the ability to differentiate into various lineages, including osteogenic, cementogenic, adipogenic, and neurogenic types–making them promising candidates for periodontal and craniofacial tissue engineering.[43]

Phenotypically, DFPCs express mesenchymal stem cell markers such as CD105, CD44, CD29, CD73, CD90, CD146, STRO-1, Notch1, and HLA-ABC, while lacking hematopoietic and endothelial markers like CD34, CD45, CD14, CD31, and CD117.[44] Their ability to form periodontal tissues has been supported through both *in vitro* and *in vivo* studies, reinforcing their role in tissue regeneration and their relevance in therapeutic applications.[44]

Stem cells from apical papilla

SCAP were first identified by Sonoyama *et al*., in 2006 from the apical tissue of immature permanent teeth.[20] These cells reside in the apical papilla at the root tips of developing teeth and play a crucial role in root maturation and dentinogenesis. Compared to DPSCs, SCAP exhibit faster proliferation, higher colony-forming efficiency, and superior mineralization potential, making them especially valuable for regenerative endodontics and craniofacial tissue engineering.[45]

SCAP express classical mesenchymal stem cell markers such as STRO-1, CD29, CD73, CD90, CD105, CD106, CD146, and CD166, while lacking hematopoietic lineage markers like CD34, CD45, and CD14.[39] A defining feature of SCAP is the expression of CD24, a specific marker not shared with other DSCs, which aids in their identification and confirms their unique lineage commitment. The co-expression of STRO-1 and CD146 is associated with a highly regenerative subpopulation, especially relevant in dental tissue repair.[39]

Human natal dental pulp stem cells

NDP-SCs are derived from the pulp tissue of natal teeth–teeth present at birth or erupting within the first month of life. These cells were first isolated and characterized by Miura *et al*., in 2003, who identified them as a unique subpopulation of MSCs with embryonic-like origin and broad regenerative potential.[22] NDP-SCs exhibit higher proliferative capacity and broader differentiation potential than adult dental stem cells like DPSCs, showing capabilities for osteogenic, adipogenic, chondrogenic, and neurogenic differentiation. Their accessibility at birth and non-invasive collection procedure makes them a promising and ethically favourable source for regenerative medicine and tissue engineering applications.

NDP-SCs express typical MSC markers such as CD73, CD90, and CD105 while lacking hematopoietic markers CD34 and CD45. They also show elevated

expression of pluripotency-associated genes including OCT-4 and NANOG, suggesting more primitive stem-like characteristics compared to DPSCs. Their high proliferation rate, multipotency, and immunomodulatory abilities underscore their potential in bone, neural, and dental tissue regeneration.[46]

Gingiva-derived mesenchymal stem cells

GMSCs were first identified by Zhang *et al*., in 2009 from the lamina propria of gingival tissue.[23] They are easily harvested from healthy or inflamed gingiva, making them highly accessible and minimally invasive for clinical use. GMSCs show robust proliferation, self-renewal, and immunomodulatory properties, which make them suitable for soft tissue regeneration, autoimmune diseases, and even systemic disorders.[47] These cells have demonstrated multilineage differentiation into osteogenic, chondrogenic, adipogenic, and neurogenic cell types, underlining their potential in regenerative medicine and tissue engineering.[47]

GMSCs express common MSC markers such as CD73, CD90, CD105, CD29, and CD44 while lacking hematopoietic markers like CD34 and CD45.[39] They also show elevated levels of CD146 and STRO-1, markers indicative of their perivascular origin. Notably, GMSCs possess unique immunoregulatory capabilities through cytokine release and T-cell suppression, distinguishing them from other oral MSC populations.[39] These traits highlight GMSCs as a promising, practical stem cell source for both dental and systemic therapeutic applications.

Dental stem cells in regenerative dentistry

In regenerative dentistry, DSCs have emerged as a promising therapeutic tool for restoring damaged dental and craniofacial structures. Their ability to differentiate into odontogenic, osteogenic, chondrogenic, and neurogenic lineages underpins their application in regenerative endodontics, periodontal regeneration, craniofacial bone repair, implantology, and experimental tooth regeneration. Preclinical and clinical studies have demonstrated their capacity to promote pulp-dentin complex formation, regenerate functional periodontium, repair critical-size bone defects, and enhance implant osseointegration. These translational advances highlight the growing clinical relevance of DSC-based therapies in modern dental practice.[48] (Table 2)

In pulp–dentin complex regeneration, preclinical and early clinical studies demonstrate that autologous or allogeneic DPSCs, when combined with scaffolds and bioactive factors, can regenerate dentin–pulp-like tissue, restore vascularity, and partially reinstate sensory function. Zhang *et al*.,[49] reported both preclinical and pilot human evidence indicating safety and functional improvement in pulp vitality using DPSC transplantation, highlighting their potential in regenerative endodontic therapies (RETs). Recent systematic reviews (2024-2025) conclude that cell transplantation and cell-homing approaches consistently outperform traditional apexification, though large-scale clinical trials remain limited.[50,51]

In periodontal regeneration, PDLSCs have demonstrated robust potential in restoring periodontal ligament, cementum, and alveolar bone. A 2024 study by You *et al*.,[52] showed that PDLSC injections not only promoted periodontal tissue repair in animal models but also modulated the oral microbiome by increasing beneficial taxa. Scaffold-free, three-dimensional PDLSC pellet constructs have recently been shown to facilitate alveolar ridge preservation and periodontal ligament-like complex formation without the need for synthetic scaffolds, indicating a promising translational strategy.[53] Reviews from 2023-2025 also highlight PDLSC-derived exosomes as a cell-free alternative with significant immunomodulatory and antimicrobial potential.[54]

For alveolar and jaw bone regeneration, systematic reviews of preclinical studies (2025) have found that DPSCs and SHED, combined with scaffolds, significantly enhance bone volume fraction, bone mineral density, and osteogenic marker expression compared to scaffolds alone.[55] In the clinical domain, ongoing randomized controlled trials are evaluating the use of autologous oral-derived MSCs with biomaterials for alveolar ridge augmentation and implant site preparation.[56]

Salivary gland regeneration is another emerging field, particularly for xerostomia secondary to Sjögren's syndrome or radiotherapy. Hu *et al*.,[57] demonstrated that DPSC-derived exosomes could restore salivary flow and glandular function in a NOD mouse model via the GPER-cAMP/PKA/CREB signalling pathway. Recent organoid and 3D bioprinting advances have enabled the creation of salivary tissue biorepositories, with oral-derived progenitors playing a key role in developing implantable salivary units.[58]

Beyond cell-based strategies, cell-free therapies involving DSC-derived exosomes and secretome products have gained traction due to their reduced immunogenicity, ease of storage, and capacity to deliver trophic, angiogenic, and antimicrobial signals. These vesicles have demonstrated efficacy in multiple animal models for pulp regeneration, periodontal healing, and salivary gland rescue, representing a scalable translational approach. Collectively, while DSC-based regenerative dentistry has matured substantially in preclinical research, clinical translation remains at an early stage. The next decade will likely focus on controlled multicentre trials, advanced biomaterial integration, and regulatory pathways to enable routine clinical application.

Dental stem cells in regenerative medicine

The definition of regenerative medicine is an emerging field of multidisciplinary research and clinical applications that focuses on the replacement, repair, or regeneration of tissues, cells, or organs to restore impaired function resulting from any cause, including ageing, disease, trauma, or congenital defects. In 1985, Y.C. Fung, a trailblazer in the fields of biomechanics and bioengineering–which are actually subfields of regenerative medicine–first used the term "tissue engineering." According to Langer and Vacanti (1993), tissue engineering is an interdisciplinary field that uses the concepts of biology and engineering to design biological replacements that preserve, enhance, or restore tissue function. Medical applications involving heart therapies[59], brain tissue regeneration[60], muscular dystrophy therapies[61], bone regeneration[62], and application in liver disease, diabetes mellitus, regenerative ocular therapy are all possible with dental stem cells.[63]

Dental stem cells as a substitute for heart tissue regeneration

DSCs have been identified as a potential option for heart regeneration due to their juvenile nature and inclination towards the cardiac lineage through the PI3-Kinase/Akt signalling pathway. Dental tissues contain stem cells or progenitors that can differentiate into a variety of cell types, including neurons, bone, cartilage, fat, and smooth muscle. They also have a high proliferative capacity and are clonogenic *in vitro*. It may be able to use multipotent stem cells in allogeneic situations because numerous research has shown that these cells are not rejected by the immune system. Furthermore, these amazing cells are readily available in large quantities and require a less intrusive isolation process than bone marrow aspiration.[64]

Medications such as anti-hypertensive or anti-arrhythmias, which primarily function to halt the progression of heart failure, are used as part of current therapy for cardiovascular illnesses. Additional cutting-edge therapies include bypass and stent surgery, which restores blood flow to the heart during ischemia in order to preserve the cardiomyocytes that remain.[65] However, the drawback

Table 2: The main biological properties of DSCs.

No.	Type of DSCs	Location	Differentiation Potential	
			In vitro	*In vivo*
1.	DPSCs	Dental pulp of permanent tooth	Odontogenic, Osteogenic, Neurogenic, Adipogenic, Myogenic, Chondrogenic	Dentin/pulp-like complex
2.	SHED	Dental pulp of deciduous tooth	Odontogenic, Osteogenic, Neurogenic, Adipogenic, Myogenic, Chondrogenic	Dentin formation, new bone formation by recruiting host murine cells
3.	PDLSCs	Periodontal ligament	Osteogenic, Cementogenic, Adipogenic, Chondrogenic, Insulin-producing cells	Cementum/periodontal ligament structure
4.	DFPCs	Dental follicle of developing tooth	Osteogenic, Adipogenic Neurogenic, Chondrogenic	Mineralized tissue structure
5.	SCAP	Apical papilla of developing tooth	Odontogenic, Osteogenic, Adipogenic, Chondrogenic, Neurogenic	Dentin/pulp-like complex
6.	GMSCs	Gingiva	Osteogenic, Adipogenic, Chondrogenic, Neurogenic, Endothelial	Connective-like tissue
7.	NDP-SCs	Dental pulp of newborn	Osteogenic, Adipogenic Chondrogenic, Myogenic Neurogenic	Pulp regeneration

of these treatments is that they are intrusive in nature and do not promote tissue regeneration. As a result, the need for innovative therapeutic strategies to lower cardiovascular disease mortality and mobility is critical. In this case, cell replacement therapy appears to be a viable option for heart repair. Moreover, a number of studies have revealed that adult cardiomyocytes are produced throughout life[66], disproving the long-held belief that the heart is a tissue devoid of the ability to regenerate itself. Nonetheless, compared to epithelial and bone marrow cells (BM), cardiomyocyte production is much lower. This led to an ongoing hunt for a different source of cells with the ability to regenerate cells. Despite genetic modification and ethical debates surrounding their use in therapeutic settings, iPSCs and ESCs have long been regarded as the best options for heart regeneration.[67] Of all the adult/ MSCs, the most research has been done on BM-MSCs. DSCs derived from dental tissue have emerged as a promising option for regenerative medicine. A number of distinct types of DSCs have now been identified in dental origin, including SHED, DPSCs, buccal mucosa, apical papilla and PDLSCs. The discovery of DSCs was initially reported by Gronthos *et al*.[68,69] Compared to isolating BM-MSCs, obtaining these cells is less invasive, simpler, and ethically acceptable. DSCs have been shown to differentiate into odontoblast/osteoblast-like cells[70], also differentiate into functional active neurons, mature melanocytes[71,72], smooth muscle cells[73], islet-like aggregates[74], and hepatic cells[75] in addition to these other known cell lineages. DSCs' remarkable potential to develop into cardiomyocytes *in vitro* and to stimulate angiogenesis in pre-clinical models[76] has recently been documented.

DSCs, particularly stem cells from SHED, show promising pluripotent capabilities due to the expression of key transcription factors like Oct-4, Sox-2, and Nanog, as well as involvement in key signalling pathways such as Wnt, TGFβ/Activin/Nodal, and BMP. These pathways play significant roles in early development and tooth formation, indicating the primitive nature of DSCs and their ease of reprogramming into iPSCs. Additionally, components like C-kit+ cells in DPSCs hint at their potential for lineage-specific applications. The PI3K-Akt signalling pathway, known for regulating cell survival, proliferation, and angiogenesis, also appears critical in guiding DSCs toward cardiomyocyte differentiation. Studies have shown that hypoxia can enhance the angiogenic capacity of DPSCs, and their intramyocardial injection in animal models improved cardiac function, likely due to angiogenesis. Furthermore, the expression of cardio genesis related genes like HAND2, GATA6, and KDR in DSCs supports their potential for cardiac regeneration, highlighting their promise as a therapeutic tool for myocardial repair. (Figure 3)

Dental stem cells as a substitute for brain tissue regeneration

DPSCs are derived from the cranial neural crest and exhibit neural traits such neurotrophin expression.[77] The diffusible peptides secreted by neurons and neuron-supporting cells (DPSCs) function as growth factors for the development, maintenance, repair, and survival of specific neuronal populations. Specifically, it has been demonstrated that neurons in the central nervous system

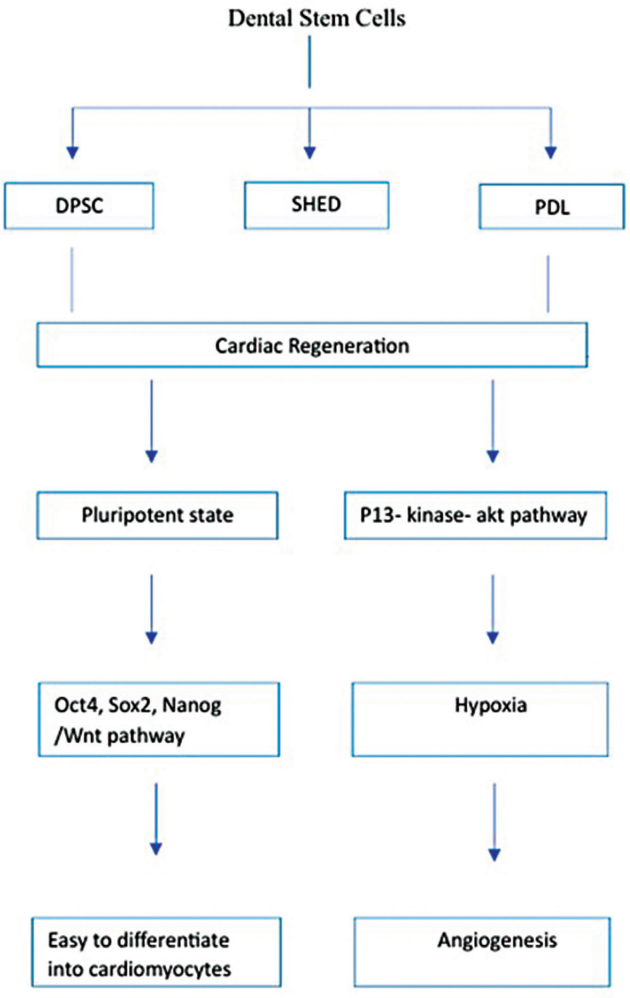

Figure 3: Diagram showing the possible therapeutic benefits of stem cells from human teeth on myocardial infarction.

(CNS), including motor neurons and dopaminergic neurons of the substantia nigra, are significantly influenced by brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and glial-cell-derived neurotrophic factor (GDNF) produced by DPSCs.[78] DPSCs have the power to affect the natural recruitment of neural stem cells and the development of neurospheres.[79] As a result, DPSCs could be a useful source for neurological diseases cell treatment.[80]

The use of DPSCS and SHEDs in models of spinal cord injury has demonstrated that the microenvironment of transplanted stem cells influences their ability to differentiate; an injured spinal cord has elevated levels of pro-inflammatory mediators that may initiate the differentiation cascade specific to oligodendrocytes. The full transected adult rat spinal cord showed a notable recovery of hind-limb locomotor capabilities upon transplantation of hDPSCs. Neuroregenerative activity were demonstrated by the hDPSCs.[81]

A cerebral artery blockage causes ischemia in a specific area of the central nervous system, which can result in stroke. By transferring differentiated neural stem cells extracted from tooth pulp, motor impairment was ameliorated and the extent of the infarct was decreased. Promising results were found in 86 therapeutic translation studies using DPSCs to treat stroke in a mouse cerebral ischemia model.[82] Porcine CD31/CD146 side population (SP) cell transplantation boosted neuronal regeneration and hastened the ischemic zone's neovascularization. The peri-infarct region was reached by Sugiyama *et al*., 88 transplanted pig CD31/CD146 SP cells, which also secreted neurotrophic factors and encouraged the migration and differentiation of neural progenitor cells in the subventricular zone. Forelimb sensorimotor function in a mouse model of focal cerebral ischemia was significantly improved by intracerebral transplantation of hDPSC. Function improvements seemed to be mediated by paracrine actions that are dependent on DPSC.[82] Following an optic nerve crush injury caused by surgery, the therapeutic advantage of implanting rat DPSCs into the vitreous body of the eye enhanced axon regeneration and neurotrophin-mediated survival of rat ganglion cells.[83]

A neurodegenerative condition known as Parkinson's disease is typified by the progressive loss of substantia nigra dopaminergic neurons, which causes a localised decrease in striatal dopamine (DA) levels. Using an indirect co-culture approach with mesencephalic cell cultures, Nesti *et al*.,[84] examined the neuroprotective effects of DPSC against MPP+ and rotenone in an *in vitro* model of Parkinson's disease. They discovered that the co-culture with DPSCs greatly reduced the toxicity caused by MPP+ or rotenone. This was likely due to the neuroprotection provided by soluble factors like NGF and BDNF that are released by DPSC. Therefore, it is possible to consider DPSC as a potential subject for research on cell-based therapy in neurodegenerative diseases.

In a rat model of spinal cord injury, DPSC showed that dental pulp-derived cell transplantation increased the survival of injured motor neurons. Neurotrophic factors, including as NGF, GDNF, and BDNF, were generated and secreted by DPSC from rats and humans, and these helped dopaminergic and sensory neurons survive. Ninety DPSC showed neuroprotective effects in *in vitro* models of Parkinson's and Alzheimer's disease. The capacity to generate and release growth factors is crucial because these factors have the power to stimulate endogenous cell types to differentiate into the specific cell types needed at the site of injury or to secrete additional neurotrophic factors from those cells in order to promote tissue regeneration. When DPSC were loaded onto poly(dl-lactic-co-glycolic acid) (PLGA) collagen and the scaffold was inserted in a model of facial nerve injury, the system allowed the reconnection of damaged axons, demonstrating its applicability also at the peripheral nervous system level for nerve injury treatment.[85]

Dental stem cells as a substitute for bone regeneration

The differentiation profiles exhibited by DPSCs resembled those observed during bone development, which makes them an intriguing model to investigate osteogenesis and the connection to scaffolds. Both *in vitro* and *in vivo*, the osteogenic differentiation capacity of DPSC has been amply proven, as evidenced by the expression of markers unique to bone within freshly produced bone and strong ALP results.[86] Immobilisation led to increased mineralization, protein secretion, and an upregulated osteo-related gene profile. Interestingly, immobilisation also caused DPSC to differentiate into osteogenic tissues without the need for induction agents in the medium.

When DPSCs were inserted into the granular depro-

teinized bovine bone (GDPB) scaffold, there was a propensity to raise the bone mineral density. In a rat calvarial critical defect model, Rat DPSCs was used in conjunction with a GDPB or beta tricalcium phosphate (βTCP) scaffold. GDPB bone scaffolds combined with DPSC demonstrated the ability to improve the process of bone regeneration when it comes to reconstructing calvarial lesions.[87] DFSCs from impacted teeth were utilised by Lucaciu *et al*.[87] To enhance bone regeneration on titanium implant surfaces. They came to the conclusion that DFSCs might be utilised to enhance bone regeneration on titanium implant surfaces after observing a spontaneous predisposition for osteogenic differentiation. DPSCs were also applied in a rat calvarial critical-sized defect model by Maraldi *et al*.[88] After eight weeks, hDPSC-seeded collagen sponges demonstrated nearly complete defect bridging. For cell treatments and regenerative medicine to be used clinically, regulation of DPSC differentiation is essential. To do this, biomaterials' topographical designs may be enhanced. The relationship between changes in pillar topography and the surface topographical parameters during DPSC attachment, morphology, proliferation, and osteogenic differentiation demonstrated increased mineralization. The results of the *in vitro* and *in vivo* investigations indicated that there is a lot of promise for the clinical use of DPSC added to scaffolds in bone restoration.[88]

Dental stem cells application for liver diseases

A permanent fibrotic alteration of the liver, liver cirrhosis can have major side effects include portal hypertension, hepatocellular cancer, and decreased liver function. The only way to stop cirrhosis from taking a more severe clinical course is still through liver transplantation. As innovative therapeutic alternatives to whole organ allografts, cell-based therapies have gained interest. SHED is a viable cell source for MSC-based therapy for patients with liver failure, both paediatric and adult. Third-molar stem cells were cultured into hepatocytes and avoided liver fibrosis and elevated albumin and bilirubin levels in an animal model of liver illness. Melatonin modulates the BMP, p38, ERK, and NF-kB pathways to enhance the hepatic development of hDPSC, as revealed by Cho *et al*.[89] Thus, they came to the conclusion that treating liver cirrhosis with melatonin and transplanted hDPSCs would be a feasible strategy.[89]

Dental stem cells application for diabetes mellitus

Diabetes is characterised by persistent hyperglycaemia, which is caused by either impaired sensitivity to insulin or autoimmune destruction of pancreatic β-cells. An alternative to the standard insulin-based therapy for diabetes may be the use of differentiated stem cells or islet transplantation to replace the lost insulin-producing cells. It has been revealed that DPSC have the capacity to develop into pancreatic cell lineages that resemble islet-like cell aggregates. According to Carnevale *et al*., insulin, pancreatic, and duodenal homeobox-1 genes are expressed by hDPSCs in response to suitable stimuli, which are linked to pancreatic β-cell formation and function. In diabetic mice, islet-like cell clusters (ICCs) generated from hDPSC and SHED were transplanted, as shown by Kanafi *et al*.[90] In experimentally diabetic mice, they observed the restoration of hyperglycaemia to the normal level. These findings raised the possibility of using dental pulp to treat diabetic patients using autologous stem cells.[90]

Dental stem cells as a substitute for regenerative ocular therapy

DSC has been effectively evaluated in corneal blindness as an autologous stem cell source. Due to the comparable embryonic origins of the cornea and DPSC, the latter successfully developed into keratocytes *in vitro*, producing a tissue-engineered corneal stromal-like tissue construct and functioning as keratocytes *in vivo* without causing overt rejection. In mouse corneal stroma, DPSC cultivated on aligned nanofiber substrate-generated tissue-engineered, stromal-like constructions recapitulated the original stromal tissue's tightly packed, aligned, parallel fibrillar collagen. These results show promise for the therapeutic use of DPSC in tissue engineering or cellular treatments for corneal stromal blindness. SHED have been found in studies to have promising effects when used for corneal epithelial regeneration. In rabbit models with complete limbal stem cell deficit, the introduction of cell sheets made of SHED, both with and without the inclusion of amniotic membrane, led to the regeneration of the corneal epithelium. SHED also express markers that are similar to those of corneal limbal stem cells. Retinal cell differentiation was observed in stem cells isolated from the periodontal ligament upon suppression of Wing-

less- related integration site (Wnt) and bone morphogenetic protein signalling, according to Huang *et al*. Third-molar-derived adult DPSCs possess the ability to develop into keratocytes, which are corneal stoma cells. Following *in vitro* differentiation, DPSC produced keratocyte-specific molecules, including keratocan and keratan sulphate proteoglycans, at both the gene and protein levels. After optic nerve damage in mice, intravitreal DPSC transplantation significantly increased neurotrophin-mediated retinal ganglion cell survival and axon regeneration.[91]

Dental stem cells banking

DSC banking requires a standardized workflow comprising donor eligibility screening, aseptic tissue handling, validated isolation/expansion, quality control, cryopreservation, post-thaw assessment, and adherence to regulatory standards (Figure 4). Proper isolation is critical for viability and purity, with methods including enzymatic digestion, explant culture, and magnetic-activated cell sorting (MACS).[92] Advances in cryopreservation media, serum-free systems, and international frameworks are enhancing safety and clinical translation.

In tissue engineering, earlier approaches relied on polymer scaffolds with dissociated tooth germ cells in animal models. Recent strategies combine DSCs with biomaterials and extracellular matrices, providing superior outcomes in dental structure regeneration.[93] Collectively, these advancements emphasize the importance of standardized isolation, storage, and banking protocols to ensure long-term clinical utility of DSCs in regenerative dentistry.

Step 1: Tooth/Tissue collection and transfer

Donor eligibility requires systemic and oral health screening to exclude bacterial or viral infections. Tooth type and developmental stage determine the suitability for banking.

• SHED: Collected from primary incisors/canines with one-third root remaining or early-extracted primary molars; excluded if necrotic, infected, or exfoliated outside clinic.

• DPSCs: Extracted from healthy impacted or orthodontically removed permanent teeth; excluded if necrotic or diseased.

• DFSCs: Obtained from developing tooth organs or impacted teeth without pathology.

• SCAP: Harvested from immature permanent teeth; excluded if roots are complete.

• PDLSCs: Isolated from periodontal ligament of freshly extracted permanent teeth.

Before extraction, antiseptic preparation with chlorhexidine is recommended. During transfer, samples must be kept moist and viable. Transport solutions include PBS, α-MEM with serum, HypoThermosol, or commercial kits, with storage ideally at 4°C. Viable yields are best when processed within 120 hours.[94]

Step 2: Cell isolation and propagation

Upon arrival in a Grade A cleanroom, samples undergo serial disinfection (PBS, povidone-iodine, sodium thiosulfate). Tissue is then dissected: pulp for DPSCs/SHED, follicles for DFSCs, apical papilla for SCAP, and ligament tissue for PDLSCs.

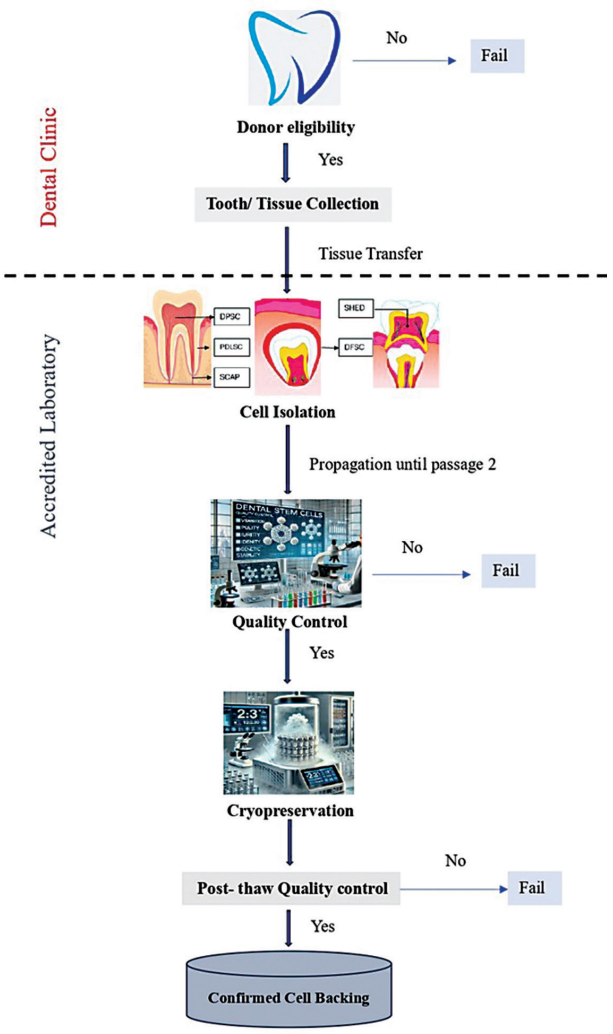

Figure 4: The outlines of dental tissue derived stem cells banking.

Two main methods are used:

• **Explant method:** Favored for clinical translation, as it reduces enzymatic stress and contamination risk.

• **Enzymatic digestion:** Uses collagenase/dispase or trypsin-EDTA for higher single-cell yield.

Cells are expanded in culture media such as α-MEM, DMEM, or DMEM/F12. α-MEM supports superior proliferation and reduced senescence. Serum-free or xeno-free formulations are preferred for clinical use.[95]

Step 3: Quality control and characterization

According to International Society for Cell & Gene Therapy (ISCT) and European Medicines Agency (EMA) guidelines, DSCs must demonstrate:

• Plastic adherence in standard culture.

• Immunophenotype: ≥95% CD105/CD73/CD90; <2% hematopoietic markers (CD45, CD34, CD14, CD19, HLA-DR).

• Trilineage differentiation into osteogenic, chondrogenic, and adipogenic lineages.

Quality control includes:

• **Viability:** ≥80% post-thaw, assessed via trypan blue, mitochondrial assays, or apoptosis markers.

• **Purity:** Sterility testing for bacteria, fungi, mycoplasma, and viral pathogens.

• **Identity:** STR profiling to avoid misidentification.

• **Genetic stability:** G-band karyotyping to confirm chromosomal integrity.[96-99]

Step 4: Cryopreservation

Several factors influence the cryostability of DSCs. The choice of cryoprotectant is critical, with conventional media consisting of 90% fetal bovine serum (FBS) and 10% dimethyl sulfoxide (DMSO). However, due to safety concerns in clinical applications, alternatives such as human serum or serum-free cryoprotectants (e.g., BAMBANKER) are being explored. Controlled cooling rates of approximately −1°C/min, followed by storage at −196°C in liquid nitrogen, are considered optimal to prevent intracellular ice crystal formation and ensure long-term viability. Thawing protocols also play an essential role in reducing cryoinjury. Advanced methods, including serum-free cryoprotectants and vitrification, have been introduced to improve cryostability and enhance the clinical translation of DSC banking.[94]

Studies have demonstrated that DPSCs and SHED retain high viability and multipotency after long-term storage. For instance, Ma *et al*.,[95] reported that DPSCs preserved for up to 24 years in liquid nitrogen maintained >70% viability and osteogenic/adipogenic differentiation potential upon thawing. Similar findings were observed in SHED, where cells cryopreserved for more than a decade showed preserved stemness markers (OCT4, NANOG) and immunomodulatory capacity.[96]

Emerging evidence also highlights the importance of epigenetic stability during long-term storage. A recent study demonstrated that DPSCs retained normal DNA methylation profiles and telomere length after >10 years of cryopreservation, supporting their functional integrity for regenerative therapies.[97] Collectively, these findings confirm that DSCs can be effectively stored long-term with preserved viability, differentiation, and epigenetic stability, strengthening their clinical relevance in regenerative dentistry.

Step 5: Post-thaw quality control

Thawing is usually performed in a 37°C water bath, though dry-heat methods reduce contamination risk. CPA removal must be gradual to reduce cytotoxicity. Post-thaw assessments include viability (>80%), metabolic assays, doubling time, and apoptosis markers, ensuring recovery for therapeutic applications.[98]

Step 6: Standardization and accreditation

DSC banks must comply with ISO 9000, Good Manufacturing Practice (GMP), ICH guidelines (Q7, Q9, Q10), FDA, and WHO regulations. These ensure consistency, sterility, safety, and traceability in therapeutic banking.[99]

Step 7: Documentation

Comprehensive records including donor data, culture history, quality assessments, and cryostorage tracking are mandatory for accreditation and regulatory approval.[99]

Conclusions and Future Directions

Dental stem cells (DSCs) represent a highly versatile, ethically accessible, and clinically promising cell population with demonstrated potential in regenerative dentistry and broad systemic applications. As summarized in this review, DSCs including DPSCs, SHED, PDLSCs, DFPCs, SCAP, NDP-SCs, and GMSCs–exhibit robust proliferative

capacity, multilineage differentiation, immunomodulatory functions, and compatibility with advanced biomaterials. Their translational relevance is increasingly supported by preclinical and early clinical evidence in pulp-dentin regeneration, periodontal repair, craniofacial bone engineering, neuroregeneration, cardiomyocyte induction, liver and pancreatic repair, and ocular therapy. Advances in DSC isolation, characterization, exosome-based therapies, 3D bioprinting, and standardized cryopreservation workflows further strengthen their role as a future cornerstone of personalized regenerative medicine. However, despite significant progress, long-term clinical validation, standardized GMP-grade banking protocols, multicentre clinical trials, and clear regulatory pathways remain essential to ensure safety, reproducibility, and therapeutic efficacy. Future research should therefore focus on large-scale human studies, molecular optimization of DSC differentiation, integration with next-generation scaffolds and organoid systems, and development of cell-free therapeutic platforms to accelerate safe clinical translation of DSC-based therapies.

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Treatment Decision for Borderline Class III Malocclusion in Adults; Orthodontic Camouflage versus Orthodontic-Orthognathic Surgery

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Abstract

Objectives: Determining the optimal treatment approach for adult borderline Class III malocclusion, whether orthodontic camouflage or orthodontic-orthognathic surgery, remains clinically challenging. This study aimed to distinguish between these two treatment options using lateral cephalometric analysis.

Methods: Pretreatment lateral cephalograms of 60 adult patients with borderline Class III malocclusion were analyzed, comprising 30 patients in the camouflage group and 30 in the surgery group. The Mann-Whitney U test was used to compare cephalometric variables between the two groups. Stepwise discriminant analysis was employed to identify the variables that best differentiated the treatment groups. An equation was then generated using the canonical discriminant function coefficients of the selected variables and a constant to calculate a critical score for treatment categorization.

Results: Stepwise discriminant analysis identified four key variables and generated the following equation: Individual score = –13.684+0.138(SN)+0.312(Wits)+0.068 (L1-MP)+0.179(H angle). A critical score of 0 was established. Patients with scores above 0 were considered suitable for orthodontic camouflage, while those with scores below 0 were better suited for orthodontic-orthognathic surgery. The overall classification accuracy of the model was 86.7%.

Conclusions: Four cephalometric variables including SN length, Wits appraisal, L1-MP angle, and H angle were effective in distinguishing appropriate treatment modalities for adult borderline Class III malocclusion patients.

Keywords: borderline Class III malocclusion, cephalometric analysis, orthodontic camouflage, orthodontic-orthognathic surgery

Introduction

Class III malocclusion is one of the most challenging dentofacial deformities to correct in dental practice. Its etiology is multifactorial, involving a complex interaction between genetic, hereditary, and environmental factors.[1] The prevalence of Class III malocclusion varies across populations, with the highest reported prevalence of 15.8% found in Southeast Asian populations, and a lower prevalence observed among European and Caucasian groups. Class III malocclusion is typically characterized by a concave facial profile resulting from mandibular prognathism, maxillary retrognathism, or a combination of both-of which the combined form is the most common presentation.[2,3] Cephalometric characteristics in these patients often show a retruded but normally sized maxilla, and a protruded mandible with increased length. Additionally, the maxillary incisors tend to be proclined and buccally tipped, whereas the mandibular incisors are typically retroclined and lingually tipped.[4]

Accurate diagnosis and treatment planning are based on comprehensive clinical examinations, study models, cephalometric analysis, and radiographic imaging, along with careful assessment of dental and skeletal relationships.[5] In adult patients, treatment of Class III malocclusion generally follows one of two approaches: orthodontic camouflage or a combination of orthodontics and orthognathic surgery. The decision depends on several factors, including the patient's chief complaint and the severity of the skeletal discrepancy. Mild to moderate discrepancies can often be managed with orthodontic camouflage, whereas more severe cases typically require orthognathic surgery.[6] Additional considerations include the cost of treatment, the invasive nature of surgery, and the patient's overall health condition.[2] However, in borderline cases, where either treatment approach may be viable, treatment planning becomes particularly controversial. Many patients are unable or unwilling to undergo surgery due to medical, financial, or psychological constraints.

Numerous studies have explored treatment decision-making in Class III malocclusion. For instance, Eslami *et al.,*[5] recommended camouflage treatment in patients with a Holdaway angle greater than 10.3° and a Wits appraisal above -5.8 mm. Rabie *et al*.,[3] similarly used the Holdaway angle as a key variable, suggesting that values above 12° are indicative of successful camouflage treatment, while lower values favor surgical intervention Benyahia *et al*.,[7] proposed a lower threshold, identifying 7.2° as the borderline value for the Holdaway angle. Other researchers, such as Eisenhauer *et al*.,[8] developed predictive equations using variables like Wits appraisal, SN length, the maxillary/mandibular (M/M) ratio, and the lower gonial angle to identify critical scores for treatment categorization. Likewise, Kochel derived an equation using Wits appraisal, the M/M ratio, and the NSAr angle, producing a critical score of 0.251.[9]

Given the variability of proposed variables and cut-off values in previous studies, the objective of this study was to identify the most decisive cephalometric variables that can reliably differentiate between treatment groups in adult borderline Class III malocclusion patients. The study further aimed to develop a discriminant function that yields a critical score to guide clinical decision-making between orthodontic camouflage and orthodontic-orthognathic surgery.

Materials and Methods

Subjects and image acquisition

This research is a retrospective study that aims to identify treatment plans for borderline Class III malocclusion patients by using pretreatment lateral cephalograms. This study was approved by the Human Experimentation Committee of the Faculty (No. 47/2024). To calculate the required minimum sample size, a pilot study was performed. The sample size calculation was done using the G*Power software program (version 3.1.9.4, University of Kiel, Germany). Considering a power of 85% and a significance level of 5% and an effect size of 0.82, the final sample was composed of 30 subjects in each group. All participants were patients attending orthodontic treatment and requiring pretreatment lateral cephalograms for diagnosis and treatment plan.

The inclusion criteria were as follows:

1. Adults aged 18 or older with skeletal Class III malocclusion
2. No previous orthodontic treatment or orthodontic-orthognathic surgery treatment
3. ANB of 0° to –5.5°
4. Wits appraisal of –10.5 mm. to. -1 mm.
5. Overjet ≤0 before treatment

Patients with syndromic or medically compromised and maxillofacial trauma history were excluded from this study.

Patients were retrospectively selected from the records of the Department of Orthodontics, Faculty of Dentistry, between 2020 and 2023. After applying the inclusion and exclusion criteria, 70 patients with skeletal Class III malocclusion remained eligible. These patients were independently categorized into either the orthodontic camouflage group or the orthodontic–orthognathic surgery group by two board-certified orthodontists. Pretreatment records, including panoramic and lateral cephalograms, intraoral and extraoral photographs, and plaster study models, were evaluated. Based solely on these diagnostic materials, and considering patient-reported symptoms, facial esthetics, and the severity of dentoskeletal discrepancies, the orthodontists independently categorized patients into either the camouflage or surgical treatment group. The final decision also considered functional and esthetic aspects as well as patient preferences. According to the sample size calculation, 30 patients from each group were required. Therefore, 30 patients were randomly selected from each category, resulting in a total sample of 60 patients for analysis.

Methods

Each patient underwent lateral cephalometric radiography (NewTom Giano, Verona, Italy). Lateral cephalograms were taken at maximum intercuspation, with the lips in a rest position and the Frankfort horizontal plane aligned according to the natural head position. All lateral cephalograms were traced digitally using Dolphin imaging software version 11.9 (Dolphin Imaging & Management Solutions, Chatsworth, Calif). The following cephalometric tracings of landmarks were made on the pretreatment lateral cephalometric radiographs: S (Sella), N (Nasion), Go (Gonion), Gn (Gnathion), Pg (Pogonion), ANS (Anterior nasal spine), PNS (Posterior nasal spine), A (Subspinale), B (Supramentale), Me (Menton), Ar (Articulare), U6 (Upper first molar occlusal plane), L6 (Lower first molar occlusal plane), U1 tip (Upper central incisor tip), U1 root (Upper central incisor root apex), L1 tip (Lower central incisor tip), L1 root (Lower central incisor root apex), N' (Soft tissue nasion), Ls (Labrale superius) and Pg' (Soft tissue pogonion) (Figure 1).

The following linear, proportional, and angular measurements were calculated:

(1) SNA angle: The angle between the sella turcica (S), the nasion (N) and the point A

(2) SNB angle: The angle between the sella turcica (S), the nasion (N) and the point B

(3) ANB angle: The relationship between the maxilla and the mandible. This measurement is obtained from the equation ANB = SNA – SNB

(4) SN: An anteroposterior length of the anterior cranial base. It is measured from the Sella turcica (S) to the nasion (N)

(5) Wits appraisal: Length of the distance AO-BO; AO (intersection between a perpendicular line from Point A and the occlusal plane); BO (intersection between a perpendicular line from Point B and the occlusal plane)

(6) NAPg: The intersection of a line from the nasion (N) to point A and point A to the pogonion (Pg)

(7) NSAr or saddle angle: An angle formed by the nasion (N), sella turcica (S) and the articulare (Ar)

(8) SN-GoGn: Divergence of the mandibular plane (Go-Gn line) relative to the anterior part of the cranial base (SN line)

(9) Gonial angle (ArGoMe): The angle formed by the articulare (Ar), the gonion (Go) and the menton (Me)

(10) Go upper (ArGoN): The angle formed by the articulare (Ar), the gonion (Go) and the nasion (N)

(11) Go lower (NGoMe): The angle formed by the nasion (N), the gonion (Go) and the menton (Me)

(12) M/M ratio: A ratio of the anteroposterior length of the maxilla (ANS-PNS) to the anteroposterior length of the mandible according to Steiner's mandibular plane (Gn-Go)

(13) L1-MP angle: An angle between the long axis of mandibular central incisor and Steiner's mandibular plane (Gn-Go)

(14) U1-L1 or interincisal angle: An angle between long axis of maxillary central incisor and long axis of mandibular central incisor

(15) Holdaway angle (H angle): An angle formed by the soft tissue H line (line tangent to upper lip and soft tissue pogonion) and the soft tissue facial plane (N-Pg)

All the measurements were made by one participant who calibrated by experienced orthodontist. A total of 60 subjects' radiographs were traced and measured twice, four weeks apart, for intra-examiner reliability. The method error in measuring was calculated by the Dahlberg's formula $ME=\sqrt{\Sigma d^2}/2n$ where d is a difference between twice measurement and n is the number of double measurements.[10] Random linear errors ranged

from 0.25 to 4.06 and errors in angular variables ranged from 0.01 to 2.09.

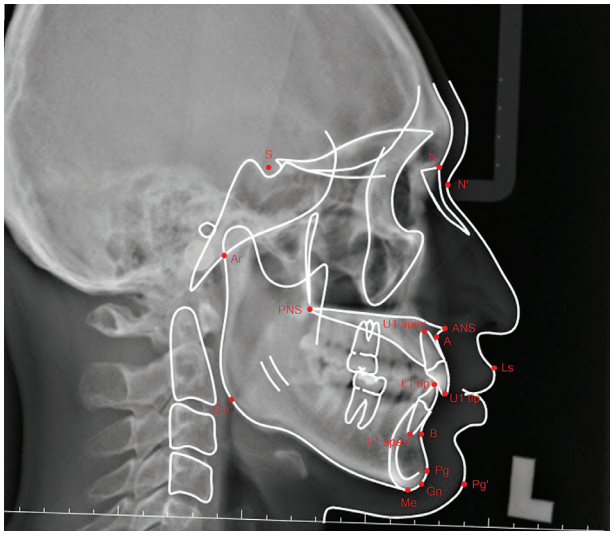

Figure 1: The lateral cephalometric reference points employed in this study included: sella (S); articulare (Ar); gonion (Go); menton (Me); pogonion (Pog); gnathion (Gn); Point B (B); root apex of the mandibular central incisor (L1 apex); incisal tip of the mandibular central incisor (L1 tip); incisal tip of the maxillary central incisor (U1 tip); root apex of the maxillary central incisor (U1 apex); Point A (A); anterior nasal spine (ANS); posterior nasal spine (PNS); nasion (N); labrale superius (Ls); soft tissue nasion (N') and soft-tissue pogonion (Pg').

Statistical analysis

Descriptive and analytical statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences), version 22.0 for Windows (IBM Corp., Armonk, NY, USA). For each variable and group, the minimum, maximum, mean, standard deviation, and median values were calculated. Descriptive statistics were used to summarize the baseline characteristics of the participants (Table 1). The Shapiro-Wilk test was applied to assess the normality of data distribution. As some variables were not normally distributed, the Mann-Whitney U test was employed to compare variables between the two groups: the orthodontic camouflage group and the orthodontic–orthognathic surgery group. A significance level of $p<0.05$ was considered statistically significant (Table 2).

In this study, stepwise discriminant analysis was used to identify the cephalometric variable that best separates the orthodontic camouflage and orthodontic-orthognathic surgery group. The canonical discriminant function coefficients were calculated with a constant for each selected variable (Table 3). This resulted in a new equation that assigns a score to each patient. The critical score corresponds to the mean value of the group centroids for both groups. Then, the classification value was evaluated (Table 4).

Results

The overall sample included 60 patients who met the inclusion and exclusion criteria. The mean age of the patients was 30.7±2.9 years. There were 27 males (45%) with a mean age of 31.2±3.0 years and 33 females (55%) with a mean age of 30.3±2.7 years. Descriptive statistics of the orthodontic camouflage group and orthodontic–orthognathic surgery group are shown in Table 1.

The Mann-Whitney U test revealed significant differences ($p<0.05$) in seven variables between the two groups (Table 2). Significant intergroup differences were observed in variables related to the sagittal skeletal relationship, including SN, SNB, ANB, Wits appraisal, and NAPg. In contrast, variables such as SNA, NSAr, SN-GoGn, Ar-Go-Me, Ar-Go-N, and N-Go-Me did not show statistically significant differences between the orthodontic camouflage group and the orthodontic-orthognathic surgery group. Additionally, the M/M ratio was not effective in distinguishing between the two groups. Regarding dental relationships, a significant difference was observed in the angle between the long axis of the mandibular central incisor and the mandibular plane (L1-MP), whereas the interincisal angle (U1-L1) did not differ significantly between groups. Additionally, a significant difference was found in the soft tissue profile, as indicated by the H angle.

According to Stellzig *et al.*,[8] discriminant analysis has been applied to determine the dentoskeletal variables that separate surgical from nonsurgical orthodontic patients. Using stepwise discriminant analysis, four highly significant variables were identified: SN, Wits appraisal, L1–MP, and H angle. The canonical discriminant function coefficients of the selected variables, along with a calculated constant, resulted in an equation that provides individual scores to categorize patients into the groups (Table 3).

Individual score = –13.684+0.138(SN)+0.312(Wits)+0.068(L1-MP)+0.179(H angle)

Table 1: Descriptive statistics (mean, standard deviation, median, minimum, and maximum) of cephalometric variables in the orthodontic camouflage group and the orthodontic-orthognathic surgery group.

Cephalometric variables	Orthodontic camouflage group (N=30)					Orthodontic-orthognathic surgery group (N=30)				
	Min	Max	Mean	SD	Median	Min	Max	Mean	SD	Median
SNA (°)	71.7	88.4	79.4	3.8	79.3	73.0	91.0	80.9	4.3	79.1
SNB (°)	75.1	88.6	81.7	3.5	81.4	74.2	92.0	84.2	4.3	83.3
ANB (°)	-4.2	-0.2	-2.3	1.0	-2.2	-5.0	-0.9	-3.3	1.3	-4.0
SN (mm)	57.0	67.5	60.8	2.5	60.3	42.5	66.8	57.7	6.7	59.0
Wits appraisal (mm)	-9.8	-3.2	-6.2	1.7	-6.3	-10.3	-2.7	-7.9	2.1	-8.7
NAPg (°)	-9.5	3.8	-3.8	3.0	-4.1	-11.3	0.9	-7.2	3.7	-9.0
NSAr (°)	110.4	128.6	117.1	4.4	117.0	112.1	129.1	117.2	4.4	115.8
SN-GoGn (°)	26.6	39.3	32.9	3.7	34.1	24.9	48.1	35.5	5.5	35.1
ArGoMe (°)	111.6	140.2	126.2	6.0	125.1	108.1	138.7	126.4	7.0	125.8
ArGoN (°)	40.8	53.2	46.3	2.9	46.6	40.1	52.7	45.5	3.3	45.6
NGoMe (°)	73.5	93.0	80.4	5.4	79.5	68.7	93.1	81.2	7.0	80.1
M/M ratio (%)	0.5	0.7	0.6	0.0	0.6	0.5	0.6	0.5	0.0	0.5
L1-MP angle (°)	72.0	96.9	87.4	5.0	88.8	69.9	97.8	77.9	6.9	76.4
Interincisal angle (°)	111.5	142.7	128.6	7.5	127.8	113.0	154.2	132.9	8.8	131.9
H angle (°)	9.0	17.4	13.7	2.5	14.1	2.2	17.3	9.2	3.4	8.6

Table 2: Significant differences between orthodontic camouflage group and orthodontic-orthognathic surgery group.

Cephalometric variables	Mann-Whitney test
SNA	0.329
SNB	0.019*
ANB	0.002*
SN	0.008*
Wits	0.0005*
NAPg	0.001*
NSAr	0.976
SNGoGn	0.059
ArGoMe	0.751
ArGoN	0.399
NGoMe	0.663
M/M ratio	0.149
L1-MP	0.000001*
Interincisal	0.059
H angle	0.000003*

*$p<0.05$

Table 3: Stepwise discriminant analysis.

Predictive variable	Canonical Discriminant Function Coefficients
SN	0.138
Wits	0.312
L1-MP	0.068
H angle	0.179

Individual score = -13.684+0.138SN+0.312Wits+0.068L1-MP+0.179H angle

Group centroid: camouflage group 1.225, surgery group –1.225 Critical score=0

Table 4: Classification result.

Original group membership	Predicted group membership	
	Orthodontic camouflage group	Orthodontic-orthognathic surgery group
Orthodontic camouflage group (n=30)	93.3% (n=28)	6.7% (n=2)
Orthodontic-orthognathic surgery group (n=30)	20.0% (n=6)	80.0% (n=24)

Sensitivity (require orthodontic-orthognathic surgery): 0.8; Specificity (correct Class III malocclusion by orthodontic camouflage): 0.93; overall accuracy: 0.867

The critical score was 0, representing the mean value of the group centroids for both groups. Each Class III malocclusion patient with an individual score above the critical score will be treated successfully by orthodontic camouflage. On the other hand, Class III malocclusion patients with an individual score below the critical score should undergo orthodontic-orthognathic surgery. The percentage of patients correctly classified by the equation was 86.7%. Two patients in the orthodontic camouflage group and six patients in the orthodontic–orthognathic surgery group were misclassified, as the discriminant equation assigned them to the opposite treatment group compared with the actual treatment received. These misclassifications underscore the limitation that, although the model showed high sensitivity (0.80) and specificity (0.93), not all cases can be perfectly distinguished (Table 4).

Discussion

The optimal treatment for borderline Class III malocclusion remains a topic of debate, as both orthodontic camouflage and orthognathic surgery are viable options. Camouflage is typically indicated for mild to moderate dentoalveolar discrepancies, whereas surgery is preferred for more severe skeletal issues.[8] Treatment decisions should consider not only cephalometric parameters but also patient esthetic concerns and expectations. Advances in appliances and biomechanics have expanded the scope of camouflage, offering less invasive alternatives. However, in cases of pronounced skeletal discrepancy, camouflage may compromise esthetic or functional outcomes, making surgery the more appropriate and stable long-term option. Treatment decisions for borderline Class III malocclusion patients are influenced by multiple factors. Key considerations include the patient's chief complaint[11], which guides clinical goals, and diagnostic tools such as study models[12] and cephalometric analysis[13] that assess skeletal and dental relationships.[14] Patient preferences, particularly concerns about surgery, and socioeconomic factors, such as cost, also play important roles. While orthodontic camouflage may appeal to those seeking non-invasive options, surgery remains necessary for more severe discrepancies. Lastly, effective communication and individualized assessment are essential for selecting the most appropriate treatment approach.

The inclusion criteria for this study were carefully defined to ensure that the sample represented borderline skeletal Class III patients for whom treatment decisions between orthodontic camouflage and orthodontic–orthognathic surgery are most critical. First, only adults aged 18 years or older were included to avoid the influence of residual craniofacial growth and to ensure that skeletal discrepancies reflected stable conditions. Second, patients with no history of orthodontic or surgical treatment were selected so that baseline cephalometric measurements would not be influenced by prior interventions. Third, the ANB angle range of 0° to –5.5° was chosen to capture mild to moderate skeletal Class III cases, which often present the greatest clinical uncertainty in treatment planning. Fourth, a Wits appraisal between –10.5 mm and –1 mm was applied to provide an additional sagittal assessment that is less affected by cranial base variations, while excluding extremely severe discrepancies requiring surgery in all cases. Finally, patients were required to present with an overjet of ≤0 mm, thereby confirming the incisor relationship characteristic of skeletal Class III malocclusion and ensuring diagnostic consistency across the study group.

Stepwise discriminant analysis is a statistical method used in classification problems, particularly when the goal is to predict a categorical outcome. It is a variation of discriminant analysis, in which predictors are evaluated and selected in a stepwise manner to find the most signi-

ficant variables that best explain the variation between the category's groups.[15] According to Stellzig, Stepwise discriminant model was used and generated 4-variable model to distinguish treatment modalities in Class III malocclusion patients which are Wits appraisal, SN, M/M ratio and lower gonial angle.[8] In Kochel's study, the degree of laterognathism was added to the model and give the result as increased in predictability for the surgical group.[9] Nevertheless, discriminant models have limitations: difficulty in identifying precise landmarks, potential omission of relevant variables, the need for large sample sizes for external validity, and reduced accuracy when group differences are subtle.[8] For present study, stepwise selected variables were SN, Wits appraisal, L1-MP and H angle. The classification power of the equation was 86.7%. Compared to the study of Stellzig, the predictive power was 92% with a greater number of sample (non-surgery group=87 patients, surgery group=88 patient).[8] In further study, relatively large samples may be needed to increase the discriminant power of the model.

The first variable extracted from the discriminant model was SN length. Meta-analysis by Gong[16] showed that anterior cranial base length was significantly smaller in Class III malocclusion than in Class I and Class II malocclusions. According to Stellzig[8], the discriminant analysis presented the different in anterior cranial base length between non-surgery and surgery group with greater SN length value in non-surgery group. However, Polat found no significant difference in anterior cranial base lengths between Class III malocclusion and normal occlusion.[17]

The second variable that best separate the treatment option of Class III malocclusion patients was Wits appraisal. According to Jacobson[18], Wits appraisal represented the severity of the anteroposterior jaw relationship. The functional occlusal plane was used as a reference for defining the relationship of the jaw. Therefore, the rotation of the cranial base will not affect the degree of jaw disharmony. Despite the precise landmark of Wits appraisal cannot clearly identified in cephalometric radiograph[7], the present study used the same investigator tracing all the radiographs to minimize the systemic error of interobserver measurement. The mean Wits appraisal value indicating a Class I skeletal relationship in the Thai population is –2.4±1.7 mm[19],whereas the values in Caucasians are –1.17±1.9 mm for males and –0.1±1.77 mm for females.[18] Previous studies by Stellzig[8] and Kochel[9] identified the Wits appraisal as the primary variable in their discriminant models to differentiate between non-surgical and surgical treatment groups. In contrast to using a discriminant function, simpler approaches such as Wits appraisal thresholds can also aid in treatment decisions. Eslami *et al*.,[5] proposed a cut-off of –5.8 mm, above which camouflage is likely to succeed, and below which surgery is more appropriate. While simpler and easier to apply, this method may lack the nuance provided by multivariate models. Nevertheless, clinicians may consider using Wits appraisal in combination with other variables in settings where full cephalometric evaluation is not feasible. Furthermore, Tseng[20] reported that a Wits appraisal below –11.18 mm was one of six criteria indicating the need for orthognathic surgery in Class III malocclusion patients.

The third variable entering the discriminant model was H angle, which is formed by the soft tissue H line and the soft tissue facial plane. Thai normal range of H angle is 15.27°±2.73°[21], while in the present study mean value H angle of non-surgery groups is 13.7° and surgery groups is 9.2°. In the literature, several studies have investigated the influence of soft tissue profile on treatment plan decisions. According to Eslami[5], borderline Class III malocclusion patients with H angle greater than 10.3° would be treated successfully by orthodontic camouflage, while patients with H angle less than 10.3° should be treated by orthodontic-orthognathic surgery. As for Rabie[3] and Benyahia[7], these studies reported this critical H angle score as 12° and 7.2° respectively. Although the critical score for the H angle varies across studies, numerous investigations have consistently demonstrated its efficacy as a discriminative variable in distinguishing between orthodontic camouflage and surgical treatment groups. As the H angle represents the soft tissue profile, it is frequently a pivotal determinant influencing patients' decisions to seek orthodontic treatment, reflecting concerns regarding facial esthetics.

Another variable demonstrating significant intergroup differences was the L1-MP angle. In this study, the mean L1-MP angles were 87.4° in the non-surgery group and 77.9° in the surgery group, both notably smaller than the reported Thai normative value of 90.1°±8.7°.[22] These findings indicate a pronounced retroclination of mandibular incisors, particularly among patients who

required surgical intervention. The lower limits for incisal movement to effectively compensate for Class III skeletal discrepancies are acknowledged as approximately 80° relative to the mandibular plane.[23] Therefore, patients whose mandibular incisors approach or surpass these limits may not achieve optimal outcomes through orthodontic camouflage alone, highlighting the significance of carefully assessing the degree of dental compensation during treatment planning. According to Tseng[20], the study found L1-MP to be the best diagnostic variable for determining treatment modalities for Class III malocclusion patients. The study suggested that borderline Class III malocclusion patients with L1-MP greater than 80.8° would be successfully treated by orthodontic camouflage. In general, mandibular incisors are relatively retroclined, while maxillary incisors are typically proclined to compensate for the skeletal discrepancy in Class III malocclusion. However, in severe Class III cases, patients exhibit a negative overjet in the incisor relationship, despite the compensatory inclination of both maxillary and mandibular incisors. The importance of L1-MP lies in the fact that patients with severely retroclined mandibular incisors prior to treatment may not achieve successful outcomes through orthodontic camouflage alone, due to anatomical limitations. Moreover, the stability of the post-treatment results may be compromised.

Population-specific variations are critical in interpreting cephalometric variables. In this Thai sample, the average SN length and Wits appraisal differed from Caucasian norms, consistent with Gong *et al*.,[16] and Chaiworawitkul's[19] reports, underscoring the need for localized data. The L1-MP angle was more retroclined than Thai normative values[22], reflecting compensatory dental patterns in Class III cases, while the H angle showed marked variability compared with other populations[24], highlighting the influence of ethnicity and cultural perceptions on soft-tissue esthetics. Beyond anatomical differences, treatment planning for Asian patients must also account for cultural and esthetic considerations. Asian populations often present with unique craniofacial characteristics such as a tendency toward bimaxillary protrusion[25], wide and prominent jaw structure compared with Caucasian populations.[26] These features can influence both cephalometric interpretations and the threshold for determining between orthodontic camouflage and orthognathic surgery.

Currently, there is no standardized protocol for selecting camouflage versus surgical treatment in borderline skeletal Class III malocclusion, making the issue controversial.[5] Camouflage is often used in adolescents and adults with mild to moderate discrepancies[27], particularly when surgery is not acceptable to the patient. This approach, involving maxillary incisor proclination and mandibular incisor retroclination, improves occlusion but does not correct the skeletal problem[23], and some patients later pursue surgery due to dissatisfaction.[28] In the context of Asian patients, these considerations become especially important. Esthetic ideals in many Asian cultures emphasize facial harmony and soft-tissue balance, which may increase the preference for surgical treatment to optimize facial profile improvement. Accordingly, the findings of the present study are particularly relevant to Asian patients, as they provide population-specific evidence to guide individualized treatment planning. Future studies comparing outcomes across different ethnic groups will be valuable in confirming the applicability of these results beyond Asian populations.

This study has several limitations. First, the sample size was relatively small (N=30 per group), which may affect the statistical power and generalizability. Second, although a digital cephalometric tracing program was utilized, human error in measurements remains a potential source of inaccuracy. To minimize this, each radiograph was traced twice by the same investigator with a one-month interval between tracings. The two variables with the highest method error were the interincisal angle and the NSAr angle. Method error in interincisal angle could be due to superimposition of both maxillary and mandibular central incisors in lateral cephalometric radiograph. The NSAr angle showed intra-observer variability, possibly due to the difficulty in identifying the Sella point, which is a constructed landmark lacking a definitive anatomical reference. Third, the retrospective design restricted the ability to control for potential confounding variables, and factors such as patient preferences, chief complaint, and socioeconomic status–important in treatment decision-making–were not included in the analysis. Lastly, as a single-center study, the findings may not be broadly generalizable. Future multi-center research with larger, more diverse populations and additional variables such as soft tissue profile and long-term outcomes is recommended to enhance the robustness and applicability of

the predictive model.

Conclusions

Among the 15 cephalometric variables analyzed, four were found to be the most effective in distinguishing between patients treated with orthodontic camouflage and those who underwent orthodontic-orthognathic surgery. These significant variables are SN length (cranial base length), Wits appraisal (sagittal jaw relationship), L1-MP (lower incisor to mandibular plane angle), and H angle (facial esthetics). The canonical discriminant function coefficients of these variables, along with a calculated constant, generate an equation that yields a critical score. This critical score effectively categorizes patients into the appropriate treatment group.

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Conflict of Interest

The authors declare no conflict of interest.

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Effectiveness of Oral Health Care in Stroke Patients with Dysphagia: A Quasi-Experimental Study

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Abstract

Objectives: To evaluate the oral health and function of hospitalized stroke patients with dysphagia and assess the effectiveness of an oral health care program during swallowing rehabilitation.

Methods: A quasi-experimental study was conducted with 46 dysphagic stroke patients randomly selected via block randomization from the rehabilitation ward of Chiang Mai Neurological Hospital, Thailand. The control group received usual daily oral hygiene care, while the intervention group followed oral health care program recommendations, including tongue brushing before swallowing therapy data collection covered demographics, oral health status, oral function (oral hygiene, tongue movement, and tongue pressure), swallowing severity, nasogastric feeding duration, and oral comfort. Descriptive and analytical statistics were used for analysis.

Results: Both groups improved swallowing ability. However, the intervention group had a significantly faster nasogastric tube removal rate than the control group (7.8±2.9 vs. 10.6±4.2 days, $p<0.05$). The intervention group also showed significant improvements in tongue coating index (57.5±15.2 vs. 76.8±9.3, $p<0.05$), diadochokinesis "ka" (3.4± 0.3 vs. 3.2±0.3 times/second, $p<0.05$), tongue pressure (15.6±5.0 vs. 11±3.8 kPa, $p<0.05$), and oral comfort (91.3% vs. 56.5%, $p<0.05$).

Conclusions: The oral health care program improves oral function and facilitates nasogastric tube removal in stroke patients undergoing swallowing rehabilitation. Healthcare providers should recommend suitable oral health programs to enhance oral function in post-stroke dysphagia rehabilitation.

Keywords: dysphagia, oral function, oral health, oral hygiene, stroke, tongue strength

Introduction

Dysphagia is common condition, over 50% of stroke patients[1], is often accompanied by hypofunction or orofacial dysfunction.[2-6] It leads to malnutrition, immune decline, and an increased risk of infection. Impaired daily activities worsen oral hygiene neglect, promoting bacterial buildup and heightening the risk of respiratory infections such as aspiration pneumonia.[7] Dysphagia significantly impacts oral, physical, and psychosocial health.[8-13] Swallowing recovery depends on multiple factors.[14,15] Oral dysphagia is primarily managed with conventional therapy, including oromotor exercises[16,17], sensory stimulation, dietary modifications, and compensatory techniques. This approach effectively help most patients discontinuing feeding tubes before discharge.[18,19]

Post-stroke patients with dysphagia receiving oral care from a multidisciplinary team tend to achieve better outcomes.[20-25] Their oral healthcare differs from other patients[20-23,26] and consists of two key components[7]: (1) oral hygiene, including mechanical cleaning and decontamination to prevent colonization and aspiration, and (2) oral function improvement through oromotor exercises and saliva stimulation to reduce aspiration risk. Combining mechanical cleaning of the teeth and tongue is more effective than tooth brushing alone.[27,28] Tongue brushing stimulates saliva production, essential in the oral preparatory phase, making it an indirect swallowing training method.[29] Studies show that tongue brushing enhances respiratory and swallowing functions by activating tongue muscles and strengthening the suprahyoid muscles, improving swallowing and coughing efficiency.[30-32] Comprehensive oral care, including tooth and tongue brushing alongside oral function training, strengthens swallowing muscles[32], enhances oral sensation and taste perception[33,34], increases nasogastric tube removal rates[26], and reduces aspiration and pneumonia incidence.[7,21,26,29] A multidisciplinary team coordinates care to optimize oral health and reduce aspiration pneumonia risk.[12,23]

Most studies on oral healthcare for stroke patients with dysphagia focus on those hospitalized during the acute phase[20,24,35], with fewer addressing care during swallowing rehabilitation.[21,26] These patients require a comprehensive oral healthcare program, including swallowing training, orofacial exercises, and oral hygiene, to improve swallowing and oral function. This study aimed to evaluate the effectiveness of oral health care program during rehabilitation, examining oral status and functions related to swallowing outcomes.

Materials and Methods

Study design and participants

This quasi-experimental study recruited stroke inpatients with dysphagia from the Rehabilitation Department of Chiang Mai Neurological Hospital, Thailand, between June 2022 and July 2023. The intervention was initiated immediately upon ward admission for patients who met the following inclusion criteria: (a) stroke diagnosis confirmed by a neurologist, (b) dysphagia assessment using the Water Swallowing Test (WST) conducted by an occupational therapist, (c) ability to undergo swallowing rehabilitation, (d) ability to communicate and follow instructions evaluated through a Basic Swallowing Evaluation conducted by an occupational therapist, and (e) daily oral hygiene maintained with tooth brushing and/or mouthwash. Exclusion criteria: (a) inability to follow instructions or uncooperative behavior, and (b) regular tongue cleaning. Block randomization assigned patients to two groups. The intervention group received daily oral hygiene care per the recommended program during hospitalization and rehabilitation, while the control group followed their usual routine.

A G*Power 3.1 analysis determined the minimum sample size, setting the effect size (ES) at 1.34 based on hospital data. With a type I error (α) of 0.05 and power (1-β) of 0.95, each group required 14 patients. To account for dropout rates, the sample size was increased to 23 per group, totaling 46 patients.

Procedures

Control group: During hospital-based dysphagia rehabilitation, patients maintained their usual daily oral hygiene -independently or with caregiver assistance- including tooth brushing and/or mouth rinsing twice a day (morning and evening).

Oral health care group: Patients and caregivers were trained by a dentist, and all participants followed a structured oral hygiene regimen before swallowing therapy and throughout the day, using recommended tools and protocols based on relevant studies[10,26,36] (Table 1). Both groups underwent oral and functional assessments before therapy and upon changes in food intake severity or rehabilitation completion. Oral hygiene adherence was

monitored through observation and patient interviews assessing oral comfort.

Measurement

Participant characteristics: Demographic data include gender, age, Body Mass Index (BMI)[37], stroke type, comorbidity history, and site of stroke, as recorded in hospital medical records. Oral care behaviors are assessed through patient and family interviews on self-oral care abilities, methods, and dental visit frequency.

Oral health status: Oral health status: A dentist assessed the patient's oral health, including the number of present teeth, functional teeth (FT; natural teeth excluding remaining roots and those with grade 3 mobility), occlusal pairs (OPs), and denture status. Masticatory performance was categorized as FT<20 or ≥20 and OPs 4 or ≥4.[38,39]

Oral function: Oral function refers to the mouth's ability to speak, chew, and swallow. Seven standard evaluation criteria were used to assess function: oral hygiene, dryness, occlusal force, tongue-lip motor function, tongue pressure, mastication, and swallowing.[38,39] A dentist trained in standardized oral function measurement conducted the evaluations. Intra reliability, measured using Cohen's Kappa Coefficient, was 0.83 and 0.85. Interrater reliability was 0.89 and 0.85, indicating excellent agreement. This study assessed three criteria related to swallowing ability in stroke patients with dysphagia.

(1) Oral hygiene: Assessed using the Tongue Coating Index (TCI)[40] as an alternative method.[38] A TCI score ≥50% indicated poor oral hygiene.[38] (2) Tongue-lip motor function: Tongue-lip motor function: Oral diadochokinesis (ODK) evaluated lip and tongue movement speed and coordination using the syllables /pa/, /ta/, and /ka/. Participants repeated each as many times as possible in 5 seconds. The syllable count was recorded via the pen dotting method, with a cut-off rate of <6 per second indicating impaired function.[6,38,39] (3) Tongue pressure: Measured using a tongue pressure measuring instrument (TPM-01, JMS Co., Ltd.). A balloon placed between the anterior hard palate and tongue was pressed with maximum force for 3 seconds.[6] The procedure was explained beforehand. TP was measured three times with rest intervals to calculate the mean. Values <30 kPa indicated reduced function.[38]

Table 1: Oral hygiene management of an oral health care program for stroke patients with dysphagia.

Oral hygiene tools	
	• Small-headed, soft-bristled toothbrush with a large handle. • High-fluoride toothpaste (1,450 ppm) with Sodium Lauryl Sulfate (SLS) free. • Tongue brush. • 0.12% Chlorhexidine gluconate solution. • Water-based oral moisturizer. • Other equipment: interdental brush, dental floss, cotton swab, and gauze.
Oral hygiene guidelines	
Positioning	• Sit or recline with the head elevated 30-45 degrees. Use a pillow to support the back or neck, keeping the neck straight.
Frequency	• At least twice daily: morning and before bedtime.
Morning – 30 minutes before swallowing rehabilitation	Step 1: remove food residues and sputum from the oral cavity with a cotton swab, gauze, or suction device. Clear as much as possible. Step 2: Brush teeth with a small-headed, soft-bristled toothbrush with a large handle. Use a pea-sized amount (0.5 cm) of high-fluoride toothpaste (>1,000 ppm fluoride), SLS-free, and employ minimal water for rinsing or use the "spit, don't rinse" technique. Spend at least 2 minutes brushing. Step 3: Use a tongue brush. Instruct the patient to extend their tongue as far as possible. Gently sweep the tongue brush outward toward the tip, brushing five times. The patient should feel slight resistance while brushing
Daytime care during the day	• Use a 0.12% chlorhexidine gluconate solution to wipe or spray the gums and oral mucosa, short duration (7-14 days). ***Stop use immediately if any adverse effects occur and inform the healthcare staff for further advice*. • Apply a water-based lubricant to moisturize dry lips.
Bed time	Perform steps 1 and 2.

Patient's self-oral care abilities: The researcher interviewed patients or caregivers about the patient's oral hygiene ability, categorizing them via the Barthel Index into three levels: dependent, requiring full assistance; independent with assistance, needing help with tasks like equipment preparation or reminders; and completely independent, managing oral hygiene alone.

Oral comfort: The researcher adapted the Numerical Rating Scale (NRS) and Faces Pain Rating Scale (FPRS) to assess oral comfort. Patients rated their oral comfort daily after swallowing rehabilitation using facial emotion icons and a color-coded system for easier decision-making. Oral comfort was classified into five levels: very low, low, neutral, good, and very good.

Severity of dysphagia: Swallowing rehabilitation data included admission date, nasogastric tube insertion and removal dates, and dysphagia severity before and after the program. An occupational therapist assessed clinical severity using the Functional Oral Intake Scale (FOIS), which consists of seven levels categorized into tube feeding (levels 1-3) and oral feeding (levels 4-7).[41] Nasogastric tube removal was based on daily clinical swallowing assessments by occupational therapists, diet progression tolerance, and physician approval, following standardized criteria for all patients.

Data collection

One day before the sample group began swallowing rehabilitation, the researcher collected demographic data, swallowing status, self-oral care abilities, and oral care behaviors from medical records and interviews, documenting them in a record form. Oral examinations were conducted, and oral functions were assessed by photographing the tongue for coating evaluation, measuring speech repetition rates, and assessing tongue pressure. During hospital rehabilitation, the intervention group received daily oral hygiene care per the oral health care program, while the control group followed their usual oral care routine. Both groups were monitored daily for oral hygiene care and assessed for oral comfort by nurses and nursing assistants. After daily rehabilitation, both groups were clinically evaluated by an occupational therapist to determine their swallowing ability, ensuring safe food texture modifications or readiness for progression. The final assessment determined whether participants had achieved safe swallowing and had their nasogastric tubes removed or were discharged despite continued dysphagia. The researcher recorded dysphagia severity, conducted oral examinations, assessed oral functions, and provided oral health care guidance for homecare. Data was recorded and entered into the researcher's password-protected computer to ensure restricted access.

Ethics

This study was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Chiang Mai University, Thailand (Approval No. 21/2565) and the Chiang Mai Neurological Hospital, Thailand (Approval Nos. EC 007-65 and EC 015-66). Participants were informed about the study's purpose, content, and their right to participate or withdraw. Informed consent was obtained from those who voluntarily agreed.

Statistical analysis

Data were analyzed using SPSS 29.0 software (IBM Corp., Armonk, NY, USA). Descriptive statistics, including percentages, frequencies, means, and standard deviations, summarized the data. The Chi-square test and T-test were used to compare categorical variables and differences between the intervention and control groups. A significance level of $p<0.05$ was used to reject the null hypothesis.

Results

A total of 46 stroke patients with dysphagia were recruited. Baseline characteristics were similar between groups, except for a history of hypertension ($p<0.05$) (Table 2). Baseline oral status also showed no significant difference ($p>0.05$) (Table 3). Before rehabilitation, all participants in both groups (100%) had poor oral hygiene, reduced tongue-lip motor function, and decreased tongue pressure, with no significant intergroup differences ($p>0.05$) (Table 3). After hospital rehabilitation, most patients in the intervention and control groups improved their swallowing ability. The majority resumed oral feeding (82.6% vs. 73.9%), while fewer required nasogastric feeding (17.4% vs. 26.1%), with no significant difference ($p>0.05$). The intervention group had a significantly shorter time to nasogastric tube removal (7.8±2.9 vs. 10.6±4.2 days; $p=0.015$). Oral comfort ratings of good to very good were significantly higher in the intervention group (91.3% vs. 56.5%; $p=0.017$) (Table 4). Post-

rehabilitation, oral function was significantly better in the intervention group, with lower TCI (57.5% vs. 76.8%), higher ODK /ka/ (3.4 vs. 3.2 times/s), and greater TP (15.6 vs. 11.0 kPa) (p<0.05) (Table 5).

Discussion

This quasi-experimental study evaluated the effectiveness of an oral health program for stroke patients with dysphagia. It assessed oral health and function, comparing tongue brushing before swallowing therapy to a control group. The intervention group showed significantly better oral hygiene, tongue pressure, posterior tongue movement, and a shorter nasogastric tube removal rate.

Participants were mostly male over 60 with ischemic stroke. Hypertension was the most common comorbidity, followed by diabetes and hyperlipidemia, consistent with other studies.[3,24,26,42,43] Patients experienced chewing difficulties due to limited functional teeth, absence of posterior occlusal pairs[38], infrequent denture use during hospitalization, and decreased self-care abilities, relying on others for oral health care. Neither group used tongue brushes despite visible tongue coating, likely due to concerns about gag reflex, aspiration, or lack of awareness of the importance of oral hygiene during hospitalization.[26] Before rehabilitation, participants exhibited reduced oral function, similar to findings in studies of dysphagic

Table 2: Baseline characteristics of participants.

	Total (N=46)	Oral health care group (n=23)	Control groups (N=23)	χ^2	p-value
Gender, n (%)				0.088	0.767
Male	25(54.4)	12(52.2)	13(56.5)		
Female	21(45.6)	11(47.8)	10(43.5)		
Age, Mean (SD)	69.1 (10.2)	67.0 (11.5)	71.2 (8.4)		0.162b
Body Mass Index, n (%)				1.099	0.577
Underweight (≤18.5)	11(23.9)	7(30.4)	4(17.4)		
Normal (18.6-22.9)	17(37.0)	8(34.8)	9(39.1)		
Overweight (≥23.0)	18(39.1)	8(34.8)	10(43.5)		
Type of stroke, n (%)				3.209	0.233a
Infarction	43(93.5)	20(87.0)	23(100)		
Hemorrhagic	3(6.5)	3(13.0)	0(0)		
Comorbidity, n (%)					
Hypertension	41 (89.1)	18 (78.3)	23 (100)	5.610	0.049[a,*]
Dyslipidemia	33 (71.7)	18 (78.3)	15 (65.2)	0.965	0.326
Diabetes mellitus	18 (39.1)	10 (43.5)	8 (34.8)	0.365	0.546
Other	7 (15.2)	4 (17.4)	3 (13.0)	0.168	1.000[a]
Site of stroke, n (%)				0.348	0.555
Left	24 (52.2)	13 (56.5)	11 (47.8)		
Right	22 (47.8)	10 (43.5)	12 (52.2)		
Self-oral care abilities, n (%)				0.817	0.665
Independent	7(15.2)	4(17.4)	3(13.0)		
Partial assist	20(43.5)	11(47.8)	9(39.1)		
Dependent	19(41.3)	8(37.8)	11(47.8)		
Method, n (%)				4.381	0.112
no	1(2.2)	0(0)	1(4.4)		
Mouthwash	3(6.5)	0(0)	3(13.0)		
Brushing teeth	42(91.3)	23(100)	19(82.6)		
Tongue brushing	0(0)	0(0)	0(0)		
Frequency, n (%)				1.037	0.595
0 time	1(2.2)	0(0)	1(4.4)		
1 time	27(58.7)	14(60.9)	13(56.5)		
2 times	18(39.1)	9(39.1)	9(39.1)		
Dental service in 1 year, yes, n (%)	0(0)	0(0)	0(0)		

n, number of samples Chi-square test, [a]Fisher's exact test, [b]independent t-test, Significant value: * p<0.05.

stroke patients using comparable or alternative methods and tools.[2,4,5,44,45] They demonstrated lower tongue strength and movement than older adults[39,46], highlighting the correlation between tongue function and swallowing performance.[47] Although these patients can safely resume swallowing, their oral function remains below typical standards for older adults. Thus, patients should be encouraged to perform regular oromotor exercises, particularly tongue-strengthening exercises, to reduce swallowing problems.[48] Likewise, studies have shown that continuous facial muscle exercises in older adults, both short-term (at least 2 months)[49] and long-term (6-12 months), improve tongue function to near-normal levels.[16,17,50]

Patients received daily 30-minute swallowing therapy from occupational therapists, combining direct and indirect techniques such as oromotor exercises, sensory stimulation, dietary modification, and compensatory techniques. Swallowing rehabilitation improves tongue function. The intervention group showed higher tongue pressure (15.6±5.0 vs. 11.0±3.8 kPa) and a greater rate of syllable 'ka' repetition (3.4±0.3 vs. 3.2±0.9 times/second, $p<0.05$), indicating an association between tongue pressure and oral motor function. This relationship is supported by previous studies[51,52], although causality cannot be confirmed. Other factors, such as age, oral health,

Table 3: Baseline of oral status and function.

	Oral health care group (N=23)	Control groups (N=23)	χ^2	*p*-value
Number of functional teeth, n (%)			0.840	0.359
< 20 functional teeth	13(56.5)	16(69.6)		
≥ 20 functional teeth	10(43.5)	7(30.4)		
Mean (SD)	17.2(7.9)	12.4(8.4)		0.069[b]
Number of occlusal pairs, n (%)			1.533	0.216
< 4 occlusal pairs	13(56.5)	17(73.9)		
≥ 4 occlusal pairs	10(43.5)	6(26.1)		
Mean (SD)	3.1(2.6)	1.9(1.8)		0.365[b]
Range (Min-Max)	(0-8)	(0-5)		
Active denture, n (%)			0.107	0.743
No	17(73.9)	16(69.6)		
Yes	6(26.1)	7(30.4)		
Active Wearing Denture), n (%)			0.258	1.000a
No	5(83.3)	5(71.4)		
Yes	1(16.7)	2(28.6)		
Oral hygiene, n (%)				
Poor (TCI ≥50%)	23(100)	23(100)		
Normal (TCI <50%)	0(0)	0(0)		
TCI, Mean (SD)	83.1(10.9)	84.4(8.3)		0.866[b]
Tongue-lip motor function, n (%)				
Low (ODK <6 times/second)	23(100)	23(100)		
Normal (ODK ≥6 times/second)	0(0)	0(0)		
ODK/pa/, times/second, Mean (SD)	1.5(0.4)	1.5(0.4)		0.877[b]
ODK/ta/, times/second, Mean (SD)	1.8(0.4)	1.8(0.4)		0.911[b]
ODK/ka/, times/second, Mean (SD)	1.8(0.4)	1.6(0.4)		0.09[b]
Tongue pressure, n (%)				
Low (TP <30 kPa)	17(73.9)	16(69.6)		
Normal (TP ≥30 kPa)	6(26.1)	7(30.4)		
TP, Mean (SD)	8.1(2.5)	6.4(1.6)		0.07[b]

Active denture, refers to patients who have dentures; Active denture wearing, refers to patients who not only have dentures but also use them during hospitalization TCI, Tongue Coating Index; ODK, Oral Diadochokinesis; TP, Tongue pressure n, number of samples; SD, standard deviation; kPa, kilopascal Chi-square test, [a]Fisher's exact test), [b]Mann-Whitney U test, Significant value: *$p<0.05$

may also contribute. Tongue brushing–requiring patients to protrude, steady, and resist while cleaning the tongue coating–mimics resistance exercises and improves range of motion. Patients in the oral health program exhibited increased tongue strength, resulting in greater protrusion beyond the lower lip and improved posterior tongue stimulation. This enhancement led to more effective pronunciation of tongue-base sounds, such as 'ka,' compared to the control group. Furthermore, enhanced strength and mobility of the tongue base improve swallowing by propelling the bolus into the pharynx, sealing the oral cavity, protecting the airway, and coordinating with pharyngeal muscles. The primary objective in dysphagia management is to ensure patients can safely transition to oral intake. Therefore, analyzing swallowing function outcomes using FOIS scores, dichotomized by tube feeding status, directly reflects this critical clinical outcome and facilitates clear outcome differentiation. This study shows that stroke patients with dysphagia in the oral health care program, which included tongue brush-

Table 4: Effects of the oral health program on the severity of dysphagia, nasogastric tube removal rate and the level of oral comfort.

		Oral health care group (N=23)	Control groups (N=23)	χ^2	*p*-value
Severity of dysphagia, n (%)					
Before	Tube feeding	23(100)	23(100)		
	Oral feeding	0(0)	0(0)		
After	Tube feeding	4(17.4)	6(26.1)	0.511	0.475
	Oral feeding	19(82.6)	17(83.9)		
Oral comfort, n (%)					
Before	Very low-neutral	18(78.3)	21(91.3)	-1.218	0.414
	Good-very good	5(21.7)	2(8.7)		
After	Very low-neutral	2(8.7)	10(43.5)	-2.657	0.017*
	Good-very good	21(91.3)	13(56.5)		
Nasogastric tube removal rate, ** **n (%)**		(n=19)	(n=17)		
1-7 days		11(57.9)	5(29.4)		
≥8 days		8(42.1)	12(70.6)		
Mean (SD)		7.8(2.9)	10(4.2)	-2.442	0.015[b*]
Median		7	10		
Range (Min-Max)		5-16	6-21		

** Nasogastric tube removal time was calculated from the initiation date of the oral care program.

n, number of sampls; SD, standard deviation Chi-square test, [b]Mann-Whitney U test, Significant value: *p<0.05

Table 5: Effects of the oral health program on the oral function.

		Oral health care group			Control groups			Z	*p*-value
		Mean	(SD)	Range	Mean	(SD)	Range		
TCI, %	Before	83.1	(10.9)	50.0-100	84.4	(8.3)	61.1-100	-0.169	0.866
	After	57.5	(15.2)	27.6-88.9	76.8	(9.3)	55.6-88.9	-4.151	<0.001*
ODK, times/second		1.5	(0.4)	0.8-2.0	1.5	0.4	0.6-2.0	-0.155	0.877
/pa/	Before	3.3	(0.4)	2.6-4.0	3.2	0.3	2.8-3.8	-1.166	0.244
	After	1.8	0.4	1.0-2.0	1.8	0.4	0.6-2.3	-0.112	0.911
/ta/	Before	3.6	0.3	3.0-4.2	3.5	0.3	3.0-4.4	-1.365	0.172
	After	1.8	0.4	1.0-2.5	1.6	0.4	0.6-2.2	-1.659	0.09
/ka/	Before	3.4	0.3	2.8-4.0	3.2	0.3	2.8-4.0	-2.132	0.033*
	After								
TP, kPa	Before	8.1	2.5	4.7-14.4	6.4	1.6	4.3-12.1	-2.741	0.07
	After	15.6	5.0	7.5-29.2	11.0	3.8	6.5-21.3	-3.175	0.01*

TCI, Tongue Coating Index; ODK, Oral Diadochokinesis; TP, Tongue pressure; kPa, kilopascal; SD, standard deviation Mann-Whitney U test, Significant value: *p<0.05

ing, resumed oral feeding (82.6%, $p>0.05$), experienced earlier nasogastric tube removal (7.8±2.9 days; $p=0.015$). Tongue brushing promotes oral hygiene, reduces coating buildup, and stimulates oral nerves and muscles, thereby strengthening the swallowing mechanism and enabling earlier resumption of feeding. Patients who performed tongue brushing before swallowing training had their tubes removed sooner[26], which is clinically important as it reduces the risks of pneumonia and malnutrition. Additionally, tongue brushing enhances sensory stimulation, improving food sensation and taste perception.[33] Izumi and Akifusa (2021) reported that tongue cleaning reduces coating, enhances taste, decreases halitosis, and stimulates the tongue and suprahyoid muscles–crucial for swallowing and speech.[32] Moreover, the oral health care program with tongue brushing reduces bacterial accumulation on the tongue, lowering aspiration risk during swallowing training, and is recommended to improve oral health and food intake in dysphagia patients.[25] While the improvement may reflect the spontaneous recovery of swallowing function[53], our findings suggest that the oral health program offers benefits beyond the natural recovery.

Study limitations include that subacute stroke patients often present cognitive and communication impairments, such as aphasia or apraxia, hindering their ability to follow instructions for assessing oral functions like repetition rate and tongue pressure. Evaluating tongue coating was challenging due to limited mouth opening, which required both photography and visual inspection. Hospital rehabilitation relied on a multidisciplinary team and caregivers; however, some caregivers were hesitant or lacked skills for tongue cleaning with a brush, and frequent caregiver changes necessitated nursing support to ensure adherence to the oral care program. This study may be adapted for stroke patients with dysphagia in various settings, including rehabilitation facilities and community care. When advanced measurement tools are unavailable or patients cannot follow complex instructions, simpler clinical assessments are feasible. For example, tongue strength can be evaluated by assessing symmetrical tongue protrusion, and tongue mobility by observing whether the tongue extends beyond the lips. These evaluations support initial assessments and facilitate periodic monitoring of rehabilitation progress. The small sample size limited statistical adjustment for confounders. Future studies should increase the sample size and use multivariable analyses to better assess factors influencing swallowing rehabilitation and oral function. Additionally, research should evaluate whether tongue range of motion affects swallowing training. The limited follow-up period constrains the interpretation of long-term outcomes. To enable comprehensive evaluation, future studies should incorporate extended follow-up durations and detailed clinical parameters, including stroke severity, length of hospital stay, duration of bed rest, present of xerostomia, and incidence of aspiration pneumonia.

Conclusions

In conclusion, rehabilitation for stroke patients with dysphagia should include daily tongue brushing as part of oral care before swallowing therapy. This practice contributes to improved oral hygiene, tongue mobility, strength, and swallowing function, facilitating earlier nasogastric tube removal. Furthermore, strengthening policy support and interdisciplinary teamwork can help integrate and promote oral care into stroke rehabilitation to optimize outcomes.

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Conflict of Interest

The authors declare no conflict of interest.

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Original Article

The Effect of Different Solvents for Gutta-Percha Removal on Cyclic Fatigue Resistance of Nickel-Titanium Retreatment File

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Abstract

Objectives: To evaluate the cyclic fatigue resistance of XP-Endo® Finisher R after immersion in various types of gutta-percha solvent.

Methods: A total of 48 new XP-Endo® Finisher R rotary files, 31 mm in length, were used in this study. These files were equally divided into four groups: the control group (not immersed in gutta-percha solvent) and three experimental groups (immersed in chloroform, eucalyptus oil and GuttaClear). The 16 mm working part of each file was exposed to solvents for 5 minutes. Then the cyclic fatigue testing was performed using a stainless steel artificial canal with a 0.5 mm inner diameter, a curvature angle of 60 degrees and a curvature radius of 5 mm. The time to fracture in seconds was recorded and then the number of cycles to fracture (NCF) was calculated. The length of each fracture fragment was also measured. Two fractured instruments from each group were randomly selected to examine the fracture surface under a scanning electron microscope. Data was analyzed by analysis of variance (ANOVA) and Bonferroni tests (p=0.05).

Results: There were no significant differences between the groups. However, the instruments immersed in eucalyptus oil showed the highest cyclic fatigue resistance followed by chloroform, control group and GuttaClear.

Conclusions: The immersion in different gutta-percha solvents had no effect on the cyclic fatigue resistance of XP-Endo® Finisher R retreatment files.

Keywords: cyclic fatigue, gutta-percha solvents, root canal retreatment, XP-Endo® Finisher R

Introduction

Nonsurgical root canal retreatment is indicated when the initial root canal treatment is unsuccessful.[1] The favorable outcome of root canal retreatment depends on the elimination of microorganism.[2] To achieve this goal, totally removal of previous root canal filling materials should be done, then the chemomechanical preparation of root canal system can be performed effectively.[3] Various methods have been proposed to remove root filling materials such as the use of heat, chemical solvents and mechanical removal by Nickel-Titanium (NiTi) rotary instruments.[4]

From previous reviews, in case of adequate root canal filling, the use of chemical solvents accompanied with NiTi rotary files can enhance the removal of root canal filling materials.[5] When comparing between the chemical solvents used for root canal retreatment, chloroform shows the most efficacy in soften root canal filling materials and make them easily removed.[6] Although chloroform is also known as carcinogen, the effects on health risk seem to be little when using as solvents during root canal.[7] According to the concerning of the toxicity of chloroform, natural gutta-percha solvents have been introduced such as eucalyptus oil and orange oil.[8] Gutta Clear (MDent, Bangkok, Thailand) is a new product of natural gutta-percha solvent, which is a citrus fruit oil-based solvent containing d-limonene has been introduced. The efficiency of d-limonene is equivalent to chloroform in dissolving gutta-percha.[9]

Moreover the development of gutta-percha solvents, various NiTi rotary file systems were also specifically designed for gutta-percha removal such as the ProTaper Universal retreatment system (Dentsply Maillefer, Switzerland), the R-Endo retreatment system (Micro-Mega, France), the D-Race retreatment system (FKG Dantaire, La Chaux-de-Fonds, Switzerland), the M-two retreatment system (VDW, Munich, Germany) and EdgeFile XR (EdgeEndo, USA). Nowadays, the XP-Endo® Finisher R (FKG Dantaire, La Chaux-de-Fonds, Switzerland) has been introduced. This NiTi rotary file was designed as ISO 30 in diameter with zero taper and produced under the heat-treated technology (MaxWire alloy), which claimed to resist the instrument fatigue. This rotary system can adapt to canal morphology that facilitates removal of root filling materials during retreatment procedures especially in curved root canals.[10]

Considering the advantages of using NiTi rotary in root canal retreatment, this can reduce treatment time and risk of root canal transportation.[11] However, using NiTi rotary instruments has the risk of separated due to torsional or cyclic fatigue.[12] Cyclic fatigue occurs when the instrument rotates in a curved canal and after a number of repeated cycles of tension and compression at the point of maximum flexure, the instrument fracture occurs.[13] During the retreatment process, the working part of rotary instruments come into contact for a few minutes with chemical solvents. This contact may influence the physical properties of NiTi rotary instruments. Previous study shows that chloroform had no effect on cyclic fatigue resistance of NiTi rotary retreatment files.[14] Since the literature is lacking of the effect of chloroform and the other solvents on the properties of heat-treated NiTi rotary instruments, this *in vitro* study aimed to evaluate the cyclic fatigue resistance of XP-Endo® Finisher R retreatment file after immersion in chloroform compare to the commonly used gutta-percha solvent such as eucalyptus oil and GuttaClear.

Materials and Methods

Sample size was calculated at the significance level of 0.05 and power of 0.95 using G * power, a sample size of 12 were obtained for each group.

A total of 48 new XP-Endo® Finisher R rotary files, 31 mm in length, were used in this study. These files were equally divided into four groups (twelve of each): the control group (not immersed in gutta-percha solvent) and three experimental groups (immersed in chloroform, eucalyptus oil and GuttaClear). In the immersion groups, the 16 mm working part of each file was exposed to solvents for 5 minutes in small glass tube.

The cyclic fatigue testing was performed using a specific device (Figure 1). This device comprised of a stainless steel artificial canal with a 0.5 mm inner diameter, a curvature angle of 60 degrees and a curvature radius of 5 mm. The handpiece was mounted on a holder. The speed and torque of endodontic motor (X-smart Plus; Densply Maillefer, Switzerland) were set at 1000 rpm and 1.5 N/cm as manufacturer's recommendation. The working length of artificial canal was set at 25 mm.

Each rotary file was rotated in the artificial canal until fracture occurred. The time to fracture in seconds was recorded. The number of cycles to fracture (NCF)

was calculated according to the following formula: NCF = time to fracture (in seconds) x rotational speed/60. The length of each fracture fragment was also measured using digital caliper. Two fractured instruments from each group were randomly selected to examine the fracture surface under a scanning electron microscope (SEM) (JEOL 6400; JEOL, Tokyo, Japan) at 200X magnification.

Statistical analysis

All statistical analyses were performed with SPSS 26.0 software (SPSS Inc, Chicago, IL USA). The Shapiro-Wilk test was used for normality testing. The analysis of variance (ANOVA) and Bonferroni tests were done to test the different of NCF and length of fracture between group. The level of significance was set at p=0.05.

Results

Descriptive statistics of NCF and the length of each fractured fragments of tested retreatment files are presented in Table 1.

There were no significant differences between the groups (p=0.556). However, the instruments immersed in eucalyptus oil showed the highest cyclic fatigue resistance followed by chloroform, control group and GuttaClear. Regarding the fragment lengths, there was no significant difference between them in terms of the mean fracture lengths (p=0.116).

The SEM analysis showed that the surfaces of retreatment files in all groups displayed the typical features of cyclic fatigue failure (Figure 2). They are characterized by a crack initiation site and fatigue zone. The fractographic analysis of cross-sectional fractured surfaces revealed crack sites at the peripheral surface, which indicated an initiation point of cyclic fatigue.

Discussion

Using Ni-Ti rotary file conjunct with chemical solvent is one of the commonly methods to remove root canal filling materials. Various types of NiTi rotary files were designed to facilitate the cleanliness of root canal during root canal retreatment. To the best of our knowledge, the present study aimed at investigations into the negative effect of various gutta-percha solvents on heat-treated NiTi rotary retreatment file.

The manufacturer claims that XP-Endo® finisher R has greater cyclic fatigue resistance. Based on the results of this study, XP-Endo® Finisher R show higher cyclic fatigue resistance than the others conventional NiTi rotary

Figure 1: Cyclic fatigue testing device.

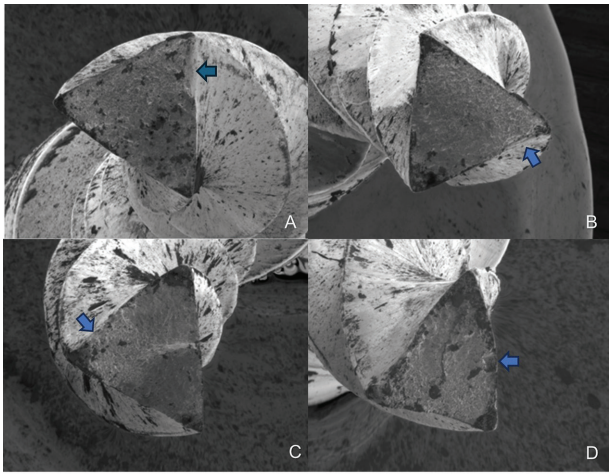

Figure 2: The fracture surfaces (200X magnification). (A) Control group; (B) Chloroform group; (C) Eucalyptus oil group; and (D) GuttaClear group. The arrows indicated crack initiation origin.

Table 1: Means ± standard deviations for NCF (cycles) and the length (mm) of fractured.

Data	Control	Chloroform	Eucalyptus oil	GuttaClear
Number of cycles to fracture (cycles)	1601.58±300.62	1636.83±292.01	1674.50±191.40	1568.75±311.41
Length (mm)	21.72±0.49	22.01±1.52	21.58±0.30	22.35±0.22

retreatment system.[14] This result can be explained by the fact that the NiTi rotary files in the previous study have the greater taper, whereas XP-Endo® Finisher R show zero taper.[15] Moreover, the design, geometrical shape and flexibility may have the effect on cyclic fatigue resistance. The retreatment rotary file systems such as ProTaper and R-Endo were designed for penetration into root filling materials. On the other hand, XP-Endo® Finisher R was designed to facilitate the removal of remnant root filling materials.[16]

In the current study, a static model was used during fatigue test. In the static model, stresses are concentrated in a particular area of the instrument which leads to change in the microstructure of the instrument. Compared to dynamic model, which the instrument moves axially, stresses are distributed along the shaft of the instrument. The differences in the distribution of stress concentration may affect the cyclic fatigue resistance of instruments.[17]

In this study, the retreatment instruments were placed in glass tube containing chloroform, eucalyptus oil, and GuttaClear to replicate the contact conditions as seen in prior research.[18] Previous studies indicate that retreatment instruments typically remove filling materials from root canals within approximately 5 minutes.[18,19] Therefore, a 5-minute immersion period was adopted to approximate clinical conditions in this study.

Our study was performed under static conditions, which do not replicate the actual clinical condition. In the clinical situation, the present of gutta-percha in root canal may affect the cyclic fatigue resistance of the files.

Various methods have been employed to assess the resistance to cyclic fatigue of NiTi instruments. In many studies, stainless steel canals have been utilized to prevent wear on the canal walls during instrumentation.[20] In this study, a device comprising a stainless steel canal with a 5 mm radius was employed to simulate curved canal. Using such devices ensures consistency in canal dimensions throughout the experiments. In this investigation, almost all the instruments fractured at the curvature, confirming the standardized positioning of the instruments within the test device.

An artificial stainless steel canal was used to standardize the anatomical variation of root canals. However, there are still no specifications or international standard block to ensure uniformity of methodology and comparable results for cyclic fatigue testing.

From the SEM image, it can be observed that crack initiation is present in the cross-sectional view. Additionally, the Ni-Ti rotary retreatment file does not exhibit unwinding in the longitudinal view, which is a characteristic of torsional fatigue. Therefore, it can be concluded that the fracture observed in this image is caused by cyclic fatigue.[21] Furthermore, a comparison between the control group and the group soaked in the solution for 5 minutes shows no change in surface condition. Thus, it can be concluded that soaking in various solutions does not affect the surface area (the cutting edge) of the material.

Conclusions

To summarize, the immersion in chloroform, eucalyptus oil and GuttaClear had no effect on the cyclic fatigue resistance of XP-Endo® Finisher R retreatment files.

Conflict of Interest

The authors declare no conflict of interest.

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Promoting Oral Health in Cleft Lip and Palate Patients: A Teledentistry and Social Media Messaging Intervention

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Abstract

Objectives: Children with cleft lip and/or palate (CLP) face unique oral hygiene challenges, placing them at a higher risk of dental caries and periodontal diseases. Teledentistry delivered via social media platforms can enhance oral health awareness and accessibility. This study evaluated the impact of a teledentistry-based program on toothbrushing behavior and clinical oral health outcomes among children with CLP.

Methods: A single-group pre-post intervention was conducted from October to December 2023 with 32 CLP children aged 6-14 years and their guardians. The intervention included onsite oral health education with entertainment activities, toothbrushing using plaque-disclosing tablets, and a social media group for guardians to share weekly photographs of plaque-disclosed teeth. Data collected included participation duration, plaque and gingival indices, and self-reported brushing behavior. Paired t-tests evaluated clinical changes, and correlation analysis examined relationships between participation levels and outcomes.

Results: Twenty-seven participants (84%) completed the 7-week program. Among these, 34% actively shared photos for 4-7 weeks, 28% for 1-3 weeks, and 22% did not engage. Self-reported improvements included increased brushing duration (p<0.001), better technique (p=0.003), and a more positive oral care attitude (p<0.001). Mean plaque and gingival indices significantly decreased from baseline (p<0.001). Participation level was not significantly associated with changes in clinical or behavioral outcomes.

Conclusions: A teledentistry-based program demonstrated potential to improve oral hygiene behaviors and clinical outcomes among children with CLP and to enhance dental care accessibility.

Keywords: cleft lip/palate, gingival index, oral health promotion, plaque index, teledentistry

Introduction

Children with cleft lip and/or palate (CLP) often face persistent challenges in achieving optimal oral hygiene due to both anatomical and functional limitations. Additional factors such as the higher prevalence of supernumerary teeth, dental crowding, and reduced dental arch space from maxillary underdevelopment can restrict access for toothbrush bristles and limit the natural cleansing action of the tongue and saliva, leading to plaque accumulation, gingival inflammation, and an increased risk of dental caries in this population.[1]

Maintaining good dental health is essential for successful orthodontic and craniofacial surgical outcomes in CLP patients. Premature tooth loss due to caries can result in alveolar bone loss, necessitating larger bone grafts and more complex surgical procedures, which may compromise long-term reconstructive results.[2] Although oral hygiene in CLP patients is often compromised, with appropriate preventive care their dental and periodontal health can be maintained at levels comparable to the general population.[3]

Teledentistry, a subset of telemedicine, integrates virtual technologies such as computers and mobile devices with the internet to provide digital communication for dental care, enabling remote consultations, diagnosis, treatment planning, and oral health promotion and prevention.[4,5] One of the early applications of teleconsultation was remote diagnosis and follow-up services. Programs implemented in inner-city childcare centers demonstrated that teledentistry could effectively screen preschool children for active dental caries.[6] A subsequent feasibility study found that dental screening of preschool children using intraoral cameras was comparable to visual oral examinations, supporting teledentistry as a viable screening method.[7] During the COVID-19 pandemic, teledentistry was used to manage cleft lip and/or palate patients through virtual clinics. Patients were classified using the Red-Amber-Green scale, which helped reduce unnecessary face-to-face appointments.[8] Teledentistry has also been utilized for health promotion and patient education, demonstrating its effectiveness in improving oral health outcomes. A meta-analysis evaluating the effect of teledentistry-based (telematic) strategies for oral health prevention and promotion found that these approaches may result in reductions in plaque index (standardized mean difference, -1.18; 95% CI, -1.54 to -0.82; low certainty) and gingival index (standardized mean difference, -2.17; 95% CI, -3.15 to -1.19; moderate certainty).[4]

Several studies have utilized digital interventions using social media messaging platforms to enhance oral hygiene behaviors. A mobile messaging "Brush Game" on WhatsApp encouraged adolescent orthodontic patients to share weekly selfies, resulting in significantly lower plaque and gingival indices.[9] Educational interventions delivered through Telegram channels improved toothbrushing frequency and clinical indicators among Iranian adolescents.[10] WhatsApp group messaging to parents of preschool children helped control the severity of early childhood caries and improve oral health literacy.[11]

While these studies demonstrate digital intervention effectiveness in general populations, children with CLP who face additional oral health challenges have not been explored. The present study aimed to assess the effectiveness of a teledentistry-augmented oral health program in improving oral hygiene behaviors and clinical outcomes in this population.

Materials and Methods

Study setting and subjects

The study used a single-group, pre-post design and was conducted at a host site in Chiang Rai, Thailand, between October and December 2023, within multinational care settings as part of a regional cleft care initiative led by Northern Womans Development Foundation (NWDF) in collaboration with Transforming Cleft's international network. Each implementation consisted of two 3-hour sessions: the first session provided oral health education for children with CLP, and the second session, scheduled seven weeks later by NWDF, was conducted for follow-up data collection.

The study was approved by the Human Experimentation Committee of the University of Phayao (Certificate of Ethical Clearance No. HREC-UP-HSST 1.3/067/66). Fifty-four children aged over 6 years were invited through the NWDF care network, which serves patients in Thailand, Laos, and Myanmar. Thirty-two participants aged 6-14 years attended on the day of the first session and were enrolled after confirming eligibility and obtaining consent.

Inclusion criteria were children with CLP whose guardians had internet access at home to support remote activities and who were able to attend both onsite sessions for baseline and follow-up data collection. Children with

additional physical disabilities that interfered with oral hygiene practices were excluded. There was no randomization or control group due to practical and ethical constraints, given the small multinational sample and unified program schedule.

Data collection

Data collection involved two methods: a short questionnaire developed to assess four items (Suppl. Material 1) and clinical assessment. The questionnaire provided an overview of self-reported brushing behavior, including duration, frequency, discipline, and technique using binary responses (Yes/No), and attitudes toward oral care measured on a 5-point Likert scale.

The questionnaire was intentionally kept short to fit within the 3-hour intervention schedule and to allow simple, direct translation for multilingual participants. Additional descriptions were provided to guardians to clarify assessment criteria for each item. Three experts, a general dentist, a periodontist, and a dental public health specialist, assessed the questionnaire's content validity, achieving an Item-Objective Congruence (IOC) index of 1.00 on all items.

Two Thai interviewers were trained by a dental public health specialist (P.S.) to ensure recording reliability, and interpreters for Lao and Myanmar participants were provided by NWDF to minimize language barriers during data collection.

Clinical parameters, including the Löe-Silness gingival index[12] and Silness-Löe plaque index[13], were assessed by a single board-certified periodontist (M.J.) to ensure consistency, while caries status (d_3mt/D_3MT) was evaluated by an orthodontist (K.N.), both independent from the intervention. The GI and PI were measured on six index teeth, with four sites per tooth (FDI tooth numbers: 16, 11, 24, 36, 31, 44). Examinations were conducted using a portable dental chair and artificial light for illumination.

Oral health promotion program

Limited resources were found for social media-based oral health promotion programs targeting children with CLP. Previous studies in non-cleft populations[9,10] have applied educational and preventive approaches incorporating guided oral hygiene instruction with plaque disclosure and weekly encouragement, showing improvements in plaque control and gingival health. The present oral health promotion program was designed with two components: an onsite oral health education session and a teledentistry-augmented component with weekly plaque disclosure.

The onsite session included a group activity on dental caries and proper hygiene practices, followed by supervised toothbrushing with half of a plaque-disclosing tablet (Dentiste Plaque Test Tablets, Thailand) to help children visualize plaque. Guardians were present to assist with brushing techniques and were shown how to take before-and-after photographs, focusing on an anterior view. All participants received a toothbrush, fluoride toothpaste (1,450 ppm fluoride), and plaque-disclosing tablets for use during the program.

The remote component involved the children's guardians in a weekly online activity. After the onsite session, guardians were invited to join a private group chat on a social media messaging platform (Facebook Messenger) titled "Brushing Activities." An anonymous LINE group was initially considered but was changed to Facebook Messenger based on participant preference.

This channel served as a tool within the teledentistry framework and was constructed based on Social Cognitive Theory[14], aiming to build children's self-efficacy in oral hygiene by helping them visualize plaque removal and practice skills consistently. Each weekend, guardians were instructed to help their child use a plaque-disclosing tablet at home and take close-up photographs of the child's teeth before and after brushing. These photographs served to support self-monitoring of cleaning effectiveness (Behavior Change Technique; BCT 2.3: Self-monitoring of behavior) and provided visual feedback to reinforce oral hygiene behavior (BCT 2.2: Feedback on behavior). Sharing these images within the group chat promoted engagement and a sense of peer involvement through group participation (BCT 3.1: Social support). Participation was monitored, and summary results were posted in the group every two weeks on Fridays to remind guardians to submit photos over the weekend (BCT 10.4: Social reward).[15]

At the end of the 7-week program, all participants were re-examined to assess changes in brushing behavior and clinical parameters from baseline. The intervention group chat was deleted after three months, with a small number of non-identifiable images retained for publication purposes.

Figure 1: (Left) Onsite oral health promotion session providing education and supervised toothbrushing, photographed by Northern Womans Development Foundation (NWDF) and used with permission. (Right) Facebook Messenger group chat for sharing disclosed plaque photographs weekly.

Statistical analysis

Statistical analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). The threshold for statistical significance was set at $p<0.05$. Descriptive statistics were used to summarize participant characteristics, presented as number (percentage) for categorical variables and mean (± standard deviation) for continuous variables. Participants' engagement in the remote component was categorized into three groups based on observed trends in weekly photo-sharing activity: no participation, moderate participation, and active participation.

Normality of data distribution was evaluated with the Shapiro-Wilk test, and homogeneity of variances with Levene's test. The McNemar test was applied to compare changes in categorical brushing behavior responses (pre- vs. post-intervention). Changes in clinical parameters (gingival and plaque indices) were assessed with paired t-tests. Additionally, Pearson correlation analyses were conducted to examine relationships between study variables.

Results

Participant characteristics

Baseline characteristics of the participants, including gender, age, ethnicity, caries experience, and participation level, are summarized in Table 1. The participants' ages ranged from 6 to 14 years, with the majority being of Lao ethnicity (59%), followed by Thai (33%) and Burmese (8%). Five participants did not attend the follow-up session, resulting in 27 out of the initial 32 participants (84%) completing the examination. Of these, 11 (34%) were classified as active participants (sharing photos in 4-7 of the weeks), 9 (28%) had moderate participation (1-3 weeks), and 7 (22%) did not share photos in the group chat.

Table 1: Baseline characteristics of the participants.

Characteristic	All (N=27)
Gender, n(%)	
Male	17 (63.0%)
Female	10 (37.0%)
Age (years)	
Mean (SD)	9.67 (2.69)
Range	6-14
Ethnicity, n(%)	
Lao	16 (59.2%)
Thai	9 (33.3%)
Burmese	2 (7.5%)
Participation, n(%)	
Active (4-7 weeks)	11 (40.7%)
Moderate (1-3 weeks)	9 (33.3%)
Absence (0 week)	7 (26.0%)
Caries status	
d_3mt and D_3MT (SD)	3.41 (3.88)
Absence of Caries $d_3/D_3=0$ (%)	6 (22.2%)

Brushing behavior

Table 2 presents the outcomes of toothbrushing behavior as reported by the guardians. The proportion

of children who brushed their teeth for more than two minutes increased from 14.8% at baseline to 92.6% at follow-up (p<0.001). Improvement in toothbrushing technique was also observed, with the percentage of children rated as having proper technique rising from 44.4% to 85.2% (p=0.003). Regarding brushing frequency, the proportion of participants who brushed at least twice daily increased from 85.2% to 96.3% (p=0.375). Participants also adopted better self-discipline in oral care, as indicated by an increase from 59.3% at baseline to 85.2% at follow-up in maintaining a regular brushing routine (p=0.065). Attitudes toward oral care also improved, with the average attitude scores increasing from 2.74 to 3.96 out of 5 (p<0.001).

Clinical parameters

Changes in clinical parameters, including the plaque index (PI) and gingival index (GI), are shown in Figure 2 and reported in detail in Supplementary Table 1. One participant from the moderate participation group was excluded from the analysis as an outlier due to pronounced increases in PI (+0.625, >2 SD) and GI (+0.500, >2 SD), whereas the overall group showed significant mean reductions in both indices.

Across all participants (n=26), PI decreased from 1.841±0.376 at baseline to 1.422±0.453 at follow-up (mean change -0.418, 95% CI -0.557 to -0.279, p<0.001), and mean GI decreased from 1.866±0.392 to 1.443±0.449 (mean change -0.423, 95% CI -0.572 to -0.273, p<0.001). Examining the subgroups, the active participation group (n=11) had the lowest baseline scores for both PI (1.738±0.397) and GI (1.757±0.422) and showed the greatest improvements, with PI decreasing to 1.259±0.454 (mean change -0.478, 95% CI -0.786 to -0.171, p=0.006) and GI decreasing to 1.236±0.430 (mean change -0.521, 95% CI -0.839 to -0.202, p=0.004). Participants in the moderate and no participation groups also showed significant reductions in PI and GI (within-group p<0.05).

Correlation analysis showed a strong positive correlation between PI and GI, and participation levels appeared to be associated with clinical improvements, although this association was not statistically significant.

Discussion

Teledentistry encompasses a range of approaches that integrate telecommunications and digital technologies into dental care.[4,16] Within the broader framework of eHealth, which encompasses all digitally supported health services, public health activities delivered via mobile devices are referred to as mHealth.[17] As part of this framework, teledentistry uses both computer-based systems and mobile applications to support services such as teleconsultation, telediagnosis, remote monitoring, and oral health education.[4,16,18]

Teledentistry interventions for prevention and promotion of oral health have increasingly used social media messaging platforms, with strategies adapted to distinct target populations. For early childhood caries prevention, LINE has been used to deliver structured educational content to parents and caregivers, supporting improved home-based oral hygiene in young children.[19] For patients with fixed orthodontic appliances, WhatsApp[20] and WeChat[21] have been used to support behavior change, including sending reminders and structured educational content to improve oral hygiene compliance. Among college students, WhatsApp has been used to deliver

Table 2: Changes in toothbrushing behavior and attitudes.

Characteristics	Baseline (N=27)	Follow-up (N=27)	p-value
Brushing behavior n(%)			
Duration (more than 2 minutes)	4 (14.8)	25 (92.6)	<.001*[a]
At least two times per day	23 (85.2)	26 (96.3)	.375[a]
Having self-discipline	16 (59.3)	23 (85.2)	.065[a]
Having proper technique	12 (44.4)	23 (85.2)	.003*[a]
Attitude toward oral care (SD)	2.74 (0.764)	3.96 (0.808)	<.001*[b]

*Indicates statistical significance at p<0.05

[a]McNemar test

[b]Paired t-test

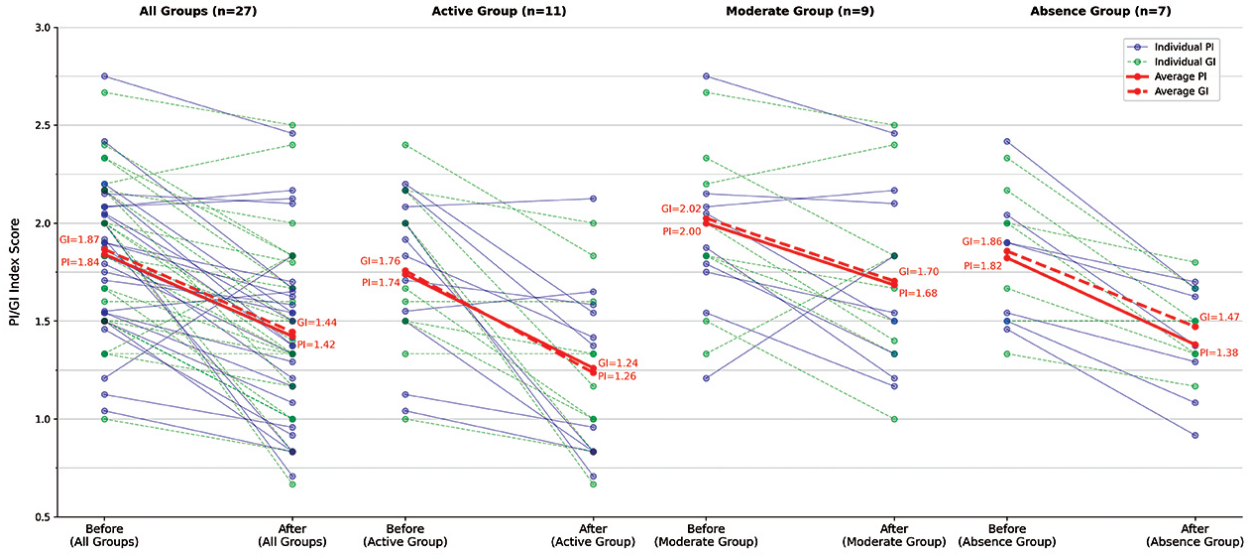

Figure 2: Changes in Plaque Index (PI) and Gingival Index (GI) scores before and after intervention. Individual PI scores are shown by blue lines, GI scores by green lines; average scores are indicated by solid (PI) and dashed (GI) red lines.

multimedia educational content aimed at enhancing oral health knowledge and daily practices.[22] The self-photograph component can be further integrated into social messaging interventions to enhance behavioral monitoring. A telegram channel has been used for weekly sharing of plaque disclosure photographs, incorporating peer interaction and competitive elements to promote engagement and adherence.[10] Similarly, a WhatsApp-based "Brush Game" required participants to submit weekly dental selfies taken before and after plaque disclosure tablet use, transforming routine plaque control into a gamified activity that encouraged consistency.[9]

Consistent with these approaches, this study utilized Facebook Messenger as a platform for guardians to photograph and share their children's plaque disclosure results weekly in children with CLP, whose oral hygiene is often compromised due to anatomical and functional limitations.[1,2] The intervention augmented a conventional oral health promotion program for children with CLP by integrating teledentistry components. The onsite component provided oral health education, oral hygiene instruction, and supervised toothbrushing demonstrations. Remote teledentistry activities incorporated self-photographs of disclosed teeth for behavioral monitoring and guardian engagement through social media messaging.

Intervention mapping is a systematic protocol for developing theory-based and evidence-based health promotion programs, intended to guide health promoters in designing structured interventions, whereas the components of such interventions can be described using the Behavior Change Techniques (BCTs) taxonomy to standardize reporting and facilitate replication of evidence-based programs.[15,23,24] Certain social messaging interventions have utilized the Health Action Process Approach (HAPA) to address both the motivational and volitional phases, as in an orthodontic care program delivered via WeChat that incorporated personalized reminders, weekly plaque-disclosing agent use, photographic self-monitoring, and targeted feedback from orthodontists.[21] A HAPA-based Telegram intervention explicitly incorporated BCTs including weekly plaque disclosure, photo uploads, peer interaction, and group encouragement to sustain engagement and support consistent oral hygiene routines.[10]

The implemented intervention incorporated four key BCTs within an SCT framework: plaque disclosure photography enabled self-monitoring and feedback on behavior (BCT 2.2, 2.3), which can improve patients' education and motivation by guiding their self-performed oral hygiene.[25] Group participation provided social support (BCT 3.1) and social recognition through participation monitoring (BCT 10.4). The program also incorporated guardian supervision through close-up photo-taking, which is known to improve children's toothbrushing skills and plaque control.[26] However, the degree of parental involvement was not assessed in this study. These BCT components aimed to strengthen self-efficacy in both parents and children by enabling them to observe

improvements and gain confidence in maintaining oral hygiene. As reported by guardians, significant increases were observed in brushing for more than 2 minutes (14.8% to 92.6%) and using proper technique (44.4% to 85.2%), with additional improvements in twice-daily brushing (85.2% to 96.3%) and self-discipline (59.3% to 85.2%), which may have lacked statistical significance due to high baseline values and a ceiling effect. Clinical parameters also showed improvements, with significant reductions in plaque and gingival indices (mean change -0.418 and -0.423, respectively).

In digital behavior change interventions, engagement can be conceptualized in two dimensions: behavioral engagement, defined as the extent of usage in terms of amount, frequency, duration, and depth of interaction, and experiential engagement, referring to the user's subjective experience of attention, interest, and affect.[27] Previous teledentistry interventions with weekly plaque disclosure photographs[9,10,21] did not quantify participation, which limits the interpretation of engagement in relation to outcomes. The present study addressed this gap by quantifying behavioral engagement as total participation weeks. Correlation analysis revealed a positive trend between the number of participation weeks and clinical improvements, but this association was not statistically significant. Although greater engagement is theoretically expected to improve outcomes, evidence for dose-response relationships in digital health interventions remains inconsistent and unable to demonstrate robustly.[28]

Participants were further categorized into three groups based on observed patterns: active participation (n=11), moderate participation (n=9), and no participation (n=7). Some individuals with no recorded participation demonstrated clinical improvements comparable to those with moderate participation. The non-participation group may have internalized their learning and continued practicing outside the digital space, or discontinued engagement because personal goals were met.[28] For instance, participants who achieved satisfactory plaque control may no longer have desired to participate in sharing self-photographs. Additionally, baseline motivation and self-efficacy, recognized as unmeasured third variables in digital interventions, may have influenced engagement patterns independently of the intervention itself, as engaged participants may have greater inclination toward healthy behaviors in general.[27] Future studies should incorporate measures of experiential engagement, motivation, and self-efficacy to better understand and adjust for these potential confounders.

The study had several limitations related to participants and research design. As part of a project organized by an external organization, the research team had limited control over participant selection and the timing of intervention and follow-up. The single-group pre-post design limits establishing causal relationships, as observed changes may not be attributed to the intervention without a control group. Instead, quantifying weekly participation served as a proxy measure for intervention exposure. The two-month assessment period was shorter than the 6-12 months reported in related studies, which may have reduced the magnitude of detectable changes and limited the ability to assess long-term effects. The small sample size (n=32, compared with n=44, 80, and 791 in previous studies[9,10,20]) also limits the generalizability of the findings. Socioeconomic variables, cultural factors, and degree of parental supervision were not collected due to the lack of standardized interpretation across nationalities and study design constraints, preventing adjustment for these potential confounding factors. Language differences require simplifying questionnaires, restricting responses to basic behavioral outcomes. Data on brushing behavior were based on guardian reports, which are susceptible to memory and social desirability bias.[10] Instead, clinical indices (PI and GI) were measured to provide an objective evaluation, as these represent the primary outcomes in teledentistry intervention studies.[4]

Despite these limitations, the teledentistry intervention combining plaque disclosure photography with guardian involvement demonstrated potential as a practical approach to improving oral health care in children with CLP. The combination of plaque disclosure and self-photography enables continuous self-monitoring of anatomically compromised areas. Regular plaque disclosure and photo sharing, coupled with remote supervision and feedback via a social media group, may reinforce proper brushing techniques and build self-efficacy for maintaining good oral hygiene practices at home. These findings suggest that integrating teledentistry into oral health programs for children with CLP is feasible across diverse care settings and may help overcome barriers to providing ongoing oral health support.

Conclusions

The teledentistry-based oral health promotion program, implemented through a social media messaging platform, demonstrated potential to improve brushing behaviors and clinical parameters among children with CLP. This approach may promote self-monitoring and enhance oral care practices, particularly in areas with limited access to dental services. Further studies with larger samples and controlled designs are needed to confirm and extend these findings.

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Conflict of Interest

The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIALS

Supplementary Material 1 – The questionnaire for assessing self-reported brushing behaviour and attitudes toward oral care (ENGLISH).

1. Self-reported brushing behaviour	
1.1 Brushing more than 2 minutes	Yes / No
1.2 Having self-discipline to brushing	
* Demonstrates an awareness of brushing time and comes to brush their teeth immediately when called.	Yes / No
1.3 Appropriate brushing technique	
* Effectively removes plaque and food debris from all areas of the mouth.	Yes / No
2. Attitudes toward oral care	
2.1 Level of attitude towards oral care	Lowest / Low / Moderate / High / Highest

*The descriptions are provided to the guardians to clarify the assessment criteria.

Supplementary Table 1: Plaque index and Gingival index at baseline and follow-up

Clinical Parameters	Subgroups	Baseline (SD)	Follow-up (SD)	Mean Change (95% CI)	*p*-value
Plaque index	All (N=26)	1.841 (0.376)	1.422 (0.453)	−0.418 (−0.557 to −0.279)	<.001*
	Active (n=11)	1.738 (0.397)	1.259 (0.454)	−0.478 (−0.786 to −0.171)	.006*
	Moderate (n=8)a	1.999 (0.363)	1.684 (0.489)	−0.314 (−0.525 to −0.103)	.010*
	Absence (n=7)	1.822 (0.348)	1.379 (0.304)	−0.442 (−0.642 to −0.243)	.002*
Gingival index	All (N=26)	1.866 (0.392)	1.443 (0.449)	−0.423 (−0.572 to −0.273)	<.001*
	Active (n=11)	1.757 (0.422)	1.236 (0.430)	−0.521 (−0.839 to −0.202)	.004*
	Moderate (n=8)*a*	2.024 (0.363)	1.704 (0.521)	−0.320 (−0.542 to −0.098)	.011*
	Absence (n=7)	1.857 (0.365)	1.471 (0.215)	−0.385 (−0.644 to −0.126)	.011*

[a]One participant was excluded from analysis as an outlier (PI change +0.625 and GI change +0.500)

*Indicates statistical significance at $p<0.05$.

Supplementary Table 2: Correlations between clinical parameters, participation levels, and toothbrushing behaviour

Variables	Clinical parameters			Activity	Toothbrushing behaviour				
	PI	GI	dmt/DMT	Participation (weeks)	Duration	Frequency	Discipline	Technique	Attitude
PI Increment									
r	1.000	.872	.379	-.046	-.233	-.244	-.041	-.272	-.227
p-value	-	<.001*	.052	.819	.242	.220	.837	.170	.254
GI Increment									
r	0.872	1.000	.364	-.151	-.218	-.245	-.050	-.181	-.274
p-value	<.001*	-	.062	.452	.274	.219	.805	.365	.167
Participation (weeks)									
r	-.046	-.151	-.095	1.000	.152	-.036	.003	.262	-.074
p-value	.819	.452	.636	-	.448	.858	.989	.188	.713

* Indicates statistical significance at $p<0.05$

r Pearson's correlation coefficient

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Prevalence and Demographics of Non-Syndromic Tooth Agenesis on the Population of Orthodontic Patients

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Abstract

Objectives: To evaluate the prevalence of tooth agenesis (TA) and its association with gender, tooth type, and location. Additionally, to investigate the clinical features of TA in non-syndromic subjects attending the postgraduate orthodontic clinic at Bangkok thonburi University, Chiang Mai branch.

Methods: This study was conducted utilizing 1337 patients, 935 females and 402 males, aged 12-45 years. TA, demographic data, and clinical features were surveyed using their complete pretreatment records, study models, panoramic radiographs, and intra-oral photographs according to gender, tooth type, location (Maxillary/Mandibular arch), and their clinical features. The level of statistical significance was set at $p<0.05$ using Chi-Square tests and descriptive statistics.

Results: The study group included 82 patients (22 males, 60 females). A total of 129 teeth were missing, not including the third molars. TA was more prevalent in the mandible. The mandibular lateral incisor was the most frequently observed missing tooth. The clinical features of TA were also classified. Microdontia was found the most frequent in maxillary arch, whereas prolonged retention was found more in mandibular arch. The most common features of maxillary intra-arch relationship were crowding, whereas spacing was more prevalent in the mandibular arch. Regarding the features of inter-arch relationship, there were deep bite, followed by anterior crossbite, anterior protrusion, posterior crossbite, and open bite respectively.

Conclusions: A prevalence of 6.13% for TA was detected in this study. There are several clinical features involved in TA both intra and inter-arch relationship.

Keywords: clinical features, inter-arch relationship, intra-arch relationship, tooth agenesis

Introduction

Tooth agenesis (TA) refers to the absence of teeth due to the absence of a tooth bud. It is one of the most common developmental anomalies in human dentition and occurs frequently across different ethnic groups, with the third molar being the most commonly missing tooth.[1] It can result from a disease, a syndrome associated with various systemic disorders (syndromic form), or from genetic abnormalities.[2,3] Additionally, environmental factors can influence TA. For instance, smoking and alcohol consumption during pregnancy are associated with a significantly higher prevalence of TA.[4,5]

TA can present in different forms, with its variability influenced by factors such as gender, age, race, and geographic location.[6] Previous studies have found that the prevalence of TA was 8.9% in Thais and the most commonly lost tooth was the mandibular second premolar.[7] The prevalence of TA ranges from 4.4% in Latin America, 5.0% in North America, 6.3% in Asia and Australia, and 13.4% in Africa.[8]

Recent studies have explored the prevalence and demographic data of TA.[3,6,9] However, there is still limited information regarding clinical features that may indicate TA. There are various types of malocclusions which are often associated with TA.[5] The following characteristics has been reported: The mandibular incisors are retroclined[10], retained primary teeth[1,11], ectopic permanent maxillary canines, transposition and rotation of teeth, and peg-shaped maxillary lateral incisors[1], disturbances in spacing of the dentition[11], shorter upper and lower dental arch lengths.[12] When retained primary teeth lack a permanent successor and are severely submerged, several clinical features may arise. These include underdevelopment of the alveolar process, absence of normal mesial drift, non-response to orthodontic forces, supra-eruption of opposing teeth, lateral open bite, and an increased occurrence of crossbites. Nonetheless, such disturbances have no long-term effects on occlusion.[13] Moreover, Becker and Shochat observed a significant shift in the dental midline toward the affected side.[14]

TA often poses a significant clinical challenge for orthodontists, as it may result in prolonged treatment duration and potentially compromised treatment outcomes in some cases.[15] Furthermore, early diagnosis of tooth agenesis is crucial, as it enhances the opportunity to address the child's dental issues from the initial oral examination through radiographic evaluation and appropriate treatment planning. Therefore, identifying the prevalence, demographic data, and clinical features of TA could be valuable for early detection and for guiding the development of future treatments.

This research was undertaken due to the current lack of comprehensive studies examining TA and its clinical features in Thai patients residing in Northern Thailand. This study aimed to evaluate the prevalence of TA and its association with gender, tooth type, and location. Additionally, to investigate the clinical features of TA in non-syndromic subjects attending the postgraduate orthodontic clinic at Bangkok Thonburi University, Chiang Mai branch.

Materials and Methods

This retrospective study included patients' records, which were collected for diagnostic purposes, routinely taken prior to orthodontic treatment at the postgraduate orthodontic clinic at Bangkokthonburi University, Chiang Mai branch from August 2016 to December 2023. The study received ethical approval from the Human Experimental Committee of the Faculty of Dentistry, Bangkokthonburi University, Thailand (No.27/2024). The sample size was calculated from G power program using prevalence of TA in Thai population from previous study[9] with an alpha=0.05 and effect size=0.11.

Three examiners created a numerical code to identify patients without specifying their names to record data of patients, the number of TA patterns in gender, tooth type, location (Maxillary/Mandibular arch), and clinical features based on intra-arch and inter-arch. The data were collected from dental history records, intraoral photographs, digital study models, and panoramic radiographs under the supervision of two orthodontists. Panoramic radiographs, using an Orthophos S 3D (Dentsply Sirona, Bensheim, Germany) device and processed with its inbuilt software (Sidexis XG 2.61, Dentsply Sirona, Bensheim, Germany), were measured twice, one week apart, to ensure reliability and reduce examination bias. Intra-rater and inter-examiner reliability was then evaluated using Kappa statistics of 20 panoramic films.

The inclusion criteria were as follows: Thai national residences in the Northern Thailand, the patients with TA of one or more teeth, the patients with complete dental history records, intra-oral photographs, digital study

models, and panoramic radiographs before treatment. The exclusion criteria were as follows: Patients with systemic diseases, syndromes, unclear diagnoses of missing teeth on radiographs, or a history of dental trauma, extraction, or surgical treatment (excluding third molar) were excluded.

Associated clinical features of TA were also evaluated, including microdontia and prolonged retention. Other clinical features relating to intra-arch relationship were torsi version, spacing, infraocclusion, supraocclusion, crowding, and transposition. The clinical features of inter-arch relationship were deep bite, anterior crossbite, anterior protrusion, posterior crossbite and open bite. All statistical analyses were conducted using IBM SPSS statistics for Windows, Version 25 (IBM Corp., Armonk, NY, USA). The independent variables are TA, whereas the dependent variables are gender, tooth type, location (Maxillary/Mandibular arch) and all clinical features. Descriptive statistics were used to present the patient characteristics. For inferential statistic, the Chi-square test was employed to evaluate the association between gender, tooth type, location, and TA. The level of statistical significance was set at $p<0.05$. The examination, evaluation, and analysis process were summarized in the workflow diagram as shown in Figure 1.

Results

Both intra-examiner and inter-examiner agreements in this study were nearly perfect (using Cohen's Kappa Coefficient statistic value equal 1). A total of 1337 previous dental history, panoramic radiographs, digital model, and intra-oral photographs of healthy patients aged 12-45 years, 935 females and 402 males were examined. Non-syndromic TA in the permanent dentition (excluding third molars and patients without study models) was diagnosed in 82 subjects, with a total of 129 missing teeth. The overall prevalence of TA was found to be 6.13%. The prevalence of TA was higher in females than males (6.42% and 5.47%, respectively) as shown in Table 1.

The most prevalent TA was lateral incisor (58.91%;

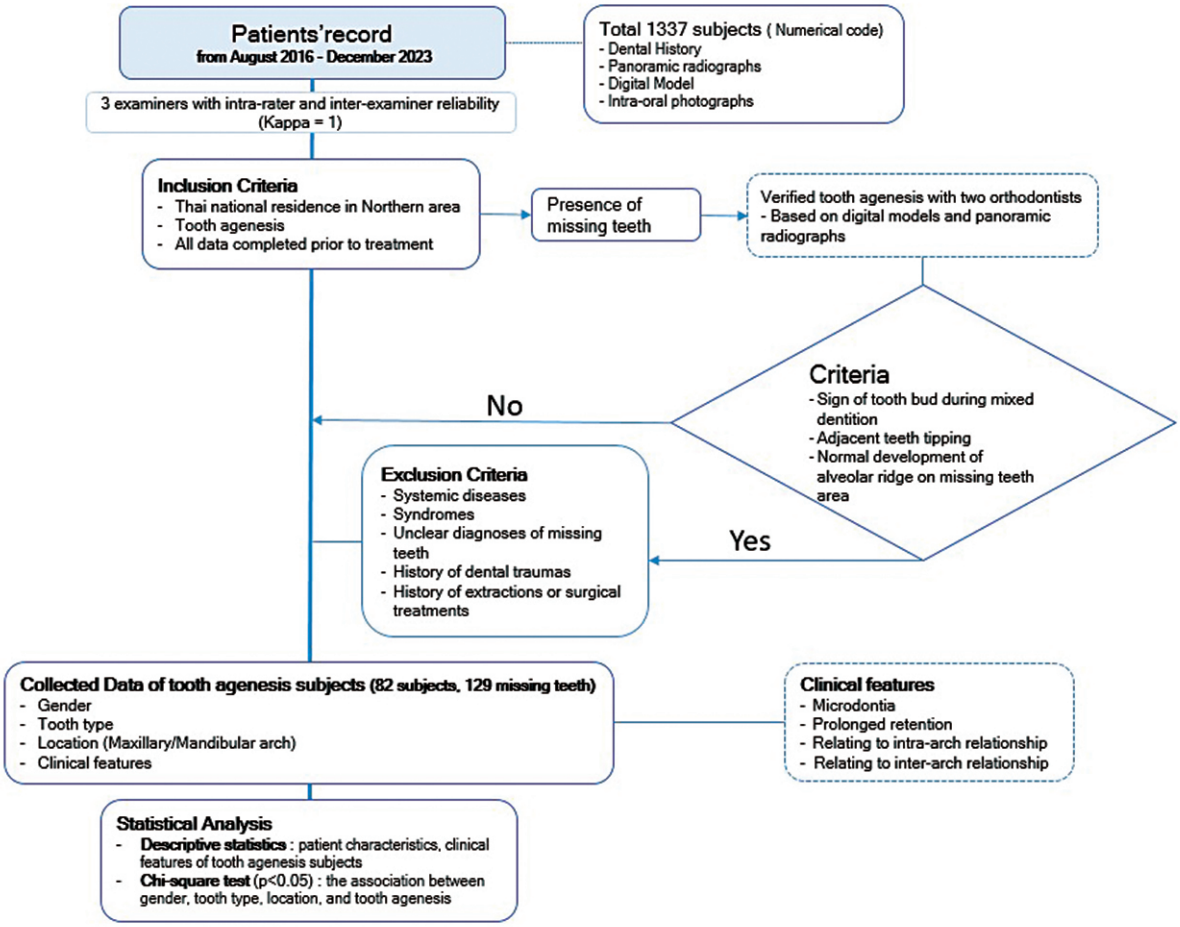

Figure 1: Workflow diagram for examination, evaluation, and analytical procedures.

n=76), followed by second premolars (23.25%; n=30), central incisors (8.53%; n=11), first premolars (6.97%; n=9), and canines (2.32%; n=3), respectively. There was no missing first and second molars. When the missing teeth were examined separately according to the dental arches, TA was more prevalent in the mandibular arch (69%) than in the maxillary arch (31%). However, no statistically significantly difference was observed between dental arches, as shown in Table 2.

Thirteen clinical features were selected for evaluation. Microdontia was most frequently observed in the maxillary arch (n=17) and was not found in the mandibular arch. Whereas prolonged retention was found more in the mandibular arch (n=7) than in the maxillary arch (n=1). Regarding the distribution of clinical features based on arch relationships, the most common feature observed in the maxillary intra-arch relationship was crowding (n=17), while spacing (n=21) was the predominant feature in the mandibular intra-arch relationship, as shown in Figure 2.

Regarding the features of inter-arch relationship between maxillary and mandibular arches, the most common feature was deep bite (32.9%), followed by anterior crossbite (21.1%), anterior protrusion and posterior crossbite (18.4% each), and open bite (9.2%), as shown in Figure 3.

Discussion

This study is an epidemiological investigation of TA observed in patients at a university postgraduate orthodontic clinic. As a result, the prevalence rate of this agenesis found in the study may not directly represent that of the general population.

While the prevalence of TA has been widely studied in various populations, it remains an area of ongoing research. In Thailand, however, research on this topic is limited. Previous research by Kanchanasevee *et al*.,[7] and Tantanapornkul[16] relied solely on data obtained from panoramic radiographs. However, this study collected data from dental history, digital dental models, intraoral photographs in addition to panoramic radiographs. Therefore, the results provided greater precision regarding missing tooth types and revealed related clinical features beyond the capabilities of panoramic radiographs alone.

Prevalence of TA in this study was 6.13% which was lower than other studies reported in other regions of Thailand. Differences in results among studies could potentially occur from variations in the demographic characteristics (e.g., regional origin, ethnicity) of the investigated populations, as well as methodological disparities such as sampling techniques and diagnostic criteria. Research has indicated that excluding the absent third molar can enhance the reliability of studies,

Table 1: Distribution of prevalence rate of tooth agenesis by gender.

	Number of Patients			
Sex	**Sample**	**Affected**	**Prevalence (%)**	***p*-value**
Male	402	22	5.47	
Female	935	60	6.42	0.53
Total	1337	82	6.13	

Table 2: Distribution of tooth agenesis by tooth type in relation to maxillary and mandibular arches.

Tooth type	Maxillary arch, n(%)	Mandibular arch, n(%)	Total, n(%)	*p*-value
Central incisor	1 (2.50)	10 (11.24)	11 (8.53)	0.171
Lateral incisor	21 (52.50)	55 (61.79)	76 (58.91)	0.321
Canine	1 (2.50)	2 (2.25)	3 (2.33)	1.000
First premolar	6 (15.00)	3 (3.37)	9 (6.98)	0.250
Second premolar	11 (27.50)	19 (21.34)	30 (23.25)	0.444
First molar	0 (0)	0 (0)	0 (0)	NA
Second molar	0 (0)	0 (0)	0 (0)	NA
Total	40 (100)	89 (100)	129 (100)	0.070

p-value from Chi-square: Comparison on affected/ non affected subjects and Maxilla/Mandible in each tooth type.

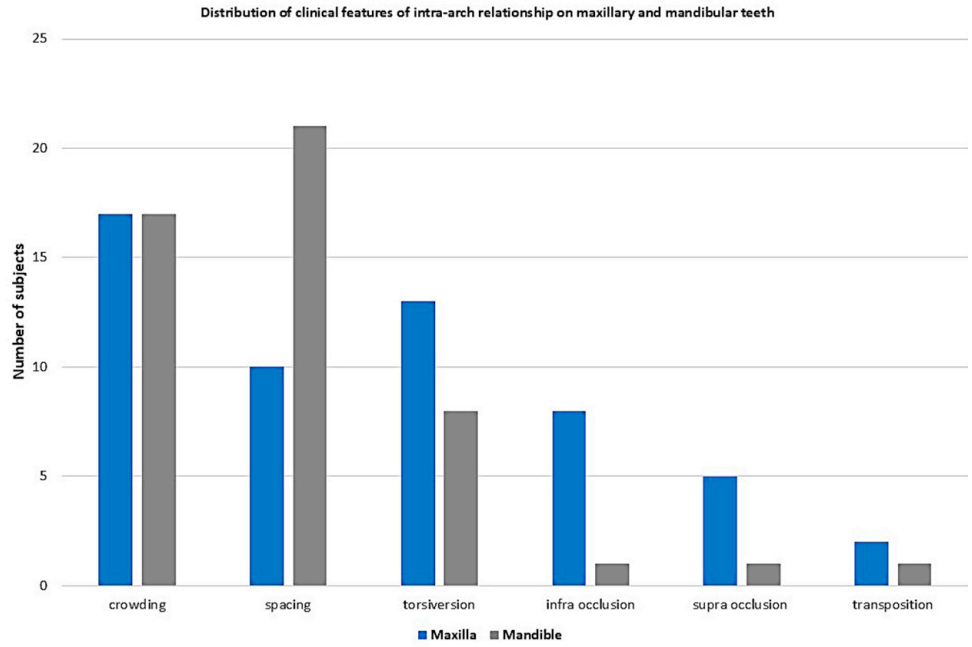

Figure 2: Distribution of clinical features of intra-arch relationship on maxillary and mandibular teeth.

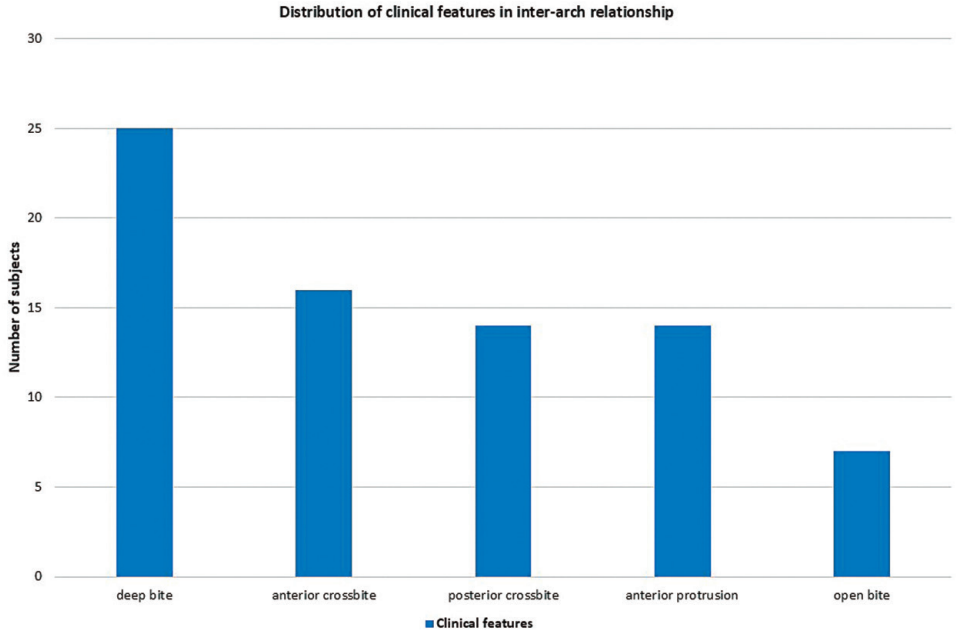

Figure 3: Distribution of clinical features in inter-arch relationship.

as the total number of patients with hypodontia rises by 27-30.6% when the third molar is considered missing.[17] Therefore, third molars were excluded from this study.

The study of Sisman *et al*[18], Hobkirk *et al*[19], Polder *et al*[20], Kanchanasevee[9], as well as the present study found that the incidence of TA was higher in females than in males, although the difference was not statistically significant. This aligns with previous findings suggesting a higher proportion of female orthodontic patients[21], with females comprised 69.9% of the sample in this study. Moreover, no statistically significant difference was observed between genders regarding the distribution of missing teeth in the maxilla and mandible. However, this study reported a nearly twofold higher prevalence of missing teeth in the mandible (68.9%) compared to the maxilla (31.1%), which is consistent with the findings of

Kanchanasevee *et al.*,[9] and Tantanapornkul.[16] These differences may be attributed to variations in jaw development (ontogenesis), particularly between the mandible and maxilla, which could contribute to the higher frequency of missing teeth and other dental anomalies in the mandibular arch.[22]

In previous articles, the most reported missing teeth are the second premolars, lateral incisors, and first premolars.[6,21,23] On the other hand, this study reported that the most prevalent missing tooth was mandibular lateral incisors followed by maxillary lateral incisors and mandibular second premolars, respectively. The absence of first and second molars was consistent with findings from previous studies on TA.[8] From an evolutionary perspective, the congenital absence of teeth in humans is not surprising, and this change is influenced by various genes. Mutations in the MSX1, PAX9, AXIN2, and EDA genes are causative factors in non-syndromic TA.[24]

TA is usually linked to several clinical features, including the location of the agenesis and the size of the neighboring teeth such as microdontia, delayed dental development, prolonged retention, and some tooth ectopia, possibly because a certain genetic mutation causes a series of different phenotypes expression.[25] Microdontia and hypodontia represent a genetic continuum of tooth size. If a tooth germ doesn't reach a critical size threshold, the tooth fails to develop, resulting in hypodontia.[26] The previous studies did not investigate the clinical features relating to tooth agenesis.[9,27,28] Whereas this study also investigated another point of view; the clinical features which are presented in terms of inter-arch and intra-arch relationships. For intra-arch relationships, the two-highest prevalence was crowding and spacing which is in agreement with previous studies which demonstrated that it is common to observe generalized spacing and rotations of the teeth next to missing mandibular second premolars.[29]

For inter-arch relationships, this study found that deep bite had the highest prevalence among the clinical features observed. Moreover, hypodontia was found to be approximately twice as common in the mandibular arch compared to the maxillary arch. This clinical feature may be related to the mismatch in the number of teeth, which affects the deficient ratio in Bolton's analysis, leading to a deep overbite and large overjet. Herrera-Atoche *et al.*, reported a tendency for the lower incisors to be retroclined, positioned more extrusively, and associated with a deeper bite compared to controls. It appears that, in patients with posterior tooth agenesis (TA), the bite deepens as a result of lower incisors extrusion.[30] The second most common inter-arch anomaly identified was anterior crossbite in this study. Wisth also found that children with hypodontia often exhibit a shorter, more retrusive maxillary arch and proclined upper incisors.[31]

Therefore, detection of TA during the mixed dentition stage allows orthodontists and pediatric dentists to implement appropriate treatment strategies, such as space maintenance or redistribution, to achieve optimal dental function and aesthetics. Further studies are recommended to explore the association between TA and dental as well as skeletal discrepancies in order to enhance clinical understanding and improve treatment planning.

Conclusions

In this study, the prevalence of permanent TA was 6.13%, which was lower than the previously reported rates of 8.6-9.7% conducted on Thai population. Single TA was the most common pattern, occurring 58.53% of affected cases, with mandibular lateral incisors being the most frequently missing teeth. We found that there are several clinical features involved with TA in both inter-arch and intra-arch relationships.

Conflict of Interest

The authors declare no conflict of interest.

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Original Article

Associations Among Oral Health Status, Caregiver Burden and Oral Health-Related Quality of Life in Dependent Older Adults

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Abstract

Objectives: To examine the associations among oral health status, caregiver burden, and oral health-related quality of life (OHRQoL) in dependent older adults.

Methods: A cross-sectional census survey was conducted with 159 dependent older adults and their primary caregivers in rural Thailand between May 2022 and June 2023. Participants were categorized as home-bound or bed-bound using Activities of Daily Living (ADL) scores. Data were collected through oral examinations, the Oral Impacts on Daily Performance (OIDP) index, and a culturally adapted caregiver burden questionnaire. Multiple logistic regression analyses were used to explore associated factors.

Results: Participants had a mean age of 79.9±10.1 years, with common comorbidities including hypertension (72.96%), hyperlipidemia (63.52%), and diabetes (35.22%). Bed-bound older adults had significantly poorer OHRQoL (mean OIDP score 15.6±12.3 vs. 10.7±10.2, p=0.020) and worse oral health status. Participants averaged 9.1 functional teeth, with only 13.21% having ≥4 occluding posterior pairs. Common problems included untreated root caries (62.26%) and xerostomia signs (37.74%). Caregiver burden was generally low (mean 18.2±11.6) but significantly higher for bed-bound caregivers (p=0.014). Greater caregiver burden was associated with poorer OHRQoL (OR=1.044, p=0.017).

Conclusions: Higher caregiver burden negatively influences OHRQoL in dependent older adults. Supporting systems for caregivers may improve oral health outcomes in rural aging populations.

Keywords: aged, caregiver, frail elderly, oral health, quality of life

Introduction

Globally, populations are aging rapidly, creating significant challenges.[1] This demographic shift raises important challenges for healthcare systems, particularly in addressing the complex needs of dependent older adults. Thailand reflects this global trend, having transitioned into an aged society in recent years, with over 20% of its population projected to be 60 or older by 2022.[2] Currently, approximately 2-3% of Thai older adults are classified as dependent, requiring assistance with activities of daily living.[3]

A significant proportion of older adults are dependent and require assistance with daily living, which impacts their overall health, especially oral health.[4] Poor oral health among dependent older adults has been linked to compromised nutrition, communication difficulties, and reduced quality of life. Previous studies have found that dependent older adults often have complex oral health issues and high rates of dental caries and root caries, particularly among those with dementia.[5,6]

In Thailand, the care system for older adults primarily relies on family support arrangements due to cultural values and limited institutional care options.[8] The traditional Thai concept of "Boon-Khun" (gratitude) and Buddhist-influenced filial piety emphasize respect and obligation toward parents and elders, reinforcing expectations that families will provide care for aging relatives.[9,10] However, with increasing numbers of older adults and declining working-age populations, Thai families face growing challenges in providing adequate care.[7]

Caregiving for dependent older adults involves multiple forms of support across different sectors. Family caregivers, primarily daughters and spouses, provide the majority of daily care within the home environment. Village health volunteers (VHVs) serve as community-based volunteers who assist with basic health monitoring as part of Thailand's primary healthcare strategy.[10] Community care managers function as professional staff who coordinate care services through the Long-Term Care (LTC) system, while local administrative organizations (LAOs) provide supplementary support services.[7] Despite these support systems, caregivers at all levels often lack adequate knowledge and skills in oral health care provision. Previous studies have found that most caregivers experience significant burden that may affect the quality of care provided, particularly for non-urgent care tasks such as oral hygiene maintenance.[12]

According to Thailand's health data center, oral health services for older adults in the LTC system show concerning utilization rates, with only 31.7% receiving oral health examinations and 29.9% receiving oral health services.[15] This gap in service delivery highlights the need for better integration of oral health care within the LTC framework, particularly for dependent older adults who face multiple barriers to accessing traditional dental services.

The burden of oral diseases significantly impacts the daily functioning and quality of life of dependent older adults. The Oral Impacts on Daily Performance (OIDP) index was selected for this study because it has been validated and culturally adapted for the Thai population[13,14], and it specifically measures the impact of oral conditions on eight daily performance activities: eating, speaking, cleaning mouth/dentures, sleeping, emotional stability, smiling, working, and social contact. This index is particularly relevant for dependent older adults as it captures functional limitations that directly affect their daily lives.

This study aimed to examine the associations among oral health status, caregiver burden, and oral health-related quality of life (OHRQoL) in dependent older adults in Phromphiram District, Phitsanulok Province, Thailand, to inform policy and practice improvements in this vulnerable population.

Materials and Methods

Study design and ethical approval

This research employed a cross-sectional survey design conducted between May 2022 and June 2023. This study obtained ethical approval from the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University, with the approval number 22/2022.

Participant recruitment

We conducted a census survey that included all dependent older adults registered in the Long-Term Care (LTC) system in Phromphiram District, Phitsanulok Province, Thailand. The study population comprised 159 dependent older adults and their 159 primary caregivers.

Participants were categorized based on their Activities of Daily Living (ADL) scores using the Barthel

Index classification:[14] scores ranging from 5 to 11 indicating home-bound status (n=112), and scores from 0 to 4 indicating bed-bound status (n=47).

Study participants

The study participants were divided into two groups: **(1) dependent older adults,** defined as individuals requiring assistance with daily activities due to functional limitations, and **(2) primary caregivers,** who were the ones spending most of their time taking care of the elderly, predominantly family members.

The inclusion criteria for dependent older adults were: 1) age 60 years or older and classified as home-bound or bed-bound with ADL score ranging from 0 to 11; 2) residing in Phromphiram District; and 3) willing to participate or having caregiver consent for participation. For caregivers, the inclusion criteria were: 1) being the primary caregiver; and 2) willing to participate in the study.

The exclusion criteria were: 1) older adults with ADL scores >11 (indicating functional independence); 2) those not residing in Phromphiram District; 3) those unwilling to participate or lacking caregiver consent; and 4) older adults or caregivers who were unable to complete all assessments.

Research instruments

The research instruments comprised two sets: one for dependent older adults and another for caregivers. The set for dependent older adults consisted of three components:

1. **Demographic questionnaire:** Including age, gender, health conditions, and functional status
2. **Oral health examination form:** Modified from Thailand's 8th National Oral Health Survey[17] which included assessment of dental caries, dental restorations, number of functional teeth and posterior occlusal pairs, prosthetic status and need, periodontal status, and oral soft tissue conditions
3. **Thai version of the Oral Impacts on Daily Performance (OIDP) index:** This validated instrument[13,14] measures the impact of oral conditions on daily performance across eight dimensions. The OIDP was chosen because it has been specifically validated for Thai populations and captures functional limitations relevant to dependent older adults.

The caregivers completed two questionnaires:

1. **Demographic questionnaire:** Including relationship to older adult, caregiving duration, and socioeconomic status
2. **The 22-item Caregiver burden assessment:** Developed by Chananchidadusadee[18] which was adapted and modified from the Zarit Burden Interview[19] and culturally tailored for the Thai population.

Data collection and data analysis

Data collection was conducted by a qualified dentist and a trained research assistant. The research assistant was responsible for conducting structured interviews, administering questionnaires, and providing translation assistance when needed, while maintaining standardization through weekly calibration sessions with the principal investigator. The qualified dentist performed all clinical oral examinations following calibration with a gold standard expert examiner, achieving inter-examiner reliability (kappa>0.80).

For older adults who could communicate, the researcher interviewed them directly using the general information questionnaire and OIDP assessment. For those unable to communicate due to cognitive impairment or severe functional limitations, demographic data were collected from caregivers only, and OIDP scores were assessed through caregiver observation and reporting.

Data were analyzed using SPSS version 25 (SPSS Inc., Chicago, IL, USA). Descriptive statistics (frequency, percentage, mean, and standard deviation) were used to summarize participant characteristics. Group comparisons (home-bound vs. bed-bound) were performed using Chi-square or Fisher's exact tests for categorical variables, and the Mann-Whitney U test for non-normally distributed continuous variables. Univariate analyses were used to identify variables associated with active dental decay and OIDP score. Factors with $p<0.05$ in univariate analyses were entered into multiple logistic regression models. Results are presented as odds ratios (OR) with 95% confidence intervals (CI). A p-value<0.05 was considered statistically significant.

Results

General characteristics and oral care practice of older adults

Table 1 presents the characteristics of dependent

older adults and their caregivers. The older adults had a mean age of 79.9 years (SD=10.1) and an average ADL score of 7.1 (SD=3.9). Common underlying conditions included hypertension (72.96%), hyperlipidemia (63.52%), and diabetes (35.22%). Most reported sufficient income for daily expenses (64.15%).

Caregivers had a mean age of 58.6 years (SD=12.7) and an average caregiving duration of 6.8 years (SD=6.1). Among the caregivers, daughters accounted for 47.17%, spouses 25.16%, and sons 11.32%, while other relatives and informal caregivers also contributed to the caregiving roles. Nearly half had underlying health conditions, and 45.91% reported sufficient income. Tooth brushing was not routinely performed by caregivers (84.91%), with most older adults brushing independently (50.31%) or rinsing after meals (33.96%). The majority had prior oral health examinations (89.94%), tooth extractions (76.10%), and home visits from health personnel (63.52%).

Oral health status of dependent older adults

The older adults had an average of 9.1 functional permanent teeth per person (SD=9.3), with significant tooth loss evident across both groups. The average number of remaining natural teeth was 12.6±10.8, indicating that participants had lost approximately 19 teeth on average. Only 13.21% had at least 4 occluding posterior pairs, which represents the minimum number recommended for adequate chewing function.[20] Home-bound individuals had more functional teeth (mean 10.0±9.7) and occluding pairs (mean 1.2±2.0) compared to bed-bound individuals (mean 7.1±8.2 teeth and 0.7±1.6 occluding pairs).

Common oral health issues among participants included untreated root caries (62.26%) and tongue coating (99.37%). Approximately one-third (32.70%) exhibited periodontal pockets of 4-5 mm, and 1.89% had pockets of 6 mm or more. Signs of xerostomia were prevalent, including dry mouth sensation (37.74%) and dental mirror adhesion to the buccal mucosa or tongue (32.70%). The significant prevalence of xerostomia signs is particularly concerning as dry mouth increases risks of dental caries, oral infections, and swallowing difficulties, potentially contributing to aspiration pneumonia.[21,22]

Caregiver burden

Assessment of caregiver burden is presented in Table 2. The overall score ranged from 0-58, with most caregivers (64.15%) reporting no burden or very little burden (scores 0-21). However, those caring for bed-bound older adults experienced significantly higher burden scores (mean 22.6±14.8) compared to those caring for home-bound adults (mean 17.2±10.0), with a greater proportion reporting moderate to severe burden (p=0.014). This difference reflects the increased physical and emotional demands of caring for individuals with higher levels of functional dependency.

Oral health-related quality of life

The assessment of oral health-related quality of life was conducted using the OIDP index scores for all 152 elderly participants. OIDP scores ranged from 0 to 48.5, with emotional stability, sleep, and oral cleaning being the most impacted daily functions.

Through frequency distribution analysis of all OIDP scores greater than 0, the 50th percentile was identified at 16.0, establishing this as the primary cut-off point. This cut-off value of 16.0 was selected based on established OIDP methodology using percentile-based categorization, which ensures statistical validity by dividing the affected population into equal halves. This threshold represents the point at which oral health issues begin to substantially interfere with normal daily activities.[23]

Consequently, OIDP scores were categorized into four groups: score 0=excellent oral health-related quality of life (2% of participants); scores 0.1-16.0=good quality of life; scores 16.1-16.375 = moderate quality of life; and scores >16.375=poor oral health-related quality of life.

Overall OIDP scores were significantly higher among bed-bound individuals (15.6±12.3) compared to home-bound participants (10.7±10.2; p=0.020), indicating worse oral health-related quality of life. Bed-bound participants showed significantly worse impacts in speaking (3.9±6.8 vs. 0.7±2.2, p<0.001), mouth cleaning (5.5±6.9 vs. 2.9±4.6, p=0.027), and sleeping (1.9±4.4 vs. 3.4±4.5, p=0.006).

Using the established cutoff score of >16.0 points to indicate unsatisfactory OHRQoL, approximately one-third (28.9%) of participants reported unsatisfactory oral health-related quality of life. While bed-bound individuals showed a higher proportion of unsatisfactory OHRQoL (65.2%) compared to home-bound individuals (34.8%), this difference was not statistically significant (p=0.118).

Factors associated with oral health and quality of life

Table 4 summarizes the multivariate logistic regression analysis. Increasing age among older adults was inversely associated with active dental decay (OR=0.934; 95% CI: 0.890-0.981, p=0.006), possibly reflecting survivor bias or tooth loss reducing caries risk. Conversely, higher caregiver age (OR=1.045; p=0.036) and higher caregiver income (OR=1.558; p=0.014) were significantly associated with increased likelihood of decay in the older adult.

Most importantly, caregiver burden scores were independently associated with unsatisfactory OIDP outcomes (OR=1.044; 95% CI: 1.008-1.082; p=0.017), indicating that for each one-point increase in caregiver burden score, the odds of the older adult having unsatisfactory oral health-related quality of life increased by 4.4%. No other control variables showed significant associations with OHRQoL outcomes.

Discussion

This study examined oral health status, caregiver burden, and factors associated with oral health and oral health-related quality of life (OHRQoL) among dependent older adults in rural Thailand. The findings revealed marked disparities between home-bound and bed-bound individuals, with the latter experiencing poorer oral health and significantly lower OHRQoL. These results underscore the importance of targeted oral health interventions for individuals with higher dependency levels and the critical role of caregiver burden in influencing oral health outcomes.

Caregiver burden and its impact

Although the average caregiver burden was relatively low (mean score = 18.2±11.6), those caring for bed-bound elders reported significantly higher strain. Cultural expectations, such as the Thai concept of 'Boon-Khun' and the broader Asian value of filial piety, may contribute

Table 1: Characteristics of participants.

Participants	Characteristics		N	%
Older Adults (n=159)	Gender	Male	48	30.19
		Female	111	69.81
	Marital status	Single	5	3.14
		Married	64	40.25
		Widowed /divorced/ separated	90	56.60
	ADL scores	0-4 (Bed-bound)	47	29.56
		5-11 (Home-bound)	112	70.44
	Health Insurance	Universal Coverage Scheme	118	74.21
		Government Officer Benefit Scheme	17	10.69
		Disability Health Benefit	24	15.09
Caregivers (n=159)	Gender	Male	41	25.79
		Female	118	74.21
	Marital status	Single	25	15.72
		Married	108	67.92
		Widowed /divorced/ separated	26	16.35
	Education attainment	No education /Primary education	97	61.0
		Secondary education	45	28.30
		≥Higher education	17	10.70
	Relationship with elderly	Daughter	75	47.17
		Wife	23	14.47
		Son	18	11.32
		Husband	17	10.69
		Others	26	16.35

Table 2: Comparing oral health status and caregiver burden scores between home-bound and bed-bound dependent older adults.

Data	Total (n = 159)	Home-bound (n = 112)	Bed-bound (n = 47)	*p*-value
Average number of decay teeth (SD)	5.44±5.88	5.70±5.98	4.87±5.67	0.297
Average number of functional teeth (SD)	9.1±9.3	10.0±9.7	7.1±8.2	0.066
Average number of remaining natural teeth (SD)	12.6±10.8	13.8±11.2	10.2±9.6	0.048*
Average number of posterior occluding pairs ± SD	1.0±1.9	1.16±2.0	0.7±1.6	0.051
Number of posterior occluding pairs				***p*-value**
0 pairs	113 (71.07%)	74 (66.07%)	39 (82.98%)	0.068
1-3 pairs	25 (15.72%)	22 (19.64%)	3 (6.39%)	
≥4 pairs	21 (13.21%)	16 (14.29%)	5 (10.64%)	
Denture status				***p*-value**
No denture	112 (70.44%)	75 (66.96%)	37 (78.72%)	0.247
Complete denture	29 (18.24%)	22 (19.64%)	7 (14.89%)	
Partial denture	18 (11.32%)	15 (13.39%)	3 (6.38%)	
Caregiver burden scores				***p*-value[b]**
0 - 21 (No burden or very little burden)	102 (64.15%)	79 (70.54%)	23 (48.94%)	0.014*
22 - 40 (Mild to moderate burden)	49 (30.82%)	30 (26.79%)	19 (40.43%)	
41 - 60 (Moderate to severe burden)	8 (5.03%)	3 (2.68%)	5 (10.64%)	
Mean ± SD	18.21±11.62	17.19±10.02	22.64±14.79	0.043*
Min - Max	0-58	2-50	0-58	

*$p<0.05$

Table 3: Comparing the Oral Impact on Daily Performance (OIDP) scores between the home-bound and bed-bound participants.

OIDP	dimension	Home-bound (n=112)			Bed-bound (n=40)[1]			*p*-value
		Mean±SD	Range	Activity affected (%)	Mean±SD	Range	Activity affected (%)	
1.	Eating	1.0±2.95	0-20	33.93	1.48±3.94	0-15	45.00	0.738
2.	Speaking	0.67±2.17	0-10	27.68	3.87±6.84	0-25	40.00	<0.001**
3.	Cleaning mouth or dentures	2.86±4.60	0-20	34.82	5.50±6.87	0-25	47.50	0.027*
4.	Sleeping	3.42±4.48	0-20	41.96	1.90±4.38	0-20	57.50	0.006*
5.	Maintaining emotional stability	1.98±2.42	0-10	45.54	2.00±2.83	0-10	65.00	0.760
6.	Smiling or showing teeth	0.37±1.50	0-12	25.89	0.38±1.75	0-10	37.50	0.557
7.	Working	0.25±1.17	0-8	16.07	0.42±2.04	0-12	30.00	0.957
8.	Social contact	0.18±0.77	0-5	22.32	0.08±0.47	0-3	35.00	0.367
Overall scores		10.73±10.22	0-45	13.24	15.62±12.26	0-53	12.73	0.020*
Quality of life level		**Home-bound (n=112)**			**Bed-bound (n=40) ¥**			***p*-value**
		N	**%**		**N**	**%**		
Satisfactoryb (0-16)		82	77.4		24	22.6		0.118
Unsatisfactory (16.1 or more)		30	65.2		16	34.8		

[1]The missing number due to the dependent older adults were not able to communicate

*$p<0.05$

**$p<0.001$

Table 4: Multivariate logistic regression analysis of the association between caregiver burden and oral health outcomes and oral health related quality of life.

Predictor	Oral health status (Having active decay teeth)			Overall OIDP Level (Unsatisfied)		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Caregiver burden scores	1.016	0.979-1.055	0.390	1.044	1.008-1.082	0.017*
Control factors						
ALD level (bed bound)	1.655	0.724-3.782	0.232	0.711	0.295-1.711	0.446
Age of older adults	0.934	0.890-0.981	0.006*	1.012	0.969-1.057	0.597
Age of caregivers	1.045	1.003-1.089	0.036*	0.978	0.941-1.018	0.276
Gender of older adults (female)	0.976	0.380-2.505	0.960	0.960	0.374-2.459	0.932
Gender of caregivers (female)	1.113	0.242-5.123	0.891	1.098	0.281-4.289	0.893
Education of caregivers (≤ primary school)	0.497	0.184-1.341	0.167	1.759	0.598-5.180	0.305
Relationship with older adults (daughter or wife)	0.442	0.115-1.695	0.234	0.955	0.273-3.343	0.943
Income of older adults	0.855	0.594-1.232	0.402	1.073	0.778-1.480	0.666
Income of caregivers	1.558	1.093-2.220	0.014*	1.199	0.862-1.667	0.282

*$p<0.05$

to underreporting of distress among caregivers.[22,23] The majority of caregivers were female (74.21%) and primarily daughters (47.17%), reflecting traditional gender roles in family caregiving which may influence their willingness to express burden.

The significant association between caregiver burden and poor OHRQoL (OR=1.044, $p=0.017$) suggests that overburdened caregivers may deprioritize routine oral care activities, focusing instead on more urgent medical needs. This finding aligns with previous research indicating that caregiver burden compromises attention to non-emergency care tasks, thereby influencing subjective oral health experiences rather than immediate clinical indicators like tooth decay.[26,27] The primary burdens reported by caregivers include physical strain from lifting and positioning patients, emotional distress from witnessing deterioration, financial constraints from reduced work capacity, and social isolation due to caregiving responsibilities. These multifaceted challenges explain why oral health care, which requires daily attention and specialized knowledge, often becomes a lower priority compared to immediate medical needs.

Barriers to oral care in dependent older adults

Most caregivers did not assist with daily oral hygiene, often due to being overwhelmed with other responsibilities or lacking confidence in providing oral care. Additional barriers include lack of training, fear of causing discomfort, and cultural beliefs that normalize tooth loss as part of aging in some Asian populations.[28,29] For bed-bound individuals, oral care becomes particularly challenging due to positioning difficulties, swallowing problems, and increased risk of aspiration.

The competency limitations of dependent older adults themselves also contribute to poor oral hygiene. Bed-bound individuals face multiple challenges including reduced manual dexterity, cognitive impairment, and physical positioning constraints that make independent oral care difficult or impossible. Specific barriers for bed-bound patients include inability to sit upright for safe oral care, difficulty with mouth opening due to muscle stiffness or pain, increased risk of choking and aspiration due to compromised swallowing reflexes, and resistance to oral care due to cognitive impairment.

Oral health status and clinical implications

The dependent older adults in this study had an average of 9.1 functional teeth, with only 13.21% having at least four occluding posterior pairs. These numbers are lower than reports from Greece (11.8 teeth)[30] and Switzerland (14.7 teeth)[31], likely reflecting disparities in oral healthcare access and public awareness. When compared to Thailand's national oral health service data, our findings highlight concerning gaps: while 31.7% of general older adults receive oral health examinations and 29.9% receive oral health services[15], dependent older adults in

LTC systems appear to have even lower access rates and worse outcomes.

The high prevalence of untreated root caries (62.26%), tongue coating (99.37%), and periodontal disease supports findings from studies in other countries reporting similar oral health issues among dependent elders.[6,32,33] The significant prevalence of xerostomia signs (37.74%) is particularly concerning as dry mouth increases risks of dental caries, oral infections, and swallowing difficulties, potentially contributing to more serious complications such as aspiration pneumonia.[21,22]

Quality of life implications and oral care protocols

The significantly worse OIDP scores among bed-bound individuals, particularly in speaking, mouth cleaning, and sleeping domains, reflect the complex interplay between functional limitations, oral health status, and daily functioning. Poor oral health in bed-bound individuals can contribute to communication difficulties, sleep disturbances due to oral discomfort, and challenges in maintaining oral hygiene, creating a cycle of declining oral health and reduced quality of life.

It is important to note that the decreased OIDP scores observed in this study may result from multiple factors beyond oral health conditions alone. Participants with underlying medical conditions such as stroke, dementia, or other neurological disorders may experience additional challenges that compound oral health impacts.

Current oral care protocols in Thailand's LTC system require significant enhancement to address the specific needs of dependent older adults. For bed-bound individuals, specialized approaches include position-modified oral care techniques for safe oral hygiene in supine or semi-recumbent positions, aspiration prevention protocols to minimize choking risks, and the use of adaptive equipment such as specialized toothbrushes and oral swabs.

The connection between oral care and systemic health outcomes is particularly important for bed-bound individuals who face increased risks of aspiration pneumonia. Professional oral care interventions have demonstrated remarkable effectiveness, with studies showing reductions in pneumonia incidence of up to 40% among high-risk elderly residents in long-term care facilities.[34,35]

Integration of oral health in long-term care

The findings support the need for better integration of oral health services within Thailand's LTC framework. Currently, dental care remains inadequately integrated, particularly in rural areas where specialized geriatric dental services are limited. Integration of oral health services within Thailand's LTC framework requires coordinated efforts including regular oral health assessments incorporated into LTC care protocols, mobile dental services to bring professional oral care directly to homebound and bed-bound individuals, comprehensive caregiver education programs, and policy integration that includes oral health indicators in LTC quality measures.

Study limitations

This study's cross-sectional design limits causal inference regarding the relationship between caregiver burden and oral health outcomes. The focus on a single rural district may limit generalizability to urban or other regional contexts. However, the findings likely reflect challenges faced by similar rural communities throughout Thailand and other middle-income countries with aging populations and family-based care systems.

Conclusions

Dependent older adults, particularly those who are bed-bound, experience significant oral health challenges and reduced quality of life that are closely linked to caregiver burden levels. Key issues include extensive tooth loss, poor oral hygiene indicators, high prevalence of oral diseases, and limited caregiver assistance with oral care. The significant association between caregiver burden and poor oral health-related quality of life highlights the critical need for caregiver support, education, and resources. Enhancing caregiver competency through comprehensive training programs, increasing dental professional involvement in LTC services, addressing cultural barriers to oral care, and developing bed-bound-specific oral care protocols are essential for improving outcomes in this vulnerable population. Policy initiatives should focus on integrating oral health services within LTC frameworks, ensuring adequate access to professional oral care for dependent older adults who cannot access traditional dental services, and establishing reimbursement mechanisms that support preventive oral health interventions.

Conflict of Interest

The authors declare no conflict of interest.

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Oral Health Literacy and Its Associated Factors of Primary School Students in Chiang Rai, Thailand

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Abstract

Objectives: To explore the level of oral health literacy (OHL) and identify factors related to the OHL of primary school students in Muang District, Chiang Rai Province, Thailand.

Methods: A cross-sectional study was conducted from May to September 2022, involving 351 Grade 6 students aged 11-12 years and their parents from 13 schools. Participants were selected using quota random sampling. Data were collected through questionnaires, with independent variables categorized according to Sørensen's classification: personal factors, situational factors, and societal and environmental factors. Additionally, the Test of Functional Health Literacy in Dentistry for Primary School Children (P-TOFHLiD) was used to assess OHL. Data analysis was performed in three stages: descriptive statistics, bivariate analysis, and multilevel logistic regression analysis.

Results: 55.8% of the sample had adequate OHL. Multilevel logistic regression further revealed that individual-level factors were significant predictors of adequate OHL. Students with a GPA of 3.00 or higher OR=2.386, 95% CI: 1.368-5.882), parental income (OR 2.233, 95% CI: 1.244-4.008), and participation in academic clubs (OR=3.409, 95% CI: 1.139-10.206) were associated with adequate OHL. School-level factors did not show a statistically significant relationship with OHL.

Conclusions: The findings highlight the impact of academic achievement, family socio-economic status, and learning environments on students' OHL. These insights can guide policymakers in designing targeted school-based OHL programs and inclusive, skill-based communication strategies for diverse student populations.

Keywords: adolescence, factors, oral health literacy, student

Introduction

Health literacy developed through effective education, empowers people to use health information for informed choices and basic health maintenance. Supporting public policies and environmental changes further enhances health literacy and encourages healthier behaviors. Health literacy is crucial for improving individual and community health outcomes and reducing disparities, empowering people to manage their health effectively.[1,2]

"Oral health" is a critical component of overall well-being. Common risk factors for oral diseases, like excessive sugar intake, also contribute to chronic non-communicable diseases (NCDs), which are a major health concern 85% of 12-year-old Thai children face daily disruptions from oral health issues, with 36% experiencing moderate to severe problems. Dental caries were identified as the most impactful in terms of size and severity.[3] Despite over two decades of oral health promotion activities by the Department of Health, Ministry of Public Health Thailand since 1998[4], tooth decay continues to be the main issue impacting Thai students' quality of life.

Sørensen's classification is a model developed through a systematic review of the literature that presents a comprehensive conceptual framework for understanding health literacy. It outlines the key processes of health literacy–accessing, understanding, evaluating, and applying health information across different stages of life. The framework enables a nuanced analysis of an individual's ability to manage their own health and classifies the determinants of health literacy into three levels: Personal factors, Situational factors, and Societal and environmental factors.[5]

Most oral health literacy (OHL) studies have focused on the elderly, adults, or parents, with limited research on elementary school children.[6] This is defined as "simply as the ability to understand health information and to understand that actions taken in youth affect health later in life, combined with the ability to access valid health information."[7] A 2020 study found that 12-year-olds with lower OHL are twice as likely to develop caries lesions compared to their peers with higher literacy levels.[8]

School-age children are at a crucial developmental stage, forming their discipline, personality, and behavior while also learning a key self-care skill.[9] Research on health literacy, particularly OHL among elementary school students in Thailand, is limited. This study aims to assess OHL and identify influencing factors, with the goal of improving oral health and quality of life for primary school children. The aim of this study was to identify the factors impacting OHL and devise the research framework based on the classification provided by Sorensen (2012).[5]

Materials and Methods

This study was reviewed by the Ethics Review Board in Human Research. Faculty of Dentistry, Chiang Mai University No. 23/2565. This cross-sectional study hypothesized that certain factors influence OHL by categorizing the independent variables into three levels, in accordance with Sørensen's classification.[5] which classifies the determinants of health literacy into three levels:

1. **Personal factors:** age[5], gender[5], ethnicity[5], income[8,10], cultural background[11], Sickness experience and use of health care system services[5,12], general knowledge (Grade or GPA)[5,13]

2. **Situational factors:** parental gender[14], parental economic status[8], parental education level[5,15-17], friend and teacher[14], use of media[18]

3. **Societal and environmental factors:** culture and language[5,11], environmental and political policy[5,19,20]

The study included sixth-grade students from public, private, and municipal schools in Mueang District, Chiang Rai Province, during the 2022 academic year. The required sample size was calculated using the Krejcie and Morgan formula[21] with known population size : $n=X^2Np(1-p) / [e^2(N-1) + X^2p(1-p)]$, when N=2,988, $X^2=3.841$ (df=1, 95% confidence level), $p=0.5$, and e=0.05. The calculation resulted in a required sample size of 341 participants. Quota random sampling was employed to ensure proportional representation across different school types.

The sampling process was conducted in two stages. First, schools were categorized by type–public, private, and municipal–and by the number of enrolled students. They were then further stratified by size: extra-large, large, medium, and small. Schools were randomly selected within each stratum, proportional to student enrollment. As a result, the final sample included 1 extra-large public school, 1 large public school, 4 medium-sized public schools, 4 small public schools, 1 extra-large private school, 1 small private school, and 1 municipal school. Within the selected schools, sixth-grade students were then randomly chosen from classrooms to participate in

the study. The inclusion criteria specified students who were currently enrolled in the sixth grade during the 2022 academic year, attended public, private, or municipal schools in Muang District, Chiang Rai Province, and were willing to participate, along with parental consent. Schools without sixth-grade students were excluded from the study.

A three-part questionnaire was used for data collection, with Part 1 focusing on student demographics like gender, age, income, GPA, cultural background, language skills, illness history, and healthcare use. The questionnaires, completed by students, were validated for content by three experts (IOC=0.98).

Part 2 of the questionnaire covered the demographic details of participants' parents, including gender, socioeconomic status, and education level. Parents completed this section, and its content validity was confirmed by three experts (IOC=1.0).

Part 3 used the Test of Functional Health Literacy in Dentistry for Primary School Children (P-TOFHLiD) to evaluate students' basic OHL. The test is a specialized assessment tool developed by Thai dentists to evaluate OHL among school-aged children. Targeting students aged 11-14 years, the test is designed to mirror standard school-based assessments, making it both familiar and accessible to its intended audience. Its primary aim is to measure functional, or basic OHL.[22]

The P-TOFHLiD employs a fluid cognitive format that challenges students to apply a combination of existing skills to solve novel or unfamiliar problems. It assesses their ability to comprehend oral health-related texts, identify key messages, and select appropriate responses. The test covers four core topics: (1) knowledge of oral organs, (2) knowledge of dental caries, (3) knowledge of oral health care, and (4) foods contributing to tooth decay. It consists of 26 items, with a total possible score of 26 points. The tool demonstrates good internal consistency, as indicated by a Cronbach's alpha coefficient of 0.808. A score of 21 or higher is considered indicative of adequate OHL, with reported sensitivity and specificity values of 0.649 and 0.587, respectively.[22] This instrument has undergone a rigorous validation process and has been tested with children in urban, semi-urban, and semi-rural settings–contexts that reflect the demographic characteristics of the current study population. In addition to its diagnostic capabilities, the tool effectively classifies OHL levels, supporting the aims of this research. Notably, it was used in the 2020 *School Oral Health Promotion Program Evaluation Research Project* conducted by the Dental Health Office, Department of Health, Ministry of Public Health, which assessed students in Grades 5 and 6 across 12 health service centers.[23] Consequently, the results obtained from sixth-grade students in Muang District, Chiang Rai Province, can be reliably compared with existing national data collected using the same instrument.

Statistical analysis

The data collected through self-administered questionnaires were analyzed using IBM SPSS Statistics, Version 26.0. The analysis was conducted in three main stages as follows:

1. Descriptive statistics

Descriptive statistics including frequency, percentage, mean, and standard deviation were used to describe the general characteristics of the participants. These included gender, age, ethnicity, type of school, household income, parental education, and scores of OHL, measured using the P-TOFHLiD tool.

2. Bivariate analysis

The Chi-square test was employed to examine the initial associations between each independent variable and the level of OHL, categorized as either adequate or inadequate. A significance level of $p<0.05$ was used as the criterion for statistical significance.

3. Multilevel logistic regression analysis

To better assess the relationship between the independent variables and OHL within the hierarchical structure of the data, a multilevel logistic regression model was employed. Variables found to be significantly associated with OHL in the bivariate analysis (Chi-square test) were included in the multilevel model. The model was structured across two levels:

Level 1 (Individual level): Included personal variables such as student gender, grade point average (GPA), history of dental check-ups within the past year, household income, and parental education level.

Level 2 (School level): Included contextual variables such as school location and type of school affiliation.

The final results were reported as Odd Ratios (OR) with 95% Confidence Intervals (95% CI) to indicate the strength and direction of association between the indepen-

dent variables and the level of OHL.

Results

Table 1 shows that the sample had a nearly equal gender distribution, with females at 50.7% and males at 49.3%. Most participants were 11-12 years old (92.6%), and 91.6% were Thai. Among parents, 62.5% were female. Parents are predominantly split between below-high school education (38.1%) and a bachelor's degree or higher (39.1%). Nearly half of the parents are aged 40-49 years (46.5%), and Nearly half of the parents are involved in personal business or freelance work (49.8%), while 33.4% are employed as government or company employees. Most students attend public schools (61.8%), with smaller numbers from private schools (17.4%) and municipal schools (20.8%). Five schools located in urban areas accounted for a total of 207 students (51%), while eight schools in rural areas comprised 144 students (49%).

Table 2 shows OHL scores for students from different school types, divided into four parts: 1) Knowledge of oral organs, 2) Knowledge of dental caries, 3) Knowledge of oral health care, and 4) Foods contributing to tooth decay. This table revealed that scores among the sample were either equal to or greater than 21 points, indicating that 196 individuals (55.8%) demonstrated adequate OHL, while 155 individuals (44.2%) exhibited inadequacy. The lowest total score observed within the sample group was 5 points, with the highest reaching 26 points, and the mean score was 20.2 points. Notably, the part foods contributing to tooth decay received the lowest average score of 3.5 points out of a potential 6 points, consistent with both the overall average total score and the average scores across different school types. When categorized by school type, the results of the health literacy assessment using the P-TOFHLiD tool revealed that municipal school students had the highest average score (22.2±2.6), followed by private school students (20.8±3.5), and public-school students (19.4±4.0). This trend was consistent across all four parts of OHL, with municipal school students scoring the highest in all part, while public school students consistently had the lowest average scores.

Table 3 identifies factors that were significantly associated with OHL. Among the **personal factors,** significant variables included student gender, student GPA, and having had a dental check-up within the past year. **Situational factors** that showed significant associations included parental gender, parental education level, parental monthly income, receipt of health knowledge through two-way communication, and participation in academic clubs. Additionally, the only **societal and environmental factor** found to be significantly associated with OHL was the use of the Thai language in everyday communication ($p<0.05$).

Table 4 presents the results of the multilevel logistic regression analysis, conducted using a fixed effects model, to identify factors associated with OHL among Grade 6 students. The analysis was conducted in two models. **Model 1** included both school-level and individual-level variables, while **Model 2** examined only individual-level variables.

Model 1: Combined school- and individual-level analysis

The results indicated that, after controlling for other factors, only individual-level variables were significantly associated with OHL. Notably, students with a GPA of 3.00 or higher were three times more likely to demonstrate adequate OHL compared to those with lower GPAs ($p=0.007$, OR=3.082, 95% CI=1.369-6.941), highlighting a positive association between academic performance and OHL.

At the school level, variables including school type (public, private, or municipal) and location (urban or rural) were not significantly associated with students' OHL ($p>0.05$).

Model 2: Individual-level analysis

When school-level variables were excluded, several individual-level factors emerged as statistically significant predictors of OHL. GPA ≥3.00 remained a significant factor ($p=0.006$, OR=2.386, 95% CI=1.368-5.882), Parental income above Thai national average was significantly associated with higher OHL ($p=0.008$, OR=2.233, 95% CI=1.244-4.008). Participation in academic clubs was also a significant predictor ($p=0.029$, OR=3.409, 95% CI=1.139-10.206).

Discussion

The primary objective of this study was to assess the level of OHL among Grade 6 students in Mueang District, Chiang Rai Province, and to identify the factors

Table 1: Characteristics of the participants (n=351).

Characteristics		n	%
Student gender	Male	173	49.3
	Female	178	50.7
Student age	9-10 years	13	3.7
	11-12 years	324	92.6
	13-16 years	13	3.7
Student ethnicity	Thai	318	91.6
	Other	29	8.4
Parental gender	Male	131	37.5
	Female	218	62.5
Parental age	< 40 years	98	34.5
	40-49 years	132	46.5
	50 years and above	54	19.0
Parental education level	Below high school	131	38.1
	High school/Vocational certificate	53	15.5
	Diploma/High vocational certificate	25	7.3
	Bachelor's Degree and Above	134	39.1
Parental occupation	Government/Company employee	114	33.4
	Personal business/Freelancer	170	49.8
	Agriculture/Not working	57	16.8
Type of school	Public schools (10 schools)	217	61.8
	Private schools (2 schools)	61	17.4
	Municipal school (1 school)	73	20.8
Location of school	Urban (5 schools)	207	59.0
	Rural (8 schools)	144	41.0

Table 2: Oral health literacy scores (P-TOFHLiD) classified by school affiliation.

P-TOFHLiD Proficiency Area	Score	Public school (n=217)	Private school (n=61)	Municipality school (n=73)	Overall (n=351)
Part 1: Knowledge of oral organs (0-7)	Min	1	1	4	1
	Max	7	7	7	7
	Mean (SD)	6.3 (1.2)	6.6 (1.0)	6.8 (0.5)	6.5 (1.1)
Part 2: Knowledge of dental caries (0-6)	Min	0	1	3	0
	Max	6	6	6	6
	Mean (SD)	4.7 (1.3)	5.0 (1.1)	5.4 (0.7)	4.9 (1.2)
Part 3: Knowledge of oral health care (0-7)	Min	0	1	3	0
	Max	7	7	7	7
	Mean (SD)	5.1 (1.5)	5.7 (1.4)	5.9 (1.1)	5.4 (1.4)
Part 4: Foods contributing to tooth decay (6)	Min	0	0	1	0
	Max	6	6	6	6
	Mean (SD)	3.3 (1.4)	3.4 (1.4)	4.0 (1.4)	3.5 (1.4)
Total score (0-26)	Min	5	6	12	5
	Max	26	26	26	26
	Mean (SD)	19.4 (4.0)	20.8 (3.5)	22.2 (2.6)	20.2 (3.8)
Level of Oral Health Literacy					
Adequate: N (%)		101 (46.5%)	41 (67.2%)	54 (74.0%)	196 (55.8%)
Inadequate: N (%)		116 (53.5%)	20 (32.8%)	19 (26.0%)	155 (44.2%)

Table 3: Chi-Square analysis of factors related to oral health literacy levels.

Factors		Categories	Inadequate OHL (n) (%)	Adequate OHL (n) (%)	Total (n) (%)	*p*-value
Personal	Student gender	Male	93 (53.8%)	80 (46.2)	173 (49.3%)	<0.001**
		Female	62 (34.8%)	116 (65.2%)	178 (50.7%)	
	Student age	9–10 years	7 (53.8%)	6 (46.2%)	13 (3.7%)	0.123
		11-12 years	140 (43.2%)	184 (56.8%)	190 (92.3)	
		13 years or more	8 (57.1%)	6 (42.9%)	134 (4.0%)	
	Student ethnicity	Thai	135 (42.5%)	183 (57.5%)	318 (91.6%)	0.093
		Other	17 (58.6%)	12 (41.4%)	29 (8.4%)	
	GPA	≤ 2.99	39 (66.1%)	20 (33.9%)	50 (19.7%)	<0.001**
		≥ 3.00	80 (33.3%)	160 (66.7%)	248 (80.3%)	
	Had toothache experience	Never	85 (48.9%)	89 (51.1%)	174 (49.9%)	0.076
		Ever	69 (39.4%)	106 (60.6%)	175 (50.1%)	
	Had check-up with a dentist in the past year	Never	28 (60.9%)	18 (39.1%)	46 (13.1%)	0.010*
		Ever	127 (41.6%)	178 (58.4%)	305 (86.9%)	
Situational	Parental gender	Male	68 (51.9%)	63 (48.1%)	131 (37.5%)	0.020*
		Female	85 (39%)	133 (61%)	218 (62.5)	
	Parental education	≤ High School	85 (51.8%)	79 (48.2%)	112 (47.8%)	0.004*
		≥ Bachelor's degree	65 (36.3%)	114 (63.7%)	231 (52.2%)	
	Parent occupation	Government officers	25 (36.2%)	44 (63.8%)	69 (19.7%)	0.139
		Others	130 (46.1%)	152 (53.9%)	282 (80.3%)	
	Parental monthly income ($\bar{x}$ Thai people = 20,723THB)	< Average	106 (52.7%)	95 (47.3%)	201 (64.6%)	<0.001**
		≥ Average	31 (28.2%)	79 (71.8%)	110 (35.4%)	
	Type of household living arrangement	Father and/or mother	23 (51.1%)	22 (48.9%)	45 (12.8%)	0.315
		Other relatives	132 (43.1%)	174 (56.9%)	306 (87.2%)	
	Health knowledge resources	Online/offline Media	76 (51.4%)	72 (48.6%)	148 (42.2%)	0.021*
		2-Way communication	79 (38.9%)	124 (61.1%)	203 (57.8%)	
	Type of club members	Academic	5 (17.2%)	24 (82.8%)	29 (8.3%)	0.002*
		Others (sports, dance)	150 (46.6%)	172 (53.4%)	322 (91.7%)	
Societal and environmental	Had health promotion activities in school	Yes	116 (42%)	160 (58%)	276 (78.6%)	0.123
		No	39 (52%)	36 (48%)	75 (21.4%)	
	Language use in everyday communication	Thai	120 (40.3%)	178 (59.7%)	298 (84.9%)	<0.001**
		Other (Ethnic & foreign languages)	35 (66%)	18 (34%)	53 (15.1%)	

*Significant $p<0.05$ **Significant $p<0.001$

associated with their health literacy. A total of 351 students participated in the study. The results showed that 56% of the students demonstrated adequate OHL, while 44% were classified as having inadequate literacy. The mean OHL score for the entire sample was 20.2, which is lower than the average score of 21.6 reported in a previous study conducted among Grade 5 and 6 students across 12 health regions in Thailand. That study also found a higher proportion, 71.4% of students with adequate oral health literacy, using the same assessment tool, the P-TOFHLiD.[4]

The analysis showed that students' OHL scored highest in knowledge about oral organs, followed by dental caries, oral health care, and lowest in knowledge about foods that cause dental caries. Notably, the domain of knowledge about foods contributing to tooth decay consistently yielded the lowest scores among students from public, private, and municipal schools. This trend aligns with a previous study.[22] These findings highlight the need for OHL programs to address all knowledge domains, with particular emphasis on improving students' understanding of the dietary factors affecting oral health. Furthermore, additional research is warranted to investigate students' dietary behaviors related to cariogenic foods in order to

Table 4: Fixed effects from multilevel logistic regression on factors associated with oral health literacy.

Factors	Model 1			Model 2		
	OR	OR (95% CI)	*p*-value	OR	OR (95% CI)	*p*-value
Intercept	0.067	0.010-0.463	0.006	0.060	0.018-0.201	<0.001
School level						
Urban school	1.030	0.370-2.866	0.955	-	-	-
Rural school (ref)	-	-	-	-	-	-
Public school	1.468	0.423-5.099	0.544	-	-	-
Private school	0.785	0.207-2.985	0.722	-	-	-
Municipality school(ref)	-	-	-	-	-	-
Individual level						
Student gender (Female)	1.372	0.772-2.439	0.280	1.309	0.746-2.299	0.344
Student's GPA (3.00 or above)	3.082	1.369-6.941	0.007**	2.386	1.368-5.882	0.006**
Had check-up with a dentist in the past one-year (Yes)	1.981	0.865-4.534	0.105	2.054	0.908-4.644	0.083
Parental gender (Female)	1.586	0.884-2.847	0.121	1.542	0.870-2.730	0.136
Parental educational level (≥ High school)	1.064	0.605-1.941	0.502	1.090	0.619-1.921	0.762
Parental income (> Thai average)	1.876	0.955-3.683	0.068	2.233	1.24 4-4.008	0.008**
Health knowledge resources (2-way communication)	1.319	0.756-2.300	0.328	1.353	0.782-2.342	0.276
Type of club members (Academic)	2.648	0.836-8.391	0.098	3.409	1.139-10.206	0.029*
Language use (Thai)	1.806	0.782-4.170	0.166	1.982	0.891-4.408	0.93

*Significant $p<0.05$ **Significant $p<0.001$

better understand the link between their knowledge in this area and actual consumption practices.

According to the factors associated with OHL, the findings showed that personal factors, especially having a Grade Point Average (GPA) of 3.00 or higher, were significantly associated with higher OHL scores. This result is consistent with previous studies.[12,13,24] In this study, all participants were Grade 6 students with the same level of formal education; therefore, academic performance, as reflected by GPA, serves as a key indicator of individual educational achievement, which may influence health literacy. Students with stronger academic performance are more likely to effectively access, comprehend, and apply health-related information. Female students demonstrated higher levels of OHL, consistent with the broader observation that females often exhibit greater interest in health-related matters compared to males.[25-27] However, after controlling for other variables in the multilevel analysis, this association was not statistically significant. This may be due to the developmental stage of the sample population, as students at this age typically show limited interest in health issues. Additionally, variations in parenting styles may also contribute to these findings.[28] Furthermore, variables related to illness experience and health service utilization, particularly dental service use, did not show a statistically significant association with OHL. This finding aligns with previous studies conducted among older adults in both the United States and Thailand, which similarly concluded that dental service use was not a significant predictor of OHL.[15,29] A likely explanation lies in the accessibility and comprehensiveness of Thailand's public health system, which routinely provides dental services and oral health education through school-based programs.[30] These services ensure that students across different literacy levels receive equitable access to oral health care and information. Therefore, differences in health literacy appear to be influenced primarily by personal factors, especially educatiaonal attainment.

Among the situational factors, Parental gender, education level, and income were initially found to be significantly associated with students' oral health literacy (OHL). However, upon further analysis, parental income emerged as the only direct and significant predictor. Families with higher income levels are more likely to provide enriched learning environments and resources, thereby increasing children's access to information,

educational materials, and supportive settings that facilitate the development of adequate OHL. Students whose parents had higher income levels and those who participated in academic clubs were 1.8 times and 2.6 times more likely, respectively, to exhibit adequate OHL. Parents with higher income levels can enhance their children's health literacy by providing access to enriched learning environments and educational resources that foster critical thinking and communication skills. Additionally, higher socioeconomic status is associated with better access to healthcare services and health education, which supports children's ability to understand and apply health information effectively. This relationship has been supported by Manganello (2008), who identified parental income as a significant predictor of adolescent health literacy, particularly due to its influence on access to health information and communication with healthcare providers.[20] Participation in academic clubs promotes reading, inquiry, and critical thinking skills that improve students' capacity to evaluate the accuracy of health information.[31] This finding aligns with Bandura's social learning theory, which emphasizes the role of enriched environments in developing knowledge and skills.[32] Health-related communication within these settings further contributes to improved OHL.[33] Consistent with prior research, structured extracurricular activities have been shown to support cognitive and social development, leading to better health-related decision-making.[34] Therefore, academic clubs play a vital role in equipping students with the competencies required to make informed health decisions and to develop overall health literacy.

However, the number of academic club participants in this study was relatively small, likely due to the limited availability of such clubs in some sampled schools. Academic clubs are typically offered only in private schools, municipal schools, or large urban public schools, reflecting broader social inequalities that influence students' opportunities for developing health literacy. Social and environmental factors were not found to be significantly associated with students' OHL in this study. One possible explanation is that, despite growing global interest and an increasing number of studies on health literacy, relatively few have focused on government policy implementation and the evaluation of health literacy strategies.[35] This highlights the need for further research to develop concrete and actionable policies aimed at promoting OHL at the systemic level.

In the multilevel analysis, individual-level factors, namely GPA, parental income, and participation in academic clubs, emerged as the most prominent determinants of OHL among students, whereas school-level factors did not demonstrate statistically significant associations. This finding may reflect systemic limitations within the Thai context, where national strategies to promote health literacy remain underdeveloped and lack clear direction, particularly in areas such as policy implementation, school-based initiatives, learning environments, and standardized national health promotion programs. These limitations may result in an inadequate response to the diverse needs of specific population groups.

Moreover, the current level of social infrastructure and support systems appears insufficient to effectively foster OHL among students. Future efforts should prioritize the development of high-quality health communication strategies that are inclusive and accessible to diverse populations. This includes strengthening the professional and communication competencies of frontline health workers and empowering the public with practical skills to access, understand, critically evaluate, and apply health information. These capabilities are essential for enhancing functional health literacy and supporting informed health decision-making in the long term.[36]

A key strength of this study is the use of the validated P-TOFHLiD instrument, specifically designed for Thai primary school children, ensuring contextual relevance and measurement reliability. The application of Sorensen's health literacy framework allowed for a multidimensional analysis of personal, situational, and societal factors. A diverse sample, multivariate and multi-level analysis further enhanced the robustness and applicability of the findings for improving OHL in adolescents. However, the cross-sectional design limits causal inferences. The study's focus on a single district may affect generalizability, and reliance on self-reported data introduces potential bias. Additionally, while predictors of OHL were identified, actual behavioral outcomes and broader sociocultural influences warrant further exploration.

Conclusions

This study aimed to identify the factors influencing OHL among sixth-grade students in Mueang District,

Chiang Rai Province. The findings revealed variations in literacy levels associated with academic performance (GPA), parental income, and participation in academic clubs. These results have implications for the development of more effective oral health promotion strategies targeting school-aged children. Accordingly, education policymakers and public health planners in other regions may apply these insights to design targeted oral health promotion programs within primary schools. In particular, integrating oral health education into the core curriculum and supporting extracurricular academic activities may help improve students' functional health literacy nationwide.

However, regional differences in language, culture, and school resources may limit the generalizability of these findings. Therefore, further research is recommended across diverse Thai populations to validate and expand upon these results.

List of Abbreviations:

OHL: Oral health literacy

GPA: Grade Point Average

IOC: The Index of Item-Objective Congruence

P-TOFHLiD: Test of Functional of Health Literacy in Dentistry for Primary school children

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Conflict of Interest

The authors declare no conflict of interest.

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Statement of Ethics

This study was reviewed by the Ethics Review Board in Human Research. Faculty of Dentistry Chiang Mai University (No. 23/2565) on April 29, 2022. Study participants have given their written informed consent.

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Clinicodemographic Study of Oral Squamous Cell Carcinoma in a Tertiary Hospital of Western Maharashtra: A Retrospective Study

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Abstract

Objectives: To identify the trends in the age group, gender, sites affected, clinical and histopathological grading in OSCC patients in a tertiary care center in a rural population.

Methods: The clinicodemographic profile of histologically confirmed 600 patients of OSCC was analyzed using descriptive statistics, correlations using the chi-square test and odds ratios between the two variables. The *p*-value less than 0.05 was considered statistically significant.

Results: The largest number of patients [158(26.3%)] were in the age group of 51 to 60 years, the mean age was 52.84 years, and the male: female ratio was 2.4:1. Seventy-four percent of patients had habits of smokeless tobacco and were from low socioeconomic status. The prevalence of oral cancer differed significantly according to habits (OR=5.15, 54.25, and 8.96 for tobacco chewing, smoking, and alcohol, respectively). Gingivobuccal sulcus was the most common site [221(36.83%)], followed by alveolus (21.34%) and tongue (15.0%). The prevalence of oral cancer significantly differed for tongue and alveolus in males and females (OR=2.34, p=0.001 for tongue, p=0.004 for alveolus). Association between buccal mucosa and/GBS and tongue tumor site and males was found to be statistically significant (p=0.001). Alveolus was also significantly associated with men (p=0.002). Morphologically, ulcerative lesion was the most common presentation [250(41.66%)], and most of the patients presented in the advanced stage (stage III and stage IV).

Conclusions: The present study highlights that the majority of OSCC cases present during the 4th to 6th decade of life, and this was due to poor lifestyle patterns, which can be prevented by avoidance of tobacco and alcohol consumption, a healthy diet, good oral and sexual hygiene, active screening, early diagnosis, and public awareness.

Keywords: clinical and histopathological grading, clinicodemographic features, oral cancer, prevention, risk factors

Introduction

Globally, oral cancer is the sixteenth most common type of cancer, with India contributing to almost one-third of the total global burden. In 2022, there were 389,485 incident cases and 188,230 deaths from oral cancer worldwide, while in India, around 143,759 new oral cancer cases and 79,979 deaths were reported.[1]

Considering global patterns and trends, the incidence and mortality rates of oral cancer in South and Southeast Asia are among the highest globally. 52% of total global deaths from oral cancer are reported from this region. The highest age-standardized incidence rates (ASIR) for lip and oral cancer in South and South-East Asia were highest in India (14.67), Sri Lanka (14.04), Bangladesh (13.61) and Pakistan (12.07) in males. The highest age-standardized mortality rates (ASMR) for lip and oral cancer were observed in India (8.17), Bangladesh (8.07) and Pakistan (7.74) for males. For developing countries like India, oral cancer poses a serious health challenge.[1,2]

Oral cancers have a multifaceted etiology with a plethora of lifestyle and environmental factors as the risk factors. Tobacco in any form, either smoking or smokeless tobacco, and alcohol consumption are major preventable risk factors. Human papillomavirus, dietary deficiencies and poor oral hygiene are minor etiological factors of oral cancer.[3] People of lower socio-economic strata of society are more commonly affected because of higher prevalence of lifestyle risk factors.[4] This high proportion is clearly associated with difficulties in accessing the health care system, with most cases eventually diagnosed at advanced clinical stages due to delayed detection and hence the chances of cure are very low. Significant survival disparities were observed among patients with oral cancer based on demographic factors and clinical characteristics. The reported overall 5-year ASRS (Age-standardized relative survival) rate is 37.2% (range, 20.9%-58.4%).[5]

Oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm of the oral cavity and represents about 90% of all oral malignancies. The aim of this retrospective study was to identify the trends in the age group, gender, the different oral sites affected, clinical and the histopathological gradings in OSCC patients in our institution, a tertiary care center.

Materials and Methods

A retrospective study of 600 patients with a histologically confirmed diagnosis of OSCC was carried out. The institutional ethical committee of Pravara Institute of Medical Sciences-Deemed University cleared the protocol (PIMS/RDC/RC/2016/191).

Study settings

This was a record-based retrospective study conducted by reviewing the clinical and treatment records of patients in the Department of Oral Medicine and Radiology in a tertiary care center of Western Maharashtra, India.

Study population

The study included patients who were diagnosed with cases of OSCC from 2009 to 2016. The inclusion criteria were defined as histopathologically confirmed, newly diagnosed cases of OSCC of the oral cavity in all age groups and with no gender differences. Patients with non-squamous histology, metastatic tumor, prior history of treatment for oral cavity malignancies, and those with incomplete data were excluded from the study.

Data pertaining to these patients was collected from the clinical case records of these patients. Data were collected in the context of age, sex, occupation, habits, site of oral cancer, duration and nature of symptoms, TNM staging (according to the 8th edition of the AJCC, 2017) and WHO histopathological grading of OSCC.

Statistical analysis

The data were analyzed using descriptive statistics such as frequency and percentage, correlations using the chi-square test and odds ratios between the two variables using IBM SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA). The obtained values were considered statistically significant if the p value was less than 0.05.

Results

Out of 600 OSCC patients, 422 (70.33 %) were males and 178 (29.67%) were females. The male: female ratio was 2.4:1. The largest number of patients (158 (26.3%)) were seen in the age group of 51 to 60 years, followed by the age group of 61 to 70 (127 (21.2%)). The youngest of all patients affected was 21 years old and the oldest was 90 years. The least number of patients (30 (5.0%)) were in the age group of 21-30 years. The mean age was 52.84 years. The prevalence of oral cancer differed significantly in the all age groups, where males were significantly affected. In

age groups of 51-60 yrs and 61-70 years, the prevalence differed but was not statistically significant (OR=3.56, 3.35 respectively).

Based on education, most of the patients were illiterate 269 (44.83%) and 172 (28.66%) patients were just literate/nongraduate. The prevalence of oral cancer differed significantly in nongraduate females as compared to males (OR=2.05).

The most frequent occupation in the present study was farming (158 (26.33%)), followed by laborers (124(20.66%)). The prevalence of oral cancer differed in female farmers (OR=5.13), laborers (OR=1.62) and self-employed (OR=9.86) as compared to male patients, but it was not statistically significant.

Based on socio-economic status, the majority of oral cancer patients belonged to lower middle and lower socio-economic classes (187 (31.16 %) and 210 (35.0 %) respectively) according to their per capita income of family. The prevalence of oral cancer differed significantly in upper middle class (OR=1.56) and middle class (OR=1.62) patients, where females are affected more frequently than males; it was statistically significant only in the upper middle class (Table 1).

The frequency of oral cancer according to tobacco habits and gender is summarized in Table 2. Most of the patients (444 (74%)) were tobacco chewers, followed by smokers (75 (12.50%)). Twenty-one patients (3.50%) were addicted to alcohol but were not consuming tobacco in any form. Only 5 patients (0.83%) had a habit of smoking, tobacco chewing and alcohol consumption.

In males, the frequency of oral cancer was highest in patients with the habit of tobacco chewing (281 (66.58%)), followed by smokers (74 (17.53%)), together accounting for 84.11%. Similarly, in females, the frequency of oral cancer was highest in tobacco chewers, accounting for a prevalence of 91.57% (163 cases). Thus, the prevalence of oral cancer differed significantly according to habits (OR=5.15, 54.25 and 8.96 for tobacco chewing, smoking and alcohol respectively). A small group of patients (16 (2.67%)) did not have any of these three habits.

The site distribution of oral cancer lesions has been listed in Table 3. The buccal mucosa and/ gingivobuccal sulcus (GBS) were the most frequently involved sites, in both males and females, accounting for 221 (36.83%) cases, (M=158 (37.44%) F=63 (35.39%)) followed by alveolus 128 cases (21.34%) (M=81 (19.19%) F=47 (26.40%)). Tongue was involved in 90 cases (15.0%) (M=78 (18.48%) F=12 (6.74%)) and floor of mouth in 52 (8.66%) patients (M=38 (9.00%) F=14 (7.86%)). Forty-six (7.67%) (M=34 (8.05%) F=12 (6.74%)) patients had involvement of palate, in 34 (5.66%) (M=21 (4.97%) F=13 (7.30%)) patients retromolar trigone, and 29 (4.83%) (M=12 (2.84%) F=17 (9.55%)) patients had lip involvement. The prevalence of oral cancer significantly differed for tongue and alveolus in males and females (OR=2.34, p=0.001 for tongue, p=0.004 for alveolus). For floor of mouth and palate also, the prevalence differed in males and females but was statistically nonsignificant.

The first symptoms noticed by the patients were ulceration (250 (41.66%)) followed by swelling (144 (24%)) and pain (112 (18.66%)). Other symptoms like difficulty with swallowing/dysphagia were experienced in 52 (8.66%) patients while reduced mouth opening was present in 42 (7%) patients (Table 4).

Duration of symptoms varied between 1-3 months in 254 (42.33%) cases, 3-6 months in 154 (25.67 %), less than one month in 112 (18.67%) and more than six months in 80 (13.33%) patients.

The majority of patients, 236 (39.33 %), were in Stage III followed by stage II in 200 (33.33%) patients, 89 (14.83%) patients were in stage IV and 75 (12.5%) patients were in stage I. Histopathologically, 277 (46.17%) cases were well differentiated carcinomas, 236 (39.33 %) were moderately differentiated, 56 (9.33%) were poorly differentiated carcinomas and 31 (5.17%) cases were nothing otherwise specified (Tables 5 and 6).

The association between the various sites of oral cancer and gender of patients is shown in Table 7. Association between buccal mucosa and/GBS and tongue tumor site and males was found to be statistically significant (p=0.001). Alveolus was also significantly associated with men (p=0.002). Lip, palate and retromolar area were more frequently involved in females than in males, but the difference was not statistically significant.

The association between various tumor sites and different age groups is reported in Table 8. The buccal mucosa was the most prevalent site in the age group of 51-60 years, with a statistically significant association between them (p=0.04). Similarly, lip and alveolus as the tumor site were significantly associated with the age group of 31-40 years (p=0.017 and p=0.03 respectively). Involvement of the palate was most prevalent in the age

group of 61-70 years and this association was highly statistically significant (p=0.008). Tongue, floor of mouth and retromolar trigone were most prevalent in the age group of 41-50 years and this association was also statistically significant.

The association between the various sites of oral cancer and habits of patients is shown in Table 9. Tongue cancer in the present study was associated with the habit of

Table 1: Prevalence of oral cancer patients according to demographic variables and gender.

Demographic Variables		Male (n=422) No. (%)	Female (n=178) No. (%)	Total no. (n=600) No. (%)	OR (95% CI)	*p*-value
Age in Years	21-30	27(6.40)	3(1.69)	30(5.0)	1	0.001**
	31-40	92(21.80)	25(14.04)	117(19.5)	3.53(2.36-5.67)	
	41-50	89(21.09)	33(18.54)	122(20.33)	3.79(2.28-6.31)	
	51-60	112(26.54)	46(25.84)	158(26.33)	3.56(1.67-7.85)	
	61-70	73(17.30)	54(30.34)	127(21.27)	3.35(1.63-6.90)	
	71and more	29(6.87)	17(9.55)	46(7.67)	0.33(0.15-11.10)	
Socio Economic Status	Upper	2(0.47)	0	2(0.33)	1	0.004**
	Upper Middle	14(3.32)	3(1.69)	17(2.83)	1.56(0.43-5.66)	
	Middle	141(33.41)	43(24.16)	184(30.67)	1.62(1.08-2.42)	
	Lower Middle	120(28.44)	67(37.64)	187(31.17)	0.63(0.43-0.92)	
	Lower	145(34.37)	65(36.52)	210(35.0)	0.92(0.64-1.33)	
Education	Illiterate	147(34.83)	122(68.54) dse3333	269(44.83)	0.24(0.17-0.36)	0.02*
	Non- Graduate	138(32.70)	34(19.10)	172(28.67)	2.05(1.34-3.14)	
	Graduate	137(32.47)	22(12.36)	159(26.50)	1	
Occupation	Farmers	142(33.65)	16(8.99)	158(26.33)	5.13(2.95-8.91)	0.001**
	Laborers	97(22.99)	27(15.17)	124(20.67)	1.62(1.02-2.57)	
	Self Employed	75(17.77)	3(1.69)	78(13.0)	9.86(3.55-27.38)	
	Home Makers	2(0.47)	113(63.48)	115(19.17)	0.003(0.0-0.11)	
	Service	58(13.74)	8(4.49)	66(11.00)	1	
	Unemployed	48(11.38)	11(6.18)	59(9.83)	1.77(0.91-3.43)	

*Statistically significant, **Highly statistically significant, OR-Odds Ratio

Table 2: Prevalence of habits among the study population.

	Gender				
Tobacco Habits	**Male (n=422) No. (%)**	**Female (n=178) No. (%)**	**Total no. (n=600) No. (%)**	**OR (95% CI)**	***p*-value**
Tobacco Chewing	281(66.59)	163(91.57)	444(74)	5.15(2.80-0.91)	0.03*
Smoking	74(17.54)	1(0.56)	75(12.50)	54.25(7.50-392.27)	0.001**
Alcohol	21(4.98)	0	21(3.50)	8.96(2.14-37.52)	0.001**
Tobacco chewing+ smoking	25(5.92)	0	25(4.17)	0.91(0.88-0.94)	0.001**
Tobacco chewing+ alcohol	14(3.32)	0	14(2.33)	0.99(0.98-1.00)	0.32
Tobacco chewing+ alcohol + smoking	5(1.18)	0	5(0.83)	0.98(0.97-0.99)	0.31
No habits	2(0.47)	14(7.87)	16(2.67)	0.18(0.06-0.49)	0.001**

*Statistically significant, **Highly statistically significant, OR-Odds Ratio

Table 3: Prevalence of sites specificity of oral cancer among the study population.

Site	Gender				
	Male (n=422) No. (%)	Female (n=178) No. (%)	Total no. (n=600) No. (%)	OR (95% CI)	*p*-value
Buccal mucosa/GBS	158(37.44)	63(35.39)	221(36.83)	0.98(0.69-1.40)	0.91
Lip	12(2.84)	17(9.55)	29(4.83)	0.80(0.40-1.56)	0.59
Tongue	78(18.48)	12(6.74)	90(15.0)	2.34(1.36-4.02)	0.001**
Floor of mouth	38(9.00)	14(7.87)	52(8.67)	1.39(0.75-2.56)	0.31
Palate	34(8.06)	12(6.74)	46(7.67)	1.32(0.72-2.44)	0.45
Alveolus	81(19.19)	47(26.40)	128(21.33)	0.55(0.37-0.81)	0.004**
Retromolar area	21(4.98)	13(7.30)	34(5.67)	0.98(0.64-1.51)	0.89

*Statistically significant, **Highly statistically significant,GBS–Gingivo- Buccal Sulcus, OR-Odds Ratio

Table 4: Presentation of symptoms in the study population.

Symptoms	Gender		
	Male No. (%)	Female No. (%)	Total No. (%)
Ulceration	166(39.34)	84(47.19)	250(41.67)
Pain	81(19.20)	31(17.42)	112(18.67)
Swelling	92(21.80)	52(29.21)	144(24)
Dysphagia	44(10.43)	8(4.49)	52(8.67)
Reduced mouth opening	39(9.23)	3(1.69)	42(7)

Table 5: Stages of oral cancer according to gender among the study population.

Stage of Cancer	Gender		
	Male No. (%)	Female No. (%)	Total No. (%)
Stage I	48(11.37)	27(15.17)	75(12.5)
Stage II	154(36.49)	46(25.84)	200(33.33)
Stage III	165(39.10)	71(39.89)	236(39.33)
Stage IV	55(13.04)	34(19.10)	89(14.83)

Table 6: Histopathological diagnosis of oral cancer among the study population.

Histopathological diagnosis	Gender		
	Male No. (%)	Female No. (%)	Total No. (%)
Well differentiated	187(44.23)	90(50.56)	277(46.20)
Moderately differentiated	175(41.38)	61(34.26)	236(39.34)
Poorly differentiated	42(9.93)	14(7.86)	56(9.34)
Not specified	18(4.26)	13(7.30)	31(5.12)

Table 7: Association between site and gender.

Site	Gender	*p*-value
Buccal mucosa and gingivobuccal sulcus	Males	0.001**
Lip	Females	0.32
Tongue	Males	0.001**
Floor of mouth	Males	0.17
Palate	Females	0.23
Alveolus	Males	0.002**
Retromolar area	Females	0.13

*Statistically significant, **Highly statistically significant

Table 8: Association between site and age groups.

Site	Age group	*p*-value
Buccal mucosa and gingivobuccal sulcus	51-60	0.014
Lip	31-40	0.017*
Tongue	41-50	0.046*
Floor of mouth	41-50	0.005**
Palate	61-70	0.008*
Alveolus	31-40	0.03*
Retromolar area	41-50	0.02*

*Statistically significant, **Highly statistically significant

Table 9: Association between site and habits.

Site	Habit	*p*-value
Buccal mucosa and gingivobuccal sulcus	Smoking	0.06
Lip	No habits	0.13
Tongue	Smoking	0.007**
Floor of mouth	Tobacco and Smoking	0.001**
Palate	Smoking and Alcohot	0.06
Alveolus	Tobacco and Smoking	0.03*
Retromolar area	No habits	0.08

*Statistically significant, **Highly -statistically significant

smoking and was statistically highly significant (p=0.007) while floor of the mouth and alveolus were significantly associated with tobacco chewing and smoking habits (p=0.001 and 0.03 respectively).

Discussion

According to the World Health Organization, oral cancer is the first and most common cancer in Indian males, while it is the fourth most common cancer in Indian females and the second most common cancer considering both sexes.[1] Tobacco use in different forms and alcohol are major and preventable etiological factors. Understanding the epidemiology and the risk factors for oral cancers can help with early identification and prompt treatment of these patients. Late detection and treatment is directly proportional to increased morbidity and mortality.

In the present study, male cases outnumbered female cases. M:F ratio was 2.4:1 which is consistent with the study by Wildt J *et al*.,[6] The higher male-to-female ratios were reported by various other studies.[7-13] The prevalence rate of oral cancer has been reported to be 20 per 1,00,000 men in the Indian subcontinent. Higher

male prevalence may be due to the vulnerability and exposure of males to different risk factors such as smoking, tobacco and sunlight.[14] Socio-cultural norms and values favor the easy availability of tobacco products to males. The common habit of consuming tobacco and betelnut as stimulants makes the male population more susceptible to oral cancers. However, in recent times, this difference in gender distribution is decreasing due to changes in modern women's social profiles and ways of life. They are more likely to be exposed to carcinogenic agents like tobacco, alcohol and high-risk HPV subtypes. It may be noted that a reverse trend has been observed in Thailand (M: F 1:1.56).[15]

According to the US National Cancer Institute SEER program, the mean age of diagnosis of oral cancer is 65 years.[16] In the present study, the mean age of the patients was 52.84 years. These findings are in accordance with the results of various previous studies.[10,12,17,18] Sankaranarayan *et al*.,[19] found that the peak-age frequency of occurrence (the fifth decade of life) in India is at least a decade earlier than that described in the western literature. Various studies in India observed an increase in the incidence of oral cancer in the younger (less than 40 years) age group.[17,20,21] In the present study, 147 (24.5%) patients were below 40 years of age. The high prevalence of addiction to tobacco and alcohol among young adult men and women may explain the stable trend in OSCC incidence in this group.

The risk of oral cancer is inversely proportional to an increasing level of education, income and occupation. The majority of oral cancer patients in the present study belonged to lower middle and lower socioeconomic classes (187 (31.16 %) and 210 (35.0 %) patients, respectively). These findings are similar to the findings of other studies.[13,22,23] The lower socioeconomic status may be a risk factor for poor oral hygiene and poor nutrition, thereby further increasing the risk of oral cancer in tobacco consumers. Most of the cases in the present study were illiterate 269 (44.83%) and just literate/nongraduate 172 (28.66%). The difference in the prevalence of oral cancer among different levels of literacy was found significant statistically (OR=2.05 p=0.02). These findings are consistent with studies by Agarwal *et al*., and Madani *et al*.[13,24]

Different occupational categories had a significant increased relative risk of cancer. In this study, the most frequent occupation was farming (158 (26.33%)), followed by laborers (124 (20.66%)), which is in accordance with some previous studies.[11,24,25] Agarwal *et al*.[13] reported laborers/ unskilled persons (68.5%) were affected significantly.This can be explained as farmers are more indulged toward tobacco addiction as it is easily available and at very affordable prices. Antoniades *et al*.,[26] reported that these patients may have SCC of the lip, which may be due to more exposure to sunlight. Thus, low education, certain occupations and low socioeconomic status are significant independent risk factors for oral cancer. Thus, the combination of high tobacco consumption, prolonged sun exposure, socioeconomic factors, and potential dietary deficiencies creates a perfect storm for oral cancer development among farmers in India.

Out of 600 cases, 584 patients were addicted to smokeless tobacco, alcohol, and smoking (single or multiple habits). Only 16 patients did not report any habit. Tobacco use is influenced by various factors, such as individual attitudes, social acceptability, availability, advertising campaigns etc. Tobacco use in India differs from that around the globe, since the dominant form of tobacco used globally is the cigarette. However, in India, chewing of tobacco and betel nut is more prevalent. Tobacco is easily available in India and the current marketing of tobacco and gutkha in small pouches, which are easily accessible at very low cost to all people. These pouches do not show the graphic images illustrating harm to the body as a result of consumption of tobacco, as well as statuary warnings unlike that on cigarette packs worldwide. Various studies in India on the prevalence of tobacco habits and their related products revealed that smokeless tobacco habits are more prevalent than smoking in both males and females.[18,27] A study on reverse smoking and its association with premalignant and malignant lesions of the palate concluded that reverse smoking induced significantly more lesions than conventional smoking, and was a major determinant of subsequent palatal cancer.[28]

Andre *et al*.,[29] observed a deleterious effect of the consumption of alcohol even with nonsmokers or casual smokers. In our study, only 6.66% patients reported habit of alcohol with or without combined habituation with smoking and tobacco. Country liquor, a form of locally brewed alcohol that has low cost and easily available, favorite in laborers and farmers. The effect of consuming tobacco and alcohol leads to a dangerous synergy of

expression of the disease. Warnakulasuriya, in his paper opined that, other than major risk factors like tobacco, alcohol, and betel quid, several emerging risk factors namely HPV infection, immunosupression, diet and nutrition, heredity and familial risk, mate drinking, marijuana (cannabis) smoking, khat chewing, medicinal nicotine use, HIV infection, and alcohol containing mouthwashes are likely to be associated with oral cancer.[3]

Epidemiological studies have shown that the sites of occurrence for oral cancer differ widely. In the present study buccal mucosa and/ gingivobuccal sulcus was the most affected sites both in males (37.44%) and females (35.39%) followed by alveolus & tongue (21.34% and 15.0% respectively). These finding are consistent with various other studies.[10,11,13,30] Most of the patients tend to keep the tobacco in the form of quid in the buccal sulcus with close proximity to gingiva and alveolus. Prolonged retention of tobacco or betel quid in the buccal pouch (to acquire the greatest effect) accelerates the percolation of carcinogens mixed with saliva, causing irritation of the adjacent mucosa and tongue resulting in the evolution of cancer at the site of contact. The findings in our study are not in agreement with the findings in some studies, where tongue and floor of mouth carcinoma are more common in western countries due to consumption of alcohol and / smoking.[31,32] Association between buccal mucosa /GBS and tongue site and males was found to be statistically significant, which was also reported by Fotedar *et al*., Singhania *et al*.,[12,33] while Smitha *et al*.,[30] found statistically significant association between buccal mucosa /GBS and females. In the present study, lip, palate and retromolar area were more frequently involved in females than in males but the association was not statistically significant.

In the present study, buccal mucosa as a site was associated with an age group of 51-60 yrs, which is similar to the findings in studies by Smitha *et al*., and Singhania *et al*.[30,33] Lip carcinoma was significantly associated with the age group of 31-40 yrs, which is not consistent with the findings of various studies where it was significantly associated with more than 60 years of age.[12,30,34,35] Floor of the mouth carcinoma was significantly associated with the age group of 41-50 years, which was also reported by other studies.[12,30,36] Tongue cancer was significantly associated with the patients in the age group of 41–50 years, similar to study by Fotedar *et al*., but is inconsistent with study by Smitha *et al*., and Selvamani *et al*.[30,37]

In India, various studies have reported that buccal mucosa carcinoma has been associated with tobacco chewing and betel nut chewing. The present study also showed this association, but was not statistically significant. The association of OSCC with alveolus and floor of mouth was statistically significant with the habit of tobacco chewing and smoking. In the present study, lip cancer was associated with patients without any habits, but this association was statistically nonsignificant. Tongue tumors in the present study were significantly associated with the habit of smoking.

Regional variations in incidence and the site of occurrence relate to the major causes, which are alcohol and smoking in Western countries, and betel quid and tobacco chewing in South and Southeast Asia.

The most common symptom at the presentation was ulceration in 250 (41.66 %) followed by swelling in 144 (24%) patients, as observed in various studies.[12,13]

The delay in diagnosis of OSCC could be correlated to patient delay, professional delay, or both and probably has some bearing on the size of the tumor presented. The time interval between the onset of symptoms and the start of treatment depends on various factors such as patient behavior, clinical course of the illness and the quality of the health services.[38] A study in Córdoba, Argentina, reported that both patients and professionals were responsible for the delay in diagnosis and indicated that the professional delay was the most associated variable to the stage of tumor.[39] In the present study, most of the patients presented within 6 months of onset of symptoms. This can be attributed to the fact that, because of poverty, illiteracy, and possibly resorting to home remedies, all lead to delays for the patients. Hence, the crux of the oral cancer problem is that many cases report late to the healthcare facility. As evident from the findings of the present study, the majority of patients, i.e. 236 (39.33 %), were in stage III, followed by 200 (33.33%) patients in stage II, 89 (14.83%) patients were in stage IV and 75 (12.5%) patients were in stage I. Similar results were found in some previous studies.[10,12,21] Detecting oral cancer in early stages when these are amenable to single modality therapies, offers the best chance of long term survival. Priorities of particular treatment modality depend on the lesion location and extent, age of patient, cosmetic and

functional outcomes, associated illnesses, and the availability of expertise.[40]

Limitations

Limitation of this study is a smaller sample size and the data is from patients of a single tertiary care center in Western Maharashtra of India and hence, could not be generalized. Hence, multicentric studies with large sample sizes are required. Also, the retrospective nature of the study posed constraints in exploring additional variable associations.

Future directions

All the patients diagnosed with OSCC in this study were either users of tobacco or alcohol or both and are reported to be in advanced stages. Screening for oral cancers in high-risk groups will help us to identify oral precancerous lesions and cancers in the early stages and future studies should recommend exploring the feasibility of cancer screening at the Primary Health Center Level. Strategies to improve public awareness about the prevention and early detection of oral cancers must be in place.

Conclusions

A clinico-demographic profile of oral cancer patients of the rural population in Western Maharashtra of India has been sketched here. The commonest age of presentation was in the 5^{th}-6^{th} decades of life with buccal mucosa and/ GBS, the most commonly affected site with male predominance. Most of the patients had habits of smokeless tobacco and were from low socioeconomic status. The majority of the cases were reported at an advanced stage that depicts the negligence of patients and health care among the population. Regional differences in the prevalence and patterns of risk factors are the main reason for variation in incidence and pattern of oral cancer. The increased prevalence of the oral cancer in the Indian subcontinent is due to the usage of various tobacco and tobacco related products (smokeless or smoking), alcohol consumption, spicy food, high exposure to sunlight due to farming, low socioeconomic status and neglect of overall oral health.

Conflict of Interest

The authors declare no conflict of interest.

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Comparative Wear Resistance of Additive, Subtractive and Prefabricated Resin Denture Teeth: An *In Vitro* Study

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Abstract

Objectives: The purpose of this research is to evaluate the wear volume of resin dentures produced through additive manufacturing, comparing them with conventional acrylic denture options available in the market.

Methods: The study involved three distinct sets of denture samples: 1) prefabricated acrylic dentures, 2) subtractive-manufactured denture teeth, and 3) additive-manufactured denture teeth. All samples were anchored in self-curing acrylic resin for wear assessment. A matching antagonist mimicking a second premolar, crafted from identical material, was developed to interact with the samples. The samples underwent occlusive force testing of 5 kg over 120,000 cycles in a chewing simulator, continuously submerged in distilled water. Measurements of wear volume and depth were obtained through a 3D profilometer. Statistical evaluations were conducted using one-way ANOVA and the post-hoc Tukey's HSD test, maintaining a significance threshold of 0.05.

Results: Significant disparities in wear volume were observed among the groups. The prefabricated acrylic denture group showed no notable difference from the subtractive-manufactured group, nor did the comparison between the additive-manufactured denture teeth. However, both the prefabricated and the additive-manufactured denture teeth groups demonstrated more considerable wear than the additive-manufactured groups ($p<0.05$).

Conclusions: Denture teeth produced from additive manufacturing process showed superior resistance to wear compared to the prefabricated and subtractive-manufactured denture teeth counterparts under conditions simulating mastication.

Keywords: additive manufacturing, chewing simulation, denture teeth, subtractive manufacturing, wear

Introduction

Complete dentures are the standard approach for rehabilitating patients with edentulous arches, comprising two essential elements: the denture base and the denture teeth.[1] For denture teeth to function effectively, they must possess sufficient strength to withstand masticatory forces, resist wear from daily use and cleaning procedures, bond securely to the base, and be biocompatible with oral tissues. Historically, materials such as porcelain, acrylic resin, and composite resin have been employed to fabricate these teeth.[2,3] Traditionally, complete dentures are fabricated through compression molding using heat-polymerized acrylic resin, which often involves multiple visits from the patient and can be associated with shrinking due to polymerization. This has prompted a transition toward digital fabrication techniques, classified as either subtractive (milling) or additive (3D printing) methods. These approaches offer advantages including shorter processing times, reduced likelihood of errors, diminished material waste.[4-8]

Despite the advantages of additive-manufactured complete dentures, their adoption in clinical circumstances is limited due to factors such as higher costs compared to traditional options, the need for specialized equipment (like intraoral scanners and 3D printers), and the necessity for training on these technologies.[8,9] Additionally, research on the properties of additive-manufactured resin materials is still inadequate. Therefore, this study aims to investigate the wear resistance of resin dentures produced through additive manufacturing process, specifically comparing the wear volume in these teeth against commercially available acrylic resin denture teeth.

Materials and Methods

The test specimens were categorized into three groups: prefabricated acrylic resin denture teeth, subtractive-manufactured resin denture teeth, additive-manufactured denture teeth. Each group consisted of nine samples, with the sample size calculated using G*Power version 3.1 to achieve a 95% statistical power based on prior research data, which indicated that a minimum of six samples per group was sufficient.[9] In the prefabricated denture teeth group, test specimens (antagonists) were prepared using commercially available acrylic resin denture teeth (Rivera alpha, Shofu, Tokyo, Japan). The setup included ten upper lateral incisor teeth attached to autopolymerized acrylic resin in a mold with the dimensions of 20 mm in diameter and 15 mm in height, with the labial surface oriented upwards for testing. The simulated chewing abrader was created using second maxillary premolar denture teeth bonded onto a mold measuring 15 mm in diameter and 12 mm in height, also secured with autopolymerized acrylic resin. The palatal cusps were the only cusps that made contact with the antagonist during the wear simulation, while the buccal cusp tips were positioned approximately 2 mm higher than the palatal cusp tips to prevent contact with the test specimen. In the subtractive-manufactured group, artificial teeth were designed featured upper lateral incisors and upper second premolars, and then exported for milling into machinable acrylic resin (Multilayer PMMA Disc, Dentsply Sirona, Bensheim, Germany) using a 5-axis milling machine (350i, imes icore GmbH, Ettlingen, Germany). This process resulted in ten upper lateral incisors and ten second premolars. The milled teeth were then polished by using polishing protocol provided by manufacturer's instruction to create smooth and shine as prepared before being affixed to the denture bases, similar to the procedure used for the acrylic resin group.

For the additive-manufactured group, the designed artificial teeth featured upper lateral incisors and upper second premolars were printed using additive-manufactured liquid composite resin (Optiprint temp, DENTONA GmbH, Singen, Germany), resulting in the production of twenty upper lateral incisors and twenty upper second premolars with a 3D printer (Asiga MAX; Asiga, Sydney, Australia). The printing layer thickness was set to 50 micrometers. After printing, the samples were then light-cured to complete the reaction using an Otoflash (Otoflash G171 Curing Light, Fona Dental, Kastrup, Denmark) for 2000 cycles under nitrogen gas, after which the artificial teeth were secured to the bases, consistent with the previous group.All samples were polished using silicon carbide wet sanding paper with grits of 600 and 1200 for one minute per piece, utilizing a rotary polishing machine (Buehler, Metaserve, Buehler Ltd., Lake Bluff, Illinois, USA). Lists of materials used in this study were shown in Table 1.

All samples were then soaked in distilled water maintained at 37°C for 24 hours prior to wear testing using a chewing simulator (CS-4.4; SD Mechatronik GmbH, Friedrichshafen, Germany). During testing, the machine moved vertically by 5 millimeters and horizontally by

2 millimeters, applying a chewing force of 5 kilograms with a frequency of 0.8 cycles per second. This setup simulated approximately six months of mastication by subjecting the samples to 120,000 chewing cycles in distilled water.[10,11] The wear of the samples was measured using a 3D laser profilometer (Keyence VR6000 series, Keyence Corporation, Osaka, Japan). The samples were scanned post-testing to calculate the lost volume and height. Data collection and analysis involved comparing the volume and height of the lost artificial teeth across the three groups. The data were analysed using One-way ANOVA and post-hoc comparisons (Tukey's HSD) using a significance level of 0.05.

Results

Following the simulation of 120,000 chewing cycles, the wear volume of the artificial teeth in all three groups was measured (Table 2). One-way ANOVA analysis demonstrated statistically significant differences in wear volume among the groups at a 0.05 significance level. Subsequent post-hoc testing with Tukey's HSD revealed that there was no significant difference in wear volume between the prefabricated denture teeth group and the subtractive-manufactured denture teeth group. However, both the prefabricated acrylic resin group and the subtractive-manufactured denture teeth group showed significantly greater wear depth and volume compared to the additive-manufactured denture teeth group at the same level of significance.

Discussion

The objective of this study was to examine the wear behavior of various denture teeth types used in complete denture construction, including a prefabricated acrylic resin group representing conventional complete denture fabrication, a subtractive-manufactured acrylic denture teeth group, and an additive-manufactured resin denture teeth group, illustrating digitally fabricated dentures. Prior research has shown that denture wear is affected by the materials employed in their construction and their opposing counterparts.[12,13] When different materials come into contact under identical conditions, they produce differing levels of wear. The results of this study reject the hypothesis that wear volume would remain unchanged when using the same material for opposing surfaces during wear simulation. The type of material influenced the extent of denture tooth wear, owing to differences in their structural and compositional properties. The acrylic resin and subtractive-manufactured acrylic denture teeth groups showed no significant difference in wear, likely due to their similar composition of polymethylmethacrylate (PMMA), which is processed under high heat and pressure to achieve comparable strength.[14] The additive-manufactured denture teeth group demonstrated the least wear, since this material contains dimethacrylate monomers, a critical component for UV-cured resins, and include inorganic fillers that enhance mechanical performance. This aligned with prior research indicating that composite-structured dentures possess superi-

Table 1: Lists of material used in this study.

Material	Compositions	Manufacturer
Rivera alpha	Cross-linkedPMMA	Shofu, Kyoto, Japan
Multilayer PMMA	Polymethylmethacrylate, Double Cross Link.	Dentsply Sirona, Benshiem, Germany
Optiprint temp	Aliphatic difunctional methacrylate 2,2'-ethylenedioxydiethyl dimethacrylate Aliphatic urethane Acrylate Phosphine oxide	DENTONA, Germany

Table 2: The average wear depth and volume loss, as well as the standard deviation of each type of material after undergoing chewing simulation.

Group	Mean wear depth ± SD (mm)	Mean volume loss ± SD (mm^3)
Prefabricated denture teeth	0.187 ± 0.022^{A}	0.286 ± 0.073^{a}
Subtractive-manufactured teeth	0.205 ± 0.093^{A}	0.215 ± 0.064^{a}
Additive-manufactured teeth	0.076 ± 0.006^{B}	0.087 ± 0.019^{b}

The same uppercase letter in the Mean wear depth column indicates no statsistically significant difference.
The same lowercase letter in the Mean volume loss column indicates no statsistically significant difference.

or wear resistance compared to traditional acrylic resin denture teeth, due to the inorganic fillers that improve their mechanical properties and overall strength.[15,16]

The wear volume and depth across all groups followed a consistent pattern, which contrasts with earlier studies that reported no significant differences in wear resistance between 3D-printed resin dentures and prefabricated denture teeth.[9,17] However, many of these previous studies focused on experiments involving metal or zirconia opposing surfaces, which may not accurately represent the conditions of complete mouth denture use. Moreover, there is limited evidence regarding how digital manufacturing techniques like subtractive manufacturing or additive manufacturing compare to conventional methods in terms of wear behavior.

This research excluded prefabricated composite resin dentures as a test group. This decision was made because the inability to standardize the shape and occlusal inclination of the opposing teeth would have led to inconsistent wear results. To simplify the measurement process, the wear simulation tests were performed on flat surfaces. The design of the wear simulation in this study was distinct from previous experiments, as it was developed to more closely imitate the conditions of actual complete denture use. Based on prior findings that 30,000 cycles equate to roughly 1.5 months of oral function[18], this study used 120,000 cycles to simulate six months of chewing. A force of 5 kg (49 N), representing the average human chewing force, was applied.[10,11] The chewing motion was mimicked using a 5 mm vertical and 2 mm horizontal movement. Throughout the simulation, distilled water was used to clear debris from the sample surfaces. However, limitations in the research equipment prevented the use of thermal cycling (alternating heating and cooling).

During the experiment, one sample from the additive-manufactured denture teeth group was excluded after it broke mid-test. The failure was characterized by both adhesive failure (separation between the different materials) and cohesive failure (fracturing within the material itself). This breakage likely resulted from inadequate adhesion between the two types of materials and a weaker bond between the individual printed layers compared to the strength within each layer. This finding aligns with research by Lim *et al*., which reported that the bond between 3D-printed resin and self-cure acrylic resin is weaker than its bond with Bis-acryl composite resin.[19] The probable cause is that methyl methacrylate does not copolymerize effectively with bifunctional monomers or light-cured resins. Thus, to improve the bonding strength for denture repairs or reinforcements, it may be necessary to enhance the mechanical and chemical surface properties of additive-manufactured parts.

The chewing simulation in this study focused on two-body wear, which involves the direct abrasive contact between two identical materials.[20,21] This models the wear on complete dentures, especially those with bilaterally balanced occlusion, that occurs during functions like chewing and swallowing. Although food can introduce three-body wear, its effect was considered minimal because modern diets are typically soft, making abrasion from food less impactful than direct tooth-on-tooth contact.[15] Therefore, the experiment was designed to measure two-body wear. The results clearly indicate that additive-manufactured denture teeth offer significantly superior wear resistance compared to traditional denture materials for complete denture applications.

Conclusions

Based on this study's findings, additive-manufactured (3D-printed) denture teeth demonstrated superior resistance to wear compared to traditional options. Following a simulated six-month period of use, the 3D-printed teeth had significantly less material loss than teeth made from subtractive-manufactured (milled) or prefabricated resin. The wear performance of the subtractive-manufactured and prefabricated acrylic resin teeth was comparable, with no statistically significant difference observed between them.

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Conflict of Interest

Authors declared no conflict of interest.

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A Comparative Study of Plaster Casts and Digital Models for Orthodontic Measurements in Different Crowding Severities

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Abstract

Objectives: This study aimed to evaluate the accuracy of linear measurements between the traditional gold-standard plaster model and digital models (cast scans and intraoral scans) across different severities of dental crowding.

Methods: Fifteen subjects requiring orthodontic treatment were divided into two groups based on crowding severity: mild (≤3.0 mm) and moderate to severe (>3.0 mm). For each subject, three types of models were generated: plaster cast (PC), digital cast scan (CS), and intraoral scan (IOS). Linear measurements were taken using digital calipers for PC and Ortho Analyzer™ software for CS and IOS. The accuracy of measurements was determined using the deviation between the plaster casts and digital models. The accuracy was analyzed using Welch's ANOVA and the Games–Howell multiple comparison test ($p<0.05$).

Results: All three model types demonstrated high intra- and inter-examiner reliability (ICC>0.97). No significant differences were found in the clinical arch length or Bolton ratios between model types. Minor, non-significant underestimations in total tooth width were observed in the digital models, particularly in maxillary teeth with moderate-to-severe crowding (maximum mean difference: 1.65 mm; $p=0.06$).

Conclusions: Both cast scans and intraoral scans are reliable alternatives to plaster casts for orthodontic measurements. While digital models slightly underestimated total tooth widths and Bolton ratios and overestimated clinical arch lengths, these variations remained within clinically acceptable limits. Operator training and scanning protocol standardization remain critical in crowded dentitions.

Keywords: accuracy, Bolton ratio, cast scan, crowding, intraoral scanner

Introduction

Accurate measurement of the arch length and tooth width is crucial in orthodontic diagnosis and treatment planning. Arch length assessments determine the space available for tooth alignment, influencing decisions such as extractions or arch expansion.[1] Similarly, the total tooth width is crucial for evaluating crowding, spacing, and arch form.[2] The Bolton ratio, which compares mesiodistal tooth widths between maxillary and mandibular teeth, is a widely accepted standard for diagnosing inter-arch tooth size discrepancies. Precise Bolton analysis facilitates the achievement of ideal overjet, overbite, and occlusal outcomes in orthodontic therapy.[3]

Plaster casts have long been considered the gold standard for dental model analysis because of their dimensional stability and high tactile accuracy.[4] They enable direct manual measurement and are widely used for visualizing occlusion, evaluating space, and constructing dental appliances. However, plaster casts are associated with several disadvantages; for example, they are susceptible to breakage, require physical storage, and are not easily duplicated or transferred between clinicians.[5] Moreover, inconsistencies in impression materials and pouring techniques can introduce errors, affecting measurement precision.[6] For these reasons, digital models obtained through cast scans or direct intraoral scanning have emerged as efficient and reliable alternatives. They offer several advantages, including enhanced data storage, easier communication, and integration with virtual treatment planning systems.[7] Both cast scans and intraoral scans produce measurements comparable to those obtained from plaster models, with high reproducibility.[8] Digital workflows also reduce chairside time and eliminate the need for physical impressions, improving patient comfort and clinical efficiency.[9]

Despite the benefits, intraoral scanners present challenges, particularly in patients with moderate-to-severe crowding. Limited access to interproximal and lingual areas, overlapping teeth, and patient movement can compromise scan accuracy.[10] In crowded cases, surface stitching errors can result in distortions, particularly in the posterior segments or narrow interproximal spaces. Jacob *et al*.,[11] reported underestimations of arch dimensions in such scenarios because of these technical limitations. Operator skill and experience are also critical factors influencing scan quality in complex cases.[12]

Numerous studies have compared the accuracy of linear measurements between digital and traditional models. Digital models offer acceptable accuracy for clinical use, especially in routine cases.[13,14] However, some investigations noted minor discrepancies in posterior tooth width measurements, attributed to scanner limitations and model curvature.[15] Overall, most studies support the clinical validity of digital methods, while acknowledging specific areas that require caution.

Few studies have specifically addressed the accuracy of digital models across varying degrees of dental crowding. Camardella *et al*.,[16] demonstrated high measurement reliability of scanned plaster casts, regardless of the severity of crowding. However, Martínez-Rodríguez *et al*.,[17] observed that measurement accuracy declined slightly in moderate-to-severe crowding, particularly in the molar regions. Therefore, this study compared the accuracy of linear measurements between plaster models and digital models across varying degrees of dental crowding to provide critical insights into their diagnostic reliability under anatomically challenging conditions. We also investigated whether the degree of dental crowding influences the accuracy of linear measurements obtained from plaster casts and digital models (cast scans and intraoral scans). In addition, we aimed to determine whether these digital models underestimate or overestimate measurements when compared with conventional methods.

The results of this study will help define clinically acceptable thresholds for measurement deviations and guide treatment planning and appliance fabrication. This study also contributes to the standardization of digital scanning protocols, particularly in crowded dentitions, and encourages further innovation in scanning technologies and software development.

Materials and Methods

This study received ethical approval (IRB No. P1-0060/2567) from the Faculty of Dentistry, Naresuan University Human Research Ethical Committee, Thailand. Fifteen subjects were selected from patients in the Department of Orthodontics at the Faculty of Dentistry, Naresuan University, Thailand, from March 2024 to September 2024, using simple random sampling. Based on a power analysis with a large effect size of 1.81, a statistical power of 0.80, and a significance level of 0.05, the minimum required sample size

for this study was determined to be 12 participants. The sample size was calculated with the G*Power software version 3.1.9.7 (Heinrich-Heine-University, Düsseldorf, Germany).

Participants with permanent dentition with fully erupted first molars to the contralateral first molars in both jaws who required orthodontic treatment, and who provided informed consent, were included in this study. Any participants with cleft lip, cleft palate, craniofacial deformity, craniofacial syndrome, or a known allergy to alginate, crystalline silica, calcium sulfate, potassium titanium fluoride, latex, or nickel, or those who did not consent to this study were excluded.

The operator (Faculty of Dentistry, Naresuan University, Thailand) performed the oral examination and intraoral scanning of each participant with a Trios 3 (3Shape, Copenhagen, Denmark) scanner, then took an impression with irreversible hydrocolloid (Alginor®; LASCOD SpA, Italy).

Intra-examiner validity and reliability testing were conducted prior to initiating intraoral scanning in the study participants. Five subjects who were not included in the study were randomly selected and examined by both the expert (Faculty of Dentistry, Naresuan University, Thailand) who is an experienced orthodontist with 5 years of clinical practice, and the operator (Faculty of Dentistry, Naresuan University, Thailand) involved in the study. Intraoral scanning was carried out with the participant seated in a dental chair. A single trained operator (Faculty of Dentistry, Naresuan University, Thailand) performed all scans following the manufacturer's recommended protocol, as shown in Figure 1.

Following intraoral scanning, the irreversible hydrocolloid impression material was prepared using a 1:1 powder-to-water ratio. The estimated mixing time was 45 s, with a total working time of 105 s and an intraoral setting time of approximately 30 s. The impressions obtained were poured using type III orthodontic stone (Sirius®; Lafarge Prestia, Thailand). The stone was mixed at a powder-to-water ratio of 100 g to 31 ml (1:3.23). The mixing, working, and setting times for the stone were approximately 1 minute, 8 minutes, and 15 minutes, respectively. The plaster dental cast was called "PC" and the digital image generated from the plaster cast using a laboratory scanner (3Shape E4, 3Shape Inc., Copenhagen, Denmark) was called "CS". The data obtained by the scanning procedure were exported as Standard Tessellation Language (STL) files and generated as digital models, which were called "IOS".

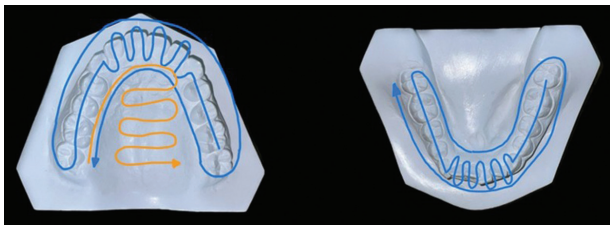

Figure 1: Scanning paths of maxillary and mandibular arches.

Measurement methods

The linear measurements included the following: 1) Clinical arch length: the total distance of the anterior and posterior segments (Figure 2). The anterior segment is the total distance between the distal side of the lateral incisor and the distal side of the contralateral lateral incisor. If a tooth exhibited crowding, labioversion, or linguoversion, the measurement was taken based on the tooth's ideal position within the alveolar bone. The posterior segment is the total distance between the mesial side of the first molar to the distal side of the lateral incisor. The posterior segment comprises the sum of the left and right sides of each arch. 2) Tooth widths: the largest distance between the mesiodistal point of each tooth. The tooth width was measured from the frontal view for anterior teeth and from the occlusal view for posterior teeth. 3) Bolton ratio[3]: a diagnostic index used in orthodontics to assess the proportional relationship between the mesiodistal widths of maxillary and mandibular teeth. It helps identify tooth size discrepancies that may affect occlusion, alignment, and inter-arch relationships during treatment planning. There are two types of Bolton ratios: anterior Bolton ratio, which compares the combined mesiodistal widths of the six anterior mandibular teeth (canine to canine) to those of the six anterior maxillary teeth, and the overall Bolton ratio, which compares the combined widths of all 12 mandibular teeth (from first molar to first molar) to the corresponding 12 maxillary teeth. The Bolton ratios were calculated using the formulas shown in Figure 3.

The linear measurements in PC were performed using a digital Vernier caliper (Mitutoyo, Tokyo, Japan). These data are regarded as the gold standard for measuring tooth widths and were the reference data. Digital images obtained from CS and IOS were transferred to the

Ortho Analyzer™ software program (Ortho Analyzer™, 3Shape, Copenhagen, Denmark) as STL files for measuring the linear measurements (Figures 4 and 5). After all the information was collected, each arch was divided into two groups based on the severity of crowding. If the amount of crowding was ≤3.0 mm, the arch was included in the mild crowding group, and those with crowding >3.0 mm were included in the moderate-to-severe crowding group.

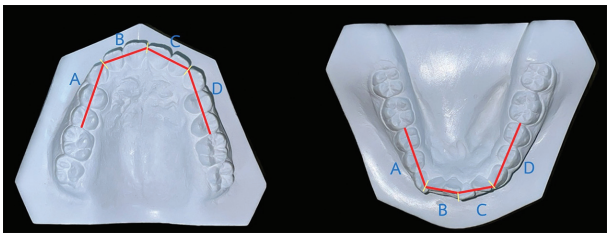

Figure 2: Clinical arch length = anterior segment (B+C) + posterior segment (A+D).

$$\text{Anterior Ratio (\%)} = \left(\frac{\text{Sum of mandibular 3–3 widths}}{\text{Sum of maxillary 3–3 widths}}\right) \text{x } 100$$

$$\text{Overall Ratio (\%)} = \left(\frac{\text{Sum of mandibular 6-6 widths}}{\text{Sum of maxillary 6-6 widths}}\right) \text{x } 100$$

Figure 3: Calculation of Bolton ratios.

Results

Intra-examiner reliability of linear measurements in plaster, scanned, and intraorally scanned models, as assessed by the two operators, showed no differences and was almost perfect for all measurements. The ICC values were 0.978 (95% CI; 0.875,0.994), 0.978 (95% CI; 0.909,0.993), 0.982 (95% CI; 0.771,0.996) for plaster models, cast scans, and intraoral scans, respectively.

The means and standard deviations (SD) of the clinical arch length (5-5) for maxillary and mandibular teeth between the mild and moderate-to-severe crowding groups are shown in Table 1. In the mild crowding group, the maxillary arch length ranged from 78.77 mm (CS) to 78.97 mm (IOS), while the mandibular arch length ranged from 67.04 mm (PC) to 67.36 mm (IOS). The mean differences between PC and IOS were minimal: -0.08 mm for maxillary arches and -0.33 mm for mandibular arches. Similarly, the mean differences between PC and CS were 0.12 mm (maxillary) and -0.26 mm (mandibular). In the moderate-to-severe crowding group, slightly larger differences were observed in the maxillary arch, with the mean clinical arch length for CS being 71.40 mm and for IOS being 71.03 mm. The mean differences reached -0.94 mm (PC-CS) and -0.57 mm (PC-IOS). In the mandibular

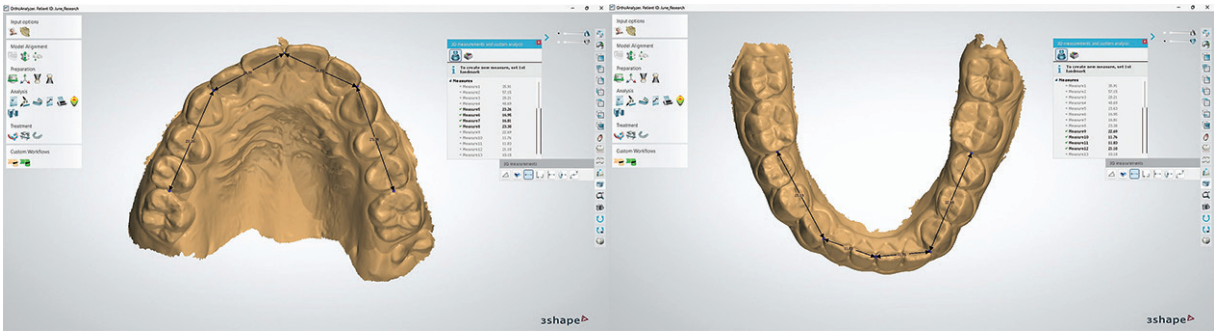

Figure 4: Clinical arch length measurement of maxillary and mandibular arches.

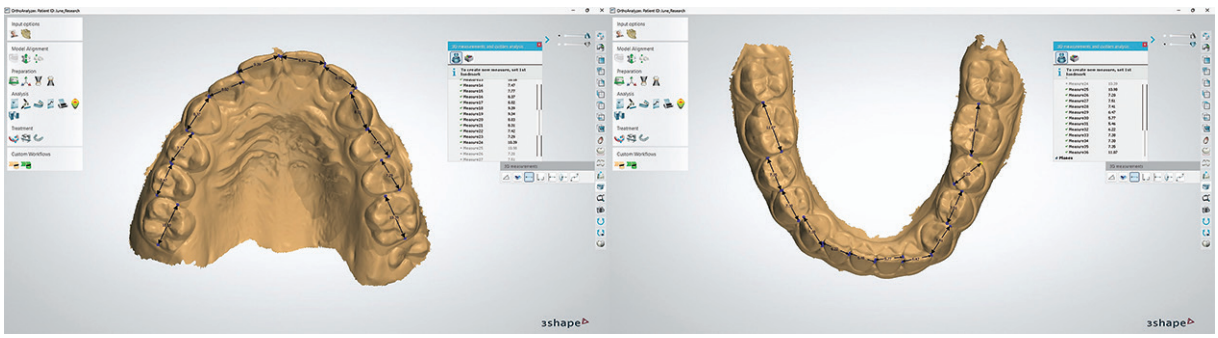

Figure 5: Tooth width measurement of maxillary and mandibular arches.

arch, values ranged from 61.82 mm (PC) to 62.08 mm (CS), with mean differences of -0.26 mm (PC-CS) and -0.18 mm (PC-IOS), closely mirroring the values of the mild crowding group.

To evaluate whether discrepancies between PC and digital models arose preferentially in the anterior or posterior portions of the arch, clinical arch length was separated into anterior and posterior segments for both mild and moderate-to-severe crowding groups (Table 1). For the anterior segment, the differences between PC and digital models were minimal in both crowding groups. In the mild group, maxillary anterior measurements showed only negligible deviations (PC-CS: +0.08 mm; PC-IOS: -0.08 mm), while the mandibular anterior segment demonstrated a uniform overestimation by both digital methods. In the moderate-to-severe group, discrepancies were slightly greater in the maxilla, with PC-CS and PC-IOS differences of -0.71 mm and -0.53 mm, respectively, whereas mandibular deviations remained small (PC-CS: -0.26 mm; PC-IOS: -0.12 mm). None of these variations reached statistical significance in pairwise comparisons (all $p>0.60$). For the posterior segment, measurements from digital models closely paralleled those from PC in both groups. In the mild group, maxillary posterior values differed only slightly (PC-CS: +0.05 mm; PC-IOS: 0.00 mm), and mandibular measurements showed similar results (PC-CS: +0.01 mm; PC-IOS: -0.06 mm). In the moderate-to-severe group, deviations were small, with the maxillary posterior segment differing by -0.23 mm (PC-CS) and -0.03 mm (PC-IOS), and the mandibular posterior segment remaining virtually unchanged (PC-CS: 0.00 mm; PC-IOS: -0.07 mm). All comparisons confirmed the absence of statistically significant differences ($p\geq0.92$).

Total tooth width (6-6) for maxillary and mandibular teeth was compared between the groups (Table 1). In the mild crowding group, the maxillary width ranged from 97.21 mm (CS) to 98.07 mm (PC), while the mandibular width ranged from 88.40 mm (IOS) to 89.09 mm (PC). Differences were minor, with mean differences between PC and IOS of 0.35 mm (maxillary) and 0.69 mm (mandibular). The mean differences between PC and CS in maxillary teeth were 0.86 mm and 0.68 mm in mandibular teeth. In the moderate-to-severe crowding group, mean differences were slightly larger in maxillary teeth: up to 1.28 mm (PC-CS) and 1.65 mm (PC-IOS). The mandibular teeth presented similar mean differences to the mild crowding group (PC-CS: 0.55 mm, PC-IOS: 0.51 mm).

Multiple comparisons using the Games-Howell test

Table 1: The difference of clinical arch length (5-5) and total tooth width (6-6) in millimeters between mild (n=10) and moderate to severe crowding groups (n=5).

			PC (mean±SD)	CS (mean±SD)	IOS (mean±SD)	Mean differences (PC-CS)	Mean differences (PC-IOS)
Anterior segment	Max	Mild	32.11±1.35	32.04±1.23	32.19±1.23	0.08	-0.08
		Mod-sev	28.13±1.28	28.85±1.36	28.67±1.19	-0.71	-0.53
	Mand	Mild	23.37±1.41	23.64±1.34	23.64±1.37	-0.27	-0.27
		Mod-sev	21.16±1.34	21.42±1.17	21.28±0.97	-0.26	-0.12
Posterior segment	Max	Mild	46.78±2.58	46.73±3.03	46.78±2.96	0.05	0.00
		Mod-sev	42.33±2.15	42.56±2.30	42.36±2.32	-0.23	-0.03
	Mand	Mild	43.66±1.93	43.65±1.97	43.72±1.98	0.01	-0.06
		Mod-sev	40.66±4.01	40.66±3.94	40.72±3.62	0.00	-0.07
Clinical arch length	Max	Mild	78.89±3.59	78.77±3.73	78.97±3.70	0.12	-0.08
		Mod-sev	70.46±1.77	71.40±2.06	71.03±2.16	-0.94	-0.57
	Mand	Mild	67.04±2.89	67.30±2.86	67.36±3.02	-0.26	-0.33
		Mod-sev	61.82±3.47	62.08±3.53	62.00±3.37	-0.26	-0.18
Total tooth width	Max	Mild	98.07±4.36	97.21±4.64	97.73±4.51	0.86	0.34
		Mod-sev	99.11±1.20	97.83±2.00	97.46±1.53	1.28	1.65
	Mand	Mild	89.09±4.09	88.41±4.12	88.40±3.66	0.68	0.69
		Mod-sev	91.00±2.32	90.45±3.40	90.49±3.24	0.55	0.51

Abbreviations: Max, maxillary arch; mand, mandibular arch; SD, standard deviation; mod-sev, moderate to severe; PC, plaster cast; CS, cast scan; IOS, intraoral scan; *p*-values were calculated by Welch ANOVA; *$p<0.05$.

Table 2: Paired pairwise statistical analysis for the difference in clinical arch length and total tooth width.

			Anterior segment			Posterior segment			Clinical arch length			Total tooth width		
			PC-CS (mm)	PC-IOS (mm)	CS-IOS (mm)	PC-CS (mm)	PC-IOS (mm)	CS-IOS (mm)	PC-CS (mm)	PC-IOS (mm)	CS-IOS (mm)	PC-CS (mm)	PC-IOS (mm)	CS-IOS (mm)
Max	Mild	mean±SD	0.07±0.58	-0.80±0.58	-0.15±0.55	0.05±1.26	0.00±1.24	-0.05±1.34	0.12±0.43	-0.08±0.40	-0.20±0.58	0.86±0.64	0.35±0.43	-0.52±0.77
		95% CI	(-1.41,1.54)	(-1.55,1.39)	(-1.55,1.26)	(-3.17,3.27)	(-3.18,3.18)	(-3.47,3.37)	(-1.31,1.08)	(-1.03,1.19)	(-1.30,1.68)	(-2.66,0.93)	(-1.54,0.85)	(-1.48,2.51)
		p-value	0.99	0.99	0.96	1.00	1.00	1.00	0.96	0.98	0.94	0.41	0.71	0.78
		effect size	0.08	-0.11	-1.01	0.05	0.00	-0.13	0.09	-0.06	-0.53	0.42	0.26	-0.54
	Mod-sev	mean±SD	-0.71±0.84	-0.53±0.78	0.18±0.81	-0.23±1.41	-0.03±1.41	0.20±1.46	-0.94±0.64	-0.57±0.55	0.38±0.84	1.28±0.52	1.65±0.50	0.37±0.72
		95% CI	(-3.11,1.68)	(-2.78,1.71)	(-2.15,2.50)	(-4.26,3.80)	(-4.08,4.02)	(-3.97,4.37)	(-1.32,3.21)	(-1.40,2.53)	(-2.80,2.04)	(-3.12,0.57)	(-3.42,0.12)	(-2.42,1.68)
		p-value	0.68	0.78	0.97	0.99	1.00	0.99	0.39	0.60	0.90	0.14	0.06	0.87
		effect size	-0.59	-0.61	0.38	-0.22	-0.03	0.47	-0.66	-0.46	0.46	1.11	1.48	0.48
Mand	Mild	mean±SD	-0.27±0.62	-0.27±0.62	0.00±0.61	0.01±0.87	-0.06±0.88	-0.07±0.88	-0.26±0.27	-0.33±0.32	-0.07±0.42	0.68±0.43	0.69±0.42	0.02±0.60
		95% CI	(-1.84,1.30)	(-1.85,1.32)	(-1.54,1.55)	(-2.22,2.24)	(-2.30,2.18)	(-2.32,2.18)	(-0.49,1.01)	(-0.56,1.21)	(-1.00,1.13)	(-1.87,0.52)	(-1.86,0.47)	(-1.54,1.51)
		p-value	0.90	0.90	1.00	1.00	1.00	1.00	0.61	0.58	0.99	0.30	0.27	1.00
		effect size	-0.76	-0.66	0.01	0.01	-0.06	-0.14	-0.31	-0.33	-0.11	0.50	0.53	0.02
	Mod-sev	mean±SD	-0.26±0.80	-0.12±0.74	0.15±0.68	0.00±2.51	-0.07±2.42	-0.06±2.39	-0.26±0.12	-0.18±0.18	0.08±0.22	0.55±1.37	0.51±1.27	-0.04±1.86
		95% CI	(-2.54,2.02)	(-2.28,2.04)	(-1.80,2.10)	(-7.19,7.18)	(-6.99,6.86)	(-6.91,6.78)	(-0.15,0.68)	(-0.46,0.83)	(-0.72,0.55)	(-5.42,4.32)	(-5.02,4.00)	(-5.29,5.37)
		p-value	0.94	0.99	0.98	1.00	1.00	1.00	0.18	0.61	0.92	0.92	0.92	1.00
		effect size	-1.17	-0.25	0.56	-0.01	-0.16	-0.13	-1.01	-0.45	0.17	0.18	0.18	-0.05

Abbreviations: Max, maxillary arch; mand, mandibular arch; mm, millimeters; mod-sev, moderate to severe; PC, plaster cast; CS, cast scan; IOS, intraoral scan
Mild group (n=10); moderate to severe group (n=5); *p*-values were calculated by Welch ANOVA; **p*<0.05.

Table 3: Paired pairwise statistical analysis for the differences in Bolton ratios in percentage.

	Anterior ratio			Overall ratio		
	PC-CS (percentage)	PC-IOS (percentage)	CS-IOS (percentage)	PC-CS (percentage)	PC-IOS (percentage)	CS-IOS (percentage)
mean±standard error	0.83±0.66	0.31±0.66	-0.52±0.94	0.29±0.51	0.08±0.43	-0.21±0.66
95% CI	(-0.91,2.57)	(-1.43,2.04)	(-2.84,1.80)	(-1.04,1.61)	(-1.03,1.20)	(-1.84,1.43)
p-value	0.45	0.89	0.84	0.84	0.98	0.95
effect size	-0.32	-0.12	0.30	-0.15	-0.05	0.14

Abbreviations: PC, plaster cast; CS, cast scan; IOS, intraoral scan *p*-values were calculated by Welch ANOVA; **p*<0.05.

revealed no significant differences in the clinical arch length of the maxillary and mandibular teeth between the methods in either the mild crowding group or the moderate-to-severe crowding group (Table 2). In the mild crowding group, *p*-values were all ≥0.58. Similar non-significant results were found in the moderate to severe group.

Comparisons for maxillary and mandibular total tooth widths also showed no statistically significant differences between methods (Table 2). In the moderate-to-severe crowding group, the PC-IOS comparison for maxillary teeth approached significance (p=0.06), with a mean difference of 1.65 mm.

Paired comparisons of anterior and overall Bolton ratios between methods showed minimal mean differences and no statistically significant results (Table 3). All *p*-values were >0.45 (e.g., PC-CS anterior: p=0.45, PC-IOS overall: p=0.98).

Discussion

This study supports the use of digital models (cast scans and intraoral scans) as reliable alternatives to traditional plaster casts in the assessment of clinical arch length, total tooth width, and Bolton ratios. The findings align with the growing body of evidence supporting the use of digital technologies in orthodontic diagnostics, although some minor inconsistencies highlight the need for further refinement.

From this study, the clinical arch length measurements demonstrated overall consistency across the three methods. In both mild and moderate-to-severe crowding groups, the mean differences between PC and digital scans were minimal, especially in the mild group. Across both crowding groups, the anterior segment showed larger variability than the posterior segment, particularly in the maxilla of moderate-to-severe cases, where deviations approached –0.7 mm. In contrast, posterior discrepancies remained close to zero regardless of arch or severity. This suggests a region-specific tendency toward overestimation in the anterior maxilla during digital measurement, while posterior values remained stable. However, these differences did not reach statistical significance. In evaluating total tooth width, slightly greater variability and underestimations were observed, particularly in maxillary measurements among patients with moderate-to-severe crowding. The greatest difference (PC vs. IOS: 1.65 mm) approached statistical significance (p=0.06), suggesting a trend toward measurement bias in complex posterior regions.

These findings are consistent with previous studies, which demonstrated that digital models offer clinically acceptable accuracy for linear measurements when compared to traditional impressions.[13,14,18] The results also support earlier studies which reported comparable linear measurements between digital and plaster models, even in cases of severe crowding.[19,20] Similarly, Lo Giudice *et al*.,[21] found that arch length values obtained from digital scans generally matched those from plaster models, but they noted slight overestimations in digital readings, especially in crowded cases. Although the maximum mean difference in total tooth width observed in this study reached 1.65 mm, this deviation is considered clinically acceptable. Prior studies have indicated that minor discrepancies in mesiodistal measurements, particularly when summed across the whole arch, typically do not compromise orthodontic diagnosis or appliance fabrication. For example, Rossini *et al*., and Grünheid *et al*., proposed that deviations of less than 0.25 mm per tooth are within acceptable limits, which would amount to up to 3 mm across a 12-tooth arch (from first molar to first

molar).[13,14] Another study reported similar levels of overestimation in digital models, ranging from 1 to 2 mm in total arch width, but the authors concluded that these discrepancies did not significantly impact clinical outcomes.[21] Together, the observed deviation in the present study falls below the generally accepted clinical threshold of 2 mm and is consistent with the literature, thus supporting the reliability of digital models, even in crowded dentitions.

Jacob *et al*.,[11] found that intraoral scanners tend to slightly underestimate arch lengths in crowded cases, likely due to distortion or scanning artifacts in interproximal areas. This discrepancy may reflect differences in scanner type, operator experience, or patient anatomy. Similar concerns were raised by Lin *et al*.,[22] who noted deviations in IOS-derived arch length exceeding 1 mm in severe anterior crowding.

Digital models tend to underestimate tooth widths in crowded cases, primarily because of technical and anatomical challenges inherent in intraoral scanning. In moderate-to-severe crowding, teeth often overlap, rotate, or are malpositioned, which limits the scanner's ability to capture the actual mesiodistal contact points of each tooth accurately.[19,23,24] Another contributing factor is the potential distortion that can occur during cast model fabrication. Despite being considered the gold standard, plaster models created from alginate impressions are subject to dimensional changes, even when poured immediately. As such, discrepancies between digital and cast models may partially originate from inaccuracies in the plaster models themselves.[25,26] Another key issue is the difficulty in identifying exact mesiodistal contact points on digital models. This becomes more problematic in crowded dentitions, where overlapping or rotated teeth hinder accurate surface reconstruction. Digital scans, when converted into STL files, represent the surfaces as hollow structures, which can limit the fidelity of proximal surfaces. Although digital software allows for flexible point selection without physical obstruction, the underlying data interpolation and stitching algorithms may still produce underestimated values in anatomically complex areas.[27] In addition, restricted access to interproximal and lingual areas in crowded arches further impairs scan accuracy, as the scanner may not fully record the narrow spaces between teeth or may misalign surface data during the stitching process.[21,23,28] Furthermore, the curvature of the dental arch in the posterior region increases the likelihood of surface stitching errors. These stitching errors are exacerbated in crowded areas, resulting in measurement inaccuracies that are more pronounced in the posterior teeth compared with the anterior region. Patient movement, saliva, and limited mouth opening can also contribute to incomplete or distorted scans in the posterior segments. Studies have consistently reported that while digital models provide clinically acceptable accuracy overall, the reliability of measurements in the posterior teeth, particularly in crowded dentitions, can be slightly reduced because of these technical limitations.[22,28-30] Despite the noted variation in maxillary posterior segments, mandibular total tooth widths showed consistent results across methods in both crowding groups. These likely reflect the more accessible and stable morphology of the mandibular arch, which leads to less variability during scanning.[16]

The slightly greater differences in the moderate-to-severe crowding group may reflect the challenges of capturing precise anatomical contours in patients with significant crowding. Despite this, the differences were small and likely not clinically significant. The comparison of PC and IOS in the maxillary teeth of the moderate-to-severe crowding group had the lowest p-value (p=0.06), suggesting a possible trend worth investigating in larger samples.

The analysis of Bolton ratios also showed negligible differences between methods, with no statistically significant values observed. These results align with numerous studies that confirmed digital platforms can reliably calculate Bolton ratios, a critical factor in assessing inter-arch tooth size.[24,31-33] However, conflicting results from Othman and Harradine raised concerns that inaccuracies in anterior regions could lead to small, yet clinically meaningful, discrepancies in digital Bolton ratios when scans are poorly aligned or affected by light reflection errors.[34] A more recent study reiterated that IOS measurements of anterior ratios could be slightly skewed if scan resolution is inadequate or if scanning is rushed in difficult-to-access interproximal spaces.[35]

Despite minor deviations in specific measurements, the differences observed in this study remained within clinically acceptable ranges. These findings indicate that such minor discrepancies are unlikely to alter clinical decisions regarding space analysis, extraction planning, or appliance design.[14,36,37] These findings also align with

those of Anh *et al*.,[38] who emphasized the influence of scan sequence and environment on IOS accuracy and recommended standardizing scanning protocols for optimal results. Beyond their demonstrated measurement reliability, digital models, including both cast scans and intraoral scans, demonstrate measurement accuracy comparable to traditional plaster casts and can be confidently used for space analysis, treatment planning, and appliance design in routine orthodontic practice. Minor deviations observed in crowded dentitions are within clinically acceptable limits and do not compromise diagnostic or therapeutic decisions. However, clinicians must remain vigilant about potential underestimations or overestimations, particularly in moderate-to-severe crowding, where anatomical and technical complexities can affect scan fidelity. Continued research and refinement of digital tools will further improve the diagnostic confidence in digital model-based orthodontic assessments.

Conclusions

This study confirms that digital models, particularly CS and IOS, offer reliable and reproducible results for most orthodontic diagnostic tasks. Nevertheless, the digital models tend to underestimate total tooth widths and Bolton ratios, while overestimating clinical arch lengths.

Implications

Digital models can be confidently used in orthodontic diagnosis and treatment planning.

However, the reliability of these models, particularly in crowded dentitions, depends on operator training and adherence to standardized scanning protocols. A limitation of the present work is that only one brand of intraoral scanner was evaluated, which may not fully represent the performance of other devices; incorporating additional scanners would strengthen the generalizability of the findings. Moreover, further studies using larger and more diverse samples may help resolve conflicting outcomes and standardize digital scanning practices across clinical settings.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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Effect of Various Roughness Parameters on Surface Wettability of 3Y-TZP Ceramic

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Abstract

Objectives: The surface wettability of zirconia has been reported to correlate with the roughness. However, most studies commonly used the arithmetical mean deviation of the roughness (Sa) to predict surface wettability. It is of interest whether other roughness parameters affect the wettability of zirconia. Thus, this study aimed to evaluate the effect of various roughness parameters on the surface wettability of 3Y-TZP.

Methods: Zirconia slabs were prepared and divided into 4 groups (n=10) based on the surface treatment as follows: no treatment (control), air abrasion (AB), etching with 48% hydrofluoric acid (HF), and etching with the mixture of 37% hydrochloric acid and 68% nitric acid (CN). The specimens were scanned using atomic force microscopy (AFM) to evaluate vertical roughness parameters (Sa, Ssk: the skewness, Sku: the kurtosis) and non-vertical roughness parameters (Rsm: the mean width of the profile elements, Sdq: the mean slope of the surface, Sdr: the rate of increase in the surface area). Wettability was determined by surface contact angle (θc) using the sessile drop technique. The data were analyzed statistically with one-way ANOVA and Tukey's test (p<0.05).

Results: The group showing statistically higher Sdq and Sdr values than that of the control group (p=0.025 and 0.019, respectively) possessed significantly lower θc (p=0.000), despite similar Sa, Ssk, Sku, and Rsm patterns (p>0.05).

Conclusions: The Sdq and Sdr parameters could affect the surface wettability of 3Y-TZP. The higher slope of the profile and the higher surface area synergistically established better wettability of 3Y-TZP.

Keywords: surface area, surface roughness, surface slope, surface wettability, zirconia

Introduction

In adhesive dentistry, achieving a strong bond between the adhesive and the tooth surface is important for successful restorations. One of the critical factors influencing bonding efficacy is surface wettability, which can be evaluated by measuring the contact angle.[1] A low contact angle indicates good wettability, signifying intimate contact and enhanced mechanical interlocking between the adhesive and adherent.[2,3] Therefore, surface wettability is a valuable parameter to predict the strength of the adhesive bond.[4,5]

One of the factors contributing to surface wettability is surface roughness. According to Wenzel's theory, wettability is increased according to increased surface roughness for hydrophilic materials, whose contact angles are less than 90°.[6] Thus, surface roughening has been indicated as a part of zirconia surface pretreatments, not only for creating the micro-mechanical interlocking but also for facilitating the wettability of the adhesive.[7] Numerical methods have been introduced to roughen zirconia surfaces, such as airborne particle abrasion and chemical etching. Air abrasion is a process involving the high-velocity impact of Al_2O_3 particles onto the ceramic surface.[8] This treatment induces surface melting of the zirconia-based materials, resulting in a roughened surface with a correspondingly higher degree of wettability.[9] Using acids like hydrofluoric acid and/or various strong acids was able to etch the zirconia surface and increase its roughness.[10-12] High-concentration HF etching can corrode both intergranular and intragranular regions of the zirconia surface[13], resulting in a roughened surface and increased wettability.[5]

Although several studies have supported the relationship between increasing roughness and improving the surface wettability of zirconia, some studies indicated that the increase in surface roughness did not improve the wettability of the zirconia surface directly.[14,15] As a matter of fact, the roughening process alters the surface roughness in three dimensions[16], other details of the roughness feature should be taken into consideration. Thus, measuring only one roughness parameter seems to be insufficient to entirely refer to the surface wettability. However, there is still limited research on the effect of the other roughness parameters of zirconia on its wettability.

Therefore, this study aimed to evaluate the effect of roughness parameters, including vertical and non-vertical planes, on the surface wettability of 3Y-TZP. Various roughness patterns on 3Y-TZP were created by airborne particle abrasion and acid etching.

Materials and Methods

Specimen preparation

Forty zirconia slabs with the dimensions of 12x12x2.5 mm^3 were prepared from partially sintered zirconia milling blanks (BruxZir® Shaded; Glidewell Laboratories, CA, USA) by using a low-speed diamond saw (IsoMet™ low-speed cutter; Buehler, IL, USA). The top and bottom surfaces of each slab were manually polished for 20 vertical strokes under running water using abrasive silicon carbide paper (2000 grit; DWetordry™, 3M). All the surfaces were rigorously rinsed with water and dried using delicate task wipers (KIMTECH™). Then, all the specimens were fully sintered in a zirconia sintering furnace according to the manufacturer's instructions at a holding temperature of 1580°C for 2.5 hours with a heating and cooling rate of 10°C/min. Due to the 20-25% shrinkage upon sintering, the final dimension of each specimen was approximately 10x10x2 mm^3.

Regarding sample size calculation, G*Power was used with the following parameters: effect size=1.87; α=0.05; power=0.80; number of groups=4. The effect size was estimated using contact angle data collected in a pilot study.

The initial contact angle measurement was carried out to allocate the specimens into 4 groups (n=10) with similar mean contact angle values. The method of contact angle measurement is described below.

Surface treatment

To create various surface roughness on zirconia, specimens in each group were treated as follows: Group 1 (control): no surface treatment, Group 2 (AB): air abrasion using 50 μm alumina particles at 2 bars pressure, applied 10 mm perpendicularly from the specimen's top surface for 10 s, followed by 10 min ultrasonic cleaning in deionized water, Group 3 (HF): etching with 48% HF, and Group 4 (CN): etching with 37% hydrochloric acid (HCl) mixed with 68% nitric acid (HNO_3) at a 4:1 ratio by volume. Materials used in this study are shown in Table 1. In group 3 and group 4, 50 μL of designated acid was dropped on the specimen's top surface and wetted the zirconia surface for 10 min, followed by rinsing

with deionized water for 3 min at ambient temperature and humidity. Since the concentrated HF is an extremely hazardous acid, the etching procedure was performed within a chemical fume hood, while the operator was protected by a long-sleeve laboratory coat, goggles, a surgical mask, and double-layer gloves. The acid waste was collected as a hazardous waste in a compatible plastic container, neutralized with a base, and disposed of according to the Enhancement of Safety Practice of Research Laboratory in Thailand (ESPReL). All the specimens were then dried and stored in a closed plastic container for 4 months before the following investigations.

Contact angle (Θc) measurement

Contact angle measurement was done by using the sessile drop technique. A 3 µL droplet of deionized water, whose conductivity was 12.9 MΩ/cm^2, was placed on top of each specimen surface and left for 1 min before the measurement using a contact angle meter (KINO/SL200KS, CAST3 system, Boston, USA). All measurements were taken at ambient temperature and humidity, which were approximately 23.6°C and 53%, respectively. The measurement was performed three times on each specimen by three locations of water dropping, which were at the center, 1 mm above the center, and 1 mm below the center of the specimen surface. Then, the average value was used as the representative contact angle of that specimen. The contact angle data were statistically analyzed using SPSS Statistics 20.0 software (IBM; Chicago, IL, USA) with one-way ANOVA and Tukey's test (p<0.05). The normality assumption required for the application of the method was verified using the Shapiro-Wilk normality test (p>0.05), while the homogeneity of variance was verified using Levene's test (p>0.05).

Surface characterization using atomic force microscopy (AFM)

The area of 5 x 5 µm^2 at the center of each specimen was scanned using AFM (PARK XE7, Park Systems, Gyeonggi, South Korea) in non-contact mode with a scan rate of 0.5 Hz to obtain three-dimensional surface images. Six surface roughness parameters, which were Sa, Ssk, Sku, Rsm, Sdq, and Sdr were obtained using analysis software (XEI 4.3, Park Systems, Gyeonggi, South Korea). The Sa, Ssk, Sku, Rsm, Sdq, and Sdr data were preliminarily tested for normality and homogeneity using the Shapiro-Wilk test and Levene's test, respectively. Accordingly, the data were statistically analyzed using one-way ANOVA, where Tukey's post-hoc tests were further used to detect statistically significant differences at α=0.05.

The definition and interpretation of each parameter are described as follows[17,18];

1) Sa refers to the arithmetical mean deviation of the roughness evaluated over the calculated 3D surface representing the mean of the average height difference for the average plane.

2) Ssk refers to the Skewness of the 3D surface texture, representing the degree of symmetry of the surface heights about the mean plane. A positive Ssk (>0) indicates the predominance of peaks, while a negative Ssk (<0) indicates valley structures.

3) Sku refers to the Kurtosis of the 3D surface texture, presenting inordinately high peaks/deep valleys if Sku>3, whereas Sku<3 indicates flat peaks and valleys. Sku=3 signifies that surface heights are normally distributed.

4) Rsm refers to the mean width, representing the mean width profile elements within the sampling length. It is used to evaluate the horizontal size of parallel grooves and grains rather than height parameters.

5) Sdq refers to the root mean square gradient, representing the steepness of the surface by indicating the mean magnitude of the local gradients (slope) of the surface. The surface is more steeply inclined as the value of the parameter Sdq becomes larger.

6) Sdr refers to the developed interfacial area ratio. This signifies the rate of increase in the surface area.

Table 1: Materials used in this study.

Agents	Manufacturer	Batch number	Composition
HF (Emsure® Hydrofluoric acid)	Merck, Darmstadt, Hesse, Germany	B1004344747	48% w/w HF
HCl (Emsure® Hydrochloric acid)	Merck, Darmstadt, Hesse, Germany	K45311117505	37% w/w HCl
HNO_3 (Gammaco™ Nitric acid)	Gammaco, Bang Kruai, Nonthaburi, Thailand	3095045	68% w/w HNO_3

Results

The mean and standard deviation of Sa, Ssk, Sku, Rsm, Sdq, and Sdr parameters, including contact angle, are shown in Table 2. All the surface treatments showed no statistically significant difference in Sa, Ssk, Sku, and Rsm compared with the control group (p>0.05). The Sdq and Sdr of the HF group showed significantly higher values than those of the control group (p=0.025 and 0.019, respectively).

For contact angle, all surface treatments caused a statistically significant decrease in contact angle, compared to the control group (p=0.000). The HF group revealed the lowest contact angle, which was statistically lower than that of the AB and CN groups (p=0.001 and 0.025, respectively).

3D AFM images of representative specimens from each group were shown in Figure 1. The control group showed a smooth surface with well-defined grain boundaries. The HF group exhibited unclear grain boundaries with nano-scale surface irregularities on the zirconia surface. In contrast, the AB group did not show the characteristic of zirconia grains, but rather the non-uniform surface with crevices. Meanwhile, the surface morphology of the CN group regarding grain boundaries rarely changed from the control group.

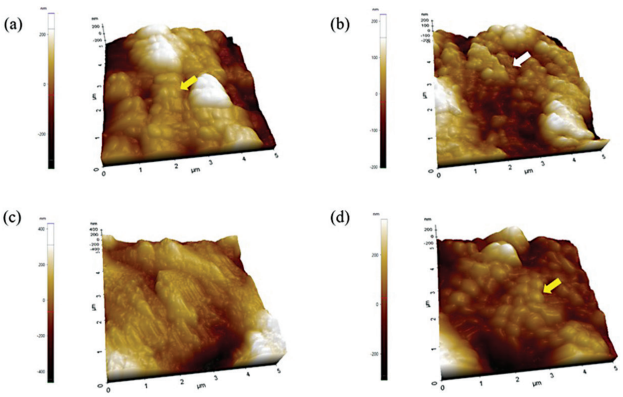

Figure 1: Representative 3D AFM images of the (a) control; (b) HF; (c) AB; and (d) CN groups showed zirconia surfaces with different morphology. The grain boundaries (yellow arrows) were clearly observed in (a) and (d), whereas they were absent in (c), whose surface was non-uniform. The nano-scale surface irregularities (white arrow) were mainly observed in (b).

Discussion

From the results of this study, it was shown that the HF group possessed the statistically highest Sdq and Sdr values, concomitant with the lowest contact angle among the studied groups. Meanwhile, AB and CN groups exhibited a higher trend of Sdq and Sdr values than that of the control group, and their contact angles were significantly lower compared to the control group.

Surface wettability is the property of a material contributed by the factors of surface topography and surface chemistry.[19] To solely investigate the influence of surface topography on the wetting behavior, the variation in surface chemistry should be eliminated. Therefore, all the specimens in this study were intentionally stored in closed containers for 4 months. This method was proposed to mask the specimens' surfaces with airborne hydrocarbon, after which the underneath surface chemistry could be shielded.[20] Given that the chemical properties of the zirconia surfaces in all groups were consistent, the measured contact angle is supposed to be solely reflected from the surface topography. However, several studies demonstrated the decreased wettability of the specimens that underwent long-term storage in the air.[21,22] It was explained that the airborne hydrocarbon absorbed on the specimen's surface decreased surface energy, thus water wettability decreased.[22,23] Therefore, the measured contact angles in this study were unavoidably higher than those expected under realistic conditions.

Table 2: Mean and standard deviations of surface roughness parameters (Sa, Ssk, Sku, Rsm, Sdq, Sdr) and surface contact angle (θ_c) of each group (n=10).

Surface treatment	Sa (μm)	Ssk	Sku	Rsm (μm)	Sdq (rad)	Sdr (%)	θ_c (°)
Control	0.091 (0.01)^a	0.120 (0.35)^a	3.315 (0.86)^a	2.016 (0.31)^a	0.526 (0.09)^a	11.425 (2.99)^a	104.368 (2.26)^a
HF	0.097 (0.02)^a	0.168 (0.37)^a	3.791 (0.73)^a	1.933 (0.30)^a	0.649 (0.09)^b	15.714 (3.20)^b	89.210 (3.68)^b
AB	0.100 (0.02)^a	-0.062 (0.29)^a	3.359 (1.03)^a	2.125 (0.72)^a	0.567 (0.10)^{ab}	12.702 (3.49)^{ab}	95.838 (2.97)^c
CN	0.093 (0.01)^a	-0.048 (0.35)^a	3.129 (0.52)^a	1.816 (0.27)^a	0.559 (0.09)^{ab}	12.733 (2.67)^{ab}	93.830 (4.50)^c

Different superscript letters indicate statistically significant differences between surface treatments (p<0.05).

To achieve zirconia surfaces with various micro- and nano-scale roughness, airborne-particle abrasion, etching with HF, and etching with a mixture of HCl and HNO_3 were employed. Air abrasion is a procedure used to treat ceramic surfaces by impacting the target surface with Al_2O_3 particles at high velocity.[8] After airborne-particle abrasion, the zirconia surface was roughened, and the typical 3Y-TZP morphology was no longer present as the well-defined grain boundaries were not observed.[24] This treatment produced a coarse surface with grooves and sharp edges.[25] On the other hand, HF is an inorganic acid of hydrogen fluoride. In the presence of water, it dissociates into its constituent ions according to the reaction $HF+H_2O=F-+H_3O+$.[26] The reaction of zirconia with HF proceeds according to equation $ZrO_2+6HF=ZrF_6H_2+2H_2O$, leading to the corrosion of zirconia grains, of which the atoms around the grain boundaries are more chemically reactive.[26] Thus, the dissolution occurs more rapidly at the grain boundaries, causing the dislodgement of the grains and the formation of irregular grooves at the boundary regions.[12] As a result, the HF-etched zirconia manifested as a rough surface with both micro- and nano-scale roughness.[5,27,28] In contrast, the HCl/HNO_3 mixture exhibited minimal etching of the zirconia substrate despite its robust oxidative capabilities and ability to dissolve common metallic oxides and hydroxides.[29]

All the treatments employed in this study could not significantly increase average surface roughness (Sa) nor change the vertical topography (Ssk, Sku) of 3Y-TZP. For air abrasion, previous numerical studies showed a significant increase in average surface roughness in both 2D (Ra)[30,31] and 3D (Sa)[9,25] measurements under similar conditions of air abrasion. However, the measurement in this study was unable to detect a significant change in surface roughness arithmetically, which is in agreement with the previous study stating that the hardness of sintered zirconia was sufficiently high to resist mechanical abrasion.[32] Nevertheless, the polished pattern was no longer seen on the surfaces of the specimens in the AB group using SEM (data not shown), indicating that the zirconia surfaces were actually abraded. Such discrepancies in the results might derive from the roughness measuring methods and protocols[33], scan rate, condition of the scanning tip, and external factors/noise[34], which influence the image resolution and roughness data. Similarly, the HF group did not exhibit a significant change in these vertical roughness parameters from the control group, which was inconsistent with the previous study.[5] Apart from the above-mentioned factors, a previous study evaluated 2D parameters, which are considered line roughness parameters and are not sensitive to individual peaks or valleys. A possible anisotropy of the surface can have a strong influence on the measurement values, rendering 2D and 3D parameters incomparable.[35]

Regarding the non-vertical roughness parameters, the average distance between peaks (Rsm) and the average slope of the surface (Sdq) were evaluated. Rsm values were shown to insignificantly differ among groups, whereas Sdq values were shown to be statistically higher in the HF group than the control group. The higher Sdq value of the HF group, indicating the steeper slopes, might derive from the dislodgement of zirconia grains along valley walls, where the acid was accumulated during the 10-min etching time. It is postulated that the steeper slopes of HF-treated zirconia form deeper and more acute-angle valleys. These morphologies might facilitate more water wettability and contribute to the significant decrease in the contact angle of the HF group. Firstly, the deeper valleys can contain more water, causing the contact angle to be lower. Secondly, the more acute angle creates a narrower groove at the bottom, which facilitates more water accumulation due to capillary action.[36] Also, this phenomenon is evident in the dip coating process, in which the frame is dipped into the liquid. It can be seen that the film of liquid is formed a little thicker at the corners of the frames, where the more acute the angle of the frame, the more liquid accumulation.[37] However, a too steep slope is not advantageous, since the water wettability could be hindered by the air pocket formation in the cavities. Saying that the Sdq of a completely level surface is 0, and a plane with gradient components of 45 degrees has an Sdq value of 1, the average inclination of slopes on zirconia surfaces in the present study could be estimated to be lower than 45 degrees (Sdq ranges from 0.526 to 0.649). These relatively small slopes can promote the complete penetration of water into surface cavities without underneath an air pocket formation.[38] Thus, it can be suggested that, within the optimal range of steepness, the steeper slope of the surface is preferable to promote water wettability according to the capillary effect.

Additionally, the HF group possesses a significantly higher surface area, which was determined by the Sdr

parameter, than the control group. The increased surface area is speculated to be acquired from the nano-scale surface irregularities on zirconia grains, as shown by AFM images. It was explained that such nano-scale roughness could suck water rapidly due to capillary action, resulting in a decreased water contact angle.[39] Moreover, the larger surface area offers a larger wetting area, as well as higher surface energies derived from the increased surface atoms.[4] Presumably, a higher surface area is also an essential prerequisite to enhance surface wettability.

AB and CN groups have similar roughness in all studied parameters; thus, their contact angles are undoubtedly statistically equivalent. However, their contact angles are significantly lower than the control, despite the insignificant differences in Sdq and Sdr values from the control group. It is noticeable that both the Sdq and Sdr values of AB and CN groups are in the upper range of those found in the control group. Therefore, it could be suspected that Sdq and Sdr contribute synergistically to reducing the contact angle.

The limitation of this study was that the surface treatments by air abrasion and acid etching altered the roughness of 3Y-TZP randomly. Although only the roughness parameters showing significant differences were used for interpreting the effect on the contact angle, the remaining parameters might play a part in surface wettability to some extent. To confirm the results of this study, roughening the zirconia surface with a more controllable method, such as a laser, would lead to the more desirable roughened surfaces, which can ensure the influence of each roughness parameter individually. Furthermore, the type of wetting fluid, regarding chemical properties and charged components, is one of the important factors influencing the resulting wettability behavior.[40] The sessile drop technique using resin adhesive in place of water revealed a significantly lower adhesive contact angle on the assintered zirconia surface.[41,42] To obtain a more clinically relevant situation, further study utilizing a 10-MDP-containing primer as the wetting fluid should be pursued.

Conclusions

Within the limitations of this study, it could be concluded that the vertical roughness parameters (Sa, Ssk, Sku) alone are insufficient predictors of surface wettability. Instead, the non-vertical roughness parameters, such as the slope of the profiles (Sdq), including the surface area (Sdr), potentially influence the wettability. The surface with higher slopes and surface area manifested as nano-scale surface irregularities is favorable in promoting water wettability on the zirconia surface. These findings can be used as a goal for zirconia surface pretreatment, of which the wettability of primer and resin cement could be maximized.

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Conflict of Interest

The authors declare no conflict of interest.

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TUGSE or OSCC? The Twisted Diagnostic Challenge: A Case Series

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Abstract

Background & Aim: Chronic, non-healing oral ulcers often straddle the fine line between benign reactive lesions and malignancies. Such lesions merit vigilant evaluation. This case series delineates two confounding clinical scenarios, initially suspected as oral squamous cell carcinoma (OSCC), which were later histologically diagnosed as Traumatic Ulcerative Granuloma with Stromal Eosinophilia (TUGSE), enhancing the pivotal role of biopsy in averting misdiagnosis.

Case Overview: Two geriatric patients presented with persistent, painful ulcerative lesions on the lateral tongue for over 7–9 months. Clinically mimicking malignancy, both lesions exhibited induration and proliferative features. Histopathological examination post-excisional biopsy confirmed TUGSE. Conservative surgical management resulted in complete healing within two months, with no recurrence.

Conclusions: Clinicians must maintain a high index of suspicion and consider TUGSE in the differential diagnosis of persistent oral ulcers. Timely histological confirmation can prevent aggressive over treatment and offer immense psychological relief to the patient.

Clinical Significance: Despite its alarming appearance, TUGSE is a benign, self-resolving entity. Its close resemblance to OSCC mandates careful clinical and histological assessment to ensure appropriate therapeutic decisions.

Keywords: osinophilic granuloma, non-healing oral ulcer, OSCC, traumatic ulcer

Introduction

Oral ulcerations persisting beyond three weeks often incite concern for malignancy. Traumatic Ulcerative Granuloma with Stromal Eosinophilia (TUGSE) is a rare, yet clinically deceptive, benign lesion of the oral mucosa that often masquerades as malignancy particularly oral squamous cell carcinoma (OSCC). First described in infants as Riga-Fede disease and later characterized in adults by Elzay in 1983, TUGSE has intrigued clinicians due to its dramatic presentation and benign course. It most commonly affects the lateral border of the tongue in older adults, with a slight male predilection, and presents as a chronic, non-healing ulcer with indurated, raised margins and a firm base features that often raise alarm for OSCC.[1]

Though frequently linked to mucosal trauma from sharp teeth, restorations, or dental appliances, many patients report no identifiable source of irritation, suggesting additional immunologic or idiopathic components in its pathogenesis. Histologically, TUGSE is hallmarked by a polymorphous inflammatory infiltrate rich in eosinophils, lymphocytes, macrophages, and large mononuclear cells extending into underlying muscle a pattern that can mimic lymphoproliferative disorders. CD30 positivity among these mononuclear cells has led to diagnostic confusion, although most immunohistochemical and molecular findings support a reactive, polyclonal nature rather than a neoplastic one.

Despite its alarming appearance, TUGSE often regresses spontaneously, particularly after biopsy and removal of causative factor, highlighting the importance of conservative management and clinician awareness. Recent systematic reviews have even noted rare occurrences on palatal mucosa and in edentulous regions, reinforcing the need for vigilance across all oral sites. Enhanced understanding of this rare entity is essential to prevent overtreatment and undue psychological stress to patients. This case series illustrates two elderly patients with clinically ominous long term ulcerations ultimately diagnosed as TUGSE. The importance of histological scrutiny in such scenarios cannot be overstated.

Case Presentations

Case 1

A 65-year-old female reported to the Department with a complaint of a gradually enlarging lesion on the right lateral border of her tongue, persisting for approximately nine months. The lesion was mildly painful, and the patient denied any para functional habits such as tongue biting, or a history of trauma and other habits like Tobacco use. She had occasionally manipulated the lesion out of concern, Patient is diabetic for past 10 years under regular medication and her medical history was non-contributory.

Clinical examination

Intraoral examination revealed a solitary, well-circumscribed, nodular lesion measuring approximately 1.0×1.5 cm on the right postero-lateral aspect of the tongue. The lesion appeared reddish-pink with a yellowish fibrinous ulcerated base and was tender on palpation. The margins were slightly everted and indurated, with a sloping edge, and a mucosal collar was noted at the periphery of the ulcer. Notably, root stumps of mandibular right molar and attrition of the opposing mandibular right first premolar was observed, suggesting chronic traumatic irritation as a potential etiological factor. For radiological assessment, orthopantomogram (Figure 1) was taken to exclude hidden sources of irritation such as impacted root tips or osseous abnormalities.

Provisional diagnosis and management

Given the clinical features persistent ulceration with induration and proliferative appearance, chronic traumatic ulcer was suspected, with OSCC considered as a differential diagnosis. After obtaining informed consent, the offending root stumps were extracted. Following informed consent and physician clearance, an excisional biopsy was performed using electrocautery, and the suspected traumatic tooth was extracted under local anaesthesia. The tissue was submitted for histopathological analysis (Figure 1).

Histopathological findings

Microscopic examination of specimen revealed parakeratinised stratified squamous epithelium exhibiting hyperplasia. The underlying connective tissue shows dense highly cellular stroma with thickened fibrous purulent membrane. Areas of chronic inflammatory cells chiefly of lymphocytes and presence of eosinophils exhibiting deep down the underlying muscles. Areas of muscular degeneration are evident. The prominent eosinophilic infiltrate observed served as a histological hallmark of TUGSE and in differentiating it from other chronic ulcerative lesions and neoplastic conditions. (H&Ex4 &x10) (Figure 2)

Figure 1: Pre-op & Post-op: Ulcerative growth with indurated margins and slough at the base and Pre-op OPG.

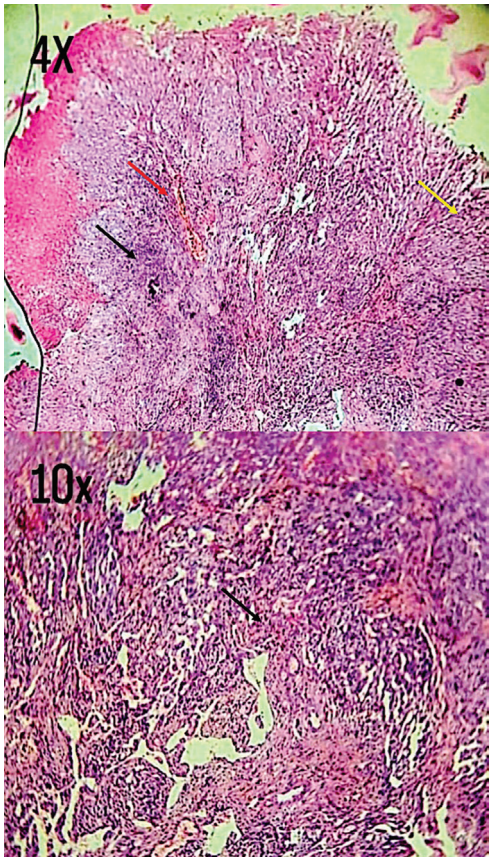

Figure 2: H&E Staining (4X & 10X). Epithelium exhibiting parakeratinized stratified squamous epithelium showing hyperplasia; In CT- Black arrow shows abundant inflammatory cells includes lymphocytes, neutrophils, eosinophils and plasma cells; Red arrow-dilated blood vessels; Yellow arrow - area of muscle degeneration.

Follow-up

The traumatic etiologic factors including sharp tooth structures and root stumps were clearly identified and addressed during the initial treatment phase. Their removal enhanced a critical role in promoting healing and preventing recurrence. The patient was monitored over a six-month follow-up period. Remarkable improvement was noted after the biopsy, with progressive reduction in inflammation and complete epithelialization by the eighth week. At the final review, the lesion had fully resolved, and the patient reported no pain, irritation, or functional limitation. No recurrence was observed (Figure 1).

Case 2

An 80-year-old male presented with a chief complaint of persistent pain on the right lateral surface of the tongue for the past seven months. The patient's medical history was unremarkable, and he reported no known systemic illnesses, or traumatic incidents. The patient is a non-smoker and has no history of tobacco use. On further inquiry, he reported no systemic illnesses, and his medical records confirmed the absence of comorbidities such as diabetes, hypertension, or immunodeficiency. The patient also denied any recent dental procedures or mechanical injury to the area.

Intraoral findings

Clinical examination revealed a solitary, non-healing, ulcerative lesion on the right dorsal-lateral tongue, measuring approximately 1.0×1.0 cm. The lesion demonstrated an ulcero-proliferative growth pattern with rolled, indurated borders, a firm base, and a floor coated with pale-yellow necrotic slough. It was located adjacent to sharp root stumps of maxillary right molar, and mandibular right molars (46 and 47) possibly altering the patient's bite and compounding tongue irritation. Along with prominent attrition on premolars 43, 44, 45, suggesting chronic trauma as a likely precipitating factor. A radiographic evaluation (orthopantomogram) was performed to rule out any residual root fragments, bone spicules, or underlying pathology that might delay healing (Figure 3).

Clinical impression and intervention

Given the ulcer's chronicity, induration, and proliferative features, a provisional diagnosis of a Chronic non healing ulcer was established, while oral squamous cell carcinoma (OSCC) remained a significant differential diagnosis. Following appropriate counseling, the sharp

lesions without recurrence. The patient was reviewed at regular intervals over a six-month period. Healing was progressive, with notable reduction in inflammation and pain by the fourth week (Figure 3).

Discussion

TUGSE, though rare, is a diagnostic chameleon that often mimics malignancy, especially OSCC, as it manifests as a rare, single, well-demarcated ulcer with indurated, often elevated margins[1], which can raise considerable clinical concern. Traumatic ulcers typically heal within two to three weeks once the source of irritation such as sharp teeth, ill-fitting dentures, or trauma is removed, although they can persist longer in immunocompromised individuals. TUGSE is a rare, benign, self-limiting lesion usually affecting the tongue or cheek, with two peak incidence periods: infancy and the fifth to seventh decades of life. It is thought to arise from repeated trauma triggering a deep inflammatory response rich in eosinophils, (Figure 5) and although histology is needed to exclude malignancy, TUGSE generally resolves spontaneously often after biopsy. In contrast, OSCC is much more common, representing over 90% of oral cancers, and is strongly associated with risk factors such as tobacco, alcohol, HPV infection, and chronic irritation. OSCC typically appears in adults over age 40, especially males, and requires biopsy for definitive diagnosis, followed by aggressive treatment (surgery, often with neck dissection, and possibly radiation and chemotherapy). Prognosis depends heavily on early detection, as many cases are already advanced and involve lymph node metastasis at diagnosis.

Experimental studies, such as those by Bhaskar and Lilly[2], support that persistent trauma to the mucosa in animal models can result in lesions histologically resembling TUGSE. Other authors, including Segura and Pindborg, suggest that an aberrant immune-mediated repair process might contribute to lesion persistence and its aggressive inflammatory profile.

Histologically, TUGSE is characterized by a deep, polymorphous inflammatory infiltrate composed of lymphocytes, eosinophils, histiocytes, and large mononuclear cells, often extending into the underlying muscle fibers. The presence of eosinophils in large numbers is a hallmark finding. The large mononuclear cells sometimes described as atypical or bizarre have raised concerns about a neoplastic process. However, immunohistochemical

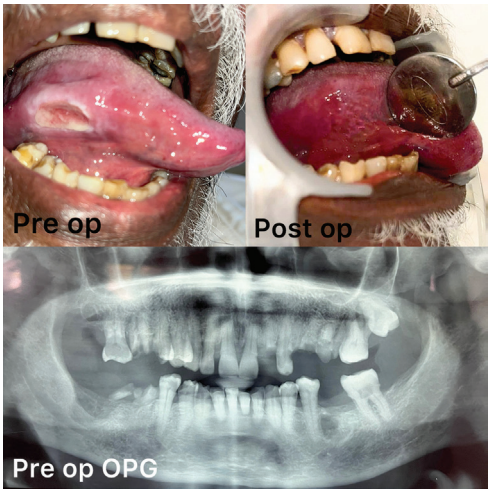

Figure 3: Pre-op & Post-op: Ulcero-proliferative lesion on the lateral tongue surface and Pre-op OPG.

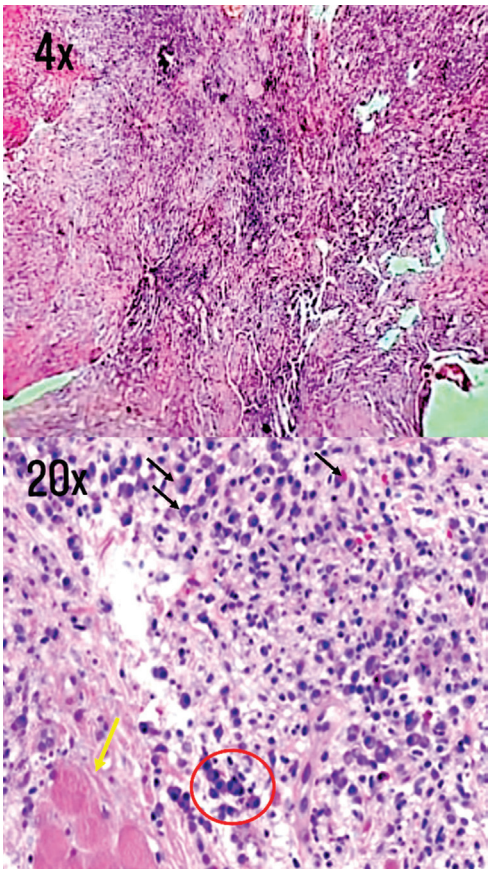

Figure 4: H&E Staining (4X & 20X). Black arrows - Numerous eosinophils into the submucosa and the underlying muscle bundle; Red circle - atypical cells; Yellow arrow - Infiltrate extending into submucosa and into the underlying muscles.

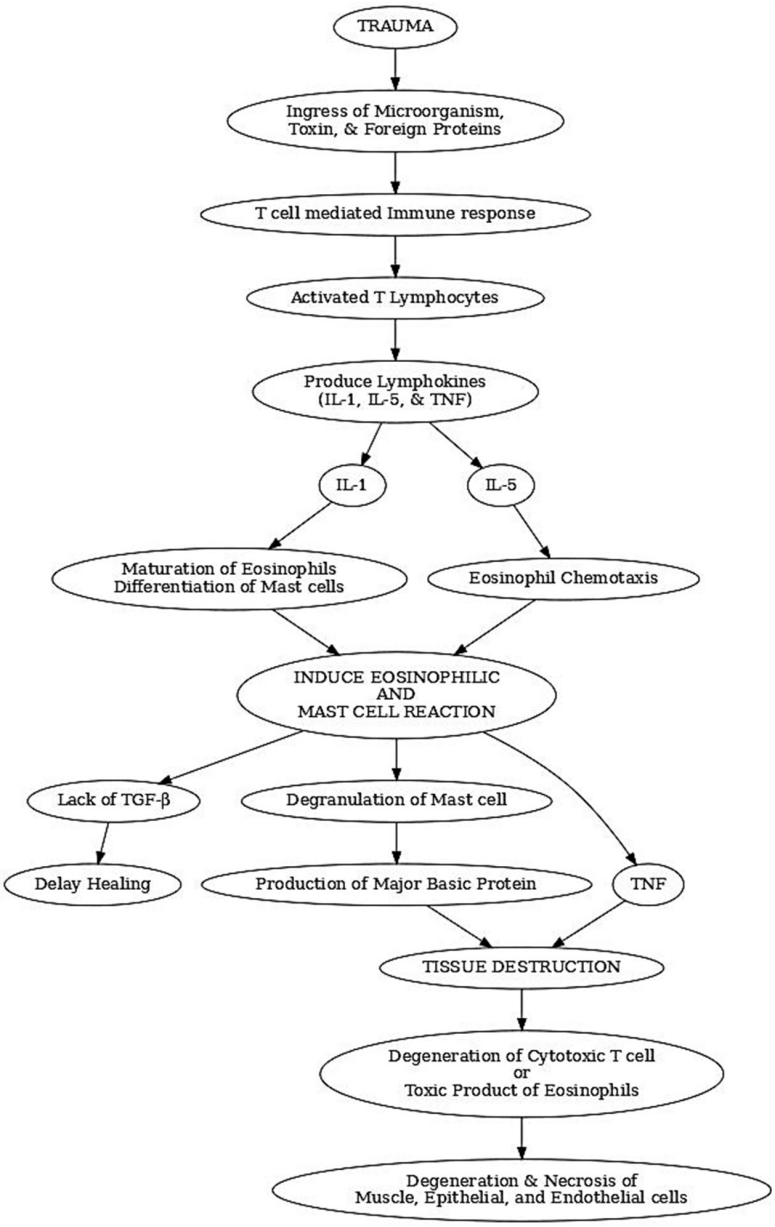

Figure 5: Pathophysiology of traumatic ulcerative granuloma with stromal eosinophilia.

root stumps were extracted, and electrocautery of the lesion was performed under local anesthesia. The specimen was sent for histopathological analysis to confirm the diagnosis and rule out malignancy (Figure 2).

Histological findings

Microscopic evaluation revealed an ulcerated surface lined by irregular, non-keratinized stratified squamous epithelium, which was non-dysplastic in nature. Beneath the epithelium, there was a diffuse and deeply penetrating inflammatory infiltrate composed predominantly of eosinophils, along with a mixture of B and T lymphocytes, macrophages, plasma cells, and occasional large atypical-appearing mononuclear cells. This infiltrate extended beyond the submucosa and infiltrated into the underlying skeletal muscle fibers, reflecting the lesion's aggressive yet reactive inflammatory pattern. On high-power magnification (H&E, 20X), the eosinophils displayed their characteristic nuclei and prominent eosinophilic granules, confirming their identity and highlighting their abundance within the deep inflammatory infiltration (Figure 4).

Follow-up

The source of trauma was successfully identified and eliminated during initial management, which likely contributed to the rapid and complete resolution of the

Table 1: Differential diagnosis of traumatic ulcerative granuloma with stromal eosinophilia.

Diagnosis	Site	Clinical Feature	Histology	IHC
Traumatic ulcerative granuloma with eosinophilia	Tongue, buccal, vestibular, palatal mucosa, retromolar area, gingiva, floor of mouth	Indurated ulcer, elevated margin, yellow base	Granuloma, dense, diffuse, polymorphic infiltrate of eosinophils, histiocytes, submucosal, muscle, salivary glands	Phenotypically regular T cells, occasionally CD30+
Squamous cell carcinoma	Lateral/tip of tongue, lower lip, retromolar area, base of oral cavity	Endophytic, infiltrative, destructive nodule, shallow ulcer, elevated margin	Epithelial differentiation, horn pearls, dyskeratosis, absent bridges, cornification, peritumoral inflammation	CK5/6+, CK19+, p63+, p40+
Atypical histiocytic granuloma	lingual mucosa and mandibular gingiva is predominant & lips.	ulceroproliferative lesions	histologically shows a histiocytic proliferation and is characterised by specific mitotic activity	CD68 marker
Lymphomatoid papulosis.	The lingual location is the most frequent, with mainly on the tongue's dorsal surface. Labial involvement from the lip commissure to the mucosal side of the lips	"ulcerated red papule" or an "inflammatory nodular lesion with an ulcerated center	infiltrate of CD 30 positive atypical lymphocytes together with a mixed inflammatory infiltrate of eosinophils, neutrophils, histiocytes and plasma cells	T-cell specific markers CD3 and UCLH1 (CD45RO)
Lymphoma (CD30+)	Reoccurring, exophytic lesions in the oral cavity	Indolent nodule or ulcer, primary cutaneous disease	Oral, soft tissue infiltrate of atypical lymphoid cells, eosinophils	MUM1+, MYC+, CD30+, loss of T cell markers
Leus (Syphilis)	Lips, tongue, pharynx	Papule at entry, indolent superficial ulcer, indurated margin, multifocal, aphthous enanthema	Intense plasma cell infiltrate, ill-defined granuloma, spirochetes	Treponema pallidum by TPPA or FTA-ABS
Muco-cutaneous ulcer (EBV+)	Oropharyngeal mucosa	Well circumscribed indolent ulcer	Inflammatory cells, atypical, large B-cell blasts, RS-like cells	EBER+, CD30+
Aphthous stomatitis	Non-keratinized labial and buccal mucosa, soft palate, inferior tongue	Shallow, round, oval, painful ulcer; fibrin pseudo-membrane, erythematous margin.	Nonspecific ulcer, extensive T cell infiltrate, elevated local TNF-α	Nonspecific

profiling generally supports a reactive origin. These cells are frequently CD30+, CD3+, CD68+, and TIA-1+, suggestive of an activated T-cell or histiocytic lineage.[3] According to Davoine *et al*.,[4] the reduced expression of cytokines such as TGF-α and TGF-β1 in eosinophils may contribute to impaired wound healing and chronicity of the lesion. Further support for a benign diagnosis comes from molecular analysis. Studies by Magro *et al*.,[3] have shown predominantly polyclonal or oligoclonal T-cell receptor gene rearrangements in most TUGSE cases. While occasional cases with clonal patterns have been documented, these tend to follow a benign, non-progressive clinical course. The differential diagnosis of TUGSE encompasses a wide range of conditions, including oral squamous cell carcinoma, CD30+ lymphoproliferative disorders (e.g., lymphomatoid papulosis), deep fungal infections, Langerhans cell histiocytosis, and granulomatous diseases such as tuberculosis and syphilis. These entities have been summarized in (Table 1).[5]

The clinical and histopathological findings in our case series align closely with those described in the literature. Similar to the majority of reported TUGSE cases, both patients in our series presented with solitary, indurated ulcers on the lateral tongue the most commonly affected site. The histological pattern, including a dense eosinophil-rich inflammatory infiltrate extending into muscle fibers are hallmark features that have been consistently documented in prior studies. The prolonged duration of ulceration (7-9 months) in both patients exceeds the typical course reported in most studies, where spontaneous

healing often occurs within weeks or shortly after biopsy. Furthermore, both patients were geriatric, a demographic shown to be vulnerable to delayed healing and heightened cancer anxiety, making evident the clinical value of timely biopsy and conservative management in this age group.

In our both cases, electrocautery was selected as the technique of choice for biopsy, not only to obtain a definitive diagnosis but also to achieve precise tissue removal with optimal hemostasis, Given the high vascularity of the tongue, managing intraoperative bleeding becomes a critical concern, particularly in elderly patients. Topical corticosteroids were initially not started, as their empirical use in a lesion with malignancy like features could have masked disease progression or delayed critical histopathological diagnosis. This conservative approach minimized patient morbidity while still achieving complete healing in both cases. Interestingly, as also reported by Wolk R *et al*.,[6] many TUGSE lesions begin to regress spontaneously following the biopsy procedure. Additional therapies topical steroids, antimicrobial rinses, or low-level laser therapy may be employed but are not typically necessary.

By consolidating clinical, histological aspects, this report contributes to the growing body of literature urging conservative management in suspected OSCC/ chronic non healing ulcer cases where TUGSE may be a differential diagnosis. Timely diagnosis not only prevents unnecessary radical surgery but also provides immense psychological relief to the patient especially in geriatric populations where anxiety about cancer is high.

Contemporary literature continues to provide valuable insights. Wolk *et al*.,[6] highlighted the consistent presence of CD30-positive T cells in TUGSE and differentiated it from lymphoproliferative disorders. Other authors like Ficarra *et al*.,[7] echo similar findings and stress the need for clinician awareness to prevent misdiagnosis and overtreatment.

Conclusions

TUGSE serve as a valuable reminder in clinical practice that not all indurated or chronic oral ulcers are malignant, even when they closely mimic the features of oral squamous cell carcinoma. As a clinician, maintaining a broad differential diagnosis and prioritizing timely histopathological evaluation are essential to avoid unnecessary aggressive interventions. The outcomes in our cases reinforce the importance of identifying and removing local traumatic factors, as well as adopting a conservative, evidence-based approach when managing suspicious lesions. Incorporating these principles into daily practice enhances diagnostic accuracy, reduces patient anxiety, and supports more personalized, minimally invasive care particularly in elderly patients with high cancer related fear.

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Intramucosal Melanocytic Nevus of Buccal Mucosa: Two Case Reports

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Abstract

Oral melanocytic nevus is a rare benign lesion in the oral cavity, arising from melanocytes and nevoid cells of neural crest origin. It appears as a blue-to-black, well-defined, round or oval lesion, 1-6 mm, usually asymptomatic. Histopathology shows nevoid cell clusters with melanin, classified into four types: junctional, compound, intramucosal (most common), and blue nevus. Differential diagnosis includes physiologic pigmentation, amalgam tattoo, oral melanotic macule, smoker's melanosis, and melanoma. Treatment involves observation or excisional biopsy for histopathology. Prognosis is good, with no malignant tendency. This case report enhances understanding of the lesion and emphasizes accurate diagnosis.

Keywords: intramucosal, melanin, nevoid cells, oral melanocytic nevus, oral pigmented lesion

Introduction

Oral melanocytic nevus is a benign mucosal lesion that occurs within the oral cavity. It may be congenital or acquired and results from the proliferation of melanocytes and nevoid cells, both of which originate from neural crest cells.[1]

While melanocytic nevi are commonly found on the skin, their presence in the oral cavity is relatively uncommon. Oral melanocytic nevi occur more frequently in females than in males and are more prevalent in Caucasian populations compared to Asians or individuals with darker skin. These lesions can be found across all age groups, with the average age of diagnosis ranging between 30 and 40 years. The most common intraoral site is the hard palate (approximately 42%)[2], followed by the buccal mucosa (17%) and the retromolar area (11%). Less commonly, they may also be observed on the gingiva, vermillion border of the lips, soft palate, and tongue.[3,4]

Clinically, oral melanocytic nevi present as asymptomatic, well-circumscribed lesions. They may appear round or oval, with a smooth surface that can be flat or slightly elevated. The coloration varies from blue or bluish gray to black. These lesions are typically small, with an average diameter ranging from 1 to 6 mm.[1,5]

Histopathologically, oral melanocytic nevi are characterized by a benign, unencapsulated proliferation of nevus cells. These cells are typically arranged in small round clusters called thèques, and they often display an ovoid or epithelioid morphology with abundant cytoplasm and intracellular melanin pigment. In deeper portions of the lesion, nevus cells usually exhibit reduced cytoplasm, lack pigmentation, and resemble lymphocytes, whereas the deepest layers commonly contain spindle-shaped nevus cells.[6] According to the World Health Organization (WHO) Classification of Head and Neck Tumours, 5th Edition (2024), oral melanocytic nevi are categorized based on the developmental stages of nevus cell proliferation into junctional, compound, and intramucosal types.[3,7,8] In addition, blue nevi, although primarily cutaneous lesions, may also occur within the oral mucosa and are further subclassified into common (dendritic) and cellular types.[9]

The junctional nevus is characterized by nests of pigmented nevus cells confined to the basal layer of the oral epithelium. The compound nevus exhibits nests of nevus cells distributed in both the epithelial layer and the superficial lamina propria. The intramucosal nevus contains nevus cell nests localized exclusively within the lamina propria, beneath the epithelium. The blue nevus consists of spindle-shaped melanocytes situated in the lamina propria. Among the latter, the common blue nevus is the most frequently encountered subtype and demonstrates an intramucosal proliferation of elongated spindle-shaped melanocytes arranged in short fascicles within the connective tissue. In contrast, the cellular blue nevus is less common and exhibits nodular proliferation composed of dendritic spindle cells intermingled with compact aggregates of larger oval-to-round melanocytes with pale cytoplasm and sparse or absent melanin pigment. Although rare, cellular blue nevi have been reported to carry a minimal yet noteworthy potential for malignant transformation.[10]

Among these subtypes, the intramucosal nevus is reported to be the most common, followed by the blue nevus.[7,11]

The differential diagnosis of oral melanocytic nevus includes distinguishing it from normal physiologic pigmentation, particularly in individuals with darker skin, vascular lesions such as hemangiomas and vascular malformations, as well as other pigmented oral lesions such as amalgam tattoos, oral melanotic macules, smoker's melanosis, and oral malignant melanoma.[12-14] Clinical examination techniques such as diascopy can be helpful in differentiating pigmented lesions from vascular lesions, as demonstrated in the cases presented herein.

In terms of management, if the lesion remains stable in size, color, and shape, and is asymptomatic, observation may be appropriate. However, if changes in the lesion are observed, if symptoms develop, or if the lesion is located in an esthetically sensitive area such as the tongue or lip, an excisional biopsy with a small margin of normal tissue is recommended to prevent recurrence. In cases where melanoma is suspected, an incisional biopsy may be indicated for histopathologic evaluation.[15]

The prognosis for oral melanocytic nevi is generally excellent. These lesions are benign with no known malignant potential, and recurrence is rare following complete surgical excision.

Case Report

Case 1

A 65-year-old Thai female patient was referred to

a dental clinic for evaluation of a red lesion on the hard palate. During the intraoral examination, an incidental finding of a black lesion was noted on the right buccal mucosa. The patient was asymptomatic and reported no prior awareness of the lesion. The palatal erythematous area was clinically suspected to be denture-related stomatitis and was unrelated to the pigmented lesion on the buccal mucosa, which was the focus of this case report.

The patient had a medical history of diabetes and osteoporosis. Current medications included insulin injections, sitagliptin, metformin, dapagliflozin, and denosumab (administered via injection every 6 months), as well as lansoprazole, caltrate, and vitamin B complex. The patient denied any known drug allergies. Extraoral examination revealed no abnormalities. Intraoral examination showed a well-defined, smooth-surfaced, darkly pigmented lesion on the right buccal mucosa, oval in shape, measuring approximately 9×5 mm² (Figure 1A).

Further evaluation of the lesion was performed using the diascopy technique, in which a glass slide was used to apply pressure to the lesion in order to differentiate it from a vascular lesion. The black lesion did not blanch under pressure suggesting a non-vascular origin (Figure 1B).

The lesion on the right buccal mucosa was excised with a narrow clinical margin of approximately 1-2 mm, which was sufficient for complete removal while preserving surrounding healthy tissue. An elliptical incision with the lesion positioned centrally was used, allowing for optimal wound closure and minimizing tension on the adjacent mucosa.[16,17] This approach is consistent with standard surgical management of benign oral melanocytic nevi (Figure 2A).

Histopathological examination of the excised specimen revealed oral mucosa covered by acanthotic parakeratinized stratified squamous epithelium. Within the lamina propria, there was a proliferation of nevoid cells containing melanin pigment granules. Nevus cells were not observed within the epithelial layer or the superficial portion of the lamina propria. No evidence of cellular atypia or mitotic activity was identified. Based on these histopathological findings, a final diagnosis of intramucosal melanocytic nevus was made (Figure 2B, 2C, 2D). In this case, the excisional biopsy served both diagnostic and therapeutic purposes. At the 7-day follow-up visit, sutures were removed, and the patient was re-evaluated one month postoperatively. The biopsy site demonstrated normal healing, with no evidence of recurrence at the right buccal mucosa (Figure 3).

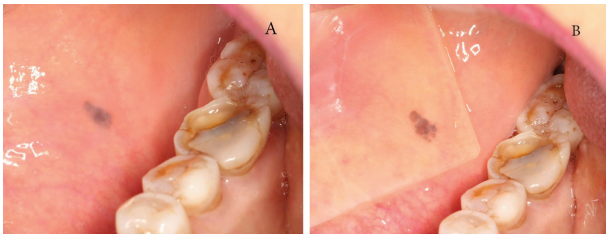

Figure 1: Intraoral examination. (A) black pigmented lesion on the right buccal mucosa; (B) diascopy test showing no blanching upon pressure with a glass slide.

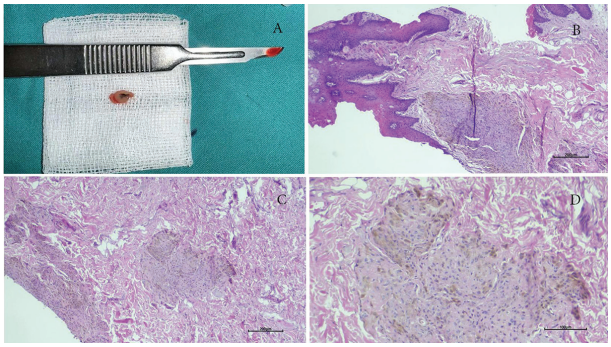

Figure 2: Excisional biopsy and histopathological findings. (A) excisional biopsy specimen showing the pigmented lesion with adjacent normal tissue; (B) the mucosa covered by acanthotic parakeratinized stratified squamous epithelium with nests of nevus cells. (hematoxylin and eosin stain, 40xmagnification); (C) nevus cell nests located within the lamina propria. (hematoxylin and eosin stain, 40x magnification); (D) nevus cell nests containing melanin pigment granules. (hematoxylin and eosin stain, 100x magnification).

Figure 3: One-month postoperative follow-up showing normal wound healing without evidence of recurrence.

Case 2

A 13-year-old Thai male patient was referred for evaluation of an incidental pigmented lesion on the right buccal mucosa, initially detected by a dentist. The patient was asymptomatic and had not previously noticed the lesion. He denied any underlying medical conditions, drug allergies, or regular medication use.

Extraoral examination revealed no abnormalities. Intraoral examination showed a brown to dark pigmented lesion on the right buccal mucosa, oval in shape, measuring approximately 3×2 mm^2. The lesion had well-defined borders, a smooth surface, and was slightly elevated (Figure 4).

Further evaluation was performed using the diascopy technique, in which a glass slide was applied to the lesion to distinguish it from a vascular lesion. The brown-black lesion did not blanch under pressure, indicating a non-vascular origin.

Excisional biopsy of the lesion was performed with a narrow clinical margin of approximately 1-2 mm, allowing complete removal while minimizing disruption to the surrounding healthy mucosa. Histopathological examination demonstrated oral mucosa lined by parakeratinized stratified squamous epithelium. The underlying connective tissue contained a proliferation of nevoid cells with melanin pigment granules, arranged in nests and occasionally in a haphazard pattern. These features were consistent with an intramucosal melanocytic nevus (Figure 5). In this case, the excisional biopsy provided both diagnostic confirmation and therapeutic benefit, ensuring lesion removal while preserving normal tissue integrity.[16,17]

Figure 4: Intra-oral examination: brown-black pigmented lesion on the right buccal mucosa.

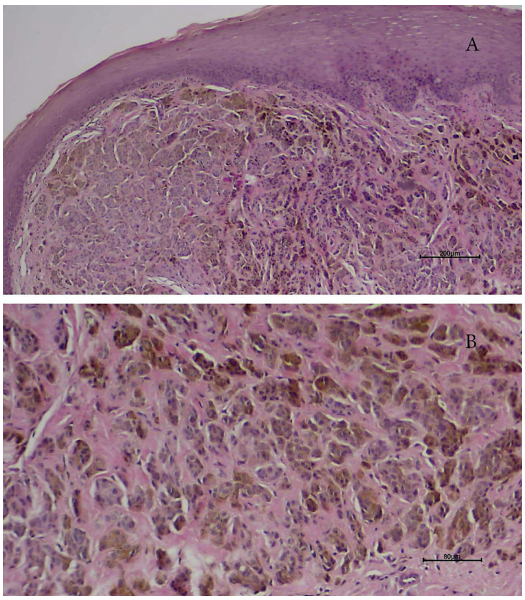

Figure 5: Histopathological characteristics of intramucosal melanocytic nevus of the oral mucosa. (A) oral mucosa covered by parakeratinized stratified squamous epithelium with nests of nevoid cells (hematoxylin and eosin stain, 40x magnification); (B) nevoid cell nests located within the connective tissue containing melanin pigment granules (hematoxylin and eosin stain, 100x magnification).

Discussion

This case report presents patients diagnosed with intramucosal melanocytic nevus located on the buccal mucosa, a relatively uncommon site for oral melanocytic nevi.

Based on the lesion's location, clinical characteristics, and histopathological findings, a definitive diagnosis of intramucosal melanocytic nevus was established. This is the most frequently encountered subtype of oral melanocytic nevus.

A study by Natarajan (2019) reported that intramucosal melanocytic nevus can occur in various locations within the oral cavity, with the most common site being the hard palate, followed by the buccal mucosa and retromolar area.[7,18]

In this case series, the lesions were located on the buccal mucosa, which aligns with previous reports of oral intramucosal melanocytic nevi.[19]

However, documented cases of intramucosal melanocytic nevus specifically involving the buccal mucosa remain limited. Therefore, this report contributes to the broader understanding of such lesions in this anatomical location.

Clinically, the lesions were asymptomatic, well-

defined, small in size, and darkly pigmented which are features consistent with the typical clinical presentation of oral melanocytic nevi. A study by Nagarajan *et al*., (2016) found that intramucosal melanocytic nevus of the oral mucosa are more commonly observed in females than in males and are typically found in middle-aged individuals.[20] This finding corresponds with the patient profile in case study 1 but differs from that in case study 2. However, the incidence of such lesions in Asian populations has not been extensively studied.

Differential diagnosis is important, as these pigmented lesions resemble other melanotic conditions in the oral cavity. In this case series, the diascopy test was utilized to distinguish the lesion from vascular lesions. Additionally, histopathological examination plays a crucial role in differentiating oral melanocytic nevi, which are benign, from oral melanoma[21], which requires precise diagnosis to avoid serious complications.

The malignant transformation rate of oral melanocytic nevi is relatively low. Studies have reported a transformation rate into oral melanoma of approximately 5.2-6.3%, with the blue nevus subtype being more commonly associated with such malignant changes.[22,23]

Limitations of this report include its nature as a case study, which prevents drawing definitive conclusions about epidemiological trends or risk factors. In addition, genetic or immunohistochemical studies were not conducted, which could have provided further confirmation of the lesion's characteristics.

From a clinical management standpoint, asymptomatic patients may be placed under observation with regular follow-ups to monitor for any changes in the lesion. Biopsy is warranted if there are suspicious changes or malignancy is suspected.[24] In this case, before performing the procedure, the patients were informed about possible management options, including close observation without biopsy or complete excision for definitive histopathological diagnosis. Both patients choose excisional biopsy, which provided both diagnostic and therapeutic benefits. This approach is supported by Tarakaji *et al*.,[25] who emphasized that although the risk of malignant transformation in oral melanocytic nevi is low, histopathological confirmation is essential to ensure diagnostic accuracy and patient safety.[25]

Dentists should educate patients about the nature of the lesion and the recommended steps if changes occur.

Conclusions

Intramucosal melanocytic nevus can occur on the buccal mucosa, although it is relatively uncommon. Diagnosis relies on thorough history-taking, clinical examination, and histopathological evaluation through biopsy. Primary treatment options include regular monitoring or complete excisional biopsy, which serves both diagnostic and therapeutic purposes. Patients should be followed up to monitor for possible recurrence. Although rare, increased understanding and documentation of oral pigmented lesions from case studies like this one can provide valuable clinical knowledge for future reference.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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ORAL SCIENCES REPORTS

Case Report

Regenerative Endodontic Therapy in a Necrotic Immature Tooth with Chronic Apical Abscess with Apical Placement of Calcium Hydroxide: A Clinical Case Report

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Abstract

Managing necrotic immature teeth is complex due to thin root walls and open apices. Regenerative endodontics has emerged to overcome these limitations. These biologically based approaches aim to restore pulp vitality and promote further root development by using dental stem cells and revascularization. A female patient aged 9 years and 4 months diagnosed with dens evaginatus, necrotic pulp, and a chronic apical abscess in an immature mandibular premolar underwent treatment with regenerative endodontic procedures (REPs). Calcium hydroxide ($Ca(OH)_2$) medication was applied to the apical region of the root canal. Over a 22-month follow-up period, the tooth remained asymptomatic, and radiographic assessments revealed periapical healing, apical closure, root lengthening, and dentinal wall thickening. The favorable outcomes observed suggest that apical placement of $Ca(OH)_2$ during REPs may be a promising approach for managing immature teeth with pulp necrosis and chronic apical abscess, supporting both disinfection and root maturation.

Keywords: calcium hydroxide, case report, immature tooth, regenerative endodontics

Introduction

Pulp necrosis in immature permanent teeth is most commonly associated with dental trauma and caries.[1] Developmental anomalies, particularly dens evaginatus (DE), may also contribute. DE is characterized by an extra cusp, often containing pulp tissue, on the occlusal or lingual surfaces of premolars and incisors[2] and has a reported prevalence of 0.5%-4.3% in Asian populations.[3] Fracture or attrition of the DE cusp may result in pulp exposure and subsequent necrosis, especially in teeth with incomplete root formation.[2] Early diagnosis and preventive intervention are therefore essential to minimize pulpal and periapical complications.[4]

Endodontic treatment of immature permanent teeth exhibiting pulp necrosis poses significant clinical challenges due to their structurally compromised root canal systems. Complications often include thin dentinal walls that are susceptible to fracture, open apices that impede conventional obturation, and inadequate root length and thickness to withstand functional forces.[5] To overcome these challenges, regenerative endodontic procedures (REPs) have been introduced as biologically informed approaches that aim to restore pulp vitality and promote continued root development through revascularization and the recruitment of dental stem cells.[6]

Effective disinfection of the root canal system is a crucial factor for the success of REPs.[6] Intracanal medications, such as antibiotic pastes and calcium hydroxide ($Ca(OH)_2$) have been commonly used to achieve microbial control.[7] Evidence suggests that the type and placement of these drugs may influence regeneration outcomes. While $Ca(OH)_2$ has been reported to promote apical closure, antibiotic pastes have increasingly been associated with enhanced root wall thickening.[8] The coronal placement of $Ca(OH)_2$ has been proposed due to its reduced cytotoxicity to apical stem cells,[9] with one study further highlighting its association with increased dentinal wall thickness.[7] This approach has been supported by several reports demonstrating favorable clinical outcomes.[10-14] However, contrasting evidence has also suggested that $Ca(OH)_2$ placed in the apical portion of the canal effectively stimulates root wall thickening.[15,16] The objective of this case report was to provide additional evidence that apical placement of $Ca(OH)_2$ within the root canal can contribute to favorable root development, including increased dentinal wall thickness, in a necrotic immature tooth with chronic apical abscess.

Case Report

A female patient aged 9 years and 4 months was referred to the University Dental Hospital. The chief complaint was a sinus tract exhibiting purulent discharge on the buccal attached gingiva associated with the mandibular right second premolar. The patient's parent reported that the tooth initially presented with a prominent occlusal tubercle that fractured and remained untreated. Following the fracture, the patient experienced recurrent buccal abscesses, with intermittent drainage through the gingival tissues. Two months prior to referral, symptomatic treatment was administered for one week at a private dental clinic, comprising antibiotics and analgesics.

The patient had no relevant medical history and was classified as American Society Anesthesiologists (ASA) Class I. Extraoral examination confirmed a lack of facial swelling or asymmetry. Clinical intraoral examination revealed that the mandibular right second premolar presented with a small, deep occlusal cavity revealing of a fractured tubercle, with no evidence of dental caries. A sinus tract opening was observed on the buccal gingiva between the mandibular right second premolar and first molar. Periodontal probing depths were within normal limits, and the tooth showed grade I mobility, without tenderness to palpation or percussion. Pulp sensibility tests elicited no response to cold or electric pulp testing (EPT).

Digital periapical radiographs were obtained and scanned using a VistaScan imaging system (Dürr Dental, Bietigheim-Bissingen, Germany). The image showed a Cvek's stage 3 root[17] with thin walls (Figure 1A). Periapical radiolucency was measured using VixWin Platinum software (Gendex Dental Systems, Hatfield, PA, USA). Gutta-percha tracing confirmed the mandibular second premolar as the origin of infection (Figure 1B). The clinical diagnosis was DE, pulp necrosis, and a chronic apical abscess. Informed consent was obtained following a comprehensive discussion of available treatment alternatives.

The treatment protocol followed clinical considerations for REPs guided by the American Association of Endodontists (AAE).[18] A right inferior alveolar nerve block was administered using 2% lidocaine with 1:100,000 epinephrine (Medicaine, Huons Co. Ltd., Seongnam-

si, Korea). Following rubber dam isolation, an access cavity was prepared. The tooth length was determined using an electronic apex locator (Root ZX, J. Morita, Kyoto, Japan) and confirmed radiographically with a size 25 K-file (Kerr Dental, Orange, CA, USA) as 17 mm. The necrotic pulp tissue removal and root canal disinfection were performed using gentle irrigation with 20 mL 2.5% sodium hypochlorite (NaOCl), normal saline solution, and 17% ethylenediaminetetraacetic acid (EDTA) for 5 minutes each, without mechanical instrumentation. $Ca(OH)_2$ was mixed with 2% lidocaine containing 1:100,000 epinephrine (Medicaine, Huons Co. Ltd., Seongnam-si, Korea) to a creamy consistency. The resulting paste was applied to the apical region of the root canal, approximately 2 mm short of the tooth length, using a lentulo spiral. A double seal was applied using Cavit (Triune Med Tec, Cambridgeshire, UK) and IRM (Dentonics, Monroe, NC, USA).

After 2 months, the patient remained asymptomatic, with no signs of pain or swelling, and complete resolution of the previously observed sinus tract. Clinical examination showed normal mobility, with no sensitivity to palpation or percussion. Following administration of an inferior alveolar nerve block with 3% mepivacaine without vasoconstrictor (Septodont, New Castle, DE, USA), the canal was re-accessed and irrigated with 17% EDTA. Bleeding was induced using a size 25 K-file to a depth of 19 mm, and controlled at the level of the cementoenamel junction (CEJ). After approximately 15 minutes, a stable blood clot was formed. A resorbable collagen matrix (CollaPlug; Zimmer Dental, Carlsbad, CA, USA) was placed over the clot, followed by a 4-mm layer of Biodentine (Septodont, Saint-Maur-des-Fossés, France) to serve as a coronal barrier. The access cavity was then restored using a resin-modified glass ionomer (Fuji II LC; GC Corp., Tokyo, Japan) and composite resin (Estelite; Tokuyama Dental, Tokyo, Japan) (Figure 1C).

At 6-, 10-, and 22-month follow-up visits following the REPs, the patient remained asymptomatic. Despite consistently negative responses to pulp sensibility testing (cold and EPT), radiographic evaluation demonstrated periapical healing and continued root development, characterized by increased root length, dentinal wall thickening, and apical closure (Figures 1D-1F). Radiopacity within the canal space was observed at 6 months, and further increased by 10 and 22 months, indicating continued calcific tissue formation. A secondary carious lesion beneath the previous restoration was identified and managed. Oral hygiene instructions were reinforced, and other teeth presenting with DE were examined and managed appropriately.

To assess changes in root wall thickness and root length, a modified method based on a previously reported protocol was employed.[7] Pre- and post-operative radiographs were analyzed using ImageJ (version 1.41; National Institutes of Health, Bethesda, MD, USA). Horizontal reference lines were established at the CEJ and at positions 3, 6, and 9 mm apical to the CEJ to represent the coronal, middle, and apical levels of the root, respectively. Root and canal widths were measured at each level, and root wall thickness was calculated by subtracting the canal width from the corresponding root width. Root length was measured from the CEJ to the apex. Measurement outcomes are summarized in Table 1 and illustrated in Figure 2.

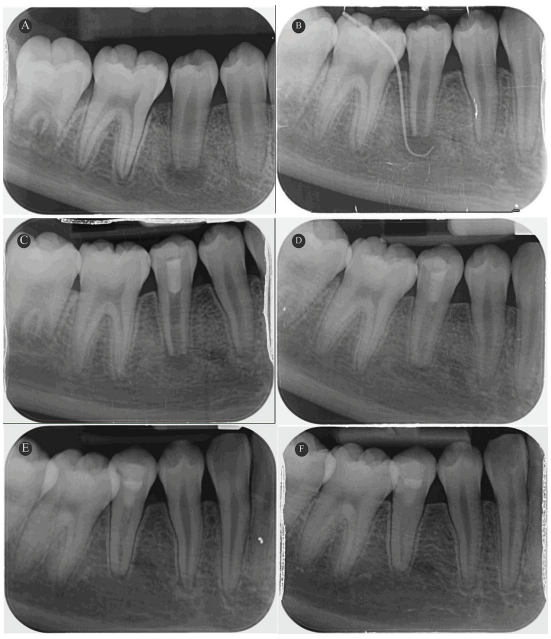

Figure 1: Periapical radiographs of the mandibular right second premolar during the regenerative endodontic treatment: (A) a preoperative periapical radiograph showed an immature root with a periapical lesion; (B) a sinus tracing radiograph; (C) a radiograph post-induced bleeding into the root canal and coronal restoration showed a reduction in the size of the periapical lesion; (D) a 6-month follow-up radiograph showed continued root development and a widening of the periodontal ligament space; (E) a 10-month follow-up radiograph showed continued root development with radiopacity in the root canal and a widening of the periodontal ligament space; and (F) a 22-month follow-up radiograph showed continued root development with a closed apex and normal periapical tissue.

Discussion

The treatment success of REPs is influenced by multiple factors, including stem cell viability, effective canal disinfection, patient age, and root morphology.[6,19,20] Favorable outcomes have been associated with patients aged 9-13 years and apical diameters >1.0 mm, both of which were true in the present case.[20] A wide apex may contribute to tissue preservation and stem cell migration, facilitating successful regeneration.[21] This case report was evaluated using the AAE's clinical and radiographic criteria[18], which define success primarily by resolution of clinical signs and symptoms and radiographic evidence of periapical healing, secondarily by increased root length and dentinal wall thickness, and finally by restoration of pulp vitality.

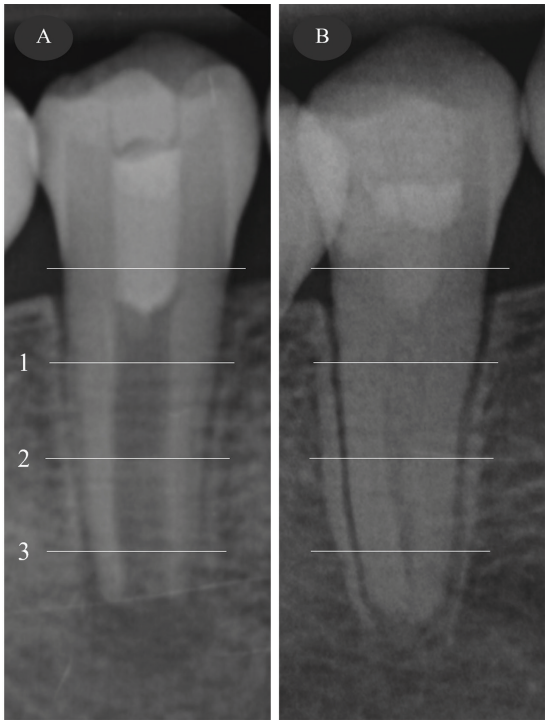

Figure 2: Images showed the three horizontal reference lines for the root and canal width measurements using ImageJ software: (A) a radiograph post-induced bleeding and (B) a 22-month follow-up radiograph. (1=coronal, 2=middle, and 3=apical)

Determining an accurate working length in immature teeth with open apices remains a considerable clinical challenge, particularly in REPs, where precise canal disinfection and intracanal medication placement must be achieved without irritating the apical stem cells. Current expert consensus recommends determining the working length using a radiograph with the endodontic file positioned 1 mm short of the radiographic apex on REPs, without specifying the file size.[6] Electronic apex locators have been used to assess the working length with increased predictability. However, their reliability was reduced when an apical foramen diameter exceeded 0.6 mm because of a reduced effective contact between the file and apical canal walls.[22] In this case report, the electronic apex locator was used as an adjunctive tool[23], and the definitive working length was verified radiographically.

Among multiple factors, persistent microbial infection is considered a primary contributor to the clinical signs and symptoms of apical periodontitis.[19] Thorough disinfection of the root canal system is therefore an essen-

Table 1: A summary of the timeline, procedural steps, and radiographic findings.

Timeline	First visit	Second visit	Third visit	Fourth visit	Fifth visit
Procedures	Disinfection	Bleeding induction	6-month follow-up	10-month follow-up	22-month follow-up
Radiographic findings					
- Root length (mm)	-	10.80	-	-	11.56
- Root wall thickness (mm) [percentage change]					
Coronal	-	2.73	-	-	3.07 [12.66]
Middle	-	1.98	-	-	2.70 [36.73]
Apical	-	1.59	-	-	2.77 [74.06]
- Apical size (mm)	-	1.60	-	-	Closed apex
- Periapical lesion characteristics	Radiolucent area	Radiolucent area	Widening PDL space	Widening PDL space	Normal
- Periapical lesion size (mm)	6.4x3.3	4.2x2.3	-	-	-

PDL = Periodontal ligament

tial step in treatment success. In this case, 2.5% NaOCl was used as an irrigant, due to its well-established antimicrobial efficacy and tissue-dissolving properties.[24] An additional final rinse with 17% EDTA was performed prior to blood clot induction to facilitate the release of growth factors from dentin, which are essential for stem cell recruitment and tissue regeneration.[25] $Ca(OH)_2$ was selected as the intracanal material for its antibacterial effect and ability to support stem cell survival and proliferation.[26-28] Previous studies have demonstrated that $Ca(OH)_2$ can induce the release of bioactive molecules from the dentin matrix, including transforming growth factor-β1 (TGF-β1), which may further contribute to regenerative success.[25,29] This paste also offers practical advantages over antibiotic-based medications, such as reduced risk of antibiotic resistance and tooth discoloration.[15,30] In this case, resolution of clinical symptoms was observed shortly after treatment, with radiographic evidence of periapical lesion reduction evident within 2 months. Complete healing of periapical tissues was confirmed by 22 months, consistent with previous reports.[12] Therefore, the primary success criterion was satisfied.

Dental anesthetic solutions, with or without a vasoconstrictor, have been used for mixing with $Ca(OH)_2$ due to their availability, sterility, and ease of handling.[31] Previous studies have demonstrated that anesthetic solutions combined with $Ca(OH)_2$ maintain pH values comparable to normal saline[32] and exhibit reduced surface tension, thereby enhancing their diffusion capacity.[33] Based on these properties, lidocaine with epinephrine was selected as the vehicle for mixing with $Ca(OH)_2$ in this case report.

As previously mentioned, $Ca(OH)_2$ medication has been consistently associated with apical closure, but not dentinal wall thickening.[8] This may be linked to the ability of $Ca(OH)_2$ to facilitate calcium deposition at the apical region and its limited effect at the dentinal wall.[8] Additionally, coronal placement of $Ca(OH)_2$ has been correlated with a greater increase in root wall thickness (53.8%) compared to placement beyond the coronal level (3.3%).[7] However, the apical placement of $Ca(OH)_2$ in the present case produced a favorable secondary outcome; radiographic evaluation showed apical closure and root wall thickening at all evaluated levels. Additionally, the root length increased from 10.80 mm to 11.56 mm, although it remained below the average adult root length of 14.5 mm.[34]

The present findings correspond with those of previous studies.[15,16] Intracanal application of $Ca(OH)_2$ in necrotic immature teeth led to periapical healing, continued root development, and a 45.4% increase in root wall thickness, compared to the 41.7% reported with antibiotic-based paste.[15] Additionally, serial intracanal applications of $Ca(OH)_2$ in teeth with pulp necrosis and acute apical abscesses facilitated continued root development, especially root wall thickness and apical closure.[16] These outcomes may reflect more effective disinfection and the stimulatory effects of $Ca(OH)_2$ on apical papilla stem cells[28,35], which are essential for continued root maturation.[35,36] Residual bacteria may hinder dentinal wall thickening, emphasizing the importance of effective microbial control during REPs.[37] In this instance, the apical application of $Ca(OH)_2$ may provide particular advantages in cases of complex or persistent infection by increasing disinfection in the apical region, thereby reducing the risk of reinfection and enhancing long-term outcomes.[19] However, further investigation is necessary to elucidate the influence of different intracanal treatments and their placement on regenerative outcomes.

At the 10- and 22-month follow-up evaluations, the treated tooth remained non-responsive to sensibility testing, indicating that the tertiary goal of REPs had not been achieved. This outcome may relate to the use of a periapical bleeding scaffold, which has been associated with delayed recovery of pulp sensibility in some cases. Mittal *et al*., reported that cold test responses may return as early as 3 months, whereas heat and EPT responses are rarely observed within 12 months.[38] Sensibility recovery has been demonstrated to be scaffold-dependent, with the highest rates observed in platelet-rich fibrin, followed by collagen, hydroxyapatite, and periapical bleeding; the latter typically showed responses around 12 months post-treatment. Additionally, radiographic evidence of radiopacity within the root canal space observed during follow-up may suggest the formation of calcific tissue, a finding reported in up to 78% of teeth treated with REPs.[39] Intracanal calcification may result from various contributing factors, including the type of scaffold and intracanal medications, especially $Ca(OH)_2$.[39,40] Nonetheless, no further intervention is typically indicated unless the tooth becomes symptomatic.[6]

A bacteria-tight coronal seal is essential for the long-term success of REPs.[6] Failure to maintain this seal may allow microbial ingress, leading to reinfection that can compromise stem cell proliferation and differentiation, ultimately impeding periapical healing and continued root development.[6] At 10- and 22-month follow-ups, a secondary carious lesion was found, probably due to an inadequate coronal seal. The restoration was changed, oral hygiene instructions were repeated, and the patient was scheduled for follow-up. These indicate the necessity of a well-adapted coronal seal and effective clinical follow-up for long-term REPs success.

Although the current case report presents favorable clinical and radiological results, it is important to acknowledge some limitations. The lack of a histological evaluation limited the assessment of the actual internal tissue organization. Additionally, as the case represents a unique instance, the generalizability of the results is limited.

Conclusions

The favorable outcomes observed suggest that apical placement of $Ca(OH)_2$ during REPs may be a promising approach for managing immature teeth with pulp necrosis and chronic apical abscess, supporting both disinfection and root maturation.

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Conflict of Interest

The authors declare that there are no conflict of interest regarding the publication of this case report.

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