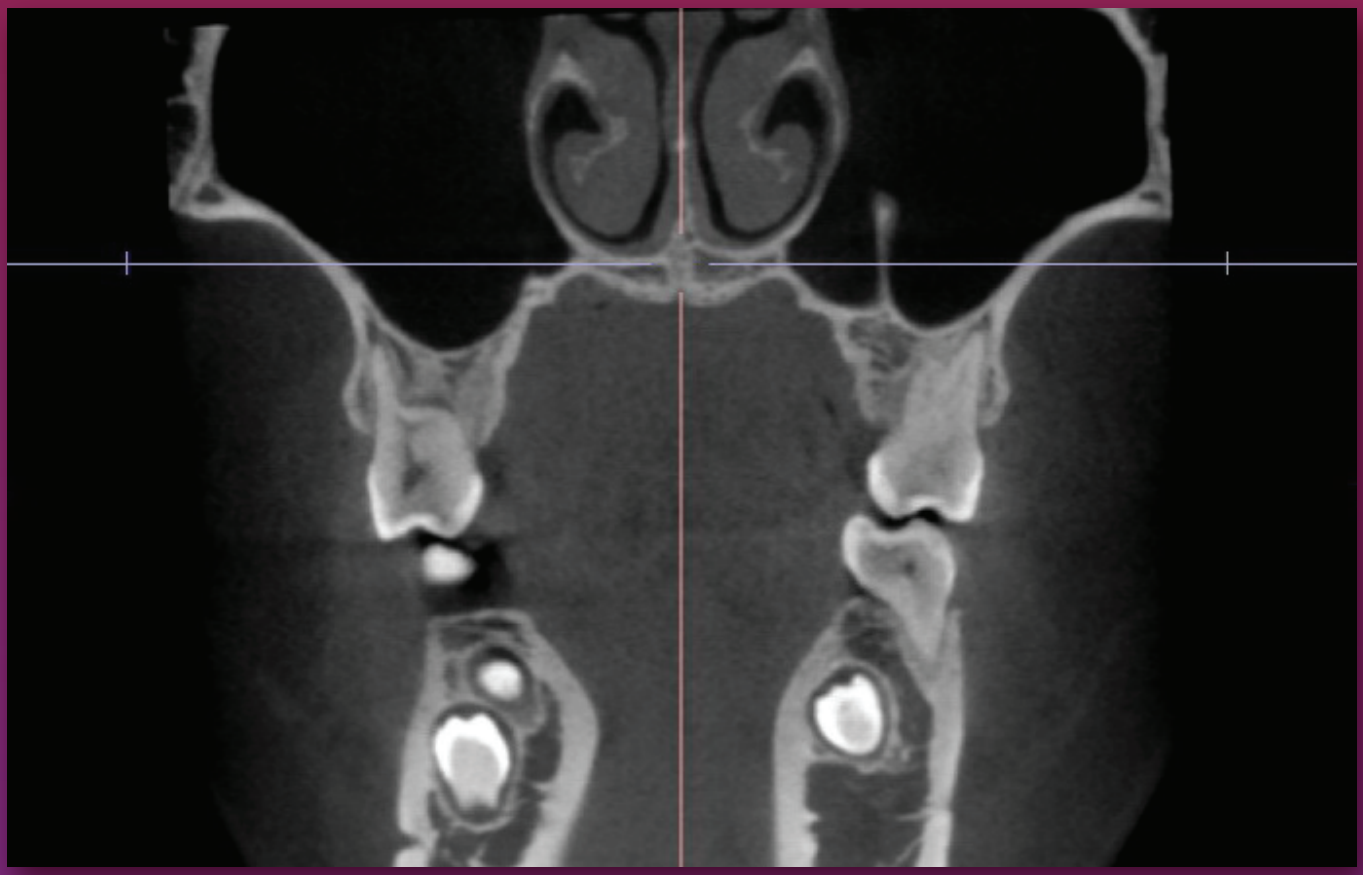
JDAT

Journal of The Dental Association of Thailand

www.jdat.org

Journal of The Dental Association of Thailand | Volume 73 Number 1 January - March 2023

ISSN 2730 - 4280



CE Quiz

Assessment of Midpalatal Suture Maturation by Cone-beam Computed Tomography in Circumpubertal Age Group

วิทยาสารทันตแพทยศาสตร์
ปีที่ 73 ฉบับที่ 1 มกราคม - มีนาคม 2566 | e-ISSN 2730-4280

ทันตแพทยสมาคมแห่งประเทศไทย
ในพระบรมราชูปถัมภ์
THE DENTAL ASSOCIATION OF THAILAND

Advisory Board

Asst. Prof. Anonknart	Bhakdinaronk
Assoc. Prof. Surasith	Kiatpongsan
Dr. Charmary	Reanamporn
Clinical Prof. Pusadee	Yotnuengnit
Lt. Gen. Nawarut	Soonthornwit
Dr. Wantana	Puthipad
Dr. Werawat	Satayanurug
Assoc. Prof. Wacharaporn	Tasachan

Board of Directors 2022 - 2025

President	Dr. Adirek	Sriwatanawongsa
President Elect	Assoc. Prof. Dr. Sirivimol	Srisawasdi
1st Vice-President	Assoc. Prof. Dr. Nirada	Dhanesuan
2nd Vice-President	Asst. Prof. Bundhit	Jirajariyavej
Treasurer	Assoc. Prof. Poranee	Berananda
Secretary General	Dr. Chavalit	Karnjanaopaswong
Deputy Secretary General	Maj. Thanasak	Thumbuntu
Foreign Secretary General	Asst. Prof. Ekachai	Chunhacheevachaloke
Editor	Dr. Ekamon	Mahapoka
Chairman of the Convention Facilities	Dr. Prinya	Pathomkulmai
Executive Committee	Assoc. Prof. Porjai	Ruangsri
	Assoc. Prof. Dr. Siriruk	Nakornchai
	Dr. Somchai	Suthirathikul
	Dr. Anuchar	Jitjaturunt
	Asst. Prof. Piriya	Cherdsatirakul
	Asst. Prof. Dr. Sutee	Suksudaj
	Dr. Terdsak	Utasri
	Dr. Thornkanok	Pruksamas
	Asst. Prof. Taksid	Charasseangpaisarn

THE DENTAL ASSOCIATION OF THAILAND
71 Ladprao 95 Wangthonglang Bangkok 10310, Thailand. Tel: 02-539-4748 Fax: 02-514-1100 E-mail: thaidentalnet@gmail.com

ทันตแพทยสมาคมแห่งประเทศไทย
ในพระบรมราชูปถัมภ์
THE DENTAL ASSOCIATION OF THAILAND

Journal published trimonthly. Foreign subscription rate US$ 200 including postage.
Publisher and artwork: Rungsilp Printing Co., Ltd
Please send manuscripts to Dr. Ekamon Mahapoka
Address: 71 Ladprao 95 Wangtonglang Bangkok 10310, Thailand E-mail: jdateditor@thaidental.or.th

ทันตแพทยสมาคมแห่งประเทศไทย
ในพระบรมราชูปถัมภ์
THE DENTAL ASSOCIATION OF THAILAND

จดหมายสารา

สวัสดีพี่น้องทันตแพทย์ร่วมวิชาชีพทุกท่านครับ

สวัสดีปีใหม่ 2566 ครับ ก้าวเข้าสู่ปีใหม่อีกครั้ง หลาย ๆ ท่านก็คงคิดเหมือนผมว่า วันเดือนปีมันช่างผ่านไปเร็วจนรู้ตัวอีกทีก็หมดไปอีกปีแล้ว โลกในยุคใหม่แม้จะเต็มไปด้วยสิ่งอำนวยความสะดวกมากมายที่ทำให้เราได้อะไรทุกอย่างมาอย่างรวดเร็วตั้งแต่ตื่นนอนจนหลับไปในหนึ่งวัน แต่ก็ปฏิเสธไม่ได้ว่าก็ต้องแลกมาด้วยความวุ่นวายหรือบางครั้งรวดเร็วจนเราตั้งตัวไม่ทัน เวลาคือสิ่งมีค่าที่แม้จะมีอยู่แต่ก็จับต้องได้ลำบากในยุคที่ทุกอย่างมีแต่ความรวดเร็ว สิ่งหนึ่งที่พวกเราพึงตระหนักคือ การบริหารเวลาให้เกิดประโยชน์สูงสุดเพื่อเมื่อผ่านไปเราจะได้มองกลับมาอย่างมีคุณค่าและน่าจดจำ จากความวุ่นวายที่พวกเราทุกคนต้องเจอทั้งภาวะโรคระบาด สงคราม ปัญหาเศรษฐกิจ ฯลฯ ผมก็อยากจะเป็นกำลังใจให้ทุกท่านก้าวข้ามผ่านไปได้อย่างสง่างามครับ แม้ว่าสถานการณ์การติดเชื้อโควิด-19 จะค่อย ๆ ดีขึ้นเรื่อย ๆ หลาย ๆ ประเทศเริ่มผ่อนคลายมาตรการควบคุมการติดเชื้อ เริ่มมีการเปิดพรมแดนให้มีการเดินทางข้ามไปมาของผู้คนเพื่อทำให้โลกกลับเข้าสู่ภาวะปกติ พวกเราในมุมของผู้ให้บริการทางการแพทย์ การระแวดระวังการติดเชื้อทั้งจากตัวเองและคนรอบข้างก็ยังเป็นสิ่งที่พึงกระทำ แม้ว่ายังไม่มีรายงานการติดเชื้อที่เกิดจากการให้บริการทางทันตกรรมก็ตาม ผมอยากขอบคุณผู้นิพนธ์ทุกท่านที่ส่งงานเข้ามาเพื่อตีพิมพ์ในวิทยาสารฯ ของเราในช่วงปีที่ผ่านมา รวมทั้งเหล่าคณาจารย์ผู้ตรวจงานนิพนธ์ทุกท่านที่ช่วยผลักดันและสนับสนุนให้เรามีวิทยาสารฯ ฉบับนี้ เพื่อเผยแพร่งานวิชาการในแวดวงทันตแพทย์ของเราและบรรลุวัตถุประสงค์การจัดทำวิทยาสารฯของเราตั้งแต่ก่อตั้งมา ในปีหน้าวิทยาสารฯ ของเราก็ยังคงพัฒนาต่อไปและหวังว่าจะเป็นประโยชน์ให้กับพวกเราไม่มากก็น้อยครับ

ขอบคุณและสวัสดีครับ

ทพ.เอกมน มหาโภคา
สาราณียกร

สำหรับหน้าที่เป็นสี โปรดเข้าชมได้ที่เว็บไซต์ www.jdat.org
For high quality coloured figures, please refer to www.jdat.org

Instruction for Authors

The Journal of the Dental Association of Thailand ***(J DENT ASSOC THAI)*** supported by the Dental Assocition of Thailand, is an online open access and peer-reviewed journal. The journal welcomes for submission on the field of Dentistry and related dental science. We publish 4 issues per year in January, April, July and October.

» Categories of the Articles «

1. Review Articles: a comprehensive article with technical knowledge collected from journals and/or textbooks which is profoundly criticized or analyzed, or tutorial with the scientific writing.

2. Case Reports: a clinically report of an update or rare case or case series related to dental field which has been carefully analyzed and criticized with scientific observation.

3. Original Articles: a research report which has never been published elsewhere and represent new significant contributions, investigations or observations, with appropriate experimental design and statistical analysis in the filed of dentistry.

» Manuscript Submission «

The Journal of the Dental Association of Thailand welcome submissions from the field of dentistry and related dental science through only online submission. The manuscript must be submitted via http://www.jdat .org. Registration by corresponding author is once required for the article's submission. We accept articles written in both English and Thai. However, for Thai article, English abstract is required whereas for English article, there is no need for Thai abstract submission. The main manuscript should be submitted as .doc (word97-2003). All figures, and tables should be submitted as separated files (1 file for each figure or table). For the acceptable file formats and resolution of image will be mentioned in 8. of manuscript preparation section.

» Scope of Article «

Journal of Dental association of Thailand (JDAT) is a quarterly peer-reviewed scientific dental journal aims to the dissemination and publication of new knowledges and researches including all field of dentistry and related dental sciences

» Manuscript Preparation «

1. For English article, use font to TH Sarabun New Style size 14 in a standard A4 paper (21.2 x 29.7 cm) with 2.5 cm margin on a four sides. The manuscript should be typewritten.

2. For Thai article, use font of TH Sarabun New Style size 14 in a standard A4 paper (21.2 x 29.7 cm) with 2.5 cm margin on a four sides. The manuscript should be typewritten with 1.5 line spacing. Thai article must also provide English abstract. All reference must be in English. For the article written in Thai, please visit the Royal Institute of Thailand (http://www.royin.go.th) for the assigned Thai medical and technical terms. The original English words must be put in the parenthesis mentioned at the first time.

3. Numbers of page must be placed on the top right corner. The length of article should be 10-12 pages including the maximum of 5 figures, 5 tables and 40 references for original articles. (The numbers of references are not limited for review article).

4. Measurement units such as length, height, weight, capacity etc. should be in metric units. Temperature should be in degree Celsius. Pressure units should be in mmHg. The hematologic measurement and clinical chemistry should follow International System Units or SI.

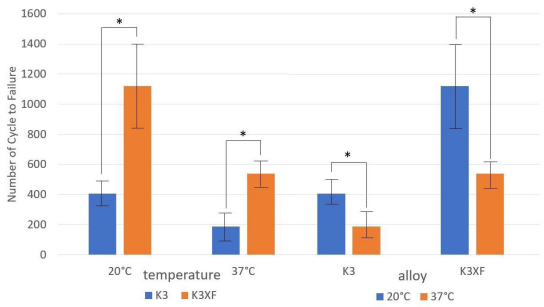
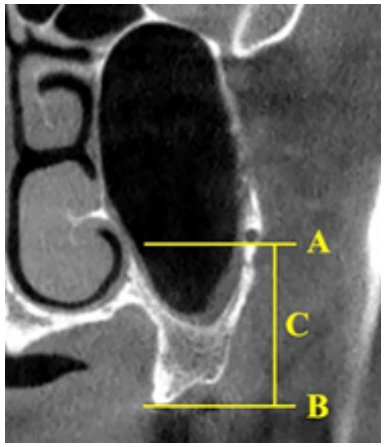
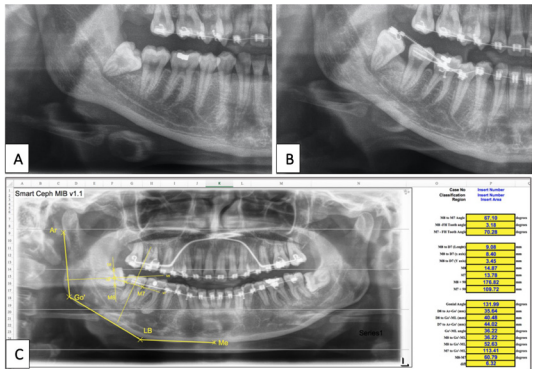
5. Standard abbreviation must be used for abbreviation and symbols. The abbreviation should not be used in the title and abstract. Full words of the abbreviation should be referred at the end of the first abbreviation in the content except the standard measurement units.

6. Position of the teeth may use full proper name such as maxillary right canine of symbols according to FDI two-digit notation and write full name in the parenthesis after the first mention such as tooth 31 (mandibular left central incisor)

7. Table: should be typed on separate sheets and number consecutively with the Arabic numbers. Table should self-explanatory and include a brief descriptive title. Footnotes to tables indicated by lower-case superscript letters are acceptable.

8. Figure : the photographs and figures must be clearly illustrated with legend and must have a high resolution and acceptable file types to meet technical evaluation of JDAT that is adapted from file submissions specifications of Pubmed (https://www.ncbi.nlm.nih.gov/pmc/pub/filespec-images/#int-disp). We classify type of figure as 3 types following: line art, halftones and combo (line art and halftone combinations) The details of description, required format, color mode and resolution requirement are given in table below.

Numbers, letters and symbols must be clear and even throughout which used in Arabic form and limited as necessary. During the submission process, all photos and tables must be submitted in the separate files. Once the manuscript is accepted, an author may be requested to resubmit the high quality photos.

Image type	Description	Example	Recommended format	Color mode	Resolution
Line art	An image which is composed of line and text and is not contained of tonal or shading areas.		tif. of eps.	Monochrome 1-bit of RGB	900-1200 dpi
Half tone	A continuous tone photograph which does not compose of text.		tif.	RGB of Graycale	300 dpi
Combo	Combination of line art and half tone.		tif. of eps.	RGB of Graycale	500-900 dpi

» Contact Address «

Editorial Staff of JDAT
The Dental Association of Thailand
71 Ladprao 95, Wangtonglang, Bangkok 10310, Thailand.
Email: jdateditor@thaidental.or.th Tel: +669-7007-0341

» Preparation of the Research Articles «

1. Title Page

The first page of the article should contain the following information

- Category of the manuscript
- Article title
- Authors' names and affiliated institutions
- Author's details (name, mailing address, E-mail, telephone and FAX number)

2. Abstract

The abstract must be typed in only paragraph. Only English abstract is required for English article. Both English and Thai abstract are required for Thai article and put in separate pages. The abstract should contain title, objectives, methods, results and conclusion continuously without heading on each section. Do not refer any documents, illustrations or tables in the abstract. The teeth must be written by its proper name not by symbol. Do not use English words in Thai abstract but translate or transliterate it into Thai words and do not put the original words in the parenthesis. English abstract must not exceed 300 words. Key words (3-5 words) are written at the end of the abstract in alphabetical order with comma (,) in-between.

3. Text

The text of the original articles should be organized in section as follows

- Introduction: indicates reasons or importances of the research, objectives, scope of the study. Introduction should review new documents in order to show the correlation of the contents in the article and original knowledge. It must also clearly indicate the hypothesis.

- Materials and Methods: indicate details of materials and methods used in the study for readers to be able to repeat such as chemical product names, types of experimental animals, details of patients including sources, sex, age etc. It must also indicate name, type, specification, and other information of materials for each method. For a research report performed in human subjects, human material samples, human participants and animal samples, authors should indicate that the study was performed according to the Experiment involving human or animal subjects such as Declaration of Helsinki 2000, available at: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/doh-oct2000/, or has been approved by the ethic committees of each institute (*ethic number is required).

- Results: Results are presentation of the discovery of experiment or researches. It should be categorized and related to the objectives of the articles. The results can be presented in various forms such as words, tables, graphs of illustrations etc. Avoid repeating the results both un tables and in paragraph =. Emphasize inly important issues.

- Discussion: The topics to be discussed include the objectives of the study, advantages and disadvantages of materials and methods. However, the important points to be especially considered are the experimental results compared directly with the concerned experimental study. It should indicate the new discovery and/or important issues including the conclusion from the study. New suggestion problems and informed in the discussion and indicate the ways to make good use of the results.

- Conclusion: indicates the brief results and the conclusion of the analysis.

- Acknowledge: indicates the institute or persons helping the authors, especially on capital sources of researches and numbers of research funds (if any).

- Conflicts of interest : for the transparency and helping the reviewers assess any potential bias. JDAT requires all authors to declare any competing commercial interests in conjunction with the submitted work.

- Reference: include every concerned document that the authors referred in the articles. Names of the journals must be abbreviated according to the journal name lists n "Index Medicus" published annually of from the website http://www.nlm.hih.gov

» Writing the References «

The references of both Thai and English articles must be written only in English. Reference system must be Vancouver reference style using Arabic numbers, making order according to the texts chronologically. Titles of the Journal must be in Bold and Italics. The publication year, issue and pages are listed respectively without volume.

Sample of references from articles in Journals

- Authors

Zhao Y, Zhu J: *In vivo* color measurement of 410 maxillary anterior teeth. ***Chin J Dent Res*** 1998;1(3):49-51.

- Institutional authors

Council in Dental Materials and Devices. New American Dental Association Specification No.27 for direct filling resins. ***J Am Dent Assoc*** 1977;94(6):1191-4

- No author

Cancer in South Africa [editorial]. ***S Afr Med J*** 1994:84:15

Sample of references from books and other monographs

- Authors being writers

Neville BW, Damn DD, Allen CM, Bouquot JE. Oral and maxillofacial pathology. Philadelphia: WB Saunder; 1995. P. 17-20

- Authors being both writer and editor

Norman IJ, Redfern SJ, editors. Mental health care for the elderly people. New York: Churchill Livestone; 1996.

- Books with authors for each separate chapter

- Books with authors for each separate chapter and also have editor

Sanders BJ, Handerson HZ, Avery DR. Pit and fissure sealants; In: McDonald RE, Avery DR, editors. Dentistry for the child and adolescent. 7th ed. St Louis: Mosby; 2000. P. 373-83.

- Institutional authors

International Organization for Standardization. ISO/TR 11405 Dental materials-Guidance on testing of adhesion to tooth structure. Geneva: ISO; 1994.

Samples of references from academic conferences

- Conference proceedings

Kimura J, Shibasaki H, editors. R The Journal of the Dental Association of Thailand (JDAT): (ISSN 2408-1434) online open access and double-blind peer review journal and also supported by the Dental Association of Thailand advances in clinical neurophysiology. Proceeding of the 10th International Congress of EMG and Clinical Neuro physiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam; Elsevier; 1996.

- Conference paper

Hotz PR. Dental plague control and caries. In: Lang PN, Attstrom R, Loe H, editors. Proceedings of the European Work shop on Mechanical Plague Control; 1998 May 9-12; Berne, Switzerland. Chicago: Quintessence Publishing; 1998. p. 25-49.

- Documents from scientific or technical reports

Fluoride and human health. WHO Monograph; 1970. Series no.59.

Samples of reference from thesis

Muandmingsuk A. The adhesion of a composite resin to etched enamel of young and old teeth [dissertation]. Texas: The University of Texas, Dental Branch at Houston; 1974.

Samples of reference from these articles are only accepted in electronic format

- Online-only Article (With doi (digital identification object number))

Rasperini G, Acunzo R, Limiroli E. Decision making in gingival rec experience. ***Clin Adv Periodontics*** 2011;1: 41-52. doi:10.1902 cap.2011.1000002.

- Online only article (without doi)

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. ***Am J Nurs*** 2002; 102(6) [cited 2002 Aug 12] Available from: http://nursingworld.org/AJN/2002/june/WaWatch.htmArticle

Samples of references from patents/petty patents

- Patent

Pagedas AC, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1.

- Petty patent

Priprem A, inventor, Khon Kaen University. Sunscreen gel and its manufacturing process. Thailand petty patent TH1003001008. 2010 Sep 20.

» Preparation of the Review articles and Case reports «

Review articles and case reports should follow the same format with separate pages for abstract, introduction, discussion, conclusion, acknowledgement and references.

» The Editorial and Peer Review Process «

The submitted manuscript will be reviewed by at least 2 qualified experts in the respective fields. In general, this process takes around 4-8 weeks before the author be noticed whether the submitted article is accepted for publication, rejected, or subject to revision before acceptance.

The author should realize the importance of correct format manuscript, which would affect the duration of the review process and the acceptance of the articles. The Editorial office will not accept a submission i the author has not supplied all parts of the manuscript as outlined in this document.

» Copyright «

Upon acceptance, copyright of the manuscript must be transferred to the Dental Association of Thailand.

PDF files of the articles are available at http://www.jdat.org

The price of addition color printing is extra charged 10000 bath/article (vat included).

Note: Color printing of selected article is considered by editorial board. (no extra charge)

» *Updated April, 2022* «

วิทยาสารทันตแพทยศาสตร์
JOURNAL OF THE DENTAL ASSOCIATION OF THAILAND

Contents

Volume 73 Number 1 January - March 2023

Original Article

วิทยาสารทันตแพทยศาสตร์

JOURNAL OF THE DENTAL ASSOCIATION OF THAILAND

Contents

Volume 73 Number 1 January - March 2023

Original Article

Front cover image:

adapted from Orientation of head position in three planes of space. coronal views.

(see *Chutasripanich.* page 14 for detail)

Original Article

A Comparison of Pulp Necrosis and Root Resorption After Auto-transplantation Between Immature Teeth and Apicoectomized Mature Teeth

Ploypailin Manovilas[1], Chootima Ratisoontorn[1], Onanong Silkosessak[2], Anchana Panichuttra[1], Kanit Dhanesuan[3]

[1]Department of Operative Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

[2]Department Radiology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

[3]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

Abstract

Success in pulp revascularization after autotransplantation tends to happen in a tooth with an incomplete root formation, while a tooth with a complete root formation needs a root canal treatment. However, recent studies showed that apicoectomy facilitated the repair and revascularization process with promising outcomes. This study aimed to compare the incidences of pulp necrosis and root resorption of autotransplanted teeth with a complete root formation which underwent apicoectomy and teeth with an incomplete root formation. Patients with a history of autotransplantation received clinical and radiographic follow-up examination. The autotransplanted teeth were divided into two groups, the incomplete root formation group and the extraoral apicoectomized complete root formation group. Pulp and periradicular outcomes (pulp healing, pulp necrosis and presence of root resorption) were determined with an additional of cone-beam computed tomography investigation. The incidence of each outcome and prognostic factors were statistically compared. The result showed that the incomplete root formation group presented 40 % (4 of 10) pulp necrosis and 10 % (1 of 10) root resorption, while the extraoral apicoectomized complete root formation group presented 77.8 % (7 of 9) pulp necrosis and 66.7 % (6 of 9) root resorption. The periradicular status between the two groups was significantly different. No prognostic factor was found to be related to pulp outcome, however apicoectomy and recipient socket were found to be related to the periradicular outcome. Autotransplanted teeth with complete root formation undergoing extraoral apicoectomy increased the risk of pulp necrosis and root resorption. A totally prepared recipient socket without remaining periodontal ligament was also found to be related to root resorption.

Keywords: Apicoectomy, Autotransplantation, Cone beam computed tomography, Revascularization, Root resorption

Received Date: Jun 17, 2022 **Revised Date:** Aug 8, 2022 **Accepted Date:** Sep 26, 2022
doi: 10.14456/jdat.2023.1

Correspondence to :
Kanit Dhanesuan, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chulalongkorn University, 34 Henri-Dunant Road, Pathumwan, Bangkok 10330, Thailand. Email: kanit.d@chula.ac.th

Introduction

Tooth autotransplantation is a surgical transposition of a tooth by extraction and replantation into another site in the same patient's mouth, also known as a controlled, aseptic avulsion and re-implantation.[1] It is one of the treatment options for patients to replace missing teeth and is preferred in children due to its adaptation to growth and developmental changes.[2]

Wound healing in autotransplantation is dependent on PDL healing, bone healing, pulp regeneration and root development.[3] Pulp revascularization is expected in an immature and developing transplanted tooth without the need for root canal treatment[4], while it is recommended that a tooth a with complete root development should undergo root canal treatment within 2 weeks after tooth transplantation to prevent root resorption.[5] According to Andreasen[6,7], 1 mm is the critical diameter of apical foramen which led to successful revascularization, while increasing the stage of root development[8] and the stage of eruption was found to be related to root resorption. However, recent case reports showed that apicoectomy of fully developed tooth facilitated the revascularization process and prevented subsequent endodontic treatment.[9,10] These were consistent with studies of extraoral apicoectomized mature teeth in dogs which showed the repair process via the ingrowth of connective tissue and pulpal revascularization.[11,12] Moreover, a recent retrospective study of extraoral root-end resection of mature teeth showed promising outcomes with a single root canal and uncomplicated root morphology.[13]

Although autotransplantation has been successfully performed for several years, only one clinical study has investigated the survival and complication of immature and root-end resected mature autotransplanted teeth.[13] Therefore, this study aimed to compare the incidence of pulp necrosis and root resorption of autotransplanted teeth with incomplete root formation and extraoral apicoectomized complete root formation at the Faculty of Dentistry, Chulalongkorn University. The finding might suggest the possibility of pulp revascularization in different root types, expand the potential applicability and create the protocol for root canal treatment in autotransplanted teeth.

Materials and methods

Patient recruitment

The Human Research Ethics Committee of Chulalongkorn University, Bangkok, Thailand (HREC-DCU 2020-070) approved the study protocol. Patients who had received tooth autotransplantation from January 2011 to May 2018 at the Faculty of Dentistry, Chulalongkorn University, were recruited and assessed via dental record history.

Inclusion criteria:

1. Patients with a history of autotransplantation and a minimum postoperative period of 1 year.

2. The patients' dental records with presurgical radiographs or immediate postsurgical radiographs.

3. At the time of surgery, the donor tooth was classified as incomplete root formation (stage 0-4 of Moorrees classification[14]) or complete root formation (stage 5-6 of Moorrees classification[14]) and underwent apicoectomy during surgery.

Data collection

Patients were divided into two groups according to donor tooth at the time of surgery as follows: (1) Teeth with incomplete root formation, (2) Teeth with complete root formation and underwent apicoectomy during surgery. Teeth with complete root formation and underwent intentional root canal treatment were excluded from this study.

The patients' demographics were collected from the hospital records. The following variables which may influence the outcome were recorded; donor tooth type, stage of root development, recipient socket, cause of tooth loss, and eruption status of donor tooth. The recipient socket was classified as a partially prepared socket, if a permanent tooth was extracted from the recipient site and the donor tooth was placed into the socket within the same visit and only need some additional socket preparation, while a totally prepared socket referred to

the recipient socket from an edentulous area or a primary tooth extraction, which operator needed to establish a new recipient socket without any remnant PDL. If the donor tooth received root canal treatment after auto-transplantation, the cause of root canal treatment was identified according to the treatment records.

Clinical and radiographic evaluation

Patients who met the inclusion criteria were contacted via phone and invited to take part in the follow-up examination from September 2020 to July 2021. A clinical examination was performed by one examiner (PM). Subjective symptoms and clinical parameters including tooth mobility[15], percussion sound, pain on percussion, sensibility test (electric pulp test and Endo-Frost), gingival index[16], periodontal pocket, soft tissue appearance (presented of sinus fistula or swelling), and restoration condition were recorded.

Two different angles of parallel periapical radiographs were taken to examine obliteration of pulp cavity, periapical lesion, the integrity of periodontal space and signs of root resorption. Patients who agreed to additional radiograph were subjected to cone beam computed tomography (CBCT) [Accuitomo 170 (J. Morita USA)] with a limited field of view (FOV) of 4x4 cm. The CBCT images were assessed using One Volume Viewer software. All examiners were reminded of the salient features of resorption lesions using sample radiographs and CBCT images before images analysis. The periapical and CBCT images were evaluated by two examiners (PM and CR) at two-week intervals.All discrepancies were resolved by a consensus between the two examiners.

Determination of pulp and periradicular outcomes

The outcomes of pulp status were categorized as pulp healing and pulp necrosis

• *Pulp healing* was defined by teeth which include all the following criteria: positivity to sensibility test (EPT or thermal) *, absence of tenderness to percussion, absence of sign and symptom of pathosis (abscess, swelling, sinus), together with a radiographic presentation of partial pulp obliteration or continue root formation (in a tooth with incomplete root formation).

* If the teeth showed any radiographic evidence including pulp obliteration or continue root formation, the teeth would be assumed as successful in pulp healing despite having a negative response to the sensibility test.[17]

• *Pulp necrosis* was defined by teeth which radiographically presented periapical radiolucency, and/or infection-related root resorption[18] with or without a response to pulp sensibility test or teeth which did not fulfill all criteria for pulp healing.

The outcome of periradicular status was to be categorized as no resorption, external inflammatory resorption, replacement resorption and external cervical resorption.

• No resorption: A tooth presented physiologic mobility with normal percussion sound. CBCT radiograph showed no sign of resorption or presented surface resorption.[19]

• External replacement resorption (ERR): CBCT radiograph showed an absence of periodontal space together with continuous replacement of loss of root substance with bone and no radiolucency.[20]

• External inflammatory resorption (EIR): CBCT radiograph showed loss of root substance, bowl-shaped resorption with adjacent periradicular radiolucency in bone.[20]

• External cervical root resorption (ECR): CBCT radiograph showed extensive irregular radiolucency extending from the cervical area into the crown and projected over the root canal outline.[21]

Statistical Analysis

Incidences of pulp necrosis and root resorption were reported by descriptive analysis as frequencies and percentages. The comparison of the incidence of each treatment outcome between teeth with incomplete root formation and teeth with complete root formation undergoing extraoral apicoectomy prior to transplantation were investigated by chi-square test or Fisher exact test as appropriate.

Quantitative prognostic factors between each treatment outcome were tested by independent *t*-test or Mann-Whitney U test. Qualitative prognostic factors between each treatment outcome were tested by chi-square

test or Fisher exact test. A *P* value < 0.5 was considered statistically significant. Statistical analysis was calculated using IBM SPSS statistics for Windows, version 22 (IBM, Armonk, New York).

Results

A total of 26 patients with 28 transplanted teeth met the inclusion criteria according to the hospital database from January 2011 to May 2018. Nine patients could not be contacted due to changing phone number or relocation. Seventeen patients with 19 transplanted teeth received follow-up examinations with an additional CBCT radiographs. Nine patients with 10 transplanted teeth were categorized in the teeth with incomplete root formation group, while 8 patients with 9 transplanted teeth were categorized in the extraoral apicoectomized complete root formation group.

The patient's demographic data and tooth variables between the two groups were not significantly different. (Table 1) The average follow-up time of incomplete root and extraoral apicoectomized complete root groups was 6.02 years and 4.72 years, respectively.

Table 1 *Patient's demographic data and tooth variable*

		Incomplete root formation	Extraoral apicoectomized complete root formation	*P*-value[†]
Gender				0.37
	Male	3	5	
	Female	7	4	
Donor tooth type				0.277
	Anterior	0	2	
	Premolar	5	4	
	Molar	5	3	
Donor root type				1
	Single root	6	5	
	Multi root	4	4	
Eruption status				0.981
	Fully erupted	6	5	
	Partial erupted	2	2	
	Unerupted	2	2	
Recipient socket				0.141
	Partially prepared socket	5	1	
	Totally prepared socket	5	8	
Age				0.115
	Mean age	16.6	20.56	
Follow up time				0.153
	Mean	6.02	4.72	

[†]*The chi-square test/Fisher exact test.*

[*]*Significant difference (p<.05).*

The incomplete root formation group presented 40 % (4 of 10) pulp necrosis, while the extraoral apicoectomized complete root formation group presented 77.8 % (7 of 9) pulp necrosis. The outcome of pulp status between the two groups was not statistically different. (Table 2)

The incomplete root formation group presented 10 % (1 of 10) root resorption. The root resorption type was defined as external cervical root resorption (Fig.1), while the external inflammatory root resorption and replacement resorption were not found in this group. The extraoral apicoectomized complete root formation group presented 66.7 % (6 of 9) root resorption. All 5 of 7 pulp necrosis teeth developed external inflammatory root resorption. (Fig.2 and 3) External cervical root resorption was also found in one tooth. (Fig.4) The periradicular status between the two groups was significantly different. (Table 2)

Table 2 *Outcome of pulp and periradicular status*

	Incomplete root formation	Extraoral apicoectomized complete root formation	*P*-value[†]
	n (% of total)	n (%) of total	
Pulp status			0.17
Pulp healing	6 (60%)	2 (22.2%)	
Pulp necrosis	4 (40%)	7 (77.8%)	
Periradicular status			0.02*
No resorption	9 (90%)	3 (33.3%)	
Root resorption	1 (10%)	6 (66.7%)	
Type of root resorption			0.286
External inflammatory resorption	-	5 (83.3%)	
External cervical resorption	1 (100%)	1 (16.7%)	
Replacement resorption	-	-	
Need of endodontic treatment			0.35
No treatment needed	5 (50%)	2 (22.2%)	
Treatment needed	5 (50%)	7 (77.8%)	

[†]*The chi-square test/Fisher exact test.*

[*]*Significant difference (p<.05).*

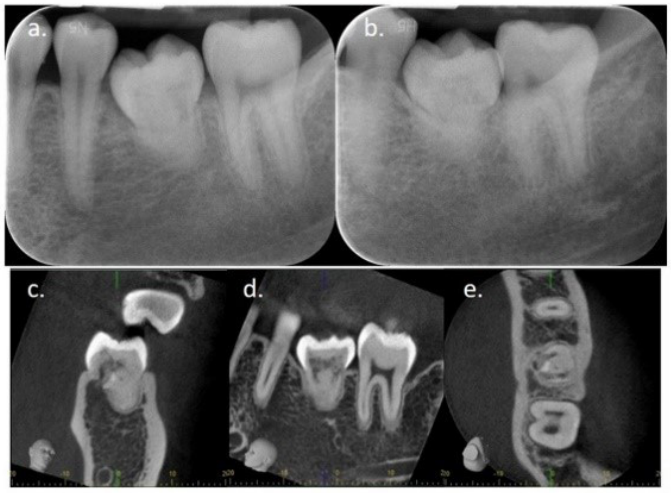


Figure 1 *Straight on (a.) and horizontal tube shift (b.) periapical radiographs of tooth 28 (left maxillary third molar) transplanted to 36 (left mandibular first molar) area at 9 years follow-up showed pulp obliteration with diffused radiolucent area in the crown. CBCT images (coronal (c.), sagittal (d.) and axial (e.)) exhibited an ECR in the reparative phase*[22]

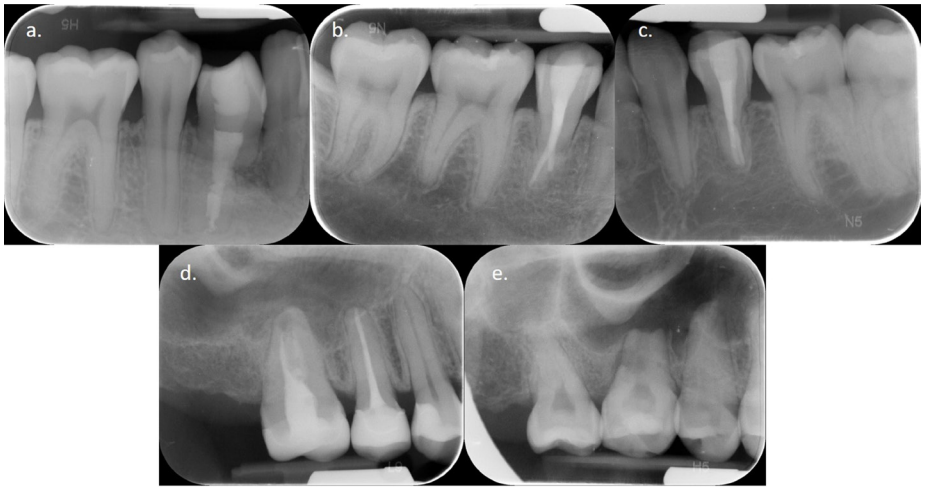


Figure 2 *Periapical radiographs of teeth in the extraoral apicoectomized complete root formation group with external inflammatory resorption (a.) Tooth 34 (left mandibular first premolar) transplanted to 44 (right mandibular first premolar) area at 4.42 years follow-up (b.) Tooth 14 (right maxillary first premolar) transplanted to 44 (right mandibular first premolar) area at 4.17 years follow-up (c.) Tooth 24 (left maxillary first premolar) transplanted to 34 (left mandibular first premolar) area at 3.75 years follow-up (d.) Tooth 47 (right mandibular second molar) transplanted to 16 (right maxillary first molar) area at 4.67 years follow-up (e.) Tooth 48 (right mandibular third molar) transplanted to 16 (right maxillary first molar) area at 4.08 years follow-up*

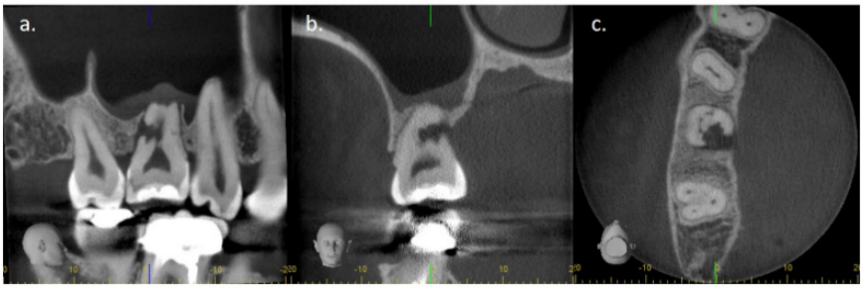


Figure 3 *CBCT radiographs of extraoral apicoectomized complete root formation tooth with external inflammatory resorption. Sagittal (a.) coronal (b.) and axial (c.) images showed extensive external inflammatory resorption. Tooth was indicated to be extracted*

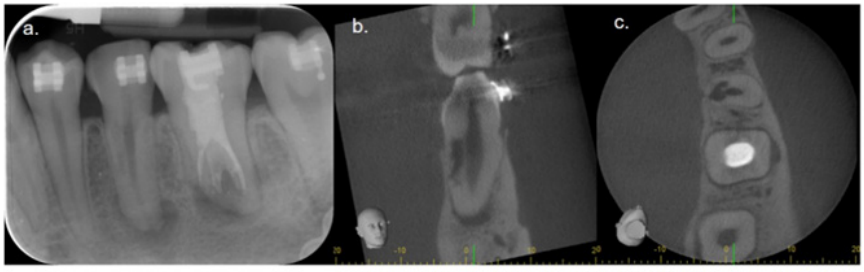


Figure 4 *Periapical radiograph (a.) and CBCT images (coronal (b.) and axial (c.)) of tooth 23 (left maxillary canine) transplanted to replace prolonged left mandibular second primary molar at 5.17 years follow-up. Extensive external cervical resorption was observed. The tooth was indicated to be extracted*

No prognostic factor (patient demographic, donor tooth type, donor root type, stage of root development, recipient socket, cause of tooth loss, eruption status of donor tooth and apicoectomy) was found to be related to pulp outcome, while apicoectomy and recipient socket were found to be related to the periradicular outcome. (Table 3)

Table 3 *Descriptive data and univariate analysis of the relationship between each prognostic factor and outcome of pulp and periradicular status*

Factor	Pulp status		*P*-value†	OR (95% CI)	Periradicular status		*P*-value†	OR (95% CI)
	Pulp healing n (% of total)	Pulp necrosis n (% of total)			No resorption n (% of total)	Root resorption n (% of total)		
Age			1	1.714 (0.228-12.89)			0.617	2.25 (0.308-16.411)
0-20	6 (46.2%)	7 (53.8%)			9 (69.2%)	4 (30.8%)		
>20	2 (33.3%)	4 (66.7%)			3 (50%)	3 (50%)		
Gender			0.059	0.082 (0.007-0.926)			0.074	0.133 (0.016-1.085)
Male	1 (12.5%)	7 (87.5%)			3 (37.5%)	5 (62.5%)		
Female	7 (63.6%)	4 (36.4%)			9 (81.8%)	2 (18.2%)		
Donor tooth type			0.237				0.906	
Anterior	1 (50%)	1 (50%)		reference	1 (50%)	1 (50%)		reference
Premolar	2 (22.2%)	7 (77.8%)		3.5 (0.145-84.694)	6 (66.7%)	3 (33.3%)		0.5 (0.023-11.088)
Molar	5 (62.5%)	3 (37.5%)		0.6 (0.027-13.582)	5 (62.5%)	3 (37.5%)		0.6 (0.027-13.582)
Donor root type			0.181	0.225 (0.032-1.584)			1	1.050 (0.159-6.924)
Single root	3 (27.3%)	8 (72.7%)			7 (63.6%)	4 (36.4%)		
Multiroot	5 (62.5%)	3 (37.5%)			5 (62.5%)	3 (37.5%)		
Eruption status			0.238				0.659	
Fully erupted	3 (27.3%)	8 (72.7%)		reference	6 (54.5%)	5 (45.5%)		reference
Partially erupted	3 (75%)	1 (25%)		0.125 (0.009-1.723)	3 (75%)	1 (25%)		0.4 (0.031-5.151)
Unerupted	2 (50%)	2 (50%)		0.375 (0.035-3.999)	3 (75%)	1 (25%)		0.4 (0.031-5.151)
Stage of root development			0.079				0.064	
Stage 2	2 (100%)	0		reference	2 (100%)	0		reference
Stage 3	4 (57.1%)	3 (42.9%)		0.417 (0.030-5.708)‡	6 (85.7%)	1 (14.3%)		1.167 (0.074-18.346)‡
Stage 4	0	1 (100%)		0.167 (0.006-4.515)‡	1 (100%)	0		0.667 (0.025-18.059)‡
Stage 5	1 (100%)	0		0.667 (0.025-18.059)‡	1 (100%)	0		0.667 (0.025-18.059)‡
Stage 6	1 (12.5%)	7 (87.5%)		0.083 (0.005-1.294)‡	2 (25%)	6 (75%)		0.143 (0.010-1.995)‡
Apicoectomy			0.17	5.250 (0.698-39.476)			0.02*	18 (1.496-216.62)
Yes	2 (22.2%)	7 (77.8%)			3 (33.3%)	6 (66.7%)		
No	6 (60%)	4 (40%)			9 (90%)	1 (10%)		
Cause of tooth loss			0.664	1.667 (0.251-11.071)			1.0	1.5 (0.195-10.032)
Agenesis	3 (37.5%)	5 (62.5%)			5 (62.5%)	3 (37.5%)		
Caries	5 (50%)	5 (50%)			7 (70%)	3 (30%)		
Recipient socket			0.319	0.222 (0.028-1.754)			0.044*	8.0 (0.78-82.052)‡
Partially prepared socket	4 (66.7%)	2 (33.3%)			6 (100%)	0 (%)		
Totally prepared socket	4 (30.8%)	9 (69.2%)			6 (46.2%)	7 (53.8%)		

†*The chi-square test/Fisher exact test.*

**Significant difference (p<.05).*

‡*Haldane's modification23 was used in sets containing zero.*

Discussion

This retrospective study was designed to compare the incidence of pulp necrosis and root resorption between an autotransplanted tooth with incomplete root formation and an autotransplanted tooth with complete root formation which underwent apicoectomy. The period of patient recruitment was from January 2011 to May 2018, due to the homogeneity of treatment protocols, before the involvement of 3D digital printing technology which improved the treatment outcome by facilitating the recipient site preparation, reducing donor tooth extra-alveolar time and avoiding PDL damage.[24] The requirement of the minimum postoperative period of 1 year is indicated because root resorption usually develops within the first year after replantation.[20] No significantly different variable was found between the two group. The average follow-up time for both groups was 5.4 years, which was enough to detect any pathology. However, the recall rate was 70 % due to loss of contact.

The transplantation was performed by residents under the supervision of maxillofacial staff. Although this led to the lack of treatment procedure homogeneity, the main concept of minimizing periodontal trauma and extra-alveolar time was still maintained. All the apicoectomy cases were supervised by KD. A tooth with a cone-shaped root was cut to achieve 1 mm. of apical foramen width, which is believed to facilitate the revascularization process.[6] In some cases, the donor's root length was longer than the recipient socket depth, the donor root had to be cut to fit into the recipient site.

Detection of apical periodontitis can be difficult, if the cortical bone was not involved.[25] Two-dimensional periapical radiograph may not detect bone changes in the cancellous bone due to its lower sensitivity compared to CBCT.[26] Cone beam computed tomography (CBCT) is a three-dimensional imaging technique that has been recommended as an additional radiograph. American Association of Endodontists and American Academy of Oral and Maxillofacial Radiology (AAE and AAOMR) joint position statement has suggested using limited FOV CBCT as an imaging modality for endodontic diagnosis and detection of periapical lesions. The statement has also suggested CBCT imaging in the localization and differentiation of inflammatory resorption defects to determine appropriate treatment and prognosis.[27] CBCT also presented superior accuracy in detecting root resorption than periapical radiograph.[28,29] This study chose the smallest FOV possible, 4x4 cm, that covered anatomical area of interest. Smaller FOV produces higher spatial resolution images with lower radiation doses compared to large FOV.[27] The benefit of using CBCT radiograph in this study is to confirm the diagnosis, type of root resorption and extent of the lesion in addition to periapical radiographs.

Pulp necrosis was found in teeth with a complete root formation which underwent apicoectomy more than in teeth with an incomplete root formation, but was not statistically significant in this study. Root development together with apical foramen diameter were known as important factors related to pulp necrosis.[30] Tooth with apical foramen larger than 1 mm. has a low risk of pulp necrosis.[6] The process of apicoectomy might enlarge apical foramen size and facilitate the ingrowth of connective tissue and blood vessel as seen in the histological study.[11,12] However, from our study, an extraoral apicoectomized tooth still achieved less pulp healing than a tooth with an incomplete root formation. This might be due to the complexity of the autotransplantation procedure. This procedure is a highly sensitive technique with several prognostic factors involved.[31] Factors affecting healing of transplanted teeth include sex[6], age of the patients[6],storage media[32], length of extra-alveolar period[32], stage of root development[7], stage of eruption[7], distance from apical foramen to pulp horn[30], and subsequent orthodontic movement[7]. In addition, stem cells from the apical papilla (SCAP) which are believed to play an important role in the healing process[33] are absent in a tooth with a complete root formation. In the previous clinical study, all the un-complicated single canal autotransplanted tooth (n=4) achieved pulp canal obliteration which was presumed as a sign of pulp healing.[34] The author suggested that the

apicoectomy technique in a single root canal gave promising outcomes.[13] In contrast with our study, 2 teeth that achieved pulp healing were a canine and a molar, while the other single canal tooth developed pulp necrosis. The contrary between the 2 studies might be inconclusive due to the small sample size. However, the previous study did not perform CBCT imaging in all samples, which might affect the interpretation of outcome since CBCT yield additional information.

This study showed the relationship between periradicular status and apicoectomy. Root resorption was statistically higher in teeth with a complete root formation which underwent the apicoectomy than in teeth with an incomplete root formation. The only case of root resorption found in the incomplete root formation group was external cervical root resorption in tooth 28 (left maxillary third molar), which was transplanted to 36 (left mandibular first molar) recipient area. Due to its divergent palatal root and insufficient bucco-lingual bone width, palatal root amputation and MTA retrograde filling were performed extraorally. The patient was unable to complete the follow-up appointment after 7 months. At 9 years follow-up examination, CBCT radiograph classified the resorption as 2Cp according to Patel classification.[35] The surgical procedure was assumed to cause a defect at the cementoenamel junction and initiated the resorptive process. (Fig. 1) The unmatched donor tooth and recipient socket could lead to endodontic complications. This can be prevented nowadays by the use of digitalized approaches such as CBCT, 3D-printed guiding templates and replicas.[24]

A tooth with pulp necrosis in an incomplete root formation group did not show any sign of inflammatory root resorption in contrast to the extraoral apicoectomized complete root formation group. In the latter group, 5 of 7 pulp necrotic teeth presented features of external inflammatory resorption. Four teeth were successfully treated and remained functional, while another didn't attended annual follow up and presented extensive root resorption at 4 years. (Fig. 2) After additional investigation with CBCT, the tooth was extracted. (Fig. 3) The risk of root resorption was found to be related to the increasing stage of root development.[7,36,37] Incomplete formed root is usually covered with thick follicle and required less traumatic force in extraction than a complete formed root with a firm attachment of the periodontal ligament.[7] Damage to the root surface together with inflammatory stimulus from necrotic infected pulp result in external inflammatory root resorption.[38] If the root surface is injured without infection, the repair process by osteoclasts and osteoblasts will replace the radicular dentin with bone which results in external replacement resorption.[39]

Another root resorption case in the extraoral apicoectomized complete root formation group was external cervical resorption. Embedded tooth 23 (left maxillary canine) was transplanted to the recipient site after tooth 75 (left mandibular second primary molar) extraction. The detection of the resorptive lesion was delayed and the tooth was extensively destructed. At 5 years follow-up, CBCT radiograph showed the resorption as 3Ap according to Patel classification[35] After a discussion with the patient's orthodontist, the tooth was extracted. (Fig. 4)

No replacement resorption was found in both groups which was assumed to result from good management of the donor tooth during surgery. The donor tooth was removed with atraumatic surgical technique and handling via its crown to preserve the PDL. The tooth was also kept in its socket during the recipient site preparation with minimal extra-alveolar time. Replacement resorption is known for its relation to the extra-alveolar period and dry storage.[32]

Due to the limitation of the retrospective study design, information about the surgical period, extra-alveolar time and storage media were not recorded. Another limitation of this study is the small sample size because apicoectomy is a novel technique in autotransplantation.[13] The incidence of pulp necrosis, root resorption and the analysis of the relationship between each prognostic factor may have a large proportion difference between the two groups, but not statistically significant due to the limitation of the small sample size. We suggest further

prospective cohort studies with a larger sample size and 3D printing technology which facilitate the surgical procedures, reduce complications from surgical techniques and control other confounding factors.

Interestingly, the recipient socket was another factor that was found to be associated with root resorption. The recipient socket was prepared slightly larger than the donor tooth by an implant drill kit or an external cooling bur. The donor tooth was periodically placed into the socket with light pressure to ensure proper fit. All teeth presented with root resorption were founded in a totally prepared socket. A tooth that was transplanted into a freshly extracted socket with partial preparation demonstrated a better outcome compared to socket preparation from the edentulous area or primary tooth extraction. Assuming some vital PDL might still be present in the former socket. Despite the importance of PDL viability on the root surface, PDL on the socket wall also facilitated the healing process as seen in the animal studies.[40,41] However, the amount of remaining PDL in a partially prepared socket in this study was inconclusive due to a lack of data.

Even though some extraoral apicoectomized complete root formation autotransplanted teeth successfully healed without any complication. This method showed more undesirable outcomes than teeth with incomplete root formation. Our study found that 80 % of an extraoral apicoectomized group without presurgical/ postsurgical root canal treatment needed more additional endodontic treatment subsequently than teeth with incomplete root formation due to pulp necrosis and root resorption, but not statistically significant. (Table 2) Root canal treatment performed in the canal with wide apical foramen or root resorption is complicated. Therefore, we suggest that extraoral apicoectomized transplanted teeth should be closely followed up.

Conclusion

Autotransplanted teeth with complete root formation undergone extraoral apicoectomy increased the risk of root resorption than autotransplanted teeth with incomplete root formation. In addition, the development of root resorption was related to a totally prepared recipient socket, which had no periodontal ligament.

Acknowledgements

This study was supported by a grant from the Faculty of Dentistry, Chulalongkorn University. The authors thank Dr. Soranun Chantarangsu and Dr.Pagaporn Pisarnturakit for statistical advice and the Faculty of Dentistry, Chulalongkorn University for research funding. The authors denied any conflicts of interest related to this study.

References

1.Ong D, Itskovich Y, Dance G. Autotransplantation: a viable treatment option for adolescent patients with significantly compromised teeth. ***Aust Dent J*** 2016;61(4):396-407.

2. Czochrowska EM, Stenvik A, Bjercke B, Zachrisson BU. Outcome of tooth transplantation: survival and success rates 17-41 years posttreatment. ***Am J Orthod Dentofacial Orthop*** 2002;121(2):110-9.

3. Tsukiboshi M. Autotransplantation of teeth: requirements for predictable success. ***Dent Traumatol*** 2002;18(4):157-80.

4. Park JH, Tai K, Hayashi D. Tooth autotransplantation as a treatment option: a review. ***J Clin Pediatr Dent*** 2010;35(2):129-35.

5. Chung WC, Tu YK, Lin YH, Lu HK. Outcomes of autotransplanted teeth with complete root formation: a systematic review and meta-analysis. ***J Clin Periodontol*** 2014;41(4):412-23.

6. Andreasen JO, Paulsen HU, Yu Z, Bayer T, Schwartz O. A long-term study of 370 autotransplanted premolars. Part II. Tooth survival and pulp healing subsequent to transplantation. ***Eur J Orthod*** 1990;12(1):14-24.

7. Andreasen JO, Paulsen HU, Yu Z, Schwartz O. A long-term study of 370 autotransplanted premolars. Part III. Periodontal healing subsequent to transplantation. ***Eur J Orthod*** 1990;12(1):25-37.

8. Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. 4. Factors related to periodontal ligament healing. ***Endod Dent Traumatol*** 1995;11 (2):76-89.

9. Jakse N, Ruckenstuhl M, Rugani P, Kirnbauer B, Sokolowski A, Ebeleseder K. Influence of Extraoral Apicoectomy on Revascularization of an Autotransplanted Tooth: A Case Report. ***J Endod*** 2018;44(8): 1298-302.

10. Gaviño Orduña JF, García García M, Dominguez P, Caviedes Bucheli J, Martin Biedma B, Abella Sans F, *et al.* Successful pulp revascularization of an autotransplantated mature premolar with

fragile fracture apicoectomy and plasma rich in growth factors: a 3-year follow-up. ***Int Endod J*** 2020;53(3):421-33.
11. Skoglund A. Vascular changes in replanted and autotransplanted apicoectomized mature teeth of dogs. ***Int J Oral Surg*** 1981;10(2):100-10.
12. Skoglund A. Pulpal changes in replanted and autotransplanted apicoectomized mature teeth of dogs. ***Int J Oral Surg*** 1981;10(2):111-21.
13. Raabe C, Bornstein MM, Ducommun J, Sendi P, von Arx T, Janner SFM. A retrospective analysis of autotransplanted teeth including an evaluation of a novel surgical technique. ***Clin Oral Investig*** 2021; 25(6):3513-25.
14. Moorrees CF, Fanning EA, Hunt EE, Jr. AGE VARIATION OF FORMATION STAGES FOR TEN PERMANENT TEETH. ***J Dent Res*** 1963;42:1490-502.
15. Andreasen JO, Paulsen HU, Yu Z, Ahlquist R, Bayer T, Schwartz O. A long-term study of 370 autotransplanted premolars. Part I. Surgical procedures and standardized techniques for monitoring healing. ***Eur J Orthod*** 1990;12(1):3-13.
16. Löe H. The Gingival Index, the Plaque Index and the Retention Index Systems. ***J Periodontol*** 1967;38(6):Suppl:610-6.
17. Bastos JV, Côrtes MIS. Pulp canal obliteration after traumatic injuries in permanent teeth - scientific fact or fiction? ***Braz Oral Res*** 2018;32(suppl 1):e75.
18. Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. 1. Diagnosis of healing complications. ***Endod Dent Traumatol*** 1995;11(2):51-8.
19. Kafourou V, Tong HJ, Day P, Houghton N, Spencer RJ, Duggal M. Outcomes and prognostic factors that influence the success of tooth autotransplantation in children and adolescents. ***Dent Traumatol*** 2017;33(5):393-9.
20. Andreasen JO, Hjorting-Hansen E. Replantation of teeth. I. Radiographic and clinical study of 110 human teeth replanted after accidental loss. ***Acta Odontol Scand*** 1966;24(3):263-86.
21. Heithersay GS. Invasive cervical resorption. ***Endodontic Topics*** 2004;7(1):73-92.
22. Patel S, Mavridou AM, Lambrechts P, Saberi N. External cervical resorption-part 1: histopathology, distribution and presentation. ***Int Endod J*** 2018;51(11):1205-23.
23. Haldane JB. The estimation and significance of the logarithm of a ratio of frequencies. ***Ann Hum Genet*** 1956;20(4):309-11.
24. Abella F, Ribas F, Roig M, González Sánchez JA, Durán-Sindreu F. Outcome of Autotransplantation of Mature Third Molars Using 3-dimensional-printed Guiding Templates and Donor Tooth Replicas. ***J Endod*** 2018;44(10):1567-74.
25. Huumonen S, Ørstavik D. Radiological aspects of apical periodontitis. ***Endodontic Topics*** 2002;1(1):3-25.
26. de Paula-Silva FW, Wu MK, Leonardo MR, da Silva LA, Wesselink PR. Accuracy of periapical radiography and cone-beam computed tomography scans in diagnosing apical periodontitis using histopathological findings as a gold standard. ***J Endod*** 2009;35(7):1009-12.
27. AAE and AAOMR Joint Position Statement: Use of Cone Beam Computed Tomography in Endodontics 2015 Update. ***J Endod*** 2015; 41(9):1393-6.
28. Patel S, Dawood A, Wilson R, Horner K, Mannocci F. The detection and management of root resorption lesions using intraoral radiography and cone beam computed tomography - an *in vivo* investigation. ***Int Endod J*** 2009;42(9):831-8.
29. Estrela C, Bueno MR, De Alencar AH, Mattar R, Valladares Neto J, Azevedo BC, *et al.* Method to evaluate inflammatory root resorption by using cone beam computed tomography. ***J Endod*** 2009;35(11):1491-7.
30. Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. 2. Factors related to pulpal healing. ***Endod Dent Traumatol*** 1995;11(2):59-68.
31. Lucas-Taulé E, Bofarull-Ballús A, Llaquet M, Mercadé M, Hernández-Alfaro F, Gargallo-Albiol J. Does Root Development Status Affect the Outcome of Tooth Autotransplantation? A Systematic Review and Meta-Analysis. ***Materials*** 2022;15:3379.
32. Andreasen JO. Effect of extra-alveolar period and storage media upon periodontal and pulpal healing after replantation of mature permanent incisors in monkeys. ***Int J Oral Surg*** 1981;10(1):43-53.
33. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. ***J Endod*** 2008;34(6):645-51.
34. Abd-Elmeguid A, ElSalhy M, Yu DC. Pulp canal obliteration after replantation of avulsed immature teeth: a systematic review. ***Dent Traumatol*** 2015;31(6):437-41.
35. Patel S, Foschi F, Mannocci F, Patel K. External cervical resorption: a three-dimensional classification. ***Int Endod J*** 2018;51(2):206-14.
36. Kristerson L. Autotransplantation of human premolars. A clinical and radiographic study of 100 teeth. ***Int J Oral Surg*** 1985;14(2):200-13.
37. Schwartz O, Bergmann P, Klausen B. Resorption of autotransplanted human teeth: a retrospective study of 291 transplantations over a period of 25 years. ***Int Endod J*** 1985;18(2):119-31.
38. Hargreaves KM, Goodis HE, Bender IB, Seltzer S. Seltzer and Bender's Dental Pulp: Quintessence Publishing Company; 2002.
39. Patel S, Saberi N. The ins and outs of root resorption. ***Br Dent J*** 2018;224(9):691-9.
40. Andreasen JO. Periodontal healing after replantation and autotransplantation of incisors in monkeys. ***Int J Oral Surg*** 1981; 10(1):54-61.
41. Shimada T. Effect of Periodontal Ligament Curetted in Alveolar Socket for Autotransplantation of Tooth in Adult Monkeys. ***JJSOI*** 1998;11(4):492-500.

CE Quiz

Original Article

Assessment of Midpalatal Suture Maturation by Cone-beam Computed Tomography in Circumpubertal Age Group

Nopparat Chutasripanich[1], Korapin Mahatumarat[1], Soontra Panmekiate[2]

[1]Department of Orthodontics, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

[2]Department of Radiology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

Abstract

This study evaluated the prevalence of midpalatal suture maturation stages in 8 to 18 years old patients and the relationship between chronological age and the suture maturation stages in a Thai population. The cone-beam computed tomography (CBCT) images of the midpalatal suture from 240 subjects (110 males, 130 females) aged 8 to 18 years were classified into five stages (A-E). The distribution of the maturation stages was determined according to chronological age and sex. Chi-square test was used to compare the prevalence of maturation stages between male and female subjects. Spearman's rank correlation analysis was performed to investigate the relationship between the maturation stage and chronological age. The results showed that the fused stages (D-E) were not seen in the prepubertal age group (8-11 years old). In the postpubertal age group (12-18 years old), the prevalence of nonfused stages (A-C) and fused stages (D-E) was 82.3% and 17.7% respectively. Stage C was the most prevalent (62.6%) in the postpubertal age group. Female showed a significantly higher prevalence of fusion than male (χ^2 = 5.434, p=0.02). The correlation coefficient between chronological age and the suture maturation was 0.325 (p<0.001). In conclusion, fusion was not observed in females under 12 years old and males under 14 years old. Thus, CBCT might be recommended to verify the suture status before performing maxillary expansion in female ≥12 years old and males ≥14 years old. Overall, chronological age had a weak positive correlation with the suture maturation.

Keywords: Chronological age, Cone-beam computed tomography, Maturation stages, Midpalatal suture, Rapid maxillary expansion

Received Date: Aug 15, 2022 **Revised Date:** Sep 8, 2022 **Accepted Date:** Oct 28, 2022
doi: 10.14456/jdat.2023.2

Correspondence to:
Korapin Mahatumarat, Department of Orthodontics, Faculty of Dentistry, Chulalongkorn University, 34 Henri Dunant Road, Pathumwan, Bangkok 10330 Thailand. Tel: 02-218-8949 Email: korapinmaha@gmail.com

Introduction

Rapid maxillary expansion (RME) is an orthopedic procedure that is routinely used in orthodontic practice for many purposes, including correction of maxillary transverse deficiency[1-4], posterior crossbite, dental crowding[1,4] and facilitating Class III correction by facemask therapy.[5] The objective of RME is to increase the transverse width of

the maxillary arch at the skeletal level by splitting the midpalatal suture.[2]

Treatment timing for RME is very important. The treatment effects of RME differ depending on the skeletal maturity of the patient.[6] As patients grow older, interdigitation of the midpalatal suture increases, making maxillary expansion more difficult.[7] Performing RME in a skeletally mature patient in which fusion of the midpalatal suture has occurred could lead to undesirable side effects such as buccal tipping of maxillary posterior teeth, alveolar bone bending, reduction of buccal bone thickness and marginal bone level, gingival recession, pain and increasing risk of relapse.[8-10] Hence surgically assisted rapid maxillary expansion (SARME) has been recommended in patients with advancing age.[11] However, there is no consensus in the literature about the time point to shift from RME to SARME.[11]

The start and the advance of fusion of the midpalatal suture vary greatly with age and sex.[12,13] There have been controversies regarding the age at which fusion of the midpalatal suture occurs. Understanding these variabilities is essential in treatment planning for RME.[7,13] For evaluation of the midpalatal suture, Angelieri *et al.*[13] classified the midpalatal suture into five stages using cone-beam computed tomography (CBCT) images. At stages A, B and C, the midpalatal suture was still open, and a conventional RME could be easily performed. At stages D and E, the midpalatal suture was partially or totally fused, hence patients in these stages might be better treated by SARME. This method has the potential to avoid undesirable effects of RME failure or unnecessary SARME, particularly in adolescents and young adults whom prognosis of RME is unpredictable. However, routine CBCT radiography of every patient is not recommended because of ethical concerns regarding unnecessary radiation exposure. Thus, a CBCT study of the midpalatal suture maturation could provide information and help in treatment planning for maxillary expansion. Unfortunately, there has been a lack of evidence regarding the maturation of the midpalatal suture and the relationship between chronological age and the midpalatal suture maturation in a Thai population. Furthermore, racial variations in the maturation have also been suggested. Therefore, the aims of this study were to evaluate 1. the prevalence of midpalatal suture maturation stages in 8 to 18 years old patients and 2. the relationship between chronological age and the suture maturation stages in a Thai population.

Materials and methods

The research protocol was approved by the Human Research Ethics Committee of Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2019-036). From 581 patients aged between 8 and 18 years old who underwent CBCT imaging at the Department of Radiology, Faculty of Dentistry, Chulalongkorn University between January 2013 and December 2018, 240 subjects (110 males, mean age 13.9± 2.8 y; 130 females, mean age 14.4±2.6 y) were consecutively selected based on the following inclusion criteria: good quality of CBCT images that displayed the entire midpalatal suture. The exclusion criteria were craniofacial syndromes, pathology in the maxilla that might affect the midpalatal suture, history of trauma in the maxillofacial region and orthodontic treatment. All CBCT images were taken for diagnosis purpose; therefore, no subjects received unjustified radiation exposure. The CBCT images were obtained using a 3D Accuitomo 170 machine (J. Morita, Kyoto, Japan) with 80-90 kV, 1-10 mA and 17.5 s exposure time. The field of view of the CBCT images was 8 x 8 or 10 x 10 cm with 0.165 or 0.25 mm voxel size. A 1-mm slice thickness was used. Infinitt® PACs software (Infinitt Healthcare Co., Ltd., Seoul, Korea) was used to adjust the patient's head position in three planes of space: in the coronal and axial views, the vertical reference line was positioned at the midsagittal plane; in the sagittal view, the horizontal reference line was adjusted so that it passed anteroposteriorly through the long axis of the palate, and positioned in the center of the supero-inferior dimension of the palate (Fig. 1).

The central cross-sectional axial slice of the midpalatal suture was used to determine the maturation stage. For subjects who exhibited a thick or curved palate,

two or more axial cross-sectional slices were assessed. All slices were saved as JPG files and randomly arranged in a Powerpoint (Microsoft Corp., Redmond, WA) presentation file with a black background; only random identification numbers were visible.

Two examiners (NC, KM) were trained and calibrated for classification and any disagreement discussed until consensus was obtained. For the main evaluation, all CBCTs were classified blindly by the principal examiner (NC). The midpalatal sutures were classified into five stages of development according to the protocol described by Angelieri *et al.*[13] (Fig. 2).

To evaluate the intra-examiner and inter-examiner agreement, 30 CBCT images were randomly selected and reclassified by the principal examiner (NC) and the second examiner (KM) 2 months after the main evaluation.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 22.0; IBM Corp, Armonk, NY). Intra-examiner and inter-examiner agreements were evaluated using weighted kappa statistics, and defined using the scale of Landis and Koch[14] (<0, poor; 0-0.20, slight; 0.21-0.40, fair; 0.41-0.60, moderate; 0.61-0.80, substantial; 0.81-1.00, almost perfect agreement). The distribution of maturation stages of the midpalatal suture according to chronological age and sex was compiled as absolute and percentage frequencies in a cross-tabs table. Chi-square test was applied to compare the prevalence of maturation stages between male and female subjects. Spearman's rank order correlation analysis was used to investigate the relationship between chronological age and midpalatal suture maturation stages. The level of significance was set at $p<0.05$ for all statistical calculations.

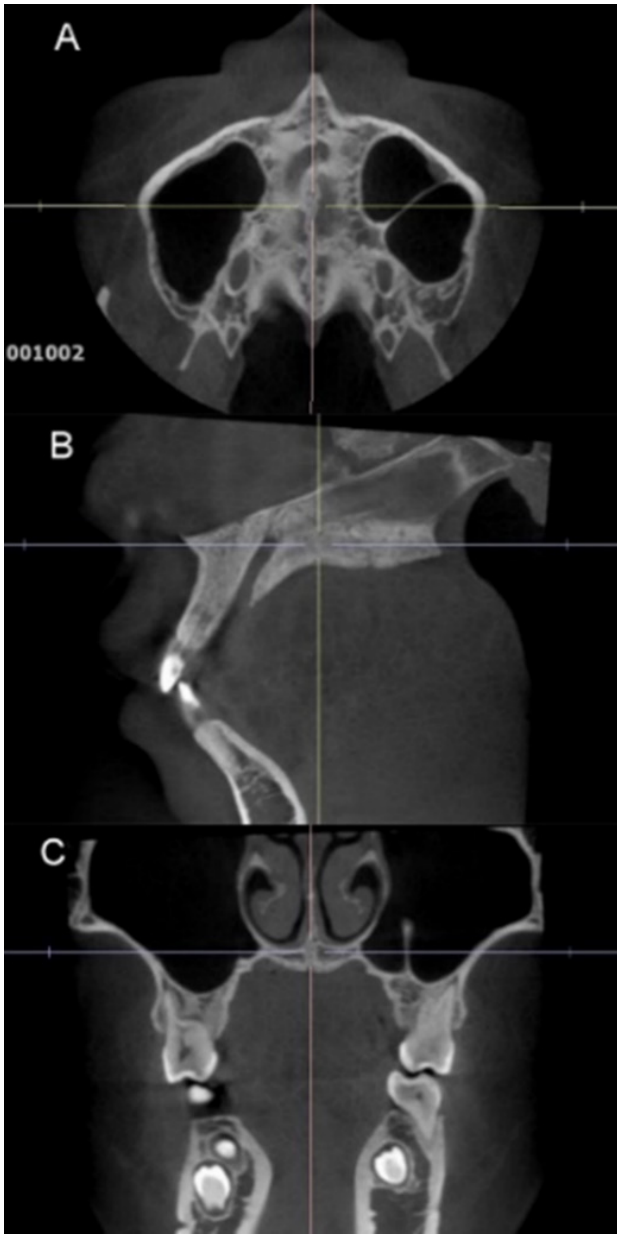


Figure 1 *Orientation of head position in three planes of space. A, axial; B, sagittal; and C, coronal views. Note that in B, the sagittal view, the horizontal line that indicates the position of the axial plane view is positioned through the center of the supero-inferior dimension of the hard palate (Infinitt® PACs software)*

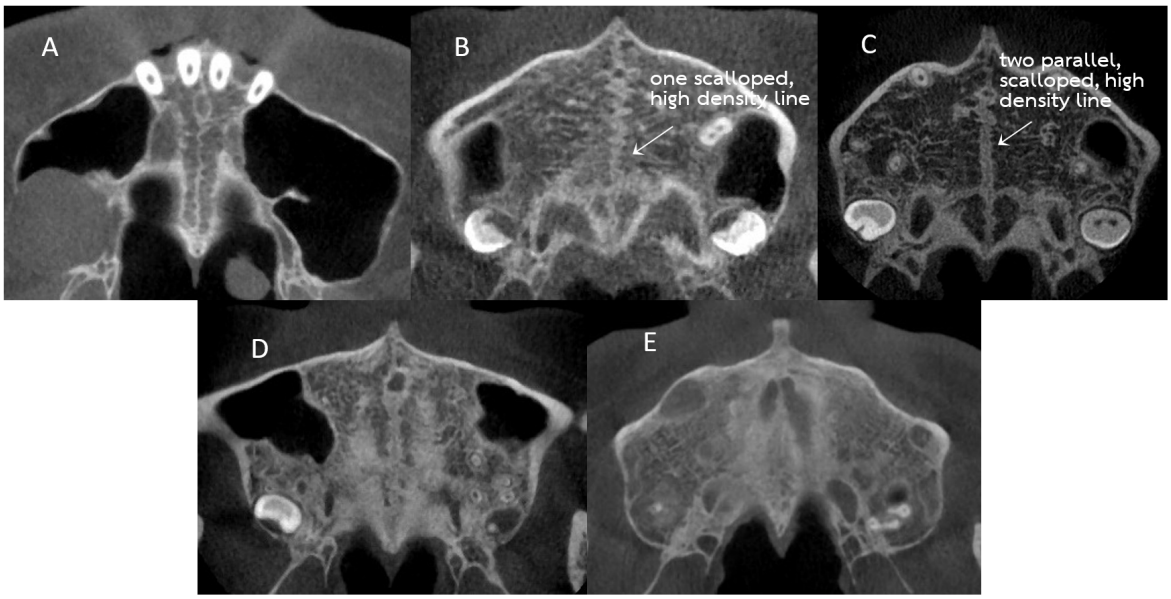


Figure 2 *Maturation stages of midpalatal suture. A) stage A, the suture is characterized by one relatively straight high-density line; B) stage B, the suture is observed as one scalloped, high density line at the midline. Stage B may present as two parallel, scalloped, high-density lines close to each other and separated by small low-density spaces in some areas. C) stage C, the suture is visualized as two parallel, scalloped, high density lines that are close to each other, separated by small low density spaces; D) stage D the suture is visualized as two scalloped, high density lines on the maxillary portion of the palate (anterior to the transverse palatine suture), but the suture cannot be identified in palatine bone (posterior to the transverse palatine suture); E) stage E, the suture cannot be identified along the maxillary and palatine bones, indicating the sutural fusion has occurred. Stage A-C (Nonfused midpalatal suture). Stage D-E (Fused midpalatal suture)*

Results

The weighted kappa coefficients for both intra-examiner and inter-examiner agreements were 0.94, demonstrating almost perfect intra-examiner and inter-examiner agreement. The distribution of the stages of midpalatal suture maturation are summarized in Table 1. The most prevalent in the study population was stage C (62.1%), followed by stage B (21.7%), stage D (10.8%), stage E (3.8%) and stage A (1.7%), respectively. The midpalatal suture was not fused in 85.4% of the total subjects. Both sexes had a higher prevalence of stage C, which was more frequent in females (females, 63.1%; males, 60.9%). Furthermore, in females, higher frequencies of stages D and E were observed (stage D, 16.2% in females, 4.5% in males; stage E, 3.8% in females, 3.6% in males). Neither females under 12 years old nor males under 14 years old had fusion of the suture. In general, the percentage of subjects who had fused midpalatal suture (stages D and E) increased with age.

Table 1 *Distribution of the midpalatal suture maturation stage by chronological age and sex*

Age (y)	Sex	MPS stage										Total
		A		B		C		D		E		
		n	%	n	%	n	%	n	%	n	%	
8	M			2	50	2	50					4
	F			1	50	1	50					2
	M+F			3	50	3	50					6

Table 1 *Distribution of the midpalatal suture maturation stage by chronological age and sex (cont.)*

Age (y)	Sex	MPS stage										Total
		A		B		C		D		E		
		n	%	n	%	n	%	n	%	n	%	
9	M			2	33.3	4	66.7					6
	F			1	20	4	80					5
	M+F			3	27.3	8	72.7					11
10	M	1	16.7	2	33.3	3	50					6
	F			2	40	3	60					5
	M+F	1	9.1	4	36.4	6	54.5					11
11	M			5	62.5	3	37.5					8
	F	1	16.7			5	83.3					6
	M+F	1	7.1	5	35.7	8	57.1					14
12	M			3	33.3	6	66.7					9
	F			4	26.7	10	66.7	1	6.7			15
	M+F			7	29.2	16	66.7	1	4.2			24
13	M			5	55.6	4	44.4					9
	F			4	36.4	6	54.5	1	9.1			11
	M+F			9	45	10	50	1	5			20
14	M	1	5.9	4	23.5	11	64.7	1	5.9			17
	F			2	9.5	15	71.4	4	19			21
	M+F	1	2.6	6	15.8	26	68.4	5	13.2			38
15	M					8	80	2	20			10
	F			1	6.3	13	81.3	2	12.5			16
	M+F			1	3.8	21	80.8	4	15.4			26
16	M			3	15.8	14	73.7			2	10.5	19
	F			2	14.3	7	50	5	35.7			14
	M+F			5	15.2	21	63.6	5	15.2	2	6.1	33
17	M			3	25	8	66.7	1	8.3			12
	F			2	12.5	8	50	4	25	2	12.5	16
	M+F			5	17.9	16	57.1	5	17.9	2	7.1	28
18	M	1	10	2	20	4	40	1	10	2	20	10
	F			2	10.5	10	52.6	4	21.1	3	15.8	19
	M+F	1	3.4	4	13.8	14	48.3	5	17.2	5	17.2	29
8-18	M	3	2.7	31	28.2	67	60.9	5	4.5	4	3.6	110
	F	1	0.8	21	16.2	82	63.1	21	16.2	5	3.8	130
	M+F	4	1.7	52	21.7	149	62.1	26	10.8	9	3.8	240

MPS, midpalatal suture; M, male; F, female

The distribution according to the midpalatal suture maturation stage is shown in Figure 3. Stage A was observed in subjects aged 10, 11, 14 and 18 years old. Stage B was observed at all ages, and the distribution tended to increase from 8 to 13 years of age, then decreased. Stage C was observed at all ages and tended to increase from 8 to 14 years of age, then decreased. Stage D was observed from 12 to 18 years of age, the distribution mainly being in the range of 14 to 18 years old. Stage E was observed from 16 to 18 years of age, mostly at 18 years of age. The distribution of stages D and E was notably in the older age group.

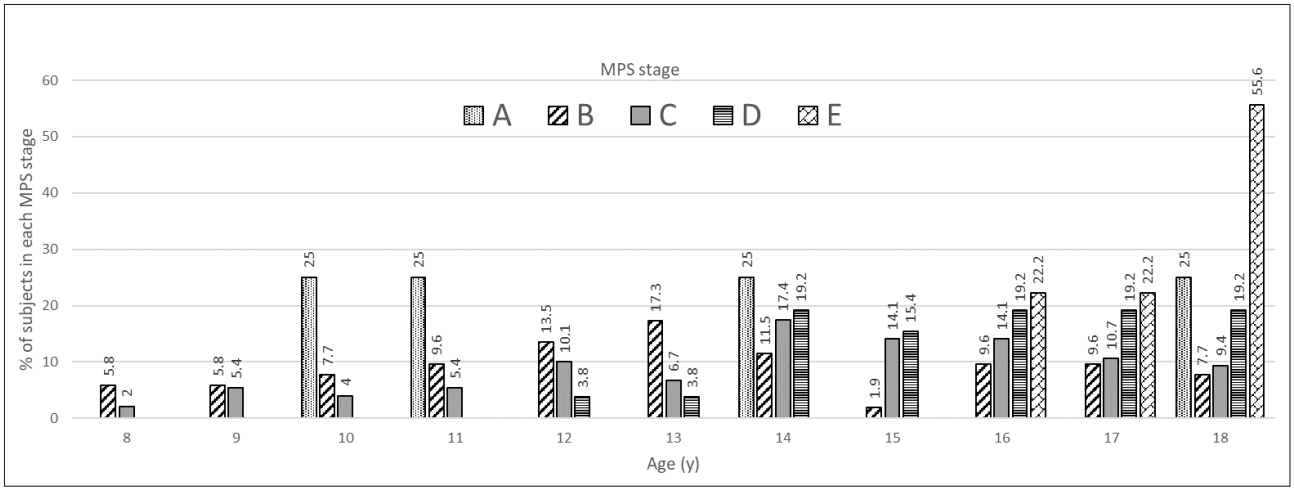


Figure 3 *Sample distribution according to the midpalatal suture maturation stage; MPS, midpalatal suture*

The comparison of the prevalence of fusion of the midpalatal suture by sex in the postpubertal age group (12-18 years old) is given in Table 2. There was a significant difference in the prevalence of fusion of the suture between the sexes. Females had a higher prevalence of fusion than males (23.2% and 10.5% in females and males respectively, χ^2 = 5.434, p=0.02).

Table 2 *Comparison of the prevalence of fusion of the midpalatal suture between the sexes in the postpubertal age group*

Sex	MPS stage		Total	Chi-square test, *p*-value
	Nonfused MPS (stage A, B, C)	Fused MPS (stage D, E)		
Male	77 (89.5%)	9 (10.5%)	86	χ^2 = 5.434,
Female	86 (76.8%)	26 (23.2%)	112	p = 0.02
Total	163 (82.3%)	35 (17.7%)	198	

MPS, midpalatal suture

In the study population, there was a weak correlation between chronological age and the midpalatal suture maturation stages (r=0.325, p<0.001). Females showed a slightly higher correlation coefficient than males (r=0.348, p<0.001 and 0.276, p=0.003 for females and males respectively).

Discussion

This study was performed to evaluate 1. the prevalence of midpalatal suture maturation stages in 8 to 18 years old patients and 2. the relationship between chronological age and the suture maturation stages in a Thai population. The results showed that fusion was

not observed in females under 12 years old and males under 14 years old, however, it was possible to find nonfused midpalatal suture in individuals older than these ages. Overall chronological age had a significant, but weak positive correlation with the maturation stage of the midpalatal suture.

Determining maturation stage of the midpalatal suture is important for RME therapy.[13,15] Many methods for assessment of the midpalatal suture have been proposed in the literature, including histological studies[7,12,16], evaluation of occlusal radiographs[17], micro-CT of autopsy material[18] and CBCT.[13] Histological and micro-CT evaluations require an invasive biopsy material, precluding its use in orthodontic patients.[15] Revelo and Fishman[17] used occlusal radiographs to assess the fusion of midpalaltal suture before RME therapy. However, the study by Wehrbein *et al.*[19] showed that an occlusal radiograph was unreliable to assess the fusion of the midpalaltal suture due to the superimposition of nearby anatomical structures. The diagnostic advantages of CBCT are its ability to visualize the midpalatal suture without such superimposition, allowing a reliable assessment of suture maturation.[20] In this study, we classified the midpalatal suture into five stages of maturation according to the method of Angelieri *et al.*[13] Although this classification method has the potential reliability and reproducibility for diagnostic purposes, it requires an extensive training program before it can be applied.[21,22] In the current study, the two examiners (NC, KM) were trained and calibrated before using this classification method, which was validated by the almost perfect intra-examiner and inter-examiner agreement.

The results of this study showed that there was a high prevalence (85.5%) of nonfused midpalatal sutures (stages A, B and C) in patients aged 8 to 18 years, consistent with the previous study by Angelieri *et al.*[13], who observed that 81.5% of subjects aged 5 to 18 years had nonfused midpalatal sutures. Furthermore, it should be noted that patients at prepubertal age (8-11 years old) had no stages D and E, indicating an absence of midpalatal suture fusion in this age group. These results are consistent with those of Angelieri *et al.*[13] and Tonello *et al.*[23], who observed a lack of subjects under 12 years of age in stages D and E. Furthermore, these findings support a previous study, which reported greater and more stable orthopedic changes when performing RME in patients under 12 years of age.[3] However, Jang *et al*.[24] observed stages D and E in some females aged 10 and 11 years old, probably because of the racial difference and the difference in classification method used in their study, in that they additionally investigated the suture on a coronal cross-sectional planar view and on volume-rendered images.

The findings of this study illustrate great variability in distribution of the maturation stages of the midpalatal suture, especially in the postpubertal age group, as subjects 12 years of age and above presented all stages of maturation. These results are consistent with Angelieri *et al.*[13], who observed all stages of maturation in subjects older than 11 years, and Ladewig *et al.*[25], who observed all stages in subjects aged 16 to 20 years. In addition, previous histological studies have also shown great variations in the ages of midpalatal suture fusion.[7,12,16,18] In the postpubertal age group (12-18 years old), it was observed that the nonfused stages (stages A, B and C) were seen in 82.3% of the subjects; this is quite similar to the study by Angelieri *et al.*[13] who observed that 75% of subjects aged 11 to 18 years were in the nonfused stages. These results demonstrated that there was a high possibility to find nonfused midpalatal suture in the postpubertal age group. Furthermore, we observed that the prevalence of fused midpalatal suture (stages D and E) gradually increased with increasing age, especially from 14 to 18 years of age, which is consistent with previous studies.[13,23,24]

The maturation of the midpalatal suture occurs earlier in females than in males, as verified in this study in which stage D was present in females from 12 years and in males from 14 years of age. These findings are consistent with previous studies[13,24] and correspond with the sex differences in the pubertal growth spurt that occurs approximately 2 years earlier in females than in males.[26] Furthermore, in the postpubertal age group, female subjects

had a significantly higher prevalence of fusion compared with male subjects. Other studies[13,24,25], although not statistically significant, also observed higher prevalence of fusion in female subjects than in male subjects. These findings have clinical relevance in that performing RME in the postpubertal age group might not be successful in some patients, particularly in female subjects. Thus, individual assessment of the suture might be considered in this age group.

In the present study, the correlation between chronological age and suture maturation stage was weak in both male and female subjects. Previous studies reported that the suture maturation stages were more consistent with skeletal age, such as hand-wrist bone age[24] and cervical vertebrae maturation (CVM)[24,27], rather than with chronological age. These indicated that the midpalatal suture maturation stages might not be reliably determined on the basis of the chronological age. However, chronological age may be a viable alternative to predict the maturation of the suture when the skeletal age cannot be assessed.[27] Shin *et al.*[28] also found that the midpalatal suture opening ratio had significant negative correlations with age, palate length, and midpalatal suture maturation stage in young adults and suggested that these parameters can be predictors of suture expansion.

Based on the results of this study, fusion of the midpalatal suture was not observed in females under 12 years old and males under 14 years old. However, the suture has not been fused in some older individuals. Thus, in order to reduce the risk of RME failure or unnecessary SARME, it would be useful to individualize assessment of the suture before performing maxillary expansion in females from the age of 12 and males from the age of 14. Female showed a significantly higher prevalence of fusion than male in the postpubertal age group. The correlation between chronological age and suture maturation stage was weak in both male and female subjects.

This study included a large sample in circumpubertal age group, i.e., from 8 to 18 years old. However, the limitation of our study was the small samples in the younger age group (8 to 11 years old), and further studies could include a larger sample size in this group. Furthermore, a longitudinal clinical study would be necessary to evaluate the clinical effectiveness of this classification method on predicting the treatment outcomes of RME. In addition, consideration should also be given to other features of anatomical resistance to maxillary expansion, such as zygomaticomaxillary, zygomaticotemporal and pterygopalatine sutures when performing RME therapy.[29,30]

Conclusions

Fusion of the midpalatal suture was not observed in females under 12 years old and males under 14 years old. However, the suture was not fused in some older individuals. Thus, from the age of 12 in females and 14 in males, CBCT might be recommended to verify the suture status before performing maxillary expansion. In the postpubertal age group, the possibility to find fusion of the suture was higher in females than in males. Overall, chronological age showed a weak correlation with the maturation stage of the midpalatal suture.

References

1. McNamara JA. Maxillary transverse deficiency. ***Am J Orthod Dentofacial Orthop*** 2000;117(5):567-70.
2. Haas AJ. The treatment of maxillary deficiency by opening the midpalatal suture. ***Angle Orthod*** 1965;35(3):200-17.
3. Wertz R, Dreskin M. Midpalatal suture opening: a normative study. ***Am J Orthod*** 1977;71(4):367-81.
4. Bishara SE, Staley RN. Maxillary expansion: clinical implications. ***Am J Orthod Dentofacial Orthop*** 1987;91(1):3-14.
5. da Silva Filho OG, Magro AC, Capelozza Filho L. Early treatment of the Class III malocclusion with rapid maxillary expansion and maxillary protraction. ***Am J Orthod Dentofacial Orthop*** 1998; 113(2):196-203.
6. Baccetti T, Franchi L, Cameron CG, McNamara JA. Treatment timing for rapid maxillary expansion. ***Angle Orthod*** 2001;71(5):343-50.
7. Melsen B. Palatal growth studied on human autopsy material. A histologic microradiographic study. ***Am J Orthod*** 1975;68(1):42-54.
8. Kılıç N, Kiki A, Oktay H. A comparison of dentoalveolar inclination treated by two palatal expanders. ***Eur J Orthod*** 2008;30(1):67-72.
9. Rungcharassaeng K, Caruso JM, Kan JYK, Kim J, Taylor G. Factors affecting buccal bone changes of maxillary posterior teeth after rapid maxillary expansion. ***Am J Orthod Dentofacial Orthop*** 2007;132(4):428.e1-8.

10. Betts N, Vanarsdall R, Barber H, Higgins-Barber K, Fonseca RJ. Diagnosis and treatment of transverse maxillary deficiency. ***Int J Adult Orthodon Orthognath Surg*** 1995;10(2):75-96.
11. Suri L, Taneja P. Surgically assisted rapid palatal expansion: a literature review. ***Am J Orthod Dentofacial Orthop*** 2008;133(2):290-302.
12. Persson M, Thilander B. Palatal suture closure in man from 15 to 35 years of age. ***Am J Orthod*** 1977;72(1):42-52.
13. Angelieri F, Cevidanes LH, Franchi L, Gonçalves JR, Benavides E, McNamara Jr JA. Midpalatal suture maturation: classification method for individual assessment before rapid maxillary expansion. ***Am J Orthod Dentofacial Orthop*** 2013;144(5):759-69.
14. Landis JR, Koch GG. The measurement of observer agreement for categorical data. ***Biometrics*** 1977;33(1):159-74.
15. Isfeld D, Lagravere M, Leon-Salazar V, Flores-Mir C. Novel methodologies and technologies to assess mid-palatal suture maturation: a systematic review. ***Head & face medicine*** 2017;13(1):13.
16. Knaup B, Yildizhan F, Wehrbein H. Age-related changes in the midpalatal suture. A histomorphometric study. ***J Orofac Orthop*** 2004;65(6):467-74.
17. Revelo B, Fishman LS. Maturational evaluation of ossification of the midpalatal suture. ***Am J Orthod Dentofacial Orthop*** 1994; 105(3):288-92.
18. Korbmacher H, Schilling A, Puschel K, Amling M, Kahl-Nieke B. Age-dependent three-dimensional microcomputed tomography analysis of the human midpalatal suture. ***J Orofac Orthop*** 2007; 68(5):364-76.
19. Wehrbein H, Yildizhan F. The mid-palatal suture in young adults. A radiological-histological investigation. ***Eur J Orthod*** 2001; 23(2):105-14.
20. Liu S, Xu T, Zou W. Effects of rapid maxillary expansion on the midpalatal suture: a systematic review. ***Eur J Orthod*** 2015;37(6):651-5.
21. Samra DA, Hadad R. Midpalatal suture: evaluation of the morphological maturation stages via bone density. ***Prog Orthod*** 2018;19(1):29.
22. Barbosa NMV, Castro ACd, Conti F, Capelozza-Filho L, Almeida-Pedrin RR, Cardoso MA. Reliability and reproducibility of the method of assessment of midpalatal suture maturation: A tomographic study. ***Angle Orthod*** 2019;89(1):71-7.
23. Tonello DL, Ladewig VM, Guedes FP, Ferreira Conti ACC, Almeida-Pedrin RR, Capelozza-Filho L. Midpalatal suture maturation in 11-to 15-year-olds: A cone-beam computed tomographic study. ***Am J Orthod Dentofacial Orthop*** 2017;152(1):42-8.
24. Jang HI, Kim SC, Chae JM, Kang KH, Cho JW, Chang NY, *et al.* Relationship between maturation indices and morphology of the midpalatal suture obtained using cone-beam computed tomography images. ***Korean J Orthod*** 2016;46(6):345-55.
25. Ladewig VM, Capelozza-Filho L, Almeida-Pedrin RR, Guedes FP, de Almeida Cardoso M, de Castro Ferreira Conti AC. Tomographic evaluation of the maturation stage of the midpalatal suture in postadolescents. ***Am J Orthod Dentofacial Orthop*** 2018;153(6): 818-24.
26. Proffit WR, Fields HW, Sarver DM. Contemporary orthodontics. Elsevier Health Sciences; 2014.
27. Angelieri F, Franchi L, Cevidanes LH, McNamara JA Jr. Diagnostic performance of skeletal maturity for the assessment of midpalatal suture maturation. ***Am J Orthod Dentofacial Orthop*** 2015;148(6): 1010-6.
28. Shin H, Hwang CJ, Lee KJ, Choi YJ, Han SS, Yu HS. Predictors of midpalatal suture expansion by miniscrew-assisted rapid palatal expansion in young adults: A preliminary study. ***Korean J Orthod*** 2019;49(6):360-71.
29. Timms DJ, Vero D. The relationship of rapid maxillary expansion to surgery with special reference to midpalatal synostosis. ***Br J Oral Surg*** 1981;19(3):180-96.
30. Chaconas SJ, Caputo AA. Observation of orthopedic force distribution produced by maxillary orthodontic appliances. ***Am J Orthod*** 1982;82(6):492-501.

Original Article

A Retrospective Comparative Study of Mandibular Stability and the Anteroposterior Dimension of the Airway between the Surgery-early and the Conventional Orthognathic Surgery after Bilateral Sagittal Split Ramus Osteotomy Setback

Patcha Chooputtipong[1] , Bancha Samruajbenjakun[2]

[1]Orthodontic Resident, Orthodontic Section, Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Songkhla, Thailand

[2]Orthodontic Section, Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Songkhla, Thailand

Abstract

The purpose of this study was to compare the skeletal and upper airway stability at 6 months post-surgical treatment between the surgery-early approach and the conventional orthognathic surgery in patients with skeletal Class III malocclusion who underwent one-jaw bilateral sagittal split ramus osteotomy setback surgery. Thirty-five patients were included and allocated into two groups based on pre-surgical orthodontic treatment: surgery-early group (n = 15) and conventional orthognathic surgery (n = 20). Lateral cephalometric radiographs were taken before surgery (T0), immediately after surgery (T1), and 6 months after surgery (T2). Independent t-test and Mann-Whitney U test were used to analyze the data between the two groups. Paired *t*-tests and Wilcoxon signed-rank tests were used to analyze the data in each group. At 6 months after surgery (T1-T2), forward, upward, and counterclockwise rotational movements of the mandible in both groups were observed with no statistically significant difference. Changes in upper airway dimensions, when compared between the pre-post surgical phase (T2-T0) revealed that the surgery-early group showed a statistically significant decrease ($p<0.05$) in the oropharynx, while the conventional orthognathic surgery group showed statistically significant decrease ($p<0.05$) in the oropharynx and hypopharynx. A comparison between the two groups at 6 months post-surgical treatment revealed no statistically significant difference. Dental movement in both groups had no statistically significant difference in either the vertical or anteroposterior movement. Compared with the conventional orthognathic surgery group, the surgery-early group showed an equal amount of mandibular movement and upper airway change at 6 months post-surgical treatment.

Keywords: Class III orthognathic surgery, Surgery-early, Upper airway

Received Date: Aug 8, 2022 **Revised Date:** Sep 16, 2022 **Accepted Date:** Dec 1, 2022

doi: 10.14456/jdat.2023.3

Correspondence to :

Bancha Samruajbenjakun, Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Songkhla 90112, Thailand. Email: samruaj@hotmail.com; Phone: 07428-7601; Fax: 07442-9875.

Introduction

In the past several years, it has been established that conventional orthognathic surgery (COS) with pre-surgical orthodontic treatment is an appropriate treatment in patients with severe skeletal discrepancies.[1] However,

the pre-surgical orthodontic phase contains leveling and aligning, and decompensation of the anterior teeth that worsen facial aesthetics with time during a period of about 15–24 months, especially among patients with skeletal Class III malocclusion where the lower lip protrudes according to the alignment of the lower teeth.[2]

The surgery-first/early approach (SEA) is a therapeutic strategy that consists of orthognathic surgery followed by post-surgical orthodontics without pre-surgical orthodontic treatment or minimal pre-surgical orthodontic treatment.[3] The surgery-first/early approach increases patient satisfaction with immediate profile improvement after surgery and shortens the total treatment time due to regional acceleratory phenomena, which include accelerated bone turnover and decreased regional mineral density resulting in faster tooth movement. Furthermore, the direction of post-surgical orthodontic treatment coincides with the natural direction of spontaneous dental compensation and muscular force.[4] Even though the surgery-first/early approach has many advantages, stable occlusion is not found immediately after surgery. More tooth movement is needed to settle the occlusion, unlike conventional orthognathic surgery. Therefore, surgical relapse due to occlusal instability is found to be greater in this group.[5]

The mandible, tongue and pharyngeal walls are closely related by their soft tissue attachments. Mandibular setback surgery can reduce pharyngeal airway volume and may lead to obstructive sleep apnea.[6,7]

Several studies have concluded that the surgery-first/early approach is a clinically acceptable and helpful approach; however, postoperative mandibular stability from post-surgical orthodontic treatment remains unclear, and none of the studies has compared post-surgical changes in the upper airway between patients in the COS and the SEA groups.

Our study aimed to assess the skeletal and upper airway stability 6 months after mandibular setback and compare the results between the COS group and the SEA group in skeletal Class III patients.

Materials and methods

Study subjects and power analysis

This retrospective study was conducted on patients with skeletal Class III who underwent surgery at the Prince of Songkla University between January 2014 and August 2020. Approval from the ethics committee was received from Prince of Songkla University(EC6303-009). The sample size was calculated using the G*Power 3.1 statistical program based on a significance level of 0.05 and a power of 80%. Power analysis showed that 19 patients were required for each group.

Inclusion criteria

- Class III patients who underwent one-jaw surgery with bilateral sagittal split ramus osteotomy (BSSRO) setback with non-extraction.
- Patients had three sets of good quality radiographs.
- The SEA group had minimal presurgical orthodontics not longer than 6 months.

The exclusion criteria included patients who underwent two-jaw surgery or genioplasty only.

The patients were subsequently allocated into two groups. The SEA group was patients who obtained initial leveling and aligning for less than 6 months (n = 15; 4 males and 11 females; mean age 27.9 ± 3.6 years). The COS group was patients who obtained complete presurgical orthodontic treatments (n = 20; 6 males and 14 females; mean age 24.9 ± 4.9 years).

Changes in the mandibular position and upper airway dimensions were retrospectively examined by measuring the lateral cephalometric radiographs (GXDP-700™, Gendex, PA, USA) at three time points: before surgery (T0), 4–6 weeks after surgery (T1), and at 6 months after surgery (T2). Dolphin Imaging software (Dolphin Imaging and Management Solutions, Chatsworth, California, USA) was used to digitize and analyze the data by one examiner who was blinded to the groups of patients.

Landmarks and reference planes

The landmarks and reference planes are shown in Figure 1 and 2. The measurements were divided into

three groups: (1) skeletal measurements: horizontal measurements, vertical measurements, and angular measurements (Fig. 1); (2) dental movements (Fig. 1); and (3) upper airway measurements (Fig. 2).

The horizontal reference plane was defined as the line that passes through the sella (S) and oriented 7 degrees inferior to the sella-nasion plane (SN7 plane). The line perpendicular to the SN7 plane through the S point was taken as the vertical reference plane (SN7 perp plane).

Pogonion (Pog) was selected to measure the mandibular position while the dental landmarks were mesial cusp tip of the upper and lower first molars. The distances between the SN7 perp plane and SN7 plane to the Pog and cusp tip were defined as horizontal measurements and vertical measurements, respectively. Rotational changes of the mandible were measured from the angle between the SN plane and the GoMe plane. Measurements of the upper airway were subdivided into three regions: nasopharynx; oropharynx; and hypopharynx. The nasopharynx was defined as the distance between the perpendicular line from the posterior nasal spine (PNS) to the upper posterior pharyngeal wall (UPW). The oropharynx was defined as the distance between the perpendicular line from the tip of the uvula (U) to the middle posterior pharyngeal wall (MPW), and the hypopharynx was defined as the distance between the perpendicular line from the vallecula (V) to the lower posterior pharyngeal wall (LPW).

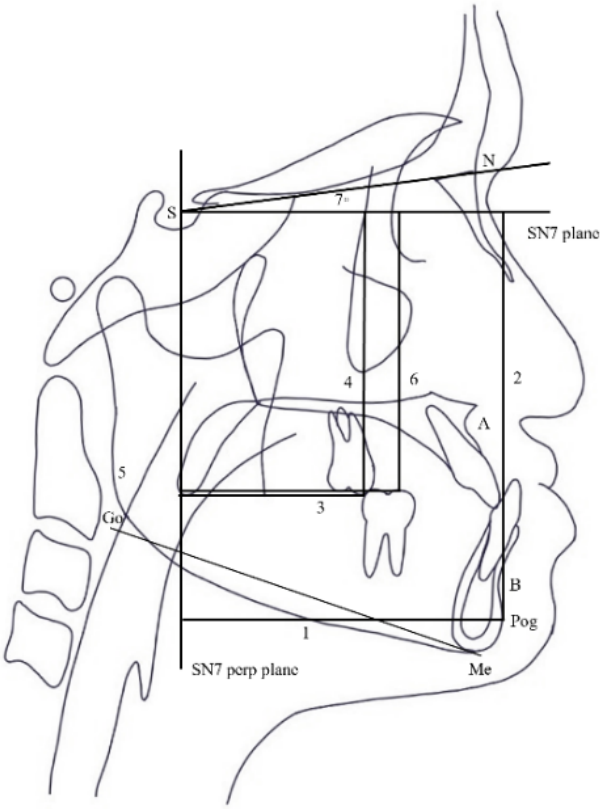


Figure 1 *Landmarks and reference planes for skeletal and dental measurements. Landmarks: S, sella; N, nasion; A, subnasale; B, supramentale; Pog, pogonion; Go, gonion; Me, menton; U6, upper molar; L6, lower molar. Reference planes: SN7 plane; perpendicular line of S to SN7 plane (SN7 perp plane). Skeletal and dental measurements (mm): 1. SN7 perp plane to Pog; 2. SN7 plane to Pog; 3. SN7 perp plane to U6; 4. SN7 plane to U6; 5. SN7 perp plane to L6; 6. SN7 plane to L6.*

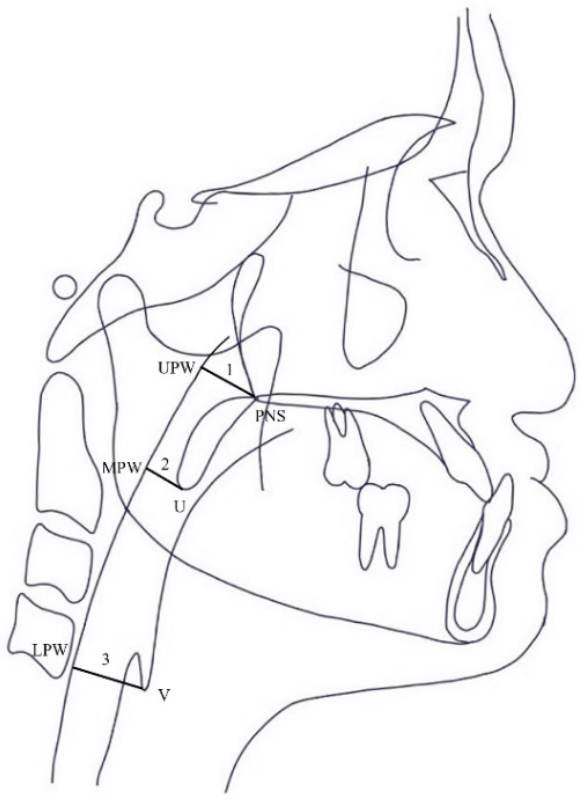


Figure 2 *Landmarks and reference planes for upper airway measurements. Landmarks: PNS, posterior nasal spine; U, uvula; V, vallecula; UPW, upper posterior pharyngeal wall; MPW, middle posterior pharyngeal wall; LPW, lower posterior pharyngeal wall. Upper airway measurements (mm): 1. PNS-UPW; 2. U-MPW; 3. V-LPW.*

Statistical analysis

Statistical analyses were performed using SPSS software, version 26 (IBM Corp., Armonk, NY, USA). Assessing the assumption of normality was accomplished by Shapiro-Wilk test. Postoperative movement of the mandible, dental, and anteroposterior changes of the upper airway in each group were analyzed using a paired t-test/Wilcoxon sign rank test, while changes between the SEA and the COS groups were determined using the independent sample *t*-test/Mann-Whitney U test. For all tests, two-sided *P* values <0.05 were considered significant.

All cephalometric measurements were repeated on ten randomly selected radiographs after a 1-month interval. The intra-observer reliability was high according to the correlation coefficients that were between 0.7916 and 0.8885.

Results

Comparison of demographic data between the SEA and the COS groups at the initial examination (T0)

No significant difference was observed in the skeletal measurements (SNA, SNB, ANB, Sn-GoMe), upper airway measurements (nasopharynx, oropharynx, hypopharynx), or overjet between the two groups (Table 1). However, overbite and interincisal angle were larger in the SEA group ($p<0.05$).

Comparison of surgical changes (T0-T1) and post-operative changes (T1-T2) in skeletal measurements

No significant difference was observed in mandibular setback distance between the SEA group (6.68 ± 3.46 mm) and the COS group (6.09 ± 3.93 mm). Rotational movement changed by 1.95 ± 2.55° in the SEA group and 1.23 ± 1.79° in the COS group with clockwise direction (Table 2). However, there was significant difference ($p<0.05$) between the groups in the vertical movement immediately after surgery. In the SEA group, downward movement (0.50 ± 1.89 mm) was observed, while upward direction (1.04 ± 2.79 mm) was observed in the COS group.

No significant difference in mandibular movement was observed at 6 months post-surgery between the two groups. The mandible moved forward in both groups: 1.44 ± 1.71 mm and 0.72 ± 2.05 mm in the SEA and the COS groups, respectively. In the vertical direction, the mandible moved upward in both the SEA group (0.59 ± 2.30 mm) and the COS group (0.97 ± 1.50 mm). The average rotational change in the counterclockwise direction was 0.52 ± 2.36° in the SEA group and 0.39 ± 1.32° in the COS group.

Comparison of the postoperative changes (T2-T0) in upper airway measurements

The anteroposterior dimension decreased with no significant difference between the two groups at the oropharynx and hypopharynx, while the nasopharynx was barely maintained through the follow-up period (Table 3).

At 6 months after surgery, the changes at the oropharynx from T0 to T2 were decreased to 1.76 ± 2.03 mm in the SEA group and 0.93 ± 2.66 mm in the COS group with statistically significant differences in SEA group ($p<0.05$).

After surgery from T0 to T2, the hypopharynx were decreased to 1.59 ± 2.43 mm in the SEA group and 1.52 ± 2.75 mm in the COS group with statistically significant differences in both groups ($p<0.05$).

Comparison of post-surgical changes (T1-T2) in dental measurements

The upper molar showed extrusion in the SEA group (0.62 ± 1.69 mm) and the COS group (0.13 ± 1.29 mm), and mesialization in the SEA group (1.09 ± 2.86 mm) and the COS group (0.47 ± 1.69 mm) without statistically significant difference between the two groups (Table 4). The lower molar presented extrusion in the SEA group (0.89 ± 2.24 mm) and the COS group (1.04 ± 1.39 mm) and distalization in the SEA group (1.38 ± 2.38 mm) and the COS group (1.26 ± 1.70 mm). However, there was no significant movement of the lower molars between the groups during this period (Table 4).

Table 1 *Comparison between the SEA and the COS groups at the initial examination (T0)*

Variable	SEA group		COS group		*p*-value
	Mean	SD	Mean	SD	
SNA	84.53	2.98	82.31	3.65	0.064
SNB	86.96	4.46	85.56	3.16	0.294
ANB	−2.41	3.64	−3.23	3.63	0.512
SN-GoMe	36.17	5.41	36.04	4.40	0.939
Overjet	−3.10	2.78	−4.31	2.66	0.201
Overbite	1.53	1.63	0.50	1.12	0.034*
Interincisal angle	132.49	7.39	122.73	7.73	0.001*
Nasopharynx	24.47	3.40	24.57	3.21	0.973
Oropharynx	11.75	2.80	11.51	3.59	0.894
Hypopharynx	15.81	3.77	17.29	3.44	0.161

nasopharynx: distance between the perpendicular line from the posterior nasal spine (PNS) to the upper posterior pharyngeal wall (UPW). oropharynx: distance between the perpendicular line from the tip of the uvula (U) to the middle posterior pharyngeal wall (MPW).

hypopharynx: distance between the perpendicular line from the vallecula (V) to the lower posterior pharyngeal wall (LPW).

**significance at p-value <0.05 (independent sample t-test)*

Table 2 *Measurement of mandibular movement*

Variable		T0		T1		T2		T0-T1		*p*-value between gr.	T1-T2		*p*-value between gr.
		Mean	SD	Mean	SD	Mean	SD	Mean	SD		Mean	SD	
SN7 perp -Pog (mm)	SEA	68.63	9.17	61.95	9.16	63.39	9.11	6.68	3.46	0.647	−1.44	1.71	0.550
	COS	69.6	8.59	63.58	7.70	64.30	7.69	6.09	3.93		−0.72	2.05	
SN7-Pog (mm)	SEA	103.7	8.06	104.28	7.85	103.69	7.62	−0.50	1.89	0.045*	0.59	2.30	0.278
	COS	103.8	8.92	102.84	8.38	101.86	8.21	1.04	2.79		0.97	1.50	
SN-GoMe (°)	SEA	36.17	5.41	38.13	4.53	37.61	5.66	−1.95	2.55	0.334	0.52	2.36	0.844
	COS	36.04	4.40	37.28	4.37	36.89	4.16	−1.23	1.79		0.39	1.32	

SN7 perp to Pog (+) backward movement of the mandible, (–) forward movement of the mandible.

SN7 to Pog (+) upward movement of the mandible, (–) downward movement of the mandible.

SN-GoMe (+) counterclockwise rotation of the mandible, (–) clockwise rotation of the mandible.

**significance at p-value <0.05 (independent sample t-test)*

Table 3 *Measurement of upper airway changes*

Variable		T0		T1		T2		T2-T0		*p*-value intra gr.	*p*-value between gr.
		Mean	SD	Mean	SD	Mean	SD	Mean	SD		
PNS-UPW (mm)	SEA	24.47	3.40	24.27	3.42	24.45	3.96	−0.02	3.50	0.983	0.784
	COS	24.57	3.20	24.37	3.20	24.30	3.30	−0.27	1.91	0.527	
U-MPW (mm)	SEA	11.75	2.80	10.14	3.54	9.99	3.99	−1.76	2.03	0.007*	0.321
	COS	11.51	3.59	10.36	3.11	10.58	3.39	−0.93	2.66	0.159	
V-LPW (mm)	SEA	15.81	3.77	15.23	3.76	14.22	4.53	−1.59	2.43	0.026*	0.941
	COS	17.29	3.44	16.06	3.48	15.77	4.67	−1.52	2.75	0.026*	

T1-T0, T2-T0 (+) increased upper airway dimension, (–) decreased upper airway dimension.

**significance at p-value <0.05 (Wilcoxon signed-rank tests)*

Table 4 *Comparison of tooth movements at post-surgical evaluation (T1-T2)*

Variable		T1		T2		T1-T2		*p*-value Intra gr.	*p*-value between gr.
		Mean	SD	Mean	SD	Mean	SD		
SN7-U6 (mm)	SEA	66.46	5.15	67.09	4.36	−0.62	1.69	0.162	0.537
	COS	66.12	4.94	66.34	5.04	−0.13	1.29	0.433	
SN7 perp-U6 (mm)	SEA	39.60	6.95	40.69	5.03	−1.09	2.86	0.201	0.714
	COS	43.09	5.08	43.52	3.39	−0.47	1.69	0.334	
SN7-L6 (mm)	SEA	68.07	5.42	68.37	5.59	−0.89	2.24	0.432	0.777
	COS	67.31	5.02	67.44	5.41	−1.04	1.39	0.506	
SN7 perp-L6 (mm)	SEA	40.43	7.09	40.49	7.16	−1.38	2.38	0.706	0.777
	COS	42.21	5.55	41.61	5.85	−1.26	1.70	0.191	

SN7-U6 (+) intrusion, (–) extrusion

SN7 perp-U6 (+) distalization, (–) mesialization

SN7-L6 (+) intrusion, (–) extrusion

SN7 perp-L6 (+) mesialization, (–) distalization

**significance at p-value <0.05*

Discussion

At the initial examination, all patients had a skeletal Class III relationship with a normal maxilla position and prognathic mandible with no difference in vertical growth to control the upper airway size.

The occlusion examinations revealed that the interincisal angle and overbite were greater in the SEA group. According to the compensatory mechanism in skeletal Class III patients, flaring of the upper incisors and lingual tipping of the lower incisors are generally observed. Therefore, in the SEA group that didn't receive a complete correction of these problems, the deep curve of Spee, flaring of the upper incisors, lingual tipping of the lower incisors, and increased overbite remained.

After surgical correction, mandibular displacement unavoidably occurs to some degree, and skeletal relapse occurs. Studies of skeletal relapse after mandibular setback for Class III patients have reported various results, including the amount of mandible setback (4.80 to 8.70 mm [mean = 6.49 mm]), forward movement (0.60 to 2.87 mm [mean = 1.49 mm]), and relapse rate (7.1% to 51.4% [mean = 22.6%]).[8] The results of this study revealed that the average amount of mandibular setback was 6.59 mm, which is close to a previous study, and the mandible moved forward with no significant difference between the groups during the post-surgical period. The average forward movement was 1.44 mm in the SEA group and 0.72 mm in the COS group, which was about 21% and 11.8% higher than the Ko *et al.*[9] study, which found a 14.3% forward movement in the SEA group and 15.7% in the COS group with no significant difference between the groups. However, the Kim *et al.*[10] study also found significant differences between the two groups and reported that the SEA group had greater relapse (3.14 mm) than the COS group (1.30 mm) due to counter-clockwise rotation of the mandible, which resulted in forward movement of Pog after correction of the occlusal interference in the SEA group.

When we compared the changes in the vertical dimension immediately after surgery, the SEA group showed a downward movement, while the COS group moved upward. This possibly occurred from a remaining deeper curve of Spee and presented dental interference in the SEA group. Six months after surgery, both groups presented an upward movement with no significant difference in distance. This is contrary to the Kim *et al.*[10]

study that found an increase in upward movement of 2.69 mm in the SEA group more than 0.93 mm in the COS group.

Clockwise rotation of the mandible during surgery in this study occurred in both groups, but didn't present a significant difference. At 6 months post-surgery, the angular measurements decreased. Counterclockwise rotation of the mandible was found in this period, but without significant difference between the two groups. The Kim *et al.*[10] study found counterclockwise rotation, but the SEA group showed increased angular movement in the counterclockwise direction that was greater than the COS group. The rotational movement might contribute to the forward and upward movement of the mandible. This indicates that the horizontal position of the mandible is equally displaced in the vertical dimension; therefore, the movement in these two directions may have the effect on rotational movement.

Changes in the mandibular position during post-surgical orthodontic treatment can be due to muscular tone, the amount of mandibular setback, fixation technique, pre-surgical orthodontic treatment, and autorotation from a settling process of the upper and lower posterior teeth.[11] The results of this study exhibited upper first molar extrusion and mesialization, and lower first molar extrusion and distalization. All findings showed no significant difference between the SEA and the COS groups. It might be the result of using Class III elastic to correct open bite on the posterior teeth.[12] In addition, there was no significant difference of mandibular and tooth movement.

According to some studies, the mandibular set-back can affect the upper airway.[6] Our findings confirm that upper airway changes need to be taken into account when planning surgical correction of dentofacial deformities. However, Park *et al.*[13] used cone-beam computed tomography which showed no significant changes of the nasopharyngeal or oropharyngeal airway in patients who underwent setback surgery. These results indicated lateral expansion of the soft tissue of the pharynx to preserve its volume. This present study showed that changes in the nasopharyngeal airway at 6 months post-surgery were not affected by mandibular setback surgery. These results are consistent with the studies by Engboonmeskul *et al.*[14]

At the 6-month post-surgical evaluation, the decrease in the anteroposterior dimension of oropharynx was founded in both groups in a ratio of 1:0.26–0.31 compared to the setback distance, but only the SEA group had a significant decrease compared with the initial examination. This agrees with the results of a study by Jeong *et al.*[15], which used CBCT superimposition to evaluate the three-dimensional morphologic changes in the upper airway space and found a decrease in the anteroposterior width of oropharyngeal region after 1-year mandibular setback surgery 14.2% and 18.0% in the CV1 and CV2 planes, respectively. However, recovery of the airway size was observed in some studies during the follow-up period.[16]

Together with the oropharynx, the hypopharynx significantly decreased in the anteroposterior dimension. A continued gradual decrease was shown in the hypopharynx and the size decreased in a ratio of 1:0.20–0.24 compared to the setback distance. This is in agreement with previous studies that found a continued reduction in the upper airway at the hypopharynx level for 2–6 years after surgery.[17,18] This occurs because this part of the upper airway is affected by the hyoid bone and muscles of the tongue that adjust to the new environment.

This study used a two-dimensional lateral cephalo-gram to analyze the pharyngeal airway space. The three-dimensional images might be a better method. However, they are more expensive and have a higher radiation dosage than lateral cephalogram. Riley and Powell[19] evaluated the reliability of CT scans and cephalograms in determining the posterior airway space and reported an acceptable result. Moreover, lateral cephalogram is widely used, less expensive, and simple for comparison with extensive normative data and with other studies.

Finally, no difference was observed between the SEA and the COS groups in the anteroposterior dimensions at all three levels of the upper airway, which is due to the similar amount of the mandibular setback.[20,21]

This study had some limitations. The small sample size possibly led to an increased type 2 error and affected the power of testing for statistical differences. The short observation period did not permit evaluation of the final complete occlusion. Further studies are needed to examine the treatment period to the end of complete orthodontic treatment to evaluate skeletal changes, especially upper airway changes.

Conclusions

At 6 months after surgery, Class III orthognathic surgery with or without presurgical orthodontic treatments showed no difference in the anteroposterior, vertical, or rotational change of the mandible, and the dimension of the upper airway. To achieve a good outcome of madibular setback surgery and prevent the occurrence of obstructive sleep apnea, careful and precise treatment plans about skeletal and soft tissue structures are needed.

Acknowledgments

We thank the Graduate School, Faculty of Dentistry, Prince of Songkla University for grant support. The authors declare no conflict of interest.

References

1. Worms FW, Isaacson RJ, Speidel TM. Surgical orthodontic treatment planning: profile analysis and mandibular surgery. ***Angle Orthod*** 1976;46(1):1–25.

2. Lee SJ, Kim TW, Nahm DS. Transverse implications of maxillary premolar extraction in Class III presurgical orthodontic treatment. ***Am J Orthod Dentofacial Orthop*** 2006;129(6):740–8.

3. Nagasaka H, Sugawara J, Kawamura H, Nanda R. "Surgery first" skeletal Class III correction using the Skeletal Anchorage System. ***J Clin Orthod*** 2009;43(2):97–105.

4. Choi JW, Lee JY, Yang SJ, Koh KS. The reliability of a surgery-first orthognathic approach without presurgical orthodontic treatment for skeletal class III dentofacial deformity. ***Ann Plast Surg*** 2015;74(3):333–41.

5. Sharma Vipul Kumar, Yadav Kirti, Tandon Pradeep. An overview of surgery-first approach: Recent advances in orthognathic surgery. ***J Orthod Sci*** 2015;4(1):9–12.

6. Kamano E, Terajima M, Kitahara T, Takahashi I. Three-dimensional analysis of changes in pharyngeal airway space after mandibular setback surgery. ***Orthod Waves*** 2017;76(1):1–8.

7. Tiner BD, Waite PD. Surgical and nonsurgical management of obstructive sleep apnea. Peterson's principles of oral and maxillofacial surgery, 2nd edn. BC Decker, Hamilton. 2004;1297–312.

8. Costa F, Robiony M, Sembronio S, Polini F, Politi M. Stability of skeletal Class III malocclusion after combined maxillary and mandibular procedures. ***Int J Adult Orthodon Orthognath Surg*** 2001;16(3):179–92.

9. Ko EW, Hsu SS, Hsieh HY, Wang YC, Huang CS, Chen YR. Comparison of progressive cephalometric changes and postsurgical stability of skeletal Class III correction with and without presurgical orthodontic treatment. ***J Oral Maxillofac Surg*** 2011;69(5):1469–77.

10. Kim JW, Lee NK, Yun PY, Moon SW, Kim YK. Postsurgical stability after mandibular setback surgery with minimal orthodontic preparation following upper premolar extraction. ***J Oral Maxillofac Surg*** 2013; 71(11):1968.e1–11.

11. Proffit WR, Phillips C, Turvey TA. Stability after mandibular setback: mandible-only versus 2-jaw surgery. ***J Oral Maxillofac Surg*** 2012; 70(7):e408–14.

12. Baek SH, Ahn HW, Kwon YH, Choi JY. Surgery-first approach in skeletal class III malocclusion treated with 2-jaw surgery: evaluation of surgical movement and postoperative orthodontic treatment. ***J Craniofac Surg*** 2010;21(2):332–8.

13. Park JW, Kim NK, Kim JW, Kim MJ, Chang YI. Volumetric, planar, and linear analyses of pharyngeal airway change on computed tomography and cephalometry after mandibular setback surgery. ***Am J Orthod Dentofacial Orthop*** 2010;138(3):292–9.

14. Engboonmeskul T, Leepong N, Chalidapongse P. Effect of surgical mandibular setback on the occurrence of obstructive sleep apnea. ***J Oral Biol Craniofac Res*** 2020;10(4):597-602.

15. Jeong S, Sung J, Kim S, Kim Y, Shin S, Kim SS. Upper airway morphologic changes after mandibular setback surgery in skeletal class III malocclusion patients measured using cone beam computed tomography superimposition. ***Int J Oral Maxillofac Surg*** 2018;47(11):1405–10.

16. Liou EJ, Chen PH, Wang YC, Yu CC, Huang CS, Chen YR. Surgery-first/early accelerated orthognathic surgery: postoperative rapid orthodontic tooth movement. ***J Oral Maxillofac Surg*** 2011;69(3):781–5.

17. Chen F, Terada K, Hua Y, Saito I. Effects of bimaxillary surgery and mandibular setback surgery on pharyngeal airway measurements in patients with Class III skeletal deformities. ***Am J Orthod Dentofacial Orthop*** 2007;131(3):372–7.

18. Greco JM, Frohberg U, Van Sickels JE. Long-term airway space changes after mandibular setback using bilateral sagittal split osteotomy. ***Int J Oral Maxillofac Surg*** 1990;19(2):103–5.

19. Riley RW, Powell NB. Maxillofacial surgery and obstructive sleep apnea syndrome. ***Otolaryngol Clin North Am*** 1990;23(4):809–826.

20. Foltan R, Hoffmannova J, Donev F, Vlk M, Sedy J, Kufa R, *et al.* The impact of Le Fort I advancement and bilateral sagittal split osteotomy setback on ventilation during sleep. ***Int J Oral Maxillofac Surg*** 2009;38(10):1036–40.

21. Kim HS, Kim GT, Kim S, Lee JW, Kim EC, Kwon YD. Three-dimensional evaluation of the pharyngeal airway using cone-beam computed tomography following bimaxillary orthognathic surgery in skeletal class III patients. ***Clin Oral Investig*** 2016;20(5):915–22.

Original Article

A Denture Cleansing Solution and Household Agents Differentially Affect the Surface Roughness of Acrylic Resin

Sita Thaworanunta[1], Naluemol Sriprasert[1], Chutimon Nanarong[2], Pichsinee Dittaratchaphong[2], Thananya Momin[2] , Thanpitcha Krisanawong[2]

[1]Prosthodontics Dept, College of Dental Medicine, Rangsit University, Pathumthani, Thailand

[2]College of Dental Medicine, Rangsit University, Pathumthani, Thailand

Abstract

This article evaluated the surface roughness of heat-cured acrylic resin before and after immersion in 4 different household agent solutions and a commercial denture cleansing solution after simulated 6-month and 12- month durations. Seventy-two specimens were fabricated from heat-cured acrylic resin and were divided into 6 groups (n=12); namely 4 household agents (100% clear vinegar, 5% acetic acid 0.1%, and 0.5% Sodium hypochlorite), a commercial denture cleansing solution, Polident® (Block Drug Company Inc, Memphis, TN38113, USA.) and tap water. The acrylic resin specimens were immersed for 10 min/cycle, 5 times/day for 36 days representing 6-month of clinical service, and continued for another 36 days representing 12-month of clinical service. The surface roughness (Ra, nm) was measured before and after simulated immersion. The data were compared using repeated ANOVA and Tukey's test. The mean difference in the Ra after the 6-month and 12-month immersions in the control group and the Polident®, 100% clear vinegar, and 5% acetic acid groups was not significantly different ($P > 0.05$). In contrast, the mean Ra in the 0.1% and 0.5% sodium hypochlorite groups was significantly higher ($P < 0.05$) after the 6-month immersion. However, the Ra increased with a diminishing value after the 12-month immersion. The Ra of the specimens immersed in 0.1% and 0.5% sodium hypochlorite was significantly increased after 6-month, which decreased by 12-month immersion. The Ra in the 100% clear vinegar and 5% acetic acid groups were not significantly different from that of the Polident® group. Therefore, 100% clear vinegar and 5% acetic acid, which are household agents, can be an alternative option for routine use. Further study should be performed to evaluate whether 0.1% and 0.5% sodium hypochlorite might be an alternative option for denture cleansing.

Keywords: Commercial denture cleansing solutions, Household agents, Heat-cured acrylic resin, Surface roughness

Received Date: Apr 26, 2022 **Revised Date:** May 23, 2022 **Accepted Date:** Nov 18, 2022
doi: 10.14456/jdat.2023.4

Correspondence to:
Naluemol Sriprasert , College of Dental Medicine, Rangsit University, 52/347 Muang-Ake, Phaholyothin Road, Lak-Hok, Muang, Pathumthani 12000 Thailand. E-mail: Naluemol.s@rsu.ac.th

Introduction

Thailand is becoming an aging society. The National Statistical Office of Thailand reported that in 2019, the aging population comprised 16.73 % of the total 66-millionThai population.[1] The Thai National Oral Health Survey by the Department of Health, Ministry of Public Health also revealed that 1 million elderly people wore complete dentures, and 4.9 million people wore removable partial dentures[2] Axe *et al.* found that denture wearers often suffered from the anxiety of further oral care problems and concerned about the esthetic problems of denture, malodor, and staining which may reveal denture wearing to others.[3] Therefore, appropriate denture cleaning is essential in plaque elimination, and maintaining good oral hygiene to eradicate all problems that denture wearers concern.

Acrylic resin was introduced as a denture base material in 1937. The reason for acrylic resins continued popularity in dentistry is the simple processing equipment required and the relatively low cost of the fabrication process.[4] The properties of a denture base which should be taken into account are biological properties, microbiological properties, and physical properties; which include surface roughness.[5] The surface roughness of a denture base is clinically meaningful and influences the amount of plaque and bacteria that accumulate on the denture.[6] *Candida albicans* is the most common opportunistic pathogen found in the oral cavity and can cause oral diseases, such as denture stomatitis. Increased porosity has been proven to increase microorganism colonization. The adhesion of *Candida albicans* to the surfaces is significantly affected by the interactions with other microorganisms in the oral cavity.[7] The acceptablesurface roughness to prevent plaque accumulation should be lower than the critical threshold of 0.2 μm.[6,8]

There are two major approaches for cleaning the denture base. The first approach is the mechanical method, such as brushing and an ultrasonic cleanser. These methods are effective in reducing and removing the biofilm.[9,10] However, brushing effectiveness can be reduced by poor manual skills of the denture wearer.

The second cleansing approach is the chemical cleaning method in which the denture base is immersed in different chemical agents, such as alkaline peroxides, alkaline hypochlorite, acids, and disinfectants.[9-11] These agents can be excellent tools because they reduce the amount of microorganisms adhering to the denture, compensate for possible limitations in brushing ability, have good acceptance by wearers, and are easy to acquire?.[12,13]

One of the chemical methods is immersion in a commercial denture cleansing solution. A good denture cleansing solution should not alter the denture properties, such as its color, dimensional stability, strength, and surface roughness.[14] In geriatric or disabled patients who are denture wearers, chemical denture cleansers can be a choice.[15]

Despite the effectiveness of commercial denture cleansing solutions, there are difficulties in finding them in the rural areas. If household agents, such as sodium hypochlorite and clear vinegar could be used to clean the denture and do not affect the surface roughness to the point of potentially increased bacteria/plaque accumulation, denture wearers would have an appropriate alternative to clean their dentures.

To evaluate the surface roughness of heat-cured acrylic resin before and after immersion in 4 different household agent solutions and a commercial denture cleansing solution for simulated 6-month and 12-month durations. The null hypothesis was that there were no significant differences in surface roughness between the immersion groups or immersion intervals.

Materials and Methods

1. Specimen preparation

The sample size calculation was performed using the G*Power 3.1.9.4® program. The calculation was performed using data from a prior study.[16]

Seventy-two 10x10x2 mm^3 disc-shaped specimens were fabricated from heat-cured acrylic resin using a stainless steel mold. The mold was designed with three layers, the upper and the lower parts were used as covers;

the center part had a 10x10x2 mm^3 disc-shaped space that was used to fabricate the specimens. These three parts were locked into one piece by screws. Therefore, all of the specimens were the same size. The mold used for preparing the test specimens was applied with separating medium. The heat-cured acrylic resin used was in the powder-liquid form. The powder and liquid was mixed at the ratio recommended by the manufacturer. When the mixture reached the dough stage, it was packed into the mold space and processed per the manufacturer's instructions. A short cure polymerization cycle (73°C for 90 min followed by 94°C for 30 min) was used. The specimens were removed from the molds and finished with 1000 and 2000 grit sandpaper, followed by a buffing polishing wheel. The other surfaces were marked with a number and left unpolished to be distinguished from the experimental surface that was measured by the surface roughness tester. Finally, the specimens were steamed in an ultrasonic cleaner.

2. Immersion procedure

A commercially available denture cleansing solution: Polident® (Block Drug Company Inc, Memphis, TN38113, USA) and 4 household agents, 0.1% and 0.5% sodium hypochlorite (Suksapan®, Thailand), 100% clear vinegar (อสร®, PFO FOOD co., ltd, Thailand), and 5% acetic acid (Suksapan®, Thailand) were used in this study. The immersion groups consisted of 4 household agents: 100% clear vinegar (pH 3.5), 5% acetic acid (pH 3.5), 0.1% and 0.5% Sodium hypochlorite (pH 7.5 and pH 8.0, respectively), the commercial denture cleansing solutions (pH 7.0) and tap water (pH 7) which served as a negative control. The pH in each group was determined using PL Precision LABORATORY® Litmas paper. The Polident® immersion solution was prepared using 1 Polident® tablet dissolved in 50 ml of tap water. The solutions were prepared in glass beakers at room temperature and the specimens were immersed horizontally.

After immersion in the respective solutions for 10 minutes/cycle, each test specimen was rinsed in running tap water for 2 min and immersed in a new respective solution, repeated for 5 cycles per day for 36 days, which is equivalent to 6-month of clinical service and continued for another 36 days representing 12-month of clinical service. When not immersed in the cleansing solutions, the specimens were stored in tap water.

3. Surface Roughness Measurement

The surface roughness (Ra) was measured at the central area of each specimen using a non-contact surface roughness tester (InfiniteFocus SL, Alicona®, Austria) at a speed of 0.5 mm/s. The speed of 0.5 mm/s was set for precisely detecting the surface roughness and the magnification of the objective lens was 50x. Each specimen was measured as an area. The Ra of each specimen was determined in three areas, and the mean Ra was calculated. The change in surface roughness was obtained by the difference in surface roughness between pre-immersion and post-immersion for 6-month and 12-month.

The specimens were divided into 6 groups (n=12). The surface roughness of the specimens in each group was measured using the non-contact surface roughness tester immediately after polishing and cleaning. The results demonstrated that the Ras of all groups were not significantly different at T0, confirming that the specimens had a similar surface roughness.

Table1 *Solutions used in the study*

Solutions	pH	Conc.	Brand	Immersion Time at room temp. (min/cycle)
Tap water (negative control)	7			10
Polident® (positive control)	7		Polident®, Inc, USA.	10
Clear vinegar	3.5	100%	อสร®,PFO FOOD., ltd, Thailand	10
Sodium hypochlorite (NaOCl)	7.5	0.1%	Suksapan®,PFO FOOD., ltd, Thailand	10
Sodium hypochlorite (NaOCl)	8.0	0.5%	Suksapan®,PFO FOOD., ltd, Thailand	10
Acetic acid	3.5	5%	Suksapan®,PFO FOOD., ltd, Thailand	10

3. Data analysis

The data analysis was performed using repeated measures analysis of variance (repeated measures ANOVA) and Post-hoc Tukey test to compare and evaluate the differences in surface roughness values between the groups. All statistical analyses were set at a significance level of < 0.05. The statistical tests were calculated using the SPSS 20.0 program (SPSS Inc., Chicago, IL, USA)

This experimental study was performed under ISO/TC212: Clinical laboratory testing and *in vitro* diagnostic test systems.

Results

The data was analyzed and confirmed to have homogeneity of variance and normality. Descriptive statistics was used to present the mean and standard deviation (S.D.) of the Ra by time (T0, T6, and T12) and by cleansing solution groups (Polident®, 100% clear vinegar, 5% Acetic acid, 0.1% sodium hypochlorite, 0.5% sodium hypochlorite, and Tap water) (Table 2). These results demonstrated no significant difference between the mean in the Ra in the control group and the Polident®, 100% clear vinegar, and 5% acetic acid groups ($P > 0.05$) after simulated the 6-month and 12-month immersions. However, the mean Ra in the 0.1% and 0.5% sodium hypochlorite groups was significantly higher ($P < 0.05$) after the 6-month immersion compared with the control group. Furthermore, the values of Ra increased with a diminishing value after the 12-month immersion in these groups. Based on these results, the null hypothesis was rejected.

Table 2 *Descriptive statistics of Ra in nm for the cleansing solution groups*

Solutions	Mean Ra (nm) ± S.D.		
	T0	T6	T12
Polident®	195.13 ± 8.92	197.84 ± 10.60^{A,1}	200.11 ± 11.12^{a,1}
100% clear vinegar	196.04 ± 8.92	195.13 ± 8.50^{A,1}	197.77 ± 8.70^{a,1}
5% Acetic acid	195.64 ± 8.94	198.25 ± 7.44^{A,1}	200.17 ± 7.44^{a,1}
0.1% NaOCl	193.72 ± 10.55	214.32 ± 13.81^{B,2}	229.07 ± 13.88^{b,2}
0.5% NaOCl	194.76 ± 7.42	218.84 ± 9.53^{B,2}	235.70 ± 11.16^{b,2}
Tap water	195.58 ± 8.69	195.80 ± 10.90^{A,1}	196.15 ± 11.00^{a,1}

** Similar superscript capital letters indicate no significant differences between groups at 6-month (left columns), similar superscript lowercase letters indicate no significant differences between groups at 12-month (right columns), and similar superscript numbers indicate no significant differences between 6-month and 12-month within each group (rows) according to Tukey's (HSD) test (p>0.05)*

The analysis of the mean in surface roughness of the heat-cured acrylic resin in 6 cleansing solutions using repeated measures ANOVA (Table 3).

Table 3 *Repeated measure ANOVA results*

Tests	Source	Type III Sum of Squares	df	Mean Square	F	*p*-value
Within-Subjects	TIME	7762.051	1	7762.051	600.311	0.000*
Contrasts	TIME * GROUP	10083.295	5	2016.659	155.967	0.000*
	Error (TIME)	853.383	66	12.930	853.383	
Between-Subjects	Intercept	8881715.333	1	8881715.333	31081.172	0.000*
Effects	GROUP	15004.404	5	3000.881	10.501	0.000*
	Error	18860.074	66	285.759		

Note: Asterisks indicate significance at the 99% confidence level

The first test was the test of within-subjects contrasts, which determined if the time used for testing (T0, T6, and T12) affected the surface roughness of the heat-cured acrylic resin. The results demonstrated that Time significantly affected the surface roughness of the heat-cured acrylic resin both directly (TIME) and indirectly (TIME * GROUP) at the 99% confidence level (Table 3).

The second test was the Between-Subjects Effects, which evaluated whether the different cleansing solutions (GROUP) (commercial denture cleansing solution, 100% clear vinegar, 5% Acetic acid, 0.1% sodium hypochlorite, 0.5% sodium hypochlorite, and Tap water) affected the surface roughness of the heat-cured acrylic resin. The results indicated that the GROUP significantly affected the surface roughness of the heat-cured acrylic resin at the 99% confidence level (Table 3).

The surface roughness of heat-cure acrylic resin immersed in groups of tap water (a negative control), commercial denture cleansing solution (a positive control), 100% clear vinegar, and 5% acetic acid had no significant difference in term of group and time. On the other hand, time and group had significant effect in groups of 0.1% and 0.5% Sodium hypochlorite

The next analysis classified the differences in the surface roughness of the heat-cured acrylic resin from the cleansing solutions (GROUP) using Tukey's test for Post-Hoc analysis.

Table 4 *The results of the Tukey's test for Post-Hoc analysis*

Solutions	N	Rate of Ra	
		Subset 1	Subset 2
Tap water	12	195.8422	-
100% clear vinegar	12	196.3142	-
Polident®	12	197.6931	-
5% Acetic acid	12	198.0206	-
0.1% NaOCl	12	-	212.3664
0.5% NaOCl	12	-	216.4336
***p*-value**		0.994	0.909

The Tukey's test for Post-Hoc analysis (Table 4) classified the treatments into 2 groups. The mean Ras in the tap water, 100% clear vinegar, 5% acetic acid, and Polident® groups were 195.8422, 196.3142, 197.6931, and 198.0206 nm, respectively, and they were not significantly different. In addition, the Ras in the 0.1% sodium hypochlorite (212.3664 nm) and the 0.5% sodium hypochlorite (216.4336 nm) were not significantly different.

Discussion

Denture cleansing is a necessary procedure that reduces the risk of oral infection and improves denture longevity. There are two methods to clean acrylic dentures: mechanical method, such as brushing, and chemical method, such as using a denture cleansing solution. Kurniawan *et al.* demonstrated that mechanical method by brushing dentures with toothpaste and chemical method by immersing in denture cleanser greatly increased the surface roughness, which causes more plaque retention.[17] Thus, this study focused on the chemical method, which is still the alternative to clean dentures to reduce biofilm formation due to their limited effect on surface roughness.

For daily use, patients soak their dentures in the cleansing solution for 10 min, then wash and store in tap water overnight. The present study simulated that situation using running tap water to clean the specimens for 2 min before immersing in a new solution in each cycle to eliminate the remaining cleansing solution. After the immersion cycles,

the specimens were stored in tap water representing soaking the denture overnight. Felipucci[18] revealed that ideally, denture cleansers should reduce or remove the biofilm without altering the physical and mechanical properties of the denture base material. However, many studies found that the daily use of denture cleansing solutions can affect the denture's mechanical and chemical properties, including the denture base material's color, surface roughness, and hardness.[9,15,17-23]

Most of the studies found that commercial denture cleansing solutions did not show any significant increase in term of surface roughness.[24,25] In our experimental study, immersing in a commercial denture cleansing solution (Polident®) was found to increase the surface roughness. Jørgensen found that Alkaline peroxides were the most commonly used cleansing solution in denture cleansers including Polident®.[26] Sodium percarbonate becomes a hydrogen peroxide when dissolved in water and releases an oxygen. The oxygen bubbles are supposed to exert a mechanical cleansing effect which is suspected to cause an increase in the surface roughness. However, the surface roughness between Polident® and tap water (a negative control) was not significantly different.

Acetic acid is one of the most important components of vinegar; which contains other by-products from the manufacturing method. Therefore, we diluted pure acetic acid to 5% which is the amount of acetic acid in clear vinegar, and used this as a comparative experimental group. The results indicated that the surface roughness in the clear vinegar and 5% acetic acid groups was not significantly different. We can also assume that these by-products do not affect the surface roughness of acrylic denture specimens.

Therefore, ester group in heat-cured acrylic resin are easily hydrolyzed with acids and formed numerous cracks on acrylic resin specimen. The number of cracks on the surface of heat cured acrylic resin causes surface irregularity and increases the roughness of the surface of the acrylic resin.[27] The results of the present study demonstrated that the surface roughness in the clear vinegar and 5% acetic acid groups was not statistically different from that of the positive control group.

Chau *et al.*[28] found that 10-minute immersion in 0.5% sodium hypochlorite eliminated microorganisms from the superficial and the inner surface of acrylic resin. de Sousa Porta *et al.*[20] stated that 0.5% sodium hypochlorite effectively reduced microorganisms without significantly changing the denture resin color or roughness. However, sodium hypochlorite significantly increased surface roughness.[23] In the present study, 0.1% and 0.5% sodium hypochlorite significantly increased the surface roughness, similar to the study by Porwal *et al.*[22] These researchers evaluated the effect of different denture cleansers on the color stability, surface hardness, and surface roughness of three denture base resin materials. The results demonstrated the most remarkable change in the surface roughness of conventional heat-cured acrylic resin when immersed in 0.5% sodium hypochlorite for 180 days. Sodium hypochlorite causes structural changes in the polymer matrix of acrylic resins. This effect could result in softening of the surface and, as a consequence, roughness could be expected to increase.[21]

Arruda *et al.* found that 0.1% sodium hypochlorite effectively removed the biofilm when used by participants with denture stomatitis.[19] Therefore, 0.1% sodium hypochlorite would be a better choice for denture cleansing compared to 0.5% sodium hypochlorite because it is less toxic and adequately removes biofilm. Göpferich and AlAmeer demonstrated that the degradation of heat cured acrylic material significantly increased when soaking in neutral or basic pH solutions.[29,30] The pH affects the degradation rates of the polymer because the breaking strength of the polymer depends markedly on the pH and is highest at neutral pH. In basic pH solutions, there is a high number of Hydroxyl ions, which is responsible for accelerating polymer degradation, thus increasing the surface roughness.[31]

The present study had limitations; the shape of the specimens did not resemble the denture shapes that reflect the patients' oral tissue anatomy. The present study focused on surface roughness only; thus, the effect of immersion on the other properties of denture base material requires further investigations.

Conclusion

The present study found that immersion in 0.1% and 0.5% sodium hypochlorite significantly increased the denture bases' surface roughness after 6- and 12-month immersions. The surface roughness in the other household groups was not significantly different from that of the commercial dentures cleansing solution and tap water groups. However, this study only evaluated 6- and 12-month immersion periods, and there may be other household agents that could be investigated in the future studies.

Acknowledgement

The authors gratefully acknowledge the financial support from the Research Institute of Rangsit University.

Reference

1. The Thai National statistical office, Demography population and Housing statistics [Internet]. 2019 [cited 2021 May 9]. Available from:http://statbbi.nso.go.th/staticreport/page/sector/en/01.aspx

2. Department of health, Ministry of public health. The Thai national oral health survey in 2017. ***Ministry of Public Health*** 2018;8(1):68-9.

3. Axe AS, Varghese R, Bosma M, Kitson N, Bradshaw DJ. Dental health professional recommendation and consumer habits in denture cleansing. ***J Prosthet Dent*** 2016;115(2):183-8.

4. Paranhos HF, Silva-Lovato CH, Souza RF, Cruz PC, Freitas KM. Effect of mechanical and chemical methods on denture biofilm accumulation. ***J. Oral Rehabil*** 2007;34(8):606–12.

5. Cervino G, Cicciù M, Herford AS, Germanà A, Fiorillo L. Biological and Chemo-Physical Features of Denture Resins. ***Materials*** 2020;13(15):3350.

6. Bollen M, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. ***Dent Mater*** 1997;13(4):258-69.

7. Al-Fouzan AF, Al-Mejrad LA, Albarrag AM. Adherence of Candida to complete denture surfaces in vitro: A comparison of conventional and CAD/CAM complete dentures. ***J Adv Prosthodent*** 2017;9(5):402-8.

8. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, van Steenberghe D. The influence of surface free energy and surface roughness on early plaque formation. An *in vivo* study in man. ***J Clin Periodontol*** 1990;17(3):138-44.

9. Paranhos HD, Bezzon OL, Davi LR, Felipucci DN, Silva CH, Pagnano VO. Effect of cleanser solutions on the color of acrylic resins associated with titanium and nickel-chromium alloys. ***Braz Oral Res*** 2014;28:1-7.

10. Shay K. Denture hygiene: a review and update. ***J Contemp Dent Pract*** 2000 Feb;1(2):28-41.

11. Gautham P, Mallikarjun M, Chakravarthy K, Kumar KR, Budege V, Bodankar N. Assessment of denture hygiene maintenance among elderly patients in Nizamabad (Telangana) population: A survey. ***J Dr NTR Univ Health*** 2016;5(4):275.

12. Jagger DC, Harrison A. Denture cleansing--the best approach. ***Br Dent J*** 1995;178(11):413-7.

13. Nishi Y, Seto K, Kamashita Y, Take C, Kurono A, Nagaoka E. Examination of denture-cleaning methods based on the quantity of microorganisms adhering to a denture. ***Gerodontology*** 2012; 29(2):259-66.

14. Sharma P, Garg S, Kalra NM. Effect of denture cleansers on surface roughness and flexural strength of heat cure denture base resin-an *in vitro* study. ***J Clin Diagnostic Res*** 2017;11(8):94.

15. Barochia J, Kamath S. Evaluation of the Effect of Denture Cleansers on the Surface roughness of hard denture base material: an *in vitro* study. ***Indian J Dent Res*** 2018;29(5):657.

16. Faul F, Erdfelder E, Lang AG, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. ***Behav Res Methods*** 2007;39(2):175-91.

17. Kurniawan AV, Dwifulqi H. Effects of brushing and immersion in denture cleanser on the surface roughness of polymethyl methacrylate. ***Sci Dent J*** 2019;3(3):75.

18. Felipucci D, Davi L, Paranhos H, Bezzon O, Silva R, Pagnano V. Effect of different cleansers on the surface of removable partial denture. ***Braz Dent J*** 2011;22(5):392-7.

19. Arruda CN, Salles MM, Badaró MM, Sorgini DB, Oliveira VC, Macedo AP, *et al.* Evaluation of biofilm removal and adverse effects on acrylic resin by diluted concentrations of sodium hypochlorite and Ricinus communis solutions. ***Gerodontology*** 2018;35(3):246-53.

20. de Sousa Porta SR, de Lucena-Ferreira SC, da Silva WJ, Del Bel Cury AA. Evaluation of sodium hypochlorite as a denture cleanser: a clinical study. ***Gerodontology*** 2015;32(4):260-6.

21. Paranhos HD, Davi LR, Peracini A, Soares RB, Lovato CH, Souza RF. Comparison of physical and mechanical properties of microwave-polymerized acrylic resin after disinfection in sodium hypochlorite solutions. ***Braz Dent J*** 2009;20:331-5.

22. Porwal A, Khandelwal M, Punia V, Sharma V. Effect of denture cleansers on color stability, surface roughness, and hardness of different denture base resins. ***Indian Prosthodont*** Soc 2017;17(1):61.

23. Sarasitsantikul B, Kunlayanapark R, Suttiat K. Effect of Sodium Hypochlorite Solution on Surface Roughness of Hard Denture Relining Resin Acrylic. ***CM Dent J*** 2014;35(2):105-14.
24. Sorgini D, Silva-Lovato C, Muglia V, Souza R, Arruda C, Paranhos H. Adverse Effects on PMMA Caused by Mechanical and Combined Methods of Denture Cleansing. ***Braz Dent J*** 2015;26(3):292-6.
25. Sharma P, Garg S, Kalra NM. Effect of Denture Cleansers on Surface Roughness and Flexural Strength of Heat Cure Denture Base Resin-An In vitro Study. ***J Clin Diagnostic Res*** 2017;11(8):94-7.
26. Budtz-Jørgensen E. Materials and methods for cleaning dentures. ***J Prosthet Dent*** 1979;42(6):619-23.
27. Sofya P, Rahmayani L, Purnama R. Effect of soft drink towards heat cured acrylic resin denture base surface roughness. ***Padj J Dent*** 2017;29(1):59-64.
28. Chau VB, Saunders TR, Pimsler M, Elfring DR. In-depth disinfection of acrylic resins. ***J Prosthet Dent*** 1995;74(3):309-13.
29. Göpferich A. Mechanisms of polymer degradation and erosion. ***Biomaterials*** 1996;17(2):103-14.
30. Al-Ameer SS. The influence of different pH of saliva and thermal cycling on the adaptation of different denture base materials. ***J Bagh Coll Dent*** 2012;24:47-53.
31. Cilli R, Pereira JC, Prakki A. Properties of dental resins submitted to pH catalysed hydrolysis. ***J Dent*** 2012;40(12):1144-50.

Original Article

Sensitivity of Brux Checker® in Grinding Bruxer

Donlatham Prommasen[1], Namrath Chatchaiyan[2], Somsak Mitrirattanakul[2]

[1]Department of Oral Diagnostic Science School of Dentistry, University of Phayao, Phayao, Thailand

[2]Department of Masticatory Science Faculty of Dentistry Mahidol University, Bangkok, Thailand

Abstract

Untreated bruxism can cause pathologic consequences to the components of the masticatory system leading to unnecessary treatments that are often complicated and costly. However, the damage could be prevented if the condition is diagnosed earlier. Bruxism patients with asymptomatic or mild jaw symptoms usually refused to admit that they grind their teeth. Therefore, a reasonably priced tool with high sensitivity that is comfortable to wear would be beneficial for early screening or diagnosing sleep bruxism. The aim of this clinical study is to investigate Bruxcore Plate (Brux Checker®) accuracy to diagnose sleep bruxism in known cases. Forty-four sleep bruxism participants with clear evidence of bruxofacets on the intraoral appliance were enrolled. Results showed that Brux Checker® had a sensitivity of 84.1% after one night of application and a sensitivity of 100% on four consecutive nights. In conclusion, at least four nights of Brux Checker® wearing is recommended for sleep bruxism diagnosis.

Keywords : Diagnostic sensitivity, Grinding bruxer, Intraoral device, Oral parafunction, Sleep bruxism

Received Date: Aug 3, 2022 **Revised Date:** Sep 5, 2022 **Accepted Date:** Oct 7, 2022
doi: 10.14456/jdat.2023.5

Correspondence to :
Namrath Chatchaiyan, Faculty of Dentistry, Department of Masticatory Science, Mahidol University, 6 Yothi Road, Ratchathewi District, Bangkok 10400 Thailand. Tel: 02-200-7856 Email: namrath.cha@mahidol.ac.th

Introduction

Bruxism is a repetitive jaw-muscle activity characterized by clenching or grinding of the teeth and/or bracing or thrusting the mandible. Bruxism has two distinct circadian manifestations: it can occur during sleep (indicated as sleep bruxism) or during wakefulness (indicated as awake bruxism).[1] Awake bruxism is a semi-voluntary bite often associated with stress about daily life or work.[1] Sleep bruxism (SB) is medically defined by the International Classification of Sleep Disorders, 3rd edition (ICSD-3) as a sleep-related movement disorder characterized by teeth grinding or clenching associated with an excessive sleep arousal activity.[2] Bruxism has been reported to be caused by forces that are transmitted during tooth contact for 20 minutes or more than two hours of tooth contact.[3]

Bruxism is a common condition. Studies showed the self-reported prevalence of 8-16% in the general adult population with similar numbers in males and females. Bruxism begins about one year after deciduous incisors appear in the oral cavity. A survey of children and adolescents found bruxism rates between 14-20% and tended to

decrease when they got older.[4] In particular, only 3% of bruxism was found in adults over 60 years of age. However, it should be considered that many older patients wearing dentures could still have bruxism. In addition, several psychotropic medications taken by the elderly could aggravate the bruxism condition.[5,6]

The etiology of bruxism is multifactorial; however, the exact cause is still unknown. There is an assumption that there may be four factors.[1,7] 1) Pathophysiological factors and the central nervous system such as genetics, neurochemicals disrupt the balance of nerve impulses in the basal ganglia, causing a disturbance in dopamine transmission similar to Parkinson's disease or brain injuries and diseases. 2) Psychosocial factors: daytime teeth grinding is associated with psychosocial conditions, especially stress.[8] 3) Factors related to drugs: drugs affecting the dopaminergic system such as amphetamine, nicotine, serotonin ecstasy, alcohol, caffeine, tobacco, selective serotonin reuptake inhibitors, benzodiazepines.[8] 4) Physical factors such as the anatomical structure of the jaw, face and oral cavity.[8]

The cause of bruxism is controversial, but it can be separated into teeth grinding while conscious and while sleeping with different disease factors. Awake bruxism can be determined by accumulated daily stress.[9] The occurrence of muscle spasms associated with sleep bruxism caused by the central nervous system and may be related to shallow sleep, also known as slight arousal (micro-arousal).[10]

Several methods were used to evaluate bruxism. A questionnaire could be a useful tool in assessing this condition. The advantage of questionnaires is being able to collect data among large populations efficiently, but the disadvantage is underestimation or overestimation in bruxism patients. Many of them could not identify themselves as bruxers, especially those who sleep alone. Thus, the questionnaire was limited in terms of accuracy.[5,11,12] Clinical examination, one of the most common methods for bruxism assessment, could be performed by looking for signs and symptoms of bruxism in the patient's oral cavity, such as wear facets, scalloped tongue and linea alba buccalis. However, these signs and symptoms should not be used in the clinical diagnosis of bruxism alone.[13-17] Measurement of muscle function or electromyography (EMG) is considered close to the standard of sleep laboratory testing.[5] However, the disadvantage involves other confounding factors that may cause interpretation error due to other orofacial movements, such as swallowing, sneezing, or coughing during sleep or sleepwalking.[5]

The gold standard for sleep bruxism evaluation is Polysomnography (Sleep laboratory), which is the most accurate but costly. Therefore, an inexpensive tool or test to diagnose bruxism with specificity, sensitivity, and accuracy that increases patient incorporation in using will benefit dentists and patients in assisting the assessment of bruxism. The assessment of bruxism by using oral devices can be divided into the following two groups:

1. Observation wear facets type:

1.1 Occlusal Splint: The result is assessed from the wear facets on the appliance.[18,19]

1.2 Bruxcore Plate (Brux Checker®): The Brux Checker® is used to clinically diagnose bruxism that facilitates to record and evaluate a patient's parafunctional activity patterns. It presents the wear marks of static and dynamic occlusal contacts, the identification of physiological or unphysiological tooth contacts, and the classification and differentiation of the dynamic occlusal scheme. The assessment of these patterns in the context of occlusion diagnostics enables the development of a precise, personalized treatment plan for each patient based on their respective current bruxism pattern.[20]

It is fabricated with a 0.51 mm thick polyvinyl chloride color-coated plate (Table 1), that is shaped according to the patient's dental anatomy, using a vacuum pressure machine.[20]

Table 1 *Demonstration of physical, chemical, mechanical and biological properties of Brux Checker® (SCHEU DENTAL GmbH, 2019)*[21]

General properties		
Properties	**Guideline**	**Value**
Form	-	Solid
Colour	-	Transparent film with a red paint layer
Odour	-	Odourless
Density	ISO 1183	1.33 g/cm^3
Water absorption after 24 h at 23°C	ISO 62 Method 1	-
Mechanical properties		
Tensile strength	ISO 527	> 42 MPa
Flectional strength	ISO 527	-
Impact strength 23°C	ISO 179	600 KJ/m^2
Notched 23°C	ISO 179	-
Yield strain	ISO 527	-
Yield stress	ISO 527	-
Elasticity	DIN 53377	+/- 4%
Elongation at tear	ISO 527	-
E-modulus	ISO 527	-
Hardness Shore D	DIN 53505	~ 78
Thermal properties		
Vicat softening point	ISO 306 / Verfahren B/50	74°C
Thermoform resistance	ISO 75	55°C
Continuous stress temperature	ISO 75	55°C
Biological properties / Biocompatibility		
The material has been tested on biocompatibility according to DIN EN ISO 10993		

Similar to an occlusal splint, the result is positive when wear facets appear after use, According to the recommendation of Prof. Sadao Sato, after one night use enabled the diagnosis of bruxism.[21,22,23]

2. Measurement of bite force type: Intra-splint force detector (ISFD) is an instrument that measures the bite force of bruxism when teeth come into contact with the tool.[24]

Even though there is a lack of standardized indications for scoring and evidence supporting its validity, the results obtained from these instruments may not reflect the current state of bruxism because of the variety of bruxism at night. The findings may not be the bruxism results and could be the disturbances associated with recording methods.[25-27] Owing to the convenience and practicality, the oral appliance has become increasingly common for bruxism assessment. Patients with bruxism may not know or refuse that they are bruxers. Therefore, inexpensive tools with high sensitivity like oral appliances are suitable for diagnosing this group of patients. This research focused on studying Bruxcore Plate (Brux Checker®)'s sensitivity test in diagnosing bruxism in known sleep bruxism patients and determining the relationship between sex, age range, and instrument (Brux Checker®) sensitivity.

Materials and method

Study design: This research was modeled as human experimental research.

Sample size calculation and Sampling method

The sample size was calculated based on the Brux Checker® determination to indicate bruxism in 80%

of patients with bruxism, 90% confidence interval, and sensitivity of Brux Checker® was equal to 0.8 ± 0.1 based on sample size for a descriptive study with dichotomous variable. Total of 44 participants were required. A random sampling method used systematic random sampling by dividing the sample into males and females and selecting from the pool within three months.

Inclusion criteria

1. Thai people between the ages of 18 - 60 years with at least two pairs of occluded posterior teeth on each side.
2. Patients who had been treated for bruxism by using an intraoral appliance in Michigan splint or flat plane stabilization splint design in which the material was heat-cured clear acrylic resin, and showing wear facets from bruxism.

Exclusion criteria

1. Having sore teeth, gums, or symptoms of periodontal disease in the past six months.
2. Parafunction of the jaw muscles resulting from other sleep disorders, medical or neurological disorders, drug or substance abuse.
3. Allergy to polyvinyl chloride and acid red 51 food coloring.

Brux Checker® fabrication and Data collection

Upon passing the inclusion criteria, participants were instructed to photograph the wear facets on the occlusal splint to confirm being the true bruxer. Then the alginate impression was taken to fabricate a dental model with dental stone. The dental model was then sent to a laboratory to construct the Brux Checker® from the polyvinyl chloride plate using vacuum pressure machine (Bio Star®). (Fig. 1) Brux Checker® was given to the participant for familiarity and observation of allergic reactions to polyvinyl chloride and acid red 51 food coloring for ten minutes. (Fig. 2) Then the participant was instructed to wear Brux Checker® at bedtime and observe for the number of nights when the wear marks on Brux Checker® first appeared. Once the recording was completed, the participant inserted the Brux Checker® on the dental model or in the box to prevent tearing and sent it back to the researcher. (Fig. 3)

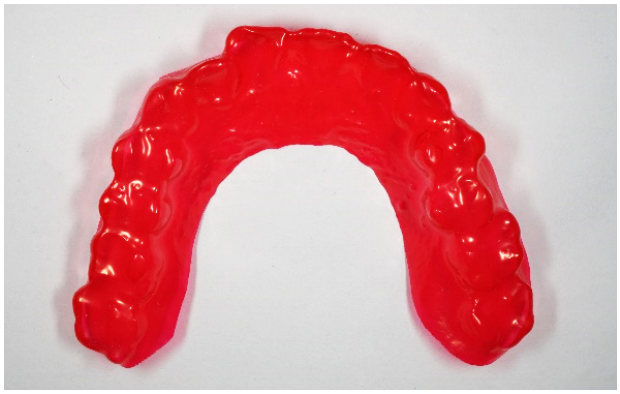


Figure 1 *Finished Brux Checker®*

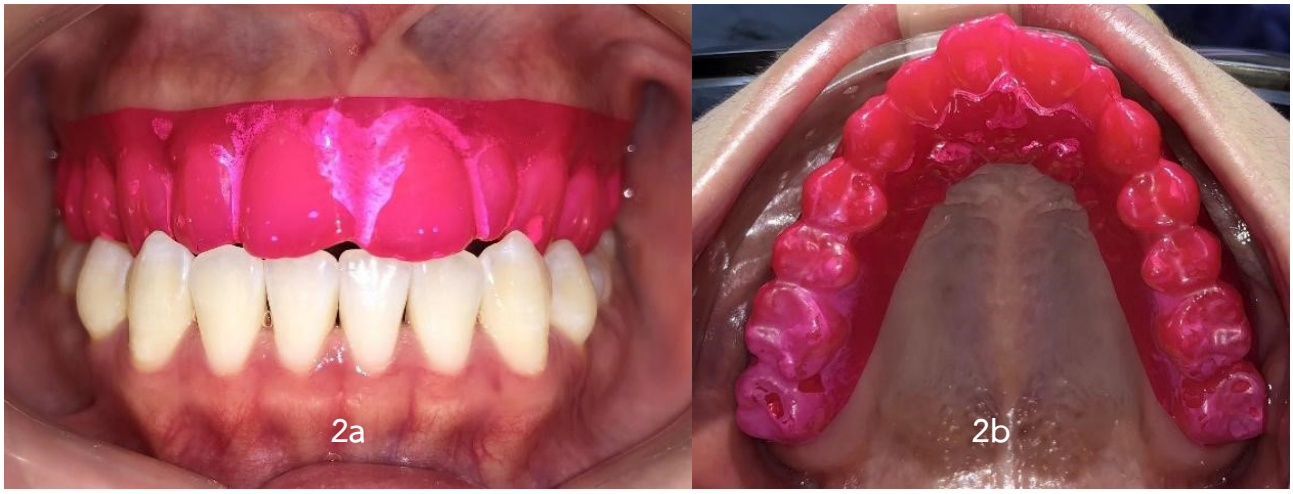


Figure 2 *Wearing a Brux Checker® in the subject; 2a in frontal view, 2b in occlusal view*

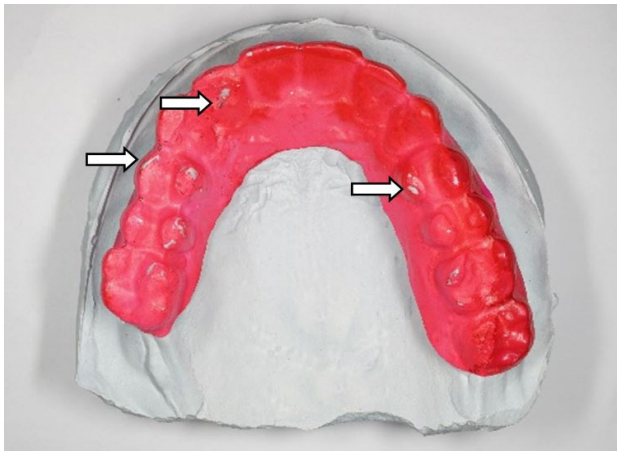


Figure 3 *Brux Checker® with wear marks*

Data analysis

The information gathered and the nominal scale data, such as sex, age range, the number of participants with wear marks on the first night and the number of nights wearing the Brux Checker® until the wear marks appeared were distributed into groups. The sensitivity of the Brux Checker® for each night was calculated based on the number of samples who reported the presence of the wear marks on the Brux Checker® after the first night of wearing it as well as the other subsequent nights that the wear marks initially appeared. The relationship between gender and Brux Checker®'s sensitivity and age range and Brux Checker®'s sensitivity were determined using Chi-square association statistics and the Fisher's Exact Test.

The study was accredited by the Human Research Ethics at the Faculty of Dentistry and Faculty of Pharmacy Mahidol University No. MU-DT/PY-IRB2018/053.140.

Results

The frequency distribution data obtained from 44 participants was analyzed in four categories: gender, age range, the presence of wear marks on the Brux Checker® on the first night, and the presence of wear marks on the Brux Checker® on other nights by using descriptive analysis. According to these data, there were 10 males (22.7%) and 34 females (77.3%). Based on the age range, there were 11 participants with the ages between 18 to 24 years old (25%) and 33 participants at the ages ranging from 25 to 60 years old (75%). (Table 2)

Table 2 *Demonstration of numbers of the participants classified by gender, age range, and the presence of wear marks on the Brux Checker® on the first night*

N = 44	Independent variable	Participants N (%)	Wear marks on the first night N (%)
Gender	Male	10 (22.7 %)	9 (20.45 %)
	Female	34 (77.3 %)	28 (63.64 %)
Age range	18 – 24 yrs.	11 (25 %)	8 (18.18 %)
	25 – 60 yrs.	33 (75 %)	29 (65.91 %)

In this study, the wear marks presented on the Brux Checker® after the first night use were found in 37 participants of which the sensitivity was 84.1%. The numbers of the positive results detected after the second and third nights were increased to three participants per night and equaled to 6.8% and were consistent with the cumulative sensitivity of 90.9% and 97.7%, respectively. On the fourth night, all participants had positive results with 100% of cumulative sensitivity. (Table 3)

Table 3 *Demonstration of the frequency of the participants with wear marks presented on the Brux Checker® from the 1st to 4th night*

Night with wear marks presented	Numbers of participants with positive wear marks N (%)	Cumulative sensitivity (%)
1st night	37 (84.1 %)	84.1
2nd night	3 (6.8 %)	90.9
3rd night	3 (6.8 %)	97.7
4th night	1 (2.2 %)	100

The expected ideal sensitivity of the diagnostic tool should approach 1. In this study, the sensitivity after using Brux Checker® for one night was 0.841 or 84.1%.

The results of Fisher's Exact Test of the data with frequency less than 5 in 1 cell of 2x2 table of total data set, revealed no significant correlation between gender, or age range and detected wear marks on the Brux Checker® used on the first night with *p*-value > 0.05 and = 0.341, respectively. (Tables 4 and 5)

Table 4 *Demonstration of the correlation between gender and detected wear marks on the Brux Checker® used on the 1st night, p-value > 0.05*

Count		Wear marks on the 1st night		Total
		Positive	Negative	
Sex	Male	9	1	10
	Female	28	6	34
Total		37	7	44

Table 5 *Demonstration of the correlation between age range and detected wear marks on the Brux Checker® used on the 1st night, p-value = 0.341*

Count		Wear marks on the 1st night		Total
		Positive	Negative	
Age range	18 – 24 yrs.	8	3	11
	25 – 60 yrs.	29	4	33
Total		37	7	44

Discussion

While the authors expected to have the sensitivity of the diagnostic tool approaching or equaling to 1 or 100%, the sensitivity of Brux Checker® was as high as 84.1% after one night of use. However, the sensitivity was 100% after four nights of use. This can be due to the variable force of teeth grinding of individuals during the week which can lead to absent or shallow wear marks on intraoral appliance in mild bruxers.[28]

Initially, the study was designed in a randomly systematically sampling manner to minimize the bias during the sample collection, and the distribution of data and study group population were expected to be normal. However, during the experiment, the number of female participants was approximately three times greater than males, and most participants in our study were in the working age. All these factors might affect data interpretation.

Interestingly, the correlation between age range and sensitivity of the Brux Checker® tested on the first night revealed that the sensitivity of Brux Checker® in diagnosis of bruxism in participants between 18 to 24-year-old was approximately four times lower than in 25 to 60-year-old participants. Firstly, this can be due to the skewed distribution of the data resulting from the great difference regarding the numbers of populations between 25 to 60-year-old and 18 to 24-year-old participants. In our study, the number of participants between 25 to 60-year-old was three times greater than the number of participants between 18 to 24-year-old. Therefore, the results may not be reflective of a true correlation between the age range and the sensitivity of the tool. Another possibility is the psychological condition from stress and an urban lifestyle which can contribute to the severity of bruxing activity during sleep.[29] In addition, most 18 to 24-year-old participants were university students in bachelor program, thus they could suffer from academic study stress and personal issues.[30] On the other hand, the 25 to 60-year-old

participants were primarily under work pressure and stress from several life-factors.[31] All of these conditions can be associated with the severity of sleep bruxism. Similar to the age range factor, the data distribution of the gender was not in normal distribution because of the difference in numbers of populations between males and females which could affect the results of data analysis. This can be due to several uncontrolled confounding variables. In the present study, there was no statistical significance between gender, age range, and bruxing activity detected on the first night. However, the correlation between these factors cannot be definitively excluded due to the above-mentioned possibilities. On the contrary, some literature suggested that gender displayed an association with bruxism.[32] Some study explained that females likely paid more attention to their oral health than males resulting in an increased incidence of bruxism in female population.[33]

The aim of the future study is to focus on the identification of correlation between factors that can impact on the presence of wear marks on the Brux Checker®, particularly gender and age range. In addition, the study population selection method should be altered in order to obtain the optimal normal distribution of the study data.

Unfortunately, there are few studies on the use of the Brux Checker® in diagnosing bruxism, and none of the studies report on how many nights to wear Brux Checker®. Only patterns of tooth wear marks have been reported in the form of horizontal grinding and vertical grinding, and the results are used for the diagnosis and treatment of bruxism.[3,17,34]

Our study found that some participants did not give positive results after the first night. Therefore, the participants should continue to wear Brux Checker® until a clear mark appeared since all participants were known bruxism cases according to wear facets on their occlusal splints.

Our hypothesis would be accepted with the confidence interval of 90%. The power of the test of the diagnostic tool was only at 80% for this study. This could be due to limitations in the funding budget and the criteria for study participant recruitment might affect the number of participants. However, if we consider the confidence interval of our study at 95-99% and increase the population size, the sensitivity and accuracy of the Brux Checker® might increase. In addition, the Polysomnography test should be applied in a future study to obtain the true negative results in the participants who do not have sleep bruxism for specificity calculation.

Conclusion

In this study, the Brux Checker® has high sensitivity at 84.1 % with 100% cumulative sensitivity after four-night usage. Thus, the authors suggest that Brux Checker® should be used at least for four consecutive nights as a preliminary bruxism diagnostic method.

Acknowledgements

The authors would like to thank Dr. Namrath Chatchaiyan, Assoc. Prof. Dr. Somsak Mitrirattanakul, and all faculty members of the Department of Masticatory Science, Mahidol University for their expert academic opinions and suggestions. Our sincere gratitude also extends to all participants in this study for their cooperation to accomplish this study.

References

1. Koyano K, Tsukiyama Y, Ichiki R, Kuwata T. Assessment of bruxism in the clinic. ***J Oral Rehabil*** 2008;35(7):495-508.

2. International Classification of Sleep Disorders. 3rd ed. Westchester, Darien, Illinois: American Academy of Sleep Medicine. Sleep related bruxism. ***AASM*** 2014:246-53.

3. Onodera K, Kawagoe T, Protacio-Quismundo C, Sasaguri K, Sato S. The use of a bruxchecker in the evaluation of different grinding patterns during sleep bruxism. ***Cranio*** 2006;24(4):292-9.

4. Sari S, Sonmez H. The relationship between occlusal factors and bruxism in permanent and mixed dentition in Turkish children. ***J Clin Pediatr Dent*** 2001;25(3):191-4.

5. Lavigne GJ, Cistulli PA, Smith MT. ***Sleep medicine for dentists.*** Chicago, IL: Quintessence. 2009;210.

6. Lavigne G, Montplaisir J. Restless legs syndrome and sleep bruxism: prevalence and association among Canadians. ***Sleep*** 1994;17(8):739-43.

7. Mohl N, Zarb G, Carlsson G, Rugh J. A Textbook of Occlusion. Chicago: Quintessence Publ. Co. Inc; 1988.

8. Van Selms M, Lobbezoo F, Wicks D, Hamburger H, Naeije M.

Craniomandibular pain, oral parafunctions, and psychological stress in a longitudinal case study. ***J Oral Rehabil*** 2004;31(8):738-45.
9. Powell R, Zander H. The frequency and distribution of tooth contact during sleep. ***J Dent Res*** 1965;44(4):713-7.
10. Powell R. Tooth contact during sleep: association with other events. ***J Dent Res*** 1965;44(5):959-67.
11. Karras SC, Wolford LM, Cottrell DA. Concurrent osteochondroma of the mandibular condyle and ipsilateral cranial base resulting in temporomandibular joint ankylosis: report of a case and review of the literature. ***J Oral Maxillofac Surg*** 1996;54(5):640-6.
12. Pollak P, Vincken W, Munro I, Cosio M. Obstructive sleep apnea caused by hemarthrosis-induced micrognathia. ***Eur J Respir Dis*** 1987;70(2):117-21.
13. Bakland LK, Christiansen EL, Strutz JM. Frequency of dental and traumatic events in the etiology of temporomandibular disorders. ***Dent Traumatol*** 1988;4(4):182-5.
14. Ross LA, Saint-Amour D, Leavitt VM, Javitt DC, Foxe JJ. Do you see what I am saying? Exploring visual enhancement of speech comprehension in noisy environments. ***Cereb Cortex*** 2007;17(5):1147-53.
15. Solomon NP, Robin DA, Luschei ES. Strength, endurance, and stability of the tongue and hand in Parkinson disease. ***J Speech Lang Hear Res*** 2000;43(1):256-67.
16. Hesse J, Van Loon L, Naeije M. Subjective pain report and the outcome of several orthopedic tests in craniomandibular disorder patients with recent pain complaints. ***J Oral Rehabil*** 1997; 24(7):483-9.
17. Tokiwa O, Park B-K, Takezawa Y, Takahashi Y, Sasaguri K, Sato S. Relationship of tooth grinding pattern during sleep bruxism and dental status. ***Cranio*** 2008; 26(4):287-93.
18. Holmgren K, Sheikholeslam A, Riise C. Effect of a full-arch maxillary occlusal splint on parafunctional activity during sleep in patients with nocturnal bruxism and signs and symptoms of craniomandibular disorders. ***J Prosthet Dent*** 1993;69(3):293-7.
19. Korioth TW, Bohlig KG, Anderson GC. Digital assessment of occlusal wear patterns on occlusal stabilization splints: a pilot study. ***J Prosthet Dent*** 1998;80(2):209-13.
20. Greven, Markus & Onodera, Kanji & Sato, Sadao. The use of the BruxChecker in the evaluation and treatment of bruxism. ***CMF*** 2015;7:249–259.
21. "Product Datasheet BRUX CHECKER®." SCHEU DENTAL GmbH, Jul 2019, http://products.scheu- dental.com/documents/5000/1-DOC/0/0/0/0/3/Datasheet_BRUXCHECKER_Original_3053.pdf
22. Shetty S, Pitti V, Babu CS, Kumar GS, Deepthi B. Bruxism: a literature review. ***J Indian Prosthodont Soc*** 2010;10(3):141-8.
23. Pierce C, Gale E. Methodological considerations concerning the use of bruxcore plates to evaluate nocturnal bruxism. ***J Dent Res*** 1989;68(6):1110-4.
24. Takeuchi H, Ikeda T, Clark GT. A piezoelectric film-based intrasplint detection method for bruxism. ***J Prosthet Dent*** 2001; 86(2):195-202.
25. Simões-Zenari M, Bitar ML. Factors associated with bruxism in children from 4-6 years. ***Pro Fono*** 2010;22(4):465-72.
26. Sugimoto K, Yoshimi H, Sasaguri K, Sato S. Occlusion factors influencing the magnitude of sleep bruxism activity. ***Cranio*** 2011; 29(2):127-37.
27. Svensson P, Jadidi F, Arima T, BAAD-HANSEN L, Sessle B. Relationships between craniofacial pain and bruxism. ***J Oral Rehabil*** 2008;35(7):524-47.
28. Manfredini D, Winocur E, Guarda-Nardini L, Paesani D, Lobbezoo F. Epidemiology of bruxism in adults: a systematic review of the literature. ***J Orofac Pain*** 2013 Spring;27(2):99-110.
29. Giraki M, Schneider C, Schäfer R, Singh P, Franz M, Raab WH, Ommerborn MA. Correlation between stress, stress-coping and current sleep bruxism. ***Head Face Med*** 2010;6:2. doi: 10.1186/1746-160X-6-2.
30. Carra MC, Bruni O, Huynh N. Topical review: sleep bruxism, headaches, and sleep-disordered breathing in children and adolescents. ***J Orofac Pain*** 2012 Fall;26(4):267-76.
31. Ahlberg J, Lobbezoo F, Ahlberg K, Manfredini D, Hublin C, Sinisalo J, *et al.* Self-reported bruxism mirrors anxiety and stress in adults. ***Med Oral Patol Oral Cir Bucal*** 2013;18(1):e7-11.
32. Nakata A, Takahashi M, Ikeda T, Hojou M, Araki S. Perceived psychosocial job stress and sleep bruxism among male and female workers. ***Community Dent Oral Epidemiol*** 2008;36(3):201-9.
33. Parveen N, Ahmed B. Oro dental health: Awareness and practices. ***J Univ Med Dent Coll*** 2011;2(2):5-10.
34. Kapusevska, Biljana & Stojanovska, Vera & Mijoska, Aneta. Use of brux checker in patients with different types of bruxism. ***Acta Stomatologica Naissi*** 2014;30:1325-1331.

Original Article

Protein Expression after Gingival Injection of mRNA Encoding Platelet-derived Growth Factors-BB in Ligature-induced Periodontitis Model in Rats

Pimphorn Meekhantong[1,2], Wichaya Wisitrasameewong[1,3,4], Noppadol Sa-Ard-Iam[4], Theeraphat Chanamuangkon[5], Somchai Yodsanga[6], Pimprapa Rerkyen[4], Rangsini Mahanonda[1,3,4]

[1]Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[2]School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand
[3]Center of Excellence in Periodontal Disease and Dental Implant, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[4]Immunology Research Center, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[5]Biomaterial Testing Center, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[6]Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

Abstract

The year 2021 marks the breakthrough of the COVID-19 mRNA vaccine as a new class of medicine. The same fundamentals of the mRNA-based vaccine could facilitate the development of mRNA-based regenerative therapy. Our research group is working on mRNA encoding growth factors for periodontal regeneration in patients with periodontitis. The objective of this study was to investigate protein expression after local administration of mRNA encoding platelet-derived growth factor-BB (PDGF) encapsulated in lipid nanoparticles (PDGF mRNA) in ligature-induced rat periodontitis. 3-0 silk was placed around the maxillary left second molar for two weeks and then removed, while the maxillary right second molar was left non-ligated. A significant bone loss analyzed by a stereomicroscope and micro-computerized tomography and gingival bleeding at the ligature sites were observed as compared to the non-ligature sites. To evaluate transfection and protein translation, different doses of PDGF mRNA including low dose (3 µg), medium dose (10 µg), high dose (30 µg), and DPBS (control) and LNPs alone were injected into rat gingiva at palatal side. The translated PDGF protein production was assessed 24 hours after the injection using enzyme-linked immunosorbent assay (ELISA). High levels of PDGF production were detected at both ligature and non-ligature sites. The mean PDGF levels in mRNA treated groups ranged from 10,912.54±1,893.94 to 51,883.91±7,415.45 pg/mg protein, whereas levels in DPBS control and LNPs alone were negligible. PDGF protein expression showed a trend of dose response but the differences between doses were not significant. Clinical findings at injected sites showed no erythema or swelling. The histological findings showed no evidence of LNPs and other foreign substances of mRNA formulation remaining in the tissues. In conclusion, this study of 24 hour-local delivery of PDGF mRNA-LNPs into rat periodontitis results in highly translated PDGF protein without profound local inflammatory response. However, further studies into the *in vivo* kinetics and therapeutic efficacy of mRNA are required.

Keywords: ligature-induced periodontitis, LNPs, mRNA, PDGF, periodontitis

Received Date: Aug 4, 2022 **Revised Date:** Sep 6, 2022 **Accepted Date:** Oct 7, 2022
doi: 10.14456/jdat.2023.6

Correspondence to:
Rangsini Mahanonda, Center of Excellence in Periodontal Disease and Dental Implant, 10th Floor Somdejya 93 Building, Faculty of Dentistry, Chulalongkorn University, 34 Henri-Dunant Road, Wangmai, Patumwan, Bangkok, 10330, Thailand. Tel: 02-218-8849 Fax: 02-218-8851
E-mail: r_mahanonda@yahoo.com

Introduction

For the past few decades, intensive research based on tissue engineering strategies for periodontal tissue regeneration has been the use of stem cells, scaffold, and signaling molecules. Many growth factors such as platelet-derived growth factors-BB (PDGF), insulin-like growth factors, fibroblast growth factors, bone-morphogenetic protein (BMP) have shown potential to regenerate the lost periodontal tissues.[1] Recombinant human PDGF-BB (rhPDGF), GEM 21S® (Osteohealth, USA), has become the first growth factor product, which was clinically approved by the U.S. FDA in 2005 for periodontal regeneration. However, one of its limitations in clinical application is the relatively short half-life of growth factors *in vivo*, which typically range from several hours to days. As a result, supraphysiologic doses or several administrations are required, and such high doses of growth factors may cause undesirable side effects and increase the cost of therapy.[2] Gene therapy is considered as an alternative approach to address the drawbacks of protein delivery. The gene therapy involves a delivery of DNA or mRNA encoding protein of interest into the cells, thus allowing cell transfection and protein translation to occur. Hence, one's own body makes the desired protein by themselves.[3]

In 2021, mRNA-based technology emerged in the field of medicines by the first mRNA vaccine against SARS-CoV-2, which was approved by the U.S. FDA.[4] This mRNA vaccine uses nucleoside-modified mRNA encoding, a spike protein antigen, which is encapsulated with lipid nanoparticles (LNPs). It is known that nucleoside modified mRNA translated more protein than unmodified mRNA by suppressing an inflammatory response, which can interfere with protein translation.[5] Encapsulation of mRNA with LNPs can protect mRNA from degradation by extracellular ribonucleases mRNA and make mRNA more stable.[6] Hence, mRNA-LNPs technology has been recognized as the most advanced platform in medicine. Now more than a billion doses of mRNA vaccine have been used around the world showing that the mRNA vaccine is both safe and effective.[7] Likewise, the same mRNA technology platform could be applied for mRNA-based therapeutics.

In the field of mRNA-based regenerative medicine, Zangi *et al.,* (2013) showed that the use of modified mRNA encoding human vascular endothelial growth factor-A (VEGF-A mRNA) in a mouse myocardial infarction model could enhance the formation of new blood vessels in infarction area and the survival rate increased as compared to controls (no mRNA administration).[8] The phase 2 study showed safety and improved heart function after direct injections of VEGF-A mRNA into the hearts of 11 patients with coronary diseases during open heart surgery, suggesting a promising clinical outcome of VEGF-A mRNA treatment for heart tissue regeneration.[9] Currently, the phase 3 clinical study of VEGF-A mRNA in a larger number of heart failure patients are ongoing, and the results are pending till next year.[10]

Our research group has studied the potential use of mRNA encoding growth factors for periodontal regeneration. High protein expression was demonstrated after 24 hour-transfection *in vitro* of primary human periodontal ligament cells and primary human gingival fibroblasts with modified mRNA encoding PDGF.[11] Furthermore, we demonstrated *in vivo* that direct injection of modified PDGF mRNA in healthy rat gingiva induced high protein translation.[12] Therefore, the present study investigated protein translation following gingival injections of PDGF mRNA in rat periodontitis. A ligature-induced periodontitis in rats was selected as a study model since it mimics the pathogenesis of periodontitis in humans caused by dental plaque accumulation around the ligature.[13] Different mRNA doses were also tested. Findings from this study will provide information regarding the use of ligature-induced periodontitis in rats for further investigation of PDGF mRNA therapeutic efficacy for periodontal regeneration.

Materials and methods

Construction of N1-methylpseudouridine - modified mRNA encoding PDGF

The nucleotide sequence of human PDGF was designed by Professor Rangsini Mahanonda and Professor Sathit Pichyangkul, and the N1-methylpseudouridine - modified mRNA encoding PDGF was synthesized by Dr. Norbert Pardi (University of Pennsylvania, USA).[14]

Ligature-induced experimental periodontitis in rats

Animal care and experimental procedures were approved by the Ethics committee at Faculty of Tropical

Medicine-Institute Animal Care and Use Committee at Mahidol University (Certificated no. FTM-IACUC 012/2019). Sprague-Dawley male rats (six weeks old) were purchased from Nomura Siam International Co.,Ltd. (Bangkok, Thailand) and adopted in individually ventilated cages with a 12-hour light/dark cycle for a week before the beginning of the experiment. Figure 1A demonstrated experimental design of ligature-induced rat periodontitis. Rats were anaesthetized by intraperitoneal injection with Zoletil (40 mg/kg) and Xylazine (5 mg/kg). Induction of experimental periodontitis in rat, silk ligatures (3-0 silk threads, Johnson & Johnson, New Brunswick, NJ, USA) were placed on the maxillary left second molar for two weeks (Fig. 1B). Suture was tied firmly with a double-knot on the buccal side of the maxillary left second molar. The maxillary right second molar was left non-ligature (Fig. 1B). The animal was fed with regular diet, received postoperative care, and the ligature was checked at one week post-ligation.

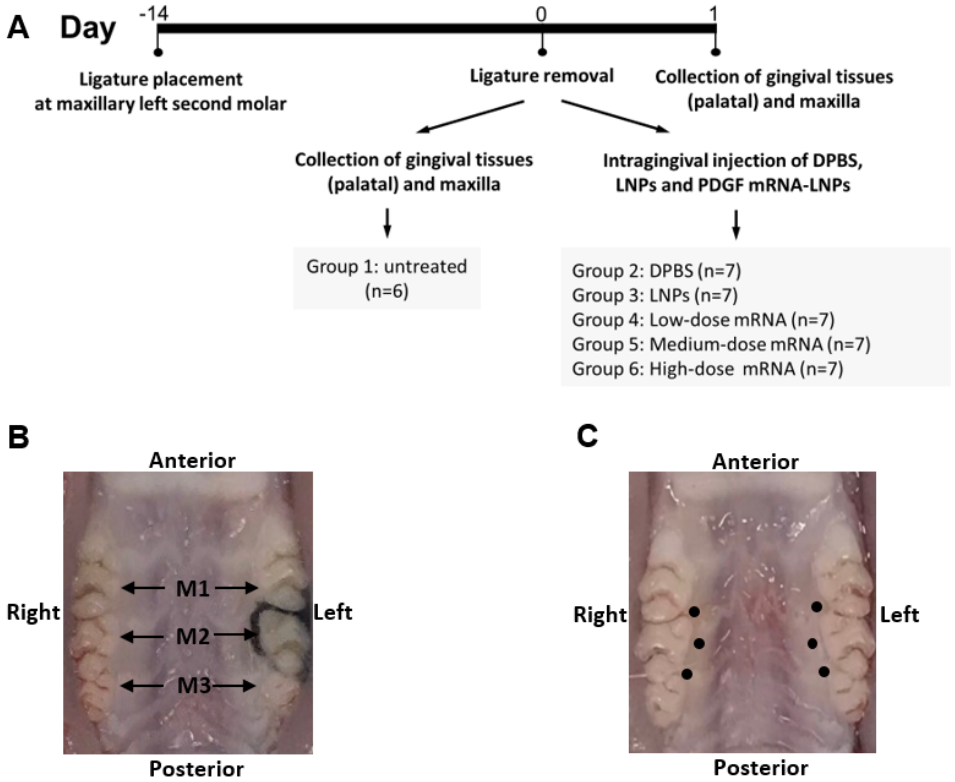


Figure 1 *Overview of the experimental design. (A) Diagram of ligature-induced rat periodontitis and the intra gingival delivery of DPBS, LNPs, and PDGF mRNA-LNPs. (B) In each rat, ligature was placed around the maxillary left second molar while the maxillary right second molar was not ligated. (C) Black dots indicate the injection sites. (DPBS = Dulbecco's phosphate-buffered saline, LNPs = lipid nanoparticles, PDGF mRNA-LNPs = N1-methylpseudouridine - modified mRNA encoding platelet derived growth factor-BB encapsulated with LNPs).*

Administration of DPBS, LNPs and N1-methylpseudouridine mRNA encoding PDGF in LNPs

All ligatures were removed after two weeks of ligation The animals were randomized into six groups (six to seven animals per group) as demonstrated in Figure 1A. The first group was immediately sacrificed and served as the untreated group. Groups 2 to 6 received different substances by intra gingival injections. The injection was performed at six sites (Fig. 1C) with the volume of 6 µl solution per site, which contained DPBS (group2, control); LNPs only (group3); 3 µg PDGF mRNA (group4, low-dose mRNA); 10 µg PDGF mRNA (group5, medium-dose mRNA); and 30 µg PDGF mRNA (group6, high-dose mRNA). A total volume of 36 µl solution was given to each rat. All animals (group 2-6) were sacrificed 24 hours after administration.

In each group, four rats were harvested for maxillae. In each maxilla, gingival tissues (palatal side) were used for measurement of protein production, while the remaining

maxillae were used for alveolar bone measurement. The other two to three rats in each group were used for micro-computerized tomography (micro-CT) imaging and subsequently for histological analysis.

Measurement of alveolar bone level

Rat maxillae were dissected and defleshed in 5% sodium hypochlorite for seven days. Pictures of these samples were taken under stereomicroscope (Olympus SZ61; Olympus Corporation, Tokyo, Japan) and analyzed alveolar bone level by ImageJ 1.52a software program (National Institutes of Health, USA). Two to three maxillae per group were scanned under a micro-CT system (Micro-CT µ35 scanco; SCANCO medical, Brüttisellen, Switzerland) and generated 3D images of the maxillae. All images of the maxillae were analyzed at the buccal and palatal sites. To identify alveolar bone loss, the mean distances from cementoenamel junction (CEJ) to alveolar bone crest (ABC) were measured at five sites on each surface (distobuccal or distopalatal line angle of maxillary first molar, mesiobuccal or mesiopalatal line angle, mid-buccal or mid-palatal, distobuccal or distopalatal line angle of maxillary second molar, mesiobuccal or mesiopalatal line angle of maxillary third molar).

Measurement of protein production

Gingival tissues at the palatal side were collected with a sulcular incision at the mesiopalatal line angle of maxillary first molar to distopalatal line angle of maxillary third molar and a horizontal incision was made approximately 2-3 mm below the gingival margin. The collected gingiva was weighed and homogenized in RIPA (extract protein) with protease inhibitor cocktail (Sigma, St. Louis, MO, USA). The homogenates were then centrifuged at 16,000 rpm for 15 minutes at 4°C. The amount of total proteins was measured by a BCA protein assay kit (PierceTM BCA Protein Assay; Thermo Scientific, Co., Ltd., Rockford, IL USA). The levels of PDGF protein, IL-1β and TNF-α were measured using enzyme-linked immunosorbent assay (ELISA) kits (Quantikine® ELISA; R&D System, Inc., Minneapolis, MN, USA). The sensitivity of the IL-1β and TNF-α was < 5 pg/ml, according to the manufacturer.

Histological evaluation

After micro-CT scanning, the maxillae were decalcified in 10 % ethylenediaminetetraacetic acid solution at 4°C for two weeks. Decalcified maxillae were dehydrated and embedded in paraffin. The serial section of specimens was performed in 7 µm thickness in mesio-distal direction and stained with hematoxylin and eosin (H&E) to analyze inflammatory cell infiltration.

Statistical analysis

All data were analyzed by the statistical software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Data between non-ligated site and ligated site were compared by Paired *T*- test. Differences among groups were analyzed by ANOVA followed by multiple comparisons with Bonferroni's post hoc test. The statistical significance was considered as *P*-value < 0.05.

Results

Ligature-induced periodontitis model in rats

In group 1, after two weeks of ligature placement, the mean alveolar bone loss of the ligature site was 0.7 ± 0.04 mm (0.63 ± 0.05 mm on buccal sides and 0.78 ± 0.07 mm on palatal sides). Whereas the mean alveolar bone loss of non-ligature sites was 0.5 ± 0.05 mm (0.35 ± 0.02 mm on buccal sides a nd 0.66 ± 0.08 mm on palatal sides) (Fig. 2A - 2C). There was significantly more bone loss at the ligature sites than at the non-ligature sites, indicating an established periodontitis model with periodontal bone loss in rats.

In group 1, both non-ligature and ligature sites showed low levels of inflammatory cytokine IL-1β < 50 pg/mg protein, with no significant differences between the two sites (Fig. 2D). TNF-α was not detected.

H&E stained sections of the ligature sites showed ulcerated epithelium, crestal bone loss and the presence of inflammatory infiltrates (Fig. 3E,3F), while non-ligature sites showed intact sulcular epithelium and some cellular infiltrates (Fig. 3B,3C). These histological findings corresponded to the 3D micro-CT imaging (Fig. 3D, 3A).

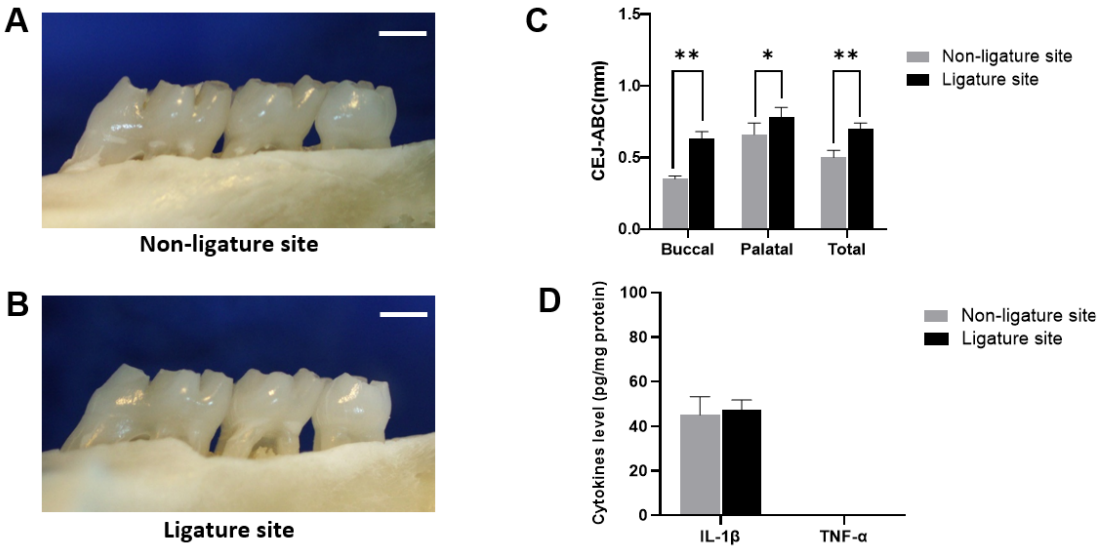


Figure 2 *Bone loss and cytokine production after 14 days of non-ligature and ligature sites in group 1. Representative stereomicroscope images from the buccal surfaces of (A) non-ligature, and (B) ligature sites (20x magnification; scale bar = 1 mm) (n=4). (C) The distance (mm) was from cementoenamel junction (CEJ) to alveolar bone crest (ABC), the linear measurement under stereo-microscope. Data shown are mean ± SE of the CEJ-ABC distance from the buccal side, palatal side, and total (the sum of buccal and palatal sides) (n = 4; *p < 0.05; ** p < 0.001, compared between non-ligature and ligature sites; Paired T- test) and (D) inflammatory cytokine levels of IL-1β and TNF-α in gingival tissues (palatal site). Data shown are mean ± SE (n = 4). (IL-1β = interleukin-1 beta, TNF-α = Tumor necrosis factor-alpha)*

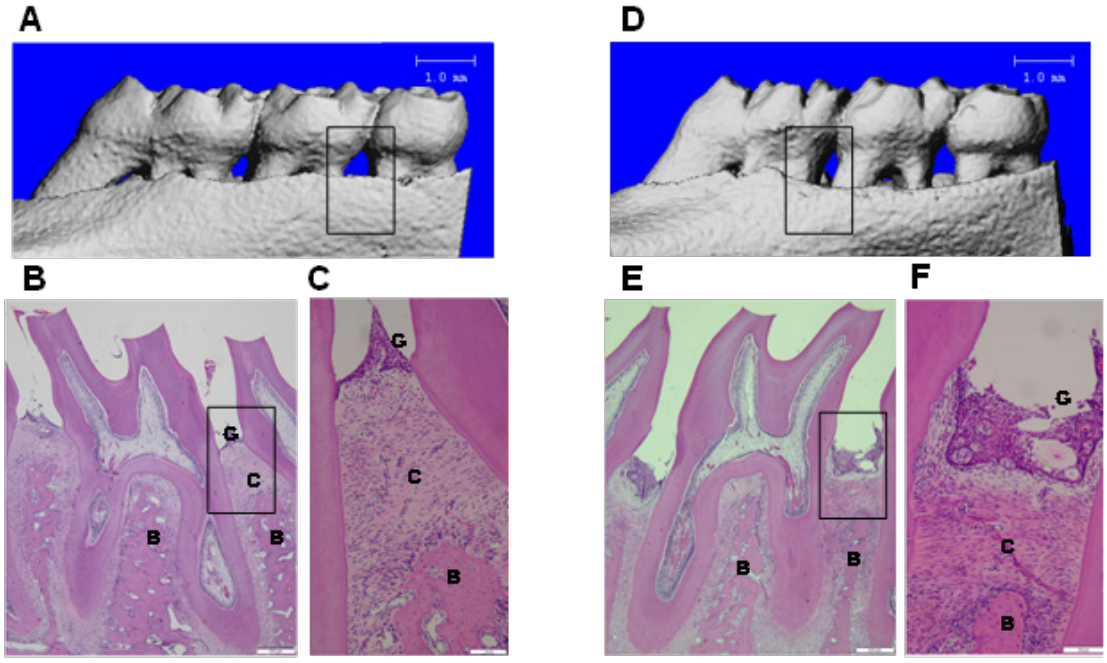


Figure 3 *Representative of micro-CT images and histological findings after 14 days of (A) non-ligature and (D) ligature sites in group 1. The corresponding H&E-stained sections of (B&C) showed intact sulcular epithelium and some inflammatory cell infiltrates whereas (E&F) showed ulcerated epithelium and the presence of inflammatory cell infiltrates in the ligature site. (B&E; original magnification x4; C&F; original magnification x20; B= alveolar bone; G= gingival epithelium; C= connective tissue)*

Expression of PDGF and inflammatory cytokines after local delivery of PDGF mRNA-LNPs in rat periodontitis model

After 24 hours of gingival injection of PDGF mRNA, clinical findings at the injected sites showed no erythema or swelling. The expression levels of translated PDGF protein tended to increase as mRNA dose increased at both non-ligature and ligature sites (group 4, 5, 6) (Fig. 4A). At each mRNA dose, there was no statistically significant difference in protein production between non-ligature and ligature sites. Negligible amount of PDGF protein was detected in DPBS control (group 2, ranging from 19.26 ± 2.01 to 24.13 ± 3.93 pg/mg protein) and LNPs (group 3, ranging from 18.79 ± 1.83 to 21.96 ± 2.3 pg/mg protein) (Fig. 4A). At the non-ligature sites, the mean PDGF protein was 51,883.91 ± 7,415.45 pg/mg protein at the high-dose mRNA; 24,666.22 ± 5,782.93 pg/mg protein at the medium-dose mRNA; and 10,912.54 ± 1.893.94 pg/mg protein at the low-dose mRNA group. It was found that the medium-dose mRNA and high-dose mRNA groups resulted in significantly higher protein production than the control and LNPs groups. In addition, the translated protein in the high-dose mRNA group was significantly higher than in the low-dose mRNA and medium-dose mRNA groups. At the ligature sites, the mean PDGF protein was 48,012.66 ± 16,063.13 pg/mg protein at the high-dose mRNA; 41,134.63 ± 10,430.55 pg/mg protein at the medium-dose mRNA; and 15,918.79 ± 7,681.11 pg/mg protein at the low-dose mRNA. It was found that the mean protein levels were significantly higher only in the high-dose mRNA-LNPs group than the control and LNPs groups.

In addition to PDGF protein production, the levels of inflammatory cytokines including IL-1β and TNF-α were assessed after injection of PDGF mRNA. TNF-α production was undetected in all groups, whereas IL-1β production was observed with the mean ranging from 72.08±13.24 to 1,458.44 ±180.51 pg/mg protein. The IL-1B level was higher in LNPs and high dose of mRNA groups compared to the other groups, however the difference was not statistically significant. (Fig. 4B).

The H&E staining showed no evidence of LNPs and other foreign substances of mRNA formulation remaining in the tissues. There was no significant inflammatory infiltration in any of the animals that received PDGF mRNA (data not shown).

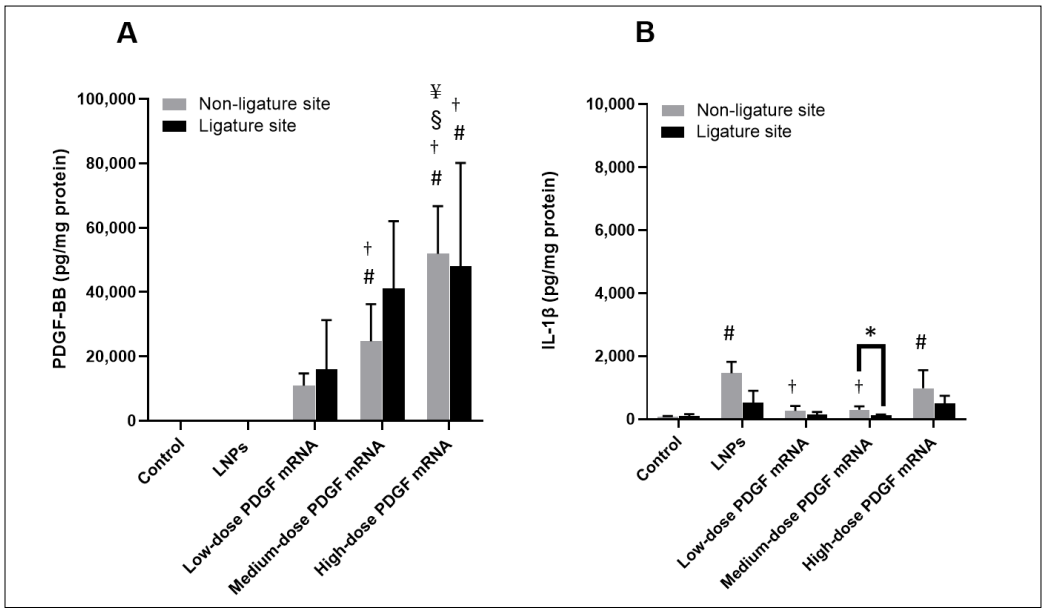


Figure 4 *Production of (A) PDGF protein and (B) IL-1β in palatal tissues after gingival injection with DPBS control, LNPs and PDGF mRNA (low, medium, and high doses) in non-ligature and ligature sites. Data shown are mean ± SE (*p<0.05, compared between non-ligature and ligature sites; #p<0.05, compared to control group; †p<0.05, compared to LNPs group; §p<0.05, compared to low-dose mRNA group; ¥p<0.05, compared to medium-dose mRNA group; one-way ANOVA and Bonferroni's post hoc tests), (DPBS = Dulbecco's phosphate-buffered saline, LNPs = lipid nanoparticles, PDGF mRNA = N1-methylpseudouridine - modified mRNA encoding platelet derived growth factor-BB encapsulated LNPs)*

Discussion

This study is the first study to explore the potential application of mRNA encoding PDGF-BB for periodontal regeneration in periodontitis. Local delivery of PDGF-BB mRNA in LNPs in ligature-induced rat periodontitis demonstrated a high production of the PDGF-BB protein. Although the translated protein showed a dose response trend, the differences in protein levels among different mRNA doses were not statistically significant.

The ligature model was used to establish periodontitis in the present study since it is a well-known approach to induce periodontitis and has been used in many relevant studies for testing efficacy of biological reagents.[15] This model is reported to be similar to human periodontitis in various aspects, as the alveolar bone resorption depends on bacterial plaque and inflammation of gingival tissue. Numerous studies of ligature-induced periodontitis in rodents showed alveolar bone loss, which is a hallmark of periodontitis.[13,15,16] In this study, the ligature-induced periodontitis model was successfully established in rats by ligation, as the alveolar bone loss at ligature sites was significantly greater than at non-ligature sites on both buccal and palatal surfaces. Our results supported previous studies that used a similar technique with 3-0 silk for 2-week ligation.[17] However, the level of inflammatory cytokine and the presence of inflammatory infiltrates were not different between the ligature site and the non-ligature site. This could possibly be explained by the timing of tissue collection for cytokine and histological analysis. Our study was conducted on day 14 after ligation, which was later than some other previous studies.[18-20] Alveolar bone loss with intense infiltration of inflammatory cells was observed at days 7-9 following ligature placement and then markedly declined.[18,19] IL-1β and TNF-α gene expression was also significantly elevated as early as days 1-3.[18,20] Those studies used real-time PCR to measure mRNA expression, whereas our study used ELISA, a reliable and appropriate method for investigating the protein levels of secreting cytokines. In addition to observation period, differences in host genetic variations on immune responses and plaque accumulation may contribute to variation in inflammation.

In a previous *in vivo* study of kinetic protein expression following local delivery of pseudouridine-modified PDGF mRNA in a healthy rat, the peak of PDGF protein translation was observed in gingival tissues 24 hours after intra gingival injection.[12] Therefore, the time frame used for protein analyses in this study was 24 hours.

The mRNA formulation used in this study was N1-methylpseudouridine-modified mRNA encapsulated with LNPs. In comparison to our previous study with pseudouridine-modified mRNA, N1-methylpseudouridine-modified mRNA encapsulated with LNPs resulted in higher protein translation and expression at the same time point (24 hours following intra gingival injection).[12] This could be explained by the use of the modified nucleobase N1-methylpseudouridine that has been shown to effectively decrease intracellular innate immune signals and, thus, improve mRNA stability.[21] In addition to the mRNA based modification, LNPs were employed to protect mRNA from RNase degradation and promote intracellular entry.[22] Because of their ease of manufacture and ability to improve mRNA translational capacity, LNPs are widely used as a carrier for mRNA, for example, in mRNA COVID-19 vaccine. The structure of LNPs consist of phospholipids, cholesterol, ionizable cationic lipids and PEGylated lipids for support, stabilization, complexation of negatively charged mRNA molecules, facilitating endosomal escape, and reducing nonspecific endocytosis by host immune cells, respectively.[23-25] However, previous pre-clinical studies reported that cationic lipid component in LNPs could activate an inflammatory response via NF-κB activation, and the production of TNF-α, IL-1β, IL-6 and IFN-γ.[26,27] In this study, we found that LNPs and high-dose mRNA groups had higher level of IL-1β in compared to other groups, however, we did not observed clinical swelling or erythema at the injected gingiva. Although, LNPs-induced inflammatory response could serve as an effective adjuvant for an mRNA-LNPs vaccine, this inflammation may be unfavorable for

tissue regeneration. Therefore, development of new types of noninflammatory delivery molecules that protect therapeutic mRNA from degradationand facilitate its cellular uptake would be required to address the issue of LNP-induced immune system activation.

Safety is a major concern when using mRNA as a therapeutic option in patients with periodontitis. The mRNA dosage used in our experiment ranges from 3 - 30 µg PDGF-BB mRNA/animal, which is considered lower than the previous study by Zangi *et al.* (2013). They injected 200 µg VEGF mRNA/animal into the myocardium of mouse myocardial infarction model. Their results showed improved heart function, no adverse effects and enhanced long term survival (1 year).[8] Of importance, it is becoming clear that COVID-19 nucleoside- modified mRNA-LNP vaccines have shown a strong safety and efficacy profile (>90%)[28] and to date, 12.68 billion doses of these vaccines have been used around the world.[29] The success of an mRNA vaccine is likely to facilitate the development of other mRNA-based therapeutic products. However, a future long-term study investigating safety and therapeutic efficacy of PDGF-BB mRNA in small and large animals is required.

Conclusion

PDGF expression was detected in gingival tissues in a periodontitis model following an intra gingival injection of PDGF mRNA-LNPs without profound local inflammatory response. A further study is required to evaluate the therapeutic effect of PDGF-BB mRNA.

Acknowledgement

This study was supported by Thailand Science Research and Innovation (DIG6280002). We would like to thank Dr. Jetsumon Prachumsri and Dr. Niwat Kangwarangsan for their advice on the animal procedures, and also Assist. Prof. Soranun Chantarangsu for statistical analysis suggestions.

References

1. Bartold PM, McCulloch CA, Narayanan AS, Pitaru S. Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. ***Periodontol 2000*** 2000;24:253-69.

2. Carragee EJ, Hurwitz EL, Weiner BK. A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. ***Spine J*** 2011;11(6):471-91.

3. Magadum A, Kaur K, Zangi L. mRNA-Based Protein Replacement Therapy for the Heart. ***Mol Ther*** 2019;27(4):785-93.

4. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, *et al.* Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N ***Engl J Med*** 2021;384(5):403-16.

5. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, *et al.* Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vectors with increased translational capacity and biological stability. ***Mol Ther*** 2008;16(11):1833-40.

6. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. ***Nature Reviews Materials*** 2021;6(12):1078-94.

7. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. ***Nat Rev Drug Discov*** 2018;17(4):261-79.

8. Zangi L, Lui KO, von Gise A, Ma Q, Ebina W, Ptaszek LM, *et al.* Modified mRNA directs the fate of heart progenitor cells and induces vascular regeneration after myocardial infarction. ***Nat Biotechnol*** 2013;31(10):898-907.

9. Anttila V, Saraste A, Knuuti J, Jaakkola P, Hedman M, Svedlund S, *et al.* Synthetic mRNA Encoding VEGF-A in Patients Undergoing Coronary Artery Bypass Grafting: Design of a Phase 2a Clinical Trial. ***Mol Ther Methods Clin Dev*** 2020;18:464-72.

10. Collén A, Bergenhem N, Carlsson L, Chien KR, Hoge S, Gan LM, *et al.* VEGFA mRNA for regenerative treatment of heart failure. ***Nat Rev Drug Discov*** 2022;21(1):79-80.

11. Surisaeng T, Mahanonda R, Sa-Ard-Iam N, Rerkyen P, Chanamuangkon T, Chanpaiboon C, editors. The use of modified mRNA encoding platelet-derived growth factor-BB as an innovation in periodontal regeneration. Proceedings of RSU International Research Conference 2020; 2020; Pathum Thani, Thailand.

12. Bhongsatiern P, Mahanonda R, Sa-Ard-Iam N, Chanamuangkon T, Wisitrasameewong W, editors. Protein expression after delivery of mRNA encoding PDGF-BB into rat gingiva: A Pilot Study. Proceedings of the 15th RSU National Graduate Research Conference; 2020; Pathum Thani, Thailand. 2020.

13. Graves DT, Fine D, Teng YT, Van Dyke TE, Hajishengallis G. The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. ***J Clin Periodontol*** 2008;35(2):89-105.

14. Pardi N, Tuyishime S, Muramatsu H, Kariko K, Mui BL, Tam YK, *et al.* Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. ***J Control Release*** 2015; 217:345-51.

15. Lin P, Niimi H, Ohsugi Y, Tsuchiya Y, Shimohira T, Komatsu K, *et al.* Application of Ligature-Induced Periodontitis in Mice to Explore the Molecular Mechanism of Periodontal Disease. ***Int J Mol Sci*** 2021;22(16).

16. Graves DT, Li J, Cochran DL. Inflammation and uncoupling as mechanisms of periodontal bone loss. ***J Dent Res*** 2011;90(2):143-53.
17. Liu YF, Wu LA, Wang J, Wen LY, Wang XJ. Micro-computerized tomography analysis of alveolar bone loss in ligature- and nicotine-induced experimental periodontitis in rats. ***J Periodontal Res*** 2010; 45(6):714-9.
18. Marchesan J, Girnary MS, Jing L, Miao MZ, Zhang S, Sun L, *et al.* An experimental murine model to study periodontitis. ***Nat Protoc*** 2018;13(10):2247-67.
19. Wu YH, Taya Y, Kuraji R, Ito H, Soeno Y, Numabe Y. Dynamic microstructural changes in alveolar bone in ligature-induced experimental periodontitis. ***Odontology*** 2020;108(3):339-49.
20. de Molon RS, Park CH, Jin Q, Sugai J, Cirelli JA. Characterization of ligature-induced experimental periodontitis. ***Microsc Res Tech*** 2018;81(12):1412-21.
21. Andries O, McCafferty S, De Smedt SC, Weiss R, Sanders NN, Kitada T. N(1)-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. ***J Control Release*** 2015;217:337-44.
22. Kowalski PS, Rudra A, Miao L, Anderson DG. Delivering the Messenger: Advances in Technologies for Therapeutic mRNA Delivery. ***Mol Ther*** 2019;27(4):710-28.
23. Kauffman KJ, Dorkin JR, Yang JH, Heartlein MW, DeRosa F, Mir FF, *et al.* Optimization of Lipid Nanoparticle Formulations for mRNA Delivery in Vivo with Fractional Factorial and Definitive Screening Designs. ***Nano Lett*** 2015;15(11):7300-6.
24. Semple SC, Leone R, Barbosa CJ, Tam YK, Lin PJC. Lipid Nanoparticle Delivery Systems to Enable mRNA-Based Therapeutics. ***Pharmaceutics*** 2022;14(2).
25. Kulkarni JA, Cullis PR, van der Meel R. Lipid Nanoparticles Enabling Gene Therapies: From Concepts to Clinical Utility. ***Nucleic Acid Ther*** 2018;28(3):146-57.
26. Ndeupen S, Qin Z, Jacobsen S, Bouteau A, Estanbouli H, Igyártó BZ. The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory. ***iScience.*** 2021; 24(12):103479.
27. Lonez C, Bessodes M, Scherman D, Vandenbranden M, Escriou V, Ruysschaert JM. Cationic lipid nanocarriers activate Toll-like receptor 2 and NLRP3 inflammasome pathways. ***Nanomedicine*** 2014;10(4):775-82.
28. Hogan MJ, Pardi N. mRNA Vaccines in the COVID-19 Pandemic and Beyond. ***Annu Rev Med*** 2022;73:17-39.
29. Ourworldindata.org [Internet]. Oxford: Coronavirus (COVID-19) vaccinations; 2021 [updated 2022 Sep 18; cited 2022 Sep 18]. Available from: https://ourworldindata.org/covid-vaccinations

Original Article

Expression of CLLD7 and CHC1L Proteins in Oral Potentially Malignant Disorders in A Group of Thais: A Preliminary Study

Sunisa Suchitanant[1], Rachai Juengsomjit[2], Sopee Poomsawat[2], Ounruean Meesakul[2], Bishwa Prakash Bhattarai[3], Boworn Klongnoi[4], Siribang-on Piboonniyom Khovidhunkit[1]

[1]Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, Thailand
[2]Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand
[3]Walailak University International College of Dentistry, Walailak University, Bangkok, Thailand
[4]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

Abstract

Chronic lymphocytic leukemia deletion 7 (CLLD7) and chromosome condensation 1-like (CHC1L) proteins are putative tumor suppressor proteins that have never been studied in oral potentially malignant disorders (OPMDs). This study aimed to evaluate the expression of these two proteins in OPMDs which encompassed oral leukoplakia, oral lichen planus (OLP), and oral lichenoid lesions (OLL). The histopathological features of oral leukoplakia were acanthosis with or without hyperkeratosis and mild to severe epithelial dysplasia. Therefore, five groups including acanthosis with or without hyperkeratosis, mild, moderate, and severe dysplasia and the last group OLP/OLL were subjected to immunohistochemistry using normal oral mucosa as a control. In each case, random areas were selected and photographed after immunohistochemistry, then at least 1000 cells were counted. For CLLD7 and CHC1L, nuclear, cytoplasmic, and/or membrane staining were considered positive. Positive cells at different locations were evaluated. SPSS version 18 was used to compare the variation of protein expression among groups with a statistical significance at $p<0.05$. CLLD7 and CHC1L proteins were expressed in all cases of NOM and OPMDs. Compared to the acanthosis group, nuclear staining of CLLD7 was significantly lower in the severe epithelial dysplasia and the OLP/OLL groups. Although increased cytoplasmic staining of CLLD7 was observed in all OPMDs groups compared to the NOM group, a statistically significant difference was observed between the mild and moderate epithelial dysplasia groups compared to the NOM group. Regarding CHC1L staining, the percentage of nuclear staining was reduced, whereas membrane staining was increased in all OPMD groups compared to the NOM group. However, a statistically significant difference was observed between the severe epithelial dysplasia and OPL/OLL groups compared to the NOM group. In conclusion, the altered subcellular localization of CLLD7 and CHC1L in OPMDs suggests that the expression of these putative tumor suppressor proteins might be dysregulated during the early malignant transformation processes of oral cancer.

Keywords: CLLD7, CHC1L, Normal oral mucosa, Oral epithelial dysplasia

Received Date: Aug 4, 2022 **Revised Date:** Sep 6, 2022 **Accepted Date:** Oct 11, 2022
doi: 10.14456/jdat.2023.7

Correspondence to :
Siribang-on Piboonniyom Khovidhunkit, Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol University, 6 Yotee Rd., Bangkok 10400, Thailand. E-mail: siribangon.pib@mahidol.edu

Introduction

The International Agency for Research on Cancer (IARC) reported that lip and oral cavity cancers accounted for 377,713 cases and 177,757 deaths worldwide in 2020.[1] In the same year, in Thailand, lip and oral cavity cancers ranked as the tenth most common cancer, 2.5 % of all new cancer cases.[2] The most common malignant tumor of the oral cavity was oral squamous cell carcinoma (OSCC).[3,4] Northeastern Thailand is the region where the incidence of oral cancer is relatively high[5,6] and strongly associated with tobacco smoking, alcohol use, and betel nut chewing habits.[7] These risk factors have some ingredients and metabolites which can gradually cause normal tissues to transform into oral potentially malignant disorders (OPMDs) and malignant tumors.[8]

As suggested by the WHO, OPMDs are any clinical presentations or conditions of tissue alteration that are hazardous to becoming cancers.[9] Therefore, early detection of OPMDs leads to confirmatory investigations and timely appropriate treatments. OPMDs consist of oral leukoplakia, oral erythroplakia, palatal lesions in reverse smokers, oral submucous fibrosis, oral lichen planus (OLP), oral lichenoid lesion (OLL), discoid lupus erythematosus, actinic cheilitis, and inherited cancer syndromes.[9] Regarding leukoplakia, it could be histopathologically diagnosed as acanthosis with or without hyperkeratosis, mild or moderate or severe epithelial dysplasia. Therefore, leukoplakia specimens were selected for this study. In addition, OLP/OLL were also included since it is one of the OPMDs.

Clinical and/or histopathological examinations are not always enough to prove the malignant potentials of OPMDs; investigations at a molecular level will complement these methods to confirm occurrences of genetic alterations more precisely.[10] This comprehensive integration is based on the biological fact that cumulative alteration of subcellular structures such as DNAs, RNAs, and proteins influences the replication and differentiation of normal cells to progress to OPMDs and invasive cancer.[11]

RCBTB proteins (Regulator of chromatin condensation 1 and Broad complex, Tramtrack and Bric à brac domain-containing proteins) belong to one of the subgroups of proteins under RCC1 (Regulator of Chromatin Condensation 1) superfamily proteins, which function as a GEF (guanine nucleotide-exchange factor) of Ran (Ras-related nuclear protein). Ran acts as a biological switch cycling between GTP-bound "on" and GDP-bound "off" states. Thus, RCC1 operating through Ran plays an essential role in cell cycle regulation, chromatin condensation, nucleocytoplasmic transport, mitotic spindle formation, and nuclear envelope assembly.[12] There are three members of RCBTB proteins, namely, CLLD7 (RCBTB1), CHC1L (RCBTB2), and IBtk (inhibitor of Bruton's tyrosine kinase), but in this study, only CLLD7 and CHC1L will be investigated.

Mabuchi *et al.* created a high-resolution physical map of chromosome 13q14 covering the critically deleted region in B-cell chronic lymphocytic leukemia[13] and identified three novel genes that were *CLLD6, CLLD7,* and *CLLD8.* The *CLLD7* gene encodes the CLLD7 protein containing the RCC1 domain at the NH2 terminus and the broad complex, tramtrack, and bric-a-brac (BTB) domain at the COOH terminus.[13] These structural components give the CLLD7 protein the official name RCBTB1 (RCC1 and BTB domain-containing protein 1).[13] In addition, Zhou and Munger investigated the biological roles of CLLD7 in different human cancer cell lines. They reported a decreasing expression of CLLD7 in human cancer cell lines, which subsided in cell apoptosis.[14] Thus, it is possible that CLLD7, as a novel protein involved in cell cycle mechanism, and transcriptional repression of several proteins, can be a tumor suppressor protein.[15]

Another novel gene, *CHC1L*, was discovered by Devilder *et al.* The gene was later designated as RCBTB2 since it contains RCC1 and BTB domains similar to CLLD7.[16] The evaluation of the mapped clones determined the *CHC1L* gene to be located on chromosome 13q14.3. Protein analysis revealed a significant resemblance between the CHC1L N-terminal amino acid sequence and the seven intradomain repeats of the RCC1, so CHC1L is

qualified to be a new member of the RCC1-related GEF family.[12] CHC1L may function in the cell cycle, nucleocytoplasmic transport, and human cell growth, possibly in the transfer of an anti-oncogenic signal.[16] Even though there is no distinct evidence to date, *CHC1L* was still considered a candidate tumor suppressor gene. Latil *et al.* in 2002, reported the loss of heterozygosity (LOH) and decreasing expression of CHC1L in human prostate cancer.[17] According to a study by Spillane *et al.,* CHC1L-deficient mice succumbed to multiple cancers, including histiocyte-rich neoplasms suggesting that CHC1L plays a role in preventing tumorigenesis.[18]

Detection of changes or abnormalities of cells from the beginning stage by the molecular technologies accompanied by conventional examination promotes effective prevention, early diagnosis, and possible treatment.[10] Our previous study analyzed the expression of CLLD7 and CHC1L proteins in OSCC specimens and compared them to the normal oral mucosa (NOM).[19] Mislocalization of CLLD7 and CHC1L proteins were found.[19] Therefore, this study aimed to assess and compare the expression of these two proteins in NOM and OPMDs, including acanthosis with or without hyperkeratosis, various degrees of epithelial dysplasia, and OLP/OLL using immunohistochemistry in biopsy specimens obtained from a group of participants in the lower part of Northeastern Thailand.

Materials and methods

This is a laboratory-based and observational cross-sectional study. Biopsy specimens were obtained from the "Development of Disease Management Model for Oral Cancer with an Integration Network of Screening, Surveillance, and Treatment in Northeast Health District" project during 2018-2019 or the Faculty of Dentistry, Mahidol University to assess the expression of CLLD7 and CHC1L in OPMDs and NOM using immunohistochemistry. The research was approved by the Committee on Human Rights Related to Human Experimentation, Faculty of Dentistry/Faculty of Pharmacy, Mahidol University (MU-DT/PY-IRB 2020/059.2909, MU-DT/PY-IRB 2019/041.0307 and MU-DT/PY-IRB 2018/025.1106). Demographic data of the participants were retrieved from the questionnaires or pathological report forms without recording the name of the participants. Only age, gender, risk factors that contributed to OPMDs and oral cancers, site, and characteristics of the lesions were recorded.

Tissue specimens

The OPMDs identified as white and/or red patches were clinically diagnosed as oral leukoplakia, OLP, or OLL by board-certified oral medicine specialists or oral surgeons who performed biopsies at the screening clinics for the OPMDs and oral cancer project. Board-certified oral and maxillofacial pathologists at the Faculty of Dentistry, Mahidol University, made histopathological diagnoses of the biopsied specimens as acanthosis with or without hyperkeratosis, various degrees of epithelial dysplasia, OLP, or OLL. Since there was no data regarding the use of the drug(s) that can induce OLL, history of dental restoration in the oral cavity, or history of liver disease, the term OLP/OLL is used in this study. The inclusion criteria of each group were as follows. 1) NOM group; male or female patients at the ages older than 15 years old who attended the Faculty of Dentistry, Mahidol University for impacted third molar removal were asked to participate. NOM specimens that served as normal controls were obtained from the flap of tissue left over from the removal of impacted third molars. These tissues were histopathologically free from inflammation and dysplasia. 2) Oral leukoplakia; male or female participants at ages equal to or older than 40 years old attended the screening for OPMDs and oral cancer in the northeastern areas of Thailand were requested to participate. The clinical diagnosis of oral leukoplakia was made according to Warnakulasuriya and colleagues, 2007.[20] Specimens included acanthosis with or without hyperkeratosis, mild, moderate or severe epithelial dysplasia. 3) OLP/OLL; male or female participants at ages equal to or older than 40 years old attended the screening for OPMDs and oral cancer in the northeastern areas were recruited. The clinical and histopathological diagnosis of OLL/OLP was according to the WHO criteria for the diagnosis of OLP/OLL, 2016.[21] Complete demographic

data and risk factors for oral cancer were also received. The specimens with incomplete data on demography and risk factors were not included in the study. Biopsy specimens with poor orientation and superficial ulceration were excluded from the study. Due to the limitation of tissue samples and immunohistochemical technique, only six samples were used in each group of NOM and OPMDs in this preliminary study. In addition, some tissue samples were too small for the staining of both proteins. Therefore, a substitution of tissue samples was made leading to different groups of tissue specimens from different participants for CLLD7 and CHC1L immunohistochemistry.

Immunohistochemical analysis

Three-µm-thick tissue sections of NOM and OPMDs were cut and mounted over aminopropyl-triethoxysilane (APES) coated slides, then deparaffinized and rehydrated. Sections were incubated with 3% H_2O_2 to block endogenous peroxidase. Antigen retrieval was performed by heating the sections in citrate buffer pH 6.0 using a microwave oven. After washing with phosphate-buffered saline pH 7.6 with 0.1% Tween 20 (PBS-T), the sections were blocked using 5% bovine serum albumin (BSA). Next, the primary antibody diluted in commercially available diluent was applied over the tissue sections. Slides were then kept inside a humidifier and incubated overnight at 4°C. The dilution of the primary antibody used for CLLD7 (ab233533, Abcam, Cambridge, UK) and CHC1L (ab175505, Abcam, Cambridge, UK) was 1:400. On the second day, after draining off the primary antibody, slides were rinsed in PBS-T with gentle agitation. A horseradish peroxidase (HRP)-conjugated secondary antibody (Dako REAL® EnVision®/HRP, Rabbit/Mouse (ENV), Dako, Denmark) was applied over the sections and incubated for 30 minutes at room temperature in a humidified environment. After thorough washing, the color was developed by incubating with freshly-made diaminobenzidine (DAB) solution. Sections were then washed and counterstained with hematoxylin before dehydration and mounting. A diluent with no primary antibody was used as the negative control reagent. Sections of the mouse brain were used as the positive controls for CLLD7 and CHC1L staining.

Evaluation of CLLD7 and CHC1L expression

For CLLD7 and CHC1L, the presence of nuclear, cytoplasmic, and/or membrane staining was considered positive. Photographs of five random fields were taken for each case using a light microscope (X400 magnification). At least 1000 cells were counted in each case. The number of positive cells at different subcellular localizations (nuclear staining, cytoplasmic staining, and membrane staining) was counted using ImageJ software, and the percentage of these cells at various subcellular locations was reported. Cells that exhibited staining with a very faint intensity which is as intense as the background were not counted. The primary investigator (S.S.) was calibrated with a board-certified oral medicine specialist (S.P.K.) to count the positive cells and identify the localization of the proteins. The intraclass correlation coefficients (ICCs) were calculated for each location of staining and it was found that the ICCs were between 0.85-0.94. After the counting, photographs were also randomly selected and re-evaluated by S.S. and S.P.K. to see if the counting was correct. If there was any discrepancy, corrections were made immediately.

Statistical analysis

Statistical analysis was performed with SPSS Statistics version 18. Demographic, clinical, and histopathological data were reported by descriptive statistics. In addition, the normality of the percentage of positive cells was analyzed by the Shapiro-Wilks test. In the case of normal distribution, Levene's Test for Equality of Variance was used. When equal variances were assumed, and at least one pair was significantly different, one-way ANOVA and Post Hoc tests were used. However, when equal variances were not assumed and at least one pair was significantly different, the Welch and Post Hoc tests were used. In cases where normal distribution was not applicable, the Kruskal-Wallis test and pairwise comparisons were used. Significant differences were established at $p \leq 0.05$.

Results

CLLD7 immunohistochemistry

The characteristics of the participants for CLLD7 immunohistochemistry are presented in Table 1. The mean age of the participants in the NOM group was approximately 20 years and in the OPMDs groups was 63.13±7.18 years. Female participants (n=24) were more common than male participants (n=12).

Data regarding the localization of CLLD7 are presented in Table 2 and Figure 1. The representative images of CLLD7 immunostaining in each group are shown in Figure 2. Membrane staining was scarcely observed in all groups which made the median number almost 0 (Table 2). Nuclear and cytoplasmic staining of CLLD7 were observed in all groups. No significant difference was observed for nuclear staining between the NOM group and all other groups (Fig. 1). However, a significant reduction was found between 1) the severe epithelial dysplasia and OLP/OLL groups compared to the acanthosis group, 2) the severe epithelial dysplasia and OLP/OLL groups compared to the mild epithelial dysplasia group, and 3) the severe epithelial dysplasia group compared to the moderate epithelial dysplasia group.

Table 1 *Characteristics of participants for CLLD7 immunohistochemistry*

Groups	Sex (M/F)	Age (Mean±SD) Range (years)	Site (n)	Associated risk factors (n)
NOM (n=6)	3/3	19.83±2.71 (15-23)	Pericoronal tissue of 3rd molar from mandible (5) Pericoronal tissue of 3rd molar from maxilla (1)	None
Acanthosis (n=6)	2/4	67.00±9.25 (50-76)	Alveolar ridge (1) Buccal mucosa (4) Tongue (1)	Smoking (3) Alcohol consumption (1) Betel nut chewing (1) Working in sunlight (4)
Mild dysplasia (n=6)	3/3	64.50±8.09 (49-71)	Buccal mucosa (1) Gingiva (2) Tongue (2) Lower lip (1)	Smoking (5) Tobacco (2) Betel nut chewing (1) Working in sunlight (1) History of cancer (1)
Moderate dysplasia (n=6)	0/6	65.67±6.62 (55-72)	Buccal mucosa (3) Edentulous area (1) Labial mucosa (1) Lower lip (1)	Smoking (3) Smokeless tobacco (3) Alcohol consumption (1) Betel nut chewing (4) Working in sunlight (2)
Severe dysplasia (n=6)	1/5	65.33±6.80 (54-72)	Buccal mucosa (3) Lower lip (1) Labial mucosa (1) Tongue (1)	Smoking (4) Smokeless tobacco (1) Alcohol consumption (1) Betel nut chewing (4) Working in sunlight (3)
OLP/OLL (n=6)	3/3	63.17±9.30 (51-73)	Buccal mucosa (6)	Smoking (4) Alcohol consumption (2) Working in sunlight (4)

NOM: normal oral mucosa; OLP/OLL: oral lichen planus/oral lichenoid lesion

Table 2 *Percentage of subcellular localization of CLLD7 and CHC1L*

Protein and localization		Category						*P*-Value
		NOM	Acanthosis	Mild Dysplasia	Moderate Dysplasia	Severe Dysplasia	OLP/OLL	
CLLD7	Nucleus	56.10±3.00 [a]	64.31±6.38 [a]	60.23±1.83 [a]	59.90±5.02 [a]	45.64±5.27 [a]	46.97±4.35 [a]	0.035[#]
	Cytoplasm	0 (0.00, 0.29) [b]	1.96 (0.23, 57.46) [b]	48.44 (29.27, 61.63) [b]	58.74 (35.08, 72.88) [b]	25.17 (18.16, 51.34) [b]	10.07 (1.91, 19.92) [b]	0.005[b]
	Membrane	0 (0.00, 0.01) [b]	0 (0.00, 0.00) [b]	0 (0.00, 0.00) [b]	0 (0.00, 0.03) [b]	0 (0.00, 0.03) [b]	0 (0.00, 0.00) [b]	0.671[†]
CHC1L	Nucleus	45.52 (36.62, 64.22) [b]	16.92 (6.31, 24.33) [b]	5.75 (2.10, 11.43) [b]	6.50 (4.80, 8.57) [b]	5.60 (0.35, 11.21) [b]	4.12 (3.75,8.89) [b]	0.003[†]
	Cytoplasm	0 (0.00, 0.33) [b]	3.27 (0.00, 12.63) [b]	2.28 (0.71, 3.44) [b]	5.18 (0.00, 9.51) [b]	2.65 (0.00, 5.83) [b]	0.80 (0.38, 1.97) [b]	0.178[†]
	Membrane	1.13 (0.00, 11.29) [b]	9.88 (2.32, 15.68) [b]	32.11 (14.64, 49.70) [b]	26.34 (21.55, 52.64) [b]	50.55 (26.27, 58.05) [b]	46.19 (32.27, 61.91) [b]	0.002[†]

[a]Mean+SEM; #One-way ANOVA test; [b]median (Q1, Q3); [†]Kruskal-Wallis test; NOM: normal oral mucosa, OLP/OLL: oral lichen planus/oral lichenoid lesions

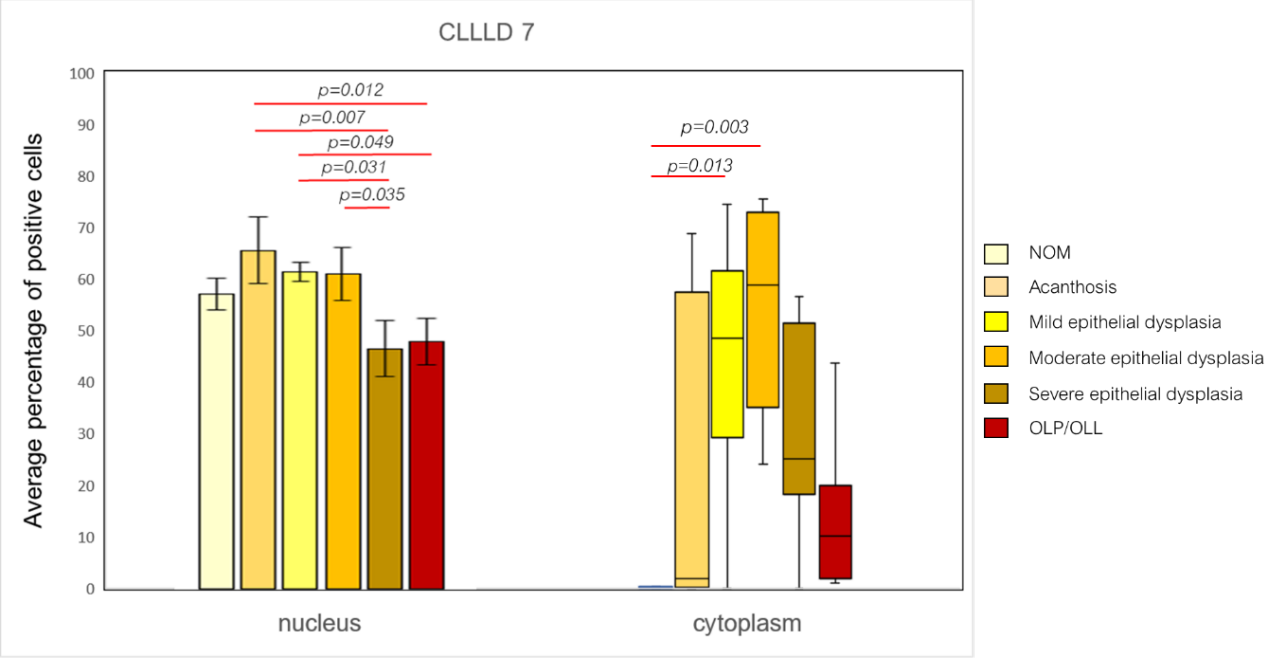


Figure 1 *Percentage of subcellular localization of CLLD 7 immunohistochemistry. No membrane staining was detected. Increased cytoplasmic staining was observed in the OPMDs groups compared to the NOM group. NOM denoted normal oral mucosa. OLP/OLL denoted oral lichen planus/oral lichenoid lesions*

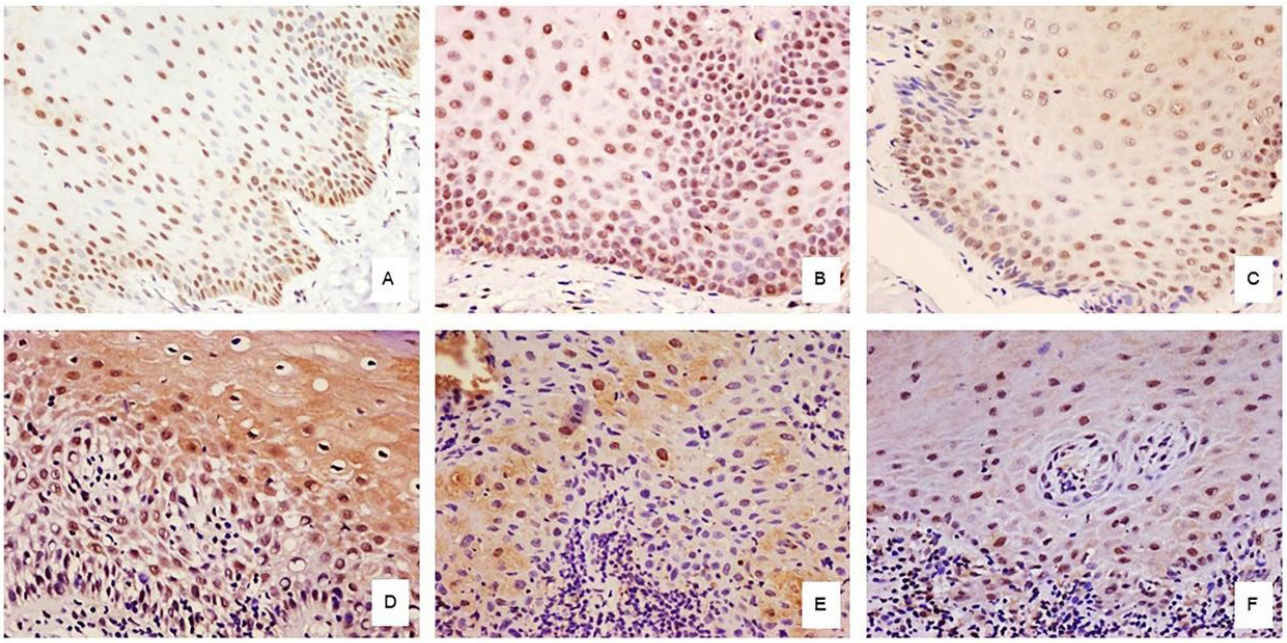


Figure 2 *Representative pictures of CLLD7 immunohistochemistry; original magnification: X400 A. Normal oral mucosa B. Acanthosis C. Mild epithelial dysplasia D. Moderate epithelial dysplasia E. Severe epithelial dysplasia F. oral lichen planus/oral lichenoid lesions. Note that cytoplasmic staining could be observed in OPMDs specimens. Positive staining was also observed in some stromal areas*

The cytoplasmic staining of CLLD7 was higher in all OPMDs compared to the NOM. However, a statistically significant increase in cytoplasmic staining was observed between the mild and moderate epithelial dysplasia groups compared to the NOM group. These data suggested some degree of changes in the localization of the CLLD7 protein in OPMDs. In addition to positive staining in the epithelial cells, positive staining was also observed in some stromal areas.

CHC1L immunohistochemistry

The biopsy specimens used for CHC1L immunostaining were not the same as those for CLLD7 due to the limited size of the biopsy specimens. Nevertheless, the mean age of each group and the sites of biopsy were similar, and this should not affect the immunohistochemical analysis.

Table 3 summarizes the characteristics of the participants for CHC1L immunohistochemistry. The mean age of the participants in the NOM group was approximately 20 years old. The mean age of the participants in the OPMDs groups was 66.63±12.95 years old. There were more female participants (n=24) than male participants (n=12).

Table 2 and Figure 3 show the data on the localization of CHC1L. Figure 4 illustrates the representative images of CHC1L staining in each group. In addition to the nuclear and cytoplasmic expression, membrane staining was detected for CHC1L. Except for the acanthosis group, the percentage of nuclear staining was significantly reduced in all OPMDs groups compared to the NOM group. Although cytoplasmic staining appeared to be increased in all OPMDs groups compared to the NOM group, a significant difference was not observed.

Interestingly, the percentage of membrane staining was higher in all OPMD groups compared to the NOM group. However, a statistically significant difference was observed between the severe epithelial dysplasia and the OLP/OLL groups compared to the NOM group. These data implied that there were changes in the localization of CHC1IL in the OPMDs groups compared to the NOM group.

Similar to CLLD7 immunohistochemistry, positive staining was also observed in some stromal areas.

Table 3 *Characteristics of participants for CHC1L immunohistochemistry*

Groups	Sex (M/F)	Age (Mean±SD) Range (years)	Site (n)	Associated risk factors (n)
NOM (n=6)	3/3	19.83±2.71 (15-23)	Pericoronal tissue of 3rd molar from mandible (5) Pericoronal tissue of 3rd molar from maxilla (1)	None
Acanthosis (n=6)	1/5	70.83±3.06 (68-76)	Buccal mucosa (5) Tongue (1)	Smoking (4) Betel nut chewing (3) Working in sunlight (3)
Mild dysplasia (n=6)	3/3	67.83±10.01 (49-79)	Buccal mucosa (2) Gingiva (1) Lower lip (2) Labial mucosa (1)	Smoking (3) Smokeless tobacco (1) Betel nut chewing (1) Working in sunlight (2) History of cancer (1)
Moderate dysplasia (n=6)	0/6	69.33±2.07 (66-72)	Buccal mucosa (3) Labial mucosa (2) Lower lip (1)	Smoking (1) Smokeless tobacco (2) Alcohol consumption (2) Betel nut chewing (4) Working in sunlight (2)
Severe dysplasia (n=6)	1/5	65.33±6.80 (54-72)	Buccal mucosa (3) Labial mucosa (1) Tongue (1) Lower lip (1)	Smoking (4) Smokeless tobacco (1) Alcohol consumption (1) Betel nut chewing (4) Working in sunlight (3)
OLP/OLL (n=6)	4/2	59.83±7.14 (51-71)	Buccal mucosa (6)	Smoking (3) Smokeless tobacco (1) Alcohol consumption (2) Betel nut chewing (1) Working in sunlight (5)

NOM: normal oral mucosa; OLP/OLL: oral lichen planus/oral lichenoid lesions

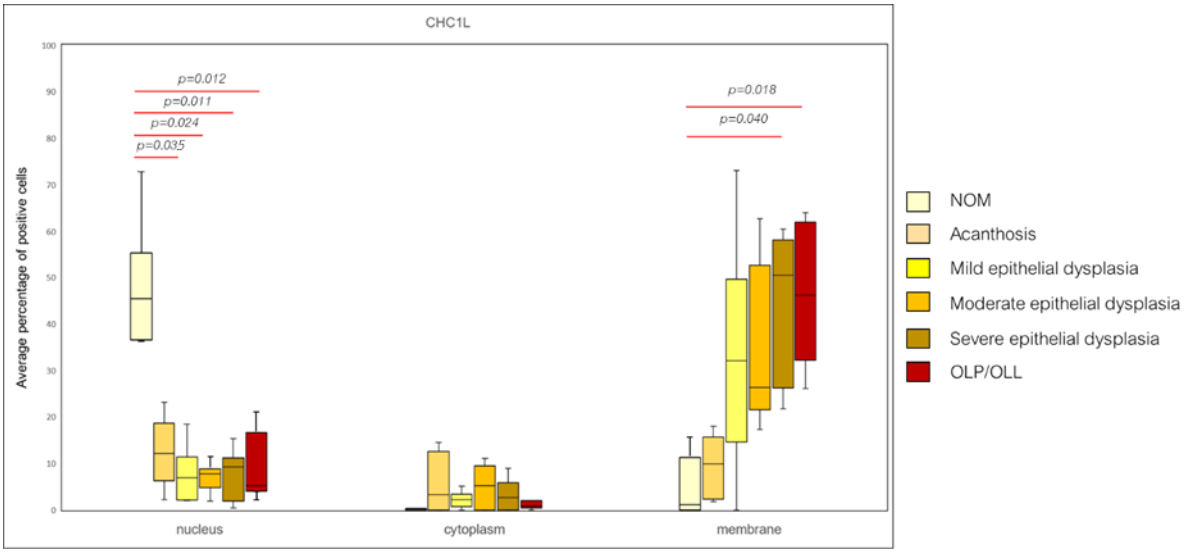


Figure 3 *Percentage of subcellular localization of CHC1L immunohistochemistry. Increase membrane staining and decrease nuclear staining were observed in the OPMDs groups compared to the NOM group. NOM denoted normal oral mucosa. OLP/OLL denoted oral lichen planus/oral lichenoid lesions*

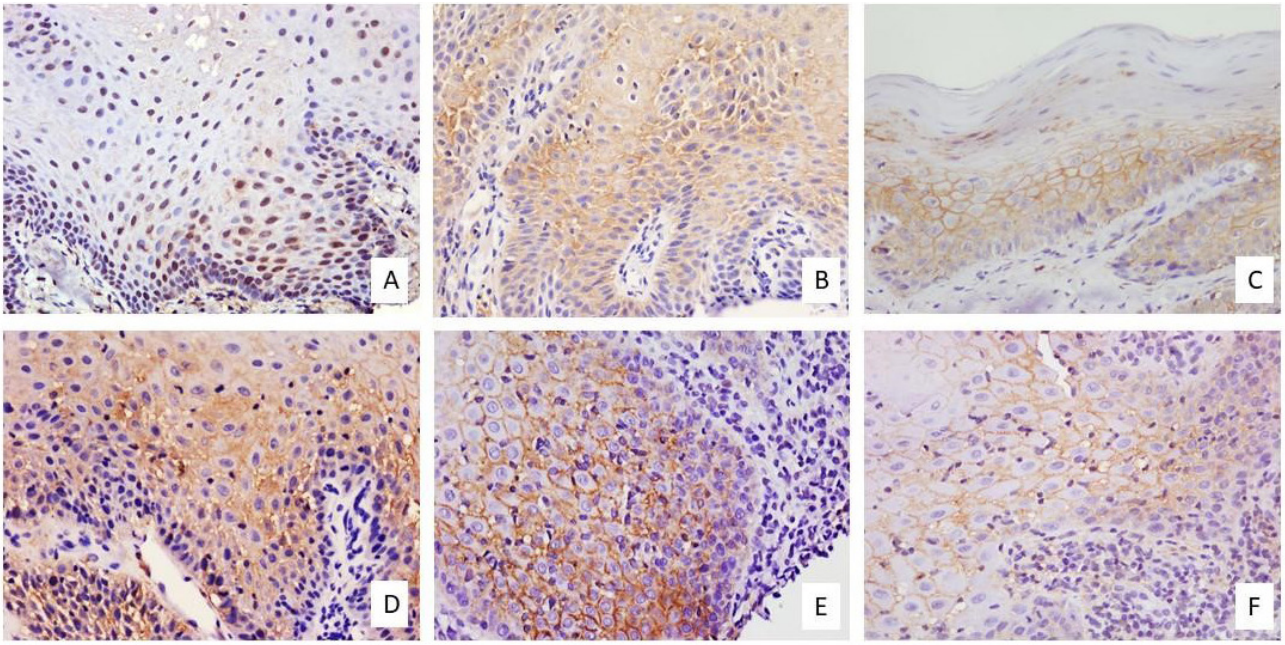


Figure 4 *Representative pictures of CHC1L immunohistochemistry, original magnification: X400 A. Normal oral mucosa B. Acanthosis C. Mild epithelial dysplasia D. Moderate epithelial dysplasia E. Severe epithelial dysplasia F. oral lichen planus. In the parabasal and prickle cell layers, membrane staining could be observed in OPMDs specimens. Positive staining was also observed in the stromal areas*

Discussion

All OPMDs participants in this research were recruited from the "Development of Disease Management Model for Oral Cancer with an Integration Network of Screening, Surveillance, and Treatment in Northeast Health District" proactive inspection project. The NOM controls were the participants who visited the Faculty of Dentistry, Mahidol University for impacted teeth removal. As presented in Tables 1 and 3, most of the participants with OPMDs lesions were from the senior population with an average age of 60. In addition, the participants exhibited the following associated risk factors; smoking, use of smokeless tobacco, alcohol consumption, prolonged exposure to sunlight during the day, and history of cancer elsewhere. Aitiwarapoj and colleagues reported that OPMDs and OSCCs occurred most frequently in the sixth and seventh decades when they surveilled 208 Thai patients with OPMDs and OSCCs at the tongue.[22] Juntanong *et al.* found that the most critical factors strongly associated with increased risk for OPMDs are smoking, alcohol consumption, and betel nut chewing.[7] Therefore, the distribution in age and characteristics of the OPMDs participants in our study are consistent with previous studies done in Thailand.

CLLD7 is a nuclear protein with the potential for tumor suppression. It has RCC1 and BTB domains, which are involved in key steps in cell division, nucleocytoplasmic transport, and protein-protein interaction.[13,23] Many previous studies pointed out that the genes at 13q14, where the CLLD7 gene is also located, were frequently deleted in different cancers.[24-26] However, those studies experimented with other tissues, but not with oral epithelium. Recently, there was a pioneer study comparing CLLD7 expression in OSCC and NOM.[19] Bhattarai *et al.* presented the mislocalization of this protein in OSCC compared to NOM. They reported that nuclear and cytoplasmic staining was observed in NOM and OSCCs, while OSCCs had a much higher cytoplasmic staining than normal mucosa.[19] It was suggested that the nuclear activity of CLLD7 may be compromised in OSCC, and the dislocation of CLLD7

may have a role in tumorigenesis. This study did not find a significant difference in nuclear staining between NOM control and other OPMDs. However, significantly different numbers of nuclear staining were appreciated in the acanthosis group compared to other groups. This may be due to the very high percentage of nuclear staining in the acanthosis group. Since only a limited number of cases were included in this study, a future study is mandatory to confirm this result.

For cytoplasmic staining of CLLD7, it was found that the average percentage of cytoplasmic staining in all OPMDs groups was higher than that of the NOM group. Still, a significant difference was observed between the mild and moderate epithelial dysplasia groups compared to the NOM group (Fig. 2). This result implied a potential sign of CLLD7 protein dislocation to the cytoplasm. Since CLLD7 must be localized in the nucleus to function properly, its localization in the cytoplasm may suggest an improper function of this protein in early oral carcinogenesis. Nevertheless, future studies are necessary to confirm this concept.

CHC1L is a candidate tumor suppressor located telomeric to the Retinoblastoma (Rb) gene on chromosome 13q14.3. It contains similar key domains to CLLD7 and, therefore, carries the same potential to be a tumor suppressor protein as CLLD7. CHC1L may play its functional role in the cell cycle, nucleocytoplasmic transportation, and human cell growth, a possible conveyer of an anti-oncogenic signal.[16] However, many studies reported the under-expression of CHC1L in tumors such as prostate cancer[17], histiocyte-rich neoplasms[18], and multiple myeloma.[27] Bhattarai *et al.* conducted a recent study to compare the expressions of CHC1L between OSCC and NOM.[19] It was suggested that the mislocalization of CHC1L as nuclear staining was reduced, whereas membrane staining was significantly increased in OSCC compared to NOM control.[19] The staining pattern in our study revealed reduced nuclear staining of CHC1L in almost all groups of OPMDs. However, increased membrane staining was appreciated in OPMDs. Furthermore, a significant difference was observed between the severe epithelial dysplasia and OLP/OLL groups compared to the NOM control group. Surprisingly, the membrane staining was gradually increased in accordance with the severity of epithelial dysplasia and was even more pronounced in the OLP/OLL group. These results were in line with the previous study of Bhattarai *et al.* that reported predominant nuclear staining in all cell layers of the normal epithelium with very few cytoplasmic and membrane staining.[19] While in OSCC, membrane staining was dominant, and only very few cells with nuclear staining were spotted.[19] Our results seemed to support the concept of protein dislocation in CHC1L proposed by Bhattarai *et al.*

Protein synthesis usually occurs in the cytosol, and the proteins are then transported to their functional sites, including the nucleus, plasma membrane, mitochondria, or other organelles. Several mechanisms are involved in the dysregulation of protein trafficking in cancer cells, causing abnormal subcellular localization of proteins. Mutation of protein-targeting signals, dysregulation of transporter machinery, aberrant endocytosis and vesicular trafficking, dysregulation of signal transduction and post-translational protein modification, alteration of protein-protein interactions, and cross-regulation of cancer-related proteins are some examples of such mechanisms.[28] Another interesting point is the mislocalization of these proteins in the OLP/OLL group. This implied that the potential dysregulation of this protein in OLP/OLL might support the concept of malignant transformation in OLP/OLL by protein mislocalization.

Another interesting point is the positive staining in the stroma. There might be some crosstalk between oral epithelial cells and the stromal cells as some studies indicated the interaction between the tumor microenvironment and its influence on the growth and metastasis of head and neck squamous cell carcinoma.[29] We believe that there might be an interaction between the stromal cells within the underlying connective tissue and the epithelial cells in dysplasia and OLP/OLL but this needs to be further elucidated in future studies.

Despite the exciting results, we acknowledge the limitations of this study in that only a small number of

specimens were included in each group. In addition, there was no investigation concerning the mechanism responsible for protein mislocalization. Therefore, future studies using molecular techniques are needed to declare the potential tumor suppressive function of these two proteins.

Conclusion

Increasing cytoplasmic expression of CLLD7 and subcellular mislocalization of CHC1L from the nucleus to the membrane were interesting. Both these proteins could be putative markers during oral malignant transformation; nevertheless, further study is still needed to confirm these results.

Acknowledgment

The authors would like to thank the Department of Oral and Maxillofacial Pathology and the Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol University, for their support. This work is supported by a Mahidol University research grant (051/2562) to Siribang-on Piboonniyom Khovidhunkit, Boworn Klongnoi, and Vanvisa Sresumatchai under the Development of Disease Management Model for Oral Cancer with an Integration Network of Screening, Surveillance, and Treatment in Northeast Health District project and in part by the Faculty of Dentistry, Mahidol University (2018).

Conflict of interest

The authors have no relevant financial or non-financial conflict of interests to disclose.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al.* Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. ***CA Cancer J Clin*** 2021;71(3):209-49.

2. World Health Organization 2020; International Agency for Cancer Research (GLOBOCAN 2020), The Global Cancer Observatory; Thailand. Available at https://gco.iarc.fr/today/data/factsheets/populations/764-thailand-fact-sheets.pdf .020.

3. Dhanuthai K, Rojanawatsirivej S, Subarnbhesaj A, Thosaporn W, Kintarak S. A multicenter study of oral malignant tumors from Thailand. ***J Oral Maxillo Path*** 2016;20(3):462.

4. El-Naggar AKC, JK. C., Grandis JR, Takata T, Slootweg PJ. WHO classification of head and neck tumours. 4th ed: International Agency for Research on Cancer (IARC); 2017.

5. Loyha K, Vatanasapt P, Promthet S, Parkin DM. Risk factors for oral cancer in northeast Thailand. ***Asian Pac J Cancer Prev*** 2012;13(10):5087-90.

6. Imsamran W. editor. Cancer in Thailand vol. IX, 2013-2015. In: Cancer Registry Unit, National Cancer Institute, Ministry of Public Health. 2015.

7. Juntanong N, Siewchaisakul P, Bradshaw P, Vatanasapt P, Chen SL, Yen AM, *et al.* Prevalence and factors associated with oral pre-malignant lesions in Northeast Thailand. ***Asian Pac J Cancer Prev*** 2016;17(8):4175-9.

8. Kumar M, Nanavati R, Modi TG, Dobariya C. Oral cancer: Etiology and risk factors: A review. ***J Cancer Res Ther*** 2016;12(2):458-63.

9. Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, Bagan JV, Gonzalez-Moles MA, Kerr AR, *et al.* Oral potentially malignant disorders: A consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. ***Oral Dis*** 2021;27(8):1862-80.

10. Nikitakis NG, Pentenero M, Georgaki M, Poh CF, Peterson DE, Edwards P, *et al.* Molecular markers associated with development and progression of potentially premalignant oral epithelial lesions: Current knowledge and future implications. ***Oral Surg Oral Med Oral Pathol Oral Radiol*** 2018;125(6):650-69.

11. Guo T, Califano JA. Molecular biology and immunology of head and neck cancer. ***Surg Onco Clinics of North Amer*** 2015;24(3):397-407.

12. Hadjebi O, Casas-Terradellas E, Garcia-Gonzalo FR, Rosa JL. The RCC1 superfamily: from genes, to function, to disease. ***Biochim Biophys Acta*** 2008;1783(8):1467-79.

13. Mabuchi H, Fujii H, Calin G, Alder H, Negrini M, Rassenti L, et al. Cloning and characterization of CLLD6, CLLD7, and CLLD8, novel candidate genes for leukemogenesis at chromosome 13q14, a region commonly deleted in B-cell chronic lymphocytic leukemia. ***Cancer Res*** 2001;61(7):2870-7.

14. Zhou X, Munger K. Clld7, a candidate tumor suppressor on chromosome 13q14, regulates pathways of DNA damage/repair and apoptosis. ***Cancer Res*** 2010;70(22):9434-43.

15. Maestro R, Piccinin S, Gasparotto D, Vukosavljevic T, Boiocchi M, Doglioni C, *et al.* Chromosome 13q deletion mapping in head and neck squamous cell carcinomas: Identification of two distinct regions of preferential loss. ***Cancer Res*** 1996;56(5):1146-50.

16. Devilder MC, Cadoret E, Chérel M, Moreau I, Rondeau G, Bézieau S, *et al.* cDNA cloning, gene characterization and 13q14.3 chromosomal assignment of CHC1-L, a chromosome condensation regulator-like guanine nucleotide exchange factor. ***Genomics*** 1998;54(1):99-106.

17. Latil A, Morant P, Fournier G, Mangin P, Berthon P, Cussenot O.

CHC1-L, a candidate gene for prostate carcinogenesis at 13q14.2, is frequently affected by loss of heterozygosity and underexpressed in human prostate cancer. ***Inter J Cancer*** 2002;99(5):689-96.
18. Spillane DR, Wang DY, Newbigging S, Wang Y, Shi CX, Cho HR, *et al.* Chromosome condensation 1-like (CHC1L) is a novel tumor suppressor involved in development of histiocyte-rich neoplasms. ***PLoS ONE*** 2015;10(8):1-12.
19. Bhattarai BP, Suppramote O, Jirawatnotai S, Meesakul O, Juengsomjit R, Janebodin K, *et al.,* editors. A preliminary study of the expression of p16INK4a, CLLD7, and CHC1L in oral squamous cell carcinoma. The 17th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT2019); 2019 July 8 - 10, 2019 (Proceeding); Pullman Khon Kaen Raja Orchid, Khon Kaen, Thailand.
20. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. ***J Oral Pathol Med*** 2007;36(10):575-80.
21. Cheng Y-SL, Gould A, Kurago Z, Fantasia J, Muller S. Diagnosis of oral lichen planus: a position paper of the American Academy of Oral and Maxillofacial Pathology. ***Oral Surg Oral Med Oral Pathol Oral Radiol*** 2016;122(3):332-54.
22. Aittiwarapoj A, Juengsomjit R, Kitkumthorn N, Lapthanasupkul P. Oral potentially malignant disorders and squamous cell carcinoma at the tongue: clinicopathological analysis in a Thai population. **Eur J Dent** 2019;13(3):376-82.
23. Solomou EE, Sfikakis PP, Kotsi P, Papaioannou M, Karali V, Vervessou E, *et al.* 13q Deletion in chronic lymphocytic leukemia: characterization of E4.5, a novel chromosome condensation regulator-like guanine nucleotide exchange factor. ***Leuk Lymphoma*** 2003; 44(9):1579-85.
24. Ripollés L, Ortega M, Ortuño F, González A, Losada J, Ojanguren J, *et al.* Genetic abnormalities and clinical outcome in chronic lymphocytic leukemia. ***Cancer Genet Cytogenet*** 2006;171(1):57-64.
25. Wada M, Okamura T, Okada M, Teramura M, Masuda M, Motoji T, *et al.* Frequent chromosome arm 13q deletion in aggressive non-Hodgkin's lymphoma. ***Leukemia*** 1999;13(5):792-8.
26. Liu Y, Hermanson M, Grander D, Merup M, Wu X, Heyman M, *et al.* 13q deletions in lymphoid malignancies. ***Blood*** 1995;86(5):1911-5.
27. Legartova S, Harnicarova-Horakova A, Bartova E, Hajek R, Pour L, Kozubek S. Expression of RAN, ZHX-2, and CHC1L genes in multiple myeloma patients and in myeloma cell lines treated with HDAC and Dnmts inhibitors. ***Neoplasma*** 2010;57(5):482-7.
28. Wang X, Li S. Protein mislocalization: mechanisms, functions and clinical applications in cancer. ***Biochim Biophys Acta*** 2014; 1846(1):13-25.
29. Koontongkaew S. The tumor microenvironment contribution to development, growth, invasion and metastasis of head and neck squamous cell carcinomas. ***J Cancer*** 2013;4(1):66-83.

Original Article

A Systematic Review of the Effectiveness of Laser Therapy in Prevention of Osteoradionecrosis of the Jaw

Thipok Sombutsirinun[1,2,3], Suwat Tanya [2,4], Sajee Sattayut[1,2]

[1]Department of Oral and Maxillofacial Surgery, Khon Kaen University, Khon Kaen, Thailand
[2]Lasers in Dentistry Research Group (LDRG), Khon Kaen University, Khon Kaen, Thailand
[3]Department of Oral and Maxillofacial Surgery, University of Phayao, Phayao, Thailand
[4]Department of Community Dentistry, Chiang Mai University, Chiang Mai Thailand

Abstract

Osteoradionecrosis of the jaw is an incurable condition. Management of complete healing of the necrotic bone and clinical full mucosal coverage is challenging. Therefore, the prevention of osteoradionecrosis of the jaw is worth considering. There was some evidence of using laser therapy for preventing osteoradionecrosis of the jaw. The purpose of this systematic review was to evaluate the effectiveness of laser therapy in the prevention of osteoradionecrosis of the jaw. A systematic review was conducted on published articles in databases of MEDLINE, Embase, Cochrane Library, Scopus, Google Scholar and Thai-Journal Citation Index Center to identify the eligible studies to compare the effectiveness to prevent osteoradionecrosis of the jaw. The latest search date was 29 May 2022. The included studies were assessed with two independent reviewers by using the Cochrane Risk of Bias Tool for randomized controlled trials or the Joanna Briggs Institute critical appraisal for case reports. Then the data was extracted by using the *Cochrane Handbook for Systematic Reviews of Interventions.* The two reviewers were calibrated. The agreement of assessment between the reviewers was 90 %. There were 24 articles included by title and abstract. Five articles were discarded because of duplication. Of 19 articles, there were four studies; one randomized controlled trial and three case reports, that met the eligible criteria. The level of the bias was low risk. In conclusion, there was a possibility of using laser therapy immediately after extraction to prevent osteoradionecrosis of the jaw by gaining faster tissue coverage. The combinations of using laser therapies; photobiomodulation and photodynamic therapy with antibiotics or pentoxifylline and tocopherol or platelet-rich fibrin allowed favorable clinical outcomes in prevention of osteoradionecrosis of the jaw.

Keywords: Osteoradionecrosis, Jaw, Laser therapy, Photobiomodulation, Photodynamic therapy

Received Date: Aug 4, 2022 **Revised Date:** Sep 13, 2022 **Accepted Date:** Oct 31, 2022
doi: 10.14456/jdat.2023.8

Correspondence to :
Sajee Sattayut, Oral and Maxillofacial Surgery, Faculty of Dentistry, Khon Kaen University, 123 Mittraphap Rd., Muang, Khon Kaen, 40002 Thailand. E mail: sajee@kku.ac.th

Introduction

It is a fact that 75% of head and neck cancer patients need radiation therapy either for primary or adjunctive therapy after surgical resection of the tumors.[1] Subsequently, patients have to undertake dental extractions or other oral surgeries. There is a risk for the patient to develop osteoradionecrosis of the jaw (ORNJ) which is one of the most unmanageable complications of radiation therapy for head and neck tumors.[2]

From the review by Marx et al. ORNJ is defined as an unhealed exposed bone for at least six months in the size of more than 1 cm^2 in an area involving the field of radiotherapy.[3] There was a systematic review and meta-analysis reporting the prevalence of ORNJ in the range of 5% to 15%.[4] According to a review by Rice *et al.* more than 70% of ORNJ occurred within the first three years after cancer treatment including radiotherapy.[5] ORNJ was commonly found in the mandible by comparison with the maxilla due to poor vascular supply and high bone density of the mandible.[5] Several factors including primary tumor sites especially at the tongue and floor of the mouth, cancer staging, radiation dose, radiotherapeutic technique, oral condition, tooth extraction, smoking, drinking and nutritional status were able to increase susceptibility to developing ORNJ.[6] The clinical presentations of ORNJ varied from an area of exposed bone intra-orally, cutaneous fistula, resorption of the inferior border of the mandible and pathological fracture.[4] Once ORNJ has occurred, it is very challenging for the medical team to regain the form and function of the jaw to the patient.

There have been several therapies used for treating ORNJ such as conservative treatments[7], hyperbaric oxygen therapy (HBO), medications comprising pentoxifylline, tocopherol and clodronate (PENTOCLO), surgery[5] and laser therapy.[6] Regarding conservative treatments including oral hygiene care and antibiotics, 40% to 60% of patients improved by these treatments.[7] From the meta-analysis of Leesomprasong *et al.* hyperbaric oxygen therapy provided no statistically significant better healing of tissue coverage of the exposed bone when compared to the no treatment group.[8] Although, PENTOCLO seemed to gain the efficacy of achieving clinical and radiographic remission of ORNJ, the result needed to be confirmed by prospective randomized studies.[9] After surgical interventions such as radical resection and immediate well-vascularized tissue flap reconstruction, only 55 of the 108 patients were free from ORNJ.[10] There was a report that proposed the benefit of laser therapy via photobiomodulation (PBM) and antimicrobial photodynamic therapy (aPDT) .[6] This is a novel treatment for ORNJ due to the prominent property of laser for promotion of wound healing. Laser therapy was used to control ORNJ because it was able to promote the healing of soft tissue coverage for exposed bone in the oral cavity.[10]

From the review as mentioned, the clinical outcome of treating ORNJ has been still unpredictable. Moreover, some procedures such as resection of the jaw and extensive surgery may compromise the quality of life of the patient. Therefore, we postulate that prevention is considered to be the best strategy for managing ORNJ. Some interventions have been introduced for the prophylaxis of ORNJ such as hyperbaric oxygen therapy and PENTOCLO. From the systematic review, there was insufficient information to demonstrate that the use of hyperbaric oxygen therapy reduced the incidence of ORNJ.[10] Regarding the use of pentoxifylline and tocopherol, there was a systematic review suggesting a lower incidence of ORNJ in the patients receiving dental extractions following radiation therapy.[11] However, both hyperbaric oxygen therapy and PENTOCLO have required a long treatment period and compliance from the patients at least 30 dives of HBO in one and a half month or nine weeks of taking pentoxifylline and tocopherol. The systematic review of El-Rabbany *et al.* reported the prevention of ORNJ by using platelet-rich plasma (PRP), fluoride gel and high content fluoride toothpaste, HBO and antibiotics. There has been no review on laser therapy preventing ORNJ.[13]

Regarding the properties of the laser, this therapy was able to increase cellular proliferation, stimulate protein synthesis, promote angiogenesis, and inhibit electrophysiological activity on the nerves.[10] Laser therapy not only enhances wound healing but also relieves pain. Therefore, it is noted that laser therapy can be a good modality for the prevention of ORNJ. This systematic review was conducted with the aim to evaluate the effectiveness of laser therapy in the prevention of osteoradionecrosis of the jaw.

Materials and Methods

The systematic review was conducted based on the assumption and methods as follows.

Objective: Our objective for this systematic review was to evaluate the effectiveness of laser therapy used for preventing ORNJ. The main process of the systematic review provided by the Cochrane Handbook for Systematic Reviews of Interventions was followed.[14] The assessment of the included article was based on the Cochrane Risk of Bias Tool for randomized controlled trials[15] or the Joanna Briggs Institute (JBI) critical appraisal for case reports.[16]

Eligibility criteria: The eligibility criteria were the following:

- Types of studies: The clinical studies or reports which had the goal to evaluate the effectiveness of laser therapy in the prevention of ORNJ in humans published from 2000 to week 5, April 2022 were included. In addition, the studies or reports must be published in Thai or English language.

- Types of participants of the studies: The studies involved patients who had a history of radiation therapy in head and neck regions that required dental extractions or oral surgeries. The studies would be excluded from the systematic review if ORNJ existed before receiving the interventions. However, it was acceptable if ORNJ occurred on a different site from the area where the intervention would be done.

- Types of interventions: The studies of interest would be the ones that used laser therapy to prevent ORNJ. Apart from that, the studies needed to provide laser parameters such as wavelength, power, and time. The interventions could be either given to patients before or after dental extractions or oral surgeries.

- Types of outcome measures: The investigators of the studies had to evaluate the complete wound healing (defined as an absence of clinical signs and symptoms of ORNJ), complete mucosal coverage with no bony exposure, no pain and patient satisfaction.

Search methods for the identification of studies. This systematic review was not registered. Our search strategy for this review was applied up to 29 May 2022, to the following electronic databases which was accessed through Khon Kaen University, namely, MEDLINE, Embase, Cochrane Library, Scopus, Google Scholar, and Thai-Journal Citation Index Center (TCI). Once the articles were identified, the reference lists of the included articles were reviewed to identify articles that may have been missed in the search. Articles published in Thai and English were included. The keywords used in the search include (Laser therapy) AND (Osteoradionecrosis), (Photobiomodulation) AND (Osteoradionecrosis), (PBM) AND (ORN), (Laser therapy) AND (Osteoradionecrosis of the jaw), (Photobiomodulation) AND (Osteoradionecrosis of the jaw), กระดูกตาย (in Thai), เลเซอร์ (in Thai). The exploration of the references of each article was also performed to include more studies.

Study selection and data extraction. Two reviewers (T.S. and S.T.) independently conducted study selection and data extraction. Before selecting publications, T.S. and S.T. calibrated the criteria of selection with S.S. Then each reviewer independently reviewed the titles and abstracts of the studies to include the eligible studies. The data extraction form was designed with guidance from the Cochrane Handbook for Systematic Reviews of Interventions version 6.3.[14] Disagreements were solved by discussion. The senior reviewer was involved when the disagreements remained uncleared. The following data was collected:- type of study, subject, control, intervention and outcome.

Assessment of risk of bias and certainty of evidence. The risk of bias was assessed in the selected studies by using either the Cochrane Risk of Bias Tool for randomized controlled trials[15] or the Joanna Briggs Institute (JBI) critical appraisal for case reports.[16] The calibration of applying these assessments was also conducted among the authors. Two reviewers (T.S. and S.T.) independently graded the studies into a "low", "unclear", or "high" risk of bias for the Cochrane Risk of Bias Tool[15] and "Yes", "No", "Unclear", or "Not applicable" for the JBI critical appraisal.[16] The reviewers solved disagreements by seeking consensus or consultation with a senior reviewer (S.S.). GRADE system was used to assess the certainty of the evidence for the main outcome. The certainty of the evidence was classified as high, moderate, low, or very low.[17-18]

Results

According to the methods of conducting this systematic review, there were 24 articles from the electronic search and no related article from the reference search.

After the removal of duplicates, there were 19 articles. These articles were independently reviewed by 2 reviewers (T.S. and S.T.) in order to screen the titles and abstracts. Out of 19 articles, 14 were discarded because they did not meet the inclusion criteria. The 14 excluded articles were related to the treatments or management of ORNJ only and had no information about prevention. Furthermore, there was one article[19] that the full paper could not be found. Finally, four articles[20-23] that met the inclusion criteria were included in our systematic review. The number of articles per process of searching, screening and selection is shown in Figure 1.

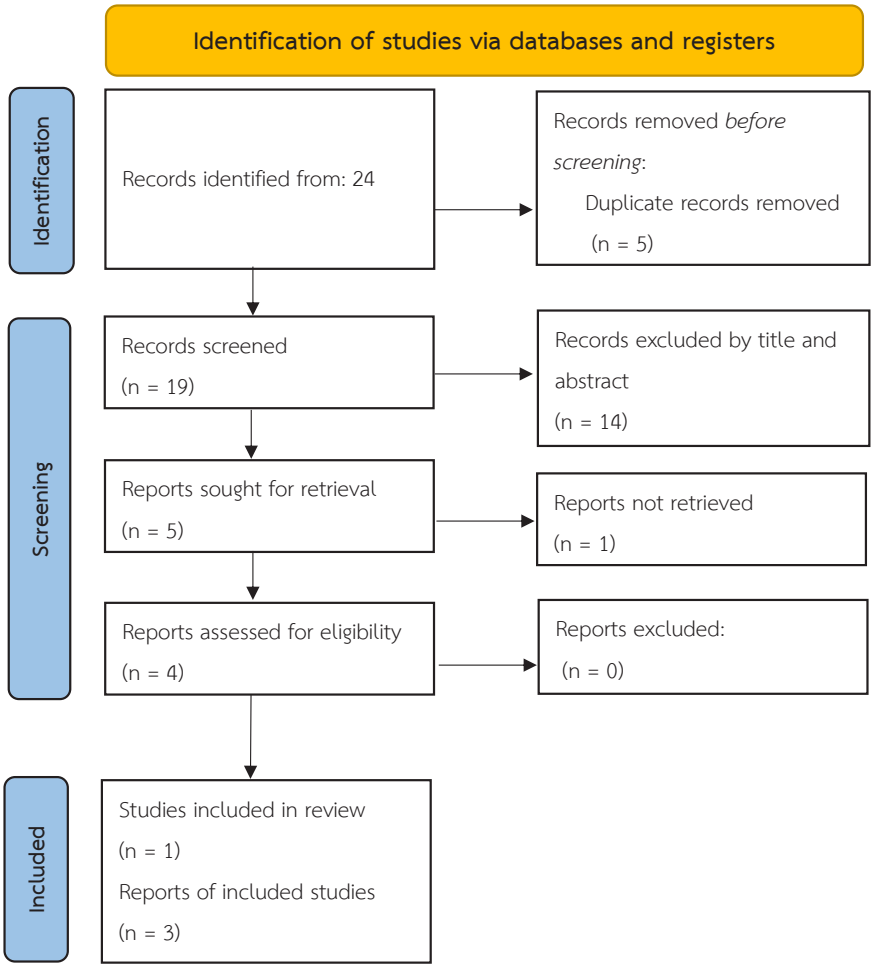


Figure 1 *The flow chart of database searching, screening, and selection. There were 5 retrieved studies (Da silva et al. 2020, Magal haes et al. 2020, Tateno RY et al. 2020, Franco T et al. 2017, Moreschi et al. 2016)*

The included articles were one randomized controlled trial[20] and three case reports.[21-23] The agreement of assessment between the two reviewers was 90%. The results of the risk of bias assessment are shown in Figure 2. The study of Da silva *et al.* which was a randomized controlled trial study showed a low risk of bias overall. This study achieved a low risk of bias in four domains and some concern in the domain of selection of the reported result (Figure 2).

The assessment of the case reports is shown in Figure 3. The evaluation of the case report of Magalhaes IA *et al.* was considered as low risk of bias as comprising 5 yes, 2 unclear, and 1 not applicable. The case report of Tateno RY *et al.* had a low risk of bias due to achieving 6 yes, 1 unclear, and 1 not applicable. The case report of Franco T *et al.* was evaluated as low risk of bias comprising 7 yes and only one unclear.

The certainty of evidence was only assessed from the included RCT (Table 10). It was a moderate certainty. The extracted data is shown in Table 2.

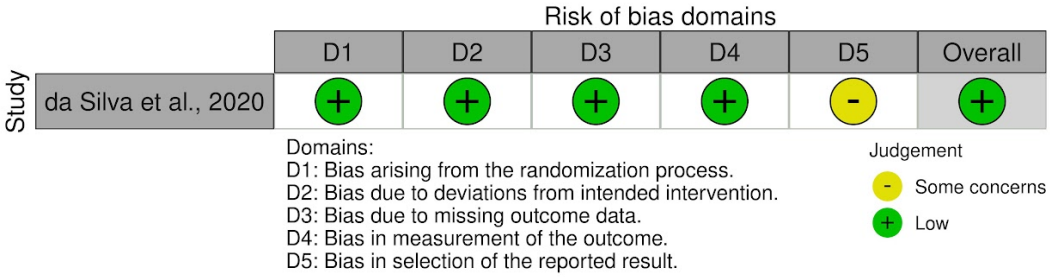


Figure 2 *The risk of bias of the randomized controlled trial evaluated by using Cochrane Risk of Bias Tool for randomized controlled trials*

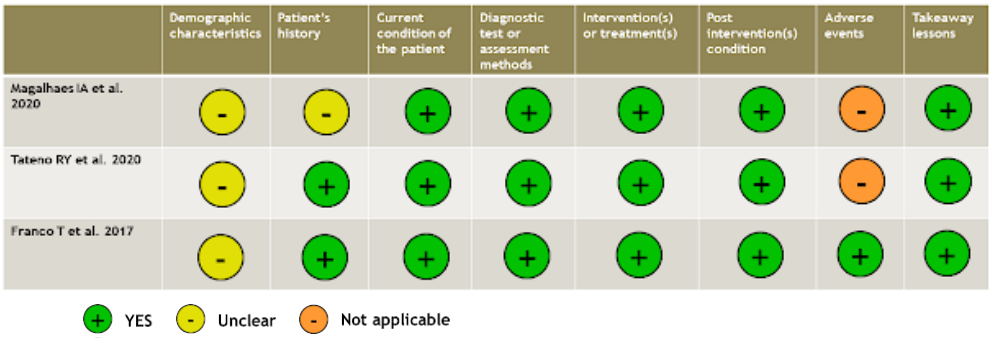


Figure 3 *The risk of bias of the case reports evaluated by using JBI critical appraisal for case reports*

Table 1 *Summary of findings: Laser therapy in prevention of osteoradionecrosis of the jaw (ORNJ)*

Laser therapy in prevention of osteoradionecrosis of the jaw (ORNJ)						
Patient or population: patients at risk of developing ORNJ **Setting:** Hospital **Intervention:** Laser therapy with antibiotics **Comparison:** sham laser with antibiotics						
Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	**№** of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with sham laser	Risk with Laser therapy				
Incidence of ORNJ (mucosal coverage)	At day 14, laser therapy significantly improved mucosal coverage 18/19 sites compared to 0/21 in the control group (RR 0.053, 95%CI 0.008 to 0.355, $p<0.001$). There is no difference in mucosal coverage on day 28.		Not estimable	40 (1 RCT)	⊕⊕⊕◯ Moderate[a]	Laser therapy (PBMT) seems to speed up the epithelization of the extraction sockets compared to sham lasers.

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).*

CI: confidence interval

GRADE Working Group grades of evidence

***High certainty:** We are very confident that the true effect lies close to that of the estimate of the effect.*

***Moderate certainty:** We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.*

***Low certainty:** Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.*

***Very low certainty:** We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.*

a. downgraded 1 level due to serious indirectness (both intervention and control groups were combined with antibiotics)

Table 2 *The summary of data extracted from the selected articles*

Study	Type of study	Participants	Control/compared treatment	Intervention including setting	Outcome
Da silva *et al.* 2020	RCT	n=40 Mean (SD) age: 57.05 (10.75) Sex (%male): 63.6%	n=21 Extraction + sham-PBMT+Amoxicillin	n=19 Extraction + PBMT using diode laser 808 nm, 40 mW, 100 J/cm^2, 70s at Day 0,Day 7, Day14 and Day 21+ Amoxicillin	PBMT had a significant positive effect on both post-operative pain (NNT=2.192, CI95%=1.372-5.445) and mucosal healing (NNT=1.056, CI95%=0.954-1.181). No incidence of ORNJ
Magalhaes *et al.* 2020	Case report	58-year-old male		A) Extraction & enucleation of periapical cyst B) Amoxicillin and Clindamycin or Metronidazole C) Diode laser (Immediately after the surgical procedure. In addition, one aPDT and three PBMT were performed weekly for 1 month) D) aPDT (using methylene blue and red light 660 nm, 100mW, 90s)	From clinical and radiographic examinations performed after 1, 4, and 12 months, there was no incidence of ORNJ.
Tateno RY *et al.* 2020	Case report	62-year-old male		A) Extraction & alveoloplasty with primary closure B) Clindamycin C) PBMT 660 nm and 808 nm, 100 mW, 1 J/point, in contact mode (Immediately after the surgical procedure was done and then once/week for 30 days) E) aPDT (using 0.01% methylene blue and red light 660 nm, 5J/point)	During follow-up of 1 year, there was no recurrence of bone necrosis or sequestration and no infection. The patient was asymptomatic and had normal oral functions.
Franco T *et al.* 2017	Case report	16-year-old female		A) PRF then suturing B) Amoxicillin with clavulanic acid, pentoxifylline, tocopherol, chlorhexidine MW C) PBMT 808nm, 100mW, 0.0028cm^2, 2 J (Immediately after surgical procedure was done)	The extraction sockets were completely healed. The patient did not report any post- operative pain, edema, or other significant side effects.

**RCT=randomized control trial, NNT (number needed to treat), PRF (platelets rich fibrin)*

In the four included articles, it was found that the investigators used the following treatments for the prevention of ORN.

1. Photobiomodulation (PBM) and amoxicillin. The study of da Silva *et al.* compared the effectiveness of PBM using diode laser and amoxicillin in the prevention of ORNJ in patients submitted to dental extraction after head and neck radiation therapy.[20] The patients were divided into two groups which were PBMT and sham-PBMT. The intervention group received PBM by using an 808 nm diode laser at 40mW, 100 J/cm^2 and 70 seconds on days 0, 7, 14, and 21. However, all patients received amoxicillin and surgical debridement to promote primary closure of the surgical site. The PBMT group showed faster mucosal healing (NNT = 1.056, CI95% = 0.954-1.181) and less post-operative pain (NNT = 2.192, CI95% = 1.372-5.445) than the other group.

2. Photobiomodulation, photodynamic therapy and antibiotics.

Magalhaes *et al.* reported on a 58-year-old male patient with a history of radiotherapy to the head and neck region exhibiting a periapical cyst and multiple root remnants.[21] The PBMT using a 660 nm diode laser at 100 mW, 35 J/cm^2, 10 seconds was immediately irradiated after the surgical procedure. The patient also received three PBMT weekly for three weeks and one aPDT by using methylene blue as a photosensitizer and 660 nm at 100mW, 90 seconds as an activator. In addition, 21 days of amoxicillin followed by seven days of clindamycin or metronidazole was prescribed. 0.12% chlorhexidine mouthwash was prescribed until the complete tissue coverage of the surgical wound. From 12 months of follow-up, there was no recurrence of ORNJ.

Tateno RY *et al.* reported on a 62-year-old male patient with a history of squamous cell carcinoma at the base of the tongue undertaking radiation therapy for 35 fractions of 2 Gy. After one year of cancer treatment, he developed generalized radiation-induced dental caries. Thereby, the dentist performed full mouth extraction.[22] The total number of 17 teeth were removed under general anesthesia. Removing bone roughness and primary closure was achieved. Immediately after the surgical procedure, PBMT by using 660 and 880nm diode lasers at 100 mW, 1 J/point together with aPDT by using methylene blue and red light 660 nm at 5 J/point were undertaken. The patient also received the combination of PBMT and aPDT once a week for 30 days. During the follow-up period of one year, there was no recurrence of bone necrosis or sequestration as well as infection. The patient was asymptomatic besides normal oral functions.

3. Photobiomodulation, platelet-rich fibrin (PRF) and antibiotics Franco T *et al.* reported on a 16-year-old female patient who was sent to the dentist for extraction of teeth 37,38 (mandibular left second and third molars) due to advanced external root resorption. She had a history of mucoepidermoid carcinoma at the left parotid gland and received conventional radiotherapy with a total dose of 70 Gy in 35 sessions five years ago.[23] The medications including 0.12% chlorhexidine mouthwash, antibiotic (amoxicillin with clavulanic acid 875mg, twice a day), pentoxifylline (400mg, twice a day) and tocopherol (1000 IU) once daily were prescribed. The patient took those medications one week prior to the surgery and continued the antibiotics for one week and pentoxifylline with tocopherol for eight weeks. After the teeth were extracted, PRF membranes collected from the patient's blood were placed in the sockets. After suturing, PBMT by using an 808 nm diode laser at 100 mW, 0.0028 cm^2, 2 J was irradiated. The extraction sockets were completely healed. The patient did not report any post-operative pain, edema, or any other significant side effects.

Discussion

Based on the assumption of our systematic review, only one randomized controlled trial met the criteria. The main result of this study showed that the mucosal healing of the group receiving PBM was faster than the other group which received sham PBM. It was noticed that both groups received amoxicillin.[20] For the case reports[21-23] showing favorable results, the combination of laser therapy either

PBM or aPDT and antibiotic medications or pentoxifylline and tocopherol or PRF was employed. However, the duration and dose of antibiotics or pentoxifylline and tocopherol taken were not less than the routine protocol.

Regarding the protocol of providing laser therapy in particular photobiomodulation, all of the studies started lasering the patients just immediately after the surgical procedure. According to the mechanism of photobiomodulation which modulates tissue by regulating cellular activity and increasing microcirculation.[24] We suggest that a pre-session of photobiomodulation be considered for improving the quality of post-radiotherapy tissue before undertaking oral surgery intervention. The aPDT has an antimicrobial effect by producing reactive oxygen species.[6] Furthermore, this mechanism does not cause bacterial resistance.[6] We postulate that using aPDT may reduce the duration of antibiotic medication taken by the patients.

Strengths and limitations of this review

This is the first systematic review evaluating the effectiveness of laser therapy in preventing ORNJ. However, meta-analysis cannot be performed because there is only one RCT that met the inclusion criteria. Before making a reliable conclusion, we emphasize that there is a need for a well-designed RCT to examine the preventive effect of laser therapy for ORNJ.

Implications for practice

There is limited evidence evaluating the effectiveness of laser therapy alone to prevent ORNJ. However, there is no report of ORNJ and clinical complications after being treated with laser therapy. This study cannot conclude that laser therapy alone prevents ORNJ due to an insufficient number of included studies. With the medium certainty evidence, this study probably recommends that pre-session and post-session of laser therapies combined with antibiotics may prevent ORNJ and promote tissue coverage in the patients who underwent extraction.

Implications for research

We strongly recommend further clinical trials assessing the effectiveness of laser therapy alone or combining the treatment with other preventive modalities to prevent ORNJ. Not only the clinical outcomes should be evaluated but the patient satisfaction should be assessed among different preventive modalities of ORNJ.

Conclusion

From this systematic review of which low risk of bias and moderate certainty of evidence, there was a possibility of using laser therapy immediately after extraction to prevent ORNJ by gaining faster tissue coverage. In the case reports, it was found some combinations of using laser therapies; photobiomodulation and photodynamic therapy with antibiotics or pentoxifylline and tocopherol or PRF. Using these combinations allowed favourable results in the prevention of ORNJ.

Sources of support: This systematic review was supported by Lasers in Dentistry Research Group (LDRG), Faculty of Dentistry, Khon Kaen University, Thailand."

Declarations of interest: There is no declaration of interest.

References

1. Alfouzan AF. Radiation therapy in head and neck cancer. ***Saudi Med J*** 2021;42(3):247–54.

2. Chronopoulos A, Zarra T, Ehrenfeld M, Otto S. Osteoradionecrosis of the jaws: definition, epidemiology, staging and clinical and radiological findings. A concise review. ***Int Dent J*** 2018;68(1):22–30.

3. Marx RE, Sawatari Y, Fortin M, Broumand V. Bisphosphonate-induced exposed bone (osteonecrosis/osteopetrosis) of the jaws: risk factors, recognition, prevention, and treatment. ***J Oral Maxillofac Surg*** 2005;63(11):1567-75.

4. Kolokythas A, Rasmussen JT, Reardon J, Feng C. Management of osteoradionecrosis of the jaws with pentoxifylline–tocopherol: a systematic review of the literature and meta-analysis. ***Int J Oral Maxillofac Surg*** 2019;48(2):173–80.

5. Rice N, Polyzois I, Ekanayake K, Omer O, Stassen LFA. The management of osteoradionecrosis of the jaws - A review. ***The surgeon*** 2015; 13(2):101–9.

6. Ribeiro GH, Minamisako MC, Rath IB da S, Santos AMB, Simões A, Pereira KCR, *et al.* Osteoradionecrosis of the jaws: case series treated with adjuvant low-level laser therapy and antimicrobial photodynamic therapy. ***J Appl Oral Sci*** 2018;26:e20170172.

7. Hwang L, Gung C, Hwang LA, Chang CH, Tai WC, Su WC. Current Management of Osteoradionecrosis of Jaw in Head and Neck Cancer. ***Int J Head Neck Sci*** 2019;3(2):92–8.

8. Leesomprasong T, Thaweedej S: The effectiveness of hyperbaric oxygen therapy for management osteoradionecrosis in Jaw: A meta-analysis. ***J Dept Med Servic*** 2021;46(1):100–6.
9. Robard L, Louis MY, Blanchard D, Babin E, Delanian S. Medical treatment of osteoradionecrosis of the mandible by PENTOCLO: Preliminary results. ***Eur Ann Otorhinolaryngol Head Neck Dis*** 2014;131(6):333–8.
10. Dai T, Tian Z, Wang Z, Qiu W, Zhang Z, He Y. Surgical management of osteoradionecrosis of the jaws. ***J Craniofac Surg*** 2015;26(2):e175–9.
11. Fritz GW, Gunsolley JC, Abubaker O, Laskin DM. Efficacy of pre- and postirradiation hyperbaric oxygen therapy in the prevention of postextraction osteoradionecrosis: a systematic review. ***J Oral Maxillofac Surg*** 2010;68(11):2653-60.
12. Aggarwal K, Goutam M, Singh M, Kharat N, Singh V, Vyas S, *et al.* Prophylactic Use of Pentoxifylline and Tocopherol in Patients Undergoing Dental Extractions Following Radiotherapy for Head and Neck Cancer. ***Niger J Surg*** 2017;23(2):130-3.
13. El-Rabbany M, Duchnay M, Raziee HR, Zych M, Tenenbaum H, Shah PS, *et al.* Interventions for preventing osteoradionecrosis of the jaws in adults receiving head and neck radiotherapy. ***Cochrane Database Syst Rev*** 201920;2019(11):CD011559.
14. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Cochrane Handbook for Systematic Reviews of Interventions* version 6.3 (updated February 2022). Cochrane, 2022. Available from www.training.cochrane.org/handbook.
15. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, *et al.* Cochrane Bias Methods Group; Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. ***BMJ*** 2011;343:d5928.
16. Munn Z, Barker TH, Moola S, Tufanaru C, Stern C, McArthur A, *et al.* Methodological quality of case series studies: an introduction to the JBI critical appraisal tool. ***JBI Evid Synth*** 2020;18(10):2127-2133.
17. Schünemann HJ, Higgins JP, Vist GE, Glasziou P, Akl EA, Skoetz N, Guyatt GH, Cochrane GRADEing Methods Group (formerly Applicability and Recommendations Methods Group) and the Cochrane Statistical Methods Group. Completing 'Summary of findings' tables and grading the certainty of the evidence. Cochrane Handbook for systematic reviews of interventions 2019:375-402.
18. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, *et al.* GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. ***J Clin Epidemiol*** 2011;64(4):383-94.
19. Moreschi C, CAPPARè P, Meleti M, Vescovi P, Bonanini M, Gherlone EF, *et al.* Low level laser therapy in non-surgical management of osteoradionecrosis of the jaws. ***Minerva Stomatol*** 2016;65(3):185-7.
20. da Silva TMV, Melo TS, de Alencar RC, Pereira JRD, Leão JC, Silva IHM, *et al.* Photobiomodulation for mucosal repair in patients submitted to dental extraction after head and neck radiation therapy: a double-blind randomized pilot study. ***Support Care Cancer*** 2021; 29(3):1347-54.
21. Magalhães IA, Forte CPF, Viana TSA, Teófilo CR, Lima Verde RMB, Magalhães DP, *et al.* Photobiomodulation and antimicrobial photodynamic therapy as adjunct in the treatment and prevention of osteoradionecrosis of the jaws: A case report. ***Photodiagnosis Photodn Ther*** 2020;31:101959.
22. Tateno RY, Palma LF, Sendyk WR, Campos L. Laser and antimicrobial photodynamic therapy for the management of delayed healing following multiple dental extractions in a post-radiotherapy patient. ***Photodiagnosis Photodyn Ther*** 2020;30:101764.
23. Franco T, Cezini M, Metropolo L, Ferreira D, Tannure P: Success of preventive approach to mandibular osteoradionecrosis in an adolescent: case report. ***Oral Surg*** 2017;10(4):e104-9.
24. Dompe C, Moncrieff L, Matys J, Grzech-Leśniak K, Kocherova I, Bryja A, et al. Photobiomodulation-Underlying Mechanism and Clinical Applications. ***J Clin Med*** 20203;9(6):1724.

Original Article

Creating A Gingival Color Database Among Thai Samples by Using Digital Images Processing

Suriyan Thammarat[1], Suttipalin Suwannakul[1], Sasitharee Nathamtong[1], Anuphan Sittichokechaiwut[1]

[1]Department of Periodontology, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand

Abstract

This research aims to analyze the digital color-coded values of clinical gingival color images and create a gingival color database using digital images to measure the clinical gingival color levels. Theories of RGB and HSB color models were applied in the present study. The research model was cross-sectional descriptive. There were two research methods: 1) Digital gingival color code analysis and 2) Digital gingival color reliability analysis. In the first part, photographs of the gingiva of 99 patients who were admitted to the dental hospital, Faculty of Dentistry, Naresuan University, were taken. Adobe Photoshop CS6 software was employed for digital color code analysis. The digital color codes of the gingiva collected from the 143 samples were then examined for their reliability. Data from the study exhibited the total numbers of digital areas from the gingival images were 3,700 areas, with slightly red being the majority, 35.51%, followed by pale pink, red, and bluish-red, for 27.46, 25.43, and 11.59%, respectively. The RGB and HSB color models revealed the frequency of one or two codes using the statistical mode. In contrast, the red code showing ten performed by the RGB model and more codes in the HSB model. The reliability analysis of digital gingiva was calculated with an alpha coefficient of 0.655, which is a moderate confidence level. The analysis of dental images in the present study shows the promising potential to create a database of gingival color. The digital image databases are created on the Windows operating system and Microsoft Access software that can display data such as frequency, percentage, gingival color code, and color samples. Performing the software, users can basically input data consisting of gingival images, color codes, filter inputs, and search the database of RGB and HSB color models.

Keywords: Digital image analysis, Gingivitis, Gingival color

Received Date: Aug 21, 2022 **Revised Date:** Oct 6, 2022 **Accepted Date:** Nov 18, 2022
doi: 10.14456/jdat.2023.9

Correspondence to :

Anuphan Sittichokechaiwut, Faculty of Dentistry, Naresuan University, Tapoe, Muang, Phitsanulok, 65000 Thailand. E-mail: anuphans@nu.ac.th

Introduction

Gingivitis and periodontitis are diseases caused by bacterial infection, which affects the periodontal organs due to antagonistic interactions between bacteria in the dental plaque and periodontal tissues via intracellular

and vascular responses. The onset and progression of periodontitis are classified as immunopathogenesis with inflammation. It can be clinically demonstrated locally or generalized. In addition, supporting factors affecting pathogenesis include environmental and genetic factors.[1]

Inflammation causes the color of the gingiva to change from normal to pale pink, starting to redden with the degree of inflammation. If the inflammation is mild, it will be slightly red, and more inflammation will be red. Gingival discoloration is caused by an increased cellular and vascular response within the gingival tissue. If the inflammation persists for a long time, it will have a bluish-red color due to congestion of venous blood.[2]

In previous studies, digital image processing was performed to assess the importance of plaque adhesion conditions on the teeth, gingival swelling, and gingival color changes associated with inflammation in Gingivitis. The researchers observed that the change in gingival color from different levels of inflammation could indicate Gingivitis.[3-6] Usually, the gingival color assessment can be assessed by a dentist, but sometimes the color assessment may vary depending on the experience of each dentist. It can cause inaccuracies in the diagnosis of Gingivitis.[7] Building a database of digital gingival color values can help develop a tool to distinguish gingival color, which would help reduce the aberration of color vision and make the gingival color assessment more accurate with the same standard.

The RGB color model is based on the principles of color theory and color mixing that uses red, green, and blue light as the primary colors to mix different colors. The color reproduction mechanism on computer monitors uses the glow of red, green, and blue phosphor dots to mix the light into different colors. It will assign three numeric values, which are the value of R, the value of G, and the value of B. The program will set a number from 0 – 255. The number 0 indicates that the beam is not firing in that area, but the higher the number, the more increasing intensity of the light.[7]

The HSB color model (HSB) is a basic color system whose mechanism of color reproduction is similar to the human color perception process. This color system divides the color composition into three parts. The first part, hue (H), indicates that color is caused by the value of light waves that hit an object and are reflected into the human eye. Hue is arranged in a color wheel ranging from 0 to 360 degrees. The second part, Saturation (S), is the intensity and fade of the color. Determined by the proportion of gray that exists in that color, it ranges from 0% (much gray) to 100% (no gray). The third part, Brightness (B), is the lightness and darkness of a color. It ranges from 0% (black) to 100% (white). [7]

A 1975 study of Ibusuki's gingival color measuring Hue, Chroma, and Value found that the gingiva of the elderly had a more purplish-blue hue and a higher chroma value, while the Value was higher among adolescents.[8] In addition, the CIELAB color system model was used to study gingival color, such as in the study of Huang JW *et al.* 2010, to develop a shade guide for pink aesthetics and to create ten gingival color shades.[9] However, the RGB color model is used to view colors in images from a typical electronic device screen. The HSB model is closer to the human color perception process, with limited studies in gingival color separation. The RGB (Red, Green, Blue) and the HSB (Hue, Saturation, Brightness) color model theories and the concept of four clinical grades of gingival color classification: Pale pink, Slightly red, Red, and Bluish-red, were used to study and build a database. This study aimed to analyze the digital color code of the gingiva on the clinical inflammatory color scale, and to create a digital image database for clinical gingival color measurement.

Materials and Methods

This study has been accredited by the Human Research Committee on Project Ethics. Naresuan University institution review board on February 27, 2019, No. COA NO.086/2019

This study was a cross-sectional descriptive of 99 Thai ethnic patients admitted to the Periodontology clinic, Faculty of Dentistry, Naresuan University, who were diagnosed with gingivitis or periodontitis. The exclusion criteria were the patient who has braces, has a metal component of a prosthesis, has a dental implant, or has hyperpigmented gingiva from the area of the second premolar to the anterior teeth onwards. The expert observers were dentists who work in periodontology and have at least five years of working experience. A Canon 700D digital camera was used to record gingival images. A tripod was used to control the distance and shooting position 80 cm away from the background wall. ISO sensitivity of 800, shutter speed (f) at f/18, exposure time of 1/40 sec, automatic white balance, and automatic head flash were set consistently. All patients were fixed at the position of the Frankfurt plane paralleling the floor. Images were taken from the frontal view. Adobe Photoshop CS6 was used to identify digital color codes. Color analysis software used in this study was newly developed by our research team. The study was conducted in two parts: 1) Digital gingival color code analysis and 2) Digital gingival color reliability analysis. For the digital gingival color codes analysis, three observers viewed images and selected the area representing the best pale pink, slightly red, red, and bluish-red from anywhere in each image (maximum of ten areas of each color). Selected areas were analyzed for the codes of RGB and HSB using Adobe Photoshop CS6. All code values were recorded and analyzed in the color analysis software. The data of frequency, percentage, highest value, lowest value, popular base, and range were described statistically. For digital gingival color reliability analysis, verifying the consistency of the representative color code values obtained from the first part is a procedure. Twelve new clinical images, (three pale pink, three slightly red, three red, and three bluish-red) selected by three experts, were used in the questionnaire, with four choices of both RGB and HSB color codes from the first part. The developed questionnaire was tested by periodontologists, general dentists, and dental students totaling 143 people. A sample of the questionnaire is shown in Figure 1. Internal reliability was analyzed using Cronbach's Alpha coefficient.

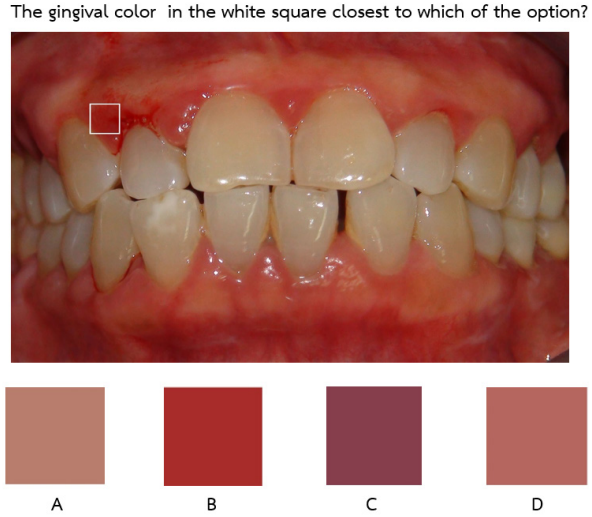


Figure 1 *Shows a sample of the questionnaire*

Results

1. Digital gingival color code analysis

All 99 participants were ethnic Thais. As for the other demographic data of the samples from this study, only the gingival images were collected in the early stages. As a result, only 31 participants could collect feedback on other sample demographic characteristics (Table 1).

The gingival images of 99 participants showed the total numbers of digital areas from the gingival images were 3,700 areas, with slightly red being the majority, 35.51 %, followed by pale pink, red, and bluish-red, for 27.46, 25.43, and 11.59 %, respectively (Table 2).

1.1 RGB color code classified according to the degree of clinical gingival color

RGB color code values classified according to the degree of clinical gingival color in the gingival images of 99 participants showed that the gingival color code is distinctly different for each clinical grade of gingival color (Table 3).

Table 1 *Demographic data of the sample (N=31)*

Demographic characteristics	Frequency	Percentage
sex		
Male	12	38.70
Female	19	61.30
Age (years)		
< 30	26	83.88
31 – 49	3	9.67
>50	2	6.45
Average = 28.16 SD = 8.97 Highest = 56 Lowest = 23		

Table 2 *Summarizing the number of areas and percentage of each gingival color chosen by the expert*

Gingival color	Area	Percentage
Pale pink	1,016	27.46
Slightly red	1,314	35.51
Red	941	25.43
Bluish-red	429	11.59
Total	3,700	100.0

Table 3 *Shows the summary of RGB color code and gingival color*

Gingival color	Min.	Mode	Max.	Range
Pale pink	109,75,39	184,125,108	204,212,150	95,137,111
Slightly red	121,52,48	181,102,94	209,166,157	88,114,109
Red	73,9,25	160,44,42 160,48,42 167,44,42 167,48,42	160,44,49 160,48,49 167,44,49 167,48,49	160,44,75 160,48,75 167,44,75 167,48,75	197,149,136	124,140,111
Bluish-red	91,27,6	135,62,76	199,143,130	108,116,124

The red code is especially the most different (Range = 124,140,111), while the slight red code was the least different (Range = 88,114,109). In addition, the visible colors are different when comparing the color vision at

all four levels of gingival color by considering only the Mode value. Pale pink is lighter than slightly red and red. The bluish-red color looks darker than purple.

However, it is worth noting that the Mode value of the red grade has up to 12 values. Looking at the color of the eyes, it can be seen that all colors are very close. It can be divided into two types of vision: reddish-orange and reddish-pink.

1.2 HSB color code classified according to the degree of clinical gingival color.

HSB color code values classified according to the degree of clinical gingival color in the gingival images of 99 participants showed that the gingival color code is distinctly different for all gingival colors, and there are 1-2 specific codes for each gingival color (Table 4).

Table 4 *Shows the summary of the HSB color code and gingival color*

Gingival color	Min.	Mode		Max.	Range
Pale pink	0°,19,32	13°,39,72		359°,59,80	359,40,48
Slightly red	0°,4,48	8°,45,68		359°,72,82	359,68,34
Red	0°,31,5	0°,47,66	0°,55,66	359°,92,78	359,61,73
Bluish-red	0°,24,5	4°,39,51	4°,39,52	359°,75,78	359,51,42

When considering only the Mode value, the visible color difference is noticeable. Pale pink looks lighter than slightly red and red, while bluish-red is the darkest.

2. Digital gingival color reliability analysis.

The sample group used to examine the reliability of the test consisted of 143 persons, including specialists, general dentists, dental students, and dental personnel. Demographic characteristics data are shown in Table 5. Cronbach's Alpha Coefficient found that when all 24 tests were taken into the analysis, the reliability score was 0.612, with the reliability value of the whole text being between 0.556 – 0.638. However, when the three low correlation tests were eliminated, the reliability value was increased to 0.655. Therefore, the visual and color-coded tests were moderately confident, with a confidence level greater than 0.610. As mentioned earlier, the exam can be used as a digital gingival color database.

Table 5 *Demographic characteristics of the sample*

demographic characteristics	Frequency	Percentage
Sex		
Male	45	31.46
Female	97	67.83
no answer	1	0.69
Age (years)		
20 - 29	75	52.44
30 - 39	60	41.95
40 - 49	6	4.19
>50	1	0.69

Table 5 *Demographic characteristics of the sample (cont.)*

demographic characteristics	Frequency	Percentage
no answer	1	0.69
Average = 29.13 SD = 5.93 Highest = 50 Lowest = 22		
expertise		
Specialized dentist	37	25.87
General dentist	64	44.76
Dental student (Class year 5-6)	37	25.87
Other dental personnel (Dental assistant)	3	2.10
no answer	2	1.40

3. Creating a Gingival color database

The color analysis program is a newly created software by our research team for collecting color-coded gingival color data in RGB and HSB models. It is run through the Microsoft Access runtime Version 2010 on Windows 8.1 and above (Windows Version 8.1). A total of 32,750 records can be recorded. The program can display frequency, percentage, gingival color code, and color samples. Using the software, users can input data consisting of gingival images, color codes, filter inputs, and search the database of RGB and HSB color models.

Discussion

The RGB color model accurately recognized the clinical gingival color with almost every color except red. The analysis showed that the RGB color code's mode was found in only one value for each of the three gingival colors. These were pale pink (Code 184,125,108), slightly red (Code 181,102,94) and bluish-purple (Code 135,62,76). The red gingival color was found to have up to 12 color code base values. Red gingival classification by RGB color system has many central tendency values; therefore, red gingival diagnosis may be inaccurate. The results supported the weakness of the RGB color model, namely its sensitivity to uneven illumination, and the difference between colors is not linear. It may cause an aberration of human color vision.[11] Another reason may be that the chances of seeing red light are higher than in other colors. Humans can generally see the light or waves of different colors, with a wavelength range of 390 – 780 nm. Different wavelengths produce different colors. For example, blue has a length of 455-492 nm; green is 492-577 nm, etc. Compared to red, with a wavelength of 622 - 780 nm, it is found that red light has a significantly longer wavelength than other colors[12], so professionals have a greater chance of seeing red than any other color.

As a result, the nominal values are more multivalued. The findings provide a better understanding of using the RGB color model in clinical gingival color diagnosis. In the case of red gingiva, the dentist may need to review or confirm the results in conjunction with the HSB color model or another color instrument. To make the diagnosis more accurate, Seshan H and Shwetha M[13] used Serif plus-6 software to help compare the differences in swelling and red gingiva before and after treatment. Alternatively, the red representing inflamed gingiva, may be compared with the alveolar mucosa, characterized by red tissue near the gingiva. More accurate confirmation of gingival inflammation was obtained by looking at the gingival color and the hemorrhages from the insertion of a periodontal probe.[14]

The HSB color model can accurately identify clinical gingival colors at all color levels. The HSB system, which analyzed four levels of gingival color, found that there were only 1-2 values for the color codes that experts agreed on: pale pink and slightly red, with only one color code: 13°, 39, 72, and 8°, 45, 68, respectively for red and bluish-red to purple with two values each. These findings show that gingival color classification by the HSB color model has very low aberrations. Therefore, this color model can be used in clinical studies or diagnoses of gingival

color. The main reason that most experts see the same color is probably because the HSB system is a primary color system consisting of Hue, Saturation, and Brightness, which is the mechanism of color reproduction that isclose to the process of receiving knowing the human color.[9] So it can be seen that the HSB color model has been used in various contextual studies; for example, a 2011 study by S. Khairunniza-Bejo and S. Kamarudin used the HSB color model to determine the sweetness of Chokanan mangoes. The study used a Keyence Machine Vision system to capture mango images with an HSB color model and then used a Digital AR2008 Abbe refractometer to obtain the sweetness to the set threshold.[15] Although the HSB color model has not been ever used for clinical gingival color analysis, there is the potential to apply it as a knowledge base to develop tools or methods for measuring and evaluating gingival color and creating a future database of gingival color.

However, a 2018 study by Edgar Chavolla[16] looked at the ability to distinguish and detect colors by different color systems using clustering and fragmentation techniques. It was found that different color systems were able to identify different colors. Each color system has different advantages and disadvantages. It was found that the RGB color system is a color system with vast color space, enabling a wide range of colors close to what the human eye sees and is compatible with electronic devices without different adjustments and can interpret mathematical results accurately.[16] Nevertheless, the system has a discrete nature within the color space, so that mistakes are easy to make when grouping and separating colors. The RGB system has no luminance processing, resulting in shadows or noise in the image and the color processing of images is easy to distort. In a color system with a separate H (Hue) value, such as HSB, HSV, or HSL, there is a discrepancy in the color values at the 0° and 360° region if a set of correction commands are inserted into these color systems. It will be able to group and separate colors well.[16] Therefore, in preparing the database, the researcher gathered information on gingival color codes for both RGB and HSB systems, as both systems have different advantages and disadvantages.

The gingival image and color code reliability in the RGB and HSB color models was within acceptable levels in most images. The alpha coefficient reliability analysis found that the value was 0.612 obtained from 24 images, indicating that the color codes from the RGB and HSB color models showed moderate or adequate reliability (an alpha coefficient greater than 0.6[10]); the tool can be used as a gingival color database. However, due to time and resource constraints in this study, only 24 items were included in the analysis, which was one reason for the low reliability. Increasing the number of questions is one way to increase the confidence score[17], where the rule of thumb must be 0.7 or higher. The higher the value, the more consistent the instrument was created.[10,18] Therefore, in order to increase the confidence in the gingival color image in the future, the number of gingival image exams should be increased. In addition, the confidence analysis can be modified with new methods such as test-retest, parallel forms, and confidence among evaluators (Interrater reliability), etc.[19], to comply with the context and limitations of the research. It will help develop tools to be more reliable.

However, in this work, three gingiva-colored images were removed. After removing the images, the confidence value rose from 0.612 to 0.655, indicating that the truncated images had a low correlation with the other images. It may be because the image that had been removed has a slightly unclear color. Understanding this point suggests future developments in gingival color measurement instruments. In measuring instrument development, especially the test, the analysis of difficulty (p) and discrimination power (r) is essential in addition to making the test quality. It also helps to check the basic properties of the tool before applying the statistical analysis in the next step.[20] However, due to the limitations of resources and the researcher's experience, only 24 initial exams (items) were created, and the difficulty and discriminant powers

of the test were not checked. Therefore, it is not possible to find confidence by other methods or other statistics such as Pearson Correlation Coefficient, Paired *T*-test, etc.

The developed RGB and HSB color databases can be used for clinical gingival color diagnosis. One of the objectives of this research is to create a digital image database to measure the level of gingival color in normal and inflamed gingiva. The created database is stored in the color analysis software. It can save data in a database, manage data, search data, and display color-coded data as users want, such as charts, frequencies, etc. The initial usage involves importing new data into a new database of images, and RGB and HSB color codes. Gingival color code range values from this present research helped to separate new codes and imported into data for each color (pale pink, slightly red, red and bluish-red). As the database grows larger, distinguishing between gingival color code range values becomes more precise and more accurate. It can potentially be used for software that could analyze the color of the gingiva from the whole image. The development of an application to analyze the color of the gingiva from the images in the smartphone may provide preliminary diagnosis and treatment recommendations for people. For example, pale pink can indicate healthy gingiva, slightly red can indicate mild gingival inflammation requiring a dentist to assess gingival health, red can indicate severe gingival inflammation needing urgent treatment and bluish-red indicates the gingiva are chronically inflamed, may have a sub-calculus and need to be treated by a dentist. In terms of clinical teaching, image databases and gingival color codes can be used in dental teaching, especially on gingival color inflammation issues. However, this newly created database has limitations in many ways. The subject needs future development, especially the ability to interpret color codes before saving the data.

In saving the gingival color into the database, it is also necessary to save the image in a room or place where the light must be controlled for the image to be equally qualified. After that, points or image color spaces are imported into Adobe Photoshop CS6 to analyze color codes in both the RGB and HSB color systems and then saved in the database. Doing so requires knowledge and programming skills as well as user time. Therefore, to save data and analyze the color of the image more efficiently. In the future, functions in the database should be developed to manipulate images and color codes independently.

The initial usage involves importing new data into a new database of the initial assessment of gingivitis, which is only part of the evaluation of gingiva inflammation. In order to accurately diagnose gingivitis and assess its severity, additional clinical examinations are required, such as measurements of bleeding on probing, probing depth, clinical attachment loss, radiograph, etc. All data obtained will be processed for diagnosis and lead to the correct course of treatment.

Conclusion

The RGB and HSB color codes classified by the clinical grade of the gingival color showed that when considering the Minimum Mode and Maximum statistics of each gingival color level, the gingival color codes were significantly different across all gingival color levels. Considering only the Mode values, there was a noticeable difference in the colors. However, it is worth noting that up to 12 of the red gingiva's mode values are in the RGB color model. Nevertheless, all the colors within the red group are very similar, so the HSB model is suitable for diagnosing accurate color separations. Although the RGB color model's color separation accuracy is less than the HSB, it is compatible with electronic devices.

The reliability of the gingiva image and the color code in the RGB and HSB color models were acceptable for almost all images in the initial instrumentation development. Therefore, the development of increased confidence and database functions to increase their potential is a plan that further research should be developed to increase accuracy and performance. When the database is accurate, and the database size is more extensive, it can be developed into an artificial intelligence system in the future.

This new research on gingival color database construction uses RGB and HSB color models to analyze the color codes to create a criterion for distinguishing pale pink, slightly red, red, and bluish-red color ranges. They are used to assess the color of gingiva in the Thai population. Nevertheless, color-coded values, especially red, still have a wide range, and the reliability is not very high. In future studies, if larger database sizes result in more accurate color-coded values, further reliability studies of this research may need to be designed to provide higher reliability.

Acknowledgements

The authors would like to thank Assist.Prof.Dr. Panwadee Bandhaya, Assoc.Prof.Dr. Danai Reabsakul, Chanoknutpha Therapong, Kanthika Phangkariya, Nuttharee Sanunchatwanich, and Wanida Muangthong.

References

1. American Academy of Periodontology. Treatment of Plaque-induced Gingivitis, Chronic Periodontitis and Other Clinical Conditions. ***J Periodontol*** 2001;72(12):1790-800.

2. Durgesh BH, Basavarajappa S, Ramakrishnaiah R, Al Kheraif AA, Divakar DD. A review on microbiological causes of periodontal disease: disease and treatment. ***Rev Med Microbiol*** 2015;26(2):53-58.

3. Ellis JS, Seymour RA, Robertson P, Butler TJ, Thomason JM. Photographic scoring of gingival overgrowth. ***J Clin Periodontol*** 2001;28(1):81-5.

4. Smith RN, Brook AH, Elcock C. The quantification of dental plaque using an image analysis system: reliability and validation. ***J Clin Periodontol*** 2001;28(12):1158-62.

5. Smith RN, Lath DL, Rawlinson A, Karmo M, Brook AH. Gingival inflammation assessment by image analysis: measurement and validation. ***Int J Dent Hyg*** 2008;6(2):137-42.

6. Seshan H, Shwetha M. Gingival inflammation assessment: Image analysis. ***J Indian Soc Periodontol*** 2012;16(2):231-4.

7. Gonzalez RC, Woods RE. digital image processing third edition. Prentice Hall Upper Saddle River, NJ 07458; 2008 401-7.

8. Ibusuki M. The color of gingiva studied by visual color matching. Part II. Kind, location, and personal difference in color of gingiva. ***Bull Tokyo Med Dent Univ*** 1975;22(4):281-92.

9. Huang JW, Chen WC, Huang TK, Fu PS, Lai PL, Tsai CF, *et al.* Using a spectrophotometric study of human gingival colour distribution to develop a shade guide. ***J Dent*** 2011;39 Suppl 3:e11-6.

10. Taber KS. The use of Cronbach's alpha when developing and reporting research instruments in science education. Research in Science Education. ***Res Sci Educ*** 2018;48(6):1273-96.

11. Garcia-Lamont F, Cervantes J, López A, Rodriguez L. Segmentation of images by color features: A survey. ***Neurocomputing*** 2018;292:1-27.

12. Approximate wavelength for the various colors. Retrieved May 25, 2021, Available from https://www.livephysics.com/physical-constants/optics-pc/wavelength-colors/

13. Seshan H, Shwetha M. Gingival inflammation assessment: Image analysis. ***J Indian Soc Periodontol*** 2012;16(2):231-4.

14. Löe H, Silness J. Periodontal disease in pregnancy. I prevalence and severity. ***Acta Odontol Scand*** 1963;21:533-51.

15. Khairunniza-Bejo S, Kamarudin S, editors. Chokanan Mango Sweetness Determination Using HSB Color Space. 2011 Third International Conference on Computational Intelligence, Modelling & Simulation; 2011 20-22 Sept. 2011. pp. 216-221.

16. Chavolla E, Zaldivar D, Cuevas E, Cisneros M. Color Spaces Advantages and Disadvantages in Image Color Clustering Segmentation. ***SCI*** 2018;730:3-22.

17. Yilmaz Kogar E, DemİRdÜZen E, Gelbal S, İNal H. Cronbach's Coefficient Alpha: A Meta-Analysis Study. ***H.U. J of Educ*** 2017;32(1): 18-32.

18. Tavakol M, Dennick R. Making sense of Cronbach's alpha. Int ***J Med Educ*** 2011;2:53-5.

19. Danner, D. Reliability – The precision of a measurement. GESIS Survey Guidelines. Mannheim, Germany: GESIS – Leibniz Institute for the Social Sciences 2016.

20. Lumbensa P. Determination of the quality of measuring and evaluation tools. in the Academic Service Project, Thasap Model (page 1-10). Yala: Faculty of Education, Yala Rajabhat University. 2016

Original Article

The Efficiency in Reducing the Dispersion Aerosols by Using Various Types of Intraoral and Extraoral Suctions

Mayurach Pipatphatsakorn[1], Anuphan Sittichokechaiwut[2], Pornsuda Norchai[2]

[1]Department of Restorative Dentistry, Faculty of Dentistry, Naresuan University, Muang, Phitsanulok, Thailand

[2]Department of Preventive Dentistry, Faculty of Dentistry, Naresuan University, Muang, Phitsanulok, Thailand

Abstract

Coronavirus disease 2019 (COVID-19) is an emerging disease that puts aerosolized dental treatments at a high risk of transmission; therefore, up-to-date knowledge of aerosol control plays an essential role in determining standard regulation in dental practice. The objective of this study was to compare the efficiency in reducing the dispersion of aerosols by using various types of intraoral and extraoral suctions. The study was conducted in a closed acrylic box. A high-speed handpiece (Airotor) was used to create aerosols. The intraoral and extraoral suctions were divided into six groups (saliva ejector, high-power suction tip, side-wing tip, dome- shaped tip, EasyPrep®, and Extraoral suction). The relative humidity in the box was monitored at 1, 5, and 10 mins with a hygrometer and was repeated three independent times. The videos were also recorded during the experiment. Results showed that the saliva ejector alone had the most aerosol diffusion outside the mouth. The mean of relative humidity was highest and was significantly higher than other groups using a saliva ejector in combination with other suctions. After 1 minute of the procedure, the mean relative humidity in the group using the saliva ejector plus extraoral suction was significantly lower than that of the group using the saliva ejector plus the high-power suction tip at *P*-value = 0.038. When the saliva ejector was used with the extraoral suction, the means relative humidity were not different between groups after 5-10 mins. In conclusion, the present study provided preliminary information for considering instruments as needed and the most effective one in reducing the dispersion of aerosols. The knowledge from this research could be used as a guideline to improve the workflow or regulation in dental practice for safety.

Keywords: Aerosol control, COVID-19, Dental aerosol, Dental aerosol reduction

Received Date: Aug 21, 2022 **Revised Date:** Oct 26, 2022 **Accepted Date:** Nov 18, 2022
doi: 10.14456/jdat.2023.10

Correspondence to:
Pornsuda Norchai, Faculty of Dentistry, Naresuan University, Tapoe, Muang, Phitsanulok, 65000 Thailand. Tel: 082-354-663
E-mail: pornsudan@nu.ac.th

Introduction

Dental procedures require various types of dental equipments, whether a high-speed handpiece, ultrasonic scaler, dental polishing tool (air polishers and air abrasion), or other tools that can produce both large droplets and small aerosols which can be smaller than 50 microns.[1] Most dental treatments involve hard tissue and require

tools to grind, cut, and drill teeth or bone. A tool is a handheld tool or the tip of a sharp trimming tool connected to the handpiece that rotates at high speed and requires water to reduce the operating temperature and the heat generated. In the operation of these instruments, microscopic droplets of water are contaminated with saliva, blood, or microorganism.[2-4] Small droplets can be dispersed into the air and contaminate the tools, equipment, clothing, and personal protective equipment. In the event of the coronavirus disease 2019 (COVID-19) outbreak, these small droplets can float and stay in the air for a period of time depending on the environment.[5,6]

Aerosols as small as 0.5 to 10 microns can enter the respiratory tract and lungs[7], making them highly susceptible to transmission of infection[1,6,8], especially a risk of infection from patient to dentist and the supporting team.[9] Therefore, it is necessary to have guidelines for dental treatment to control the spread of aerosols and infection effectively and strictly. The dispersion of these aerosols has not yet to be reported how far they can disperse, making disinfection on all surfaces and ventilation systems within the dental clinic imperative. However, the current standard protocol in clinical practice may not be effective enough to prevent the spread of COVID-19, despite tremendous screening efforts to evaluate if the patients are in the latent period without any symptoms or not providing accurate information to screening.

The use of an ultrasonic scaling instrument has been reported to produce aerosol dispersion as far as 18 inches, even with no water and extraoral testing.[8] Using a high-speed handpiece can create a wide diffusion area of aerosols which can deposit around the working area.[10] However, to date, there has been no study comparing the control of aerosol distribution in devices currently used during the COVID-19 outbreak. Based on current knowledge, it is believed that aerosols produced by dental treatments are airborne and may remain for several hours.[7,8] As for the diffusion distance, there has been no definitive research on how far the aerosols spread. During the COVID-19 outbreak, it has been suggested to use aerosol diffusion control equipment plus the tools and equipment regularly used under normal circumstances. A wide variety of additional tools and equipments are available in the market with different features. However, there has been no proper research indicating the comparative efficiency of controlling micro-aerosols' dispersion by these instruments and devices.

Since droplets can remain in the air for up to 30 minutes, there is a high risk of infection if they are contaminated with bacteria or viruses6, especially if the operators immediately remove masks and protective clothing in the working area after performing the treatment. For the control of aerosols in the dental area that aerosols disperse, in some clinics or hospitals, only a saliva ejector or together with a high-power suction attached to the dental chair is used. This can only reduce the dispersion of aerosols to a certain amount. However, if a high-volume evacuator is used with a high-volume evacuator power of 100 cubic feet of air per minute, the aerosols can be reduced by more than 90 percent.[7,9,11] However, high-volume evacuators, both intraoral and extraoral, are not in the standard protocol for dental treatments and are not commonly used in dental clinics or hospitals. Moreover, some types of the high-volume evacuators require additional installation resulting in increased costs. In Thailand, these tools are imported or fabricated for commercial purposes. Nevertheless, it is not widely used because the comparative efficacy has yet to be dicovered. Therefore, in this study, seven different tools available in the market were selected and tested for their effectiveness in reducing aerosol diffusion.

Direct measurement of aerosol concentrations is technically difficult. The aerosol measurement, including Laser scattering technology, has been adopted, with a pump-suction sampling method to real-time detect and calculate the number of suspended particles with different particle sizes in the air. Although the newly developed novel aerosol measurement methods, such as "handheld particle counters", are available in the market, they are expensive.[12] Therefore, the hygrometer was used in this study to measure liquid dental aerosols

because of its cost-effectiveness rather than using direct particle aerosol detection.

Materials and Methods

This experiment tested the reduction of dental aerosols by various types of intraoral and extraoral suctions (Free arm forte-S, Tokyo Giken, INC.). A high-speed handpiece (Airotor) (TwinPower Turbine4H® handpieces, J.Morita MFG. CORP.) was used to generate small aerosols by connecting to the water system of the dental treatment unit (Actus 9000, Siamdent). The experiment was conducted in a closed system using a clear acrylic box. The experimental groups were divided into six groups, as shown in Table 1.

Table 1 *Shows the experimental groups on the efficiency in reducing the dispersion of fine aerosols by six different types of intraoral and extraoral devices*

Type of aerosol suction devices	Experimental groups					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Saliva Ejector	+	+	+	+	+	+
High-power suction + straight tip		+				
High-power suction + side-wing tip			+			
High-power suction + dome- shaped tip				+		
High-power suction + EasyPrep®					+	
Extra oral suction						+

Before testing, the relative humidity in the box was measured. The test was carried out using an aerosol generator in the upper front teeth area. The hygrometer (Humidity Thermometer DT-321S, Eastern energy co.,ltd) and the instruments inside the acrylic were positioned at the same place in all groups. The time required to perform the experiment and collect data was 1, 5, and 10 mins, while the aerosol generator and the tested devices were operated simultaneously. Images and video clips were collected and observed during the experiment (PXW-Z150 4K camcorder, Sony). The relative humidity inside the acrylic box was measured using a hygrometer before every experiment in the acrylic box. The experiments were carried out in triplicate in each group. Statistical analysis was performed using SPSS (IBM SPSS Statistics for Windows, Version 23.0) and statistical significance was set at 0.05. Mean and standard deviation were calculated for the relative humidity. One-way ANOVA and Scheffe's test were used to compare the relative humidity means between the experimental groups.

Results

The results from the simulation using a high-speed handpiece (Airotor) on the upper front teeth adjacent to the lip, where aerosol diffusion occurs most, together with various types of aerosol suction tools. Observation of Images and video clips showed that using a saliva ejector alone in the control group showed a large amount of aerosol diffusion outside the mouth. When using other types of aerosol suction tools with the tip closest to the aerosol source, it was found that high-power suction combined with a straight tip, high-power suction with a side-wing tip, and high-power suction with EasyPrep® were able to reduce the amount of aerosol dispersion significantly. The aerosols were sucked back into the oral cavity when the high-power suction connected with the EasyPrep® inside the oral cavity was used. The use of high-power suction with the dome-shaped tip and the use of extraoral suction, which had the large diameter of the tips, also made it possible to suck the aerosol back into the tools, especially when the tool was placed close to the source

of aerosols. However, if the tool's tip was far from the aerosol source, it could not absorb aerosols properly, and aerosols started spreading out of the mouth. Moreover, the large tip size interfered with work and vision more, as shown in Figure 1.

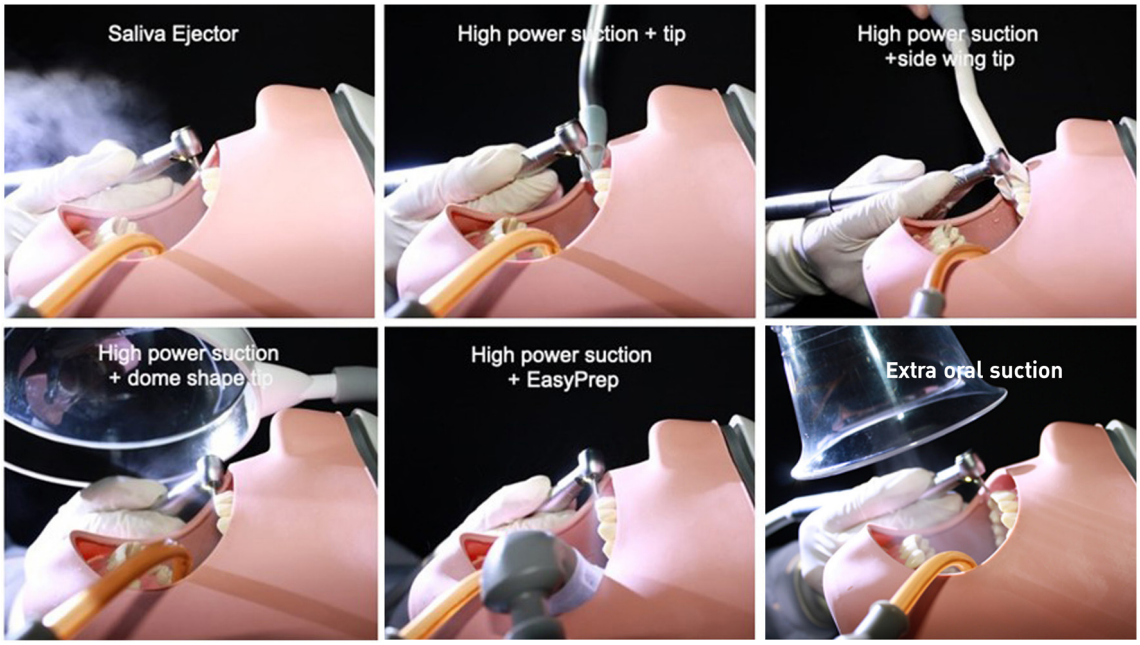


Figure 1 *The reduction of aerosol diffusion caused by a high-speed handpiece (Airotor) by various types of intraoral and extraoral suction tools*

The relative humidity inside the acrylic box measured by a hygrometer at 1, 5, and 10 mins showed that the group of saliva ejector alone had a mean relative humidity at 10 min increased from the mean relative humidity at the beginning of the experiment. For the group using the saliva ejector in combination with other groups of aerosol suctions, the data showed that the mean relative humidity at 10 min decreased from the mean relative humidity at the beginning, as shown in Table 2.

Table 2 *The results of the mean relative humidity (percent) generated by fine aerosols from the high-speed handpiece (Airotor), classified by type of aerosol suction device used and time of use.*

Type of aerosol suction devices	The mean relative humidity (percent)			
	Mean ± SD			
	0 minute	1 minute	5 minutes	10 minutes
Saliva ejector	74.23±0.19	78.28±3.11	77.86±3.48	75.85±3.11
Saliva ejector and High power suction tip	74.23±0.65	68.69±1.28	63.45±1.36	65.17±2.38
Saliva ejector and High power suction side- wing tip	73.75±0.84	67.85±1.36	61.80±2.10	60.68±2.55
Saliva ejector and High power suction dome-shaped tip	74.56±0.80	67.04±0.36	62.65±3.03	61.05±2.74
Saliva ejector and EasyPrep®	74.38±0.60	67.89±1.82	61.22±1.80	59.82±1.86
Saliva ejector and Extra oral suction	74.12±0.82	62.32±2.18	63.15±2.24	63.71±2.28

From the statistical analysis to compare the average relative humidity from the aerosol suction device in each group, it was found that at the start of the experiment (min 0), the means relative humidity of all groups were not different from the group using saliva ejector alone as a control group. In comparing the average relative humidity

from each group of aerosol suctions when the experiment was conducted for 1 min, the mean relative humidity of the saliva ejector alone showed the highest value and a statistically significant difference from all groups. In addition, it was found that the mean relative humidity of the group using the saliva ejector combined with the high-power suction was significantly higher than that of the group using the saliva ejector combined with extraoral suction at 1 min (P-value = 0.038). Comparison of the average relative humidity from each group of aerosol suctions at 5, and 10 mins showed that the saliva ejector group had a statistically higher value than that of others as shown in Table 3.

Table 3 *The comparison of the average relative humidity classified by the type of aerosol suction devices and the duration of the experiment. (*Statistically significant differences (P<0.05; ANOVA test and Scheffe's test)*

Duration	Control group	Experimental groups	*P*-value	95% Confidence Interval	
				Lower Bound	Upper Bound
0 minute	Saliva ejector	Saliva ejector and High-power suction tip	1.000	-2.214	2.214
		Saliva ejector and High-power suction side- wing tip	0.977	-1.731	2.698
		Saliva ejector and High-power suction dome- shaped tip	0.996	-2.538	1.891
		Saliva ejector and EasyPrep®	1.000	-2.358	2.071
		Saliva ejector and Extra oral suction	1.000	-2.108	2.321
1 minute	Saliva ejector	Saliva ejector and High-power suction tip	0.002*	3.517	15.656
		Saliva ejector and High-power suction side- wing tip	0.001*	4.360	16.500
		Saliva ejector and High-power suction dome- shaped tip	<0.001*	5.164	17.303
		Saliva ejector and EasyPrep®	0.001*	4.317	16.456
		Saliva ejector and Extra oral suction	<0.001*	9.884	22.023
	Saliva ejector and High-power suction tip	Saliva ejector	0.002*	-15.656	-3.517
		Saliva ejector and High-power suction side- wing tip	0.997	-5.226	6.913
		Saliva ejector and High-power suction dome- shaped tip	0.943	-4.423	7.716
		Saliva ejector and EasyPrep®	0.998	-5.270	6.870
		Saliva ejector and Extra oral suction	0.038*	0.297	12.436
5 minutes	Saliva ejector	Saliva ejector and High-power suction tip	<0.001*	6.550	22.264
		Saliva ejector and High-power suction side- wing tip	<0.001*	8.203	23.917
		Saliva ejector and High-power suction dome- shaped tip	<0.001*	7.357	23.070
		Saliva ejector and EasyPrep®	<0.001*	8.780	24.494
		Saliva ejector and Extra oral suction	<0.001*	6.853	22.567
10 minutes	Saliva ejector	Saliva ejector and High-power suction tip	0.008*	2.575	18.785
		Saliva ejector and High-power suction side- wing tip	<0.001*	7.065	23.275
		Saliva ejector and High-power suction dome- shaped tip	<0.001*	6.695	22.905
		Saliva ejector and EasyPrep®	<0.001*	7.918	24.129
		Saliva ejector and Extra oral suction	0.003*	4.031	20.242

Discussion

The most effective method to select devices for reducing the amount of aerosol dispersion caused by dental treatments is controversial. Currently, manufacturers provide many devices in the market as accessories during the COVID-19 pandemic, with the expectation that the efficiency of aerosol removal can be significantly enhanced. Most of the tools currently used in dental clinics are intraoral suctions, which are small, easy, and quickly mobilized. In addition, there is a tool for reducing the aerosol from outside the mouth to be used as well. Some of these aerosol removal devices are expensive and may be inconvenient. Therefore, it is still being determined whether it is worth using or not. In the present study, various devices widely used to reduce saliva and aerosols during the COVID-19 outbreak were tested.

Relative humidity is a ratio of atmospheric moisture relative to the amount that would be present if the air was saturated. This displays it as a percentage of the total amount needed for the air to be fully saturated at the same temperature. Measuring relative humidity is not complicated by using a hygrometer. Digital humidity hygrometers have a higher degree of accuracy than analog hygrometers, and they do not need to be recalibrated. Absolute humidity measures the weight of water vapor per unit volume of air. The absolute humidity unit is given as g / m^3., units of grams of water vapor per cubic meter of air, since the absolute humidity of the air is calculated by dividing the mass of water contained in the air by the volume occupied by the quantity of air concerned. So, it is rather complicated than the relative humidity measurement. To quantify the aerosol remaining in the air in this study, we decided to use the simple, cheap, but still reliable and acceptable method.

From the results of this study, it was found that at 10 minutes after using the aerosol-generating tool, the decline in relative humidity percentage was consistent with other studies.[7,9-11] When the relative humidity was measured at 5 and 10 mins after the procedure, no tools tested in the present study significantly reduced the relative humidity. However, using the saliva ejector alone is not recommended since this device is ineffective in reducing the aerosols. The Extraoral suction and high-power suction combined with a dome-shaped tip had the same relative humidity reduction efficiency as other types of aerosol reduction tools, and they can reduce aerosols effectively when placed near the aerosol source. However, they have a limitation: the tool's tip is oversized and must be placed as close to the aerosol source as possible, impeding the dentist's work and vision. Therefore, it is difficult to use effectively compared with the High-power suction with a straight tip, high-power suction with a side-wing tip, or high-power suction with EasyPrep®, since these devices are small and can be placed near the source of aerosol easily in the oral cavity. Moreover, they do not obscure the vision or obstruct the dentist's work, thereby reducing the amount of aerosols greatly.

For the effective removal of aerosols generated by dental procedures, a saliva ejector is recommended for only suctioning of water and saliva. For aerosols, the use a high-power suction combined with a tip that can be placed as close as possible to the aerosol source is recommended. Aslam et al. suggested that the high-power suction should be connected to a large tip at least 8 mm in diameter.[13] Although Shahdad *et al,* 2020 and Noordien *et al,* 2021 showed that an extraoral suction could reduce contamination by aerosols, droplets, and splatter.[14,15] Our present study recommended that an extraoral suction could be used as an additional option to enhance the elimination of aerosol diffusion from the procedure. Shahdad *et al,* 2020 also recommended that four-handed dentistry and the appropriate use of rubber dam should remain the primary mitigating factors.[14] Lloro *et al* 2021 showed that the percentage contamination reductions were highest on the operator face-shield. They recommended standard protective gear such as goggles, face shield, and surgical gloves for maximum safety.[16] Piela et al studied aerosol generated particles using ultrasonic scaling and high-speed handpiece in 2022. They evaluated the efficacy of different high-volume and low-volume suction devices in preventing particle escape during procedures. They found that the

use of any suction device tested resulted in a significant reduction in particle counts compared with no suction. Our present study also showed that all tested devices could effectively reduce aerosols.[17]

The previous study revealed no significant difference in splatter and aerosol reduction between Isolite illuminated isolation system (Isolite Systems) and a saliva ejector.[18] Since those devices were attached to the high-volume suction, they may work similarly to the high-volume evacuator (HVE). The HVE has effectively reduced 90 % of aerosols and spatter from the operation site.[7,19,20] In contrast, this study showed a significant difference in aerosol reduction of saliva ejectors compared to other suction devices. All types of suction tips used in this study were attached to the HVE except saliva ejector—this causes low efficiency in aerosol reduction. This study's findings showed that suction systems attached to HVE can remove a large volume of aerosols within a short period, similar to other studies.[7,19,20] Moreover, the extraoral suction at 1-minute showed significant aerosol reduction compared to other suction tips attached to HVE. The extraoral suction can suck up a large volume of air and aerosols since it has a wide-mouth suction hood and power level of the device. However, after 5 and 10 minutes, the effectiveness of decreasing the aerosols of the extraoral suction was not different from other suction tips attached to HVE.

In the actual clinical situation, the placement of the aerosol-generating instruments in each position is different, resulting in the pattern, direction, and distribution of aerosols from the instruments. The results of this study can be applied to the appropriate positioning of the aerosol reduction tools by choosing to place them in the most critical dispersed position to minimize the spread and contamination as much as possible. Although no definitive study has reported the effect of 2019 coronavirus infection and susceptibility to dental aerosol contamination, this study showed that the aerosol control from each instrument had the potential to control dispersion. However, the quantity and the distribution of pathogens in aerosols are related to many factors, such as the types of procedures performed, tools, positions, and the efficient air circulation system. Therefore, further studies of those factors are still needed, including the accumulation of viruses in closed areas of the dental treatment room and a study of the mean probabilities of how many more people will spread through dental work, known as the Reproductive number, R0 or R naught.[21]

This study was done in a closed system and without airflow to determine the true potential of the instrument. However, in actual practice, the ventilation system may spread germs and droplets farther and longer in the air. It can be seen from several reports that infected people were linked to locations in the enclosed space, not adequately ventilated, in crowded condition, staying in the place for a long time, and without personal protection. Therefore, various methods must be combined to reduce the risk of infection. Improving ventilation systems in the building and dental treatment rooms may decrease the concentration of germs in the air and reduce the spread of contaminated droplets in the air by means of bringing sufficient fresh air from the outside to fill the working area and have exhaust air to be disposed of, resulting in better air quality.[22-24] Even though we know the effectiveness of suction devices in aerosol reduction, the range of the suction tip to the operating sites and the proper direction to obtain the best practice to control the aerosol dispersion of each working area in the mouth still need to be determined in the future.

Conclusion

The most effective method to reduce the amount of aerosol dispersion caused by dental treatment is still doubtful whether it is worth using. From the results of this study, it was found that the use of an inexpensive high-power suction tip in combination with the saliva ejector can reduce the relative humidity well. Notably, based on the results of this study, the saliva ejector alone is ineffective in reducing dental aerosols. Although the extraoral suction has the potential to reduce the aerosols, it has a limitation and is challenging to be used effectively. Therefore, in terms

of investment cost-effectiveness, using extraoral aerosol suction devices could only be an alternative to enhance the efficiency and could not be used as the primary replacement for other devices.

Acknowledgement

This research plan was successfully accomplished by receiving research grants from the Thai National Research Office and contribution of the Faculty of Dentistry in providing assistance, equipment, and location. We also thank Mr. Soraphong Wongnoi, Ms. Niratcha Chaisomboon, and Ms. Nawarat Koomyat, who kindly participated as research assistants.

References

1. Harrel SK, Molinari J. Aerosols and splatter in dentistry: a brief review of the literature and infection control implications. ***J Am Dent Assoc*** 2004;135(4):429-37.

2. Barabari P, Moharamzadeh K. Novel Coronavirus (COVID-19) and Dentistry-A Comprehensive Review of Literature. ***Dent J*** 2020;8(2):1-18.

3. Meng L, Hua F. Coronavirus Disease 2019 (COVID-19): Emerging and Future Challenges for Dental and Oral Medicine. ***J Dent Res*** 2020;99(5):481-7.

4. Spagnuolo G, De Vito D, Rengo S, Tatullo M. COVID-19 Outbreak: An Overview on Dentistry. ***Int J Env Res Pub Health*** 2020; 17(6):2094.

5. Cottone JA. Practical infection control in dentistry. Making the pieces fit. Part 1. ***Tex Dent J*** 1987;104(9):7-10.

6. Hinds WC. Aerosol technology: properties, behavior, and measurement of airborne particles: John Wiley & Sons; 1999.

7. Micik RE, Miller RL, Mazzarella MA, Ryge G. Studies on dental aerobiology. I. Bacterial aerosols generated during dental procedures. ***J Dent Res*** 1969;48(1):49-56.

8. Harrel SK, Barnes JB, Rivera-Hidalgo F. Aerosol and splatter contamination from the operative site during ultrasonic scaling. ***J Am Dent Assoc*** 1998;129(9):1241-9.

9. Kohn WG, Collins AS, Cleveland JL, Harte JA, Eklund KJ, Malvitz DM. Guidelines for infection control in dental health-care settings--2003. ***MMWR Recomm Rep*** 2003;52(RR-17):1-61.

10. Bentley CD, Burkhart NW, Crawford JJ. Evaluating spatter and aerosol contamination during dental procedures. ***J Am Dent Assoc*** 1994;125(5):579-84.

11. Klyn SL, Cummings DE, Richardson BW, Davis RD. Reduction of bacteria-containing spray produced during ultrasonic scaling. ***Gen Dent*** 2001;49(6):648-52.

12. Somsen GA, van Rijn CJM, Kooij S, Bem RA. Measurement of small droplet aerosol concentrations in public spaces using handheld particle counters. ***Phys Fluids (1994)*** 2020;32(12):121707.

13. Aurangjeb AM, Zaman T, Badruddoza M. Practice of Dental Surgeons about Dental Splatter and Aerosol. ***City Dent Coll J*** 2013;10(2):10-6.

14. Shahdad S, Patel T, Hindocha A, Cagney N, Mueller JD, Seoudi N, *et al.* The efficacy of an extraoral scavenging device on reduction of splatter contamination during dental aerosol generating procedures: an exploratory study. British dental journal. 2020:1-10.

15. Noordien N, Mulder-van Staden S. *In Vivo* Study of Aerosol, Droplets and Splatter Reduction in Dentistry. ***Viruses*** 2021;13(10).

16. Lloro V, Giovannoni ML. Perioral Aerosol Sequestration Suction Device Effectively Reduces Biological Cross-Contamination in Dental Procedures. ***Eur J Dent*** 2021;15(2):340-6.

17. Piela K, Watson P, Donnelly R, Goulding M, Henriquez FL, MacKay W, et al. Aerosol reduction efficacy of different intra-oral suction devices during ultrasonic scaling and high-speed handpiece use. ***BMC oral health*** 2022;22(1):388.

18. Holloman JL, Mauriello SM, Pimenta L, Arnold RR. Comparison of suction device with saliva ejector for aerosol and spatter reduction during ultrasonic scaling. ***J Am Dent Assoc (1939)*** 2015;146(1):27-33.

19. Harrel SK, Barnes JB, Rivera-Hidalgo F. Reduction of aerosols produced by ultrasonic scalers. ***Journal of periodontology*** 1996; 67(1):28-32.

20. Jacks ME. A laboratory comparison of evacuation devices on aerosol reduction. ***Journal of dental hygiene : JDH*** 2002;76(3):202-6.

21. Achaiah NC, Subbarajasetty SB, Shetty RM. R(0) and R(e) of COVID-19: Can We Predict When the Pandemic Outbreak will be Contained? ***Indian J Crit Care Med*** 2020;24(11):1125–7.

22. Yue L. Ventilation in the Dental Clinic: An Effective Measure to Control Droplets and Aerosols during the Coronavirus Pandemic and Beyond. ***Chin J Dent Res*** 2020;23(2):105-7.

23. Bourouiba L. Turbulent Gas Clouds and Respiratory Pathogen Emissions: Potential Implications for Reducing Transmission of COVID-19. ***Jama*** 2020;323(18):1837-8.

24. Peng X, Xu X, Li Y, Cheng L, Zhou X, Ren B. Transmission routes of 2019-nCoV and controls in dental practice. ***Int J Oral Sci*** 2020;12(1):9.

CONTINUING EDUCATION QUIZ

Journal of The Dental Association of Thailand

Year 2023 Volume 73 Issue 1 January - March 2023

Assessment of Midpalatal Suture Maturation by Cone-beam Computed Tomography in Circumpubertal Age Group

Nopparat Chutasripanich[1], Korapin Mahatumarat[1], Soontra Panmekiate[2]

[1]Department of Orthodontics, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

[2]Department of Radiology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

1. การศึกษานี้ใช้ภาพถ่ายรังสี CBCT ระนาบใดในการประเมินการเจริญเติบโตของ midpalatal suture
   - ก. Coronal view
   - ข. Sagittal view
   - ค. Cross-sectional axial view
   - ง. ถูกทุกข้อ

2. Stage ใดที่พบว่า midpalatal suture มีการเชื่อมกันแล้ว
   - ก. stage C
   - ข. stage D
   - ค. stage E
   - ง. stage D, E

3. Midpalatal suture stage ใดที่สามารถทำการขยายด้วยวิธี conventional rapid maxillary expansion ได้
   - ก. stage A
   - ข. stage B
   - ค. stage C
   - ง. ถูกทุกข้อ

4. ในกลุ่ม postpubertal age group (12-18 ปี) ข้อใดกล่าวได้ถูกต้อง
   - ก. พบ stage C มากที่สุด (62.6%)
   - ข. 82.3% ของผู้ป่วยพบว่า midpalatal suture ยังอยู่ใน nonfused stages (stage A-C)
   - ค. พบ fused stage (stage D-E) ในผู้หญิงมากกว่าในผู้ชาย
   - ง. ถูกทุกข้อ

5. ควรพิจารณาส่งถ่ายภาพรังสี CBCT ก่อนขยายกระดูกขากรรไกรบนในผู้ป่วยอายุเท่าใด
   - ก. อายุมากกว่า 12 ปี
   - ข. อายุน้อยกว่า 12 ปี
   - ค. ผู้หญิงอายุ 12 ปีขึ้นไป, ผู้ชายอายุ 14 ปีขึ้นไป
   - ง. ผู้หญิงอายุ 14 ปีขึ้นไป, ผู้ชายอายุ 12 ปีขึ้นไป

กรุณา ลงทะเบียนหรือ Login ใน www.cdec.or.th เพื่อตอบคำถามและรับคะแนน 3 หน่วยกิจกรรม