JOURNAL OF DENTISTRY
Shiraz University of Medical Sciences

Contents

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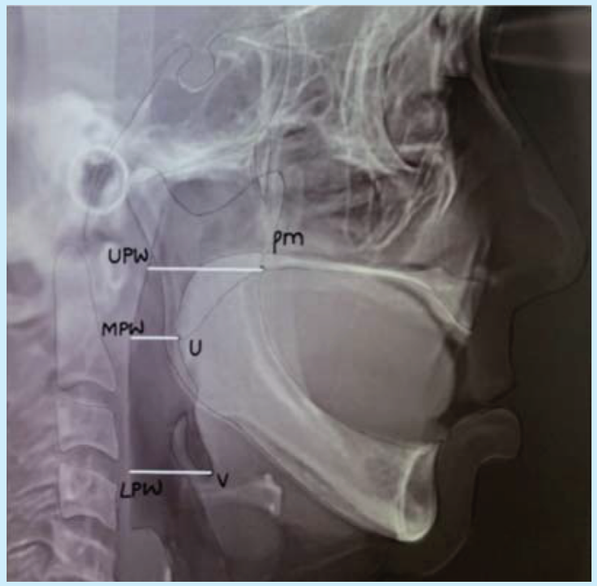

Cephalometric variables depicting Airway space

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Introduction should be brief and end with a statement of the aim of the study or hypotheses tested. Describe and cite only the most relevant earlier studies. Relevant background information, including critical review of available literature, is essential for any experimental study but avoid presentation of an extensive review of the field.

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A description of the statistical methods used to analyze the data should also be included, together with any tests of reproducibility of measurement. Reference should be made to any permission given by related committees of ethical practice. Authors should have considered the ethical aspects of their research in human and animal study, and mention it.

Results should concisely present the finding. Tables are the most useful means of presenting summarized descriptive data.Tables and figures should not include data that can be given in the text in one or two sentences.

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In *conclusion*, bring together the topic sections or experimental findings into a rational combination of current opinion/ knowledge, adding up your own views based on your study.

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REFERENCES

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List should be double- spaced at the end of the article in numeric sequence.We recommend ***Vancouver style*** (described in the British Medical Journal (1991) 302: 338-41)

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Contents

Literature Review

Application of Artificial Intelligence in Clinical Dentistry, a Comprehensive Review of Literature

Kimia Ghods [1], DMD Student; Arash Azizi [2], DMD, MScD; Aryan Jafari [3], DMD Student; Kian Ghods [4], PhD Student;

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KEY WORDS
Artificial Intelligence;
Dentistry;
Machine learning;
Deep learning;
Diagnostic System;

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ABSTRACT

Statement of the Problem: In recent years, the use of artificial intelligence (AI) has become increasingly popular in dentistry because it facilitates the process of diagnosis and clinical decision-making. However, AI holds multiple prominent drawbacks, which restrict its wide application today. It is necessary for dentists to be aware of AI's pros and cons before its implementation.

Purpose: Therefore, the present study was conducted to comprehensively review various applications of AI in all dental branches along with its advantages and disadvantages.

Materials and Method: For this review article, a complete query was carried out on PubMed and Google Scholar databases and the studies published during 2010-2022 were collected using the keywords "Artificial Intelligence", "Dentistry," "Machine learning," "Deep learning," and "Diagnostic System." Ultimately, 116 relevant articles focused on artificial intelligence in dentistry were selected and evaluated.

Results: In new research AI applications in detecting dental abnormalities and oral malignancies based on radiographic view and histopathological features, designing dental implants and crowns, determining tooth preparation finishing line, analyzing growth patterns, estimating biological age, predicting the viability of dental pulp stem cells, analyzing the gene expression of periapical lesions, forensic dentistry, and predicting the success rate of treatments, have been mentioned. Despite AI's benefits in clinical dentistry, three controversial challenges including ease of use, financial return on investment, and evidence of performance exist and need to be managed.

Conclusion: As evidenced by the obtained results, the most crucial progression of AI is in oral malignancies' diagnostic systems. However, AI's newest advancements in various branches of dentistry require further scientific work before being applied to clinical practice. Moreover, the immense use of AI in clinical dentistry is only achievable when its challenges are appropriately managed.

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Cite this article as: Ghods K, Azizi A, Jafari A, Ghods K. Application of Artificial Intelligence in Clinical Dentistry, a Comprehensive Review of literature. *J Dent Shiraz Univ Med Sci.* December 2023; 24(4): 356-371.

Introduction

John McCarthy introduced the term artificial intelligence (AI) in 1956, and it is defined as " a field of science and engineering involved with the machine understanding of what's usually known as intelligent behavior, and with the creation of artifacts that manifest such behavior" [1] In other words, AI is a technology that enables machines to perform tasks usually performed by humans [2].

Numerous forms of AI in dentistry have been introd-

uced in the last two decades. The application of AI in dentistry initiated with one of its most prevalent types, which was machine learning (ML). In ML, the aim was to design a machine/ system using algorithms in a way that it could learn and operate without explicitly planning and dictating each action. [3-4]. Along with rapid advancements of AI in dentistry, another type of AI named artificial neural networks (ANN) was introduced. This algorithm sought to develop information processing inspired by the human brain's neural network. In other words, the neural network helped train computers to respond appropriately to events, instead of dictating what needs to be done. Each neuron in this network is a processing element and solves different problems along with other processing elements [5-6]. Probably the newest development of AI systems in dentistry is deep learning (DL). This system uses several different layers of neural networks. Each of these layers analyzes parts of the input information. Therefore, this mechanism predicts outcomes based on unlabeled and unstructured data [6-9]. Figure 1 shows a schematic view of AI, ML, ANN, and DL.

Numerous studies have reported multiple advantages achieved using AI technology in dentistry. The most eminent advantage of AI is probably its capability to integrate and cross-link data collected from imagery techniques with non-imagery data, including clinical records and general and dental history data, to result in better diagnosis. Nevertheless, the immense application of AI is still a matter of discussion, as its possible drawbacks have been mentioned in new research [2]. Further, in this review article, we will discuss applications of AI in different branches of dentistry along with its challenges.

Materials and Method

A query on some databases, such as PubMed and Google Scholar, was carried out using "Artificial Intelligence", "Dentistry", "Machine learning", "Deep learning", and "Diagnostic Systems "as keywords. In view of the fact that the application of AI in dentistry has started in the last 2 decades, articles published between the years 2010 and 2022 were selected. Moreover, based on the topic of the article and subject of interest, mentioned keywords were defined. Searches were limited to domestic and foreign journals and reference books. The inclusion criteria were the year of publication and the relevance of the title and purpose of the articles to the research topic. The exclusion criteria were studies with additional information, irrelevant topic, studies before 2010, and case reports. Accordingly, 153 English-written articles were selected and studied. All authors collaborated in evaluating the selected articles. An AI specialist, who is the fourth author of the article, initially appraised the articles to choose articles containing valuable, correct, and complete information regarding AI technology. After this phase, 16 articles were excluded from the study. Subsequently, the other three authors evaluated the articles on basis of the accuracy and eloquence of information, and methodology of the study presented in each article. Concerning this phase, 21 articles were excluded. In Figure 2, the methodology of the study is summarized.

Results

According to the mentioned process of methodology, 116 articles were selected for final analysis and article writing. Among 116 articles evaluated, 2 articles were focused on the overall concept of AI and its subtitles,

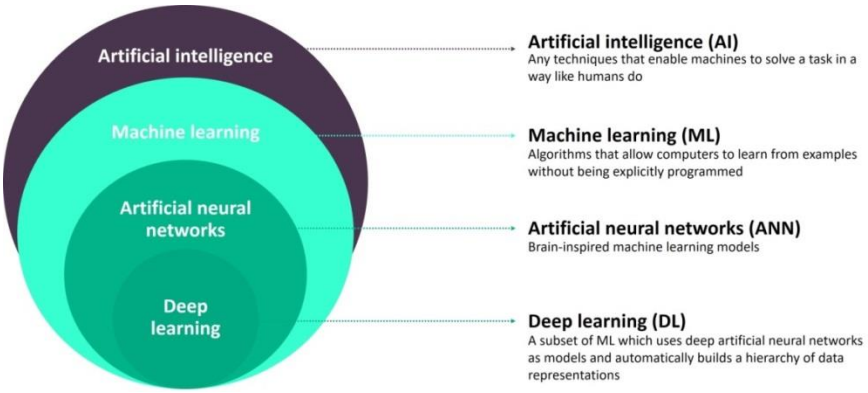

Figure 1: A schematic view of AI, ML, ANN, and DL

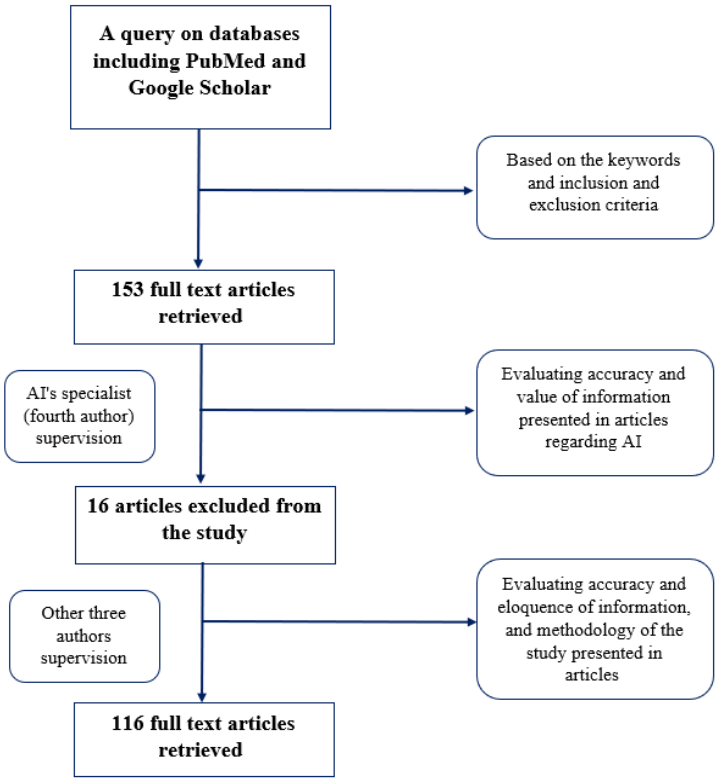

Figure 2: A schematic chart of article's methodology

and 114 other articles were focused on the application of AI in different branches of dentistry. Out of the 114 articles, 16 articles were related to dental pathology, 2 articles were regarding dental diagnosis systems, 16 articles were about dental radiology, 7 articles were related to restorative dentistry, 14 articles were regarding periodontology, 15 articles were about endodontics, 19 articles were related to orthodontics, 7 articles were about prosthodontics, 2 articles were regarding pediatric dentistry, 2 articles were related to dental surgery and 14 articles had a multidisciplinary approach covering various branches of dentistry.

Discussion

Nowadays, AI's influence in all branches of dentistry is evident. In the following, we discuss the impact of AI on each branch separately.

Pretreatment phase

AI technology can perform simple tasks, including booking and coordinating regular appointments according to the convenience of the patients and dentists, and managing the paperwork and insurance. Furthermore, AI can assist in more complicated tasks such as alerting the dentists about any allergies or medical conditions that the patient may have, and also setting up regular reminders for patients who are on tobacco or smoking cessation programs. It is also observed that AI is beneficial in providing emergency teleassistance in cases of dental emergencies when the dental health care professional cannot be contacted, with higher precision and fewer errors [10]. On the other hand, due to the Covid-19 pandemic, AI technology can screen patients and secure dental staff's health [11]. In a study by McCall *et al*. [11], it was elucidated that AI systems can collate data regarding several key risk factors of Covid-19 in order to aid in screening people and monitoring social media, newsfeeds, or airline ticketing systems across the world. The risk factors included in this study were the transmissibility of the virus and risk populations, the natural history of infection, the incubation period, the mortality rate, and the characteristics of the organism in charge. However, the type of AI required to perform this task is not specified [11].

Diagnosis and treatment plan

Probably the most prominent effect of AI in dentistry is in the process of diagnosis and decision-making. Many studies have confirmed AI systems can easily integrate data collected from the patient and present a good outcome that helps the dentist with her final diagnosis and treatment plan [12-16]. In order to accomplish this mis-

sion, AI systems initially need to be trained with a vast amount of data collected from reliable sources. In other words, valid information about the possible mutual relationship between medications, systemic conditions, and clinical dental manifestations is gathered from textbooks and previous clinical experiences to train AI systems. Moreover, AI learns to consider the effects of a patient's past dental and medical history, patient preferences, and imagery data all together on the final dental outcome and presents the intended treatment plan. In this case, it can be almost assured that all the factors affecting the patient's dental condition have been concluded to deliver the treatment plan [12-16]. Nevertheless, it should be noted that AI application for automated interpretation in practice is extremely costly and requires the dentist's constant careful inspection [17].

Radiology

Different forms of AI are used in maxillofacial radiography. For instance, ANN can be applied for classification, detection, and segmentation in the field of radiology. The classification includes a broad spectrum from the detection of the absence or presence of pathology to classifying the type of detected malignancy. Detection is identified as a process of determining intended disease expansion in the tissue or vital anatomical structures. Segmentation is a process that segments different anatomical structures or pathologies in images obtained with various modalities, including plain radiography, CT, MR, and ultrasound images [18-20].

To use AI, especially DL, in radiology, it is necessary to provide a considerable high-quality amount of data to train the system**.** Therefore, the radiologist must go through a data curation process. It means that the raw data collected from radiographs should be first anonymized. In the second phase, the representativeness of the data is checked which defines as choosing a set of data that adequately replicates the larger group of patients. In the third phase, standardization of the data format is conducted in order to ease the information process for the AI system. Minimization of noise and other radiographic errors in the data occurs in the fourth phase. In the fifth phase, the segmentation of the region of interest in order to be scanned by the system is run. Ultimately, in the last phase, the radiologist enters descriptive information regarding the meaning of an image into the system to train the AI. As it is observed, this process is time consumable [21-22].

After entering the required data into the system, data augmentation is completed. In this step, the input data is modified to alter and change the data representation while keeping the label the same. In most cases, augmentation is achieved by blurring or skewing an image, modifying the contrast or resolution, flipping or rotating the image, adjusting zoom, and changing the location of a lesion. By this means, the efficacy and accuracy of the results presented by AI are enhanced [21].

Following data augmentation, the input data divides into three data sets, including training, validation, and test data sets. The training data set is used to train and adjust the parameters of the learning model. The validation data are used to monitor the model's performance during training and search for the proper model. The test data are used to evaluate the final performance of the advanced model. In the end, a developed valid model is defined [21-22].

AI can diagnose a broad range of diseases, including dental caries, periodontal disease, osteosclerosis, odontogenic cysts and tumors, and diseases of the maxillary sinus or temporomandibular joints on a radiology image. In other words, AI technology can suggest a list of differential diagnoses by providing the patient's clinical and radiological records [17].

Recent studies have investigated the use of DL to diagnose dental caries, periodontal disease, vertical root fracture, periapical pathosis, and cysts and tumors of jawbone on radiology images [23-26]. Moreover, a study performed by Chang HJ *et al.* [27] showed that the novel hybrid intelligent system combined with DL architecture and the conventional computer-aided diagnosis (CAD) approach demonstrates high accuracy and excellent reliability in the automatic diagnosis of periodontal bone loss and staging periodontitis. In this research, DL models were applied to specify radiographic CEJ level and bone level. After the automatic detection of periodontal bone loss, the staging of periodontitis was carried out by CAD. In this step, the percentage rate analysis of the radiographic bone loss combined the tooth long-axis with the periodontal bone and CEJ levels. Subsequently, CAD receives this information to stage periodontitis automatically.

In panoramic and CBCT imaging, the DL technique can detect and classify teeth stages with automated

CAD outputs, assist dentists in decision-making, and reduce charting time by automatically digitally filling patients' records [28-29]. On the other hand, DL with the CAD system could provide information to dentists for the early detection of osteoporosis based on their panoramic images. In this process, AI detects the reduction in mandibular cortical width and the degree of erosion of the mandibular lower cortex [30-31]. Another usage of DL in radiology is an automatic estimation of bone age done by setting regions of interest in the hands and wrists on radiographs [32].

AI can also automatically determine 3D cephalometric landmarks based on an inputted algorithm [33]. For instance, in a study conducted by Neelapu *et al.* [34], 20 landmarks were targeted for automatic detection, of which 12 landmarks exist on the mid-sagittal. Due to the complex detection of these landmarks by a radiologist, this technology improved the outcome and resulted in a better and faster diagnosis.

DL and ML techniques can enhance the quality of usual films taken by radiologists. In other words, AI technology attempts to reduce the errors, particularly noises, in the film [35]. In research by Zhang K *et al.* [35], it was explained that ANN models could be trained to reduce the noise in the radiographic film. In order to achieve this aim, ANN models were trained with 400 images of size 180×180. The training images included both before and after the Gaussian de-noising process images. Therefore, in this scenario, ANN models learn the method used in the Gaussian de-noising process and can be beneficial in de-noising of input radiographic films.

Moreover, AI can reduce artifacts caused by metal restorations, including crowns and implants on CT and CBCT images. The proposed methodology for this goal is similar to the methodology used in reducing the noise in the radiographic films mentioned above [35].

Forensic dentistry is a science that often necessitates accurate and detailed information displayed on dental radiographs. According to poor dental image quality and the possible presence of artifacts and other errors, the application of AI can overcome these difficulties and lead to greater clinical decisions [37].

Periodontology

AI technology has prominently advanced in the field of periodontology in recent years. A number of studies have reported the benefits of AI systems in assessing periodontal health and diagnosis of disease [38-40]. For instance, Lee *et al.* [41] claimed that using DL technology can be more effective than ANN to detect periodontally compromised teeth on periapical radiographs. Moreover, it has been shown the accuracy of detection was higher in premolars than in molars, which are most likely related to the simpler anatomy of the roots in premolar teeth.

On the other hand, another study performed by Yauney *et al.* [42] showed that ML models could be trained to correlate information derived from clinical examination, dental radiographs, and patient's medical history to diagnose the disease automatically in the future.

One of the newest advancements of AI technology in periodontology is the ability of this system to detect the severity of periodontal disease. In a study conducted by Papantanopoulos *et al.* [43], it was mentioned that ANN could distinguish between aggressive and chronic periodontitis by assessing immunologic parameters in patients. The accuracy of this methodology was 90-98%, which is statistically valuable.

A novel systematic review study revealed that the accuracy of AI applications in detecting dental plaque is 73.6% to 99%. Nonetheless, the accuracy of this technology in the detection of periodontitis based on intraoral radiographs is reported to be between 74% and 78.20%. Therefore, with the development of AI, it is expected to witness the usage of this technology as a powerful diagnostic tool in periodontology [44].

AI technology has made an enormous impact on dental implantology science [45]. AI technology is applied to optimize the design of dental implants. In this case, AI models modify the dental implant's porosity, length, and diameter, leading to minimized stress at the implant-bone interface and improved dental implant design [46]. On the other hand, multiple studies have reported that models can predict dental implant success and osteointegration [47-49]. However, the input data differed among various studies [47-49].

Bone level and the amount of bone that supports dental implants are critical factors in treatment success. It is suggested AI technology, specifically DL, can identify the bone level on radiograph images. This finding can significantly reduce failure in the field of implantology [50].

Probably, the latest advancement of AI is its ability to identify dental implant brands and the stage of treatment explicitly to ensure efficient care. In one research by Sukegawa *et al.* [51], the accuracy of DL models in identifying dental implant brands and the stage of treatment based on panoramic radiographic films was surprisingly high. Furthermore, it was noted that multi-task learning facilitated analysis accuracy.

Oral Pathology

Oral pathologies, including oral lesions and malignancies, are among the most concerning dental problems that need early detection. Nowadays, AI technology has eminently progressed to aid in diagnosing oral pathologies [52].

In a study performed by Mahmood *et al.* [52], 11 studies that applied AI technology to point out malignant cells based on their distinct histopathologic characteristics were supervised. These studies reported the high accuracy rate of AI technology in detecting different oral lesions, as shown in Table 1 [53-63]. Hence, due to the high risk of bias and probability of accuracy overestimation, AI technology cannot fully be trusted to detect oral pathologies and needs further advancements.

In an exciting review study, different AI techniques for identifying cancerous oral lesions were examined. In the first method, AI algorithms were trained on hematoxylin and eosin (H&E)-stained tissue sections to detect oral squamous cell carcinoma's specific characteristics, including keratin pearls. In the second method, AI models were trained to overlap cancerous areas in pathologic sections taken from different patients to define the similarities. These similarities were specified as cancer's characteristics for the AI's interpretation accordingly. The results showed a negligible variance

Table 1: Accuracy of AI technology in the detection of oral pathologies

Author(s)	AI Technology	Oral Lesion	Study Method	Accuracy of Methodology
Baik *et al.* [53]	Trained algorithm	Oral Epithelial Dysplasia	Training a sequence of classifiers using morphometric data calculated on nuclei from 29 normal, 5 carcinomas in situ and 28 SCC specimens	80% at the cellular level and 75% at the tissue level
Krishnan *et al.* [54]	Hybrid Intelligent System	Oral Submucosa Fibrosis	Grading the histopathological tissue sections into normal, Oral submucosal fibrosis without Dysplasia and Oral submucosal fibrosis with Dysplasia	95.7%
Krishnan *et al.* [55]	ANN	Oral Submucosa Fibrosis	Segmentation of collagen fibers in the subepithelial connective tissue	91.64%
Krishnan *et al.* [56]	ML	Oral Submucosa Fibrosis	Segmentation and classification of subepithelial connective tissue cells except endothelial cells in oral mucosa of normal and oral submucosa fibrosis conditions	88.89%
Krishnan *et al.* [57]	Trained algorithm	Oral Submucosa Fibrosis	Delineation of the epithelial layer from histological images in discriminating normal and oral submucous fibrosis	98%
Mookiah *et al.* [58]	ANN	Oral Submucosa Fibrosis	Detection of textural features of the oral mucosal epithelium to discriminate between normal and oral submucous fibrosis	96.43%
Das *et al.* [59]	ANN	Oral Squamous Cell Carcinoma	Identification of architectural variations of epithelial layers and the presence of keratin pearls in microscopic view	96.88%
Rahman *et al.* [60]	ML	Oral Squamous Cell Carcinoma	Analyzing abnormality based on textural features present in squamous cell carcinoma histological slides	100%
Sun *et al.* [61]	ML	Oral Squamous Cell Carcinoma	Automatic color-based feature extraction system for parameter estimation of oral cancer from optical microscopic images	Not mentioned
Lorsakul *et al.* [62]	Trained algorithm	Oral Squamous Cell Carcinoma	Simultaneous analysis of multiple biomarker expressions within a single tissue section stained with an immunohistochemistry duplex assay	91.64%
Fouad *et al.* [63]	ML	Oropharyngeal Squamous Cell Carcinoma	Segmentation of oropharyngeal cancer tissue into epithelial and stromal regions	81%

between the two techniques, and an acceptable statistical result using either method was observed [64]. AI technology may also be used to follow up the healing process of the oral lesion based on their radiographic view [65]. For instance, Yang *et al.* [65] exhibited that ANN can be used to compare pairs of periapical radiographs before and after treatment and classifies the healing process of lesions. In this study, 196 periapical images before and after dental treatment with one of the labels of "getting better", "getting worse" or "have no explicit change" were extracted to train ANN models.

Prosthodontics

AI technology in the field of fixed prostheses has advanced extensively. In the modern world, AI models are used to enhance crown designs with better marginal adaptation, prediction of crown longevity, and color reproduction [66].

One of the most important reasons for the crown's failures is a lack of marginal adaption. With the evolution of AI technology, the accuracy of marginal adaptation has significantly increased [67]. For instance, a study revealed that using intrinsic AI and algorithms for automatic tracing of the margin line in the implant-abutment through subgingival could improve marginal adaptation by 96.2%. Nevertheless, this finding was exclusively related to monolithic zirconia crowns and might not be valid for other types of crowns. Due to the absence of studies regarding AI's efficacy in marginal adaptation improvement in crowns excepting monolithic zirconia crowns, the mentioned methodology can be tested in other types of crowns in future research [68].

AI technology can also be used as an assessment tool. Yamaguchi *et al.* [69] evaluated the probability of CAD/CAM composite resin crowns' debonding using DL. In this study, 2-dimensional images captured from 3-dimensional stereolithography models of a die scanned by a 3-dimensional oral scanner were used. A total of 8640 images from 24 cases including 12 trouble-free and 12 with the debonding problem were collated to train the system. The results showed that AI technology presents a good performance in assessing the likelihood of composite resin crowns' debonding.

The human eye has many visual errors and gets tired quickly, which can cause the shade selection process of a crown difficult. Researchers have attempted to implement a system that can keep its accuracy in the long term for many years. Fortunately, in a study, it has been witnessed ANN produces greater accuracy in color reproduction within the given color space than the traditional visual approach. In order to train ANN models, 43 metal ceramic samples were produced by mixing proportional porcelain powders. Following AI's training phase, the color reproduction of 10 different maxillary incisors was done in both AI and visual approaches. In the end, color distributions of target teeth and fabricated metal ceramic specimens by AI were compared [70].

Identifying dental arch based on Kennedy's classification is crucial for correct treatment planning in edentulous patients. It is seen utilizing ANN can lead to prompt and precise detection. Moreover, one study reported that this accurate classification of a patient's dental arch is higher in the maxilla than the mandible [71].

It should be noted the side effects of prosthetic treatments would appear in long term. Therefore, the high success rate of AI technology mentioned in current studies cannot be reliable, and further research in the future is indeed needed [72].

Restorative Dentistry

In all likelihood, the most impressive role of AI in restorative dentistry is the detection of caries. Most studies have shown a significant impact of AI in caries detection and screening by several methods [73-75]. For instance, Geetha *et al.* [76] revealed ANN could distinguish normal tooth anatomy from dental caries based on oral images taken from the patient. This technology may also detect interproximal caries that are challenging for dentists to detect. The reported accuracy using AI technology was 97.1%. However, no studies have shown that AI technology can predict the severity of caries, and further studies are required.

AI can also ascertain dental restorations on dental radiographs. In a study by Abdalla-Aslan R *et al.* [77], it was observed that AI models could specify dental restoration and separate different restorations by shape and distribution of grey values on dental radiographs.

Since streptococcus mutans (*S.mutans)* constitute the majority of dental caries' construction, selecting an appropriate caries excavation method can lead to better caries removement and subsequently lesser *S.mutans* in the mouth post-treatment. In an interesting study conducted by Javed *et al.* [78], trained ANN was employed to predict post-*S.mutans* based on pre-*S.mutans* and the

chosen caries excavation method. Three disparate caries excavation methods including excavation with carbide bur, polymer bur, and spoon excavator were tested. The results showed a high preciosity of 99%. This outcome can assist the dentist in the proper selection of caries excavation method.

Other advantages of AI technology are determining post-operative sensitivity and predicting dental restoration failure. A controversial study was conducted to train AI in order to predict the probability of post-operative sensitivity based on key factors including the type of restorative material, the location of the carious defect, and the depth of the carious defect. Training the AI was led by information provided in questionnaires completed by dentists regarding the considered origin of 213 cases of post-operative sensitivity. Since the training of AI models was based on the clinical experience of dentists, the result is not completely reliable [79]. In another study performed by Aliaga *et al.* [80], it was seen that a case-based learning model could be used to select the appropriate restorative material and hence, predict the longevity of the restoration. This technique appeared to be a valid method in practice.

Dental Surgery

ANN has played a significant role in dental surgeries. It is highlighted that orthognathic surgeries can be massively transforming by using AI technology [73]. For example, in a study by Lu *et al.* [81], it was witnessed that ANN models improved the accuracy of orthognathic surgery's outcome considered by oral surgeons. In order to accomplish this aim, 30 pre-treatment facial images of orthognathic surgery cases were evaluated by surgeons and predicted post-surgery facial images were produced. In the next step, trained ANN models were applied to modify the predicted post-surgery results. Comparing the actual post-surgery facial images with pre- and post-AI modification of predicted facial images proved that AI intervention enhances the accuracy by more than 80%. Therefore, AI's application can prominently affect decision-making and treatment planning in orthognathic surgeries [81].

Nonetheless, the efficacy of orthognathic surgery can also be checked by AI using pre- and post-treatment images of patients [82-83]. For instance, a study by Patcas *et al.* [82] showed that trained AI models could be applied to score facial attractiveness and age appearance based on pre-and post-orthognathic treatment photographs of the patient. However, the accuracy of this methodology was not mentioned. On the other hand, another work conducted by the same author revealed that AI technology with the same training methodology could be utilized to score facial attractiveness in cleft patients based on pre-and post-treatment frontal and profile images. Ultimately, AI evaluation results were compared to human ratings and no statistical difference was observed [83].

One of the most prevalent side effects of third molar extraction is paresthesia due to surrounding nerve damage. However, this error can easily be avoided by carefully detecting the nerve's position based on the patient's panoramic images. Moreover, DL systems are now frequently employed to predict the possibility of the inferior alveolar nerve damage during the mandibular third molar extraction using panoramic radiographic images before the surgery. In other words, DL models precisely detect the inferior alveolar nerve position on the panoramic images. Based on the proximity of inferior alveolar nerve to mandibular third molar, DL systems predict the possibility of nerve damage during the surgery. The accuracy of AI technology in assessing the possibility of nerve damage after dental extractions has been reported to be 82% [84].

Several oral lesions require complicated surgeries. These extensive surgeries may involve a majority of the mouth and subsequently damage vital structures and cause irreversible harm. Therefore, AI technology has aided in precisely detecting oral lesions' location on panoramic radiographs before the surgery and increasing the treatment's success rate. In one study, the accuracy of AI was 90.36% in the detection of ameloblastoma and odontogenic keratocytes on dental images [85].

Orthodontics

In the modern world, AI technology has rooted in the field of orthodontics. In other words, various orthodontic procedures have been changed due to AI advancements [86-88]. Cephalometric tracing and interpretation are among the most critical stages in orthodontic treatment planning which also has been transformed subsequent to AI's emersion [73]. However, various studies demonstrate contradictory results regarding AI's superiority rather than the gold standard technique [86-88]. For instance, research conducted by Kim *et al.* [88]

manifested a high accuracy rate of AI's application in cephalometric interpretation compared to manual marking and diagnosis. On the other hand, a study conducted by Kunz *et al.* [86] exhibited that no statistically significant differences exist between humans' gold standard and the AI's predictions in cephalometric analysis.

Since the detection of anatomic landmarks is a key to successful orthodontic treatment, multiple studies have mentioned AI's ability to detect these landmarks on different modalities of radiographs. The accuracy of AI in different radiographic modalities is demonstrated in Table 2 [88-91].

Another essential element in designing a treatment plan for an orthodontic patient is analyzing growth patterns and estimating biological age. Determination of a patient's developmental stage can quickly be done using various methods. One of the most applicable methods is appraising cervical vertebrae stages based on a lateral cephalometric radiograph [92]. In a study performed by Kök *et al.* [93], it was cited that ANN could detect different stages of cervical vertebrae growth from stage 1 to 4 and stage 6. However, it is expressed that the accuracy of this technique in the detection of stage 5 remarkably decreases, and it is preferred to use other methods instead.

Another methodology to estimate the patient's age is identifying the dental stage of the patient based on panoramic radiographs. This method determines the possible age range of the patient. In a study, the accuracy rate of ANN, based on the later methodology, was reported to be 94.15% [94].

AI technology has also developed to predict orthodontic treatment needs in the future. For instance, Thanathornwong *et al.* [95] showed AI models could be used to assess the possible future needs for orthodontic treatment in patients with permanent teeth. For this aim, AI models were trained by data acquired from patients between the ages of 14 to 19 with orthodontic treatment needs. Various variables including overjet, overbite, crossbite, and so on were examined and inputted into the system. Therefore, the system was trained to correlate the mentioned variables to future orthodontic needs and present a reliable outcome. In this study, the accuracy rate was 93%-95%. Moreover, Shin *et al.* [96] exhibited that the DL method can also evaluate future orthodontic treatment needs based on dentofacial dysmorphisms and a malocclusion observed on patients' cephalograms. This study reported a high accuracy rate of 95% as well.

Table 2: Accuracy rate of correct anatomic landmark identification on different radiographic modalities

Author(s)	AI Technology	Cephalometric Image	Accuracy Rate
Dobratulin *et al.* [89]	DL	Lateral Cephalometric Radiograph	92%
Kim *et al.* [90]	ANN	CBCT Images	80.4%
Muraev *et al.* [91]	ANN	Frontal Cephalometric Radiograph	82%

AI technology can also be applied in severe complicated cases of orthodontic treatments [97]. Wang *et al.* [98] showed that DL models could detect asymmetry of the maxilla in patients with unilateral cleft lip and palate on CBCT images. Moreover, Chen *et al.* [99] introduced a novel ML methodology to identify maxillary structure variation in unilateral canine impaction based on CBCT images.

Endodontics

Since endodontic treatment aims to retain the teeth in their functional position and prevent any subsequent complications, AI advancement in this field can be exceptionally effective [100]. The frequent benefits of AI application in endodontics are mentioned as analyzing root canal anatomy, detecting root fractures, and periapical lesions, precise assessment of working length, predicting the viability of dental pulp stem cells, and predicting the success rate of retreatment procedures [101].

An accurate working length determination is one of the most important keys to a successful endodontic treatment. Radiographic images are the most prevalent method employed to measure working length precisely [102]. Recently, some studies have reported that ANN models have aided endodontists in correct working length measurement by automatic apical foramen identification on a periapical image. The accuracy of ANN models was between 93 and 95% [103-104].

An essential advantage of AI is detecting periapical lesions and vertical root fractures prior to treatment [102]. Although AI technology can identify periapical abnormalities in different radiographic modalities, authors have a controversy about this technique's accuracy [105-107]. Therefore, the possible application of AI for periapical lesions detection in the future should be fur-

ther examined [102]. On the other hand, a few studies declared a high accuracy rate of AI in detecting vertical root fractures based on patients' radiographic images [108-109]. In one study, this accuracy rate was reported to be 70% in periapical radiographs and 96.6% in CBCT images [108]. This finding can prevent failures before treatment initiation [102].

Teeth manifest various root anatomies in different people and races. Hence, complete knowledge of these variations is crucial for dentists in treatment. In recent years, frequent subcategories of AI have aided dentists in the flawless diagnosis of root canal anatomy [102]. For instance, Hiraiwa *et al.* [107] studied the application of DL to detect the presence or absence of an extra root in the distal root of the mandibular first molar on panoramic images. The gold standard in this study was the dentist's observation and assessment based on CBCT images. The results showed an accuracy of 86.9% compared to the gold standard method. Therefore, with the application of AI, errors caused by a misdiagnosis of root canal anatomy are decreased [102].

A novel research topic that has attracted much attention worldwide is predicting the viability of dental pulp stem cells. Due to stem cells' ability to differentiate into various cells, maintaining these cells' vitality is crucial [110]. In a study conducted by Bindal *et al.* [110], it was reported hybrid intelligent system could efficiently estimate the impact of platelet concentrates on the proliferation of stem cells derived from the human tooth. In order to train the system, the data collected from dental pulp stem cells' viability rate subsequent to stem cells' culturing in three different cultures enriched with platelets were used.

After endodontic treatment, frequent variables can impress the outcome. Lately, ML has been used to assess multiple variables influencing endodontic surgery outcomes and ultimately predict treatment's success rate. The variables employed by different studies to train ML models include tooth type, lesion size, type of bone defect, root filling density, root-filling length, and the apical extension of post, the age, and the sex. The results of these studies suggest the application of ML in assessing the prognosis of endodontic treatment [111-112].

Surprisingly, AI has been used in genetics when it comes to endodontics [113]. In a study by Poswar *et al.* [113], it was observed that ANN could be used to analyze the gene expression of the two most common periapical lesions, radicular cysts, and periapical granulomas. This study showed that not only the inflammatory nature but also other biological processes might differentiate radicular cysts and periapical granulomas.

Pediatric Dentistry

Pediatric patients require unique conservations and treatments due to the presence of deciduous teeth. Deciduous teeth have smaller crowns with huge pulps and can be missed or over-retained and interfere with the expected evolution and eruption of permanent teeth. Therefore, early diagnosis of deciduous teeth problems is critical [114].

A study conducted by Zakirulla *et al.* [114] concluded that ANN models could be used to detect dental caries and impacted teeth on different modalities of radiographic images in a pediatric patient. Moreover, Kılıc *et al.* [115] used the DL approach for the automatic detection and numbering of deciduous teeth in children based on panoramic radiographs. The accuracy rate reported in this study was 95%.

Disadvantages of AI

Despite the mentioned advantages of AI, it should be noted that AI still has three major disadvantages to encounter. First, this novel technology should be simplified to use. In this case, the need for dentists to pass training courses to learn to work with AI systems is eliminated. Second, the required equipment is not economically viable. Third, since the function of AI is yet not completely identified, the presented outcomes cannot be fully trusted, and an experienced dentist must supervise its results. Therefore, prior to the immense application of AI in dental practice, mentioned cons of AI need to be efficiently managed [116].

Conclusion

With the significant development of AI, all branches of dentistry have also benefited from its desirable changes. Today, integrating these two sciences has led to improved detection of dental abnormalities and oral malignancies based on radiographic view and histopathological features, enhanced designing of dental implants and crowns, precise tooth preparation finishing line determination, accurate analysis of patient's growth patterns, correct biological age estimation, better prediction of the viability of dental pulp stem cells, gene expres-

sion of periapical lesions analysis, and reliable prediction of the success rate of different dental treatments. These mentioned outcomes can ultimately result in enhanced diagnosis and treatment planning, reduced time waste, and greater patient satisfaction. However, it should be noted that this new technology has important challenges to face. Therefore, the application of AI in clinical dentistry can be considered a major contributing factor to higher success rates and fewer treatment failures in the future only after solving its basic problems.

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The authors declare that they have no conflicts of interest.

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Systematic Review

An Overview about New Methods in Management of Gag Reflex during Dental Treatment: A Systematic Review

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ABSTRACT

Statement of the Problem: Management of gag reflex is a challenging process during many dental treatments. Various studies have been carried out to evaluate different pharmacological and non-pharmacological techniques to control gagging.

Purpose: The aim of this study is to review the available evidence on methods proposed for managing the gag reflex.

Materials and Method: This systematic review adheres to the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines. A comprehensive search was conducted in English and Persian based on articles published from 2015 to 2022 (February) in PubMed, Scopus, Science Direct, Web of Science, Google Scholar, ISC and SID. All studies were first screened based on their title and abstract. The quality assessment of articles was carried out by two independent authors. Then, risk of bias evaluation was conducted according to Cochrane parameters.

Results: In total, 1704 studies were identified via search. After reviewing title and abstract, 16 studies found eligible based on inclusion and exclusion criteria. Following quality and risk of bias assessment, 9 studies included in the systematic review.

Conclusion: Based on the finding of this review, distraction techniques, nitrous oxide, and low-level laser therapy were found effective in management of gag reflex. The dentist should consider gag reflex management based on the type of dental treatment, gag severity, patient's age, and available capabilities.

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Introduction

The Gag reflex is an innate response of human body that protects the respiratory and gastrointestinal systems against external stimuli [1]. Stimulation of sensory receptors in the posterior area of the mouth and oropharynx causes signals across the cranial nerves 5, 9, and 10, triggering a reflex. Often, the response manifests as spasmodic muscular contractions, which are essentially an attempt to expel an external stimulus [2]. The gag reflex severity and the stimulus that initiates it vary significantly across different individuals and over time [3]. Although the gag reflex is present in the majority of people, the severe form is only experienced in a few. There is a statistically significant association between gag reflex during dental treatment and feminine gender, poor educational level, and dental anxiety [4]. It becomes less severe with age, especially after the age of four, as the child's chewing, swallowing, and breathing capacities develops [5].

Dental treatment is more difficult in people who suffer from severe gag reflex. Numerous dental procedures, including dental impression, third molar extraction, endodontic therapy, and intraoral radiography of the posterior teeth, might elicit the gag reflex [6]. Additionally,

gagging might complicate some diagnostic and medical procedures, such as endoscopy [7]. Gag reflex can be induced by a variety of stimuli, including sonic vibration created by rotational devices, the smell, and taste of dental materials, direct physical stimulation of the posterior parts of the mouth, viewing of equipment, and in some circumstances, even envisioning dental treatment [6]. The neuronal connections between the gag reflex center and the cerebral cortex explain phenomena such as inducing gag with mental images and relieving gag reflex by diverting the patient's attention [8].

Numerous approaches for managing the gag reflex during dental treatment have been developed. Gag reflex management strategies are generally classed as pharmacological or non-pharmacological [9]. Local anesthetic, general anesthesia, sedatives, and herbal medications are examples of pharmacological approaches. Non-pharmacological techniques such as behavioral therapy, hypnosis, acupuncture, and laser therapy are also mentioned [10]. Numerous studies have used different indexes for quantification of the severity of gag reflex, including the gag severity index, the gag prevention index, the gag problem assessment, and the visual analogous scale as well as measuring the depth of swap penetration into the soft palate [11-14].

Despite the conduction of several researches, there is currently no reliable clinical guidance to assist dentists in making clinical decisions in situations of gag reflex. Most systematic reviews on this subject have been done before 2015 including a review in the Cochrane database of systematic reviews conducted in 2015, which evaluated only one eligible study for the final review [6]. This review has been updated in 2019, however, still has evaluated little evidence on this subject. Several clinical trials have done from that time and have introduced new methods in their research. These trials justify the need for new systematic reviews.

The purpose of this study was to conduct a systematic review of the available evidence, evaluating new methods proposed for managing the gag reflex during dental treatment so that it could be used as a clinical guide during dental treatment.

Materials and Method

The current study adheres to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines published in 2020 [15]. The study was aimed to find the randomized clinical trials, which have been published between 2015 till 2022(February) and evaluated the effect of an intervention on the severity of gag reflex during dental treatment (according to 4W question method). At the first step, we developed a protocol for conducting the systematic review. The protocol included databases and search strategy, screening techniques, inclusion and exclusion criteria, data extraction, and assessment techniques.

Search strategy

Articles published since 2015 to 2022 (February) were searched in electronic databases such as PubMed, Scopus, Science Direct, Web of Science, Google Scholar, ISC, and SID. The search was undertaken in both Persian and English. To avoid publication bias, an attempt was made to evaluate papers, dissertations, and projects, both print and non-print sources (ProQuest dissertations and theses, Irandoc dissertations). Medical subheadings (MeSH) were used to incorporate certain keywords, which are prevalent in medical papers. The key words included gag reflex, gagging, and the truncation of [dent] and [prosth]. The words: glucose amino glycan and genes were excluded from the search, because in some studies the term “gag” was used as an abbreviation form of the above words.

In all, 1704 studies were included in the screening step. Then, papers were screened based on their title and abstract according to the following inclusion and exclusion criteria.

The inclusion criteria were defined as randomized clinical trials with a control group in which participants did not have a specific systemic disease or were not using a specific medicine that may impact the intensity of the gag. This study made no distinction between age groups, genders, or races, and all comparable studies were evaluated.

The exclusion criteria were defined as non-clinical or non-randomized research and studies in which individuals had a specific systemic disease or mental handicap were excluded.

Initial screening

At this step, after deleting duplicates, titles and abstracts of all studies were reviewed. Following initial screening 16 related studies were identified eligible from the 1704 articles. Selected records were entered into Endnote

software (Thomson Reuters, New York, USA). Two independent reviewers carried out initial screening. A total of 15 out of the 16 studies listed above were journal articles, while 1 was a dissertation. Additionally, 11 articles were written in English and 5 in Persian. The complete text of these articles was prepared for further assessment. To obtain all of the information necessary to evaluate the studies, the authors of some studies were contacted via e-mail during the evaluation process.

Data extraction and quality assessment

The checklists for each research comprised information of the eligible studies using the PICOS criteria (population, intervention, comparison, outcome, study) [16]. To minimize bias in the research evaluation, the 16 papers were numbered sequentially according to their publication year. Each study's title, first author's name, and year of publication were recorded. Moreover, the e-mail address of the corresponding author was written in the checklist of each article for additional information. Following that, two authors assessed each study independently. If there was a disagreement over assessment, the third author was consulted.

The Cochran checklist was used to evaluate the risk of bias in any qualifying article [17]. Each article was evaluated for possible bias in case selection, randomization, blinding, and reporting of results using Cochran's criteria, and the risk of bias was classified into three categories: low risk, high risk, and unclear. Finally, a critical review of qualified articles was conducted, and conclusions were drawn from various gag reflex management techniques. The process of searching and selecting literature is illustrated in Figure 1.

Table 1 summarizes data of 16 studies in the quality assessment stage. Out of 16 eligible articles, in eight articles, the maxillary impression was made using alginate [18-25]. Maxillary and mandibular alginate impression was taken in two studies [26-27]. The other two studies examined the periapical radiographs of patients, one from maxillary teeth [28] and the other from the mandible [29]. One research examined the intensity of gag experienced during various dental surgical procedures performed under general anesthesia [30]. The type

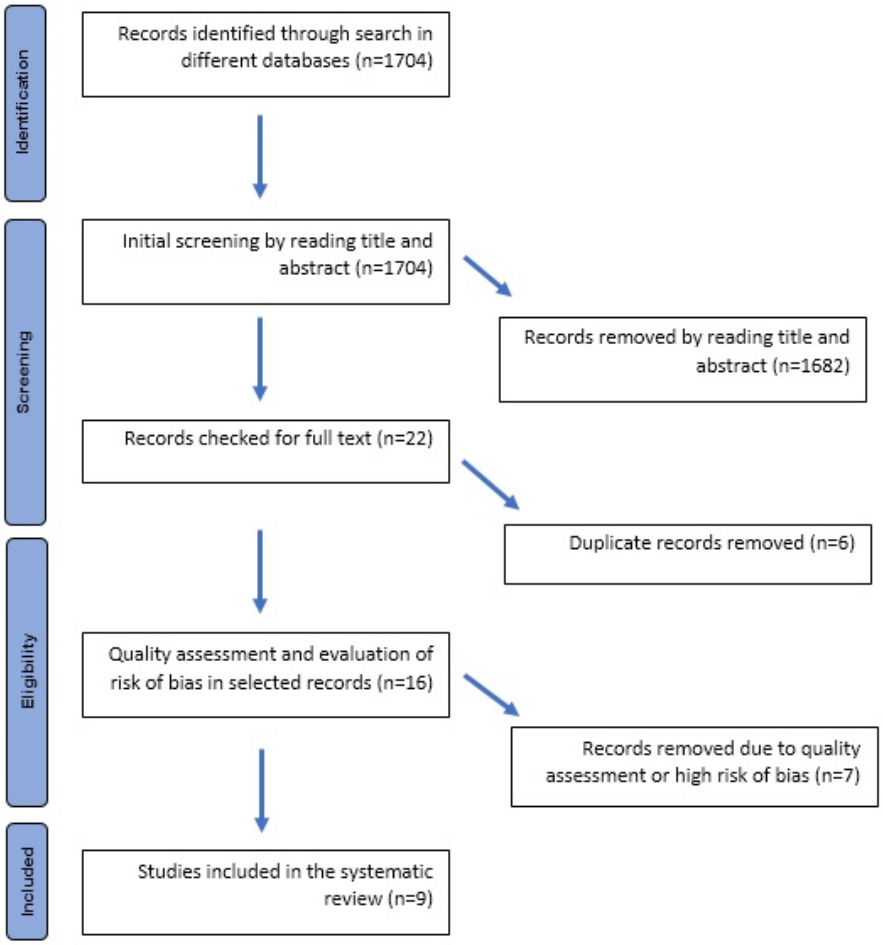

Figure 1: Flow diagram of search and selection of studies in the systematic review

Table 1: Demographic specifications of studies in the first screening stage

Row	Author, year	Age	Gender	Dental treatment	Intervention	Included?*
1	Mustafa *et al.* [18] 2021	25.6	M: 24% F: 76%	Maxillary impression	Distraction technique music	N
2	Kulkarni *et al.* [26] 2021	5-12	NM	Maxillary and mandibular impression	Distraction technique Colored game	Y
3	Dixit *et al.* [19] 2020	5-10	NM	Maxillary impression	Distraction technique Colored puzzle	Y
4	Jawdekar *et al.* [20] 2020	6-12	M: 50% F: 50%	Maxillary impression	Ear plug and temporal tap	Y
5	Shin *et al.* [30] 2020	15-74	NM	NM	IV sedation Propofol	N
6	Balouch *et al.* [21] 2020	25-56	M: 46% F: 54%	Maxillary impression	Metoclopramide	N
7	Yamamoto *et al.* [31] 2018	17-70	M: 62% F: 38%	NM	IV sedation Propofol and midazolam	N
8	Debs *et al.* [22] 2017	5-11	M: 52% F: 48%	Maxillary impression	Distraction technique Colored game	Y
9	Goel *et al.* [23] 2017	4-14	M: 42% F: 58%	Maxillary impression	Low-level laser therapy	Y
10	Kamran *et al.* [27] 2016	21.6	M: 50% F: 50%	Maxillary and mandibular impression	Adding Lidocaine to impression material	N
11	Elbay *et al.* [28] 2016	6-12	M: 68% F: 32%	Radiography from maxilla	Low-level laser therapy	Y
12	Veaux *et al.* [29] 2016	14-42	M: 50% F: 50%	Radiography from mandible	Nitrous oxide sedation	Y
13	Shadmehr *et al.* [32] 2016	NM	NM	Oral examination	Tannic acid patch	Y
14	Fakhrzadeh *et al.* [24] 2015	NM	M: 54% F: 46%	Maxillary impression	Benzocaine topical anes-thesia	N
15	Rahshenas *et al.* [33] 2015	28	M: 36% F: 64%	Oral examination	Acupressure	Y
16	Ebadi *et al.* [25] 2015	24	F: 100%	Maxillary impression	Acupuncture	N

*: included in the systematic review? NM: not mentioned N: no Y: yes

of dental therapy was not specified in one research [31]. The remaining two studies examined the degree of gag reflex following oral examination [32-33].

Six of the sixteen studies involved children with an average age of fewer than 14 years [19, 21-23,26,28]. The age distribution was not given in one paper [32]. The remaining studies were conducted on adults with a mean age of more than 20 years.

Only one research was done exclusively on females in the preceding sixteen papers [25]. Three studies did not mention the research population's gender makeup [19, 26, 32]. In other studies, the target group included both men and women.

Risk of bias assessment

Figure 2 contains an overview of the risk of bias assessment. Different biases were evaluated in the present study including:

1. Selection bias (Randomization)

Among the evaluated studies, five were recognized as having a high probability of bias in the randomization method [20, 25, 27, 30-31]. Only three of the sixteen researches have mentioned their method of randomization [19, 21, 28].

2. Selection bias (Allocation concealment)

Four of the studies had a high risk of bias in allocation concealment [18, 20, 30-31].

3. Attrition bias

In the study by Ebadi *et al.* [25] on the effects of acupuncture on gag reflex, ten participants were excluded due to their inability to bear impression. This is an illustration of attrition bias. It may be stated that the intervention's effect was overestimated positively in this study.

4. Reporting bias

In the study by Kamran *et al.* [27], the gag reflex severity was measured by the gag severity index before the intervention, whereas the index after the intervention was declared the gag prevention index. This incident exemplifies reporting bias. It is probable that changing the index make the findings more significant. To evaluate the intervention's effect adequately, the indices used for comparison must be identical.

Row	Author, year	Selection bias Randomization	Selection bias Allocation concealment	Attrition bias	Reporting bias	Blinding
1	Mustafa [18] 2021	-	X	-	+	NP
2	Kulkarni [26] 2021	+	+	-	+	NP
3	Dixit [19] 2020	+	+	+	+	SB
4	Samaleti [20] 2020	+	-	-	+	NP
5	Shin [30] 2020	X	X	+	+	NP
6	Balouch [21] 2020	X	X	-	+	NP
7	Yamamoto [31] 2018	X	X	-	+	NP
8	Debs [22] 2017	+	+	-	+	NP
9	Goel [23] 2017	-	-	-	+	NP
10	Kamran [27] 2016	X	X	-	X	SB
11	Elbay [28] 2016	+	+	+	+	DB
12	Veaux [29] 2016	-	-	-	+	NP
13	Shadmehr [32] 2016	-	-	+	+	DB
14	Fakhrzadeh [24] 2015	-	-	-	-	SB
15	Rahshenas [33] 2015	+	-	+	+	DB
16	Ebadi [25] 2015	X	X	X	+	NP

+ : Low risk, X : High risk, - : Unclear, NP: not possible, SB: single blinded, DB: double blinded

Figure 2: Risk of bias assessment

5. Blinding

Blinding is not achievable in some experimental trials. For instance, it was impossible to blind the subject and the intervener in the research that compare the intensity of gag caused by conventional and digital impressions [34]. Only three of the papers included in this evaluation were double-blinded [28, 32-33].

Results

After quality and risk of bias assessment, nine eligible studies with low risk of bias were included in the final review, and their results were analyzed [19, 21-23, 26, 28-29, 32-33]. Table 2 summarizes the findings of the studies included in this systematic review.

Non-pharmacological intervention

Mental Distraction techniques

Kulkarni *et al.* [26] assessed the effect of intellectual colored game on gag reflex. They revealed that the anxiety level was statistically lower following the game. In this article, no information was provided about the randomization method, sample size determination, and gender makeup of the population.

In the study of Dixit *et al.* [19], the effect of puzzle game on gag reflex and anxiety level was investigated.

Table 2: Summary of studies included in the systematic review

Row	Authors	Year	Objective	Sample Size	Statistical Analysis	Variables	Main Results
1	Kulkarni *et al.* [26]	2021	To evaluate the effect of the Intellectual colored game on the severity of gag reflex and anxiety.	50	Wilcoxon signed-rank test, Mann Whitney test	GPI FIS	Intellectual colored game is effective in lowering gag severity and anxiety level in test group relative to the control group.
2	Dixit *et al*. [19]	2020	Evaluate the effect of interactive distraction method to manage gag reflex during impression.	48	SPSS, Chi square test	FIS GISS GSI	Interactive distraction method can effectively manage gag reflex in children.
3	Jawdekar *et al* [21]	2020	To compare earplug and temporal tap technique with distraction technique on gagging.	30	SPSS, Chi square test, Friedman test, Mann Whitney U test	GPI 5- point patient reported scale	Earplug and temporal tap technique did not reduce gag reflex but led to a better experience.
4	Debs *et al*. [22]	2017	To evaluate the effect of intellectual colored game on the severity of gag reflex.	41	Descriptive statistics, SPSS, Fisher's exact test, Friedman test	GPI FIS	There was a statistically significant decrease in GPI and FIS after intellectual color game.
5	Goel *et al*. [23]	2017	To determine the effect of LLLT on PC6 acupuncture point on the severity of gag reflex.	40	SPSS, Spearman correlations, Wilcoxon signed-rank test, Mann- Whitney U test	GSI Modified child dental anxiety scale, Pulse rate oxygen saturation	LLLT is useful in reducing anxiety level and severity of gagging. After LLLT, O2 saturation increased and pulse rate declined.
6	Elbay *et al*. [28]	2016	To investigate the efficacy of LLLT on lowering gag reflex.	25	SPSS, McNemar test	Corah dental anxiety scale, GS score	LLLT is effective in reducing gag reflex. There was no significant correlation between gag severity and anxiety level.
7	Veaux *et al*. [29]	2016	To compare different concentrations of nitrous oxide on lowering gag reflex.	14	Wilcoxon signed-rank test, Mann Whitney test	PGS MDAS GSI VAS	Increasing in Nitrous oxide concentration from 30 t0 70% is effective in controlling gag. With 70% concentration, all patients having severe gag reflex could tolerate the test.
8	Shadmehr *et al*. [32]	2016	To assess the tannic acid patch effect on reduction of gagging.	88	Wilcoxon signed-rank test, Mann Whitney test	Gog reflex intensity	Both statistical analyses showed significant reduction in gag severity in the test group.
9	Rahshenas *et al*. [33]	2015	To evaluate the effect of acupressure on severity of gag.	75	Wilcoxon signed-rank test, Mann Whitney test, Kruskal walis test	Glasscow scale	There was a statistically significant decrease in gag severity of case group 2 relative to the control group and case group 1(placebo).

GPI: gag prevention index; GSI: gag severity index; VAS: visual analogue scale; FIS: facial image scale; MDAS: modified dental anxiety scale

All participants in the case group could tolerate impression, unlike those in the control group. The severity of gagging and level of anxiety were also decreased in the intervention group.

In the study of Debs *et al.* [22], gag reflex severity and anxiety level were significantly lower following intellectual colored game. A significant association between gag severity and anxiety level illustrates the fact that the child's participation in game may boost his confidence, by releasing serotonin and endorphin [22, 35].

Acupressure techniques

Jawdekar *et al.* [21] evaluated the efficacy of acupressure using earplug on gag severity. According to this study, gagging was not significantly different between case and control group. It is asserted that this technique might be beneficial in suppressing gag reflex mediated by auriculotemporal nerve. However, earplug has no effect on gagging mediated by glossopharyngeal nerve,

which is most responsible for gagging during dental treatments [36].

In the study of Rahshenas *et al.* [33], gag severity was compared between case group, placebo group, and no-intervention group. The reduction of gag reflex was not statistically different between placebo and non-intervention group. However, significant decrease in gag severity was stated following acupressure on palm region.

Laser therapy

Goel *et al.* [23] assessed the effect of low-level laser therapy (LLLT) on PC6 point during impression making. Increased level of oxygen saturation and decreased gag severity and pulse rate was revealed following intervention.

Elbay *et al.* [28] evaluated the effect of LLLT on PC6 during radiography. Gag severity was significantly lower in the case group; however, the anxiety level showed no significant difference. It can be inferred that the mechanism of laser action is due to nerve stimulation and is irrelevant of anxiety level [37].

Pharmacological intervention

Nitrous oxide

The study of Veaux *et al.* [29] investigated the effect of nitrous oxide on gag reflex. According to their results, the severity of gag was declined relative to the increase in nitrous oxide dosage. Although 50% nitrous oxide effective in most (86%) participants, all subjects could tolerate taking radiographs by the use of 70% concentration.

Herbal medicine

In the article of Shadmehr *et al.* [32], tannic acid patch application on the palate was resulted in gag reflex reduction. However, same reduction was noted in the placebo group. This result can be attributed to the psychological effect of the intervention. Thus, herbal medicines effect in gag reflex management is still contradictory. Other organic remedies like salt have been advocated, but there is not enough evidence to support such methods [36].

Discussion

According to research performed during our study, the majority of approaches for controlling gag reflex during dental treatment have focused on distraction techniques and diverting the patient's attention away from the ongoing therapy (Table 1). According to the methodology of these studies, it appears that these approaches are more helpful in people with a mild to moderate gag reflex. Thus, the existing data support the efficacy of distraction methods in reducing gag reflex associated with dental treatment, particularly in children. Among different distraction techniques, intellectual games are found the most effective [19, 22, 26]. However, other methods like listening to music have shown lower impact on gag. In the study of Mustafa *et al.*[18], it has been shown that listening to music reduces the anxiety during dental treatment, although there is not enough evidence to verify that music reduces gag severity. It can be inferred that, playing games can better involve children attention and are more effective in mitigating gagging.

In the systematic reviews published prior to 2015, the level of evidence on gag reflex management techniques was evaluated low, and their available data did not support the efficacy of any particular therapy [6, 8, 38]. However, based on the findings of our review, scientific data supports the efficacy of distraction techniques, nitrous oxide, and LLLT.

The investigation on usefulness of nitrous oxide in alleviating gag reflex verifies this method's efficacy [29]. Similar findings have been observed in Chidiac *et al.* [39] research. Due to the common use of it in dentistry and the fact that it is safe and widely accepted among patients, present data support the adoption of this strategy. Additionally, this method can be used easily during routine dental procedures like taking intraoral radiographs. Whilst, other types of medical interventions like intravenous (IV) sedation sound not logical to be used routinely.

Among the high-quality evidence studies, two examined the use of LLLT on PC6 accupoint for effective gag reflex control [23, 28]. Both above studies used diode laser on the same palm region. Goel *et al.* [23] have used laser with the power of 0.5mW, wavelength 940 nm, energy 4J, and 3-4mm away from the tissue for 1 minute. While Elbay *et al.* [28], applied laser with a continuous wavelength of 810 nm, having 1 cm distance from the target area and 4J energy density for 14 seconds. Due to the extensive usage of lasers in dental clinics, it appears that, this less invasive technology might be useful in reducing gag reflex associated with dental treatment. The study of Soltani *et al.* [40], also

shows significant reduction in gag after LLLT application on the same point.

There is little evidence to support the use of local anesthetics, mouthwashes, or the addition of anesthetics to the impression material for controlling gag reflex effectively. None of the studies conducted using these approaches were determined to be of sufficient quality for final assessment. In the studies performed by Bassi *et al.* [8], and Means *et al.* [41], the use of superficial topical anesthetic has shown no discernible impact and has even recorded instances of increased gag intensity following its administration. Based on the findings of our review, applying topical anesthesia has no significant effect on reducing the gag reflex.

Systemic medications for the suppression of the gag reflex, such as intravenous sedation, are more frequently used in patients with moderate to severe gag reflex. The available evidence on the method and dosage of sedation required for effective gag reflex management is insufficient and requires additional investigation. Now, the only accessible pharmaceutical approaches are general anesthesia and intravenous sedation, which are impractical except in the situations of specific treatments with severe gag reflex.

The results of studies on the efficacy of acupuncture and acupressure for controlling gag reflex are likewise conflicting. Based on the current available data, acupressure and laser-therapy techniques performed on PC6 accupoint on the palm region is found effective in suppressing gag reflex [23, 28, 33, 42]. However, other accupoint like external auditory canal was not effective [21, 43]. Additional controlled research is required in this area. Since the counterintuitive efficiency of these approaches has occasionally been linked to distraction effects, future research should compare the effectiveness of acupuncture to other gag reflex treatment strategies, particularly attention-diversion methods.

Conclusion

According to the findings of this systematic review, scientific data supports the efficacy of distraction techniques, nitrous oxide, and LLLT. In general, there is still no one-size-fits-all technique for managing gag reflex during dental treatment. The dentist should manage gag reflex in accordance with the type of dental treatment, the patient's level of gag reflex, the patient's age, and available capabilities, and facilities.

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Ethical committee code of the research: IR.MUQ.REC.1400.120.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Original Article

Effect of Prosthetic Rehabilitation on Airway Space in Edentulous Patients with Obstructive Sleep Apnea: A Preliminary Observational Study

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ABSTRACT

Statement of the Problem: The common causes of obstructive sleep apnea (OSA) are identified as anatomic and/or functional abnormality in the oral cavity, oropharynx, velopharynx, and hypopharynx leading to compromised airway space and increased collapsibility.

Purpose: This study was conducted to evaluate the effect of implant-supported mandibular complete denture in improving the airway space among completely edentulous patients with OSA and compare it with conventional complete denture.

Materials and Method: In this observational study, completely edentulous individuals were screened with snoring, tiredness, observed apnea, high blood pressure, body mass index, age, neck circumference, and gender (STOP-Bang) questionnaire to evaluate the incidence of OSA. Ten mild-moderate patients were included as study participants. Lateral cephalograms (L1) made at the edentulous state was considered baseline. They were rehabilitated with complete denture prosthesis. One week after denture insertion, two implants were placed in the edentulous mandibular arch. Delayed loading protocol was followed. Lateral cephalogram (L2) was made 6 months after complete denture insertion and 6 months after implant-supported prosthesis (L3). Cephalometric tracings were used to evaluate change in upper airway space (UAS), middle airway space (MAS), and lower airway space (LAS). Repeated measures ANOVA was used to evaluate statistical significance in the airway measurements made at the three intervals. Post hoc Tukey HSD and Bonferroni test were used to assess if the differences obtained were truly significant.

Results: Statistical analysis revealed significant differences in UAS, MAS and LAS between L1, L2 and L3 ($p< 0.05$). Post hoc Tukey HSD indicated that UAS increased significantly at all three intervals followed by LAS and MAS respectively ($\alpha=.05$). Post hoc Bonferroni test indicated that implant-supported mandibular complete dentures had a significant improvement in airway space when compared to conventional complete dentures ($\alpha=.05$).

Conclusion: Implant-supported mandibular complete denture could be effective in edentulous patients with mild-moderate OSA.

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Introduction

The common causes of obstructive sleep apnea (OSA) are identified as anatomic and/or functional abnormality in the oral cavity, oropharynx, velopharynx, and hypopharynx leading to compromised airway space and increased collapsibility [1]. Several studies have

proved that edentulism can be considered as one of the exacerbating factors that could worsen OSA [1-2]. Complete edentulism causes changes in the postural position of mandible, muscle tone, tongue posture and vertical dimension, thereby affecting airway dimensions. These morphological changes cause challenges in treating such edentulous sleep apneic patients [2-4]; however, it is underdiagnosed and not characterized in elderly edentulous. Diagnosing and treating OSA is important to improve quality of life, reduce morbidity, and mortality [5]. Alternatives to the standard method of using polysomnography to diagnose OSA are becoming increasingly popular due to its expense and/or limited availability [6]. Questionnaires are appropriate tools for quick chair side prediction [7]. Though computed tomography and magnetic resonance imaging have been regarded as the best method of anatomic evaluation, they require more technical expertise, and expenses. In this regard, cephalometric analysis can play a significant role in identifying patients at risk for OSA [8]. Numerous cephalometric parameters have been associated with its occurrence and it is a simple, safe, and economic method to depict the relevant anatomy of the airway spaces [8]. Oral appliances like mandibular repositioning device and tongue retainers gained recognition in treating dentulous sleep apneic patients. However, in edentulous patients the retention is not enough to accommodate the appliances intraorally [9]. Complete dentures have been used in edentulous sleep apneic patients to increase the airway space and reduce apneic episodes [6]. Owing to short follow up, the effects of wearing dentures is inconclusive [10]. Moreover, a lack of stability and retention together with residual bone resorption would decrease the chewing ability in patients wearing conventional complete dentures. Implant-supported dentures provide greater retention, stability, and quality of life for edentulous patients [11]. Considering the benefits conferred by implant-supported complete dentures, the present study aimed to evaluate the impact of implant-supported mandibular complete denture in improving airway spaces in completely edentulous individuals with mild-moderate OSA and compared it with conventional complete denture prosthesis. The null hypothesis stated that there was no difference in airway space dimensions among mild-moderate sleep apneic edentulous patients rehabilitated with complete dentures and implant-supported mandibular complete denture.

Materials and Method

Formal Ethical approval was obtained from the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (SRIHER, DU) (REFERENCE NO: CSP/19/NOV/81/420). Patients with completely edentulous arches who visited the Department of Prosthodontics were explained about the study and an informed consent was obtained. They were screened using the snoring, tiredness, observed apnea, high blood pressure, body mass index, age, neck circumference, and gender (STOP-Bang) questionnaire, which is specifically developed to meet the need for a reliable, concise, and easy-to-use screening tool [9,12]. It consists of eight dichotomous (yes/no) questions related to the clinical features of OSA. The patients were able to respond to most of the questions. When they were not aware of their behaviour during sleep, spouse or children answered those questions. For each question, answering "yes" scores 1, a "no" response scores 0, and the total score ranges from 0 to 8. Patients with a STOP-Bang score of 0 to 2 can be classified as low risk for moderate to severe OSA, scores in the midrange (3 or 4) as moderate risk for moderate to severe OSA, whereas those with a score of 5 to 8 can be classified as high risk for moderate to severe OSA (Table 1) [12]. Adopting 95% power in calculating sample size (G*Power 3.1.9.2) and

Table 1: STOP-BANG Sleep Apnea Questionnaire

Stop		
Do you SNORE loudly (louder than talking or loud enough to be heard through closed doors)?	Yes	No
Do you often feel TIRED, fatigued, or sleepy during daytime?	Yes	No
Has anyone OBSERVED you stop breathing during your sleep?	Yes	No
Do you have or are you being treated for high blood PRESSURE?	Yes	No
Bang		
BMI more than 35kg/m?	Yes	No
AGE over 50 years old?	Yes	No
NECK circumference > 16 inches (40 cm)?	Yes	No
GENDER: Male?	Yes	No
Total Score		

High Risk of OSA: Yes 5-8
Intermediate risk of OSA: Yes 3-4
Low risk of OSA: Yes 0-2
*Chung, F., Abdullah, H., & Liao, P. (2016). STOP-Bang Questionnaire. Chest, 149(3), 631-638. https://doi.org/10.1378/chest.15-0903

regarding the study of Milosevic *et al*. [13], ten patients aged between 50-65 years, who were first time denture wearers with well-formed residual ridges in Class 1 relation and characterized into low-mode-rate risk category were included as study participants. Patients with history of any metabolic syndrome, surgery of tongue, palate or upper airways, skeletal l class III relationship, grossly resorbed residual alveolar ridges, and musculoskeletal disorders were excluded from the study. Standardised lateral cephalograms (X-ray device-CARESTREAM) were made for all the study participants at edentulous state and was considered baseline (L1). The participants were further screened using lateral cephalograms to eliminate nasal obstruction or pharyngeal tumours.

Cephalometric variables depicting the upper airway space (UAS), middle airway space (MAS), and lower airway space (LAS) were traced by the principal investigator following the standard procedure. UAS was defined as the distance between the pterygomaxillary and upper pharyngeal wall (pm‑UPW), MAS was the distance between the tip of the uvula and middle pharyngeal wall (U‑MPW), and LAS was the distance between the vallecula and the lower pharyngeal wall (V‑LPW) (Figure 1). The dimensions were measured in millimetres (mm) by both the investigators and tabulated. Subsequently, conventional complete dentures with bilateral balanced occlusion were fabricated by a single operator and inserted. Twenty-four-hour review was scheduled for post-operative adjustment. One week following the denture insertion, implant placement was planned in the canine region (B and D position) on the mandibular arch. The mandibular complete denture was duplicated using acrylic resin (Dental Products of India - clear heat cure) to function as a guide. A full thickness mucoperiosteal flap was elevated after administration of local anaesthesia. Sequential osteotomy was performed and dental implants of size 3.3*11.5mm (BIOLINE, India) were torque wrenched into the osteotomy sites, after which cover screws were placed. The flap was approximated using simple interrupted sutures. Study population was reviewed after twenty-four hours and denture bases were relieved to prevent discomfort.

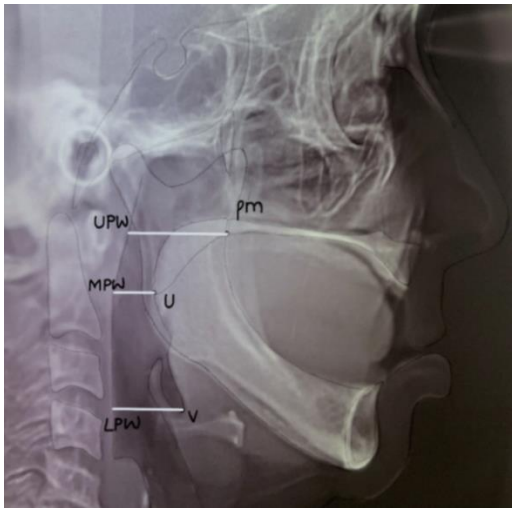

Figure 1: Cephalometric variables depicting Airway space

Six months after complete denture insertion, study participants were recalled and lateral cephalogram (L2) was made to evaluate the change in airway dimensions brought about by complete dentures. Mandibular complete dentures were then converted to implant-supported denture using ball attachments (BIO-T1201, BIO-T1202) and corresponding metal caps (BIO-T3001). Occlusal prematurities were eliminated and the study participants were reviewed after 24 hours. Six months after implant-supported prosthesis, the airway spaces were re-evaluated with lateral cephalogram (L3). Although the effects of oral appliances on the airway are discernible within six to eight weeks, we evaluated after six months to enhance patient compliance with dentures. The collected data was analysed using SPSS software version 21. Repeated measures ANOVA was used to assess the mean difference between the values obtained at three different time intervals. In the above statistical tool, $p< 0.05$ was considered statistically significant. Intraclass correlation coefficient test was used to assess the inter-observer variability.

Results

The Repeated measures ANOVA measured the mean difference between the airway dimensions (in mm) on lateral cephalograms made at three time intervals. On evaluating the UAS, MAS and LAS, the mean difference between L1- L2, L2 - L3 and L1- L3 was statistically significant with a $p<0.05$ as indicated in Tables 2-4. Intraclass correlation coefficient test assessing the inter-observer variability revealed an average value of 0.828 indicating high similarity as seen in Table 5. Intragroup Post hoc analysis using Tukey HSD indicated that the difference between UAS and MAS, MAS and LAS, LAS and UAS was statistically significant at all three intervals as seen in Table 6. However, the difference in

Table 2: Assessment of upper airway space dimensions (UAS) in mm

Time intervals	Mean	Std. Deviation	N	*p* value
L1	2.60	.36	10	
L2	2.80	.31	10	0.00
L3	2.88	.30	10	

*p< .050 statistically significant
[a]L1 indicates edentulous state-baseline
[b]L2 indicates 6 months after complete denture rehabilitation
[c]L3 indicates 6 months after implant-supported mandibular complete rehabilitation
[d]N refers to number of samples

Table 3: Assessment of middle airway space dimensions (MAS) in mm

Time intervals	Mean	Std. Deviation	N	*p* value
L1	1.03	.20	10	
L2	1.21	.12	10	0.00
L3	1.40	.11	10	

* p<.050 statistically significant
[a] L1 indicates edentulous state-baseline
[b] L2 indicates 6 months after complete denture rehabilitation
[c] L3 indicates 6 months after implant-supported mandibular complete rehabilitation
[d] N refers to number of samples

Table 4: Assessment of lower airway space dimensions (LAS) in mm

Time Intervals	Mean	Std. Deviation	N	*p* value
L1	1.56	.35	10	
L2	1.63	.21	10	0.00
L3	1.78	.13	10	

* p<.050 statistically significant
[a] L1 indicates edentulous state-baseline
[b] L2 indicates 6 months after complete denture rehabilitation
[c] L3 indicates 6 months after implant-supported mandibular complete rehabilitation
[d] N refers to number of samples

Table 5: Intraclass correlation coefficient

	Intraclass Correlation	95% Confidence Interval		F Test with True Value 0	
		Lower Bound	Upper Bound	Value	Sig
Single Measures	.16	.06	.30	5.22	.00
Average Measures	.83	.63	.92	5.22	.00

UAS was statistically more significant followed by LAS and MAS (α=.05). Intergroup Post hoc- analysis using Bonferroni test indicated that the difference in the UAS and MAS obtained at L2 and L3 was truly significant (α =.05) when compared with L1 as seen in Table 7. However, the differences in LAS were truly significant only at L3.

Table 6: Post hoc Tukey HSD (Intragroup comparison)

Dependent Variable	Airway space comparison		Mean Difference (I-J) in mm	Sig.
L1	Upper	Middle	1.57*	.00
		Lower	1.04*	.00
	Middle	Upper	-1.57*	.00
		Lower	-.59*	.01
	Lower	Upper	-1.046*	.00
		Middle	.53*	.01
L2	Upper	Middle	1.58*	.00
		Lower	1.17*	.00
	Middle	Upper	-1.58*	.00
		Lower	-.41*	.00
	Lower	Upper	-1.17*	.00
		Middle	.41*	.00
L3	Upper	Middle	1.48*	.00
		Lower	1.10*	.00
	Middle	Upper	-1.48*	.00
		Lower	-.38*	.00
	Lower	Upper	-1.10*	.00
		Middle	.38*	.00

*p<. 050 statistically significant
[a]L1 indicates edentulous state-baseline
[b]L2 indicates 6 months after complete denture rehabilitation
[c]L3 indicates 6 months after implant-supported mandibular complete rehabilitation

Table 7: Post hoc Bonferroni test (Intergroup comparison)

Comparison between airway spaces at the three time intervals (L1, L2 and L3)			Mean Difference in mm	Sig.
UAS	L1	L2	-.20*	.00
		L3	-.29*	.00
	L2	L1	.20*	.00
		L3	-.09*	.00
	L3	L1	.29*	.00
		L2	.09*	.00
MAS	L1	L2	-.19*	.04
		L3	-.37*	.00
	L2	L1	.19*	.04
		L3	-.19*	.00
	L3	L1	.37*	.00
		L2	.19*	.00
LAS	L1	L2	-.07	1.00
		L3	-.23	.25
	L2	L1	.07	1.00
		L3	-.16*	.03
	L3	L1	.23	.25
		L2	.16*	.03

*p<.050 statistically significant
[a] L1 indicates edentulous state-baseline
[b] L2 indicates 6 months after complete denture rehabilitation
[c] L3 indicates 6 months after implant-supported mandibular complete rehabilitation
[d] UAS indicates upper airway space
[e] MAS indicates middle airway space
[f] LAS indicates lower airway space

Discussion

Complete edentulism results in reduction of lower facial height, mandibular rotation, and impaired neuromuscular reflexes favouring upper airway collapse [2-4,11,14-15]. These changes along with abnormal tongue position

predispose edentulous patients to OSA. Although an overnight polysomnography is the gold standard diagnostic test, it is time consuming, labour-intensive, expensive and requires the expertise of sleep medicine specialists [5,16-17]. Thus, a simple chairside screening tool is required to assist in identification. The STOP-Bang questionnaire has demonstrated high sensitivity in detection across various populations [9]. In this study, it has assisted in identifying patients with risk for OSA.

Oral appliances were found to be more satisfactory for mild to moderate OSA patients and contraindicated in patients categorised with severe OSA [17]. Complete dentures have been used as an oral appliance for patients with mild-moderate OSA [18-19]. They improved airway space through positional changes in mandible, tongue, and soft tissue. However, the results of studies assessing the effectiveness of complete dentures on airway space done in the past were inconclusive [18,20]. This study aimed to evaluate the effect of complete dentures on the airway space for a period of six months and the improvement in airway space with implant-supported complete dentures was assessed in the same study population. Both male and female study participants had worn complete dentures fabricated using conventional method and routine materials thus providing a homogenous and representative sample [18]. Airway space dimensions were measured on lateral cephalograms by two investigators. The cephalometric method, despite being a static two-dimensional method to evaluate anatomical structures of the head and neck, has been useful in assessing airway space. Repeated measures ANOVA revealed a statistically significant increase in the UAS, MAS, and LAS dimensions in L2 made six months after insertion of complete denture when compared with dimensions in L1 made at completely edentulous state with a $p< 0.05$ (Tables 2-4). We speculate that the increase in airway dimensions could have occurred due to the positional changes of the mandible. Complete dentures help re-establish maxilla-mandibular relationship. This in turn helps in restoring the tongue to its normal position and prevents blocking of airways. Additionally, the tonicity and function of the surrounding soft tissues are restored, which helps reduce pharyngeal collapsibility [18]. The results of the present study were in correlation with the findings of Gao *et al.* [21], who confirmed the positive effect of complete dentures on enlarging upper airway. Erovigni *et al.* [22] also stated that complete dentures increased the diameters of the velopharynx, oropharynx in the sagittal plane as well as at the uvula level.

Although complete dentures are the first option for rehabilitating edentulous patients, the mandibular complete denture lacks sufficient retention and stability. This affects patient compliance in wearing complete dentures, deteriorating its positive influence on airway space. Implant-supported mandibular denture had evolved as a viable option for edentulous patients with improved retention and quality of life [11]. In this study, mandibular complete dentures of the participants were converted into an implant-supported denture. Lateral cephalograms (L3) obtained six months after implant-supported prosthesis assessed the effect of implant-supported mandibular complete denture on the airway space. The values obtained with L3 were compared with the values obtained at L1 and L2. Post Hoc Bonferroni test indicated that the differences in UAS, MAS and LAS were found to be statistically significant after wearing implant-supported mandibular complete dentures (L3) when compared with conventional complete dentures (L2) and at edentulous state (L1) at $p< 0.05$ (Table 7). This increase, when compared to conventional complete dentures, could have been due to better retention, stability, patient comfort, and compliance, which in turn contributes to improved muscle tone and function [23]. Post Hoc Tukey HSD assessed the difference in the airway dimensions at the three-time intervals between both complete denture and implant-supported mandibular denture. It indicated a significant increase in UAS followed by LAS and MAS with implant-supported mandibular complete denture (L3) (Table 6). Upper airway collapse has been found to be more common in patients with OSA [2]. Thus, the results of this study indicate that implant-supported dentures itself can act as an oral appliance to increase airway space in edentulous sleep apneic patients. Hoekema *et al.* [24] identified mandibular advancement device, which was retained with a single implant, to be effective in edentulous sleep apneic patients. Moreover, the quality of life and sleep characteristics substantially improved with prosthetic rehabilitation in completely edentulous patients who had mild-moderate OSA.

In this study, the edentulous population benefitted

from both complete dentures and implant-supported mandibular complete dentures. Hence, the follow-up period evaluated the impact of both the modes of prosthetic rehabilitation on the airway space. No other study has assessed the effect of implant-supported mandibular complete dentures on the airway space dimension among edentulous patients. While this observational study included ten participants for evaluation, a randomized controlled trial with a larger sample size can help in validating the results. The cephalometric tool used in this study evaluated the antero-posterior diameter of the airway. Subsequently, a three-dimensional evaluation with a longer follow-up in edentulous individuals might assist in drawing conclusions that are more definitive.

Conclusion

Within the limitations of this study, the following conclusions were drawn. Implant-supported mandibular complete dentures improved airway space dimensions when compared with conventional complete dentures in edentulous patients with mild-moderate OSA at the end of twelve months. Among the three airway dimensions, the increase in UAS was statistically significant when compared to MAS and LAS for both modes of rehabilitation indicating a positive influence of complete dentures on airway space.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Original Article

Antifungal Effect of Probiotic *Lactobacillus casei* on Drug-Resistant Oral *Candida albicans* Isolated from Patients with Hematological Malignancy: An *in vitro* Study

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ABSTRACT

Statement of the Problem: *Candida albicans* (*C. albicans*) is recognized as the most common opportunistic pathogen in patients with an impaired immune system, and due to the frequent use of antifungal medicine, a variety of drug-resistant species are developing. Probiotics are a part of the human microbiome and natural competitors of Candida by producing lactic acid, low pH, and other secreted metabolites. The role of probiotics in preventing fungal infections has always been discussed.

Purpose: This study aimed to investigate the antifungal effect of *Lactobacillus casei* (*L. casei*) on fluconazole- and amphotericin B-resistant *C. albicans* species isolated from the oral cavity of acute myeloid leukemia patients.

Materials and Method: In this experimental study, eight strains of fluconazole- and amphotericin B-resistant *C. albicans* were used. The antifungal effects of probiotic *L. casei* and nystatin were measured by the co-aggregation method 1, 2, and 4 h after beginning the study. After each hour of exposure, *C. albicans* and *L. casei* colonies were counted.

Results: *L. casei* had a significant ability to aggregate with both fluconazole- and amphotericin B-resistant *C. albicans* in all designated intervals, which increased with time. In the first hour of the study, no significant difference was observed between the effects of *L. casei* on the two drug-resistant strains. However, as time passed, it had a more significant antifungal effect on fluconazole, compared to amphotericin B resistant species (p Value<0.001). Cell counts showed that the number of fungal cells decreased significantly as time passed (p< 0.001).

Conclusion: *L. casei* had a significant ability to aggregate with both drug-resistant *C. albicans* species and showed higher antifungal activity on fluconazole-resistant than amphotericin B-resistant species.

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Introduction

Candida albicans (*C. albicans*) is recognized as the most common opportunistic pathogen in impaired immune system patients, and its prevalence is increasing with the growing number of immunocompromised people [1-2]. It is clear that patients with a weakened immune system, due to chemotherapy and radiotherapy for cancer treatment, are highly prone to oral candidiasis. The quality of these patients' life is completely affected by fungal infections, and they may experience severe, invasive, and even life-threatening side effects following the entry of Candida species into their bloodstream (candidemia) [1-4]. The colonization of *C. albicans* in immune-deficient patients, especially in hematological malignancies, can affect the final treatment results [5]. Currently, following the frequent, long-term, and proph-

ylactic use of antifungal medications in patients with candidiasis, the variety of drug-resistant Candida strains is increasing. Therefore, finding an alternative way to control and prevent this group of fungal infections is suggested [4,6]. Some studies [7-10] show that Candida species colonization is reduced following the consumption of foods containing probiotics [7]. Lactobacillus species are among probiotics that inhibit the formation and growth of Candida biofilm and reduce the symptoms of infection. The role of probiotics in preventing infectivity is not fully understood, and it is currently hypothesized that probiotics inhibit the growth of fungal cells by competing for food, occupying the sites required for Candida attachment, and producing antimicrobial agents. Probiotics can also improve the mucosal immune system [8-10]. Therefore, this study aims to investigate the role of probiotic *Lactobacillus casei* (*L. casei*) in the prevention of fungal lesions in the form of co-aggregation.

Materials and Method

L. casei and culture conditions

In this *in vitro* experimental study, the probiotic *L. casei* (PTCC number 1608) was used, provided by the Iranian Research Organization for Science and Technology. The *L. casei* was cultured under anaerobic conditions in Man Rogosaf Sharpe (MRS, SIGMA, USA) medium at 37˚C for 24 h. The isolated *L. casei* colonies were transferred to the MRS medium (5 ml) and incubated at 37˚C for 48 h. After the incubation, *L. casei* was stocked at 20˚C in glycerol. 1 ml of *L. casei* was relocated to 5 ml of MRS broth medium for recultivation. Finally, L-cysteine (50 µl) was added. The microtubes were located in a shaker incubator (16B; KTG laboratory equipment) at 37˚C for 20 h [8].

C. albicans and culture conditions

A microbiologist isolated the *C. albicans* from the oral cavity of patients with acute myeloid leukemia admitted to the Taleghani Hospital of Shahid Beheshti University (Tehran, Iran). These patients did not show any improvement in oral lesions following the administration of fluconazole and amphotericin B, either clinically or microscopically (in terms of colony count, cell numbers, and fungal wall destruction). Sampling was done with two swabs prepared from the oral lesions caused by Candida species. They were placed in 1 cc of distilled water and transferred to the laboratory. A direct slide was prepared from one swab and the second swab was prepared on Saburo Dextrose Agar (SDA, SIGMA, USA) medium. Minimum inhibitory concentration results were determined according to the CLSI-M27-S3 (Clinical & Laboratory Standards Institute) and showed that the strains were resistant to fluconazole and amphotericin B (13). Candida species were stored at –70˚C in Tryptic Soy Broth (SIGMA, USA). To start the study, Candida species were cultured in SDA and transferred to Saburo Dextrose Broth (SDB) for 24 h at 37˚C. Eight strains of fluconazole- and amphotericin B-resistant *C. albicans* were used, and the antifungal effects of *L. casei* (study groups) were measured, in comparison with nystatin (control groups), by the co-aggregation method 1, 2, and 4 h after the study began. The study groups included (1) *L. casei* and fluconazole-resistant *C.albicans*, (2) *L.casei* and amphotericin B resistant *C. albicans*, (3) nystatin and fluconazole-resistant *C.albicans*, and (4) nystatin and amphotericin B resistant *C. albicans* [8].

Co-aggregation of *C. albicans* and *L. casei*

The co-aggregation was assessed by a spectrophotometer (PD-303; Apple model). At first, the mixture of *L. casei* and *C. albicans* was incubated for 1, 2, and 4 h. Then, the co-aggregation ratio was determined consistent with the methodology proposed by Salari *et al.* [8] Initially, the detached colonies of 24-h *L. casei* were moved to 5 ml MRS broth medium and were incubated in a shaker incubator (84 RPM; 24 h; 37˚C) in anaerobic carriage. The *C. albicans* was isolated from SDA and cultured in SDB at 37˚C for 24 h. Then, the microtubes containing *L. casei* and *C. albicans* were centrifuged (855 RPM; 10 min, 25˚C). The supernatant was removed and the sediment was washed three times in phosphate buffer saline (PBS, SIGMA, USA) and suspended in 10 mmol/L PBS (at pH=7). The optical absorbance was measured by a spectrophotometer at the specific wavelength of 600 (OD600 nm) nanometers equivalent to the McFarland standard (10^8 CFU/mL for *Lactobacilli* and 10^6 CFU/mL for Candida species). In total, 1ml of each *L. casei* and 1 ml of *C. albicans* were totally mixed and incubated in a shaker incubator (100 rpm; 37˚C; for 1, 2, and 4 h). Then, the optical density (OD) measurement was performed with spectrophotometer at OD600 nm. Each experiment was repeated three times [8].

The percentage of co-aggregation was calculated ba-

sed on the following equation:

$$C - \text{aggregation}\%: [(OD_0 - OD_h)/OD_0] \times 100$$

OD_0: the absorption amount of the complex suspension of *L. casei* with *C. albicans* at the beginning of the trail (0 h).

OD_h: the absorption amount of the complex solutions at various times

L. casei and _C. albicans_ cell counts

To evaluate the cell number of *C.albicans* and *L.casei* 1, 2, and 4h after the study began (after measuring the absorbance of the solution by the spectrophotometer OD600nm), some of the solution containing *L.casei* and *C.albicans* was removed with an inoculation needle and transferred to the culture medium. After 24h, the number of cells was determined by counting the colonies of each species separately and calculating the number of cells by calculating each colony in a certain coefficient of dilution (coefficient of dilution was prepared based on the diameter of the needle loop). Small colonies were belonged to *L.casei* and larger colonies revealed *C.albicans*.

Data analysis

Data were analyzed by the SPSS 11 software through one-way ANOVA and Tukey HSD tests.

Results

Co-aggregation percentage between _L. casei_ and two drug-resistant _C. albicans_ in 1, 2, and 4 hours of study

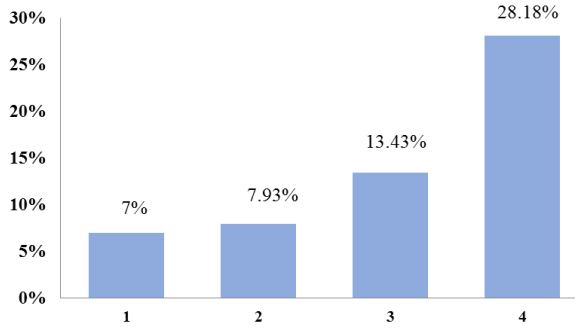
The normality of data distribution was checked by Kolmogorov-Smirnov test. Based on the test results, the distribution of all data was normal. One-way ANOVA results showed that in all three times (1, 2, and 4 h), *L. casei* had a significant ability to aggregate with drug-resistant *C. albicans*, which increased with time ($p< 0.001$).

First hour of the study

There was no significant difference between the co-aggregation degree of *L.casei* with fluconazole-resistant and amphotericin B-resistant *C. albicans*, and the antifungal effect was almost similar. At the same time, compared to nystatin, *L. casei* had a significantly lower antifungal effect on amphotericin B resistant species ($p< 0.001$). On the other hand, nystatin had a slightly more antifungal effect on fluconazole-resistant species when comp-ared to *L. casei*, but the difference was not significant (Figure 1).

Second hour of the study

Herein, a significant difference was monitored between the co-aggregation percentage of *L. casei* with a fluconazole-resistant, compared to amphotericin B-resistant *C. albicans* ($p< 0.001$). Moreover, *L. casei* had greater co-aggregation ability with fluconazole-resistant species than amphotericin B-resistant strains. At this time, no significant difference was observed between the antifungal results of *L. casei* and nystatin on amphotericin B and fluconazole-resistant species. However, the findings revealed that in amphotericin B-resistant species, the effect of nystatin was slightly better, and in fluconazole-resistant species, *L. casei* had an antifungal effect similar to that of nystatin (Figure 2).

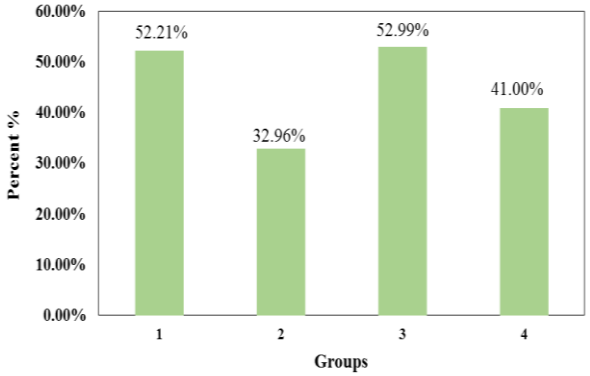


Group 1: *L. casei* and fluconazole-resistant *C. albicans*
Group 2: *L. casei* and amphotericin B-resistant *C. albicans*
Group 3: Nystatin and fluconazole-resistant *C. albicans*
Group 4: Nystatin and amphotericin B-resistant *C. albicans*

Figure 1: Co-aggregation percentage of *L. casei* with drug-resistant *C. albicans* species in comparison with Nystatin, in the first hour of the study

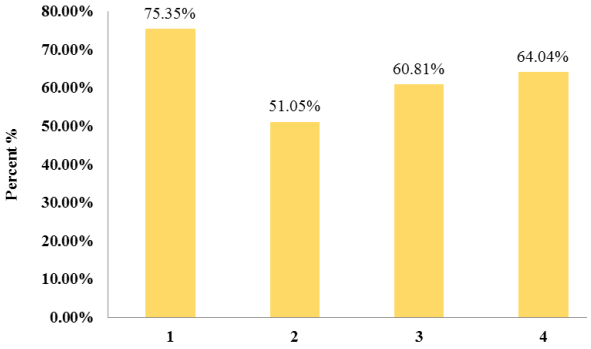
Fourth hour of the study

In the fourth hour, *L. casei* had a significantly higher co-aggregation rate with fluconazole-resistant than amphotericin B-resistant species ($p< 0.001$). Furthermore, compared to nystatin, *L. casei* had a significantly greater



Group 1: *L. casei* and fluconazole-resistant *C. albicans*
Group 2: *L. casei* and amphotericin B-resistant *C. albicans*
Group 3: Nystatin and fluconazole-resistant *C. albicans*
Group 4: Nystatin and amphotericin B-resistant *C. albicans*

Figure 2: Co-aggregation percentage of *L. casei* with drug-resistant *C. albicans* species in comparison to Nystatin, in the second hour of the study



Group 1: *L. casei* and fluconazole-resistant *C. albicans*
Group 2: *L. casei* and amphotericin B-resistant *C. albicans*
Group 3: Nystatin and fluconazole-resistant *C. albicans*
Group 4: Nystatin and amphotericin B-resistant *C. albicans*

Figure 3: Co-aggregation percentage of *L. casei* with drug-resistant *C. albicans* species in comparison to Nystatin, in the fourth hour of the study

antifungal effect on fluconazole-resistant species ($p< 0.001$). However, this comparison was completely reversed in the *L. casei* and nystatin groups on amphotericin B-resistant *C. albicans*, leading to a better effect of nystatin than *L. casei* ($p< 0.001$). At this stage, the co-aggregation of *L. casei* with fluconazole-resistant strains was better than all groups and had a significant antifungal effect, in comparison with other groups of the study ($p< 0.001$) (Figure 3).

Cell counting of *Lactobacilli casei* and drug-resistant *C. albicans* after 1, 2, and 4 hours of exposure

Cell number of *L. casei* and resistant *C. albicans* after 1, 2, and 4 hours of exposure showed that the amount of *L. casei* and drug-resistant *C. albicans* cells decreased significantly over time ($p< 0.001$). It was also found that the amount of fluconazole-resistant *C. albicans* and *L. casei* cells decreased more, in comparison with *L. casei* and amphotericin B-resistant cells in the fourth hour of the study ($p< 0.001$) (Figure 4).

Discussion

Due to the increasing prevalence of oral candidiasis in immunodeficient patients and the development of drug-resistant Candida species, the use of prophylactic components, such as probiotics, for preventing fungal infections has always been proposed [11-14]. The role of probiotics in this field is generally recognized, and various studies have revealed its role in reducing the signs and symptoms of oral candidiasis [15-16]. Several experiences have revealed antifungal effects of different probiotics on oral *C. albicans* [8-9,17-18]. This study tested the antifungal effect of probiotic *L. casei* on fluconazole- and amphotericin B-resistant *C. albicans* by evaluating the degree of co-aggregation and the colony counting method. In the study of Salari *et al.* [8], the ability of co-aggregation was seen between *L .acidophilus* and *L .plantarum* with all tested Candida strains, and their co-aggregation percentage increased over time. The highest co-aggregation rate was observed between *C. krusei* and two probiotics, which was lower than that of *C. albicans*. In a study by Jørgensen *et al.* [19], *L. reuteri* with *C. tropicalis* and *C. krusei* had the greatest co-aggregation ratio. Chew *et al.* [20] also stated that *L. reuteri* showed a mainly greater co-aggregation ratio versus *C. glabrata* species, in comparison with *L. rhamnosus.* Therefore, it appears that co-aggregation levels are exclusive for each species of Lactobacilli [8]. In the next part of this study, colony counting was performed 1, 2, and 4 hours after the study began. Based on counti-

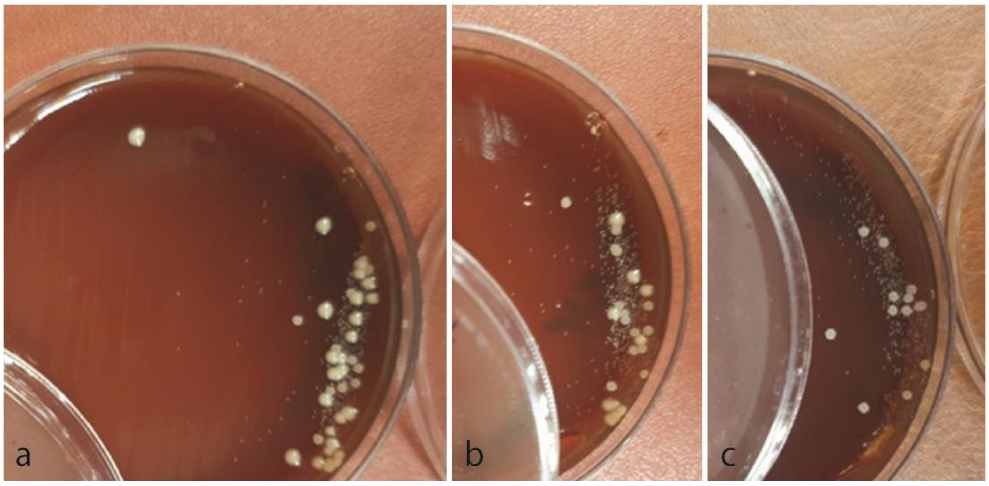


Figure 4: **a:** Smaller colonies were belonged to *L. casei* and larger colonies revealed *C. albicans*. control group, **b:** *L. casei* and amphotericin B resistant *C. albicans*, ***c:*** *L. casei* and fluconazole resistant *C. albicans*

ng drug resistant *C. albicans* colonies, it was found that the number of cells reduced significantly over time, and the number of fluconazole-resistant colonies decreased more significantly, in comparison with amphotericin B-resistant colonies in the fourth hour of the study ($p< 0.001$). It was consistent with the co-aggregation results, which showed that in the fourth hour, *L. casei* had greater co-aggregation ability with fluconazole-resistant strains than amphotericin B-resistant strains. The findings of a study by Srivastava *et al.* [21], which tested the inhibitory effect of *L. plantarum* on *C. albicans* and *Streptococcus mutans* (*S. mutans*) by counting the colonies, showed that *L. plantarum* significantly stopped the formation of *C. albicans* biofilm alone and in combination with *S. mutans*. Probiotics inhibit *C. albicans* activity in different ways, including the inhibition of fungal adhesion to surfaces, production of acids, bacteriocins, biosurfactants, as well as hydrogen peroxide, and co-aggregation. Co-aggregation is one of the hallmarks of primary biofilm formation since it involves an adhesion-receptor interaction between microbial cell surfaces. In fact, the reduction in cell-dependent adhesion is probably due to the simultaneous co-aggregation of *L. casei* and *C. albicans* species. Interestingly, good initial adhesion of certain strains of probiotics, such as *L. gasseri*, *L. cryspatus*, or *L. vaginalis*, is not compatible with a good inhibition of *C. albicans* adhesion. It indicates that *C. albicans* adhesion is minimized due to the changes in the surface of epithelial cells or the effect of probiotics on the adhesion ability of the pathogen [16,22]. Previous study has also shown that *L. casei* has the ability to produce Interleukin-12, which stimulates natural killer cells (first line of defense) to respond against the infection and their proper function plays an important role in the prognosis of the disease [18]. In a study by Villena *et al* [9], *L. casei* was found to prevent the damage caused by inflammatory responses by producing interleukin-10 (IL-10). The IL-10 is a potent anti-inflammatory regulatory cytokine that has a beneficial effect on the host's response against fungal infections. In the early stages of infection, cytokines can interfere with the antifungal function of phagocytes and the production of other proinflammatory cytokines. However, in the later stages of infection, the increase in IL-10 production may help relieve the inflammatory response. Therefore, the use of this probiotic as a supplement with the mechanism of increasing IL-10 prevents the damage caused by inflammatory responses [9]. The difficulty of Lactobacillus bacteria growth in culture medium and the sensitivity of probiotics to environmental conditions were some of the possible limitations. A novel feature of our survey was the comparison between the antifungal effects of *L. casei* on fluconazole- and amphotericin B-resistant *C. albicans.* These species were isolated from the oral cavity of AML patients. The incidence of oral candidiasis is associated immunity reduction followed by a chemotherapy regimen in patients with hematological malignancies.

Conclusion

The findings revealed that *L. casei* had a significant ability to co-aggregate with both drug-resistant *C. albicans* species and showed higher antifungal activity on fluconazole-resistant strains than amphotericin B-resistant species. Further investigations are recommended for assessing the antifungal properties of other Lactobacillus species on drug-resistant *C. albicans* and recognizing the precise mechanisms of their action, as well as performing antifungal susceptibility patterns in models of infected animal.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Original Article

Comparative Diagnostic Accuracy of VistaCam IX Proxi and Bitewing Radiography for Detection of Interproximal Caries

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Dental Caries;
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ABSTRACT

Statement of the Problem: Early detection of caries and the extent of carious lesions for appropriate treatment planning are very important and lead to introduction of new diagnostic tools.

Purpose: This study aimed to compare the diagnostic accuracy of VistaCam IX Proxi and bitewing radiography for detection of posterior interproximal caries.

Materials and Method: This *in vitro* study was performed on 40 extracted posterior teeth without cavitated carious lesions. Bitewing radiographs were obtained, infrared (IR) examination was performed, and the teeth were sectioned for histopathological analysis under a stereomicroscope as the gold standard for detection of caries and determination of the extent of carious lesions. Data were analyzed with Cohen's kappa statistic, and Wilcoxon rank sum test.

Results: The specificity of VistaCam IX Proxi and bitewing radiography was 71.4% and 87.7%, respectively. Their sensitivity was 100% and 40% for enamel caries, 72.8% and 54.5% for external half dentin caries, and 82.3% and 58.8% for internal half dentin caries, respectively (p= 0.048).

Conclusion: Bitewing radiography had a higher specificity and lower rate of false positive results. However, VistaCam IX Proxi had higher sensitivity for caries detection with lower rate of false negative results. Considering the higher sensitivity and significantly lower frequency of false negative results by VistaCam IX Proxi, it may be reliably used for caries detection specially enamel caries, and can serve as an adjunct to bitewing radiography.

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Introduction

In spite of scientific advances and the improved status of public health, dental caries has remained a noticeable dilemma [1]. The development course of caries is both preventable and stoppable. In the case of early detection of caries, noninvasive methods including antimicrobial therapy, fluoride therapy, low-level laser therapy, and diet modification, may be used to stop or even reverse the caries process [2]. The most practical diagnostic tools used in clinical practices are radiological and clinical examination. Although both of these diagnostic tools feature high specificity, they manifest low sensitivity. As a result, some incipient caries may be missed. Given the impossibility of direct observation and contact with the adjacent teeth, the detection of interproximal caries is accompanied by many difficulties. Thus, interproximal carious lesions are hardly detectable in their initial stages [3-4].

In recent years, a number of techniques have been presented in order to improve the detectability of interproximal caries without the need for radiography, including laser fluorescence and fiber optic transillumina-

tion [3-4]. The use of infrared (IR) and near IR wavelengths is a new technology based on digital imaging fiber optic transillumination for caries detection. The main difference between these two techniques is that while visible light is used in digital imaging fiber optic transillumination, the other system employs invisible light characterized by a long wavelength [5-9]. Some of the new systems introduced to the market for this purpose are replaceable Proxi head for VistaCam IX intra-oral camera (Durr Dental, Bietigheim-bissingem, Germany) and Diagnocam (Kavo, Biberach, Germany) [3, 10]. This device benefits from 2IR LED (850 nm wavelength) and the optical output part of this device measures 7×9mm. Following the radiation of light to the distal and mesial surfaces of the adjacent teeth, the radiation passes through the transparent enamel structure and is scattered by the carious lesion and enamel [11-12]. The CCD receptor receives the reflected and scattered lights. This phenomenon leads to the development of some white points on the image compared to healthy enamel. The image is then displayed by DBSWIN or VISTA SOFT programs [3-4, 6, 11-12]. Concerning the IR reflection and absorption spectrum, a number of scholars have recommended that waves ranging from 1300 to 1700 nm show the best potential of revealing caries in this technology. One can attribute this phenomenon to favorable absorption and low scattering within the above range, leading to the provision of superb contrast for differentiation between the sound enamel and carious lesions [13-14]. Nonetheless, when choosing the characteristics of the intra-oral devices for *in vivo* circumstances, one should consider the effect of water on/within the surface of enamel as the most important effective parameter. Thus, the appropriate wavelength used to capture the best diagnostic image is 850 nm [4]. Evidence suggests that one can use IR images in order to detect demineralization beneath sealants, buccal surface caries, and secondary caries beneath composite restorations and determine the extent/severity of occlusal caries and also the extent of water loss of the structure of teeth in the course of demineralization [10, 15-22]. This system is supposed to serve as a superb diagnostic system, in particular for the follow-up of patients at high risk of caries, children, pregnant women, and patients suffering large torus, an extreme gag reflex, as well as the sites, which are hardly examinable through radiography [10]. Early detection of caries is highly important in order to prevent invasive treatments, especially in patients for whom radiography is contraindicated. This investigation aimed to study the accuracy of VistaCam IX Proxi with IR light at a wavelength of 850 nm compared to bitewing radiography for detecting interproximal caries.

Materials and Method

The present *in vitro* diagnostic investigation was carried out on 40 extracted human permanent premolars and molars. The inclusion criterion of the study was sound teeth characterized by discoloration/non-cavitated incipient caries, which were unobservable directly when the teeth were in contact with each other. Teeth with cavitated lesions or restorations were excluded. By immersing in 1% sodium hypochlorite solution for 12 h, the collected teeth were disinfected, which were then stored in saline. Using two silicon blocks, which were in contact with one another, the selected teeth were mounted in such a way that the contact area between every two teeth simulated the clinical intraoral position of the teeth. Bitewing radiographs were obtained under similar conditions using a photostimulable phosphor plate receptor (ACTEON, France) and an intra-oral radiography unit (GENDEX, USA). Then, using VistaCam IX (Durr Dental, Bietigheim-bissingem, Germany), IR images were obtained from the proximal surfaces of the mounted teeth. Therefore, after drying the teeth, they were placed within a medium in a dimly lit room in order to simulate the oral cavity. Subsequently, the camera and also its special holder were adjusted over the occlusal surface at the contact area between the two teeth, and the image of interest was taken from the teeth in accordance with the instructions presented by the manufacturer (Figure 1). After image acquisition and briefing the observers regarding the correct observation of each series of images and the enhancement techniques, the images were evaluated by two oral and maxillofacial radiologists. Their opinions regarding the absence or presence of caries and the extent of interproximal lesions were recorded separately for each interproximal region using the criteria defined as (0) no caries at the contact area, (1) caries found in the enamel, (2) caries found in the outer half of dentin, and (3) caries found in the inner half of dentin.

Figure 1: Vistacam IX Proxi's real time image

After two weeks, the images were observed again by the same observers, and the intra- and inter-observer reliability values were calculated. Among the results, cases with a higher level of agreement between the two observations were set aside, and the differences were discussed by the two observers until a consensus was reached. After reaching a consensus, individual responses were recorded for the final comparison. SCANORA software (Soredex, Tuusula, Finland) was used to observe and interpret the radiographic images, and DBSWIN software (Durr Dental, Bietigheim-bissingem, Germany)was utilized to observe and interpret the IR images. The teeth were then assessed by histological examination as the gold standard. For the same purpose, by employing a saw (Isomet; Buehler, USA), a minimum of three sections were prepared from each tooth at the site of its carious lesion. Then, the prepared sections were converted to microscopic slides observed via a stereomicroscope (Olympus, szx9, Japan) (see Figure 2). Absence or presence of caries and the extent of lesions were recorded by considering the defined criteria as (0) minimum caries, (1) caries found at the level of enamel, (2) caries found in the external half of dentin, (3) caries found in the internal half of dentin.

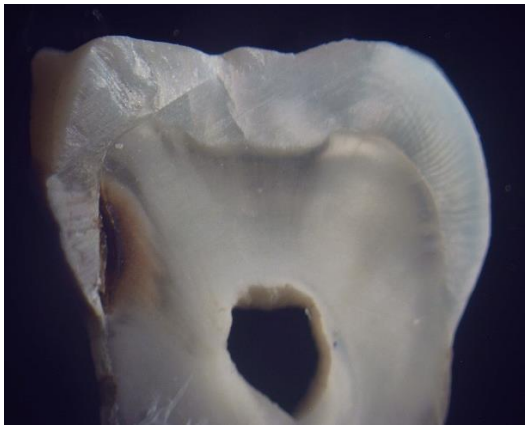

Figure 2: Histopathological analysis (gold standard)

Next, a comparison was made between the results obtained by using each diagnostic tool and between the histological analysis results as the gold standard. As soon as data collection was completed, the data pertaining to the two observers were compared, and then the kappa coefficient of agreement between the two observers was estimated at two different time points. The relative and absolute frequency of the correct diagnoses obtained using each diagnostic tool were also reported. In addition, the kappa coefficient of agreement between the two techniques was estimated via the standard technique and reported. It is noteworthy that the sensitivity of each tool (bitewing radiography and VistaCam IX Proxi) was determined based on the extension of caries and reported. Eventually, the specificity value of each diagnostic tool for the detection of caries-free teeth was determined and reported. While the kappa statistic was used to analyze the agreements, the Wilcoxon rank sum test was utilized to make a comparison between the results obtained using the two diagnostic tools and the corresponding value of the gold standard. To analyze the obtained data, SPSS 21 was utilized. The Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RIDS.REC.1395.365) verified the research protocol.

Results

According to the results obtained through histological analyses as the gold standard, 7 out of 40 teeth evaluated in this study had absolutely no caries, while five teeth had enamel caries, 17 had caries in the internal half of dentin, and 11 had caries in the external half of dentin. The value of the intra-observer agreement for the first observer was 0.85, while it was 0.68 for the second observer when employing VistaCam. In addition, it was 0.69 for the second observer and 0.79 for the first observer in bitewing (see Table 1).

The inter-observer agreement calculated for the first observation of VistaCam iX Proxi images was 0.82, while it was 0.56 for the second observation. The value of the inter-observer agreement for the results acquired from the first and the second bitewing assessments were 0.76 and 0.72, respectively (see Table 2).

Given the acquired agreement coefficients, in the

Table 1: Intra observer agreement

		VistaCam results					bitewing results				
		Sound	**Enamel caries**	**External half dentin caries**	**Internal half dentin caries**	**Total**	**Sound**	**Enamel caries**	**External half dentin caries**	**Internal half dentin caries**	**Total**
First observer	Sound	5	1	0	0	6	10	1	0	0	11
	Enamel caries	0	7	0	0	7	1	4	0	0	5
	External half dentin caries	0	0	9	3	12	0	0	10	2	12
	Internal half dentin caries	0	0	0	15	15	0	0	2	10	12
Total		5	8	9	18	40	11	5	12	12	40
Kappa agreement						0.85					0.79
Second observer	Sound	4	2	0	0	6	9	2	0	0	11
	Enamel caries	0	6	0	0	6	1	3	2	0	6
	External half dentin caries	0	0	8	5	13	0	0	8	1	9
	Internal half dentin caries	0	0	2	13	15	0	0	3	11	14
Total		4	8	10	18	40	10	5	13	12	40
Kappa agreement						0.68					0.69

first observation of both VistaCam IX Proxi images and bitewing radiographs, the first and second observers showed a higher agreement.

At last, the results acquired from the first observation were selected, and the disagreements between the two observers were resolved by consultation. As the final result was obtained from VistaCam IX Proxi and bitewing radiographs, a comparison was made between this result and the corresponding gold standard. To compare the final results with those obtained via the histological analysis as the gold standard, the kappa agreement coefficient and the Wilcoxon rank sum test were applied. The results obtained from comparing VistaCam IX Proxi and the gold standard are as follows (Table 3).

As Table 3 indicates, the value of the total agreement coefficient obtained for VistaCam was 0.718. Of a total of seven teeth without caries, VistaCam reported 1, 1, and 5 cases as cases with caries in the external half of dentin, cases with enamel caries, and sound, respectively. Therefore, its specificity for caries-free surfaces was 71.4%, and the reported percentage of false positive results was 28.5% approximately. In addition, among of dentin, one was reported as sound. As a result, the positive predictive value of VistaCam was calculated as 83.3%. All five samples featuring enamel caries were diagnosed correctly. Thus, the VistaCam sensitivity was 100% for the surfaces suffering enamel caries, while the

Table 2: Inter observer agreement

		VistaCam results					bitewing results				
		Sound	**Enamel caries**	**External half dentin caries**	**Internal half dentin caries**	**Total**	**Sound**	**Enamel caries**	**External half dentin caries**	**Internal half dentin caries**	**Total**
First observation	Sound	5	1	0	0	6	10	1	0	0	11
	Enamel caries	1	5	1	0	7	1	4	0	0	5
	External half dentin caries	0	0	11	1	12	0	1	8	3	12
	Internal half dentin caries	0	0	1	14	15	0	0	1	11	12
Total		6	6	13	15	40	11	6	9	14	40
Kappa agreement						0.82					0.76
Second observation	Sound	2	3	0	0	5	9	2	0	0	11
	Enamel caries	2	5	1	0	8	1	3	1	0	5
	External half dentin caries	0	0	6	3	9	0	0	10	2	12
	Internal half dentin caries	0	0	3	15	18	0	0	2	10	12
Total		4	8	10	18	40	10	5	13	12	40
Kappa agreement						0.56					0.72

Table 3: Comparison the results of VistaCam ix proxy and DIAGNOdent with gold standard

		Gold standard				Total
		Sound	Enamel caries	External half dentin caries	Internal half dentin caries	
VistaCam	Sound	5	1	1	0	7
	Enamel caries	0	5	0	0	5
	External half dentin caries	1	1	8	1	11
	Internal half dentin caries	0	0	3	14	17
Total		6	7	12	15	40
Bitewing	Sound	6	0	0	1	7
	Enamel caries	2	2	1	0	5
	External half dentin caries	3	1	6	1	11
	Internal half dentin caries	0	2	5	10	17
Total		11	5	12	12	40

reported percentage of false negative results was 0%. In addition, one sample with caries in the external half of dentin and one sound sample were reported among those enamel caries. Thus, the positive predictive percentage of the surfaces characterized by enamel caries was 71.4%. Among the eleven samples featuring caries in the external half of dentin, VistaCam correctly detected 8 cases with caries found in the external half of dentin, 1 with enamel caries, 1 without caries, and 1 with caries situated in the external half of dentin. Therefore, its sensitivity was 72.7% for the surfaces with caries in the external half of dentin, while the percentage of false negative results was 9%. Furthermore, one sample without caries and three samples with caries situated in the internal half of dentin were wrongly detected as those with caries situated in the external half of dentin by VistaCam. As a result, the positive predictive value of VistaCam reported for those surfaces that featured caries in the external half of dentin was 66.6%. Of 17 samples with caries situated in the internal half of dentin, the VistaCam correctly detected 14 cases; 3 cases were diagnosed with caries situated in the external half of dentin. As a result, the sensitivity of the device for surfaces characterized by caries in the internal half of dentin was 82.3%. On the other hand, it wrongly reported one sample with caries situated in the external half of dentin as having caries found in the internal half of dentin. Therefore, its positive predictive value for surfaces with caries situated in the internal half of dentin was 93.3%.

As Table 3 shows, the total agreement coefficient of bitewing radiography was equal to 0.449. Of the seven samples without caries detected on bitewing radiographs, six were correctly detected as sound and one had caries situated in the internal half of dentin. As a result, the estimated specificity of bitewing images for sound surfaces was 87.6%, while the percentage of false positive results was equal to 14.2%. In addition, two samples with enamel caries and three samples with caries in the external half of dentin were detected as sound on bitewing radiographs. As a result, the estimated negative predictive value of bitewing radiography was 54.5%. Two out of the five samples characterized by enamel caries on bitewing images were properly reported to have enamel caries, 1 was reported with caries in the external half of dentin, and 22 were reported as sound. As a result, the calculated sensitivity of bitewing radiography for enamel caries was equal to 40%; moreover, the percentage of false negative results was equal to 40%. In addition, bitewing radiography incorrectly reported one sample with caries found in the external half of dentin and also two samples with caries situated in the internal half of dentin as sound. As a result, the estimated positive predictive value of bitewing radiography for the surfaces featuring enamel caries was 40%. Of the 11 samples featuring caries in the external half of dentin, six were correctly diagnosed, one had enamel caries, three did not have caries, and one featured caries in the internal half of dentin. As a result, the calculated sensitivity of bitewing radiography for the surfaces featuring caries in the external half of dentin was 54.5% approximately, and the percentage of false negative results was equal to 27.2%. From another viewpoint, one sample featuring enamel caries and five samples featuring caries in the internal half of dentin were wrongly reported to have caries situated in the external half of dentin. Thus, the positive predictive value of bitewing radiography for the surfaces featuring caries in the external half of dentin was 50%. Of 17 samples with caries situated in the internal half of dentin, two, five, and ten samples were reported to have enamel caries,

caries situated in the external half of dentin, and caries situated in the internal half of dentin (the latter being properly reported), respectively. As a result, the estimated sensitivity of bitewing radiography for the surfaces featuring caries in the internal half of dentin was 58.8%. Also, one sample without caries and one sample with caries in the external half of dentin were wrongly reported to have caries situated in the internal half of dentin. As a result, the estimated positive predictive value of bitewing radiography for the surfaces featuring caries in the internal half of dentin was 83.3%. The results of both VistaCam IX Proxi and bitewing radiography had significant differences compared to the gold standard (p= 0.048).

Discussion

At present, with regard to the reversibility of caries if detected early before cavitation, and the reduction of caries prevalence in some parts of the world, finding more precise methods for caries detection has become increasingly important. Furthermore, various imaging methods are available to evaluate dental caries. Different studies in recent years have assessed the accuracy of various imaging systems and reported controversial results [15-22]. Based on studies, the different representation of demineralized tissue in comparison with other changes, such as developmental lesions, pigmentation, cracks, scales, and fluorosis, is an advantage of the IR images. Furthermore, since IR images are real-time, this characteristic enables the clinician to diagnose carious lesions, which could have remained undetected on bitewing radiographs. Nonetheless, the IR images also have a number of disadvantages, including inaccurate inspection of the depth of caries and distance from the pulp and incapability to examine the periodontal structure around the teeth [10, 15-22]. Nevertheless, IR images have the ability to reveal proximal lesions [23]. The present investigation was carried out in order to compare and assess the diagnostic precision of VistaCam IX Proxi, which takes advantage of IR light in order to detect caries and bitewing radiography for the detection of proximal caries. The obtained results indicate that the estimated specificity of VistaCam IX Proxi for sound surfaces was equal to 71.4%, while it was 85.7% for bitewing radiography. This suggests that it is less likely that false-positive results occur in sound samples when using VistaCam compared to bitewing radiography, and it has a higher probability for correct detection of sound surfaces; thus, unnecessary treatments would be prevented. Previous studies have obtained contradictory results on this topic. For example, in an *in vitro* study by Maia Ama *et al.* (2011) comparing the IR radiation imaging and bitewing radiography techniques for the detection of incipient caries, it was observed that the specificity of images obtained by IR was higher than that of bitewing radiography [24]. However, in their *in vivo* investigation, Russotto *et al*. (2016) used IR images to detect proximal caries. They suggested that bitewing images were more specific than IR images [25]. Furthermore, Gokhan Ozkan *et al*. (2017) evaluated IR images for the detection of dentin proximal caries *in vivo*. Eventually, they found that the specificity of bitewing images was higher than that of IR images [3]. Schwendicke *et al*. [26] performed a systematic review and concluded that the specificity of bitewing radiography was higher than that of IR images. Considering enamel caries, since the determination of the extent of caries in the internal and external half of enamel would have no significant effect on preventive treatment planning, the extent of enamel lesions was not specified in this study, and such lesions were generally categorized as enamel caries. Considering enamel caries, while in VistaCam IX Proxi, the sensitivity was 100%, the sensitivity of bitewing radiography was 40%. This finding reflects the considerably higher sensitivity of VistaCam IX Proxi for the detection of incipient enamel carious lesions, which were reversible as well. Taking advantage of non-invasive approaches without the need for employing ionizing radiation will be favorable in preventive dentistry and the follow-up of high-risk patients. With regard to caries located in the external half of dentin, the sensitivity of bitewing radiography and VistaCam IX Proxi was 54.5% and 72.7%, respectively. This indicates the higher diagnostic accuracy and sensitivity of VistaCam in comparison with bitewing radiography for the follow-up of patients and detecting the extent of carious lesions. False negative responses in IR images are less likely to occur, while the probability of proper detection of caries situated in the external half of dentin is higher. With regard to caries situated in the internal half of dentin, the sensitivity of bitewing radiography and VistaCam IX Proxi was

58.8% and 82.3%, respectively, which indicates the higher sensitivity of the latter for more precise determination of the extent of caries. This finding suggests that the false negative results may hardly occur in IR images, while the probability of proper detection of caries in the internal half of dentin would be higher. Furthermore, investigation of the false detection of caries by bitewing radiography suggested that the majority of incorrect results were related to the cases where the extent of caries was underreported. As a justification, the minimum extent of demineralization of tooth structure required to be detectable on radiographs is around 35-40%. Therefore, the sensitivity of radiography is lower, and its results are typically underestimated. Furthermore, the comparison of the two modalities with the gold standard suggested a correlation coefficient of 0.449 for bitewing radiography and 0.718 for VistaCam. This reflects the higher capability of VistaCam IX Proxi in accurate diagnosis of the extent of caries. The results of previous studies on this topic are controversial. Maia Ama *et al*. (2011) made a comparison between the diagnostic accuracies of bitewing radiography and IR images for the detection of initial interproximal caries. They found that the IR images showed a higher sensitivity for caries diagnosis compared to bitewing radiography [24]. Russotto *et al*. evaluated IR images for the diagnosis of interproximal caries *in vivo*. They found that the IR images resulted in much more sensitivity for the diagnosis of interproximal caries. In addition, the occurrence of false positive responses was more probable, which approved the results of this investigation [25]. Nonetheless, Kuhnisch *et al*. (2016) evaluated the validity of IR radiation for the diagnosis of dentin interproximal caries. They showed that IR images and bitewing radiography had the same diagnostic accuracy for caries extended to dentin [9]. In their *in vivo* investigation of the diagnostic accuracy of IR radiation for caries diagnosis, Sochtig *et al.* (2014) observed that both techniques had the same detection accuracy for the diagnosis of occlusal and proximal caries [27]. Hakki Baltacioglu *et al*. (2017) made a comparison between these two diagnostic modalities for the detection of interproximal caries. They found no significant difference between the images obtained by the two techniques and suggested that IR radiation could be used as a suitable method with acceptable accuracy for caries detection [5]. Also, in their *in vitro* investigation, Abogazalah *et al.* (2017) compared these two techniques for the diagnosis of non-cavitated proximal caries. They found out that both techniques were of the same diagnostic accuracy for the diagnosis of non-cavitated proximal caries [28]. Jablonski-Momeni *et al*. (2017) evaluated VistaCam IX Proxi for the diagnosis of enamel caries. They observed no meaningful differences between the diagnostic accuracies of IR images and bitewing radiography for the diagnosis of enamel proximal caries [4]. Variations in the results of earlier investigations and the current findings are attributable to different conditions of the samples, such as performing the study only on initial enamel or dentin caries, different experimental conditions *(in vivo/ in vitro*), use of conventional or digital radiography with different sensors, different wavelengths of IR radiation, and employing other methods as the gold standard. Based on the obtained results and its availability, VistaCam IX Proxi seems to be an appropriate modality for the detection of caries with/without radiography. It is capable of improving the course of treatment and follow-up of high-risk patients, especially those with incipient caries. About limitations, this study was *in vitro* with limited sample size, and it is better to compare more devices and diagnostic methods with each other.

Conclusion

With regard to its significantly low percentage of false negative responses and high sensitivity in the diagnosis of dental caries, VistaCam IX Proxi is suitable for caries detection, especially enamel caries, and can be a valuable adjunct to the bitewing radiography technique in the clinical setting. VistaCam IX Proxi is also applicable for preventive measures and follow-up of pediatric patients and individuals for whom radiography is difficult or contraindicated.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Original Article

Correlation of Clinical and Histopathological Features of Salivary Pleomorphic Adenoma

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ABSTRACT

Statement of the Problem: Salivary gland tumors represent about 3% of the head and neck tumors. Pleomorphic adenoma (PA) is the most common benign salivary gland tumor.

Purpose: This study was conducted to investigate and describe some clinical and histopathological aspects of salivary pleomorphic adenomas with special reference to the epithelial and mesenchymal components.

Materials and Method: In this retrospective study, one hundred tissue samples diagnosed as PA were sourced from archival tissue blocks between 2009 and 2019 in this retrospective study. Some clinical and demographic features, including age, sex, tumor size, and tumor location were recorded. This study included only samples taken by excisional biopsy. Then, the samples were histologically classified into three subtypes according to the proportion of epithelial and stromal components. The demographic and clinicopathological variables were statistically analyzed using Chi-square test or Fisher's exact test, considering a significance level of 5% ($p< .05$).

Results: In the present study, most cases (61%) were found in females, representing a female–male ratio of 1.6:1. The peak incidence was seen in the 4th and 5th decades of life. In 87% of cases, PA occurred in major salivary glands. There was a significant difference between the age of the patient and squamous metaplasia ($p= 0.036$). There was also a significant difference between the size of tumor and the amount of myxoid stroma ($p= 0.021$). Extensive myxoid stroma was mostly seen in tumors larger than 3.37cm ($p= 0.001$). In addition, there was a statistically significant difference between capsular invasion and the development of squamous metaplasia ($p= 0.001$).

Conclusion: In this study, there was a significant correlation between the gland type and capsular features and between the size of tumor and rate of squamous metaplasia. A detailed clinical and histopathological analysis of PAs may provide a better insight to the pathophysiology of the lesion, tumor cell differentiation, and prognostic factors.

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Introduction

Salivary gland tumors represent about 3% of the head and neck tumors [1-2]. Pleomorphic adenoma (PA) is the most common benign salivary gland tumor, making up 40%-70% of all salivary gland tumors [3]. PA is mostly found in females and the average age at presentation is about 43 years [4]. The vast majority of cases arise in major salivary glands, predominantly the parotid gland. The palate is the most common site of minor salivary gland affected by PA. Histologically, PA is a

mixed tumor composed of epithelial and myoepithelial components arranged in different morphologic patterns surrounded by a fibrous capsule [3]. The tumor stroma may appear as myxoid, chondroid, osseous and hyalinized. Depending upon which component predominates, PA can be classified into (I) cellular (either epithelial or myoepithelial cell rich) type, (II) mixed or classic type, and (III) stroma-rich (myxoid) type. However, it has been suggested that this classification lacks any clinical significance [5]. The epithelial tissue appears as ducts, strands, tubules, or solid sheets; it is classified into ductal-like cells and neoplastic myoepithelial cells. Myoepithelial cells appear as plasmacytoid, clear and spindle cells, squamous, sebaceous, and adipose metaplasia. Keratin pearl formation is another phenomenon, which is associated with squamous metaplasia. The stroma can be found as myxoid, chondroid, chondromyxoid, osseous, hyalinized, and fibrous tissue [6]. Due to some factors such as histological variability, common features to other salivary gland neoplasms and the variations in epithelial and stroma components, the detailed knowledge of histological patterns of PA may contribute to an accurate diagnosis of this tumor [6]. In the current study, we proposed to describe some clinical and histopathological aspects of salivary pleomorphic adenomas with special reference to the epithelial and mesenchymal components and to compare with those reported in prior studies.

Materials and Method

This retrospective study was approved by the local Research Ethics Committee (Protocol #IR.UMSHA.REC. 1397.290). One hundred tissue samples diagnosed as PA were recruited from archival tissue blocks between 2009 and 2019. This study included only samples taken by excisional biopsy. An anatomical pathologist and an oral pathologist reviewed all histopathologic slides to confirm the diagnosis. As several slides were prepared for each sample, all slides were reviewed carefully and then histological classifications of subtypes were provided. Some clinical and demographic features, including age, sex, tumor size, and tumor location (type of involved gland) were recorded. Then, the samples were histologically classified into three subtypes according to the proportion of epithelial and stromal components including Subtype I or cellular type, Subtype II or classic subtype, and Subtype III or myxoid subtype [6]. Then, the data were analyzed by descriptive analysis using SPSS (Statistical Package for Social Sciences) program, version 20. In addition, the clinical and histopathological variables were statistically analyzed using Chi-square test or Fisher's exact test, considering a significance level of 5% ($p< .05$).

Results

In the present study, 61 cases (61%) were found in females, representing a female–male ratio of 1.6:1. The tumors were distributed in a wide age range from 6 to 88 years, with a mean age of 38 years ± 16. In 6 cases, PA occurred in less than 16 years of age. The peak incidence was seen in the 4th and 5th decades of life. In 87% of cases, PA occurred in major salivary glands (77 cases in parotid). The hard palate was the most affected minor salivary gland (n=5), followed by the soft palate (n=3). The clinical and demographic data are presented in Table 1. Besides, all microscopic features are described in Table 2. Considering the histological subtype, Subtype I was observed in 32 cases, subtype II in 54 cases and subtype III in 14 cases. Clinical and histopathological characteristics of PAs have been compared based on affected salivary gland type. A detailed summary are summarized in Table 3. In addition, there was a significant difference between the age of the patient and

Table 1: A summary of clinical parameters of 100 cases with pleomorphic adenoma

Clinical parameters	Pleomorphic adenomas (No.100)
Gender	
Female	61
Male	39
Female-to-Male ratio	1.6:1
Age(y)	
Mean age (range)	38(6-88)
≤38	57
>38	43
Diameter(cm)	
Mean (range)	3.37(1-9)
≤ 2	32
>2	68
Location	
Minor glands	13
Hard palate	5
Soft palate	3
Lip	2
Buccal	2
Oropharynx	1
Major glands	87
Parotid	77
Submandibular	10

Table 2: A summary of histopathological features of 100 cases with pleomorphic adenoma

Histological parameters	Number of cases
Subtype	
I	32
II	54
III	14
Lack of capsule/focal absence of the capsule	
Subtype I	5 (31.3%)
Subtype II	7 (43.8%)
Subtype III	4 (25 %)
Components and histologic findings	
Duct-like structure	95
Squamous metaplasia	39
Clear cells	28
Plasmacytoid-like cells	26
Keratin pearl	24
Spindle-shaped cells	18
Cystic formation	17
Adipose tissue	14
Myxoid stroma	73
Hyalinized stroma	46
Chondroid stroma	41
Osteoid stroma	36
Satellite Nodule	9
Pseudopedia	7
Mitosis	6

Table 3: Comparison of Demographic and Clinicopathological characteristics based on affected salivary gland type

Demographic and Clinicopathological parameters	Major salivary gland cases	Minor salivary gland cases	*p* Value
Gender			
Female	57	4	0.019*
Male	30	9	
Age(y)			
≤38	59	9	0.596
>38	28	4	
Size(cm)			
≤ 2	25	7	0.071
>2	62	6	
Capsule			
Lack /focal absence	9	7	0.001*
Complete	78	6	
Capsular invasion			
Yes	20	2	0.418
No	67	11	
Histopathologic subtype			
Cellular	30	2	0.205
Classic	44	10	
Stroma-rich	13	1	
Histologic components			
Squamous metaplasia	29	10	0.004*
Keratin pearl	18	6	0.054
Cystic formation	16	1	0.306
Hyalinized areas	35	11	0.003*
Plasmacytoma	24	2	0.286
Spindle cells	4	0	0.568
Clear cells	21	7	0.033*
Satellite nodules	8	1	0.669

*Results are statistically significant

squamous metaplasia (p= 0.036). Squamous metaplasia was frequently found in patients older than 38 years. There was also a significant difference between the size of tumor and the amount of myxoid stroma (p= 0.021). Extensive myxoid stroma was mostly seen in tumors larger than 3.37 cm (p= 0.001). In addition, there was a statistically significant difference between capsular invasion and the development of squamous metaplasia (p= 0.001). Figure 1(A-I) shows the histopathological features of tumor.

Discussion

PA is the most common benign salivary gland tumor. In addition, it is more common in females and commonly presents in the 5th and 6th decades of life [7-8]. In the present case series, the lesion was also, more common in females (61%) but 57% of the patients were aged 38 years and younger. Similar to other studies, in the current study, parotid gland was the most common affected salivary gland, followed by minor salivary glands [8-9]. The hallmark of PA is its histological diversity, which is composed of epithelial and stromal/mesenchymal component [10]. In accordance to a previously published paper, the present study showed myxoid stroma as the most frequent mesenchymal content, followed by hyaline, and chondroid stroma [9]. Other studies have indicated fibrous stroma as the most frequent stromal pattern, followed by myxoid stroma [11-12]. In the current study, lack of capsule or focal absence of the capsule was frequently manifested in Subtype II (43.8%). A previous meta-analysis has suggested that the stroma-rich tumors show a focal tumor capsule disruption and the formation of satellite nodules [13]. Similar to the study conducted by Wu *et al*. [6], in the present study, Subtype II (54%) was the most frequent histologic category, followed by Subtype I (32%). A previously published study found Subtype II (52%) as the most common histologic subtype, followed by Subtype III (28%) [14]. In a study carried out by Stennert *et al*. [15], Subtype III was reported as the most frequent subtype (51%) and Subtype II as the less frequent (14%) type. However, other studies have demonstrated Subtype I as the less common histologic subtype [14, 16]. Squamous (epidermoid) cells and keratin pearl formation are also shown in PAs [17]. Squamous metaplasia occurs in about 20- 25% of all PAs [18]. In the current study,

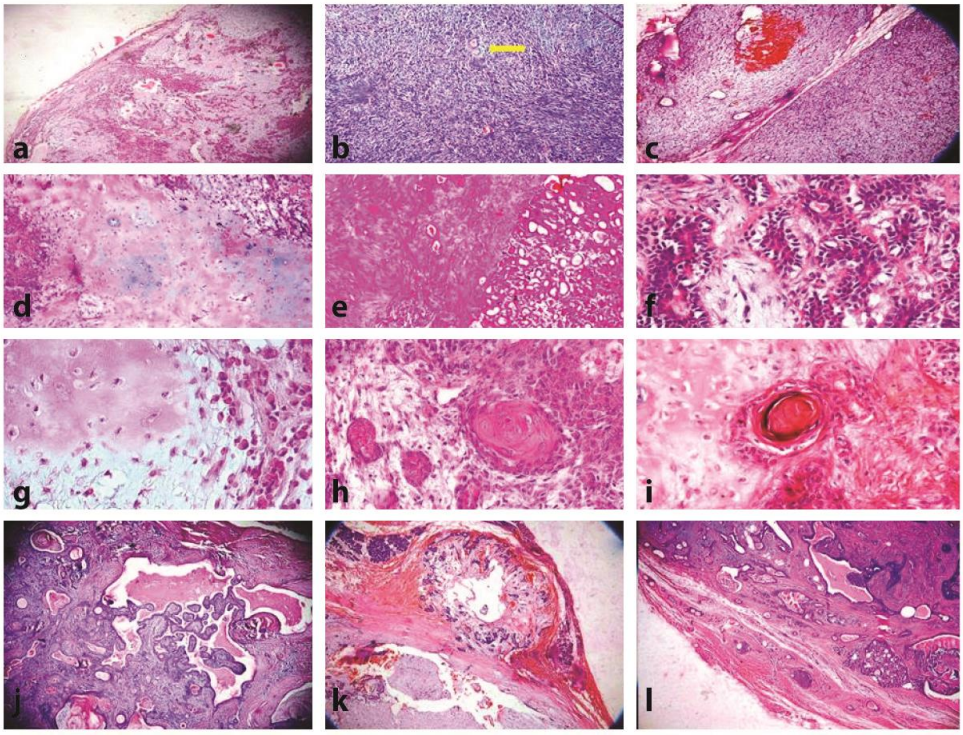

Figure 1: Histological patterns of PA, **a:** Classic type (100×), **b:** Cell-rich type. Remnant of a salivary duct is evident (100×), **c:** The myxoid component (100×), **d:** Areas of osseous and chondroid stroma(X100×), **e:** Extensive hyalinized area adjacent to the ductal pattern of tumor (100×), **f:** Clear myoepithelial cells (400×), **g:** Clusters of plasmacytoid-like cells (on the right) adjacent to chondroid and myxomatous stromal areas (X400), **h:** Squamous metaplasia (400×), **i:** Keratin pearl formation (400×), **j:** Cystic areas with papillary projections (100×), **k:** Pseudopedia adjacent to the main tumor capsule (100×), **l:** Capsular invasion(100×)

squamous metaplasia was indicated in 39% of cases, mostly in major salivary gland tumors. In the present study, in accordance with previous research, the squamous metaplasia was associated with capsular invasion ($p< 0.000$) [19]. It has been suggested that trauma, ischemia, and tissue repair after infarction are the origin of squamous metaplasia [17]. A Previous investigation has shown that artery ligation results gradual dedifferentiation and hyperplasia of the acinar-intercalated duct system. Overtime, tonofilaments and desmosomes appear in the luminal and abluminal myoepithelial cells. Finally, keratinization of central cells happens [20]. The presence of squamous epithelium has been reported in a number of reactive or tumoral conditions such as necrotizing sialometaplasia, chronic sialoadenitis, Warthin's tumor, basal cell adenoma, and mucoepidermoid carcinoma [18]. It has been suggested that squamous metaplasia may increase the risk of development of squamous cell carcinoma [21]. In the present study, plasmacytoid-like cells were found in 26% of cases. A previously published study demonstrated that luminal cells are the origin of plasmacytoid-like cells [22]. A detailed study has indicated that plasmacytoid-like cells are in transition from one type of cell to another [5]. Other phenotype change is spindle shaped cells, which has been suggested as the possibility of epithelial-mesenchymal transition (EMT) phenomenon. This hypothesis has been raised from the previous investigations that considered the myoepithelial cells as the neoplastic cells. In addition, myxochondroid, osseous, or collagenous stromal variations have been suggested as their products. Therefore, EMT phenomenon may explain the dynamic transitions of tumor cells [23]. A previously published paper reported that in PA samples, plasmacytoid-like cells, and spindle cells express WT1, an important promoter of EMT [23]. Besides, in PA samples, E-cadherin expression was weak or absent in plasmacytoid-like cells and was negative in spindle cells [24]. These findings may suggest that plasmacytoid-like cells and spindle cells are capable of EMT. Thus, it could be concluded that plasmacytoid-like cell rich PAs and/or spindle cells rich PAs are more susceptible to malignant transformation. Therefore, it is advisable to be more careful in PA samples with higher frequency of plasmacytoid-like cells and spindle cells.

The age of patient, tumor size and location, income-

plete excision or capsular violation can increase the risk of recurrence. We found a significant association between tumor size and myxomatous areas. Tumor size has been considered as a critical variable to predict of the malignant transformation in a salivary gland tumor [25]. On the other hand, myxoid areas are associated with more capsular disruption [23]. The capsular invasion and extension of tumor into the surrounding tissues through the capsule have been observed and called as satellite nodules. Pseudopodia and satellite nodules have been considered as the recognized causes of PA recurrence [25]. Interestingly, pseudopodia and satellite nodules have mostly been indicated in cellular type (Subtype I) PAs [15]. In other study, pseudopodia and satellite nodules were found in 46% of Subtype I cases and 18% of Subtype III cases, respectively [14]. In the present study, pseudopodia were detected in 7% of cases and satellite nodules were found in 9% of cases. Pseudopodia were found in 28% of Stennert *et al.*'s case series [15], in 40% of Zbären and Stauffer study [14], and in 20% of Grasso *et al.*'s samples [26]. Similar to other studies, we did not find any correlation between satellite nodules and pseudopodia and PA subtype [7, 26].

Conclusion

The present study demonstrated the diversity of histological characteristics of PAs. According to the results, duct-like structure was the most frequent histological finding. Besides, there was a significant correlation between the gland type and capsular features and between the size of tumor and the rate of squamous metaplasia. However, a larger sample size needs to be assessed in order to predict the possibility of malignant transformation. A detailed clinical and histopathological analysis of PAs may provide a better insight to the pathophysiology of the lesion, tumor cell differentiation, and prognostic factors.

Acknowledgement

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Original Article

Effect of Repeated Use of Different Types of Scan Bodies on Transfer Accuracy of Implant Position

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ABSTRACT

Statement of the Problem: Some components of implant treatment are reusable. Therefore, possible changes during fixation, removal, and sterilization process should be tested. Many studies have examined the reuse of implant parts, but the impact of repeated use of scan bodies on the accuracy of implant position has not been well investigated.

Purpose: The aim of this *in vitro* study was to compare the effect of repeated use of two different types of scan bodies on the accuracy of implant position.

Materials and Method: In this *in vitro* experimental study, two acrylic resin maxillary models, each with two implant analogues inserted at the site of missing first and second molars were used. Two types of scan bodies including titanium and polyetheretherketone (PEEK) were used for digital impression. Then they were ten times removed and autoclaved for sterilization. The first scan was considered as a reference to be compared with the other next nine scans. Values of linear distance between two scan bodies, diameter changes of each scan body, and three-dimensional linear displacement (ΔR) were measured. These values were compared between the two types of scan bodies using *t*-test (α=.05).

Results: There was significant difference between titanium and PEEK scan bodies regarding inter-implant distance variation (p=.006) and diameter change (p< .001) in repeated use. However, for the ΔR, there was no significant difference between them (p= 0.759).

Conclusion: The results demonstrated that type of scan body could affect the accuracy of implant position transfer after repeated use. PEEK scan body performed better after 9 cycles of reuse in comparison with titanium scan body.

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Introduction

The prerequisite for long term success of osseointegration is to provide passively fitting restorations [1-2]. Many controversies exists about the clinically accepted misfit level, but to keep away from long term complications, 150µm limit was recommended [3]. Accuracy of transferring implant position from the mouth to laboratory is widely discussed and considered as a determinant factor for final passive fit of restoration [4]. With the advancement of technology, the computer-aided design (CAD) and computer-aided manufacturing (CAM) process and digital impression have been introduced. Digital impressions improved efficiency as it offered advantages of reduced risk of deformation during transfer and the laboratory phases. Furthermore, by digital technology patient comfort and acceptance increased [5].

However, in conventional impressions, transfer problems may occur as a result of shrinkage, detachment of the impression material from the tray, different layer thickness, and the impression deforming [6].

The latest technology in this field is the intraoral scanners used for digital workflow. These scanners are easier to use than conventional techniques and are the first choice of many dentists [7]. With digital impression technique, there should be no need for commonly used impression copings, but a need for scan bodies, which are used to transfer 3D information about the position and direction of the implants to the virtual cast [8]. Scan bodies are reliably used for digital impressions [9-10]. They are either monolithic components or a combination of different materials, as aluminum alloy, titanium alloy, polyetheretherketone (PEEK), and various resins [11-12]. Despite the great variability of design and forms of scan bodies, they all consist of three distinct components including scan region, body, and base that form the most apical portion. The scan region is the part, which is scanned, the body extends from the scan region to the base, and the base is the part, which is seated into the connection. The scan region and body usually are made of same material [13-14]. Characteristics of scan bodies including connection type, design, dimension, material, reusability, and compatibility between the surface of scan body and software influence the accuracy of position transferring [15].

Cost is an important item in choosing a product for health management [16]. Due to financial issues, many clinicians had to reuse medical equipment [17-18]. In dentistry, there are studies that have concentrated on the reuse of procedural components in orthodontic, endodontic, surgical, and implant treatments [17, 19-25]. Since implant treatment is relatively expensive, some components are reused. Therefore, possible changes during fixation, removal, and sterilization process should be tested. Their effective performance should also be evaluated [26]. Many studies have examined the reuse of implant parts [27], but the impact of repeated use of scan bodies on the veracity of implant position, has not been well investigated. Two research groups had evaluated the reuse of impression copings and scan bodies [28-29]. They recommended the reuse of these parts after cleaning and sterilization. Since PEEK is capable of deformation due to reuse and sterilization process [13], the purpose of this *in vitro* study is to evaluate the effect of repeated use of two different types of scan bodies on the accuracy of implant position. The null hypothesis was that the accuracy of titanium and PEEK scan bodies are equal after repeated use.

Materials and Method

In this *in vitro* study, two acrylic resin maxillary models, each with two implant analogues (4.3mm diameter× 11mm length) (Replace Select, Nobel Biocare, Zurich, Switzerland) inserted at the site of missing first and second molars were used. Analogues were fixed into its corresponding holes using auto polymerizing acrylic resin (Technovit 4000, Heraeus, Hanau, Germany). To ensure that the resin polymerization process is completed, the process was stopped for a week. Then, two types of two-pieces scan bodies, both compatible with the implant system, were used. One was titanium based (Doowom, Arum, Daejeon, Korea) and the other was PEEK based (Nt-trading, Scan body 3D-Guide, Karlsruhe, Germany). The connection was in titanium for both (Figure 1). They were attached to implant analogues and torqued to 10N.cm. The models were scanned with intra

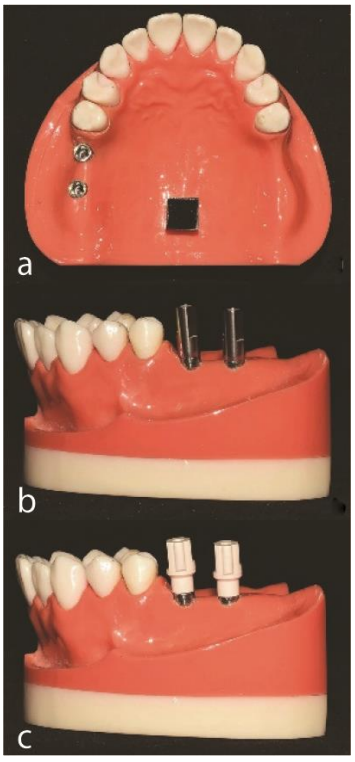

Figure 1: a: Maxillary model with two internal connection implant analogues; **b:** Titanium implant scan bodies attached to the implant analogues; **c:** Polyetheretherketone (PEEK) implant scan bodies attached to the implant analogues

oral scanner (Trios, 3shape, Copenhagen, Denmark). Then the scan bodies were removed and autoclaved for sterilization. The cleaning project was carried based on the manufacturer's instructions, which included scrubbing the interior and exterior sides of the scan bodies using a soft-bristled nylon brush for 2 minutes. The copings were then dried on absorbent paper. After that, they were packed and sterilized, using a steam autoclave (Steam Sterilizer A35-B, WEBECO Gmbh & Co, Selmsdorf, Germany) according to DIN standard 13060 and the standard protocol, suggested for the sterilization of surgical and dental equipment [30]. The process consisted of sterilizing at 134˚C for 10 minutes and then drying for 15 minutes. This process was repeated 9 times for each type of the scan bodies. All scans were performed by an experienced operator, after enough trial scans to find the best scan strategy of the model. The first scan of each type was considered as a reference to be compared with the other next nine scans. For measuring the accuracy of the implant position, the trueness of the scan bodies was evaluated by comparing each scan with the reference one. All scans were saved as standard tessellation language (STL) files.

Measurements for all digital datasets were undertaken using GOM software (ATOS Core 80; GOM GmbH, Braunschweig, Germany). Each scan was superimposed to the reference scan (Ref) based on the geometry of remaining teeth with local best-fit option in the software. A CNC milled cube was attached to the model in order to define three-dimensional (3D)-coordinate origin (point 0,0) in all scans. A cylinder and a plane best fitted to the external and occlusal surface of each scan body were defined. The central axis of each cylinder was specified and its intersection with the occlusal plane was marked as point A and B for anterior and posterior implants, respectively. Three-dimensional position of each point (R) was calculated with x, y, and z coordinates ($\sqrt{X^2 + Y^2 + Z^2}$) and ΔR was defined as $R–R_{Ref}$. Inter-implant distance variation was obtained by calculating the linear distance between the two scan bodies from point A to point B (Figure 2). Diameter changes of the cylinder best fitted to the scan body were calculated to indicate the diameter changes of each scan body.

All statistical analyses were performed using SPSS 18.0.0 (SPSS Inc., Chicago, IL). The *p* Values less than 0.05 were considered statistically significant. A sample size of 10 in each group achieve 75.288% power to reject the null hypothesis. All tests were two-sided. Shapiro-Wilk test was applied to examine the normality assumption. The mean and standard deviation values were reported for dependent variables including inter-implant distance variation, diameter change, and ΔR. T-test was considered to compare the two scan bodies.

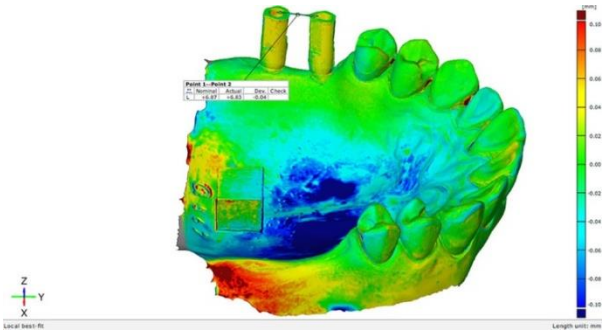

Figure 2: Inter-implant distance measurement

Results

Ten scans were obtained from repeated use of each type of scan body. The mean and standard deviation for inter-implant distance variation, diameter change, and ΔR are presented in Table 1. The results indicated that there was significant difference between titanium and PEEK scan bodies regarding inter-implant distance variation (p= .006) and diameter change (p< .001) in repeated use of them. The inter-implant distance variations were more in titanium than PEEK scan bodies (mean difference = 0.021mm), while the titanium scan bodies had less diameter changes than PEEK ones after repeated use (mean difference= 0.037mm). However, for the ΔR, there was no significant difference between titanium and PEEK scan bodies (p= 0.759). Figures 3 to 5 show the graphical representation of inter-implant distances, changes in diameter of scan bodies, and ΔR with respect to autoclave cycles.

Discussion

The results of this study rejected the null hypothesis. It

Table 1: Descriptive values for inter-implant distance variations, diameter changes, and ΔR of the groups

Outcomes	Scan body		*p* Value
	Titanium	PEEK	
Inter Implant Distance changes	0.032±0.016	0.011±0.012	p= .006
Diameter changes	0.029±0.020	0.066±0.014	p< .001
ΔR	0.069±0.052	0.080±0.044	p= 0.759

Data are expressed as mean±SD. PEEK: polyetheretherketone

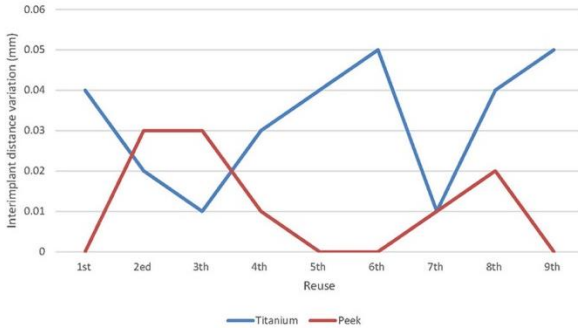

Figure 3: Comparison of inter-implant distance variation between titanium and polyetheretherketone (PEEK) scan bodies during repeated use

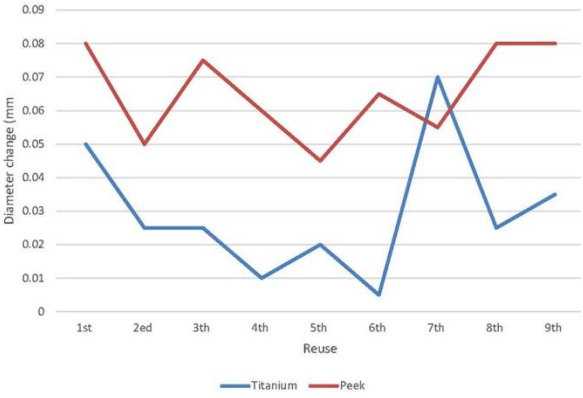

Figure 4: Comparison of diameter change between titanium and polyetheretherketone (PEEK) scan bodies during repeated use

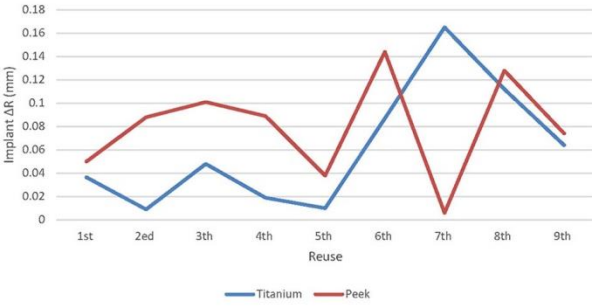

Figure 5: Comparison of ΔR between titanium and polyetheretherketone (PEEK) scan bodies during repeated use

was demonstrated that the inter-implant distance variations were more in titanium than PEEK scan bodies. The results further indicated that titanium scan bodies had less diameter changes than PEEK scan bodies after repeated use. However, regarding the three-dimensional linear displacement (ΔR) there was no significant difference between them.

There are studies that have focused on the possibility of reusing some implant parts [17, 25, 28, 29, 31-33]. The effects of reused cover screws on clinical outcomes were evaluated by Schwartz *et al.* [17]. They gathered that despite reusing cover screws could result in surface properties alteration, it would not adversely affect clinical outcomes. A systematic review indicated that common method used for cleaning and sterilization of healing abutments and cover screws might not result in the complete removal of contaminants. However, it would not cause any biologic or mechanical complications [34]. Other studies evaluating reuse of implant components have shown that cleaning, sterilization, and mechanical changes during insertion and removal can alter the surface morphology of implants, which result in variations in differentiation and osteoblastic growth [25,35]. However, it is not likely to be an issue with impression copings, as they have no constant contact with hard and soft tissues [25]. Browne *et al.* [31] reported that by sterilizing used impression copings, they did not show any particular deformation and were comparable to new copings. Alikhasi *et al.* [28] and Babu *et al.* [32] also indicated that impression copings could be cleaned, sterilized, and reused up to 10 and 12 cycles, respectively, without meaningfully decreasing the impression accuracy. In another study, a more number of reusing cycles was studied. Gallardo *et al.* [33] evaluated the effect of reusing and changing impression copings on impression accuracy. They concluded that after 30 times of cleaning and sterilization, impression copings that were modified by airborne-particle abrasion and polyvinyl siloxane (PVS) adhesive showed less impression inaccuracy than that unmodified impression copings. However, they were all still clinically acceptable [33].

Sawyers *et al.* [29] investigated the effect of several using of impression copings and scan bodies on implant cast accuracy. In that study, an implant stone cast with two bone level internal connection implant analogues was used to make ten conventional and ten digital impressions. They reported no significant differences between the impressions by reusing impression copings or scan bodies up to 10 times [29]. However, their measurement method was different from present study. They used a non-implant related reference point, the right mandibular canine tip, in relation to the linear z-axis measurement. In the present study, superimposing of the scans was based on best fit of the teeth. Moreover, the exact point of intersection between cylinder axis and the upper plane was defined for measurements. Moreover, it is worth mentioning that contrary to the present study,

Sawyers *et al.* [29] simulated indirect scanning using a laboratory scanner. Thus, the scan bodies were detached, removed from the cast, and reattached without being subjected to sterilization. Their results might not be generalized to reuse of scan bodies in direct intraoral scanning. Stimmelmayr *et al.* [15] evaluated the reproducibility of scan body fit on both stone and polymer models by reusing the scan bodies up to 10 times. They reported a better repositioning ability of the scan body on lab analogues than on original implants. They suggested that companies should reduce machining tolerance to increase the renewable fit of the scan bodies in the original implants.

The optical properties of the scan region material might potentially affect the number of points detected by a scanner [36], while the mechanical properties of the base material could influence the fit and wear resistance of the scan body connection, particularly when reusing the scan body [37]. In present study, the more inter-implant distance variations in titanium scan bodies may be related to their more reflective surface. The more diameter changes in PEEK scan bodies with titanium connection could be due to the surface deterioration during sterilization process and the reason why no significant difference was found in three-dimensional linear displacement (ΔR) between these two scan bodies might be because of their same titanium connection. Arcuri *et al.* [37] investigated the effect of implant scan body material on the accuracy of full-arch digital impression. Three intraoral scan bodies including PEEK, titanium, and PEEK with a titanium base were used. They showed that intraoral scan accuracy was influenced by the scan body material. PEEK exhibited the best outcomes on both angular and linear measurements, followed by titanium, and PEEK with a titanium base was the less accurate [37]. They explained that the worst performance of the PEEK with a titanium base scan body could be attributed to the possible microscopic mismatch between its two components. Their different study method in which scan bodies were not reused could have possibly lead to different results [37].

The effect of sterilization cycles on dimensional stability of PEEK have been studied for medical devices. Kumlar *et al.* [38] reported 6% decrease in lateral dimension of a clip after 30 cycles of sterilization. However, as dimensional change is highly related to the shape of device. The result of mentioned study could not be extended to scan bodies.

Some factors such as inter-implant distance and diameter changes have an equal effect on single-unit restoration. In the case of using multi-unit restorations, the effects of these factors are dissimilar [10, 12]. In our study two implants were inserted; with the change in the diameter (smaller or bigger diameter of scan body), the center of the scan body remains constant and there is no problem in the path of insertion of the two-unit restorations. However, inter-implant distance has a direct effect on the insertion of the two-unit restorations. Therefore, the lower inter-implant distance variation in PEEK is clinically more important.

In this study, a colorful map of deviation of the scans from the reference scan was also presented. The mean diameter changes of titanium and PEEK scan bodies were 0.03 and 0.06 mm, respectively. However, this deviation was consistent during repeated use in both types of scan bodies. All expected changes after repeated use is assumed to be from displacement or deformation of scan body. Because of consistent changes of diameter, it could be assumed that the changes in position are related to displacement rather than deformation of scan bodies. The inter-implant distance variations were higher in titanium compared to PEEK scan bodies. This higher variation in titanium scan bodies might be attributed to undesired reflective properties of the metal. Neither the scan body nor the scanner manufacturer's guideline recommended using opacifiers. However, it seems that dusting the scan region with a light coating of titanium dioxide powder, or sandblasting it with alumina powder would logically reduce surface reflection during scan, which might lead to higher accuracy. Arcuri *et al.* [37] evaluated the effect of scan body material and reported that PEEK scan bodies have the highest accuracy followed by titanium and titanium-PEEK. The material of matting surface could also influence the fitting of scan body to the implant and be responsible for wear after repeated tightening. In this study both scan bodies have titanium base and differences was related to the material of body and scan region of scan bodies.

The main aim of this study was to investigate the effect of repeated use of two different types of scan bodies on the accuracy of implant position. Accordingly, an intraoral scanner, instead of a more accurate scanner

like a desktop scanner or an industrial one, was used to make the first (reference) scan. Based on the literature, digital impression on dental implants provides a comparable accuracy compared with conventional impression technique [39]. Digital impressions using scan bodies are shown to have similar accuracy as conventional impressions [40-41]. Therefore, digital impression using an intraoral scanner was considered as an acceptable method. Indeed, regardless of how accurate the first scan was, we aimed to assess possible changes of the next cycles compared with the first one. It is significant to corroborate that the outcomes were limited to 10 cycles of reusing the scan bodies and might not be suitable to clinical situations. Accordingly, more research is needed to compare the effect of repeated use of other different types of scan bodies with a more number of reusing cycles on the accuracy of implant position.

Conclusion

Due to the limitations of this study, it is concluded that the type of scan body could affect the accuracy of implant position transfer after repeated use. PEEK scan body had a better performance after 10 cycles of reuse in comparison with titanium scan body.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Original Article

Effect of Two Remineralizing Agents on Dentin Microhardness of Non-Caries Lesions

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ABSTRACT

Statement of the Problem: The prevalence of non-carious dentin lesions is on the rise mainly due to improved life expectancy. Successful management of these lesions is often challenging, and given that dentin can be remineralized, adverse consequences due to progression of these lesions can be prevented or minimized as such.

Purpose: This study aimed to assess the effect of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) and Remin-Pro remineralizing agents on dentin microhardness of non-carious dentin lesions.

Materials and Method: This *in vitro*, experimental study evaluated 36 extracted sound human premolars. The teeth were decoronated at the cementoenamel junction. Enamel was removed, and dentin was exposed at the cervical third of the buccal surface. The primary microhardness of dentin was then measured. The teeth, standardized in terms of dentin microhardness, then underwent demineralization by acid etching and were subjected to microhardness test again. They were then randomized into three groups for treatment with CPP-ACP, Remin-Pro, and artificial saliva (control), and dentin microhardness was measured for the third time after treatment. Data were analyzed using ANOVA.

Results: Within group comparisons showed a significant difference in microhardness at the three time points in all three groups ($p< 0.005$). Between-group comparisons revealed that the microhardness of the three groups was not significantly different at baseline or after demineralization. However, the microhardness of the three groups was significantly different after the intervention ($p= 0.000$). Pairwise comparisons revealed significantly higher microhardness in the CPP-ACP group than the other two groups ($p= 0.003$). Remin-Pro and the control groups were not significantly different in this respect ($p= 0.340$).

Conclusion: CPP-ACP can be used for remineralization of non-caries dentin lesions; however, Remin-Pro does not appear to be effective for this purpose.

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Introduction

The prevalence of non-carious dentin lesions is on the rise mainly due to improved life expectancy. These lesions have been reported in 56% of males over 75 years of age [1]. Also, they have a higher prevalence in patients with a history of head and neck radiotherapy [1]. These lesions can adversely affect the long-term survival of the teeth [1]. Non-caries dentin lesions are multifactorial, and can lead to tooth hypersensitivity, plaque accumulation, and caries development. If not treated, they can compromise the structural integrity of the teeth and can even affect the pulp vitality. Successful man-

agement of these lesions is often challenging, and given that dentin can be remineralized in these lesions, adverse consequences due to progression of these lesions can be prevented or minimized as such [2].

Due to the significance of conservative dentistry, researchers have long been in search of enamel and dentin remineralizing agents [3-5]. Remineralization of tooth structure often occurs as the result of an increase in the levels of calcium, HPO4, and fluoride ions as well as the buffering agents in the saliva [6-7].

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is derived from the milk protein and is used as a remineralizing agent. The optimal efficacy of CPP-ACP for remineralization of enamel lesions has been well documented [8]. CPP bonds to enamel and releases calcium and phosphate whenever required. The released calcium and phosphate ions penetrate into the enamel crystals and increase the density and hardness of hydroxyapatite crystals [9-10].

Remin-Pro is another remineralizing agent, which unlike CPP-ACP, contains calcium and phosphate in the form of hydroxyapatite. It also contains fluoride and Xylitol. Remin-Pro Forte is a newer generation of Remin-Pro, which also contains ginger and turmeric extracts. It not only remineralizes the tooth structure, but also affects the soft tissue. It has been confirmed that Remin-Pro can positively enhance enamel remineralization [11-13]. However, studies regarding its efficacy for dentin remineralization are limited [14]. Both CPP-ACP and Remin-Pro remineralize the enamel structure by deposition of calcium and phosphate ions, and reinforcing the structure of hydroxyapatite crystals. Thus, they may be able to remineralize the dentin as well, due to the presence of hydroxyapatite in dentin structure [15].

The microhardness of enamel and dentin indicates the mineral content of their surface. Several studies have measured microhardness as an indicator for the degree of mineralization of enamel and dentin [11-12,14]. Thus, this study aimed to assess whether it is effective to use CPP-ACP and Remin-Pro remineralizing agents on dentin microhardness in non-caries dentin lesions or not.

Materials and Method

Samples collection

This *in vitro* experimental study evaluated 36 human premolar teeth extracted as part of orthodontic treatment or due to poor periodontal prognosis. The teeth were sound and had no carious lesion, restoration, crack, wear, or hypoplasia. The study protocol was approved by the Ethics Committee of our university (IR.IAU.DENTAL.REC.1398.001). The sample size was calculated to be 12 in each group according to a study by Liang *et al* [16], assuming alpha=0.05, beta=0.2, standard deviation of the mean microhardness to be 65, and effect size of 0.57 using one-way ANOVA power analysis feature of PASS 11 software.

Samples preparation

The teeth had been extracted within the past 1 month, and had been stored in 0.1% thymol solution at room temperature. Tissue residues were removed by a scalpel, and the teeth were cleaned with a prophy brush and low-speed hand-piece under running water. The teeth were then decoronated by a diamond disc and stored in distilled water. Next, enamel of the cervical third of the buccal surface of the teeth was removed by a diamond bur to expose dentin. For the purpose of standardization of specimens, the exposed dentin was polished with 600-grit abrasive discs (Sof-Lex; 3M ESPE, St. Paul, MN, USA). The specimens were then mounted in auto-polymerizing acrylic resin in the form of blocks measuring 2×3cm such that the dentin surface remained exposed.

Vickers microhardness test

The baseline dentin microhardness of each mounted specimen was then measured by a digital Vickers microhardness tester (Bareiss, USA), and all specimens were standardized regarding baseline microhardness as much as possible. The microhardness was measured at 3 points, and the mean value was calculated and reported as the mean Vickers hardness number.

Demineralization procedure of samples

Next, the specimens were randomized into three groups (n=12) of CPP-ACP (GC Tooth Mousse; GC America Inc.; USA), Remin-Pro (Voco GmbH, Germany), and artificial saliva (control) by block randomization. To induce demineralization, the specimens were etched with 37% phosphoric acid (Morva-Etch, Iran) for 15 s [16], rinsed with saline for 1 min, and stored in a buffering solution with a pH of 7.4 at room temperature [14]. All specimens underwent microhardness test again after the etching process.

Intervention

Next, the specimens were treated with CPP-ACP and Re-

min-Pro according to the manufacturers' instructions for 5 min daily, for a total of 15 days [17-18]. The control specimens were stored in artificial saliva at 37°C for 15 days after the second microhardness test. It should be noted that all specimens in the CPP-ACP and Remin-Pro groups were stored in artificial saliva at the time intervals between the interventions. The composition of the artificial saliva was water, glycine, sodium hyaluronate, propylene glycol, lysine, and proline. The artificial saliva was refreshed daily. The final microhardness of the specimens was measured in all three groups after 15 days using the same Vickers microhardness tester as explained earlier.

Statistical analysis

Repeated measures ANOVA was used to compare the microhardness at different time points within the three groups. One-way ANOVA was applied for pairwise comparisons. All statistical analyses were performed using SPSS version 25 at 0.05 level of significance.

Results

Table 1 presents the measures of central dispersion for the microhardness (Vickers hardness number) of the three groups. Repeated measures ANOVA showed a significant difference in microhardness at different time points within each of the three groups (p= 0.001).

Between-group comparison of microhardness revealed a significant difference in microhardness of the three groups as well (p< 0.05). Thus, microhardness of the three groups was compared with each other separately at each time point using ANOVA. The results showed no significant difference in microhardness among the three groups at baseline (p> 0.05), or after acid etching and demineralization (p= 0.482).

However, the difference in microhardness of the three groups was significant in the final measurement after the intervention (p= 0.000). Thus, one-way ANOVA was applied for pairwise comparisons of microhardness of the groups at each time point. The results showed significant differences in microhardness between the control (group 1) and the CPP-ACP (group 2) (p= 0.003) and Remin-Pro (group 3) (p= 0.000) such that the group 2 had significantly higher microhardness than the other two groups.

However, the difference in this respect was not significant between the group 3 and control (p= 0.340). Neither the CPP-ACP nor the Remin-Pro could return the baseline microhardness of the specimens.

Discussion

This study assessed the effect of CPP-ACP and Remin-Pro remineralizing agents on dentin micro-hardness of non-caries lesions. Remin-Pro and the control groups were not significantly different in this regard. Hydroxyapatite is the main constituent of Remin-Pro. The GC Tooth Mousse contains nano-scale CPP-ACP, which may explain simpler deposition of ions in-between the collagen fibers in this group while the hydroxyapatite crystals in the composition of Remin-Pro cannot remineralize the dentin as well as they remineralize the enamel. Since the dentin structure is different from the enamel structure in terms of presence of dentinal tubules and collagen fibers, it appears that some modifications are required to be made in the structure of hydroxyapatite crystals in the composition of Remin-Pro in order to be able to induce dentin remineralization.

The mineral content of enamel and dentin determines their microhardness [19]. Thus, microhardness test was performed in this study to assess the remineralization of dentin. The microhardness test is widely used for

Table 1: Measures of central dispersion for the microhardness (Vickers hardness number) of the three groups. CPP-ACP: Casein phosphopeptide amorphous calcium phosphate

Group	Time point	Maximum	Minimum	Mean±std. deviation
Group 1 (Control)	Baseline	40.60	12.00	25.00±5.9
	After acid etching	24.80	12.30	16.89±3.7
	Final	16.40	6.90	11.70±2.65
Group 2 (CPP-ACP)	Baseline	40.60	14.80	27.73±6.32
	After acid etching	40.60	10.20	16.48±6.62
	After intervention	22.20	4.00	14.17±4.30
Group 3 (Remin-Pro)	Baseline	40.10	14.20	31.05±9.96
	After acid etching	25.80	8.10	15.57±3.72
	After intervention	15.10	5.70	10.66±2.03
p Value	0.000			

assessment of enamel remineralization. For instance, Denelon *et al*. [20] used the microhardness test to assess enamel remineralization by CPP-ACP, and Kamath *et al*. [18] used the microhardness test to assess the remineralizing capacity of Remin-Pro. Despite the availability of studies on the efficacy of CPP-ACP and Remin-Pro for enamel remineralization, studies on their efficacy for dentin remineralization are limited. Poggio *et al.* [21] evaluated the remineralization of enamel and dentin with CPP-ACP using atomic force microscopy and scanning electron microscopy. They reported that CPP-ACP had a significant protective effect against enamel and dentin demineralization; however, this effect was greater for the enamel. Studies regarding the effects of CPP-ACP and Remin-Pro on the enamel are controversial. Some authors have reported higher efficacy of CPP-ACP [11] while others have shown the equal efficacy of the two for enamel remineralization [12,22].

In the present study, 37% phosphoric acid was used for demineralization since it affects the hydroxyapatite and weakens the mineral structure of dentin, simulating demineralization in the clinical setting [14,16]. Remineralization occurs as the result of penetration of calcium and phosphate ions in-between the crystals, causing their recrystallization. Naturally, these ions are provided by the saliva [23]. Fluoride is the most widely known agent to enhance the remineralization process and prevent enamel demineralization [24]. At present, the efficacy of dairy products for enhancement of remineralization and prevention of demineralization has been the topic of many investigations. Both GC Tooth Mousse and Remin-Pro are recommended for patients with tooth hypersensitivity and high risk of enamel erosion in order to enhance enamel remineralization [12,17].

Greater efficacy of CPP-ACP for dentin remineralization in the present study may be due to the more controlled release of calcium and phosphate ions from the CPP-ACP paste compared with Remin-Pro. In other words, CPP-ACP bonds to areas in higher need of calcium and phosphate ions, and gradually releases these ions over time [9-10]. It should be noted that despite the higher efficacy of CPP-ACP for dentin remineralization compared with the other two groups, all groups experienced a reduction in microhardness during the experiment, which has not been reported by studies conducted on the enamel [12,20,24]. This finding may be attributed to several reasons such as lower percentage of minerals in dentin, and the differences in the structure of enamel and dentin, causing enhanced demineralization of dentin over time. This is also the case in dentin caries since carious lesions have higher speed of progression in dentin, especially in root dentin.

It should be noted that this study had an in vitro design. Thus, generalization of results to the clinical setting must be done with caution.

Conclusion

Within the study limitations, the results showed that the CPP-ACP (in GC Tooth Mousse) can reinforce the remineralization of demineralized dentin while Remin-Pro did not show similar effect. In the future, this study can be performed on patients with non-caries dentin lesions (like erosion) as an *in vivo* study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Original Article

Effect of Incorporating Titanium Dioxide Nanoparticles into White Portland Cement, White Mineral Trioxide Aggregate, and Calcium Enriched Mixture Cement on the Push-out Bond Strength to Furcal Area Dentin

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ABSTRACT

Statement of the Problem: Bond strength of furcation repair materials is an essential factor in clinical success. Studies on the effect of adding titanium dioxide (TiO_2) nanoparticles on the push-out bond strength of commonly used endodontic cements for furcation perforation repair is limited.

Purpose: This study aimed to evaluate the effect of adding TiO_2 nanoparticles to white Portland cement (PC), white mineral trioxide aggregate (MTA), and calcium enriched mixture cement (CEM) on their push-out bond strengths.

Materials and Method: In this *in vitro* study, 120 endodontically treated molars were assigned to six groups (n=20) based on the material used to repair the perforation. In three groups, the cements (white PC, white MTA, and CEM) were placed in pure form, and in the three remaining groups, 1 weight % of TiO_2 was added. The push-out bond strength was measured using a universal testing machine at a strain rate of 0.5 mm/min. Data were analyzed using one-way ANOVA and post hoc Games-Howell test ($p< 0.05$).

Results: One-way ANOVA showed significant differences in the mean bond strength values between the six groups ($p= 0.002$). The post hoc Games-Howell test showed that the bond strengths in MTA+TiO_2 and PC+TiO_2 groups were significantly higher than those in MTA and PC groups, respectively. However, there was no significant difference in the bond strength between CEM and CEM+ TiO_2 groups.

Conclusion: The incorporation of TiO_2 into MTA and PC increased their push-out bond strength. However, it did not affect the push-out bond strength of CEM cement.

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Introduction

Mineral trioxide aggregate (MTA), Portland cement (PC), and calcium enriched mixture (CEM) cement are widely used as biomaterials in the perforation repair, root-canal obturation, pulpotomy, and apexification procedures [1-2]. MTA, as a bioactive material, forms a hydroxyapatite or carbonate apatite layer on its surface in the presence of tissue fluid, due to hydration. This interfacial layer creates a chemical bond between MTA and the furcation wall [3-4], which improves the sealing ability and marginal adaptation of MTA [5]. The main composition of PC and MTA is similar [6]. PC's ability to seal furcal perforations is similar to or even more effective than MTA [7]. PC has been suggested as a proper substitute for MTA due to its lower price, better sealing ability, and biocompatibility [8].

CEM has recently been introduced and consists of a variety of calcium compounds and is chemically different from MTA and PC [9]. Its surface is capable of producing hydroxyapatite with exogenous and endogenous sources. It is similar to MTA in expansion during the setting reaction and has been reported to be better than

MTA regarding film thickness, flow, and sealing capability [9-10].

Various ingredients including calcium chloride [11], zeolite-silver-zinc compound [12], propylene glycol [12], disodium hydrogen phosphate [13], chlorhexidine [14], and titanium dioxide (TiO_2) [15] have been added to MTA in order to improve its clinical properties, reduce the setting time, and increase its compressive and push-out bond strengths. They have been successful in enhancing some of its properties and unsuccessful in some others. For example, adding 5% calcium chloride and K-Y gel have reduced the setting time [11]; adding 0.12% chlorhexidine have increased compressive strength; and the combination of zeolite-silver-zinc have reduced its compressive strength [16].

TiO_2 is a metal oxide widely used in everyday life, including its use in wastewater treatment, accelerating chemical reactions, anti-fog layers, self-cleaning glass, and cosmetic products. The superb photoelectric photocatalytic and hydrophilic properties of TiO_2 nanoparticles as a unique property and its mechanical properties, such as density, melting point, and high elasticity coefficient, result in their consideration as a suitable additive for drugs and biomaterials to increase their efficiency [17]. Incorporating these nanoparticles into glass-ionomers [18] and acrylic resins [19] has improved their mechanical properties in a dose-dependent manner. Mouthwashes containing this compound have antibacterial effects against *streptococcus mutans* and *streptococcus sanguis* [20].

Samiei *et al.* [15, 21] showed that adding 1 w% of TiO_2 did not adversely affect MTA's biocompatibility and improved its compressive and push-out bond strengths; however, it increased the working and setting time. Since studies on the effect of adding TiO_2 nanoparticles to three commonly used endodontic cements on their push-out bond strength is limited, this study aimed to evaluate the impact of incorporating 1 w% of TiO_2 nanoparticles to white PC, white MTA, and CEM cement on their push-out bond strength in furcation area dentin.

Materials and Method

In this *in vitro* study, 120 mandibular first molars were selected from the archives of extracted teeth in Department of Oral and Maxillofacial Surgery that had been extracted for periodontal reasons. The protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (NO: IR.TBZMED.REC.1395.627). The inclusion criteria consisted of absence of cracks, fractures, and caries in the furcation and cervical areas, absence of anomalies in the shape and size of the teeth, and complete formation of tooth roots.

The soft tissues were removed using a hand scaler, and the samples were stored in a physiological saline solution for a maximum of three months until the initiation of the study. The samples were autoclaved immediately before the study began.

The tooth crowns were removed with a diamond disc (Teezkavan, Tehran, Iran) from the CEJ. The teeth were fixed in acrylic resin molds (Acropars, Tehran, Iran) with the furcation area and 3 mm apical to the furcation area exposed to create a space under the furcation to place a matrix to pack cements to repair perforations.

Perforations were created using a $\frac{1}{2}$ round diamond bur (Teezkavan, Tehran, Iran) perpendicular to the furcation area floor and parallel to the tooth long axis. The perforation site was then enlarged using a #4 Peesoreamer (Dentsply Maillefer, Ballaigues, Switzerland) so that the perforation measured 3 mm in diameter. The height of the perforation walls was measured using a periodontal probe. The excess thickness was removed with a diamond disk to leave a height of 2 mm in that area in all the samples. All the samples were irrigated using normal saline solution to eliminate the debris remaining from the working and cutting steps. The samples were divided into six groups (n=20) based on the material used to repair each perforation.

In the group 1, MTA (Angelus Dental Industry Products, Londrina, Brazil) was prepared based on manufacturer's instructions and located in the perforation area by employing a special carrier. After eliminating excess MTA from the pulp, a wet cotton swab dipped in normal saline solution was placed on the furcation repair material. In the group 2, MTA was mixed with 1 w% of TiO_2 and placed in the perforation area similar to that in the group 1.

In the group 3, according to the manufacturer's instructions, a 1:1 ratio of the powder and liquid of CEM cement (BioniqueDent, Tehran, Iran) was mixed and placed in the perforation area; then, wet cotton pellet was placed on the furcation area. In the group 4, 1 w% of TiO_2 nanoparticle powder was added to CEM cement

powder. The rest of the steps were similar to phases performed in the group 3. In the groups 5 and 6, the furcation area perforations were repaired with PC and PC mixed with 1 w% of TiO_2, respectively. In all the samples, a piece of wet cotton was placed on the cement in the furcation area. Then, the samples were incubated at 37ºC for 72 hours for the complete setting of the cements.

Subsequently, the samples were fixed in a steel holder, which was screwed by an aligning device and fixed to its special place on the universal testing machine (Hounsfield Test Equipment, Model H5KS, Surrey, UK). A vertical force was applied using a metal rod 2 mm in diameter at a crosshead speed of 0.5 mm/min directly in the middle of the furcation area cements (Figure1). The maximum force applied to the cement was recorded in Newton before displacement. The push-out bond strength was calculated in MPa by dividing force (N) by the surface area (mm^2). The following equation was used to calculate the bonded surface area: $A= 2\pi r \times h$, in which r is the radius of the perforation area and h is the height of the cement in the furcation area in millimeters. After carrying out the push-out bond strength test, the samples were divided into two sections longitudinally using high-speed diamond discs under continuous water and air spray. The failure modes were evaluated under a Nikon stereomicroscope (SMZ 1000, Tokyo, Japan) at 40× magnification [15-16].

One-way ANOVA was used to compare the bond strengths. Post hoc Games-Howell tests were used for two-by-two comparisons of the groups. Statistical significance was set at $p< 0.05$.

Figure 1: A specimen under push-out test using universal testing machine

Results

The mean and standard deviation of push-out bond strength values are presented in Table 1. The Shapiro-Wilk test showed that the data were distributed normally in all the groups ($p> 0.05$). One-way ANOVA was used to compare the bond strengths, which showed significant differences in the mean bond strength values between the six groups ($p= 0.002$). Post hoc Games-Howell test was used for two-by-two comparisons of the groups due to non-homogeneity of variances ($p= 0.03$). The results showed that the bond strengths in groups 2 and 6 were significantly higher than that in the groups 1 and 5. However, there was no significant difference in bond strength between the groups 3 and 4. The MTA+TiO_2 combination exhibited the highest bond strength. The PC+TiO_2 combination ranked second among the six groups. The CEM cement and CEM cement+TiO_2 combination ranked third in terms of bond strength. The MTA and PC alone exhibited the lowest bond strength (Figure 2).

Evaluation of failure patterns before and after adding TiO_2 to MTA and PC revealed changes in the failure pattern percentage in the form of a decrease in adhesive failure percentage and an increase in cohesive and mixed pattern percentages. In the case of CEM cement, similar to push-out results, there was no significant change in failure patterns before and after incorporating TiO_2.

Discussion

Iatrogenic perforation is one of the challenges endodon-

Table 1: Mean and Standard Deviation (SD) of Push-out bond strength values in study groups

Groups	Push-out Bond Strength (MPa)	
	Mean	SD
MTA	20.60[a]	9.01
MTA + TiO_2	37.60[b]	18.71
CEM	20.30[a]	13.03
CEM + TiO_2	26.10[a]	14.82
PC	22.20[a]	11.23
PC + TiO_2	30.80[b]	14.11

Different superscripts mean statistically significant differences ($p< 0.05$)
MTA: Mineral trioxide aggregate
TiO_2: Titanium dioxide
CEM: Calcium Enriched Mixture
PC: Portland cement

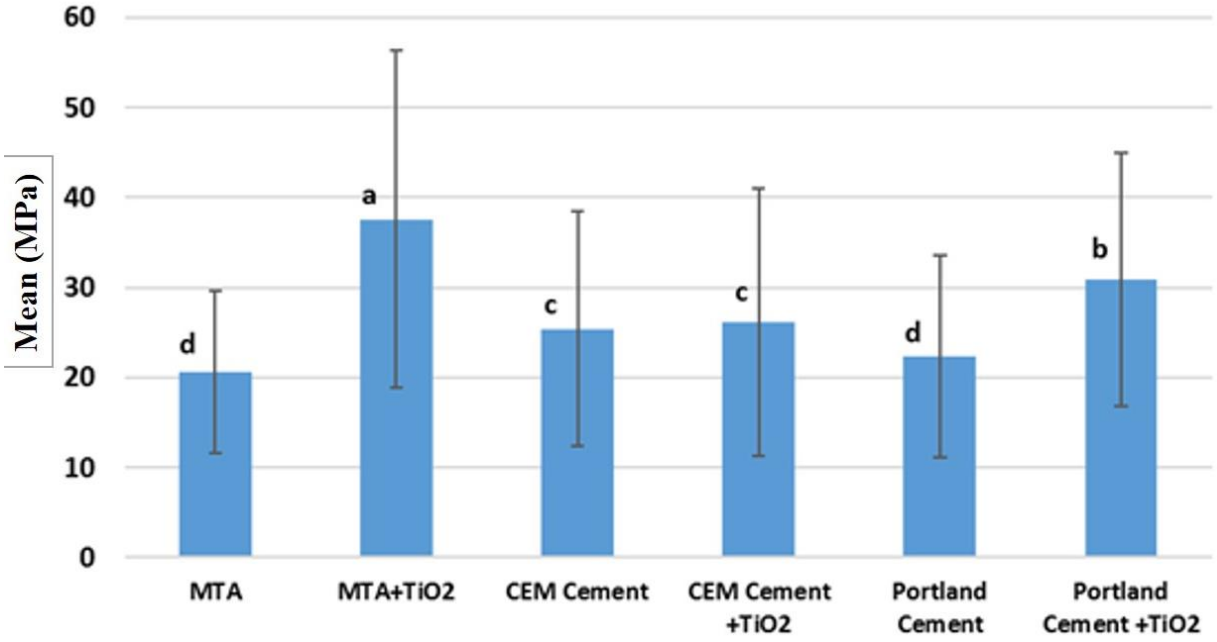

Figure 2: Bar chart showing comparison among study groups. Different letters means statistically significant differences ($p< 0.05$) MTA: Mineral trioxide aggregate, TiO_2: Titanium dioxide, CEM: Calcium Enriched Mixture

tists face during root canal treatment and is of great importance due to its proximity to the gingival sulcus area and attachment loss, followed by bone loss [22]. The success of furcation perforation repair depends on the provision of a properly sealed crown restoration and the repair material's resistance to displacement under masticatory forces and the condensing forces of the permanent restoration [23]. Amalgam condensation forces might reach 3.7–11.3 MPa, which is sufficient to remove the repair material from the furcation area [24]. Therefore, the bond strength of furcation repair material is an essential factor in clinical success. Various methods, such as tensile, compressive, and push-out bond strength tests have been used to investigate the bond strength. The push-out strength test is reliable, practical, and readily available [25-26].

MTA and CEM cement are among the materials that have exhibited successful outcomes in repairing furcation perforations since they are compatible with PDL and radiopaque, and have excellent stability, sealing ability, and marginal adaptation [7, 27]. On the other hand, a change in the host tissue pH affects the physicochemical properties of these materials, leading to the loss of hardness and sealing ability, and a decrease in compressive strength [22, 28]. In this study, to increase the efficiency of these materials, TiO_2 nanoparticles were added. Because of the unique photoelectric properties and mechanical properties such as high elasticity, TiO_2 nanoparticles are a suitable material to increase the efficiency and compressive strength of geometric materials [15, 17]. This study aimed to evaluate the effect of incorporating TiO_2 nanoparticles to white PC, white MTA, and CEM cement on the push-out bond strength in furcation perforation repair.

The results showed that, in general, all three substances exhibited the same bond strength in their pure form, with no significant difference between them, consistent with Tavasoli *et al*. [29] and lotfi *et al.* [30]. However, Sahebi *et al*. [31] demonstrated higher bond strength for CEM in comparison to MTA. On the other hand, Adl *et al*. [32] and Mohammadian *et al*. [33] reported higher bond strength for MTA in comparison to CEM as root end filling material. The noteworthy point is that, according to Ertas *et al*. [34] bond strength of MTA differs between various commercial brands. They showed that the push-out bond of the ProRoot MTA was statistically higher than MTA-Angelus. Furthermore, there was no significant difference between the bond strength of CEM cement and MTA-Angelus [34]. MTA-Angelus consists of 80% PC and 20% Bi_2O_3 and does not contain calcium sulfate, TiO_2, P_2O_5, and FeO that are present in the composition of ProRoot MTA [9, 34]. Furthermore, Regarding PC, Amoroso-Silva *et al*. [35] demonstrated that push-out bond

strength of PC with 20% ZrO was similar to MTA. However, push-out bond strength of PC with 20% calcium tungstate was lower than MTA. It has been demonstrated that the retention of these materials to the dentinal walls depends on the water/powder ratio, temperature, humidity, the quantity of air trapped in the mixture, and the particle size of the materials, which might explain the different results obtained in different studies [5].

As an interesting finding in the present study, adding TiO_2 to the MTA and PC increased the push-out bond strength. While, incorporation of TiO_2 into CEM cement did not affect its push-out bond strength. Similarly, Bichile *et al*. [36] demonstrated that TiO_2 added to MTA lead to a significant increase in its push-out bond strength. This increase in push-out bond strength in MTA is because of its unique pozzolanic activity property. Due to pozzolanic activity, the highly active TiO_2 nanoparticles quickly consume $Ca(OH)_2$. This reaction is favorable to form a denser structure of hydroxyapatite [36]. Hence, two events take place in this reaction. First, the amount of free $Ca(OH)_2$ is eventually decreased. Second, when this reaction is taking place, there is an increase in the formation of calcium silicate hydrate and calcium aluminate hydrate. These hydration products are effective in increasing the overall strength of the material [36-38]. Furthermore, TiO2 nanoparticles would fill spaces between cement particles, producing smaller pores to increase the physical strength. Therefore, it is confirmed that the addition of nanoparticles to cement mortars improved their strength characteristics [37-38]. This is also in accordance with Samiei *et al*. [15,21] who concluded that addition of TiO2 nanoparticles in 1% weight ratio to MTA increases its push-out bond strength, compressive strength, working time and setting time.

Interestingly, Feng *et al*. [39] demonstrated similar results as observed for PC when combined with 1% weight TiO2 nanoparticles. They showed that achievement of good nanomodification with 1% weight TiO2 increased the amount of cementitious phase, decreased the microporosity and amount of internal microcracks and defects, and lead to the formation of a denser microstructure with reduced nanoroughness. They concluded that admixing TiO2 nanoparticles into PC not only lead to denser hardened cement paste but also altered the morphology and chemical compositions of cement hydration products [39].

On the other hand, according to the findings of the present study, the incorporation of TiO_2 into MTA and PC gave rise to similar results, and CEM cement exhibited a different result, which might be attributed to the difference in the chemical composition of MTA and PC from CEM cement. According to previous reports [9-10, 40-41], PC and MTA have the same composition and are composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate, and bismuth oxide. However, CEM cement is chemically different from MTA. CEM is composed of calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, hydroxide sulfate, and calcium chloride, and unlike MTA, it does not contain bismuth oxide. MTA contains calcium, silicon, and bismuth elements. However, CEM contains calcium, phosphorus, and sulfur elements. Although these two types of cement have different compositions, they have similar applications [9-10, 40-41].

Considering the limitations of the present study, further studies are recommended to compare TiO_2 nanoparticle modified CEM, MTA, and PC as a root-end filling material. Further studies are also recommended for long-term comparisons of these materials mixed with TiO_2.

Conclusion

The incorporation of TiO_2 into MTA and PC increased their push-out bond strengths. However, adding TiO_2 to the CEM did not affect its push-out bond strength.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Original Article

Molecular Identification of *Candida albicans* in Endodontic Retreatment Cases by SYBR Green I Real-time Polymerase Chain Reaction and its Association with Endodontic Symptoms

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ABSTRACT

Statement of the Problem: Recent microbiological studies have expressed ever-increasing concerns about *Candida albicans* as a causal factor in the failure of endodontic treatments. Real-time quantitative polymerase chain reaction (qPCR), including the SYBR Green I system, is a technique in which a fluorescent dye is incorporated into the double-stranded DNA that is produced during DNA polymerase activity.

Purpose: This study aimed to determine the relative prevalence of *Candida albicans* in root canals of retreatment cases and its association with endodontic symptoms.

Materials and Method: In the present cross-sectional/analytical study fifty subjects were selected. Clinical features and radiographic status of the teeth were also evaluated. After access cavity preparation, the retrieved material and dentinal chips removed from the root canal were transferred into 1.5-mL microtubes, followed by storage at -20ºC until used for DNA extraction. A DNeasy Tissue Kit was used to extract DNA using the DNeasy protocol for animal tissues. Master Plus SYBR Green I (Jena Bioscience, Germany) was used in a Rotor-gene Real-time PCR System for real-time PCR. The relationship between the presence of *Candida albicans* and the clinical and radiographic features were analyzed using McNemar's test.

Results: There was a significant relationship between the radiographic findings in endodontically treated teeth and the presence of *Candida albicans*. However, there was no significant relationship between the presence of *Candida albicans* and any of the clinical symptoms.

Conclusion: In spite of the limitations of this study, we concluded that *Candida albicans* was associated with root canal infections in endodontic retreatment cases, but there was no relationship between root canal infections and the clinical symptoms.

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Introduction

The presence of microorganisms in the root canals is the leading cause of unsuccessful endodontic treatment [1]. Fungi have been discovered in multiple investigations [2–5] on endodontic infections. According to a recent meta-analysis [6], fungal pathogens prevalence in root canal infections was 9.11 percent, with 9.0% in treatment and 9.3% in retreatment cases. In research using culture techniques, this prevalence in primary and retreatment endodontic cases were 6.3% and 7.5%, while in studies using molecular techniques, it increased to 12.5% and 16.0% respectively [6]. *Candida albicans*

(C.albicans), *Candida tropicalis*, and *Candida kefys* are the most frequently isolated fungi from infected root canals, respectively [7].

Candida species related infections are poorly understood in endodontics. Nonetheless, accumulating evidence suggests that the *Candida* biome is important to the pathogenesis of endodontic problems [8-10]. *C.albicans* has a strong affinity for hydroxyapatite and can strongly attach to untreated or ethylenediaminetetraacetic acid/sodium hypochlorite-treated dentin [11-12]. The smear layer promotes *C.albicans* dentin attachment [15-16], most plausible as a result of the calcium ions and uncovered collagen on the dentinal surface. *Candida* can attach to collagen types IV, and I with a preference for dentinal collagen [17-20]. In addition, calcium ions affect *Candida*'s capacity to proliferate and bind to proteins in the extracellular matrix [15-16, 21]. *C.albicans* owns the exceptional capacity to thoroughly enter tubules owing to its thigmotropic qualities. According to these characteristics, yeast is classified as a dentinophilic microorganism [22].

Several studies also indicate that *Candida* plays a significant role in root canal related infections [22-24]. Being a microaerophilic eukaryote, it is metabolically adapted to live in the hard and arid root canal environment. Multiple studies have demonstrated that *in vitro*; *C.albicans* could utilize dentin as a source of nourishment colonizing both canal walls and dentinal tubules, if they did not have any other food supplements [22-24]. A smear layer generated by equipment helped *C.albicans* enter dentinal tubules *in vivo* [25]. Therefore, *C.albicans* protected existence within the tubules, away from equipment and irrigants, may cause endodontic infections to persist [17, 26]. Consequently, *C.albicans* is frequently detected in endodontic infections that are resistant to root canal therapy [27]. There have also been reports of the isolation of pure *Candida* cultures from root canal infections [24,28-29].

Studies based on culture methods are a further factor to consider. If the correct culture medium is not utilized and the samples are not examined for a minimum of 3 days, the low number of fungi in the infected root canal system may take up to 3 days to proliferate as colony forming units [6]. The yeast frequency is likely to be underestimated if laboratory personnel do not use wrong culture media (such as Sabouraud agar or chromogenic agar) and/or only review data for up to 24 hours. However, using molecular mycological techniques like polymerase chain reaction (PCR) with specific universal primers can identify all kinds of fungal flora, even the uncultivable forms, regardless of the endodontic ecosystem's population size [6].

The conventional PCR method detects the presence or absence of a target microbe, but does not quantify its concentration. In addition, this approach requires post-amplification processing to segregate and identify particular PCR products [30]. Modifications to the standard PCR procedure can be used to circumvent the limitations. In the real-time quantitative PCR (qPCR) method, which incorporates the SYBR Green I system, a fluorescent dye is incorporated into the double-stranded DNA produced by DNA polymerase activity. The generation of a fluorescent signal during amplification enables the identification and quantification of products in real time [30]. There is limited data available about the relationship between *C. albicans* and endodontic symptoms, thus this study seeks to figure out the frequency of *C.albicans* in root canals of retreatment cases and its association with endodontic symptoms.

Materials and Method

The current cross-sectional/analytical study included 50 patients referred for endodontic retreatment to the Department of Endodontics at the Tabriz Faculty of Dentistry due to reasons such as replacement of restorations and prosthesis or clinical signs and symptoms of root canal treatment failure. The study excluded patients who had taken corticosteroids or antibiotics during the preceding three months, as well as patients with systemic diseases. Additionally, teeth with more than 4 mm of probing depth and teeth that were impossible to isolate were excluded. For each tooth, the clinical manifestations of pain, sensitivity to periapical tests, sinus tracts, and swelling were documented. Using periapical radiographs taken with paralleling technique, the radiographic health of the teeth was also evaluated and recorded. Each case of periapical radiolucency or lack thereof was documented. The coronal repairs, intracanal posts, and carious lesions removed. After creating access cavities and isolating the teeth with a rubber dam, the teeth were disinfected with 5.25% sodium hypochlorite and rinsed with normal saline solution.

Table 1: Control samples used

No	*Candida* standard strain (ATCC 36232) DNA (ng)	Human DNA (ng)
S1	100	0
S2	10	90
S3	10	0
S4	10	22.5
S5	1	99
S6	1	25
S7	0.1	99.9
S8	0.1	0
S9	0.1	25
S10	0.01	99.99
S11	0.01	0
S12	0.01	25

Root canal treatments and sample collection were conducted under aseptic circumstances. To clean out the canal filling materials, we used Gates-Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland) and Hedstrom (MANI, Yohara, Japan) instead of chemical solvents. The extracted materials and dentinal chips were placed in 1.5-mL microtubes and stored at -20oC until DNA extraction.

The ATCC 36232 strain of *C.albicans* was obtained and cultured on Sabouraud dextrose agar to evaluate the procedure and the specificity of the PCR tests used in this study. Extracted DNA from the reference strain was combined with human genomic DNA in the proportions shown in Table 1. The study employed twelve different concentrations of the retrieved DNA.

Extraction of nucleic acids

A powdering method using liquid nitrogen was used on approximately 50 mg of the tissues removed from each tooth. The DNeasy Tissue Kit (Qiagen, Germany) was used to extract DNA from animal tissues using the DNeasy technique. 25 mg of each sample's powder was used to get the DNA, and the concentration was measured using UV absorption spectrophotometry at 260 nm (Biophotometer plus, Eppendorf, Germany).

Oligonucleotide primers

CANAL-F (5- TTTCTCTCGCCCCGTGTGGGT-3) as well as CANAL-R (5- GGCAGCTCTACCTTCAACGCCA-3) were built by Primer3 Design software (http://frodo.wi.mit.edu/) and based on the Heat Shock Protein 60 gene, (accession number AF085694), which targets a 294 bp of mitochondrial DNA of *C.albicans*.

A pair of primers (5'-TGTCCACCTTCCAGCAGATGT-3', 5'-CACCTTCACCGTTCCAGT-TTT-3') built ona chromosomally encoded ß actin gene was employed for the control amplification of a 249-bp mammalian sequence [31]. Bioneer Co., Ltd. (S. Korea) was the source of primers.

PCR test

The conventional PCR assay was applied in a Perimus 96 thermocycling unit (Peqlab, Germany). The reaction mixture (25µL) consisted of $MgCl_2$ (4 mM), 1 M of MdNTP mix (Bioline), 2 mM of each primer, 0.2 U Taq DNA polymerase (Bioline), and 2µL of extracted DNA. The thermocycling procedure consisted of 5 minutes at 95°C for initial denaturation of DNA and 30 cycles thereafter as follows: 95°C for 30 seconds (the denaturation phase), 60°C for one minute (the annealing phase), and 72°C for 30 seconds (the extension phase) and 10 minutes for final extension at 72°C. The traditional PCR products were separated on a 2% (w/v) agarose gel in TAE (Tris base, acetic acid and EDTA) buffer, followed by staining with ethidium bromide. A 50-bp DNA ladder (Fermentase, Vilnius, Lithuania) was utilized as a size marker. The gel photos were taken using a Syngene gel documentation system.

The real-time PCR technique was applied using Master Plus SYBR Green I (Jena Bioscience, Germany) in a Rotor-gene Real-time PCR System (Corbett Life Science, Australia). A total of 20 µL of reaction mix contained 2 µL of Master Plus SYBR Green I, 1 µL of each primer (35 nM) and 2.5 µL of the template (10 ng). In the non-template controls, the template was replaced by double-distilled water (Cinnagen Co, Tehran, Iran). The PCR cycling protocol was run as before. Concerning the melting curve, a thermal gradient was applied from 60°C to 95°C at 0.5°C/5 s. The efficacy of each reaction was determined using this formula: $E= (10^{(1/slope)}-1)$. All the reactions were performed twice.

Statistical examination

Using SAS, the data were statistically evaluated (Ver. 9.2). Using McNemar's test, the relationship between the presence of *C.albicans* and clinical and radiographic characteristics was analyzed.

Results

Genomic DNA was detected as a high-intensity band in both the control samples (ATCC 36232) and the patient-extracted samples. Each sample's concentration of DNA ranged between 20 and 50 g/mL.

PCR assay

The control samples' DNA was taken out and added to

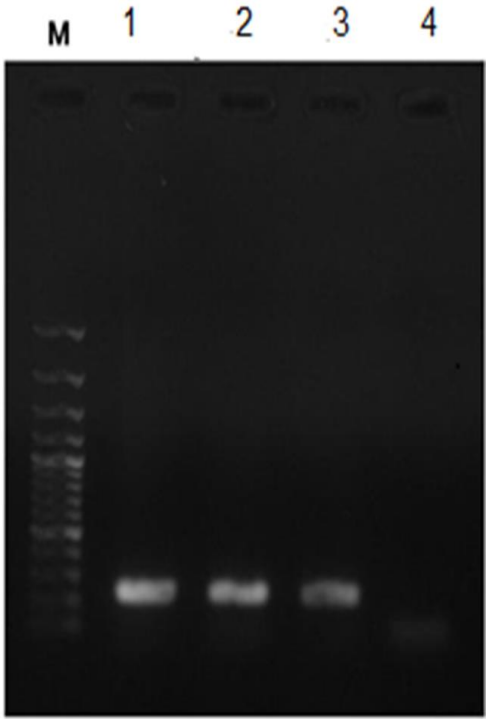

Figure 1: PCR amplification products with *Candida albicans* specific primer pair M: DNA ladder; 1: control sample (S1) containing 100 ng of *Candida* standard strain DNA; 2: control sample (S2) containing 10 ng, 90 ng, of *Candida* standard strain DNA and human genomic DNA respectively; 3: control sample (S5) containing 1 ng, 99 ng of *Candida* standard strain DNA and human genomic DNA; 4: control sample (S7) containing 0.1 ng, 99.9 ng of *Candida* standard strain DNA and human genomic DNA respectively

the PCR mix so that it could be used as a template to test how specific the primer pair was. As expected, the control sample and four patient samples (8%) showed a specific band with 294bp (Figure 1). Containing 0.01ng C. albicans DNA, the PCR test came back negative (Table 1).

In order to quantitate, the human genomic DNA and *C.albicans* template DNA were serial diluted by 10 times, starting at 100ng/µL. SYBR Green I real-time PCR at 60°C was used to examine the primer pair (Figure 2).

Moreover, the formula for the threshold cycle against DNA concentration at various dilutions was y=-3.36x +28.31. The estimated regression coefficient was 0.99 (Figure 3). The response showed that there was 0.1ng of *C.albicans* DNA. The analysis of the melting curve showed a certain amplicon with a Tm value of 87.5°C (Figure 4).

After the melting curve examination, there was no peak in the control samples. Dissociation curves showed a single peak that represented the PCR product's melting point of 87.5°C. The results presented above showed no primer dimers and selective amplification.

In 50 patient samples, four positive reactions were seen when the DNA from the study samples was added to the reaction. The positive samples had *C.albicans* DNA in concentrations as high as 4.5ng and as low as 0.42ng, respectively. The melting curve analysis of the positive samples showed the same particular amplicon. After adding various concentrations of human DNA to the extracted DNA of the *C.albicans* control specimen, the real-time PCR showed that the amount of *C.albicans* DNA was similar to what was planned. The PCR assay's effectiveness was assessed to be greater than 90%. After 36 thermal cycles, PCR with 100ng of control DNA of all the human control samples showed weak fluorescence. For the *C.albicans* control, each reaction showed 100ng of genomic DNA, with a mean Ct of 17.5.

Relationship to the clinical manifestations

C.albicans was found in four (eight percent) of the fifty

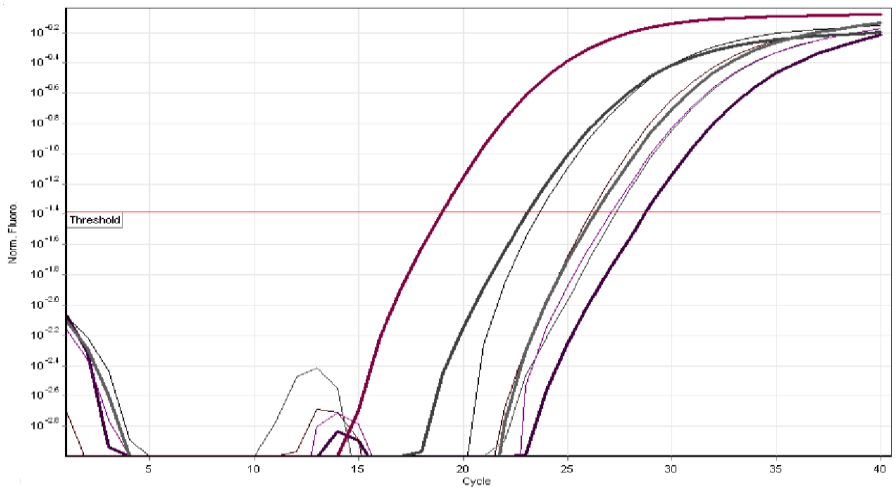

Figure 2: Amplification plots of SYBR green I real time PCR for a selection of control samples (thick lines) and patient samples (thin lines)

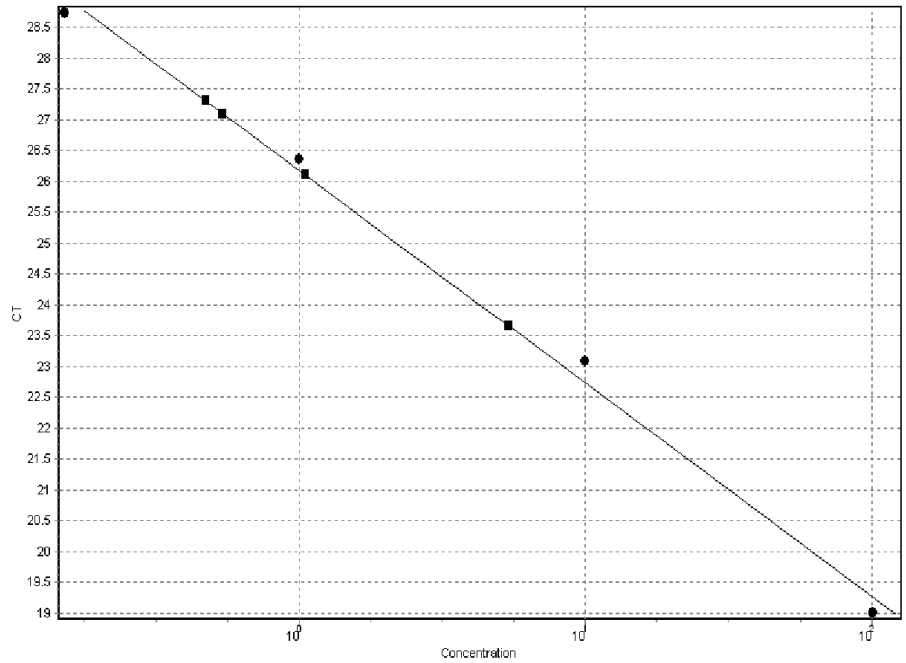

Figure 3: Standard curve for the SYBR green I real time PCR amplification of *Candida albicans* DNA. A plot of Ct value against the logarithm of 100, 10, 1 and 0.1 ng of extracted DNA from control samples (circles) and patient samples (squares) are indicated

cases, which were the only cases with apical radiolucency. Apical radiolucency and the presence of *C.albicans* were found to have a highly significant correlation (p<0.0001), according to the statistical analysis. On the other hand, there was not a significant correlation found between the presence of *C.albicans* and any of the clinical symptoms, since only one of the twenty-two patients with symptoms was *C.albicans* positive (p> 0.05).

Discussion

In this study, SYBR Green I Real-time PCR was used with the aim of determining the prevalence of *C.albicans* in root canals of retreatment cases and its association with endodontic symptoms. Another factor is scientists' microbiological cultivation methods, which may yield different results. If the correct culture medium is not utilized and the samples are not examined for at leastthree days, the low number of fungi in root canal may take up to 3 days to proliferate as colony-forming units. If laboratory personnel do not use the right culture media and only review culture data for 24 hours, yeast frequency may be underestimated. However, using molecular mycological techniques like PCR with specific universal primers can identify all kinds of fungal flora,

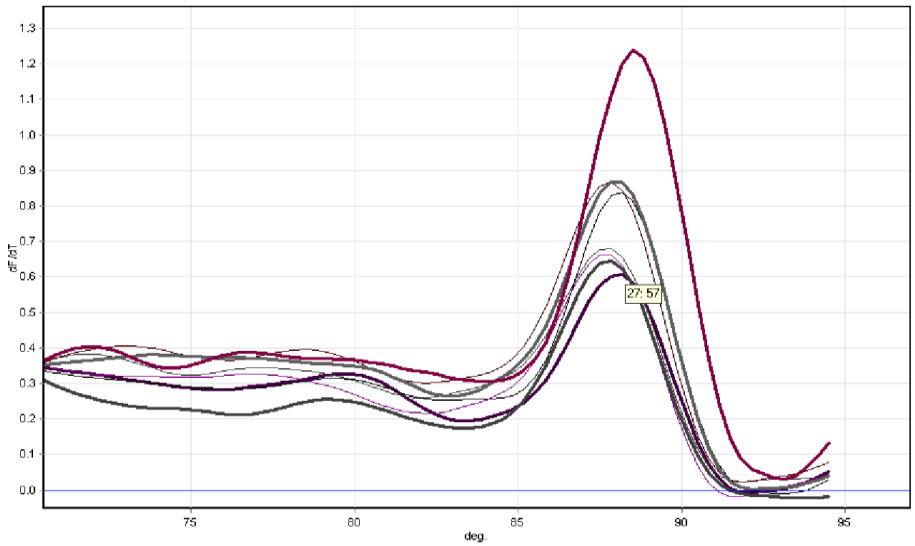

Figure 4: Dissociation curve analysis of SYBR green I real time PCR products of a selection of control samples (thick lines) and patient samples (thin lines)

even the uncultivable forms, regardless of the endodontic ecosystem's population size [6].

The conventional PCR method detects the presence or absence of a target microbe, but does not quantify its concentration. In addition, this approach requires post-amplification processing to segregate and identify particular PCR products. Modifications to the standard PCR procedure can be used to circumvent the limitations. In the real-time qPCR method, which incorporates the SYBR Green I system, a fluorescent dye is incorporated into the double-stranded DNA produced by DNA polymerase activity. The generation of a fluorescent signal during amplification enables the identification and quantification of products in real time [30,32].

The real-time PCR sensitivity to detect *C.albicans* was evaluated with the use of samples containing different amounts of this agent and human genomic DNA. A paper has reported sensitive TaqMan real-time PCR assays that are capable of detecting as low as 0.5pg of *C. albicans* DNA in clinical blood samples [33]. The detection limit of SYBR Green I real-time PCR in this study was 0.1 ng, measuring *C.albicans* DNA in dental tissue mixtures.

The method used in this research was specific enough to make a distinction between *C.albicans* and human genomic DNA. The presence of different ingredients in dental tissues collected from endodontic retreatment cases, such as collagen and gutta-percha, might inhibit DNA polymerase, resulting in false negative results. We tried to minimize polymerase activity inhibition by these ingredients with the use of a silica-based method used to extract and purify DNA.

Candida species endodontic infections are poorly understood. Yet, growing research suggests that the *Candida* biome is relevant to endodontic issues [8-10]. *C.albicans* has a strong affinity for hydroxyapatite and can strongly attach to untreated or ethylenediaminetetraacetic acid/sodium hypochlorite-treated dentine surfaces [11-12]. The smear layer's exposed calcium ions and collagen let *C.albicans* stick to human dentin [15-16]. Root canal irrigants and medicaments that have poor anti-fungal properties might promote yeast proliferation in root canals [27]. Endodontics uses calcium hydroxide predominantly intracanally. *C.albicans* thrives over a wide pH range (3.0- 8.0) and as a result, it is resistant to calcium hydroxide [27]. *C.albicans* also uses Ca^{2+} from calcium hydroxide for growth [27]. Thus, root canal-resistant endodontic infections commonly contain *C.albicans* [27].

We detected *C.albicans* in 8% of cases, and there was a significant relationship between the presence of radiolucency and *C.albicans*, consistent with other studies. Waltimo *et al.* [5] found fungi in 7% of the samples in resistant chronic apical periodontitis cases, using the culture techniques, 80% of which were *C.albicans*. Pinheiro *et al*. [34] applied advanced microbiological techniques to anaerobic species and a prevalence rate of 3.3% for *C.albicans* in root canals of endodontically treated teeth with periapical lesions. No significant relationship was found between the radiographic status of the endodontically treated and the presence of any specific bacterial species (p> 0.05) [34]. Ashraf *et al.* [35] studied cases of root canal retreatment, with and without periapical lesions, using a culture technique in order to evaluate the amount of *C.albicans*; 36.7% of the cases with periapical lesions had *C.albicans*, while only 13.3% of the cases without periapical lesions had *C.albicans*. There was a significant difference between the cases with and without periapical lesions [35]. These findings were consistent with those of our study; we also found a significant association between the *C.albicans* and periapical radiolucency. The coexistence of *C.albicans* and *Enterococcus faecalis* has been hypothesized to exacerbate periapical lesions by increasing the virulence of the biofilm [38]. It has been suggested that the *C.albicans* are good biofilm formers [36], and the coexistence of it with *Enterococcus faecalis* specifically accelerated osteoclastic bone resorption and inhibited osteoblastic bone growth. TNF-α and IL-6 are two examples of the inflammatory cytokines that the synergistic effects increase [37].

Dumani *et al*. [38] using PCR technique, demonstrated that *C.albicans* was present in 11% of retreated root canals with apical periodontitis in a Turkish patient group. Egan *et al.* [39] reported a prevalence rate of 16 % for yeasts in the retreatment cases with apical periodontitis, using a culture technique. Employing molecular biology techniques, researchers directly analyzed the endodontic microflora of patients from various parts of the world and discovered substantial variations in the presence of several key species [40-41]. Therefore, the differences in the prevalence of *C.albicans* in these stu-

dies might be attributed to geographical differences [42].

It is feasible for *C.albicans* to survive endodontic treatment and grow because of its ability to penetrate dentin, develop biofilm, and withstand routinely used intracanal antimicrobials. The likelihood of a fungal infection should therefore be considered by clinicians when retreating endodontic failures. These circumstances might necessitate further treatments, like the use of intracanal medicaments like chlorhexidine gel or alexidine digluconate [27]. In addition to having antifungal properties, recently created bioceramic sealers also have the capacity to reduce them [43-44]. For application in root canal obturation, novel calcium-silicate (Ca-Si) compounds that are set in wet conditions have drawn interest. They produce alkaline conditions through their hydration processes, which have antimicrobial effects, but more crucially, these materials stimulate intratubular biomineralization [45-46]. As root canals were filled, precipitates containing Ca-Si-based hydroxyapatite were seen inside the tubules of the dentin; these micromechanical biomineralized structures were thought to have beneficial retention [47-48]. It is interesting to note that additional research revealed that these biomineralized precipitates had trapped dead microorganisms inside dentinal tubules [49].

The current investigation found no evidence of a connection between endodontic symptoms and *C.albicans*. It might be either the nature of endodontic symptoms is multifactorial and not limited to only one organism or the current study's sample size is small. There is a limited amount of information available on this subject, thus more research is necessary.

Conclusion

Our research indicates that the SYBR Green I real-time PCR could also be applied for molecular identification of *C.albicans* in the endodontic retreatment cases. Under the limitations of this study, we concluded that *C.albicans* was associated with root canal infections in endodontic retreatment cases. There was a significant relationship between the radiographic status of the endodontic treatment and the presence of *C.albicans*. This microorganism might be a potent pathogen in apical lesions. However, there was no significant relationship between the presence of *C.albicans* and the clinical symptoms, and more research is needed in this regard.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Case Report

Concurrent Odontogenic Keratocyst and Odontoma: Report of an Unusual and Rare Entity

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KEY WORDS
Odontogenic Cysts;
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ABSTRACT

Hybrid lesions of jaws are rare entities defined as two different lesions co-occurring in the same location, with identical histopathological origin. Ameloblastoma, calcifying cystic odontogenic tumor and odontoma are among the most common lesions that have been reported to combine with other lesions. In this study, a hybrid lesion of odontogenic keratocyst (OKC) and odontoma in the mandible of a forty-five years old male reported. Additional to the rarity of this hybrid lesion, the present case had unique radiologic features, including atypical location and extension of the lesion and profound knife-edge root resorption of the teeth in the area, which was not a common feature for any of the two lesions. The surgical procedure was marsupialization to reduce the size of the lesion. As a result of the surgery, the healing of the surgical wound was uneventful. In addition, careful follow-up for the patient was conducted, which had no recurrence till now (after 15 months).

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Introduction

The odontogenic keratocyst (OKC) is a benign but aggressive intraosseous tumor drawn from the remains of the original tooth germ or dental lamina [1]. OKC can develop in association with an unerupted tooth or as a solitary entity in bone [2]. This cyst demonstrates a propensity for aggressive behavior and a relatively high recurrence rate compared to the other odontogenic cysts [1]. Ameloblastomatous change and malignant transformation of this cyst were also reported [3].

OKCs are classified as central (intraosseous) versus peripheral or mucosal forms. The central form is further subclassified as parakeratotic, orthokeratotic, or mixed [4]. There is a predilection for posterior jaws and the mandible is affected two to four times more often than the maxilla [5]. Clinically, OKCs usually cause no symptoms and manifestations, although mild swelling may occur and is the most common clinical manifestation [1]. Pain may also occur in case of secondary infection [5]. Radiographically, they appear as unilocular or multilocular radiolucencies with a corticated or sclerotic, reactive bony rim. The margin of the lesion may be smooth or scalloped. Sometimes, it may have destructive borders, invading the adjacent structures [6].

Odontomas are the most prevalent odontogenic hamartoma of the jaws, with a frequency of 35%–76%,

characterized by their non-aggressive behavior [7]. These tumors produce both the epithelial and mesenchymal components of the dental apparatus with complete, mature differentiation. They produce Enamel, dentin, cementum, and pulp [8]. Odontoma is classified into two main types including compound and complex. The more common type, compound odontoma, is consisted of several tooth particles, while the complex type is a heterogeneous mass of dental tissue [9]. The predominant location for odontoma is anterior of the maxilla and posterior of the mandible in compound and complex odontoma, respectively [7].

Co-occurrence of two different lesions in the jaw is categorized into two types including collisional and hybrid lesions. The collisional lesions are tumors with different histological origins, which exclusively co-exist in the same region. However, the hybrid lesions, which are rare, are diverse tumoral demonstrations of the same histopathological source [9].

Extensive lesions in the jaws need to be completely investigated to make a distinction between their less aggressive compartment and the threatening compartment, so as to choose an appropriate surgical approach for their treatment. The treatment plan and the prognosis of OKC and odontoma are completely different. Therefore, precise diagnosis of the hybrid lesion is necessary.

To the best of our knowledge so far, only two cases have reported the co-occurrence of OKC and odontoma [6, 10], therefore the present case would be the third case reporting of such co-occurrence in the literature.

Case Presentation

A 45-year-old male was referred to the Oral and Maxillofacial Radiology Department by a surgeon. The patient had attended dental school to fill his left maxillary tooth. After taking a panoramic radiograph, a mixed radiopaque-radiolucent lesion was detected incidentally in the mandible. The patient had no history of pain/ tenderness or previous surgical manipulation in his mandible. Also, he had no systemic disease. However, the intraoral clinical examination revealed a mild swelling on the left side of the buccal and palatal cortices. Cone-beam computed tomography of the mandible was prescribed for the patient to establish a thorough radiographic assessment. The cone-beam computed tomography images demonstrated a large mixed radiolucent-radiopaque lesion. The radiolucent compartment had extended in the periapical area of mandibular teeth from the mesial aspect of the left second molar tooth to the distal part of the right canine, crossing the midline. In addition, root divergence of the canine and first premolar teeth in the left side was noticed. A non-homogenous radiopaque mass with denticle-like density was detected interdentally between the canine and the first premolar teeth on the left side (Figure 1). The superior border of the lesion was scalloping between the teeth, especially on the right side.

Extreme knife-edge root resorption in all involved teeth in the lesion was evident. Thinning, expansion and loss of continuity of both buccal and lingual cortices, thinning and endosteal erosion of the mandible's inferior border were also detected. (Figures 2-4) Moreover, involvement and displacement of the inferior alveolar nerve canal, resulting in loss of cortical boundaries was noted and mental foramen could not be followed.

An incisional biopsy of the mandible was carried out under general anesthesia. The specimen received in formalin solution and consisted two separate bottles.

The first bottle contained multiple pieces of irregular

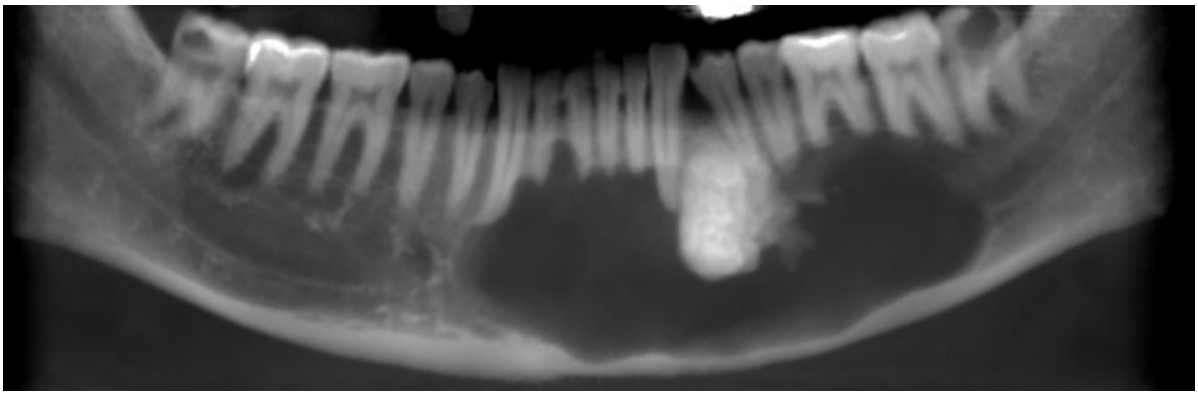

Figure 1: The reconstructed panoramic view showing the extension of the lesion and the radiopacity is also clearly detected

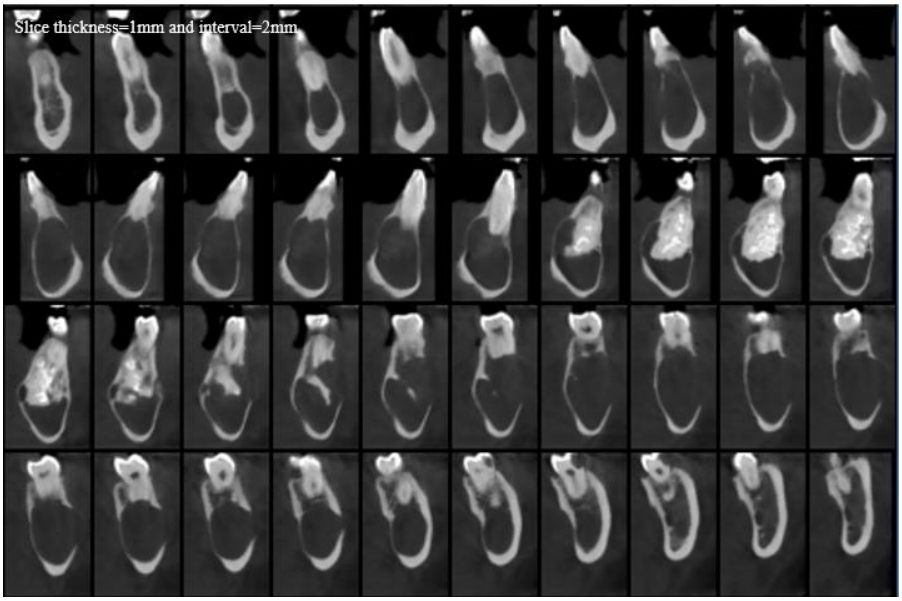

Figure 2: Cone beam computed tomography scan shows thinning of both buccal and lingual cortices and loss of continuity of buccal cortex

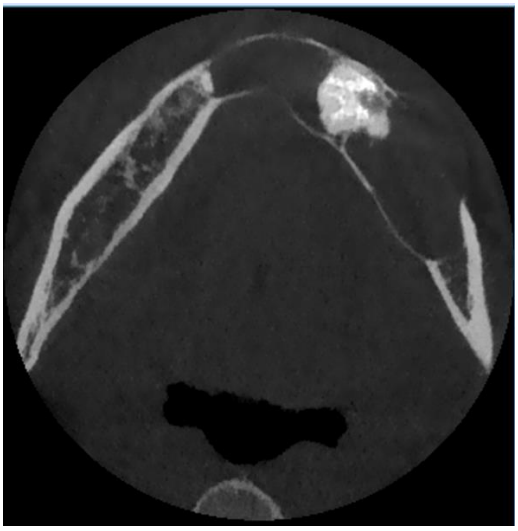

Figure 3: Axial image shows the expansion of the lesion, discontinuity of the buccal cortex, thinning of both cortices and radiopacity of complex odontoma

creamy-brown elastic tissues and the second bottle contained three pieces of irregular creamy bony hard tissues. The histopathological examination revealed odontogenic epithelium and basophilic enamel matrix. They also disclosed the cystic space-lined connective layer of stratified epithelium with a prominent basal cell layer and the absence of rete ridges. The connective tissue showed an inflammatory response. Eosin-stained odontogenic components with adjacent clear spaces were visible, composed of dental hard tissue and mesenchyme distributed in fibrous tissue.

Mature tubular dentin with incremental lines randomly placed unstructured sheets of eosin-stained hard tissue component, and cementum-like structures in the periphery were seen. A thorough histopathological examination diagnosed an inflamed OKC associated with complex odontoma (Figure 5).

The surgical procedure was marsupialization to reduce the size of the lesion. As a result of the surgery, the healing of the surgical wound was uneventful (Figure 6). The patient was kept under observation at regular intervals to detect any recurrence.

Discussion

OKC is a common developmental cyst, possibly originating from remnants of epithelium, whether from tooth

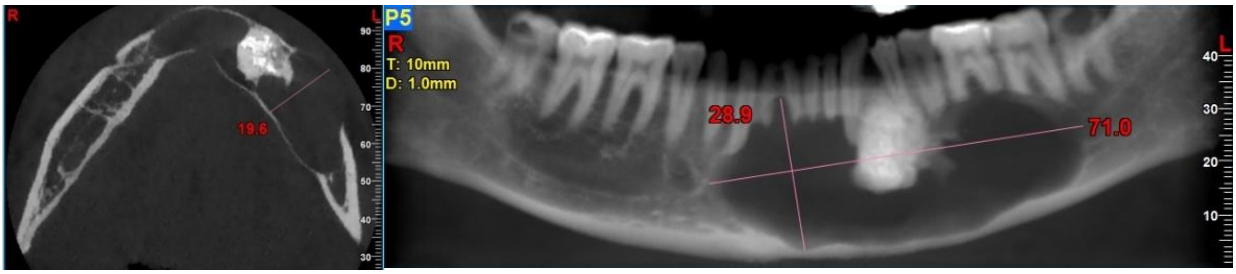

Figure 4: Dimensions of the lesions in axial and reconstructed panoramic images

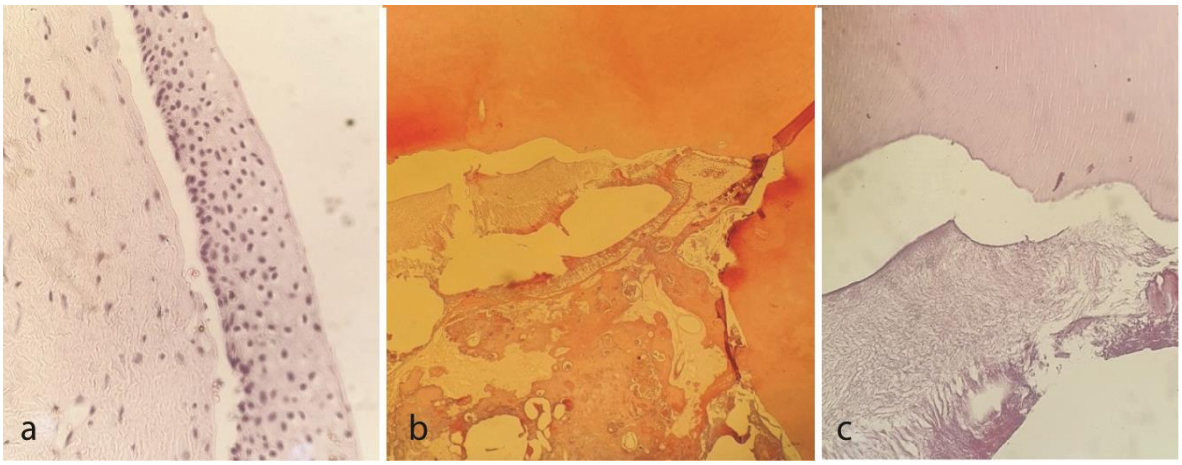

Figure 5: Images were taken at magnification of 200× (A), 100× (B), 200× (C) Eosin-stained odontogenic components with adjacent clear spaces, **a:** Dental hard tissue and mesenchyme, distributed in fibrous tissue, **b:** Mature tubular dentin with incremental lines, **c:** Randomly placed unstructured eosin-stained hard tissue component and cementum like structures in periphery

germ or dental lamina. Although in previous classifications of WHO, OKC was considered to have a dual manner as a cyst or tumor, in the most recent category, it is exclusively viewed as a cyst [1]. This cyst has aggressive behavior and a high recurrence rate (%5-%62.5) [1]. In addition, ameloblastomatous change and malignant transformation of OKC are possible [3]. Several hybrid lesions have been reported to date.

Ameloblastoma, calcifying cystic odontogenic tumor and odontoma are among the most common lesions that have been reported to combine with other lesions [6, 9, 11]. The most common lesions that have been reported to co-occur with odontoma are calcifying odontogenic cyst, adenomatoid odontogenic tumor, ameloblas-

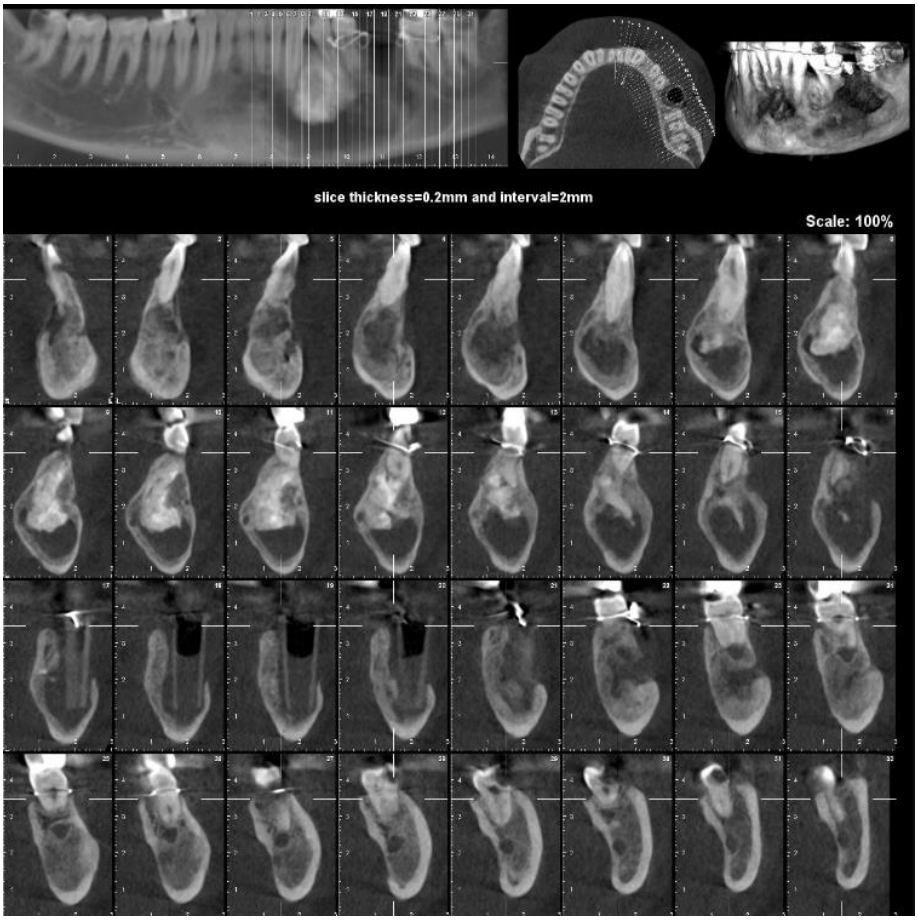

Figure 6: The follow up CBCT image after 15 months illustrated healing and reduction of size of the lesion

tic fibroma and ameloblastic fibro-odontoma [9, 12-13]. Limited reports of combined odontoma with botryoid odontogenic cyst, glandular odontogenic cyst, and cemento-ossifying fibroma were also found [9, 14-15].

To the best of our knowledge, there were only two reports of combined OKC with odontoma in literature till now. Bang *et al.* [10] reported a case of combined OKC, which interestingly co-occurred with erupting odontoma. Moreover, Kulkarni *et al.* [6] reported an orthokeratinized odontogenic cyst with complex odontoma in the maxilla, which has caused suffering in the patient's breathing.

The co-occurrence of OKC with odontoma is rare and worthy of attention. Additionally, our present case was unique regarding its location. The extension of OKC in the present case was predominantly in the anterior mandible crossing the midline, while OKC is mainly found in the posterior of the mandible [1]. Also, the accompanying complex odontoma was located interdentally between the roots of the anterior teeth, which is an atypical location for a complex odontoma. The other unusual feature of the present case was profound, knife-edge resorption of the teeth in the region. The study conducted by Kitisubkanchana *et al.* [11] revealed that the frequency of root resorption in OKC was %7 and OKC usually causes mild or no root resorption. The possible justification for these divergent features is the pluripotentiality of the odontogenic epithelium, leading to unusual odontogenic lesions' behaviors [3].

A clinical point worth mentioning is that if the treatment plan for teeth involved by OKC is extraction, the surgical procedure should be conducted as atraumatic as possible to prevent the cyst's rupture, because injury to the cyst and its rupture leads to the superimposition of infection and systemic symptoms in the patient [1]. However, in the present case, the teeth were kept.

The high recurrence rate, and ameloblastomatous, and malignant transformations of OKC impose the surgeon to conduct a thorough presurgical assessment of this cyst. In hybrid cases of OKC, the evaluation should not be underemphasized due to the other less aggressive compartment, which was a complex odontoma in the present case. Moreover, conservative marsupialization of this cyst is the least aggressive surgical treatment for this lesion, which was conducted, in the present case.

A written consent was obtained from the patient before imaging to use the patient's images in research works.

Conclusion

Extensive lesions in the jaws need to be completely evaluated to differentiate between their minimally invasive compartment and the threatening one, in order to select the appropriate surgical approach for surgeons. The current lesion is a rare case of an extensive OKC associated with a complex odontoma for which, marsupialization was performed due to the large size of the lesion. Healing was uneventful and showed bone regeneration in the surgical defect. The patient has been followed up for over 15 months and no signs of recurrence have been detected so far.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Case Report

Metastatic Adenocarcinoma of Mandible with Unknown Primary Origin (CUP Syndrome): A Rare Case Report

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ABSTRACT

Metastatic lesions of the jaws are a diagnostic challenge because of their scarcity and uncharacteristic clinical-radiographic features. Carcinoma of unknown primary origin (CUP) is characterized by the existence of metastatic disease with no recognized primary neoplasm after a comprehensive work-up. CUP shows a poor prognosis with limited treatment choices. This paper presents a 64-year-old male with a chief complaint of paresthesia of the chin and lower lip. Panoramic radiography showed an ill-defined radiolucency in the left mandibular molar area and the residue of the first molar root. Microscopic examination demonstrated features of mucin-producing adenocarcinoma and was not similar to common neoplasms of the jaw. The whole-body scan revealed multiple osseous uptakes. CDX2 was diffusely positive. However, in the end, the origin of the primary tumor was not determined. Considering the aforementioned data, the diagnosis of metastatic adenocarcinoma with unknown primary origin was made. CUP of the oral cavity is an extremely rare event. The possibility of metastasis should be raised in a patient who complains of paresthesia. Awareness of the clinical and histopathologic features of these malignancies is crucial for clinicians and pathologists to have a proper diagnosis.

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Introduction

Jaw metastasis has great importance because it may be the only sign of an undiagnosed primary malignancy. Clinical signs and symptoms are variable, and the lesion may be asymptomatic. The most common radiographic feature is an ill-defined radiolucency [1]. Metastatic lesions of the jaws show posterior mandibular predilection. They are more common in men and the elderly [2]. Factors other than the frequency of neoplasms may be present in oral metastasis, such as biological behavior of cancer, aggressiveness, and a tendency for particular regions of oral cavity [3-4]. CUP is a diverse group of malignancies characterized by the existence of metastatic disease with no recognized primary neoplasm at initial presentation. It comprises 2–3% of all epithelial cancers [5-6]. CUP shows a poor prognosis with limited treatment choices [6]. Dental practitioners have a vital role in the diagnosis of cancer patients, mainly those with oral metastasis and silent primary malignancies [2]. This paper presents a case of metastatic adenocarcinoma with unknown primary origin of the left molar region of the mandible affecting a 64-year-old man.

Case Presentation

A 64-year-old man with a chief complaint of paresthesia of the chin and lower lip area was referred to a private dental clinic (Tehran, Iran) in May 2018. He had a history of Wegener's granulomatosis and was taking meth

Figure 1: Panoramic radiograph shows an ill-defined radiolucent lesion in area of #18, #19 with bone sclerosis, residue of first molar root and resorption of mesial root of #18

otrexate, prednisone, and Calcium D. Extra-oral examination was normal and the intraoral examination revealed mild buccal expansion without any mucosal erosion or ulcer. There was no cervical lymphadenopathy. Panoramic radiography revealed a radiolucent lesion with ill-defined borders in areas #18 and #19 with bone sclerosis and residue of the first molar root. Resorption of the mesial root of the second left molar is also evident (Figure 1). Due to numb chin syndrome and radiographic features, a provisional diagnosis of the inflammatory periapical lesion, lymphoma, aggressive central giant cell granuloma, and odontogenic carcinoma were made, and an incisional biopsy was performed under local anesthesia. Histopathologic examination showed a malignant epithelial neoplasm composed of cribriform and ductal architectures lined by pseudo-stratified columnar epithelium with vesicular nuclei. Tumoral giant cells and goblet cells existed. The stroma was fibrous to mucoid and contained many foamy histiocytes. Tumoral islands were admixed with bony trabeculae. Hemorrhage, chronic inflammatory cell infiltration, and cholesterol clefts were also seen (Figure 2). Based on microscopic features and the intraosseous nature of the lesion, an overall diagnosis of adenocarcinoma was rendered. A whole-body scan and immunohistochemical (IHC) staining for CK7, CK20, Napsin A, CDX2, and TTF-1 was recommended to rule out a metastatic tumor. The whole-body scan demonstrated multiple osseous uptakes involving the skull, left rib, right sacroiliac (SI) joint and proximal femur. The prostate-specific antigen (PSA) was at the normal limit. IHC was negative for CK7, TTF-1, and Napsin A. CK20 showed a patchy reaction and CDX2 was diffusely positive (Figure 3).

Findings were more compatible with metastatic mucin-producing adenocarcinoma of gastrointestinal tract (GI) origin, especially the colon, and the patient was

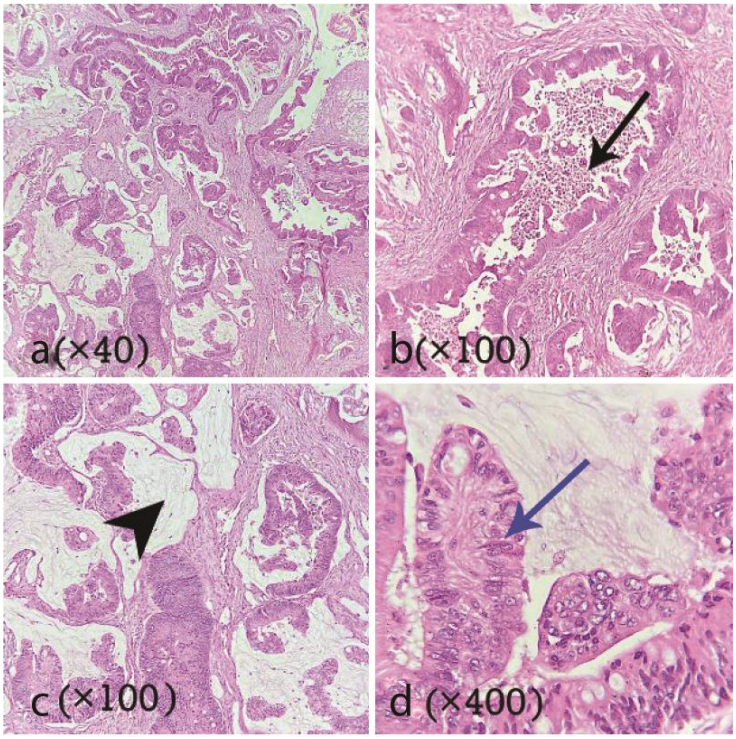

Figure 2: a, b: Histopathologic sections demonstrate ductal structures (black arrow) lined by pseudo-stratified columnar epithelium, **c:** Mucoid stroma (arrowhead), **d:** Tall columnar cells with vesicular nuclei (blue arrow)

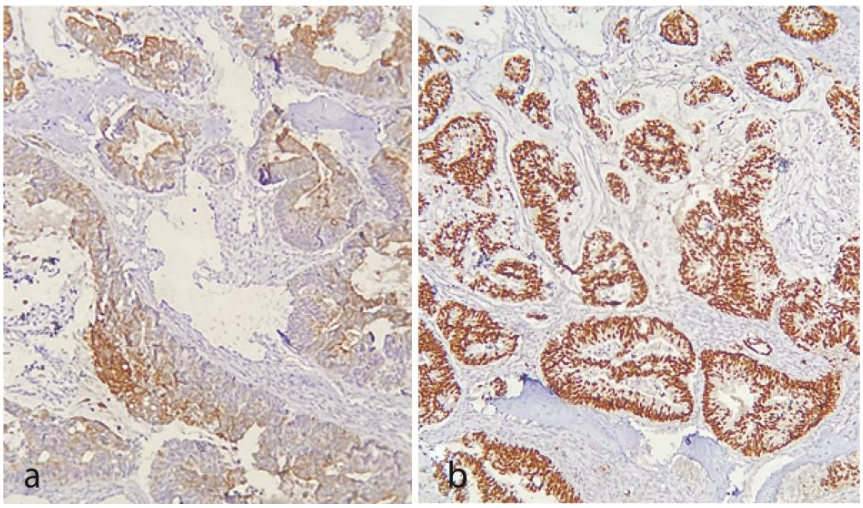

Figure 3: a: CK20 showed a patchy reaction (100×), **b:** CDX2 was diffusely and strongly positive (100×)

referred to gastric endoscopy and intestinal colonoscopy. No gastrointestinal (GI) problems were seen in these assessments. Thyroid, liver, and kidney function tests, blood tests, and chest radiography were also normal.

Complete workup including positron emission tomography (PET) scan was performed but the documents are not available. Based on the aforementioned data and oncologist consultation, the diagnosis of metastatic adenocarcinoma with unknown primary origin was made and he underwent chemotherapy based on a CUP syndrome protocol. Unfortunately, he passed away in May 2019.

Discussion

Approximately 23% of oral metastases are the first indication of occult cancer elsewhere in the body [2]. Clinical signs and symptoms are variable and include bony swelling with tenderness, pain, ulcer, hemorrhage, paresthesia, pathological fracture, tooth mobility, trismus, and numb chin, but it may be asymptomatic [1]. A numbed chin is a major diagnostic indication of metastatic disease [7-8]. As a result, the possibility of metastasis should be raised in a patient who complains of paresthesia [7].

CUP comprises 2–3% of all epithelial cancers. The two main microscopic patterns include adenocarcinomas and undifferentiated carcinomas, although squamous cell carcinoma (SCC), neuroendocrine carcinoma, and uncommon histopathology comprise the remaining 10% [5]. The primary neoplasm may stay tiny and consequently escape clinical recognition or it may be vanished after seeding the metastasis or has been removed by the body's defenses [6]. The most frequent primary locations recognized at autopsy include the lung, the pancreas, the GI (colon, stomach, bile duct, and liver), and the urogenital tract [5]. Metastases in lymph nodes, lungs, liver, or bone are the most common clinical manifestation of CUP. The majority of cases show disseminated metastasis. Clinical symptoms related to the involved organ and the extent of metastasis. In asymptomatic patients, it may be discovered incidentally through radiography [9]. IHC assessment helps determine the type of tumor [5-6]. The expression of cytokeratins (CKs) is very useful in determining the subtype. For instance, the profiles CDX2+, CK20+, and CK7- are typical of colon cancer, although the profiles CK7+, WT1+, PAX8+, and CK20- are characteristic of ovarian cancer [10]. CDX2 is the most well-known marker for the diagnosis of metastases originating from the GI. Moreover, the expression pattern is also significant. Colorectal malignancies demonstrate uniform positive stain; other site carcinomas show variable or focal staining [11]. In the present case, almost all neoplastic cells were positive for CDX2. Napsin A indicates pulmonary origin and co-expression with TTF1 is highly specific for pulmonary adenocarcinomas [11] that both of them were negative in current case. GATA3 is a sensitive marker for breast and urothelial carcinomas [11]. Asking about family history is important [6]. It has been stated that there is a familial cancer predisposition in CUP cases [10] and the metastatic site shows a familial clustering pattern. On the other hand, CUP patients' relatives reveal an increased risk of CUP, malignancies of the lung, pancreas, or colon themselves

[5]. Liver and kidney function tests, blood tests, chest radiography, mammography, or a PSA test should also be evaluated [6]. Bronchoscopy or colonoscopy should be done only when IHC findings or clinical features are highly indicative of lung or colon malignancies. TP53, K-RAS, and CDKN2A are most mutated genes [5]. An integrated PET-computed tomography (CT) is also needed to find the location of primary cancer [5]. Salem *et al.* [12] reported a 75-year-old woman with cervical lymphadenopathy that clinical and CT did not reveal any other site involvement. Lymph node biopsy showed metastatic SCC. F-18 FDG PET/CT imaging demonstrated a small lesion in uvula and biopsy showed SCC of uvula.

In head and neck region, the most common occult primary site is the oropharynx and hypopharynx respectively [13]. On the other hand, cervical lymph nodes are the majority of CUP cases in this region [13-14]. SCC includes 53–77% of all head and neck CUPs [14]. The level of the metastasis can give evidences to predict the site of the primary lesion. In the case of SCC, metastasis to levels I, II, and/or III apparently shows that the primary origin is in the oral cavity or oropharynx. Moreover, in the case of adenocarcinoma, metastasis to level IV or V suggests that the primary neoplasm is located in the thyroid gland or thoraco-abdominal area [13]. In metastatic SCC cases, screening for association with human papillomavirus (HPV) and Epstein-Barr virus (EBV) is a beneficial diagnostic method to define the unknown primary location [15]. EBV positivity supports the nasopharyngeal origin. The evidence of HPV and P16 overexpression suggests the oropharyngeal origin. In some cases, the primary location has never been recognized. However, it maybe finds during follow up after initial treatment [13]. Oriyama *et al*. [13] described a 69-year-old female patient with metastatic cervical lymph node in which maxillary primary intraosseous carcinoma was found 6 months after the initial treatment. However, Aro *et al*. [14] mentioned that diagnosis of a primary later in the follow up does not affect the survival.

Small proportions of CUP patients show a favorable prognosis, and attain meaningful improved survival. Nevertheless, most patients include the unfavorable subtype [16]. If there is a single metastasis, local radical surgery or radiotherapy is recommended [9]. However, more than 75% of CUP patients show multiple metastases, and systemic chemotherapy is recommended [9]. The majority of patients do not respond to chemotherapy and the medium overall survival rate is estimated about 6 to 10 months [11]. Conway *et al.* [16] stated that this one-size-fits-all attitude does not reflect the heterogeneity of these neoplasms, and underline the necessity for enhanced treatment stratification. Informed consent was obtained from the patient for publishing his radiography.

Conclusion

In conclusion, CUP of the oral cavity is an extremely rare event with poor prognosis. Numb-chin syndrome can be the first clinical sign of a disseminated metastasis. Oral and maxillofacial surgeons and pathologists should be familiar with this entity.

Conflict of Interest

The authors declare that they have no conflict of interest.

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