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UTICAJA DVAJU EKSTRAKCIONIH TERAPIJSKIH PROTOKOLA PRILIKOM LEČENJA MALOKLUZIJE II/1 KLASE NA ANGULACIJU DONJIH TREĆIH MOLARA U RAZVOJU

THE EFFECT OF TWO DIFFERENT EXTRACTION PROTOCOLS IN THE TREATMENT OF CLASS II, DIVISION 1 MALOCCLUSION ON THE ANGULATION OF DEVELOPING MANDIBULAR THIRD MOLARS

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Sažetak

Uvod: *Ekstrakcije premolara su česte u lečenju malokluzije II/1 klase.*
Cilj rada *je da se ispita nulta hipoteza o nepostojanju statistički značajnih razlika u promeni angulacije donjih trećih molara u razvoju između grupe pacijenata sa malokluzijom II/1 klase lečenih ekstrakcijom gornjih prvih i donjih drugih premolara i grupe pacijenata lečenih ekstrakcijom gornjih drugih premolara.*
Materijal i metod: *Ispitivanje je obuhvatilo ortopantomografske snimke snimljene pre početka i nakon završetka ortodontske terapije dvadeset osam pacijenata uzrasta od petnaest godina do dvadeset dve godine sa malokluzijom II/1 klase koji su podeljeni u dve grupe. Grupu I (n = 14) činili su pacijenti lečeni ekstrakcijom samo gornjih drugih premolara, a grupu II (n = 14) pacijenti lečeni ekstrakcijom gornjih prvih, donjih drugih premolara. Statistička analiza sprovedena je primenom deskriptivne statistike i odgovarajućih inferencijalnih testova (Studentov t-test, Pearsonov χ^2 test i Mann–Whitney test), uz nivo statističke značajnosti $p < 0,05$.*
Rezultati: *Promena srednje vrednosti angulacije donjih trećih molara u razvoju u grupi pacijenata lečenih ekstrakcijom gornih prvih i donjih drugih premolara je pozitivna i statistički značajno se razlikuje od promene srednje vrednosti angulacije donjih trećih molara u grupi pacijenata lečenih ekstrakcijom gornjih drugih premolara bez ekstrakcije u donjem nizu zuba ($p < 0,01$). U obe ispitivane grupe nije pronađena statistički značajna promena angulacije donjih trećih molara leve i desne strane. Procenat zuba kod kojih je došlo do povećanja angulacije je 32,14% u prvoj grupi, dok je u drugoj 71,43%.*
Zaključak: *Nulta hipoteza je odbačena uz zaključak da terapija malokluzije II/1 klase vođena ekstrakcijom gornjih prvih i donjih drugih premolara povećava mogućnost nicanja donjih trećih molara jer dovodi do statistički značajnije promene angulacije donjih trećih molara u razvoju.*

Ključne reči: *ekstrakciona terapija, malokluzija II/1 klase, angulacija donjih trećih molara*

Abstract

Introduction: *The extraction of premolars is a common therapeutic approach in the treatment of Class II, Division 1 malocclusion.*
Aim: *This study aimed to test the null hypothesis that there is no statistically significant difference in the change in angulation of developing mandibular third molars between patients with Class II, Division 1 malocclusion treated with the extraction of maxillary first and mandibular second premolars and those treated with the extraction of maxillary second premolars.*
Materials and Methods: *The study included orthopantomographic radiographs taken before and after orthodontic treatment in 28 patients aged 15–22 years with Class II, Division 1 malocclusion. The patients were divided into Group I (n = 14), treated with the extraction of maxillary second premolars only, and Group II (n = 14), treated with extraction of maxillary first and mandibular second premolars. Statistical analysis was performed using descriptive statistics and appropriate inferential tests (Student's t-test, Pearson's χ^2 test, and Mann–Whitney test), with the level of statistical significance set at $p < 0.05$.*
Results: *The mean change in angulation of developing mandibular third molars in group II were positive and significantly different from that observed in Group I ($p < 0.01$). No statistically significant differences were found between the left and right sides within either group. The percentage of lower third molars showing an increase in angulation was 32.14% in Group I and 71.43% in Group II.*
Conclusion: *The null hypothesis was rejected. Orthodontic treatment of Class II, Division 1 malocclusion involving the extraction of maxillary first and mandibular second premolars increase the likelihood of mandibular third molar eruption by producing a significantly greater change in their angulation.*

Key words: *extraction treatment, malocclusion, Class II, Division 1, lower third molar angulation*

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Introduction

Third molars are the last teeth in the dental arches. They erupt last and frequently lack sufficient space. The prevalence of their impaction in the European population is relatively high, around 25%, while in the Asian populations it exceeds 40%[1]. The etiology of third molar impaction remains not fully understood. Although adequate space is considered one of the most important factors, it does not guarantee eruption in all cases. The developmental pathway of these teeth shows considerable variability in terms of formation, position, calcification, and ultimately eruption[2].

A large number of orthodontists and oral surgeons believe that third molars generate an ananteriorly directed force that may contribute to late mandibular crowding[3] and therefore recommend their prophylactic removal[4]. Although third molars are not typically included in active orthodontic treatment, they represent an important factor to be considered during treatment planning[5]. While a substantial body of literature addresses the causes of third molar impaction[6,7], fewer studies have focused on predicting their eruption potential[7,8].

From a clinical perspective, there is a strong tendency to preserve as many teeth as possible; therefore, the influence of premolar extractions on the eruption and positioning of third molars represents an important orthodontic concern. Despite its clinical relevance, relatively few studies have addressed this topic, and those available provide inconsistent conclusions[8–10]. To date, no study has specifically investigated the effect of Class II, Division 1 malocclusion treatment involving the extraction of the maxillary first and mandibular second premolars on the angulation of mandibular third molars.

Class II Division 1 malocclusion is relatively common. Several treatment protocols are available depending on patient age, sagittal jaw relationships, growth pattern, incisor inclination, degree of crowding, and facial profile. Both non-extraction and extraction approaches are used, with premolar extractions performed either in the maxillary arch alone or in both arches[11]. Given the distal occlusion, extraction of maxillary first and mandibular second premolars is often preferred to achieve a Class I molar relationship at the end of treatment.

The aim of this study was to evaluate the effect of two different extraction-based treatment protocols for Class II, Division 1 malocclusion on the angulation of developing mandibular third molars.

Aim

The performed study aimed to test the null hypothesis that there is no statistically significant difference in the change of angulation of developing mandibular third molars between patients with Class II Division 1 malocclusion treated with the extraction of maxillary first and mandibular second premolars and those treated with the extraction of maxillary second premolars only.

Materials and Methods

Ethical approval of the study was obtained from the Ethical Committee of the Clinic for Dental Medicine in Niš with reference No. 14/6-2023-2 EO. It was a retrospective longitudinal cohort study.

The study included 28 patients (8 males, 20 females) aged 15 to 22 years with Class II Division 1 malocclusion, treated at the Clinic for Dental Medicine. All patients were divided into two groups according to the treatment protocol. Group I (n = 14; 5 males, 9 females; mean age 17.79 ± 2.78 years) consisted of patients treated with the extraction of maxillary second premolars only. Group II (n = 14; 3 males, 11 females; mean age 17.07 ± 2.37 years) included patients treated with the extraction of maxillary first and mandibular second premolars (Table1). The duration of treatment in the entire sample ranged from 26 to 39 months.

The effect of mandibular second premolar extraction on the angulation of mandibular third molars was assessed using orthopantomographic (OPG) radiographs taken before the start and after the completion of orthodontic treatment (Figure1).

Strict inclusion and exclusion criteria were applied during patient selection.

Inclusion criteria: High-quality orthopantomograms obtained before and after treatment; radiographs taken no more than one month prior to treatment initiation and within seven days after treatment completion; presence of Class II, Division 1 malocclusion with a half-cusp Class II molar relationship prior to treatment (assessed using study models); presence of mandibular third molar germs at least at Nolla stage 5 (crown almost completed)[12]; and complete closure of

extraction spaces at the end of treatment. Exclusion criteria: Patients with syndromes.

Orthopantomogram radiographs were obtained under standardized conditions using the same equipment, Sirona Axeos CBCT Ceph (Sirona Dental System GmbH, Bensheim, Germany) and Sidexis 4 software, Galileos Viewer (Dentsply Sirona, USA).

The influence of the two treatment approaches on the angulation of mandibular third molars was assessed using OPG analysis according to the methods described by Tarazona et al.8 and Jain et al.[9].

Contours of the mandibular ramus, corpus, sigmoid notch, and mandibular third molars were traced on transparent acetate paper (A4 size, 90 g/m²) using a 0.50 mm technical pencil. A line connecting the lowest points of the sigmoid notch bilaterally was used as the reference line. The angulation of mandibular third molars was determined relative to this line by measuring the external angle formed between the reference line and the long axis of the mandibular third molar, according to Jain et al.[9].

In cases where the mandibular third molar had not reached Nolla stage 6, the long axis of the tooth was determined as shown in Figure 1. In cases where the mandibular third molar had reached Nolla stage 6 or higher, the long axis was determined as shown in Figure 2.

All angles were measured using an angle grinder by the same investigator (orthodontist).

The long axis of mandibular third molars was determined depending on the stage of tooth development. In early developmental stages, when only the crown was formed, the long axis was constructed by connecting the midpoint of the line defined by the most convex points of the mesial and distal crown contours with the midpoint of the line defined by the mesial and distal cervical regions of the developing root (Figure 2).

In cases of completed root development, the long axis of the tooth was determined by connecting the midpoint of the line defined by the most convex points of the mesial and distal crown contours with the midpoint of the line defined by the apices of the mesial and distal roots of the third molar (Figure 3).

Changes in angulation of mandibular third molars were calculated by subtracting pre-treatment values from post-treatment values.

All measurements were performed twice using an angle grinder by the same examiner, and their mean values were used for statistical analysis.

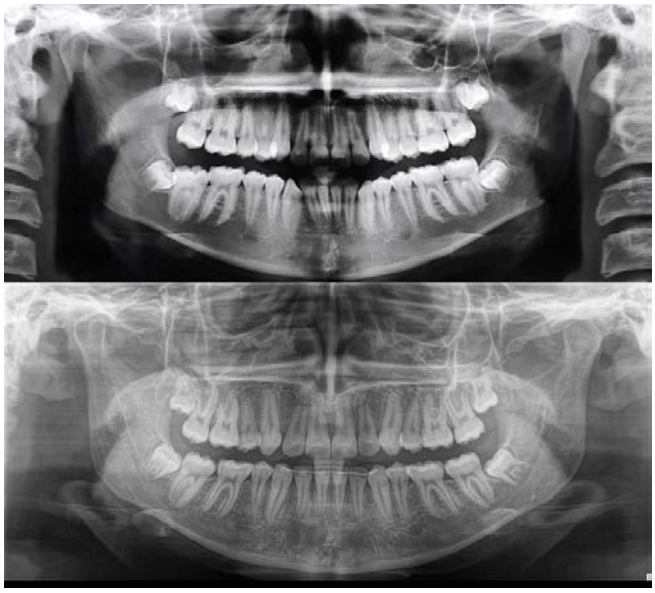

Figure 1. Pre- and post-treatment orthopantomograms of a patient treated with the extraction of the upper first and lower second premolars

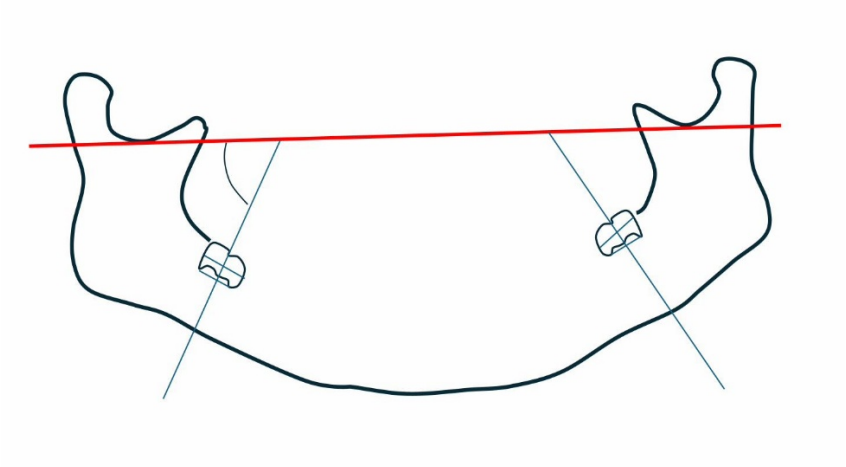

Figure 2. Determination of the long axis of the third molar at the developmental stage when only the crown is formed. The external angle formed between the long axis of the molar and the line connecting the lowest points of the sigmoid notch was measured to assess the inclination of developing mandibular third molars

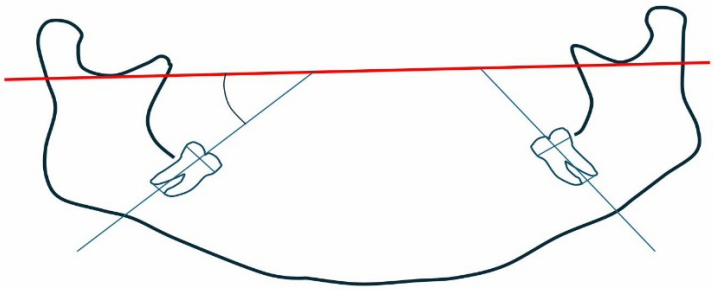

Figure 3. Determination of the long axis of the third molar at the developmental stage when both the crown and root are formed. The external angle formed between the long axis of the molar and the line connecting the lowest points of the sigmoid notch was measured to assess the inclination of developing mandibular third molars

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA; version 15.0 for Windows). Data analysis included the application of descriptive statistics and appropriate inferential tests. The Student's t-test for independent samples was used to compare mean values between two independent groups.

The Pearson χ^2 test was applied to analyze differences in the distribution of categorical variables. The Mann–Whitney test was used to assess differences in age between the groups. The level of statistical significance was set at $p < 0.05$.

Results

In the present study, intra-observer variability and measurement reproducibility were assessed by re-measuring 10 randomly selected OPG radiographs after a 7-day interval. The method error was calculated using Dahlberg's formula[13].

To evaluate the reliability of repeated measurements, the method error was compared with biological variability. The reliability of repeated measurements of the angulation of developing mandibular third molars ranged from 0.94 to 0.96.

The effects of Class II, Division 1 malocclusion treatment with the extraction of maxillary first and mandibular second premolars, as well as treatment with the extraction of maxillary second premolars, on the angulation of developing mandibular third molars are presented in Table 1. The mean value of the measured angle increased by 6.43 ± 9.45 in Group II, whereas in Group I, without mandibular premolar extractions, it decreased by -1.11 ± 8.71, with a statistically significant difference between the groups ($p < 0.01$) (Table 2).

Following the extraction of mandibular second premolars in Group II, the mean angulation of mandibular third molars increased on both the right and left sides (6.43 ± 8.05 and 6.43 ± 10.99, respectively). In Group I without mandibular premolar extractions, the mean angulation slightly decreased on both sides (-0.64 ± 8.48 and -1.57 ± 9.23, respectively), with no statistically significant difference between the right and left sides in either group (Table 3).

Among all evaluated mandibular third molars in Group I, angulation increased in 32.14% of cases, decreased in 46.43%, and remained unchanged in 21.43%. In Group II, angulation increased in 71.43% of third molars, decreased in 17.86%, and remained unchanged in 10.71% of cases (Table 4).

Table 1. Mean age of patients by groups

	Group I	Group II
	17.79 ± 2.78 (16.00)	17.07 ± 2.37 (16.00)

Data are presented as mean ± standard deviation (median)

Table 2. Descriptive parameters of changes in the angulation of mandibular third molars in the examined groups

	Group I	Group II
Changes in the angulation of mandibular third molars	-1.11 ± 8.71 (0.00)	6.43 ± 9.45 (5.00) *

Data are presented as mean ± standard deviation (median) * – $p<0.01$

Table 3. Descriptive parameters of changes in the angulation of left and right mandibular third molars in the examined groups

Lower third molars	Group I	Group II
Right	-0.64 ± 8.48 (-1.50)	6.43 ± 8.05 (6.50)
Left	-1.57 ± 9.23 (0.00)	6.43 ± 10.99 (3.50)

Data are presented as mean ± standard deviation (median)

Table 4. Distribution of changes in angulation of mandibular third molars by group

Changes in the angulation of mandibular third molars		Group I		Group II
Increase	9	32.14%	20	71.43%
No change	6	21.43%	3	10.71%
Decrease	13	46.43%	5	17.86%
Total	28	100.00%	28	100.00%

Data are presented as frequencies and percentages

Discussion

The extraction approach represents a common therapeutic option in orthodontics, with premolars being the teeth most frequently extracted. Their extraction enables the resolution of dental crowding, the retraction of anterior teeth, the improvement of the facial profile, and the establishment of proper sagittal relationships.

Before deciding on premolar extraction, it is essential to consider the presence, position, and morphology of third molars. The issue of their eruption and proper alignment is particularly pronounced in the mandible, as third molars are located within a limited space between the distal surface of the second molar and the mandibular ramus. Nance et al. reported that unerupted third molars are more likely to reach the occlusal plane when positioned more vertically, compared to those with greater mesial inclination[14]. Literature data also suggest that the extraction of mandibular premolars may favorably influence the inclination of mandibular third molars and increase the likelihood of their proper positioning in the dental arch[2,8,15].

Impaction of mandibular third molars may lead to numerous complications, including late mandibular crowding, pericoronitis, and periodontal damage to adjacent second molars, while their surgical removal is often complex and associated with prolonged recovery[16]. The present study evaluated the effect of two extraction protocols of Class II, Division 1 malocclusion with and without the extraction of mandibular premolars on the angulation of mandibular third molars.

Based on the comparison of angulation values before and after orthodontic treatment, a positive mean change in angulation was observed in Group II with the extraction of maxillary first and mandibular second premolars, whereas a negative change was observed in Group I without mandibular premolar extractions, with a statistically significant difference between the groups ($p < 0.01$). These findings indicate that the extraction of mandibular second premolars has a favorable effect on the uprighting and eruption of mandibular third molars. This is consistent with the findings of Tarazona et al.[8], Benkaddour et al.[15], and Yadav et al.[2], but contrasts with studies by Russell et al.[17], Di Giovanni et al.[18], and Miclotte et al.[19], who did not find a statistically significant improvement in third molar angulation following premolar extractions. Miclotte et al.[19] reported that premolar extraction might positively influence the eruption space and vertical position of third molars, but found no association with their uprighting. In contrast, Hammouda et al.[20] observed a positive effect on the uprighting of third molars regardless of whether the first or second mandibular premolars were extracted.

Yadav et al. reported a mean angulation change of 14.00 ± 12.66° the extraction of mandibular second premolars, which is considerably higher than the results obtained in the present study (6.43 ± 9.45°)[2]. Although lower, the value observed in this study is still clinically significant, given that Russell et al. identified a 5° change as the threshold for clinical relevance in third molar angulation[17].

In interpreting these findings, it is important to note that the reference plane used in this study was the line connecting the lowest points of the sigmoid notches, which is considered relatively stable within the examined age range[8]. However, other studies have used different reference planes on OPG radiographs, which may partly explain discrepancies in results.

Orthodontic extraction of mandibular premolars has a positive effect on the eruption of mandibular third molars, not only by influencing their inclination but also by increasing retromolar space.

The greatest increase in retromolar space occurs between the ages of 10 and 12 years, after which growth slows until approximately 16 years of age[21]. The mean age of patients in both groups in the present study was approximately 17 years, with no statistically significant difference between groups, indicating limited residual growth in the observed region.

Björk et al.[22] in a longitudinal study conducted using the implant method, demonstrated that premolar extraction is associated with significant resorption at the anterior border of the mandibular ramus, which further increases retromolar space and facilitates third molar eruption.

In the present study, no statistically significant differences were found between the left and right sides in either group, which is consistent with the findings of Benkaddour et al.[15], but differs from those of Yadav et al.[2], who reported greater uprighting on the right side in the extraction group.

Our results also showed that uprighting of mandibular third molars occurred in 32.14% of teeth in Group I, compared to 71.43% in Group II, with the extraction of mandibular second premolars, consistent with the findings of Gohilot et al.[21]. This further supports the positive influence of mandibular second

premolar extraction on third molar uprighting and eruption.

Although retromolar growth is nearly complete in the age group included in this study, Richardson noted that pre-eruptive rotational movements of mandibular third molars may still occur[23]. This may explain the positive angulation changes observed in approximately one-third of teeth in Group I.

Nevertheless, the overall mean change in angulation in Group I was slightly negative, consistent with the findings of Saysel et al.[24], but differing from Yadav et al.[2], who reported slight improvement in angulation in cases without extraction of mandibular premolars. In general, both positive and negative changes in third molar angulation in cases without mandibular premolar extractions are minimal—typically only a few degrees—and are unlikely to significantly influence eruption outcomes.

Conclusion

Based on the obtained results, the null hypothesis was rejected.

Extraction of mandibular second premolars in the treatment of Class II, Division 1 malocclusion positively affects the angulation of unerupted mandibular third molars, with no significant difference between the left and right sides. In the group of patients treated without the extraction of mandibular premolars, the mean change in angulation of mandibular third molars was slightly negative.

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Conflict of Interest

The authors declare no conflict of interest.

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GENETIČKA OSETLJIVOST ČULA UKUSA I BRZINA LUČENJA PLJUVAČKE KOD ORALNE SUBMUKOZNE FIBROZE I DIJABETES MELITUSA TIPA 2: KOMPARATIVNA STUDIJA PRESEKA

GENETIC TASTE SENSITIVITY AND SALIVARY FLOW RATES IN ORAL SUBMUCOUS FIBROSIS AND TYPE 2 DIABETES: A COMPARATIVE CROSS-SECTIONAL STUDY

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Sažetak

Uvod: *Izmenjena percepcija ukusa i smanjena funkcija pljuvačnih žlezda često se javljaju kod sistemskih i oralnih oboljenja kao što su oralna submukozna fibroza (OSMF) i dijabetes melitus tip 2 (engl. type 2 diabetes mellitus – T2DM). Genetska osetljivost na gorko jedinjenje fenil-tiokarbamid (engl. phenylthiocarbamide – PTC), posredovana TAS2R38 receptorima, može pružiti uvid u promene povezane sa bolešću.*
Materijal i metode: *Devedeset ispitanika bilo je ravnomerno podeljeno u tri grupe: OSMF, T2DM i zdrava kontrolna grupa. Osetljivost na ukus testirana je primenom standardizovanih PTC traka koje su bile postavljene na dorzalnu površinu jezika deset sekundi i ocenjena na devetostepenoj skali (1–2 = neosetljivi na ukus, 3–7 = osetljivi, 8–9 = superosetljivi). Nestimulisana pljuvačka se prikupljala pomoću ispljuvavanja koje je trajalo tri minuta, dok se stimulisana pljuvačka dobijala žvakanjem komadića lateksa. Beležen je protok pljuvačke (mL/min). Podaci su analizirani u programu SPSS v22; vrednost p < 0,05 smatrala se statistički značajnom nakon primene odgovarajućih parametarskih i neparametarskih testova.*
Rezultati: *Ispostavilo se da pacijenti sa OSMF-om imaju značajno niže vrednosti PTC-a (1,2 ± 0,5) i manji protok pljuvačke (stimulisana: 2,3 ± 0,2 mL/min, nestimulisana: 0,5 ± 0,1 mL/min) nego ispitanici iz kontrolne grupe. Pacijenti sa T2DM-om imali su smanjeno lučenje pljuvačke (stimulisana: 1,6 ± 0,3 mL/min, nestimulisana: 0,4 ± 0,2 mL/min), ali su zadržali umerenu osetljivost na ukus (5,6 ± 1,3). Pokazalo se da su kod ispitanika iz zdrave kontrolne grupe najviše vrednosti (7,8 ± 0,7) i najveće vrednosti protoka pljuvačke.*
Zaključak: *I OSMF i T2DM povezani su sa poremećenom percepcijom ukusa i funkcijom pljuvačnih žlezda. Testiranje fenil-tiokarbamidom može predstavljati jednostavan dopunski alat u oralnoj dijagnostici. Takođe, uključivanje genotipizacije receptora TAS2R38 moglo bi unaprediti buduća istraživanja.*

Ključne reči: *dijabetes melitus, oralna submukozna fibroza, fenil-tiokarbamid, pljuvačka, percepcija ukusa*

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Abstract

Introduction: *Altered taste perception and reduced salivary gland function are frequently reported in systemic and oral diseases, including oral submucous fibrosis (OSMF) and type 2 diabetes mellitus (T2DM). Genetic sensitivity to the bitter compound phenylthiocarbamide (PTC), mediated by TAS2R38 receptors, may provide insights into disease-related changes.*
Materials and Methods: *Ninety participants were equally divided into three groups (OSMF, T2DM, and healthy controls). Taste sensitivity was tested using standardized PTC strips placed on the dorsal tongue for 10 seconds and scored on a 9-point scale (1–2 = non-tasters, 3–7 = tasters, 8–9 = super-tasters). Unstimulated saliva was collected by the spitting method for three minutes, while stimulated saliva was obtained by chewing latex pieces. Flow rates (mL/min) were recorded. Data were analyzed using SPSS v22, with p < 0.05 considered significant, and appropriate parametric and non-parametric tests.*
Results: *Patients with OSMF showed markedly lower PTC scores (1.2 ± 0.5) and salivary flow (stimulated: 2.3 ± 0.2 mL/min; unstimulated: 0.5 ± 0.1 mL/min) compared with controls. Patients with T2DM had reduced salivary secretion (stimulated: 1.6 ± 0.3 mL/min; unstimulated: 0.4 ± 0.2 mL/min) but retained moderate taste sensitivity (5.6 ± 1.3). Healthy controls demonstrated the highest scores (7.8 ± 0.7) and flow rates.*
Conclusion: *Both OSMF and T2DM are associated with impaired taste perception and salivary function. Phenylthiocarbamide testing may be a simple adjunctive tool in oral diagnostics, while integration of TAS2R38 genotyping could enhance future research.*

Key words: *diabetes mellitus, oral submucous fibrosis, phenylthiocarbamide, saliva, taste perception*

Introduction

Taste receptor cells, located within taste buds of the mouth, throat, larynx, and esophagus, play a crucial role in gustatory perception. In patients with oral submucous fibrosis (OSMF), taste dysfunction may arise from chronic inflammation, reduced blood supply to the tongue, and impaired saliva production, leading to injury of the receptor cells or atrophy of taste buds[1,2,3].

Phenylthiocarbamide (PTC) was first synthesized by Arthur Fox in 1931 and has since been used as a classic genetic marker in population studies[4]. The TAS2R38 gene, discovered in 2003, encodes bitter taste receptors responsible for PTC perception. Two common alleles—one associated with the "taster" phenotype and the other with the "non-taster" phenotype—along with several rare alleles, contribute to inter-individual variability in bitter sensitivity[5,6].

In type 2 diabetes mellitus (T2DM), vascular changes, neuropathy, and impaired immunity can contribute to taste perception disturbances. Reduced salivary secretion, altered composition, and damage to sensory nerve endings in papillae may further exacerbate these changes[7]. Salivary parameters such as flow rate, pH, and protein composition play an important role in gustatory function, as diminished secretion is often associated with impaired taste[8].

Given these associations, the present study aimed to evaluate PTC-mediated genetic taste sensitivity and salivary flow dynamics in OSMF and T2DM patients, and to compare them with healthy controls.

Materials and Methods

This comparative cross-sectional study included 90 participants aged 25–80 years, equally distributed into three groups: patients with oral submucous fibrosis (OSMF), patients with type 2 diabetes mellitus (T2DM), and healthy controls. Participants were selected based on defined eligibility criteria. Individuals with oral submucous fibrosis (OSMF) who did not have any associated systemic illness were included, along with patients diagnosed with type 2 diabetes mellitus who were free from oral lesions and did not report any adverse habits. Healthy participants without any systemic disease or oral pathology were also recruited. Exclusion criteria comprised pregnant individuals, patients with syndromic conditions, and those with systemic, neoplastic, or neurological disorders. Additionally, individuals who were unwilling to participate or those presenting with potential confounding factors such as tobacco or alcohol use, or the intake of medications known to influence taste perception, were excluded from the study.

Taste perception was assessed using PTC paper strips placed on the dorsal tongue for 10 seconds. Participants rated bitterness on a 9-point scale. Scores 1–2 = non-tasters, 3–7 = tasters, 8–9 = super-tasters (Figure 1). In this study, only phenotypic assessment of TAS2R38-mediated bitter taste sensitivity was performed using PTC strips; direct genotyping was not included.

The unstimulated saliva flow rate was detected under controlled temperature and humidity at 11:00, using the spitting method. Unstimulated whole saliva (UWS) was collected for 3 minutes: the undisturbed subject, sitting in a comfortable position, swallowed residual saliva present in the mouth before the beginning of the collection, and then, with the head down and mouth slightly open, saliva was allowed to drip from the lower lip into a pre-weighed, sterile plastic test tube. In the last few seconds of the 3 min, saliva accumulated in the mouth was spat out into the plastic funnel. No other conscious movements of the oral musculature were made during the collection.

Stimulated saliva was obtained with latex pieces (1.0 cm thickness and 12 mm diameter) previously sterilized. Patients expelled saliva into another plastic beaker for 3 minutes (Figure 2).

Data were entered in MS Excel and analyzed using SPSS v22. Descriptive statistics were expressed as mean ± SD or median (IQR), depending on data distribution. Normality was assessed using the Shapiro–Wilk test. ANOVA, Dunnett's, Tukey's, Kruskal–Wallis, and Mann–Whitney U tests were applied where appropriate. A p-value < 0.05 was considered statistically significant.

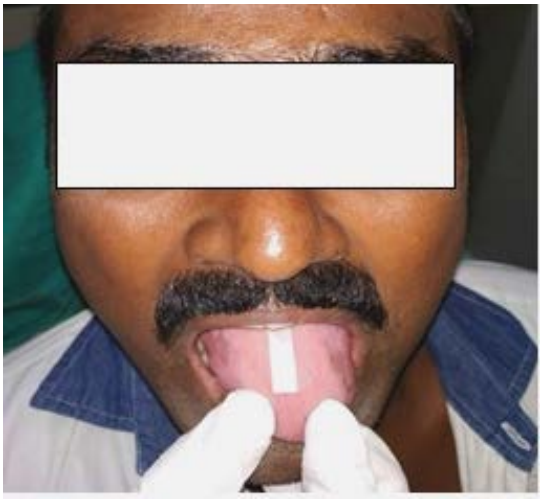

Figure 1. Image showing examination of taste perception using PTC paper strip

Figure 2. Image showing a collection of patient's saliva samples

Results

In this study, we examined 90 respondents aged 25 to 80 years, divided into 3 groups: OSMF, T2DM, and healthy, with each group comprising 30 respondents.

The mean stimulated salivary flow rates were 2.3 ± 0.2 mL/min for OSMF patients, 1.6 ± 0.3 mL/min for T2DM patients, and 3.5 ± 0.4 mL/min for healthy controls (Table 1).

The unstimulated salivary flow rate in OSMF patients was recorded as 0.5 ± 0.1 mL/min, T2DM patients 0.4 ± 0.2 mL/min, and healthy participants 1.1 ± 0.3 mL/min (Table 2).

PTC taste perception and salivary flow rates were significantly different among the groups. When comparing taste perception among three groups using PTC strips, OSMF patients recorded the lowest scores (mean 1.2 ± 0.5), indicating a non-taster phenotype. T2DM patients scored 5.6 ± 1.3, while healthy controls showed the highest sensitivity (7.8 ± 0.7) (Table 3, Figure 3).

Among all evaluated mandibular third molars in Group I, angulation increased in 32.14% of cases, decreased in 46.43%, and remained unchanged in 21.43%. In Group II, angulation increased in 71.43% of third molars, decreased in 17.86%, and remained unchanged in 10.71% of cases (Table 4).

Table 1. Showing stimulated salivary flow rates in all three groups

Variable	Group	**Mean**	**SD**	Median	IQR	Min.	Max.	P-value	O vs. D	O vs. H	D vs. H
Stimulated saliva	OSMF	2.3	0.2	2.3	.3	1.8	2.6	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	T2DM	1.6	0.3	1.6	.5	1.0	2.2				
	Healthy	3.5	0.4	3.5	.6	2.7	4.0				

Table 2. Unstimulated salivary flow rates in all three groups

Variable	Group	Mean	SD	**Median**	**IQR**	Min.	Max.	P-value	O vs. D	O vs. H	D vs. H
Unstimulated saliva	OSMF	0.5	0.1	0.5	.1	.3	.7	**< 0.001**	**0.012**	**< 0.001**	**< 0.001**
	T2DM	0.4	0.2	0.4	.2	.2	1.0				
	Healthy	1.1	0.3	1.2	.5	.7	1.5				

Table 3. Comparison of taste perception among the three groups

Variable	Group	Mean	SD	**Median**	**IQR**	Min.	Max.	P-value	O vs. D	O vs. H	D vs. H
Score	OSMF	1.2	0.5	1.0	0.0	1.0	3.0	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	T2DM	5.6	1.3	6.0	1.3	2.0	7.0				
	Healthy	7.8	0.7	8.0	1.0	6.0	9.0				

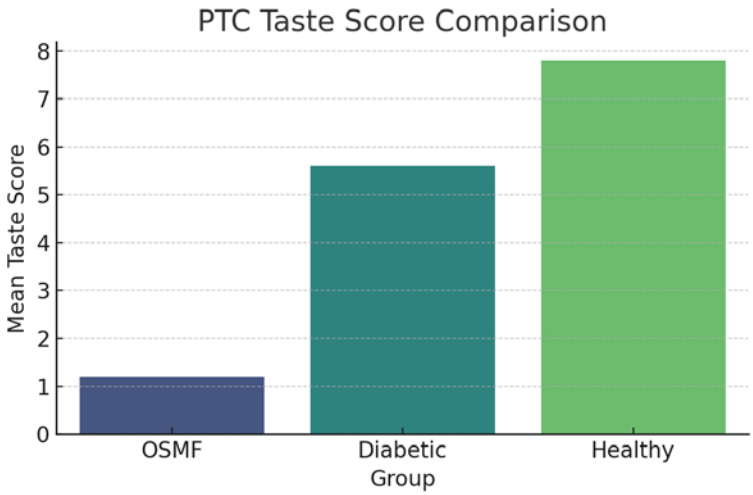

Figure 3. PTC taste score comparison by group

Discussion

This study demonstrates significant impairments in gustatory perception and salivary function among patients with OSMF and T2DM compared with healthy controls. Although both groups exhibited reduced salivary flow, the pattern of taste dysfunction differed, reflecting the combined effects of genetic predisposition, salivary physiology, and disease-specific pathology.

Taste Physiology and Salivary Contribution

Taste buds, located primarily on the tongue papillae, contain receptor cells that transmit chemical signals via cranial nerves VII, IX, and X[6,9]. Saliva is essential for dissolving tastants, transporting them to receptor sites, and maintaining taste bud function. Reduced secretion impairs this process, causing receptor damage, atrophy of taste buds, and ultimately diminished sensitivity[10,11]. In this study, both OSMF and T2DM patients showed significantly reduced stimulated and unstimulated salivary flow compared with controls, consistent with earlier findings by Kadher et al., who reported markedly reduced secretion in OSMF patients[12]. In diabetes, hyposalivation may be aggravated by hyperglycemia, neuropathy, and microvascular dysfunction, with severity correlating with glycemic control[7,13].

TAS2R38 Genetic Variation and Phenotypic Testing

Phenylthiocarbamide strips were used in this study as a phenotypic tool to assess genetic taste sensitivity. Phenylthiocarbamide, also known as phenylthiourea, belongs to the thiourea class of organosulfur compounds and is notable for its highly variable taste perception—ranging from very bitter to tasteless—depending on individual genetic makeup[14]. This inter-individual variability is a classic Mendelian trait, primarily mediated by polymorphisms in the TAS2R38 gene[4,14].

The TAS2R38 gene, located on chromosome 7q, encodes the T2R38 bitter receptor protein. PTC binds to the apical microvilli of taste receptors encoded by this gene, triggering G-protein–coupled signaling via gustducin and initiating a cascade that produces the sensation of bitterness[15]. Three nonsynonymous polymorphisms (A49P, V262A, I296V) give rise to two predominant haplotypes: PAV, associated with the taster phenotype, and AVI, associated with the non-taster phenotype. Individuals homozygous for PAV/PAV perceive strong bitterness, AVI/AVI homozygotes are insensitive, and heterozygotes (PAV/AVI) exhibit intermediate sensitivity. Importantly, TAS2R38 is one of the few taste receptor genes with a consistent genotype–phenotype correlation for both PTC and PROP (6-n-propylthiouracil) sensitivity[16,17].

In this study, phenotypic testing was performed using PTC strips. Although this approach is validated and widely used to evaluate genetic taste variation[10], it cannot fully differentiate between true genetic non-tasters and individuals whose reduced sensitivity results from disease-related local changes. Nevertheless, prior research has demonstrated strong concordance between PTC phenotype and TAS2R38 genotype18, supporting the clinical relevance of phenotypic testing as a practical and accessible method in oral diagnostic research.

Impact of OSMF on Taste Perception

OSMF is a chronic, progressive, and irreversible condition characterized by epithelial atrophy and submucosal fibrosis. Chronic areca nut chewing contributes to local cytotoxicity through the release of alkaloids, flavonoids, polyphenols, and nitrosamines, which undergo nitrosation and generate oxidative stress[10,19]. These biochemical alterations, combined with fibrosis, compromise salivary gland function and directly impair taste buds, resulting in diminished gustatory sensitivity. In this study, OSMF patients frequently exhibited a non-taster phenotype, aligning with previous findings of altered perception across sweet, sour, salty, and bitter modalities, with advanced stages particularly affecting salt sensitivity[2,11,20,21].

Impact of T2DM on Taste Perception

In contrast, T2DM patients retained partial PTC sensitivity despite reduced salivary flow. This suggests that TAS2R38 receptor function may remain relatively intact, with taste deficits arising instead from neuropathy, vascular compromise, and salivary gland dysfunction[7,13,22]. These findings highlight distinct pathogenic mechanisms in OSMF and diabetes.

Confounding Factors

Taste and salivary function can be influenced by age, sex, medications, nutrition, and lifestyle habits[8]. Although the present study minimized comorbidities through exclusion criteria, residual confounding cannot be entirely excluded. Larger studies with stratification and multivariate analysis are needed to address these influences more robustly.

Relationship between Salivary Flow and Taste

This study confirmed that both OSMF and T2DM groups had significantly lower salivary flow than controls. Patients with the lowest salivary secretion also tended to exhibit the poorest PTC sensitivity, supporting the hypothesis that saliva plays a critical role in maintaining taste function[16]. However, the persistence of moderate PTC sensitivity in T2DM patients despite hyposalivation suggests that factors beyond saliva, such as neuropathy, may modulate taste in T2DM[6].

Clinical Implications

Altered taste perception and xerostomia can significantly affect dietary choices, reduce nutritional intake, and impair quality of life[23]. For dental practitioners, recognizing these deficits can aid in counseling patients, guiding dietary modifications, and preventing malnutrition-related complications. Early detection of taste and salivary changes may also serve as a marker for disease progression or severity in OSMF and T2DM.

Certain limitations should be considered when interpreting these findings. The absence of TAS2R38 genotyping limits the ability to distinguish between genetic influences and disease-related changes in taste perception. The cross-sectional nature of the study also restricts any inference of causal relationships. In addition, the relatively small sample size may affect the generalizability of the results, and despite careful selection criteria, the possibility of residual confounding cannot be completely excluded.

Conclusion

This study highlights that patients with OSMF show marked impairment in bitter taste perception, largely presenting as non-tasters, while those with T2DM retain partial sensitivity despite reduced salivary flow. These findings suggest that local pathological changes such as fibrosis and salivary alteration, play a dominant role in OSMF, whereas neuropathic and metabolic factors contribute to diabetes. Although only phenotypic testing was performed, the results underscore the clinical relevance of taste assessment as an early indicator of disease impact. Future studies integrating TAS2R38 genotyping and salivary profiling are essential to better differentiate genetic predisposition from disease-related impairments and to improve nutritional and quality-of-life outcomes in these patients.

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MUKOGINGIVALNI PRISTUP POSTAVLJANJU IMPLANTANATA U MASKILARNOJ REGIJI: PRIKAZ NIZA SLUČAJEVA

MUCOGINGIVAL APPROACH TO IMPLANT PLACEMENT IN THE MAXILLARY AREA: A CASE SERIES

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Sažetak

Uvod: *Postavljanje implantata u maksilarnoj regiji predstavlja jedinstven hirurški i protetički izazov zbog visokih zahteva u pogledu estetike i čestog prisustva deficita kostiju i mekih tkiva. Nedavno predloženi mukogingivalni pristup kombinuje principe regenerativne i mukogingivalne hirurgije sa ugradnjom implantata, s ciljem optimizacije ishoda periimplantantnih mekih tkiva.*
Cilj: *Cilj rada bio je da se opišu hirurški protokol i kratkoročni klinički ishodi triju slučajeva ugradnje implantata primenom mukogingivalnog pristupa u različitim kliničkim scenarijima: ranoodloženom, odloženom i pri neposrednom postavljanju.*
Metode: *Tri pacijenta sa jednostrukom bezubosti u maksilarnoj regiji lečena su primenom mukogingivalnog pristupa, koji je uključivao split-full-split koronarno pomereni bukalni režanj, transplantaciju vezivnog tkiva i protetičko kondicioniranje mekih tkiva. Svim pacijentima su ugrađeni transmukozni implantati (TRI Octa, Švajcarska).*
Rezultati: *Svi implantati su uspešno integrisani u kost i održali su stabilne nivoe krestalne kosti u periodu od šest do dvanaest meseci. Periimplantantna mukoza je pokazala zadovoljavajuću debljinu, visinu i popunjenost papila; takođe, ishodi u pogledu estetike bili su povoljni.*
Zaključak: *Mukogingivalni pristup predstavlja svestranu i efikasnu tehniku postavljanja implantata u maksilarnoj regiji, budući da istovremeno omogućava rešavanje deficita mekih tkiva i korekciju susednih mukogingivalnih problema. Da bi se ovi rezultati potvrdili, potrebno je sprovesti dalje kontrolisane kliničke studije sa dužim periodom praćenja.*

Ključne reči: *mukogongivalni pristup, neposredna ugradnja implantata, estetska zona, augmentacija mekih tkiva, transplantat vezivnog tkiva, koronarno pomereni režanj*

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Abstract

Introduction: *Implant placement in the maxillary region presents unique surgical and prosthetic challenges due to high esthetic demands and the frequent presence of bone and soft-tissue deficiencies. The recently proposed mucogingival approach combines principles of regenerative and mucogingival surgery with implant placement to optimize peri-implant soft-tissue outcomes.*
Aim: *The paper aimed to describe the surgical protocol and short-term clinical outcomes of three implant cases placed with the mucogingival approach in different clinical scenarios: early-delayed, delayed, and immediate placement.*
Methods: *Three patients with single-tooth edentulism in the maxillary region were treated with mucogingival approach, involving a split-full-split coronally advanced buccal flap, connective-tissue grafting, and prosthetic soft-tissue conditioning. All patients received transmucosal implants (TRI Octa, Switzerland).*
Results: *All implants osseointegrated successfully and maintained stable crestal bone levels at 6–12 months. The peri-implant mucosa exhibited satisfactory thickness, height, and papillary fill, with favorable esthetic outcomes.*
Conclusion: *The mucogingival approach is a versatile and effective technique for implant placement in the maxillary area, enabling simultaneous management of soft-tissue deficiencies and correction of adjacent mucogingival problems. Controlled clinical studies with longer follow-ups are warranted to further validate these findings.*

Key words: *mucogingival approach, immediate implant placement, esthetic zone, soft-tissue augmentation, connective-tissue graft, coronally advanced flap*

Introduction

The replacement of missing teeth with dental implants is a predictable and evidence-based procedure yielding high long-term success rates and patient satisfaction. However, implant placement in the maxillary area continues to challenge clinicians due to the complex interplay between bone anatomy, soft-tissue architecture, and esthetic expectations[1].

Traditionally, the "esthetic zone" refers to the anterior maxilla, though patients' perception increasingly extends it to the mandibular anterior region and even to the premolar areas. Implants in these areas may be placed at various time points following extraction—immediate, early, or delayed—each with distinct biological and esthetic risks. Immediate placement, while reducing treatment time, is prone to esthetic complications, particularly when buccal bone or mucosal integrity is compromised[2].

The mucogingival approach, recently introduced by Zucchelli et al., as an alternative to flapless immediate placement, aims to combine the benefits of open surgical visibility with mucogingival and regenerative principles[3–4]. It enables correction of hard- and soft-tissue defects, coronal advancement of the gingival margin, and prosthetic soft-tissue conditioning. This technique is particularly advantageous in cases with damaged buccal bone walls or adjacent mucogingival defects, where traditional flapless immediate placement is contraindicated[5].

The present case series demonstrates the clinical application of the mucogingival approach in three different implant placement scenarios, evaluating its short-term outcomes in terms of peri-implant soft-tissue stability and esthetics.

Materials and Methods

Case Selection

Three systemically healthy patients (two males, one female; mean age = 41 years) requiring single implants in the anterior maxilla were included. Inclusion criteria:

(1) age > 18 years; (2) American Society of Anesthesiologists (ASA) I–II status; (3) good oral hygiene (full mouth plaque score (FMPS) < 20%, full mouth bleeding score (FMBS) < 20%).

Exclusion criteria: pregnancy or lactation, untreated periodontitis, bisphosphonate or oncologic therapy, and heavy smoking (> 10 cigarettes/day)[6].

Treatment planning was prosthetically driven using cone beam computed tomography (CBCT) analysis. The straightforward, advanced, complex (SAC) classification developed by the International Team for Implantology (ITI) was applied to assess surgical difficulty and risk[7]. Patients provided informed consent after receiving detailed explanations of surgical and prosthetic procedures.

A standardized medication protocol included amoxicillin 1 g/8 h for 5 days, ibuprofen as needed, and 0.12% chlorhexidine rinses twice daily for 7 days.

Surgical Protocol

Flap Design

Following local anesthesia (4% articaine + 1:200,000 adrenaline), a split-full-split coronally advanced mucogingival flap[8] was designed. The flap included at least one adjacent papilla mesial and distal to the edentulous site. Oblique submarginal incisions extended from the gingival margin of neighboring teeth toward the mucosa, at a distance corresponding to the desired coronal advancement.

The supracrestal portion was elevated in split thickness, followed by full-thickness reflection over 2–3 mm of bone exposure, and a final apical split to release muscular tension. Deep and superficial incisions were made parallel to the bone and mucosal surface, respectively, to achieve passive flap mobility. Anatomical papillae were de-epithelialized to create a vascular bed for surgical papilla adaptation.

Final suturing used external sling sutures (5-0 PGA and polypropylene, 12 mm needle), proceeding from the periphery toward the center of the flap.

Connective-Tissue Harvesting

Soft-tissue augmentation employed extraorally de-epithelialized free gingival grafts from the lateral palate, offering superior consistency and absence of glandular tissue even in thin palates[9]. The combined flap + graft thickness was standardized at ≈ 2 mm.

Graft width extended to neighboring papillae, and vertical dimension exceeded the buccal crest by 2–3 mm. The graft was sutured either to the anatomical papillae or to the inner

surface of the flap using 6-0 PGA sutures, depending on tissue resilience.

Implant Placement

All cases utilized transmucosal, tissue-level implants (TRI Octa, Switzerland). These implants have a polished collar favoring mucosal adhesion and an implant–abutment junction located coronally, away from the crestal bone[10].

Implants were placed free-hand following the CBCT plan, with osseointegratable (rough) surface 3–4 mm apical to the gingival margin of contralateral teeth, and within the alveolar contour of adjacent teeth. When achieved, a minimum torque > 35 Newton centimeters (Ncm) allowed immediate provisionalization.

Prosthetic Phase

Prosthetic soft-tissue conditioning commenced immediately after implant placement. The first provisional restoration (in the immediate case) was a composite crown relined on a titanium base; subsequent provisionals were polymethyl methacrylate (PMMA) screw-retained crowns.

Provisional contours were modified over 3–6 months according to the esthetic biological contour concept (EBC), guiding papillary fill and gingival margin position[11]. The implant stability at the timepoint of final restoration delivery was objectively measured using the implant stability quotient scale (ISQ), which was above 70 for all implants. Final restorations were screw-retained zirconia crowns cemented extraorally on titanium bases. The final esthetic outcome was evaluated utilizing the pink and white esthetic scores (PES/WES), with all implant restorations presenting high scores—above[12].

Results

Case 1—Early-Delayed Placement (Tooth 12)

A 39-year-old male presented after the extraction of tooth 12 due to recurrent infection and fistula. Tooth 13 displayed a Cairo I recession. The case was classified as complex according to the SAC classification. Four months post-extraction, a mucogingival flap was elevated, and the site was prepared via osseodensification (Densah burs). A TRI Octa implant was inserted with > 40 Ncm torque, positioned 3–4 mm below the gingival margin. A 1 mm titanium-base composite provisional was fabricated and immediately loaded.

Three months later, a PMMA provisional refined soft-tissue contours; at five months, a zirconia crown was delivered. Stable bone level and papillary fill were observed with high patient satisfaction.

Case 1 (Figures 1–19) Early-delayed implant placement and soft-tissue maturation

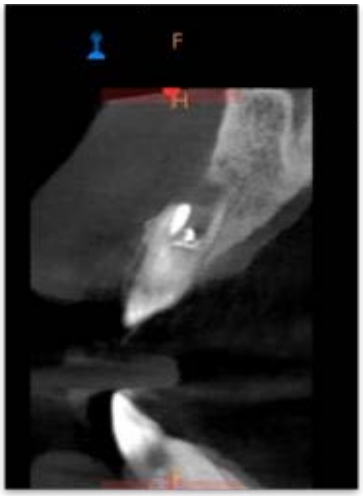

Figure 1*. CBCT imaging*

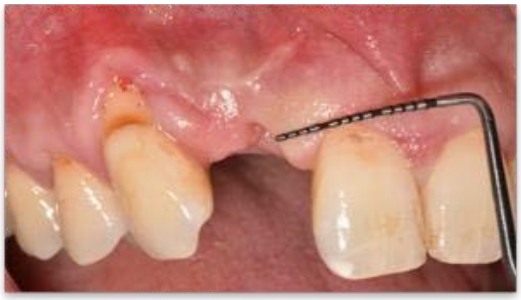

Figure 2. Flap design

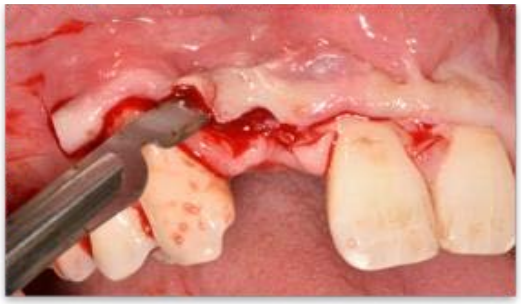

Figure 3. Split-full-split ffap elevation

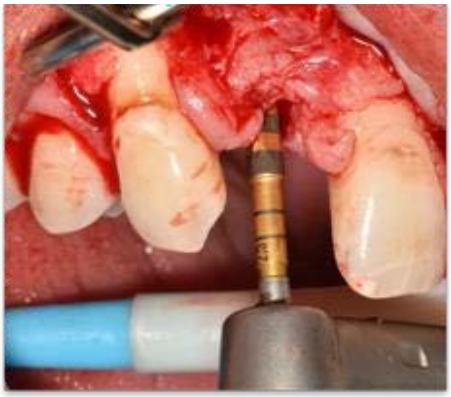

Figure 4. Osseodensification

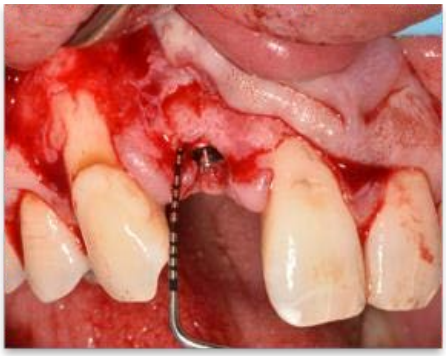

Figure 5. Implant placement

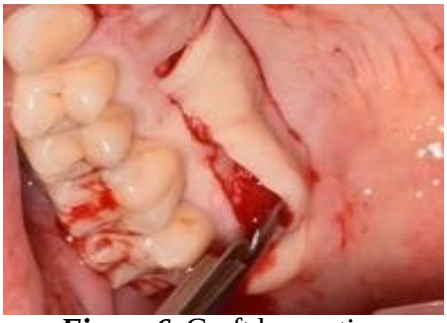

Figure 6. Graft harvesting

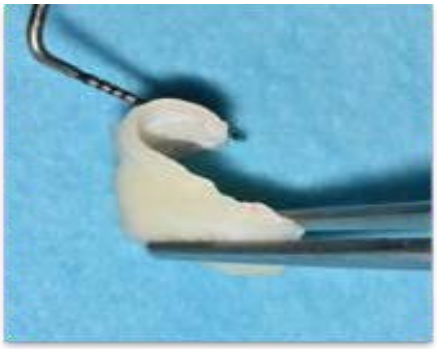

Figure 7. Graft plasticity

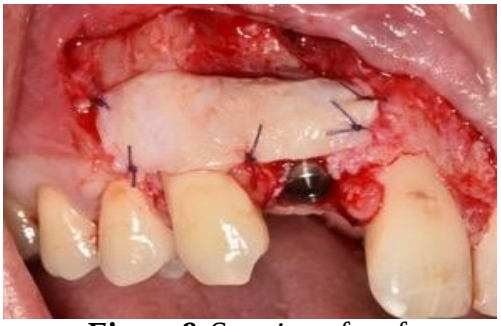

Figure 8. Suturing of graft

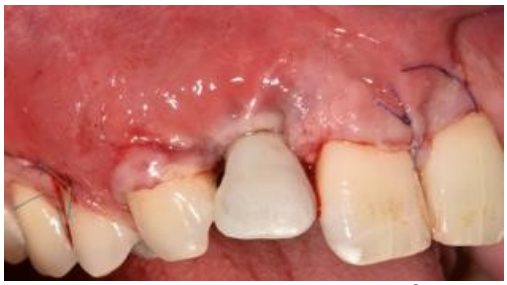

Figure 9. Flap sutured with first provisional

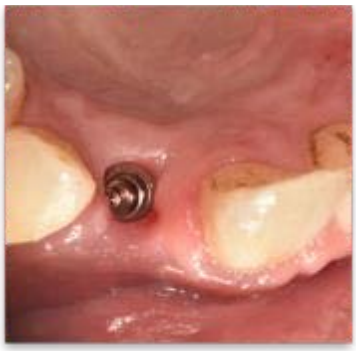

Figure 10. Emergence profile 3 months later

Figure 11. PMMA provisional

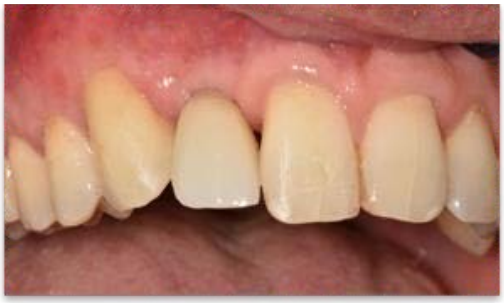

Figure 12. Second provisional in situ

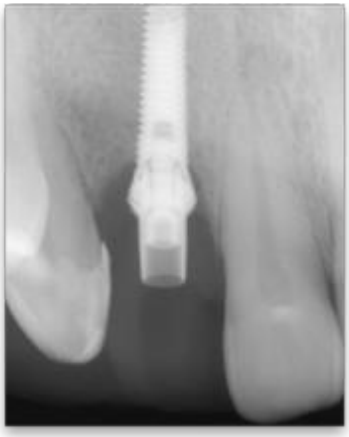

Figure 13. RVG image

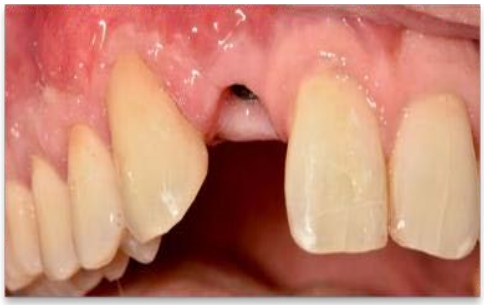

Figure 14. Emergence profile 5 moths later – frontal view

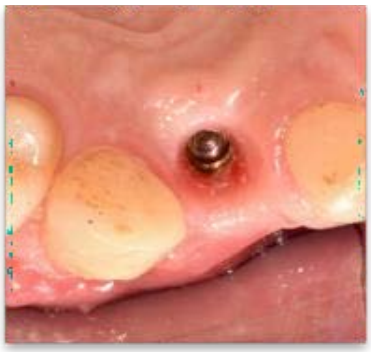

Figure 15. Occlusal view

Figure 16. Final Zr restoration

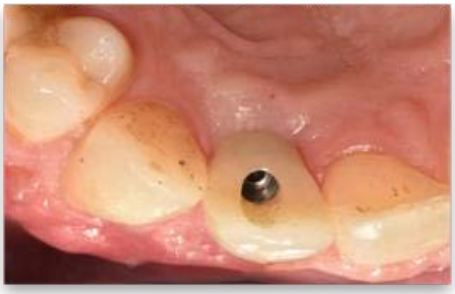

Figure 17. Occlusal view

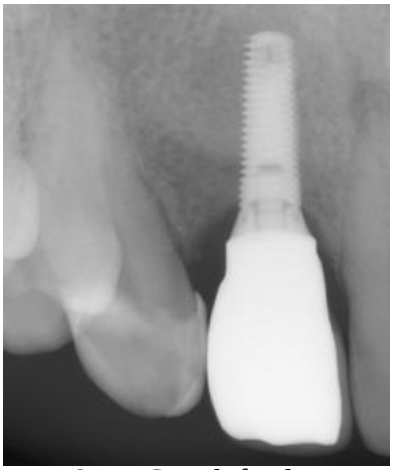

Figure 18. RVG with final restoration

Figure 19. Final restoration in upper arch

Case 2—Delayed Placement with Combined Augmentation (Tooth 24)

A 44-year-old male with a history of controlled periodontitis required implant placement at site 24, extracted 8 months earlier. Cone beam computed tomography revealed a horizontal-vertical defect and adjacent canine recession. This was an advanced SAC case.

A mucogingival split-full-split flap was elevated, and a transmucosal implant was placed 3 mm below the gingival margin, leaving coronal threads exposed. Buccal dehiscence was managed with an allograft (Maxxeus, USA) and an overlying connective-tissue graft acting as both membrane and soft-tissue thickening element (bilaminar technique)[12].

No immediate provisional was placed. At 4 months, partial root coverage of the canine and satisfactory buccal volume were achieved. Sequential PMMA provisionals over 5 months yielded improved papillary architecture before final zirconia crown delivery.

Case 2 (Figures 20–44) Bilaminar approach and prosthetic conditioning of peri-implant mucosa

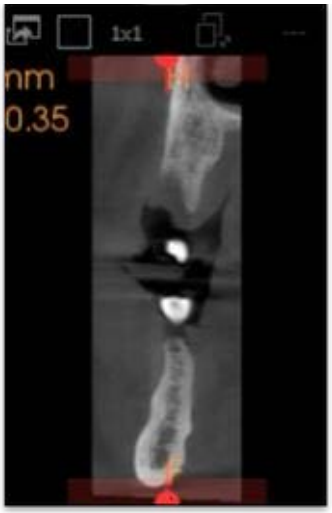

Figure 20. CBCT imaging

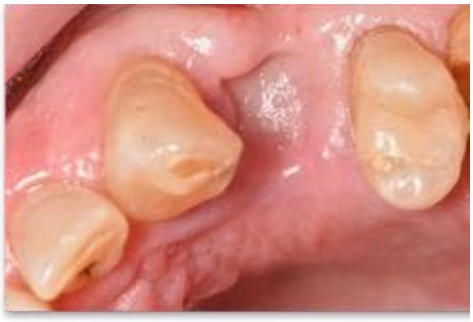

Figure 21. Occlusal pre-op view

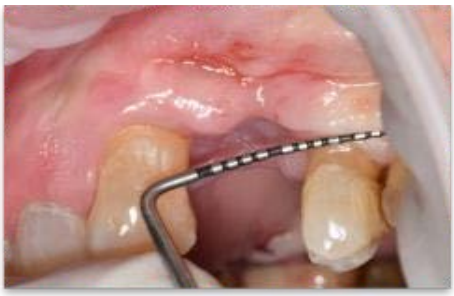

Figure 22. Flap design

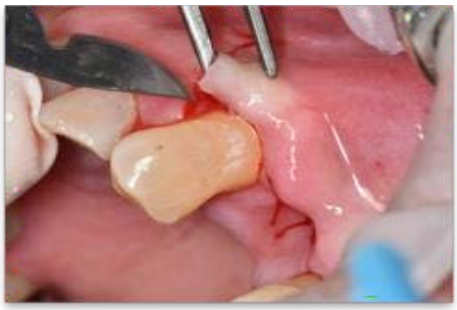

Figure 23. Split-full-split elevation

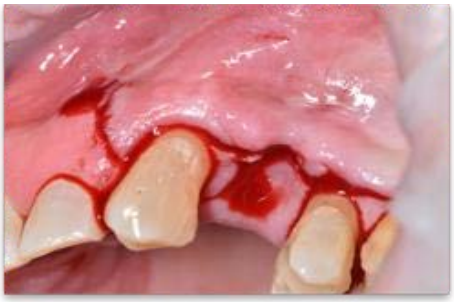

Figure 24. The ffap - occlusal view

Figure 25. Implant placement

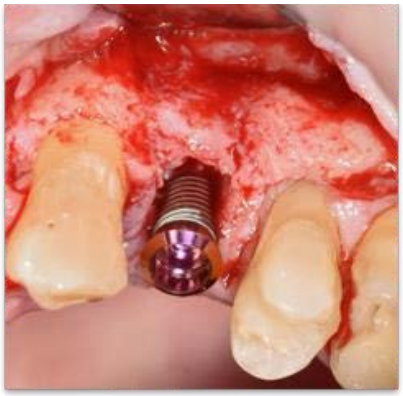

Figure 26. Final implant position

Figure 27. Anatomical papillae de-epithelization

Figure 28. Coronal advancement of ffap

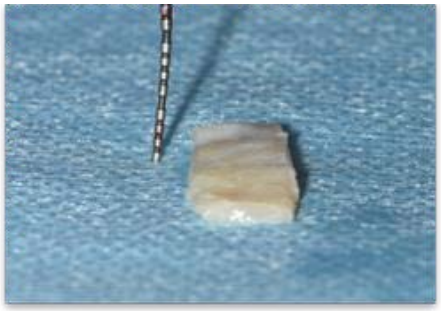

Figure 29. Graft thickness

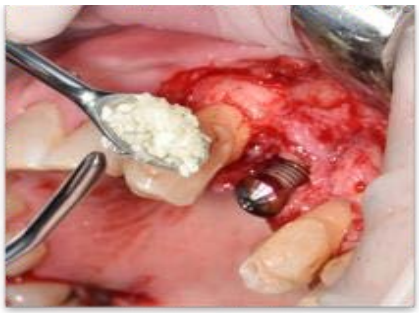

Figure 30. Bone graft introduction

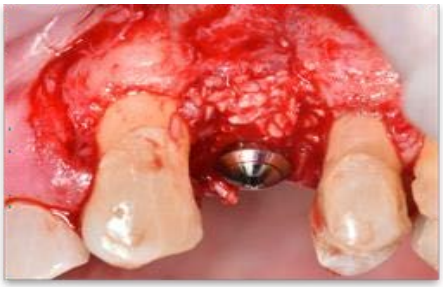

Figure 31. Defect filled up to the bony envelope

Figure 32. Soft tissue graft try-on

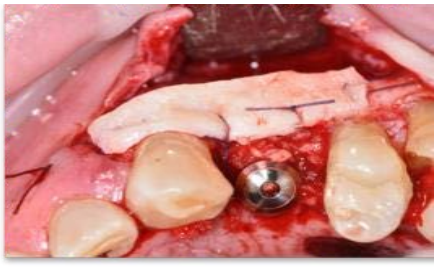

Figure 33. External matress sutures

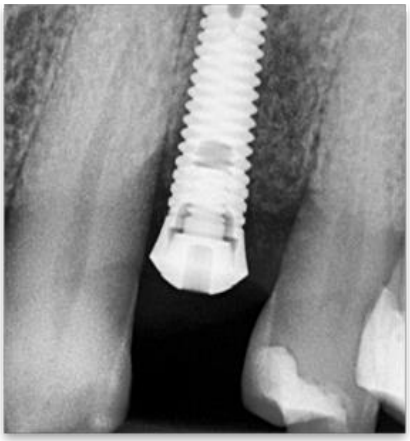

Figure 34. RVG post-op

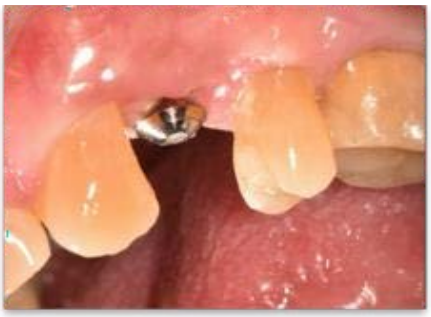

Figure 35. Lateral view 4 months post-op

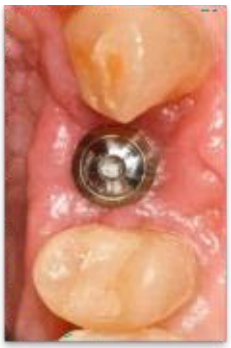

Figure 36. Occlusal view

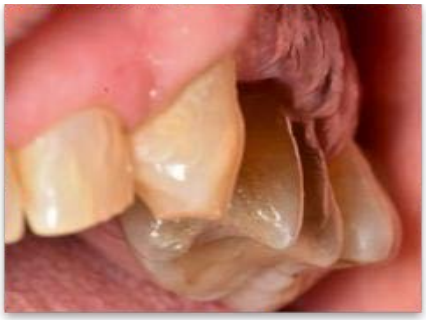

Figure 37. Frontal view

Figure 38. PMMA provisional

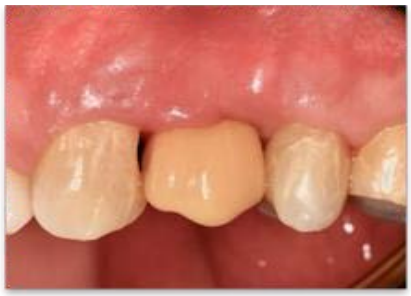

Figure 39. Prosthetic conditioning

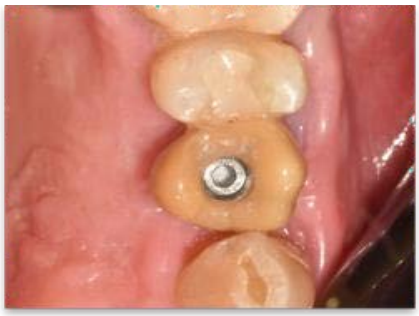

Figure 40. Occlusal view

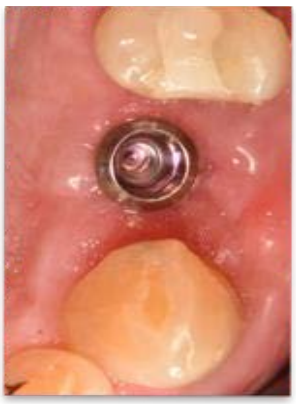

Figure 41. Emergence profile 5 months later

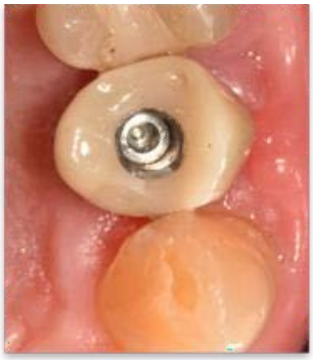

Figure 42. Final Zr restoration - occlusal view

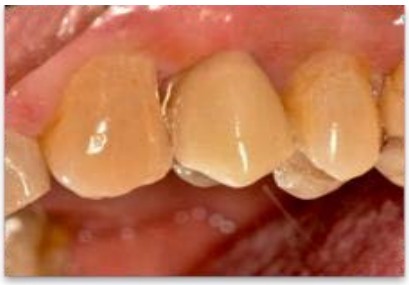

Figure 43. Lateral view

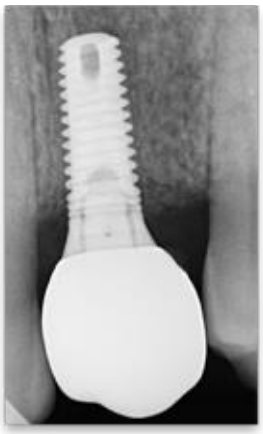

Figure 44. RVG with final restoration

Case 3—Immediate Placement with Buccal Defect (Tooth 12)

A 35-year-old female presented with a compromised tooth 12 due to failed endodontic treatment and recurrent pain. Cone beam computed tomography confirmed a complete loss of buccal bone but intact apical and interradicular bone. This was a complex SAC case.

A variable-thickness buccal mucogingival flap enabled atraumatic extraction and socket debridement. A TRI Octa implant was placed free-hand, with rough surface 3–4 mm below the gingival margin. Buccal reconstruction included allograft bone and a slow-resorbing pericardium collagen membrane fixed with pins[13].

An extraorally de-epithelialized palatal graft was sutured submarginally to the inner buccal flap using horizontal mattress sutures for optimal adaptation.

Immediate loading was deferred, and a temporary Maryland bridge was provided. After 4 months, a PMMA provisional was inserted and progressively modified every 1–2 months to stimulate tissue maturation. At 6 months, a final zirconia crown exhibited complete papilla fill, healthy peri-implant tissues, and radiographically stable bone.

Case 3 (Figures 45–76) Immediate implant placement with simultaneous hard- and soft-tissue reconstruction

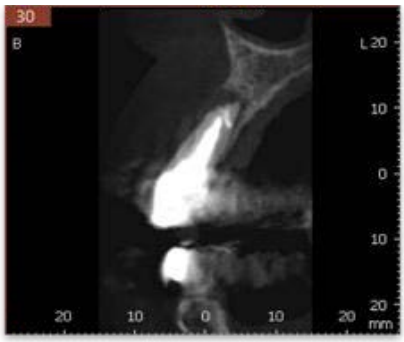

Figure 45. CBCT imaging

Figure 46. Frontal view

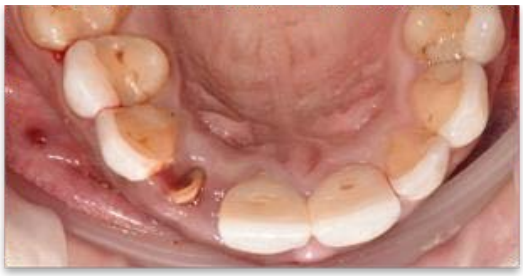

Figure 47. Occlusal view

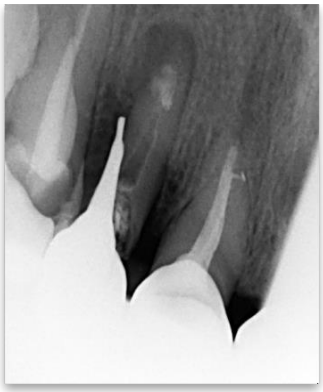

Figure 48. RVG pre-op

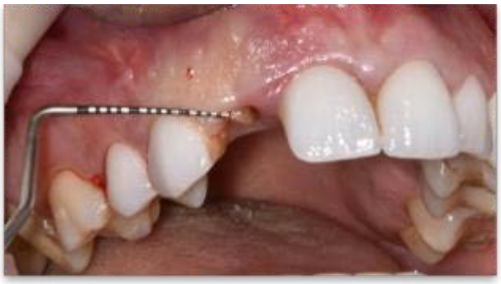

Figure 49. Flap design

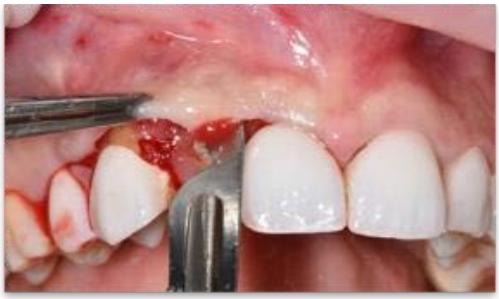

Figure 50. Split thickness elevation

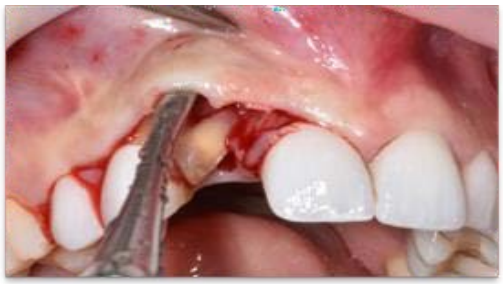

Figure 51. Full thickness elevation

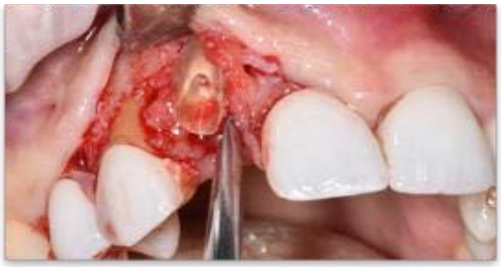

Figure 52. Extraction of root

Figure 53. Coronal advancement

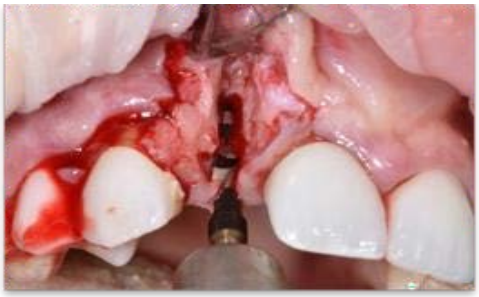

Figure 54. Osteotomy

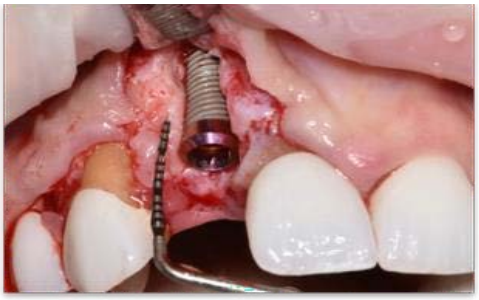

Figure 55. Implant placed

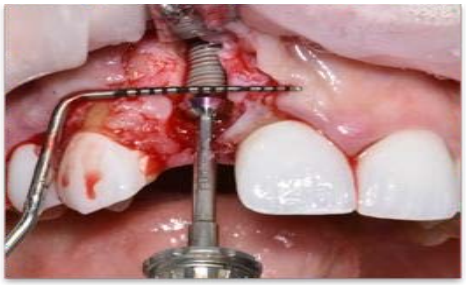

Figure 56. Within bony envelope

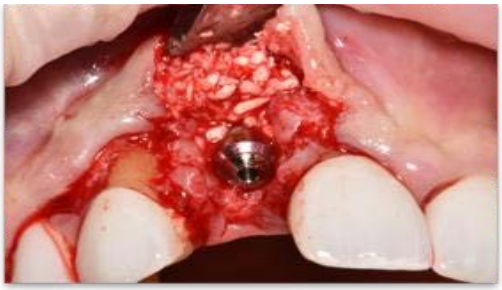

Figure 57. Membrane fixation

Figure 58. Harvested graft

Figure 59. Occlusal view

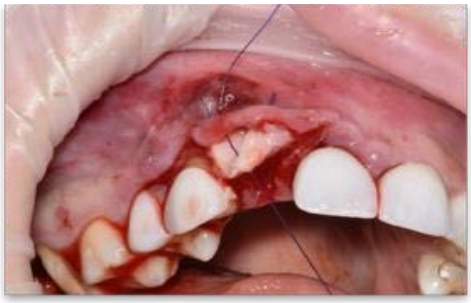

Figure 60. Graft sutured to inner side of ffap

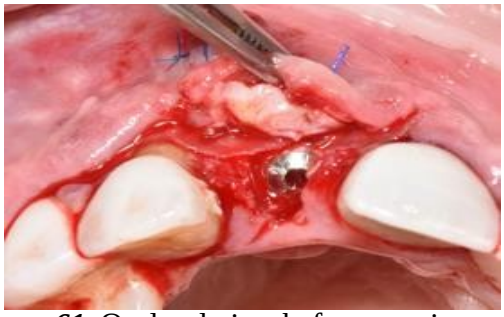

Figure 61. Occlusal view before suturing

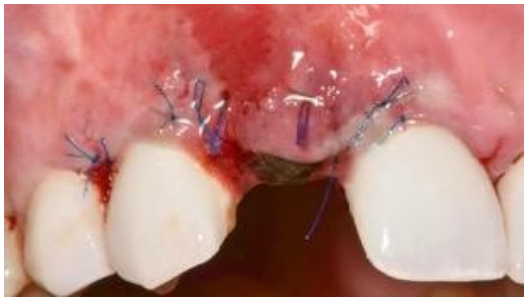

Figure 62. Frontal view with sutured ffap

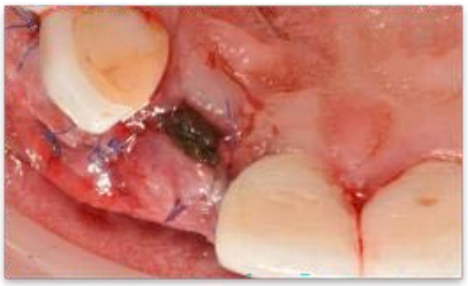

Figure 63. Occlusal view

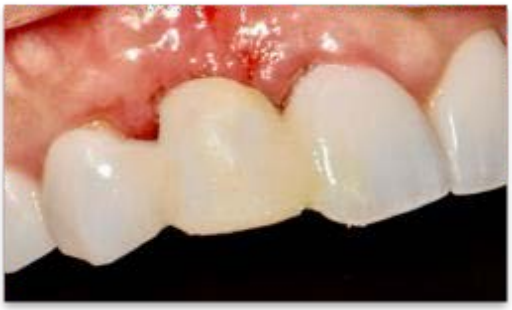

Figure 64. Maryland bridge

Figure 65. PMMA provisional to be screwed-in 4 monts later

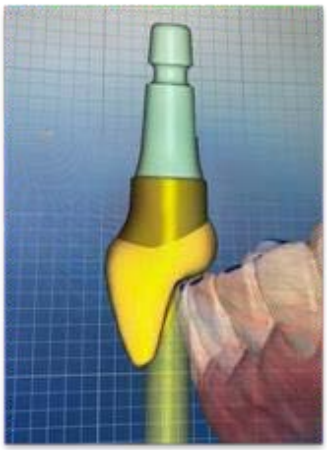

Figure 66. Design according to EBC concept

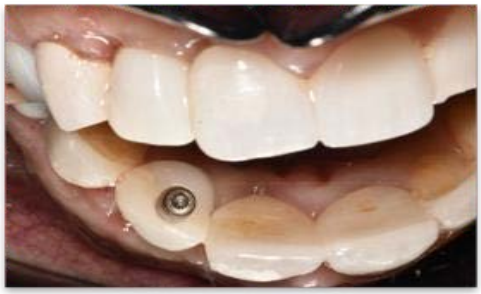

Figure 67. Occlusal view

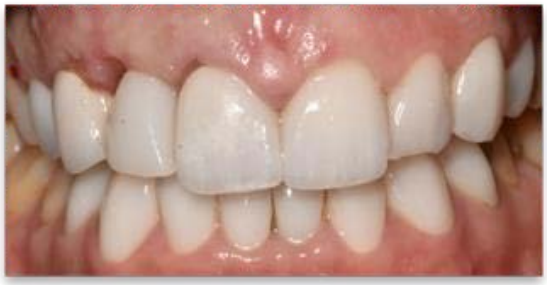

Figure 68. Frontal view with insufficient papillae

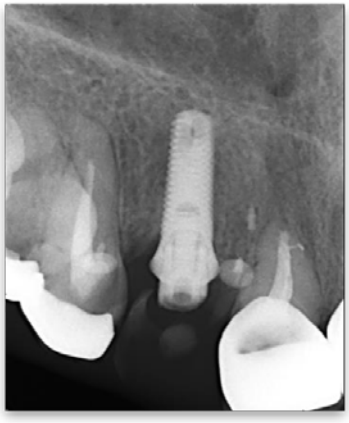

Figure 69. RVG with provisional

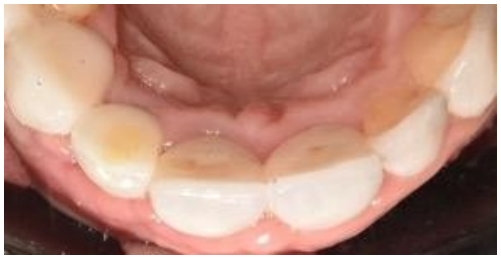

Figure 70. Occlusal view

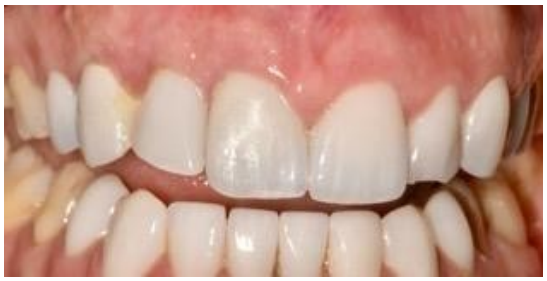

Figure 71. Papillae fill c months later

Figure 72. Emergence profile - frontal view

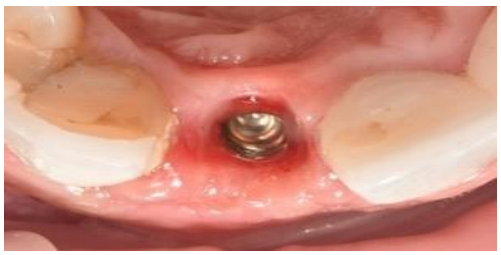

Figure 73. Occlusal view

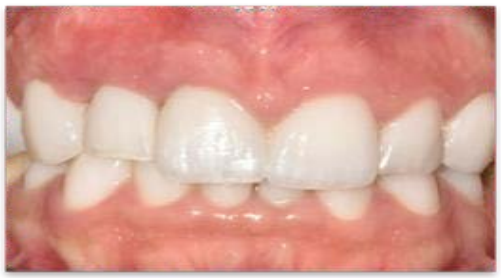

Figure 74. Final Zr restoration in dental arch - frontal view

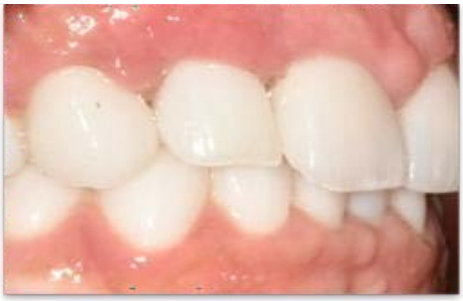

Figure 75 Lateral view

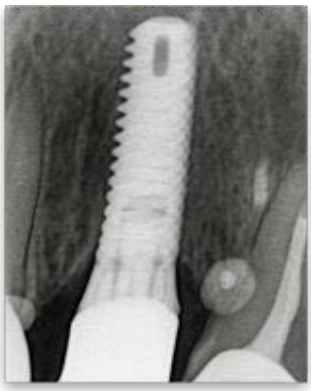

Figure 76. RVG with final Zr restoration

Discussion

The mucogingival approach integrates mucogingival and regenerative concepts into implant surgery, offering visibility, control, and the ability to simultaneously address bone and soft-tissue deficiencies[3–4,14]. Its main objectives include: 1) correction of neighboring gingival recessions, 2) visualization and augmentation of dehiscences, 3) coronal repositioning of the gingival margin, and 4) facilitation of prosthetic soft-tissue conditioning.

Raising a flap, especially in the esthetic zone, has traditionally been viewed as a risk factor for buccal bone resorption; however, current evidence indicates that buccal resorption primarily results from loss of bundle bone after extraction rather than from flap elevation itself. Moreover, damaged buccal bone is no longer considered a contraindication for immediate implant placement[14,15]. A well-designed mucogingival flap, when properly managed, does not increase morbidity and provides superior control of soft-tissue positioning compared to flapless protocols.

Immediate provisionalization plays a pivotal biological role, maintaining clot stability and graft adaptation, while also serving as a mechanical template for coronal tissue migration. In contrast, delayed loading may risk partial recession or papilla loss due to flap contraction during healing.

Implant positioning remains a conditio sine qua non for success with this approach. While free-hand placement demands high surgical expertise, guided implant surgery can further enhance accuracy, allowing the clinician to focus on soft-tissue management. When primary stability is inadequate or extensive regeneration is required, delayed loading remains a valid alternative, albeit with longer conditioning periods.

It was of paramount importance to evaluate and compare the clinical outcomes across the three clinical cases to conclude how this approach performs in different scenarios. In the present series, all three implants demonstrated uneventful healing, stable bone levels, and esthetically harmonious results after 6–12 months. The mucogingival approach proved adaptable to different timing protocols—immediate, early-delayed, and delayed—while consistently enabling correction of adjacent mucogingival anomalies[3,11].

Conclusion

The mucogingival approach represents an efficient and biologically sound technique for implant placement in the esthetic zone, particularly in cases with buccal bone or soft-tissue deficiencies and adjacent recession defects. It combines the precision of regenerative surgery with mucogingival and prosthetic conditioning principles, achieving predictable esthetic and functional outcomes.

Short-term results from this series indicate stable crestal bone levels, healthy peri-implant mucosa, and high patient satisfaction. Further prospective, randomized clinical trials with larger cohorts and extended follow-up are needed to substantiate its long-term advantages over traditional flapless and two-stage approaches.

Limitations

This study has several limitations that should be acknowledged.

First, it was based on a small number of cases (n = 3), which limits the generalizability of the findings and prevents statistical comparison or subgroup analysis.

Second, the included clinical cases were heterogeneous in terms of defect characteristics and treatment complexity, which may have influenced clinical outcomes

and reduced the ability to draw uniform conclusions across cases.

Third, the follow-up period was relatively short, and therefore, the long-term stability of the reported clinical results cannot be fully evaluated.

Despite these limitations, the present case series provides valuable preliminary clinical observations and may serve as a basis for future studies with larger and more homogeneous cohorts and longer follow-up.

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ODONTOMETRIJSKA KORELACIJA DUŽINE I VELIČINE ZUBA U PROCENI TELESNE VISINE: IZ PERSPEKTIVE FORENZIČKE NAUKE

ODONTOMETRIC CORRELATION OF TOOTH LENGTH AND SIZE FOR STATURE ESTIMATION: A FORENSIC PERSPECTIVE

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Sažetak

Uvod: *Dentalna morfometrija predstavlja primarnu metodu forenzičke identifikacije, posebno u slučajevima kada su skeletni ostaci nepotpuni ili kada nisu dostupni.*
Cilj: *Cilj ove studije bio je da ispita odnos između visine i različitih odontometrijskih parametara maksilarnog zubnog luka, uključujući interkaninu širinu (engl. Intercanine Width – IC), interpremolarnu širinu (engl. Interpremolar Width – IPW), kombinovanu meziodistalnu širinu (engl. Combined Mesiodistal Width –CW) anteriornih zuba, kao i individualne dužine zuba.*
Materijal i metode: *Ova studija preseka obuhvatila je šezdeset ispitanika (trideset muškaraca i trideset žena) starih od osamnaest do trideset godina, kojima su uzeti maksilarni zubni modeli. Odontometrijski podaci su dokumentovani pomoću digitalnog kalipera. Visina je merena standardnim stadiometrom. Pearsonova korelaciona analiza korišćena je za procenu povezanosti između visine i odontometrijskih karakteristika, dok je regresiona analiza primenjena za izradu prediktivne jednačine za procenu visine.*
Rezultati: *Pearsonova korelaciona analiza pokazala je značajnu pozitivnu korelaciju između interkanine širine i visine ($r = 0,489$; $p < 0,001$), dok je kombinacija ICW + IPW pokazala slabiju ali statistički značajnu povezanost ($r = 0,375$; $p = 0,003$). Ostali odontometrijski parametri nisu pokazali statistički značajnu korelaciju ($p > 0,05$). Kada je reč o individualnim dužinama zuba, samo je kod maksilarnog levog drugog premolara (zuba 25) zabeležena značajna negativna korelacija sa visinom ($r = −0,374$; $p = 0,038$). Regresiona analiza je identifikovala interkaninu širinu kao najbolji prediktor visine (visina = 129,234 + 2,165 × ICW; $R^2 = 0,25$; SEE = 6,7 cm).*
Zaključak: *Činjenica da su dimenzije zuba 25 i interkanina širina pokazale značajnu povezanost sa visinom zuba ukazuje na to da maksilarna odontometrijska merenja mogu služiti kao dodatno sredstvo prilikom forenzičke procene visine u slučajevima kada nema dugih kostiju.*

Ključne reči: *forenzička antropologija, forenzička odontologija, procena telesne visine, dužina zuba, odontometrijska analiza*

Abstract

Introduction: *Dental morphometrics is an important tool in forensic identification, especially when skeletal remains are incomplete or unavailable.*
Aim: *This study aimed to investigate the relationship between stature and various odontometric parameters of the maxillary arch, including intercanine width (IC), interpremolar width (IP), combined mesiodistal width (CW) of anterior teeth, and individual tooth lengths.*
Materials and Methods: *This cross-sectional study included 60 participants (30 males and 30 females) aged 18 to 30 years. Maxillary dental casts were acquired, and odontometric data were documented with a digital vernier caliper. Height was assessed utilizing a conventional stadiometer. Pearson's correlation analysis was employed to assess the link between height and odontometric characteristics, while regression analysis was conducted to construct a predictive equation for height estimation.*
Results: *Pearson's correlation analysis showed a significant positive correlation between intercanine width and stature ($r = 0.489$, $p < 0.000$), while IC + IP demonstrated a weaker but significant association ($r = 0.375$, $p = 0.003$). Other odontometric parameters showed no significant correlation ($p > 0.05$). Among individual tooth lengths, only the maxillary left second premolar (tooth 25) showed a significant negative correlation with stature ($r = −0.374$, $p = 0.038$). Regression analysis identified intercanine width as the strongest predictor of height (Height = 129.234 + 2.165 × IC; $R^2 = 0.25$; SEE = 6.7 cm).*
Conclusion: *The dimensions of tooth 25 and IC width exhibited a substantial correlation with stature, indicating that maxillary odontometric measurements may function as additional tools for forensic stature estimation in the absence of long bones.*

Key words: *forensic anthropology, forensic odontology, stature estimation, tooth length, odontometric analysis*

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Introduction

Dental morphometrics involves the quantitative evaluation of tooth dimensions and morphology, serving as a crucial tool in forensic odontology for individual identification[1]. By examining dental features, one can estimate a person's biological profile. In forensic human identification, two primary methodologies are typically utilized: comparative techniques and constructive procedures. Comparative approaches depend on the correlation of antemortem records, which include dental information from a living individual, such as dental charts, radiographs, and DNA profiles, with postmortem records that are detailed documents generated through the examination of a deceased person's teeth, focusing on the identification of key identifiers. Nevertheless, in numerous forensic scenarios, particularly when antemortem data is lacking or insufficient, these procedures become impractical. In these cases, constructive or reconstructive identification methods, which entail creating a biological profile through assessments of sex, age, ancestry, and stature, are essential[2,3]. Consequently, calculating height from odontometric characteristics is an essential component of biological profiling, offering a significant tool for human identification when direct comparison methods are unavailable[4].

One essential anthropometric characteristic that helps determine a person's physical identity is stature, or height[5]. Stature is considered one of the primary factors in forensic anthropology, alongside age, sex, and ancestry, and is essential for the preliminary evaluation and reconstructive identification of skeletal remains[6]. A basic anthropometric characteristic, stature indicates a person's height when standing up straight and has a clear proportionate relationship with several anatomical features, such as the foot, trunk, long bones, and cranial and facial bones[7]. Long bones are typically utilized to estimate stature in cases involving skeletal remains due to their strong correlation with height. In instances of decomposed, fractured, or damaged bodies, it is crucial to apply the proportional relationship between stature and various anatomical elements, including the head, face, trunk, and extremities, to aid in personal identification[8].

Teeth are highly resistant, especially under adverse circumstances such as high temperatures, and are often detected after the rest of the body has disintegrated[9]. Dental morphometrics can provide a reliable estimate of an individual's stature since teeth are one of the most chemically stable components of the human body. This is particularly relevant in forensic and archaeological contexts, as teeth are frequently recovered, even when other skeletal remains are absent[10]. Consequently, dental and oral characteristics are crucial for determining significant factors such as age, sex, and ancestry via odontometric analysis[11].

Extensive study has examined the use of permanent dental morphometrics to estimate physical stature, including measurements such as tooth length, crown length (CL), mesiodistal breadth, and labiolingual width, while seeking to correlate these metrics with face features[12,13]. Nonetheless, there has been a paucity of research on evaluating stature based on tooth structures like crown length, root length, and mesiodistal or buccolingual widths, which can be used to study how they relate to height. This study aimed to examine the relationship between height and odontometric features, encompassing intercanine width (IC), interpremolar width (IP) of the maxillary arch, as well as the mesiodistal crown width and tooth length of all permanent maxillary teeth up to the second molars, in addition to the combined mesiodistal width (CW) of all permanent maxillary anterior teeth, and the various combinations of IC + IP, IC + CW, IP + CW, and IC + IP + CW.

The emphasis on maxillary teeth arises from their enhanced preservation, accessibility, and measuring accuracy in forensic examination. The maxillary teeth, particularly the canines and premolars, exhibit distinct anatomical features that facilitate reliable odontometric evaluation on both physical specimens and radiographs[14]. They demonstrate greater resistance to postmortem deterioration, facilitate precise measurement by callipers and orthopantomograms, and reveal significant correlations with craniofacial proportions pertinent to stature estimation[15]. Therefore, focusing on the maxillary arch improves precision, consistency, and forensic relevance in stature assessment. The goal is to develop dependable methods for assessing stature using oral parameters. The findings of this study could significantly increase the efficacy of forensic and archaeological investigations by contributing to enhancement of identification possibilities when complete skeleton remains are not available.

The null hypothesis of the study includes no statistically significant relationship between height and odontometric features, including IC width, IP width, mesiodistal crown width,

tooth length of permanent maxillary teeth up to the second molars, CW of permanent maxillary anterior teeth, or their combinations (IC + IP, IC + CW, IP + CW, IC + IP + CW).

Previous research examined the correlation between odontometric measurements and stature, revealing moderate associations between factors such as intercanine width, mesiodistal tooth size, and crown measurements and height across several populations. Nevertheless, the majority of research has concentrated on a small number of dental variables, and evidence assessing numerous maxillary odontometric features simultaneously remains limited.

The present study aimed to evaluate the correlation between height and several maxillary odontometric parameters, including intercanine width (IC), interpremolar width (IP), mesiodistal crown width, and tooth length of permanent maxillary teeth up to the second molars, as well as the combined mesiodistal width of maxillary anterior teeth and their combinations, to assess their efficacy in stature estimation.

Materials and Methods

This cross-sectional research included 60 participants recruited from the Institute's Outpatient Department. Informed consent was gathered from all participants following an explanation of the study's objectives and methodologies. Confidentiality and anonymity were ensured, and participation was voluntary, with the option to withdraw at any moment. All procedures followed the ethical standards of the Institutional Research Committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (approval number IECVDC/23/UG01/OP/IVT/90) of the Institutional Ethics Committee, Vishnu Dental College, India). The sample size of 60 was based on convenience sampling of participants who met the inclusion criteria during the study period. Because of the exploratory nature of the research and the limited availability of radiographic records meeting quality standards, the study was considered preliminary/pilot in design. The participants were drawn from a relatively homogenous population, patients of South Indian ancestry attending the Outpatient Department of Vishnu Dental College. Age and sex distribution were recorded but not stratified for analysis due to the limited sample size. Subjects who visited the dental hospital for regular dental checkups were selected for this study based on the following inclusion criteria: provided informed consent; fully erupted tooth; radiographs without any pathologies; radiographs with adequate density, contrast, and clarity; and the tooth with complete root formation. Exclusion criteria were as follows: blurred images and artifacts caused by metallic objects; missing teeth, attrited teeth, root-canal-treat teeth; periodontally compromised teeth, developmental anomalies, under orthodontic treatment; cleft palate; crowding and spacing; and any dental or surgical procedure that might change or obstruct a tooth's natural shape or accurate measurement of its size.

The individual mesiodistal crown width and tooth length of all permanent maxillary teeth till socond molars, maxillary IC width, maxillary IP width, CW of all permanent maxillary anteriors, IC + IP, IC + CW, IP + CW, IC + IP + CW, and stature (H) were measured and documented. A digital vernier calliper (Insize Digital Calliper, China) with an accuracy of 0.01 mm was utilized to measure the parameters. Following the completion of each patient's examination, the vernier calliper was sanitized using an antiseptic solution (surfacept). The sharp tines facilitated access to the interproximal portions of the teeth. The display was utilized to measure and document the distance between the tines.

The horizontal distance between the cusp points of the maxillary right canine and the cusp tip of the left canine was utilized to determine the IC width (Figure 1), while the IP width was derived from the horizontal distance between the buccal cusp tip of the maxillary first premolar from the right to the left side (Figure 2). The mesiodistal width of teeth 17 to 27 was measured at the anatomical contact locations using equipment aligned with the occlusal plane, directly on the subject. The CW of the maxillary anterior teeth was calculated by summing the individual mesiodistal widths of these teeth (Figure 3).

An orthopantomogram (OPG) was employed to ascertain tooth length. A Satelec X Mind Panoceph OPG machine equipped with photostimulable phosphor plates from Soredex was utilized to acquire panoramic radiographs for each participant. A single trained operator acquired all radiographs to reduce magnification and distortion errors, using uniform exposure settings. The system's magnification factor was calibrated according to manufacturer specifications, and measurements were taken with the same software to ensure consistency and accuracy.

The head posture was standardized using the machine's built-in cephalostat and laser alignment guides to ensure consistent orientation across all participants. The final images were processed using Scanora software and subsequently saved in DICOM format. Utilising OPG pictures, the lengths of individual maxillary teeth, excluding the third molars, were measured from the apex of the root to the tip of the buccal cusp for posterior teeth and to the tip of the incisal surface for anterior teeth (Figure 4).

Stature was calculated as the vertical distance from the apex of the head to the floor. The subject's posture should be standardized with the individual standing with an erect spine, heels together, and head in the Frankfurt plane. The height was measured in front of a scale calibrated to 0.1 cm on an immovable vertical plane. All measurements were conducted by a single examiner to eliminate interobserver error. Measurements were conducted with an accuracy of 0.01 mm for odontometric parameters and 0.1 cm for stature, employing millimetres (mm) and centimetres (cm), respectively.

Ten randomly selected radiographs and clinical measurements were reevaluated by the same examiner after a two-week interval. The Intraclass Correlation Coefficient (ICC) was calculated and showed high reliability (ICC = 0.91 for odontometric measurements and ICC = 0.93 for stature), indicating excellent reproducibility. All measurements in the present study were performed by a single experienced examiner to maintain procedural uniformity and minimize variability due to differing measurement techniques. To address this, the examiner was blinded to participants' stature during odontometric assessments, and each measurement was taken twice on separate occasions to enhance consistency.

Statistical Analysis

Data were analysed using IBM SPSS Statistics version 29.0 (New York, USA). Normality of data distribution was verified using the Shapiro–Wilk test, confirming that all variables followed a normal distribution ($p > 0.05$). Descriptive statistics (mean, standard deviation, minimum, maximum) were computed for age, sex, height, and odontometric parameters. Pearson's correlation coefficient (r) was used to evaluate relationships between stature and odontometric parameters. Multiple linear regression analysis was conducted to identify the most significant predictors of stature. The standard error of estimate (SEE) and coefficient of determination (R^2) were calculated to assess model accuracy and predictive precision. A scatter plot illustrating the correlation between predicted and actual stature values was generated to visually demonstrate model performance. A p-value ≤ 0.05 was considered statistically significant.

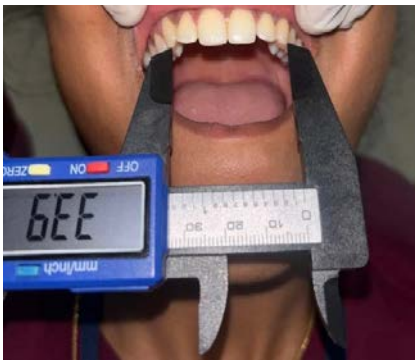

Figure 1. Measurement of inter canine width using a digital vernier caliper

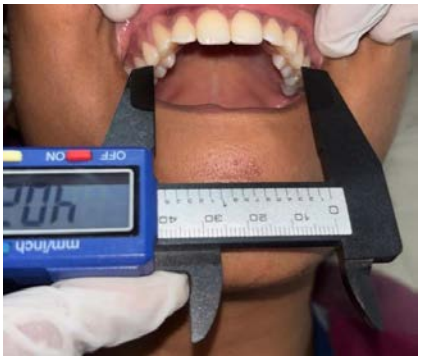

Figure 2. Measurement of inter premolar width using a digital vernier caliper

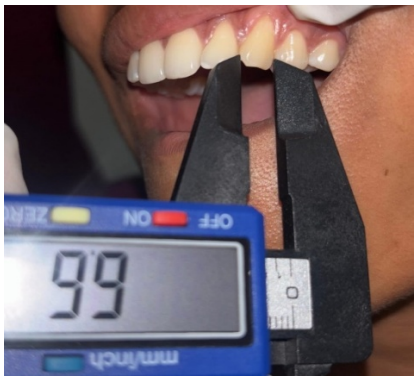

Figure 3. Measurement of mesiodistal width of individual tooth

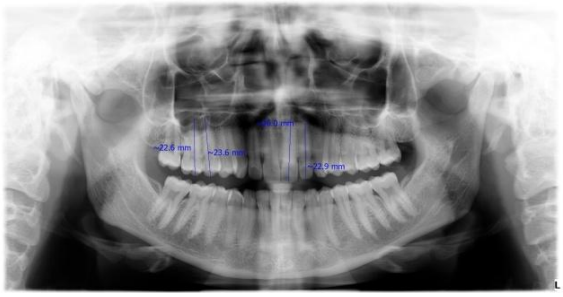

Figure 4. Measurement of tooth length on an orthopantomogram (OPG)

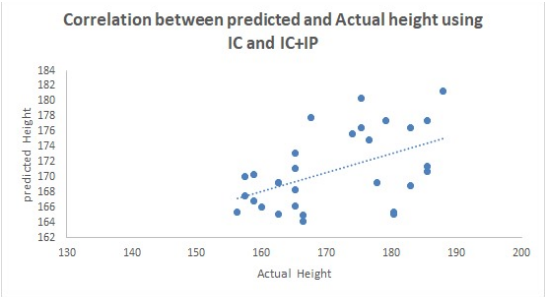

Figure 5. Correlation between predicted and actual height using IC and IC + IP

Results

A total of 60 participants were included in the study, comprising 30 males (50%) and 30 females (50%). The mean age of the participants was 22.8 ± 3.4 years, ranging from 18 to 30 years. The mean height of the participants was 160.0 ± 8.2 cm, ranging from 145 cm to 178 cm (Table 1). Pearson's correlation coefficient (r) was calculated to examine the relationship between stature and tooth width (seven odontometric characteristics) across all subjects, irrespective of sex distinctions. The Pearson correlation analysis revealed a statistically significant positive correlation between IC width and stature ($r = 0.489$, $p < 0.000$), suggesting a moderate and meaningful association. The combined parameter IC + IP demonstrated a statistically significant correlation with stature ($r = 0.375$, $p = 0.003$), but to a lesser extent. Other variables, such as IP width, CW, and their combinations (IC + CW, IP + CW, IC + IP + CW), showed no statistically significant correlations with stature ($p > 0.05$) (Table 2).

The multiple linear regression analysis examined the relationship between the independent variables IC and IC + IP with the dependent variable height. The model revealed that IC was a statistically significant predictor of Height ($B = 2.165$, $p = 0.005$), indicating that for every one mm increase in IC, Height increased by approximately 2.17 cm. The standardized Beta coefficient (0.683) further suggested that IC had a strong positive influence on Height. In contrast, the variable IC + IP showed no significant association with Height ($B = -0.437$, $p = 0.352$), suggesting that this predictor did not meaningfully contribute to explaining the variance in Height. Collinearity statistics revealed moderate multicollinearity between the two predictors (VIF = 4.246), which falls within acceptable limits but may still warrant further attention. Overall, IC emerged as the key predictor in this model, while IC + IP did not show a significant independent effect (Table 3).

Model 1: Stature prediction using IC and IC + IP

Height (cm) = 129.234 + 2.165 (IC) − 0.437(IC + IP)

$R^2 = 0.25$, SEE = 6.7 cm

IC was a statistically significant predictor ($B = 2.165$, $p = 0.005$), whereas IC + IP was not significant ($p = 0.352$). This indicates that each 1 mm increase in intercanine width corresponds to an approximate 2.17 cm increase in stature.

Model 2: Stature prediction using tooth 25 length

Height (cm) = 211.247 − 1.550 (tooth 25 length)

$R^2 = 0.140$, SEE = 7.2 cm. The negative regression coefficient indicates that greater tooth length was associated with slightly lower stature ($B = – 1.550$, $p = 0.038$).

Pearson's correlation analysis of tooth length and height found that tooth 25 (maxillary left second premolar) had a statistically significant negative correlation with stature ($r = − 0.374$, $p = 0.038$). This demonstrates a moderate inverse association, which means that when the length of tooth 25 increases, stature tends to decrease to a moderate extent. No other teeth showed statistically significant correlations (Table 4). The multiple linear regression analysis investigated the relationship between Height and tooth length, indicating that the length of 25 (maxillary left second premolar) served as a statistically significant predictor of Height. ($B = 211.247$, $p = 0.005$) (Table 5). Correlation between predicted and actual height using IC and IC + IP was done and represented (Figure 5). This displays a scatter plot illustrating the correlation between predicted height and actual

height utilizing IC and IC + IP data. In this picture, each point denotes an individual participant, with actual height represented on the x-axis and predicted height on the y-axis. The dotted regression line illustrates the positive correlation between the variables, signifying that when actual height rises, the predicted height similarly tends to rise. The ascending trend in the scatter plot indicates the positive correlation observed in the statistical study.

Table 1. Mean values and standard deviations for the principal odontometric parameters

Parameter	**Mean ± SD**	**Minimum**	**Maximum**	**Units**
Height (H)	160.0 ± 8.2	145.0	178.0	cm
Intercanine width (IC)	32.5 ± 2.4	28.0	37.0	mm
Interpremolar width (IP)	38.9 ± 3.1	33.0	45.0	mm
Combined mesiodistal width (CW)	42.8 ± 2.9	38.0	48.0	mm
Tooth 25 length	21.5 ± 1.9	18.0	25.0	mm

Table 2. Correlation between Height and tooth width using Pearson's correlation

Variables	**r value**	**P-value**
Stature	0.130	0.321
IP	0.130	0.130
IC	0.489	0.000**
CW	−0.101	0.442
IC + IP	0.375	0.003*
IC + CW	0.188	0.151
IP + CW	−0.133	0.312
IC + IP + CW	0.205	0.116

Table 3. Multiple linear regression analysis between Height and tooth width

R^2 = 0.251								
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	129.234	17.995		7.182	0.000		
	IC	2.165	0.749	0.683	2.890	0.005	0.236	4.246
	IC + IP	−0.437	0.466	−0.222	−0.938	0.352	0.236	4.246
a. Dependent Variable: Height								

Table 4. Correlation between Height and tooth length using Pearson's Correlation

Variables	HEIGHT	
	r value	p-value
11	−0.108	0.562
12	−0.235	0.203
13	−0.243	0.188
14	−0.212	0.251
15	−0.262	0.154
16	−0.192	0.302
17	−0.288	0.117
21	−0.102	0.585
22	−0.194	0.295
23	−0.193	0.299
24	−0.204	0.271
25	−0.374*	0.038*
26	−0.034	0.856
27	−0.274	0.137

Table 5. Multiple linear regression analysis between Height and tooth length

R^2 = 0.140						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	211.247	18.449		11.450	0.000
	25	−1.550	0.713	−0.374	−2.174	0.038
a. Dependent Variable: Height						

Discussion

Forensic odontology is a crucial field within forensic science that utilizes dental knowledge for legal enquiries, especially in the identification of human remains. Teeth, owing to their remarkable resilience, frequently endure conditions that obliterate other tissues, rendering them essential for constructing biological profiles when remains are fragmented16. Although long bones are generally employed for stature estimation, teeth provide a significant alternative when such bones are inaccessible. Their morphometric characteristics namely length, width, and arch dimensions, exhibit significant associations with height[17]. This study demonstrates that IC width and the length of tooth 25 (maxillary left second premolar) are statistically significant predictors of height, emphasizing the value of dental morphometrics, specifically arch width and individual tooth length as dependable adjuncts for forensic stature estimation, particularly in situations of fragmented or incomplete remains.

The present study findings indicate that the null hypothesis was rejected only for inter canine width and partially for the length of tooth 25. A moderate and statistically significant correlation between inter canine width and stature was found, however no such correlation was observed for the combined metric IC + IP (Table 3). This might be because there is a lack of homogeneity in the IP measurements taken from the same population due to individual differences in arch shape or alignment, which has a greater impact on premolar placement than canines. While intercanine width is biologically linked to

craniofacial growth and overall body size, the relationship identified with the second premolar lacks a clear anatomical reason and may be due to random variation in this small pilot sample. It is possible that the individual predictive power of composite measures is reduced due to the overlapping variance shown by the moderate multicollinearity between IC and IP.

Our results correspond with the study conducted by Yadav et al., which also recognized canine width as a crucial predictor of stature[18]. Our findings align with the research from Jani et al., which demonstrated a significant correlation between height and IC ($r = 0.223$, $p = 0.025$), although IP and combined odontometric measurements (IC + IP, IC + CW, etc.) showed no significant correlation. Their regression model, founded on IC, exhibited reliability when utilized on a test dataset[19]. Collectively, our findings highlight the significance of intercanine width as a reliable odontometric indicator for stature assessment, especially in forensic scenarios where other skeletal components are absent.

Revathy et al. conducted a study that revealed a feeble statistically significant association between height and the total mesiodistal width of maxillary anterior teeth ($r = 0.091$ for females and 0.093 overall), with no significant correlation observed in males. Their regression models had restricted predictive power ($R^2 = 0.04–0.057$), underscoring the hypothesis that odontometric traits, such as IC, could serve as significant supplementary indicators for stature assessment, particularly when long bones are unavailable[20].

Hossain et al. investigated the correlation between the size of a person's tooth crown and their height in a group of people from Southeast Asia. Crown length, mesiodistal breadth of central incisors and canines, and individuals' height were found to be statistically significantly yet moderately correlated ($r = 0.35−0.45$). Combining measurements, especially the sum of crown length and mesiodistal breadth of central incisors and canines, yielded the best correlation ($r = 0.537$). Nevertheless, even with these statistical correlations, the regression models revealed a large discrepancy between the real and estimated heights, with inaccuracies reaching a maximum of 16.5 cm[21].

Our study identified the length of tooth 25 as a statistically significant predictor of stature ($B = 211.247$, $p = 0.000$) (Table 4), highlighting the possible application of individual tooth lengths in height estimation. This corresponds with the results of Narayanan et al., who identified a notable association between height and various tooth lengths, with correlation coefficients (r) between 0.12 and 0.57. Their research found maxillary molars, premolars, and canines, specifically the right second molar and first premolar, exhibited the most significant correlations with stature. Although their investigation incorporated numerous teeth in regression modelling, our findings underscore that even a single tooth, such as the upper second premolar, can function as a dependable metric for stature assessment, particularly in instances with scant dental remains[22]. These findings enhance the increasing evidence favoring the utilization of tooth length in forensic stature assessment in the absence of skeletal remains.

Our study findings contradict those of Rao et al., who investigated four odontometric parameters: IC width, IP width, arc length, and CW of anterior teeth across three Asian ethnicities (Malay, Indian, Chinese). Notably, the analysis revealed that only arc length had a significant link with stature ($r = 0.287$, $p = 0.002$), while IC and IP demonstrated no significant correlation. Their regression model utilizing arc length produced a weak R^2 of 0.08, indicating restricted explanatory capacity[23]. The absence of significance for IC in their study, in contrast to the moderate correlation identified in ours, may be ascribed to ethnic diversity or disparities in sample selection.

The regression models in this study exhibited limited explanatory and predictive power, with R^2 values of 0.25 for Model 1 and 0.14 for Model 2. The values suggest that a limited proportion of the variance in the outcome was explained, indicating that the predictive accuracy of the models requires cautious interpretation. The inclusion of both IC and IC + IP in the same regression model results in multicollinearity, given that IC is contained within the IC + IP composite variable. The observed VIF of approximately 4.2 indicates moderate collinearity, which should be recognized. Multicollinearity can increase standard errors, diminish statistical precision, and lead to unstable regression coefficients.

The observed negative association between the length of tooth 25 and stature is biologically unexpected and may reflect random variation rather than a true anatomical relationship, especially given the limited sample size and modest effect size.

This finding must be interpreted with caution and confirmed in bigger, population-based studies.

Our findings also differ from those of Panigrahi and Babu, who specifically assessed the second maxillary inter premolar distance and its association with height. Their findings demonstrated minimal correlation coefficients ($r = 0.05$ for males and $r = 0.13$ for females), suggesting that interpremolar distance is not a reliable predictor for stature estimation[24]. In contrast to our findings, which emphasize the forensic significance of IC, their data reveal the heterogeneity in predictive capacity across several dental characteristics. This emphasizes the necessity for population-specific and parameter-specific validation in the application of dental measurements for forensic stature assessment. Previous studies done by Rao et al.[23], Panigrahi and Babu[24] have reported weaker or differing results, which are mainly attributed to population variation; other factors, such as limited sample size, methodological differences, and measurement inconsistencies, may also account for these discrepancies. Identifying these alternative influences facilitates a more proportionate interpretation of variations among studies.

Although the sample size in this pilot study was limited for sex-stratified evaluation, it is crucial to note that sex has a considerable influence on both stature and odontometric dimensions. By combining male and female individuals, the study may have missed any sex-specific trends, which should be noted when interpreting the results. Future research with bigger sample sizes should include sex-based stratification or adjustment to increase analytical accuracy.

The enormous demographic diversity of India leads to variations in stature based on geographical location, caste, and tribe. Given that stature is affected by genetic, environmental, and climatic variables, it is imperative to utilize population-specific regression models to enhance precision in forensic identification. Our results endorse the utilisation of IC width and individual tooth length (25) as potentially useful predictors for estimating age that requires validation in larger samples.

Limitations

This study's primary limitation is its relatively small sample size and focus on a single population group, potentially restricting the generalizability of the regression models. The use of OPGs presents potential magnification and distortion errors, even with standardization and calibration efforts in place. The presence of moderate collinearity between IC width and IP width complicates regression analysis and may affect the accuracy of predictive models. Future multicentric studies with larger and more diverse populations are necessary to validate these preliminary findings and enhance the generalizability of odontometric parameters for stature estimation across various demographic groups.

Conclusion

This research established that IC width and the length of tooth 25 are statistically significant odontometric indicators that exhibit moderate correlations with stature. These parameters may function as useful adjuncts in forensic stature estimation, especially in cases where skeletal remains are incomplete or absent. Given the limited sample size and moderate strength of associations, these findings warrant cautious interpretation and should be considered preliminary. Subsequent studies must confirm these findings in larger and more heterogeneous populations, utilizing standardized reliability evaluations and methodological improvements to reduce radiographic distortion and improve measurement accuracy. Creating strong, population-specific regression models will enhance the forensic utility of odontometric parameters in human identification.

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Ethics approval and consent to participate: All procedures followed the ethical standards of the Institutional Research Committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (approval number IECVDC/23/UG01/OP/IVT/90) of the Institutional Ethics Committee, Vishnu Dental College, India). Informed consent was gathered from all participants following an explanation of the study's objectives and methodologies. Confidentiality and anonymity were ensured, and participation was voluntary, with the option to withdraw at any moment.

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DETEKCIJA I HISTOPATOLOŠKI NALAZI HUMANOG PAPILOMAVIRUSA U ORALNIM LEZIJAMA KOJE SU POTENCIJALNO PREMALIGNE KOD OSOBA KOJE ŽIVE SA HIV-om U SUPSAHARSKOJ POPULACIJI

HUMAN PAPILLOMAVIRUS DETECTION AND HISTOPATHOLOGICAL FINDINGS IN POTENTIALLY PREMALIGNANT ORAL LESIONS AMONG PEOPLE LIVING WITH HIV IN A SUB-SAHARAN POPULATION

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Sažetak

Pozadina: *Mada je humani papiloma virus (HPV) povezan sa onkogenim procesima, povezanost između virusnih onkoproteina i disfunkcionalnog p16 tumor-supresorskog proteina u oralnim premalignim lezijama ostaje upitna.*
Cilj istraživanja*: Ovo istraživanje je sprovedeno radi utvrđivanja prisustva proteina p16 u klinički suspektnim oralnim premalignim lezijama kod osoba koje žive sa virusom humane imunodeficijencije (engl. Human Immunodeficiency Virus – HIV).*
Materijal i metode: *Ispitano je devet od dvadeset četiri oralnih uzoraka dobijenih biopsijom sa displastičnim ili papilomatoznim histopatološkim promenama. Ispitani su i normalni uzorci, i to pomoću bojenja hematoksilinom i eozinom. Prisustvo HPV-a procenjivalo se na osnovu metode lančane reakcije polimeraze (engl. Polymerase Chain Reaction – PCR) i ekspresije proteina p16 određene imunohistohemijskom analizom.*
Rezultati: *Šest uzoraka (66,7%) bilo je pozitivno na HPV. Detektovani su različiti tipovi HPV-a, među kojima su i tip 16, 18, 35, 45 i 68. Kod svih papiloma, osim kod jednog, zapažene su mešovite infekcije. U svim tkivima, uključujući ona u kojima nije dokazano prisustvo HPV DNK metodom PCR, stepen imunohistohemijske pozitivnosti bio je različit. Rezultati studije su pokazali da se u ovoj populaciji HPV prisutan u pljuvački razlikuje od onog detektovanog u tkivu.*
Zaključak: *U ovoj maloj eksploratornoj deskriptivnoj studiji visokorizični tipovi HPV-a detektovani su u tkivima koja pokazuju prekomernu ekspresiju proteina p16. Ovakvi rezultati ukazuju na moguću ulogu HPV-a i proteina p16 u nastanku oralnih lezija kod osoba koje žive sa HIV-om. Međutim, da bi se ova zapažanja potvrdila, treba sprovesti obimnija istraživanja.*

Ključne reči: *humani papiloma virus, oralne lezije, osobe koje žive sa HIV-om, kliničkopatološki, premaligno, leukoplakija, eritroplakija*

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Abstract

Background: *Although human papillomavirus (HPV) is associated with oncogenic processes, a correlation between viral oncoproteins and a dysfunctional p16 tumor suppressor protein in oral premalignant lesions is questionable.*
Aim: *We embarked on a study to determine p16 positivity in clinically suspicious oral premalignant lesions seen in people living with human Immunodeficiency virus (HIV).*
Materials and Methods*: We examined 9/24 oral biopsy specimens with dysplastic or papillomatous histopathological changes, as well as normal specimens, using hematoxylin and eosin staining to assess HPV presence via polymerase chain reaction (PCR) and p16 expression by immunohistochemistry.*
Results*: Six specimens (66.7%) were HPV-positive. Various HPV types, including 16, 18, 35, 45, and 68, were detected. All papillomas had mixed infections except one. Varying degrees of immunohistochemical positivity on all tissues, including those that did not demonstrate the presence of HPV DNA on PCR, were seen. Study results showed that in this population, HPV shed in saliva differed from that seen in tissue.*
Conclusions: *In this small exploratory descriptive study, high-risk HPVs were detected in tissue showing p16 overexpression. These findings suggest a possible role for HPV and p16 in oral lesions among PLWHIV, but larger studies are needed to confirm these observations.*

Key words: *human papillomavirus, oral lesions, people living with HIV, clinicopathological, pre-malignant, leukoplakia, erythroplakia*

Introduction

Precancerous lesions are defined as ‘‘structurally altered tissue clinically or histologically with a higher risk to transform into malignancy when compared to similar but normal ones[1].’’ In the oral cavity, erythroplakia, leukoplakia, and palatal keratosis from reverse smoking are usually categorized as precancerous lesions[2]. However, conditions such as submucous fibrosis, actinic keratosis, lichen planus, discoid lupus erythematosus have been reported as premalignant by some authors[3]. The term precancerous is interpreted as indicative of a definite malignant transformation pathway, even though research has shown this is not always the case[4]. The potential confusion caused by this nomenclature has led to a shift toward the use of the term potentially malignant disorders. Due to the numerous possible causes, there is a wide range of potentially malignant conditions that vary by geographic location and possibly lifestyle[5,6].

In the oral cavity, leukoplakia is the most common “precancerous” lesion, with a global prevalence ranging from 1.49%–2.6% and a predisposition for men[7,8]. Submucous fibrosis is more common among areca and beetle nut chewers, hence a high prevalence in those parts of Asia with a penchant for these[6,9].

In Sub-Saharan Africa, research into oral premalignant lesions is scanty, yet the population lifestyle and composition are rapidly changing[10,11]. With the high prevalence of human immunodeficiency virus (HIV)[12] in the region, the burden and variety of “potentially pre-malignant lesions” within this subcontinent need to be investigated. In this study, we set out to determine the clinical nature of “possible pre-malignant lesions”, their corresponding histopathological picture, the presence of human papillomavirus (HPV) in saliva/tissue using polymerase chain reaction (PCR), and p16 (a marker of HPV related malignant tissue transformation) staining among people living with HIV (PLWHIV). The study findings ought to raise awareness about the importance of oral health beyond dental caries and periodontal diseases in people living with HIV and on long-term highly active antiretroviral medication in developing countries. The findings may contribute to a better understanding of HPV-related oral lesions in this population and underscore the potential importance of incorporating oral mucosal screening in long-term HIV care, particularly given the elevated prevalence of tobacco and alcohol use among PLWHIV compared to the general population[13,14].

Materials and Methods

Study design, site, and population

This work was part of a larger single-institutional prospective study conducted between December 2022 and August 2023. The parent study was conducted in a large, low-resource setting serving approximately 80,000 PLWHIV at the Mulago ISS clinic affiliated with Makerere University through its Makerere Joint AIDS Program (MJAP), in Kampala, Uganda. The MJAP provides care to over 180,000 PLWHIV across 20 clinics in urban and rural Uganda. The clinic has a retention rate of 93% for patients on antiretroviral therapy (ART) over one year. All participants had been given an opt-in provision for subsequent contact by way of providing a phone number in case of any follow-up. Of 95 patients with suspicious oral lesions identified during the first month of the parent study, 32 returned for follow-up within two weeks. Six participants had lesions that resolved spontaneously, and two declined to provide biopsy specimens. The HPV PCR analysis was carried out on the remaining 24 tissues and saliva.

Measurements and variables

During the study period, data, including age, sex, site involved, and history of tobacco and alcohol use, were captured using the National Health and Nutrition Examination Survey (NHANES) tool[15]. The oral examinations were done by dentists who had undergone multimedia training. Additionally, a senior oral health clinician was on site to discuss and resolve unclear cases. Patient records were maintained at MJAP, and only data abstraction forms used were kept under lock and key at the Department of Anatomy, Makerere University College of Health Sciences.

As part of the standard of care, participants with suspected premalignant oral lesions were called back after 2 weeks for review; in cases where the lesions had not spontaneously resolved, a biopsy was taken. Suspected premalignant lesions constituted visible or palpable changes in the oral mucosa that are not typical of normal mucosa and could not be easily scraped off. These included abnormal tissue texture or color, ulcerative lesions, red or pink patches, white patches, and mixed red and white changes. The affected mucosal specimens were taken after written consent from participants at the MJAP clinic. Each specimen was sectioned into two, with one

piece preserved in 10% buffered formalin immediately, while the other was preserved with RNA later on ice and subsequently stored at -20 °C. The formalin-fixed specimens were processed and read at the Department of Pathology, Mulago National Referral Hospital, for haematoxylin and eosin and P16 staining.

However, before taking a biopsy, 5 mL of unstimulated saliva was collected from the participants and transported to the Department of Anatomy on ice. Two mL of which was used for DNA extraction and subsequent PCR analysis for HPV detection as previously described[16].

Typing HPV PCR

All HPV positive samples in the study pool were subjected to further PCR-based typing using the previously described Sotlar method[17], with the following modifications. Human papillomavius DNA was amplified using a single consensus forward primer, GP-E6-3 F [GGGWG KKACT GAAAT CGGT], and two consensus reverse primers, GP-E7-5B [CTGAG CTGTC ARNTA ATTGC TCA] and GP-E7-6B – [TCCTC TGAGT YGYCT AATTG CTC]. These primers gave a 602 bp to 666 bp PCR product visualized on 2% agarose gel containing ethidium bromide using the ebox machine (Vilber E-box, Vilber, Deutschland GmbH, Wielandstrasse 2, Germany). Then, four second-round primer-cocktail sets were used for the genotyping[17]. Both first and second round PCRs were performed in a final volume of 50 μL, and each PCR mixture contained 9 mM Tris-HCl (pH 9.0), 2.0 mM MgCl2, 0.2 mM of each dNTP, 320 nM of each of the primers, and 1.25 U of DreamTaq polymerase under the same conditions as already published[18]. The amplifications were carried out using a thermal cycler, a Simply Amp Thermocycler (Applied Biosystems, Waltham, MA, USA), following the above modified Sotlar method parameters[17]. The typing was aided by the ebox analyse software (Vilber E-box, Vilber, Deutschland GmbH Wielandstrasse 2, Germany), that was used to measure the band base pair weights in reference to a 100 bp ladder using the previously published reference levels to identify the oncogenic (Hpv_16, Hpv_18, Hpv_31, Hpv_33, Hpv_35, Hpv_39, Hpv_45, Hpv_51, Hpv_52, Hpv_56, Hpv_58, Hpv_59, Hpv_66, Hpv_68), and non-oncogenic (Hpv_11/6, Hpv_42, Hpv_43, Hpv_44) types of HPV[17]. The data from the typing were captured in an Excel sheet as either present or absent for each of the four primer cocktail sets per sample, as described[17].

P16 staining methods

The p16 staining procedure was done using the Benchmark Ultra Ventana automated stainer (Ventana Medical Systems, Tucson, AZ, USA using the Optiview DAB IHC detection system. Five-micron-thick tissue sections were placed on charged slides and incubated overnight in a hot-air oven at 50 °C. The sections were deparaffinized in the auto stainer and antigen unmasked at 100 °C for 48 minutes. Pre-primary peroxidase inhibitor was applied, followed by primary antibody for 12 minutes. Counterstain (hematoxylin) was applied for 4 minutes, and post-counterstain bluing was done for 4 minutes. Sections were reported to be positive when there was continuous staining of cells of the basal and parabasal cell layers of the squamous epithelium. Sections were scored as negative when there was no staining in the squamous epithelium or when the staining was focal, non-continuous, or not in the basal and parabasal cells. A known p16-positive tissue of squamous cell carcinoma was used as a positive control. The biopsies were scored as positive when more than 5% of cells (cut-off) stained positively.

Statistical analysis

Parametric tests were run to compare the group that returned for biopsy and those that did not, with p set at 0.05. These variables included gender, age, HPV status, and alcohol and tobacco use history.

Ethical considerations

The study was cleared by the Makerere University School of Medicine research and ethics committee, SOMREC (REC REF 2022-451), and the Uganda National Council of Science and technology (HS2541ES) as part of a larger study “Oral Papillomavirus, Microbiota and Cancer in People Living with HIV (OHPVMC)”.

Results

A total of 95 clinically suspicious lesions were included in the study, of which 93 were suspected to be leukoplakia, and only two were erythroplakia. There was nearly equal distribution in terms of gender, with females constituting 52.7% and an overall mean age of 46.6 ± 10.0 years (Table 1). Some of the different manifestations of potentially premalignant lesions seen in our overall study population are shown in Figure 1. Of the 95 participants who were contacted, only 32 responded during the two-week period when biopsies were taken. Most participants wanted to return on a day coinciding with their MJAP drug refill clinic visit, explaining the low turnout. Out of the 32 who returned, lesions had cleared in six participants. One participant reported that she normally developed changes in her oral mucosa after eating deep-fried silver fish, but these changes would resolve on their own after a few days. Two participants declined to have a biopsy after the procedure was explained to them. The main reason was the thought of having a mouth wound, yet whatever we had pointed out wasn't, and may never bother them.

Captions a1 and a2 show a participant with a traumatic palatal ulcer that resolved within two weeks; Caption b shows white lesion caused by tobacco packing in the buccal aspect; Caption c depicts widespread white lesions in the buccal and gingiva areas; In caption d, buccal mucosal changes are observed and the patient denied any tobacco use; Captions e and g illustrate different participants with widespread white lesions on the palate; Caption f presents a lesion suspected to be erythroplakia based on clinical findings.

Table 1. Comparison of the participants who returned for biopsies and those who didn't

		Came back and accepted to be part of this study arm	Didn't come back	Significance
Gender	Male	10	35	$\chi^2 = 3.46$, p = 0.63
	Female	20	30	2 males declined biopsy
Age	Mean	46.63	46.52	P = 0.96
HPV Status	HPV detected	13	34	$\chi^2 = 1.42$, p = 0.49
	HPV not detected	17	31	
Tobacco use	Yes	4	12	$\chi^2 = 0.38$, p = 0.54
	No	26	53	
Alcohol use	Yes	14	24	$\chi^2 = 2.27$, p = 0.13
	No	16	41	

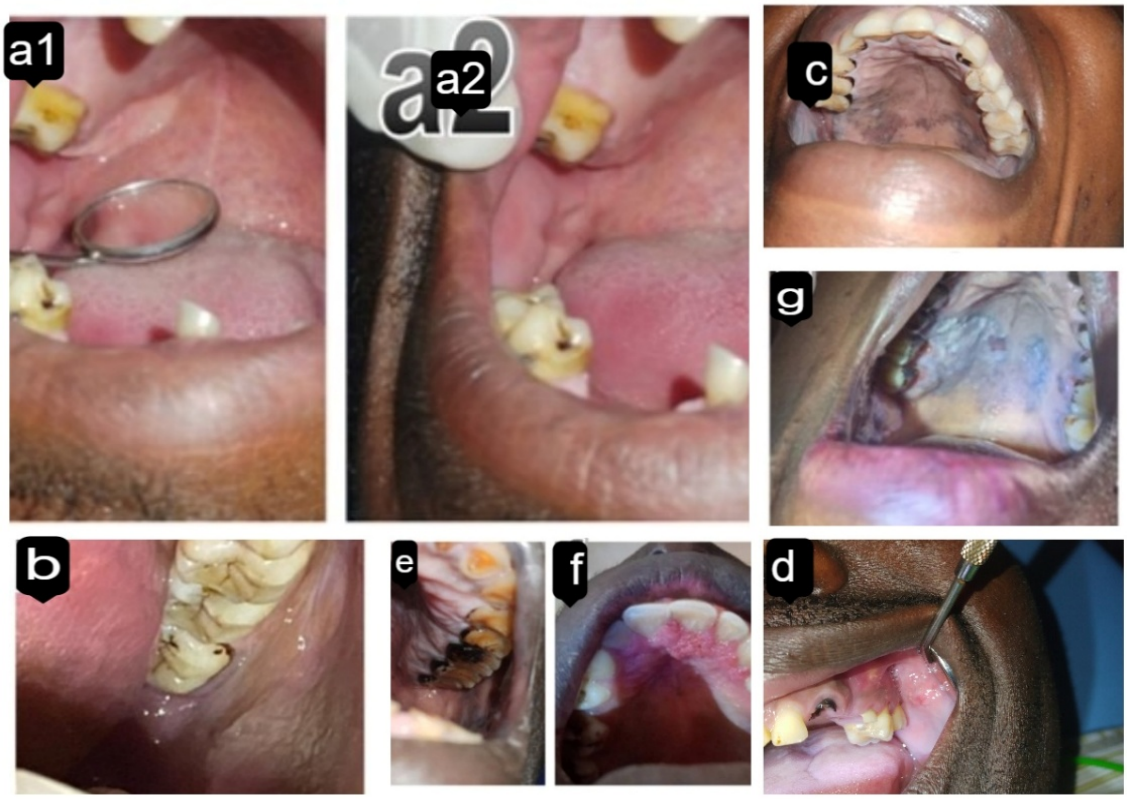

Figure 1. A variety of oral lesions observed among the 95 participants identified during the initial screening with clinically suspicious lesions

Four participants had dysplastic changes. Of these, one had severe dysplasia/intraepithelial neoplasia (shown in Figure 2) and the rest had what was considered mild dysplastic changes. Five patients had papillomatous changes seen in the tissue. In both the dysplastic and papillomatous groups, there was an equal sex distribution. We had one case of chronic inflammation and one that had insufficient tissue for histology. In comparison, the participants who were unable to return and those biopsied were not different, as shown in Table 1.

Human papillomavirus PCR

An assessment of all 24 samples, which had either normal or histological abnormality, was done to determine their HPV status and the HPV types they contain, both in the tissue and saliva. As shown in Table 2, all samples with histological change and HPV positivity (6/9, 66.67%) had one or more oncogenic HPV types. It is important to note that the participant with carcinoma in situ had HPV 6817 in their saliva sample as opposed to the types 45 and 35 seen in the biopsy sample (tested by PCR16). Additionally, this carcinoma in situ sample (see number 1 in Table 2) had two oncogenic HPV types in the extracted tissue DNA. Histologically normal tissue had HPV as well (cases 11, 18, 19, 21, 22, and 23), but had better concordance with what was found in saliva, as can be seen in Table 2.

The results demonstrated varying patterns of p16 immunopositivity across all nine cases analyzed (Figure 3), although only six of these were HPV-positive by tissue PCR (Table 2).

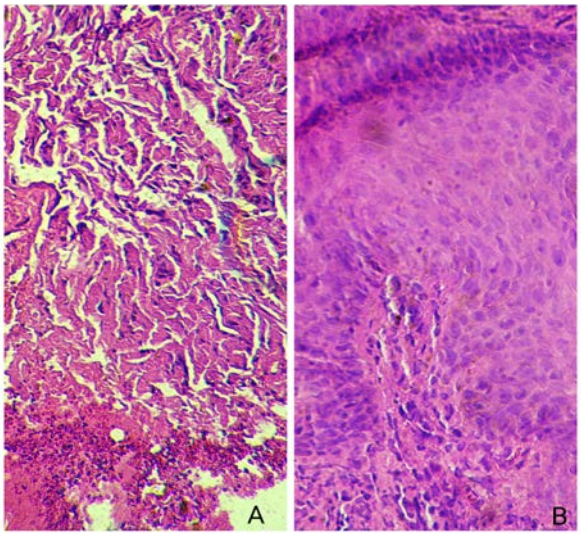

Figure 2. Shows severe dysplasia/intraepithelial neoplasia from one participant (Hematoxylin and eosin micrograph showing carcinoma in situ in the squamous epithelium x200)

Table 2. Biopsy, histopathology, and HPV status in the sample

No	Diagnosis	HPV	HPV in tissue	HPV in Saliva
1	Severe dysplasia/Carcinoma *in situ*	Positive	35, 45	68
2	Mild dysplasia	Negative		None detected
3	Papilloma	Positive	18, 68	None detected
4	Mild dysplasia	Positive	16, 18	None detected
5	Papilloma	Positive	18	18, 42, 44, 66, 68
6	Papilloma	Positive	18, 68	31, 42, 45
7	Papilloma	Positive	18, 35,45, 68	31,45, 58, 68
8	Papilloma	Negative		None detected
9	Mild dysplasia	Negative		None detected
10	Normal mucosa	Negative		None detected
11	Normal mucosa	Positive	35	35
12	Insufficient tissue	Negative		None detected
13	Normal mucosa	Negative		None detected
14	Normal mucosa	Negative		None detected
15	Normal mucosa	Negative		None detected
16	Normal mucosa	Positive	35,66	66
17	Normal mucosa	Negative		None detected
18	Normal mucosa	Positive	68	68
19	Normal mucosa	Positive	39	39
20	Normal mucosa	Negative		None detected
21	Normal mucosa	Positive	35	35
22	Normal mucosa	Positive	35, 68, 39	35, 39
23	Normal mucosa	Positive	35	35
24	Chronic inflammation	Positive	11	None detected

p16 immunohistochemistry staining

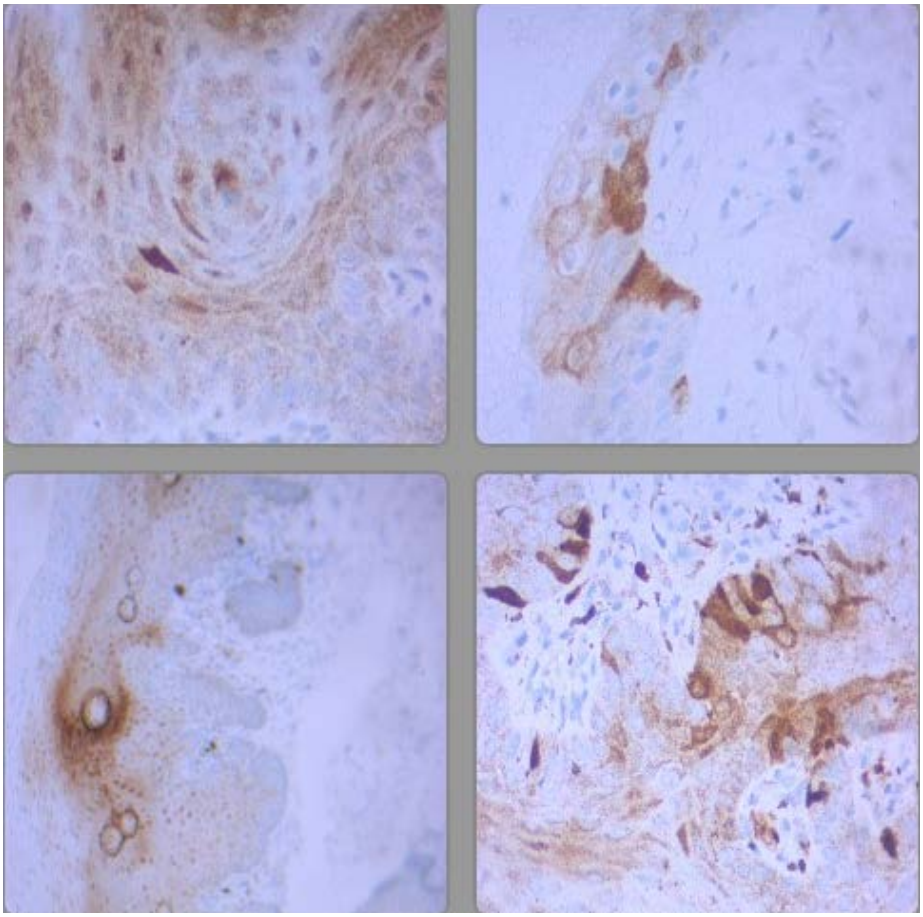

Figure 3. Shows the immunohistochemical staining using p16 (p16 positive staining in the squamous epithelium x200 showing different levels of positivity)

Discussion

Uganda has one of the highest numbers of PLWHIV as a percentage of the total population[12], and the use of tobacco products among PLWHIV has been reported to be higher than that of their negative counterparts[13]. Likewise, Uganda in the Eastern African region features as one with the highest prevalence of current alcohol users[19]. Unfortunately, alcohol use is widely reported among PLWHIV, and the factors associated with its misuse are varied[20]. Additionally, human papillomavirus (HPV) infection rates reported in the general population are quite variable, ranging from as low as 2.6% to up to 20%[21]. Some HPVs have been reported to be associated with benign oral lesions such as common wart (verruca), condyloma acuminatum, squamous cell papilloma, and focal epithelial hyperplasia[22]. However, there are reports of integration of some high-risk HPVs in normal mucosa, for which smoking and alcohol consumption seemingly increase the likelihood of this happening[21]. As can be seen in Table 2, many normal mucosa had HPV 35 and 39, which is in line with some existing studies[21]. However, other reports have shown that potentially malignant HPVs are detectable in malignant and pre-malignant but not normal tissue[23]. Longer-term usage of HAART has been associated with an increase in HPV associated lesions among PLWHIV[24], and as such, we anticipated that the same may be the case with our population. Furthermore, oral HPV infection of both low and high risk has been reported to be more prevalent among PLWHIV when compared to negative individuals[25]. Unfortunately, we didn't have a control group; therefore, a better-designed study with appropriate controls would go a long way in enlightening us on what is happening in our population. Additionally, only 24 samples were analysed for HPV presence of such a large cohort. It is possible that only those participants who were related to the facts presented to them came forward for the biopsies. This would represent a special group, and thus these results may overestimate the prevalence of HPV in both tissue and saliva. However, this study could be a pilot, and more versatile research may confirm or refute the significance of these findings.

Detection of high-risk HPVs has been reported to rise with increased detection of potentially malignant oral lesions[25]. It is against this background that we looked at clinically suspicious oral lesions in our PLWHIV cohort and decided to recall them for biopsy. Although only 32% were able to turn up within the two-week, we felt they were representative of the others, as shown in Table 1. We got one carcinoma in situ and five participants with papillomatous changes. It is noteworthy to report that the papillomatous changes were detected in what appeared as flat, whitish mucosal oral lesions. The fact that we had many squamous papillomatous changes is not surprising since a study from Mexico reported them as the most common oral lesions seen in PLWHIV[26]. The one case of carcinoma in situ and four mild dysplastic cases seen in our study were fewer than what was seen among PLWHIV from the US, which may point to differences in risk factors and behaviour[25].

Worth noting were the differences in the HPV types detected in tissue samples and those detected in the saliva from the same participant, as shown in Table 2. The participant with the carcinoma in situ had HPV35 and HPV45 in the extracted DNA from the lesion tissue, but was shedding a different oncogenic HPV68 in saliva. A similar observation was made with participants three to seven. Participants five and seven had HPV18 and HPV68 detected in the extracted DNA from tissue and saliva, respectively. The detection of HPV in tissue may be indicative of integration from previous infections. Although HPV DNA integration plays a big role in the eventual progression of the infection to cancer, there is still controversy on the role of integration in pre-malignant oral lesions[27,28]. It has been noted that integration of the HPV genome into the human host genome leads to disruption of some of the HPV regulatory regions. For HPV16, this commonly occurs at the splitting of the E2 region that regulates the transcription of the E6 and E7 regions of the viral genome. This disruption leads to overexpression of the HPV oncoproteins E6 and E7, which in turn inactivate the retinoblastoma (RB) pathway, causing TP53 degradation and P16 upregulation, leading to HPV-associated squamous cell carcinoma of the oropharynx[29]. However, the fact that many dysplastic lesions that are HPV positive do not progress to cancers and many cancers have no pre-malignant phases points to the challenges in ascribing the role of HPV in the process[27,30]. We therefore need to do more on the available tissue and clinical follow-up if we are to show the effect of detecting HPV in potentially pre-malignant lesions. We need to conduct longitudinal studies to assess the progression of oral premalignant lesions to cancer in people living with HIV. Seven of the cases with normal mucosa had HPV, and some of these, like cases 11, 16, 21,

and 22, had oncogenic types in tissue, as seen in Table 2. Therefore, normal mucosa doesn't preclude the detection of oncogenic HPVs.

Interestingly, although the tissue HPV PCR test was positive for fourteen cases, the p16 immunostaining demonstrated different patterns of positivity in both abnormal and normal tissue. This suggests that p16 expression may not always correlate with HPV infection status, as p16 can be upregulated through mechanisms other than HPV infection, such as cellular stress or other oncogenic processes[31]. Therefore, the use of p16 immunostaining as a surrogate marker for HPV infection should be interpreted cautiously[32]. Additional molecular or clinical data may be necessary to confirm the association between p16 expression and HPV status in these cases.

Limitations of the study

The return rate for the second visit was so low that the results may not be as representative. However, the study's findings can justify resource allocation for oral health screening programs targeting people living with HIV. Just like hypertension and diabetes screening have brought immense survival benefits for PLWHIV, it is likely that oral health screening will add to the quality of life and long-term survival.

Conclusion

Our small cohort of PLWHIV showed high-risk HPV types in both saliva and tissue, with associated p16 overexpression in oral lesions with dysplastic features. Although the findings are preliminary, they highlight the potential value of incorporating HPV and p16 assessment in future oral cancer screening research among HIV-positive individuals. Larger, controlled studies are needed to validate these observations and assess their relevance for the development of screening guidelines.

Transparency statement

The data reported in this manuscript were collected as part of a larger data collection among a cohort of PLHIV. This particular report focuses on participants who had clinically suspicious lesions for potentially pre-malignant oral lesions who returned for biopsy of lesions. The corresponding author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been reported and explained.

Data availability

Data is available on request from William Buwembo by email (william.buwembo@mak.ac.ug)

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Conflict of Interest

The authors declare no conflict of interest.

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PREVALENCIJA MIŠIĆNO-SKELETNIH POREMEĆAJA MEĐU SPECIJALIZANTIMA NA STOMATOLOŠKOM FAKULTETU U SKOPLJU

PREVALENCE OF MUSCULOSKELETAL DISORDERS AMONG RESIDENTS AT THE FACULTY OF DENTISTRY IN SKOPJE

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Sažetak

Cilj: *Cilj ove studije bio je da utvrdi prevalenciju i distribuciju mišićno-skeletnih poremećaja (MSP) među specijalizantima na Stomatološkom fakultetu u Skoplju.*
Materijal i metode: *Studija je obuhvatila 70 specijalizanata stomatologije sa Stomatološkog fakulteta u Skoplju koji su započeli specijalizaciju u akademskoj 2022/2023. godini. Svaki ispitanik je dobio upitnik koji se sastojao od modifikovanog standardizovanog Nordijskog upitnika i nekoliko pitanja osmišljenih za potrebe ove studije. Za unos podataka i statističku analizu korišćen je program Microsoft Excel, uz primenu odgovarajuće formule za $\chi 2$ test.*
Rezultati: *Među ispitanicima su bile 42 žene (60%) i 28 muškaraca (40%). Većina ispitanika imala je između 31 godine i 40 godina. Prevalencija mišićno-skeletnih poremećaja među specijalizantima stomatologije u prethodnih dvanaest meseci iznosila je 52,9% (37 od 70 ispitanika); dakle, više od polovine ispitanika prijavilo je prisustvo MSP-a. Najčešće zahvaćena regija tela bio je vrat, koji je navelo 27 ispitanika (73%). Slede donji deo leđa – kod 23 ispitanika (62,2%); ramena – kod 20 ispitanika (54,1%); ruke – kod 13 ispitanika (35,1%); noge – kod 10 (27%) ispitanika.*
Zaključak: *Pokazalo se da je stopa pojave mišićno-skeletnih poremećaja među specijalizantima stomatologije u porastu, kao i da je mišićno-skeletni bol prisutan u velikoj meri. Stoga, treba naglasiti značaj održavanja pravilnog položaja tela u toku stomatoloških procedura i redovne fizičke aktivnosti kao preventivnih mera kada je reč o pomenutim poremećajima.*

Ključne reči: *mišićno-skeletni poremećaji, stomatologija, ergonomija, bol*

Abstract

Objective: *This study aimed to determine the prevalence and distribution of musculoskeletal disorders (MSDs) among residents at the Faculty of Dentistry in Skopje.*
Materials and Methods*: The study included 70 dental residents from the Faculty of Dentistry in Skopje who commenced their specialization during the 2022/2023 academic year. Each participant received a questionnaire consisting of a Modified Standardized Nordic Questionnaire and several self-developed questions. Data entry and statistical analysis were performed using Microsoft Excel, applying the appropriate formula for the Chi-square test.*
Results: *Of all respondents, 42 were female (60%), and 28 were male (40%). The majority of respondents were between 31 and 40 years of age. The prevalence of musculoskeletal disorders among dental residents over the past 12 months was 52.9% (37 out of 70 participants), indicating that more than half reported experiencing MSDs. The most commonly affected body region was the neck, reported by 27 participants (73%), followed by the lower back in 23 participants (62.2%), the shoulders in 20 (54.1%), the arms in 13 (35.1%), and the legs in 10 (27%).*
Conclusion: *The occurrence of musculoskeletal disorders among dental residents is on the rise, with a significant presence of musculoskeletal pain. Therefore, the importance of maintaining proper posture during dental procedures and engaging in regular physical activity should be emphasized as preventive measures against these disorders.*

Key words: *musculoskeletal disorders, dentistry, ergonomics, pain*

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Introduction

Dentistry is a profession that requires a high degree of precision and a broad range of skills. Dentists are exposed to numerous occupational risk factors arising from prolonged dental procedures, extended working hours, and frequent exposure to various bodily fluids. Musculoskeletal disorders (MSDs) are among the most common occupational health problems affecting dentists, primarily resulting from poor working posture, intensive physical exertion, repetitive movements, and psychological variables such as occupational stress and high mental workload[1].

Musculoskeletal disorders develop progressively and may lead to loss of musculoskeletal function, accompanied by pain and limited mobility. If not diagnosed and treated in a timely manner, they may result in disability and impaired daily functioning[2]. Musculoskeletal disorders are highly prevalent among dentists worldwide, with reported rates ranging from 54% to as high as 96% of individuals who have experienced pain in at least one body region during their professional lifetime[3]. The most commonly affected regions are the neck, shoulders, and back, while pain is the most frequently reported symptom associated with these disorders[4–12].

Data from the literature indicate that pain caused by these disorders significantly interferes with daily activities, affects the quality of dental procedures and patient care, and may sometimes compel dentists to postpone or even discontinue treatment. Several studies have shown that MSDs can lead to disability and represent a major reason why dentists prematurely end their professional careers or change professions[13–16].

The aim of this study was to determine the prevalence and distribution of MSDs among dental residents at the Faculty of Dental Medicine in Skopje.

Materials and Methods

To achieve the stated objectives, this study included 70 dental residents from various specialties at the Faculty of Dentistry, Ss. Cyril and Methodius University in Skopje, who commenced their specialization during the 2022/2023 academic year. Each participant received a questionnaire consisting of the Modified Standardized Nordic Questionnaire[17] and several self-developed questions, which covered basic demographic data, work-related characteristics, the occurrence of MSDs, and aspects of the participants' lifestyle. Incomplete questionnaires were excluded from the study.

Data entry and statistical analysis were performed using Microsoft Excel, applying the appropriate formula for the Chi-square test to examine the association between musculoskeletal disorders and variables such as gender, age, type of dental specialty, working patterns, and computer use. A p-value of < 0.05 was considered statistically significant.

Results

Of all respondents, 42 were female (60%) and 28 were male (40%). The majority of respondents were between 31 and 40 years of age (56.8%).

The prevalence of musculoskeletal disorders (MSDs) among dental residents at the Faculty of Dentistry in Skopje during the past 12 months was 52.9% (37 out of 70 participants), indicating that more than half of the respondents reported MSDs. The most commonly affected body region was the neck, reported by 27 participants (73%), followed by the lower back in 23 participants (62.2%), shoulders in 20 (54.1%), arms in 13 (35.1%), and legs in 10 (27%) (Table 1).

The most common working posture among participants was a combination of sitting and standing, reported by 60% of respondents, while 17.1% preferred working in a sitting position, and 22.9% in a standing position. Most participants reported working more than 40 hours per week (44.3%), and spending approximately one hour per day using a computer (57.1%). Additionally, 75.7% of respondents reported engaging in regular physical activity during the workweek.

The intensity of pain was most often of average character across all body regions. Among the respondents, 31 reported neck pain, of whom 4 (12.9%) experienced mild pain, 22 (71%) average, and 5 (16.1%) severe pain. Twenty-six respondents reported shoulder pain: 6 (23.1%) mild pain, 15 (57.7%) average, and 5 (19.2%) severe pain. Twenty-eight respondents reported lower back pain: 3 (10.7%) mild pain, 15 (53.6%) average, and 10 (35.7%) severe pain. Twenty-four respondents reported arm pain: 12 (50%) mild, 10 (41.7%) average, and 2 (8.3%) severe pain. Twenty-one participants reported leg pain: 6 (28.6%) mild, 10 (47.6%) average, and 5 (23.8%) severe pain (Table 2).

Table 2. Distribution of participants according to age, gender, MSD in the last 12 months, type of dentistry, physician consultations, and intensity of pain in different regions, altered duty due to MSD, daily usage of computers, and physical activities

Table 1. Site of musculoskeletal pain in study subjects in the last 12 months

Site of musculoskeletal pain	**Number (%)**
Neck	27 (73%)
Shoulder	20 (54.1%)
Lower back	23 (62.2%)
Hand/wrist	13 (35.1%)
Legs	10 (27%)

Table 2. Distribution of participants according to age, gender, MSD in the last 12 months, type of dentistry, physician consultations, and intensity of pain in different regions, altered duty due to MSD, daily usage of computers, and physical activities

Variable	**Results**	**Number (%)**
Age	< 30 years	17 (24.3%)
	31–40 years	41 (58.6%)
	41–50 years	11 (15.7%)
	> 50 years	1 (1.4%)
Sex	Male	28 (40%)
	Female	42 (60%)
Type of dentistry	Standing	16 (22.9%)
	Sitting	12 (17.1%)
	Combination	42 (60%)
MSD in the last 12 months	Yes	37 (52.9%)
	No	33 (47.1%)
Neck pain intensity (31 responses)	Mild pain	4 (12.9%)
	Average pain	22 (71%)
	Severe pain	5 (16.1%)
Shoulder pain intensity (26 responses)	Mild pain	6 (23.1%)
	Average pain	15 (57.7%)
	Severe pain	5 (19.2%)

Lower back pain intensity (28 responses)	Mild pain	3 (10.7%)
	Average pain	15 (53.6%)
	Severe pain	10 (35.7%)
Arm pain intensity (24 responses)	Mild pain	12 (50%)
	Average pain	10 (41.7%)
	Severe pain	2 (8.3%)
Leg pain intensity (21 responses)	Mild pain	6 (28.6%)
	Average pain	10 (47.6%)
	Severe pain	5 (23.8%)
Physician consultations for MSD	No	39 (55.7%)
	Yes	31 (44.3%)
Altered duty due to MSD	No	66 (94.3%)
	Yes	4 (5.7%)
Daily usage of computers	0 hours	17 (24.3%)
	1 hour	40 (57.1%)
	2–4 hours	9 (12.9%)
	> 4 hours	4 (5.7%)
Physical activities	Yes	53 (75.7%)
	No	17 (24.3%)

The most common pain management strategies were medication therapy 28 (75.7%) and physical therapy 24 (64.9%) (Figure 1). Most participants did not seek professional medical help for their condition 39 (55.7%) and did not change profession due to MSDs 66 (94.3%).
Figure 1.

The sociodemographic variables of the study participants in relation to the presence of MSDs are presented in Table 3. The Chi-square test showed no statistically significant association between the occurrence of MSDs and gender ($p = 0.9$), age ($p = 0.6$), or type of dental specialty ($p = 0.5$).

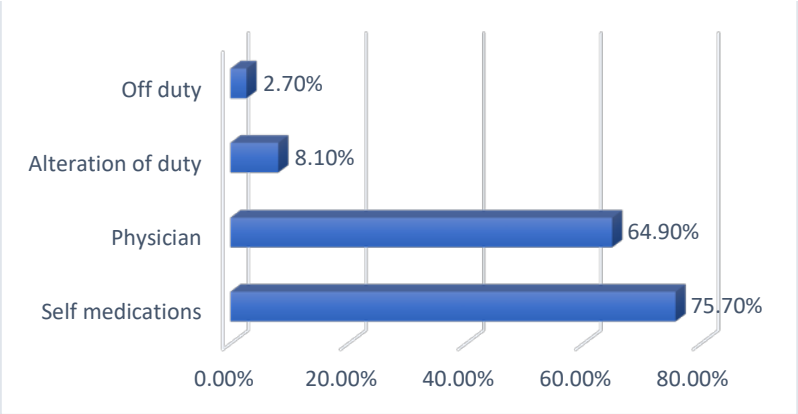

Figure 1. The most common pain manegement strategies

Table 3. Sociodemographic variables of the study participants in relation to the presence of musculoskeletal disorders

Sociodemographic variables	Presence of MSD	P-value
Age		
< 30	8 (21.6%)	
31–40	21 (56.8%)	0.6
41–50	7 (18.9%)	
> 50	1 (2.7%)	
Sex (%)		
Male	15 (40.5%)	0.9
Female	22 (59.5%)	
Types of specialization (%)		
Endodontics and restorative dentistry	3 (50%)	
Oral surgery	16 (57.1%)	
Orthodontics	11 (64.7%)	0.5
Prosthodontics	4 (55.5%)	
Pediatric dentistry	1 (20%)	
Oral medicine and periodontology	2 (28.6%)	
Type of dentistry (%)		
Sitting	7 (18.9%)	
Standing	7 (18.9%)	0.7
Combination	23 (62.2%)	
Usage of computers/laptops (%)		
0 hours	8 (47%)	
1 hour	23 (57.5%)	0.6
2–4 hours	5 (55.5%)	
> 4 hours	1 (25%)	

**Statistically significant

Discussion

Musculoskeletal disorders (MSDs) are among the most common occupational diseases affecting dentists worldwide. The present study aimed to determine the prevalence of MSDs among dental residents at the Faculty of Dental Medicine in Skopje.

Analysis of the obtained data revealed that the prevalence of MSDs among the participants was 52.9% (37 out of 70 residents), indicating that more than half of the respondents reported musculoskeletal pain. Similar findings were reported by Kumar et al.[18], where the prevalence of MSDs among dentists was 58.3%. The prevalence observed in our study was lower compared with studies conducted among dentists in Thessaloniki, Greece (62%)[19], Qatar (78.6%)[20], Germany (92%)[21], and Agadir, Morocco (100%)[22].

Conversely, lower prevalence rates were found among dentists in Kuwait (48%)[16] and Peshawar, Pakistan (47%)[23].

In the present study, the most commonly reported regions of musculoskeletal pain were the neck (73%), lower back (62.2%), and shoulders (54.1%), consistent with the findings of Holzgreve et al.[24], who also identified the neck as the most frequently affected area. Similarly, Eddhaoui et al.[20] found that the most affected anatomical regions during the past 12 months were the neck (22.3%), shoulders (21.4%), and lower back (19.8%), which aligns with our observations.

Due to the unequal number of participants from different specialties, it was not possible to accurately determine the prevalence of musculoskeletal disorders among residents specializing in different fields. The study conducted by Muralidharan et al.[25], reported that all orthodontists (100%) experienced neck-related musculoskeletal pain. In contrast, Kumar et al.[18] found that among various dental specialties, endodontists exhibited the highest prevalence of musculoskeletal pain (88.02%), followed by oral surgeons.

In our study, the majority of participants (60%) preferred a combined working posture alternating between sitting and standing, though no statistically significant difference was observed between those who worked exclusively in a sitting or standing position. These findings are consistent with those of Kumar et al.[18], where 65.6% of respondents reported a combined posture in dental practice. However, in the study by Dabholkar et al.[26], only 5% of dentists preferred a combined posture. Other studies have reported that a seated working posture is the most commonly used among dental practitioners[27,28].

Regarding physical activity, 75.7% of participants in our study reported engaging in regular exercise during the workweek. Kierklo et al.[28] found that 68% of respondents performed physical activity, but mostly after the onset of musculoskeletal pain, while only 10% maintained regular exercise habits. In the study by Banfaida et al.[22], 40.3% of respondents reported not engaging in any physical activity.

When managing MSD-related pain, most participants in our study relied on pharmacological therapy (75.7%) and physical therapy (64.9%), whereas only 8.1% reported taking sick leave due to MSD-related issues. A small proportion (5.7%) reported changing or considering a career change due to the inability to continue practicing dentistry. Eddhaoui et al.[20] similarly found that 84.8% of dentists treated MSD-related problems with medication, emphasizing the high dependence on pharmacological measures while neglecting physical therapy and adherence to ergonomic principles during dental work.

Overall, female participants in our study (59.5%) reported a higher prevalence of MSDs compared to males (40.5%), though this difference was not statistically significant.

Although well-established ergonomic guidelines and principles exist for dental practice, their reinforcement and practical application in daily clinical work remain essential. The consistent implementation of ergonomic measures has a direct impact on dentists' health and the longevity of their professional careers.

The findings of this study corroborate numerous international studies highlighting the high global prevalence of musculoskeletal disorders, particularly among female dentists, and suggest that the frequency of these disorders has remained largely unchanged over the past two decades.

Conclusion

The occurrence of musculoskeletal disorders among dental residents is increasing, with confirmed presence of musculoskeletal pain. A higher prevalence of these disorders was observed among female participants, although these differences were not statistically significant. Regular engagement in physical activities, such as sports and exercise throughout the week, reduces the risk of developing musculoskeletal disorders. Therefore, the importance of maintaining proper posture during dental procedures and promoting regular physical activity should be strongly emphasized as key preventive measures.

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NOVI PRISTUP DEPIGMENTACIJI GINGIVE: VITAMINI C I E ZA POBOLJŠANO ZARASTANJE I SMANJENJE REPIGMENTACIJE

A NOVEL APPROACH TO GINGIVAL DEPIGMENTATION: VITAMINS C AND E FOR ENHANCED HEALING AND REDUCED RE-PIGMENTATION

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Sažetak

Uvod: *Gingivalna hiperpigmentacija, premda benigna, često predstavlja značajan estetski problem za pacijente, posebno u slučajevima izraženog prikazivanja gingive prilikom osmehivanja. Dostupne su različite tehnike depigmentacije, uključujući i hirurške i hemijske pristupe, koji se primenjuju u velikoj meri. Vitamini C i E, poznati po svojim antioksidativnim i depigmentacionim svojstvima, mogu imati pomoćnu ulogu u poboljšanju zarastanja rane i smanjivanju sinteze melanina.*
Materijal i metode: *Pacijent muškog pola, star devetnaest godina, sistemski zdrav, javio se zbog „crne gingive“ koja je zahvatala anteriornu maksilarnu gingivu. Na kliničkom pregledu uočena je difuzna pigmentacija melanina od desnog do levog prvog premolara. Sprovedena je kombinovana procedura depigmentacije skalpelom i dijamantskim borom u lokalnoj anesteziji, s lokalnom aplikacijom vitamina E i C. Propisana je analgetska vodica za ispiranje usta. Vitamin C je primenjen zbog svog potencijalnog inhibitornog dejstva na melanogenezu.*
Rezultati*: Zarastanje je proteklo bez komplikacija; pritom, ostvaren je odličan estetski rezultat. Na kontroli obavljenoj nedelju dana nakon operacije nisu uočene nikakve komplikacije.*
Zaključak: *Lokalna i sistemska primena vitamina C i E posle gingivalne depigmentacije može doprineti unapređenju zarastanja i smanjenju postoperativne hiperpigmentacije. Ovaj prikaz slučaja ukazuje na uspešan estetski rezultat koji se može postići kombinacijom hirurškog i hemijskog pristupa u tretmanu gingivalne pigmentacije melanina.*

Ključne reči: *zubni karijes, studenti, oralno zdravlje, stavovi*

Abstract

Introduction: *Gingival hyperpigmentation, although benign, often presents a significant aesthetic concern for patients, particularly in cases with excessive gingival display. Among the various available depigmentation techniques, both surgical and chemical approaches are widely used. Vitamins C and E, known for their antioxidant and depigmentation properties, may offer adjunctive benefits in enhancing wound healing and reducing melanin synthesis.*
Materials and Methods: *A 19-year-old systemically healthy male presented with a complaint of “black gums” affecting the anterior maxillary gingiva. Clinical examination revealed diffuse melanin pigmentation from the right to the left first premolar. A combined depigmentation procedure using a scalpel and diamond bur was carried out under local anesthesia, with topical application of vitamins E and C. Analgesic mouthwash was prescribed. Vitamin C was incorporated for its melanin-inhibiting potential.*
Results: *Healing was uneventful, with excellent aesthetic improvement and no complications observed at the 1-week follow-up.*
Conclusion: *The adjunctive use of vitamins C and E following gingival depigmentation may enhance healing and reduce postoperative hyperpigmentation. This case highlights a successful aesthetic outcome achieved through a combination of surgical and chemical approaches for managing gingival melanin pigmentation.*

Key words: *aesthetics, ascorbic acid, vitamin E, antioxidants, melanin*

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Introduction

Gingival health and aesthetics are crucial key factors for a pleasing and harmonious smile, with pigmentation playing a vital role. The hyperpigmentation of the gingiva is esthetically not very pleasing and may have a psychological influence on individuals possessing it. This consequence is further exacerbated in patients with a "gummy smile" or overexposed gingiva. A typical macroanatomical characteristic of healthy gingiva is its pink color which varies in shade and is influenced by the degree of vascularization, gingival epithelial thickness, stratum layer keratinization, and level of melanin pigmentation present in the epithelium. Gingival pigmentation manifests as diffuse, dark, purplish discoloration or unevenly shaped brown and light brown or black patches, striae or strands[1].

Melanin, carotene, reduced hemoglobin and oxyhemoglobin, bilirubin, iron, and/or pathological diseases and conditions contribute to the pigmentation of the gingiva; however, gingival hyperpigmentation occurs because of the pronounced production of melanin pigments by the hyperactivity of melanocytes in the gingival epithelium. The cascade of pigmentation consists of three phases: the activation of melanocytes, the synthesis of melanin, and the expression of melanin. Furthermore, it can be influenced by exogenous causes such as drugs, tobacco smoking, amalgam tattoos, or heavy metal deposition, or may be endogenous because of endocrine disorders, syndromes, chronic irritation, or neoplasia[2].

Gingival depigmentation (GD) is a plastic periodontal modality that eliminates or minimizes heightened melanosis. There are various techniques available for gingival depigmentation that involve removing the pigmented layer surgically or chemically. The surgical method consists of partial thickness excision/surgical excision with a scalpel, electrocautery, laser therapy, gingival abrasion/surgical stripping, and cryotherapy. In addition, chemical agents or nonsurgical methods include gingival peeling procedures, which employ ascorbic acid (vitamin C), phenolic compounds, salicylic acid, glycolic acid, trichloroacetic acid, and alcohol. It can also be reduced by masking the pigmented gingiva with grafts from minimally pigmented areas as free gingival autografts or by using acellular dermal matrix allografts[3].

Among chemical depigmentation agents, ascorbic acid (vitamin C) has been recognized as a hydrophilic antioxidant and a vital nutrient for collagen biosynthesis. It contributes to the elimination of hyperpigmentation by chelating copper ions at the tyrosinase active site, which suppresses the enzymatic activity of tyrosinase, thereby decreasing melanin production[4]. It can downregulate the formation of dopaquinone, a progenitor involved in melanin biosynthesis, thus suppressing melanin formation[3]. Furthermore, vitamin E (α-tocopherol, α-Toc) is a lipophilic antioxidant that influences skin pigmentation by interfering with lipid peroxidation of melanocyte membranes, increasing the intracellular glutathione content, and inhibiting tyrosinase activity. Additionally, α-tocopherol has been reported to upregulate the expression of basic fibroblast growth factor and stimulate the formation of type I collagen, which is crucial for periodontal wound repair and tissue regeneration processes[5].

However, studies on the depigmentation effect of vitamin E on gingival hyperpigmentation are scarce. Therefore, in this case report, the depigmentation effects of topical application of vitamin C and vitamin E after a surgical depigmentation procedure were evaluated and compared. This case report also compared the gingival stripping depigmentation procedures performed with a blade bur and a diamond bur.

Materials and Methods

A 19-year-old male patient, who was systemically healthy, attended the Department of Periodontology with a chief complaint of blackish discoloration of gums with a Dummett oral pigmentation index (DOPI) score of 4 and was deeply pigmented, as shown in Figure 1. His gums showed marked pigmentation bilaterally up to the first premolar region during the intraoral examination. The patient had a history of excessive pigmentation since childhood. The patient also had maxillary and mandibular anterior crowding and desired to undergo orthodontic treatment, but wanted the elimination of excessive pigmentation prior to the placement of orthodontic braces. Extraoral examinations revealed a fair skin complexion. An intraoral examination revealed a healthy periodontium with mild gingival inflammation around the marginal and papillary gingiva.

To eliminate gingival inflammation and return the gingiva to a healthy state, phase 1 therapy was carried out 15 days prior to

scheduling the surgical procedure. During this period, the patient was advised to maintain optimal levels of oral hygiene.

Surgical Procedure

To perform GD, a scalpel/blade technique and a gingival abrasion technique using a bur were planned along with the topical application of vitamins C and E. Informed written consent was obtained from the patient following a thorough explanation of the surgical procedure. Laboratory investigations, familial predispositions, and detailed systemic examinations were carried out to identify potential limitations to surgery.

A split-mouth surgical design was planned for the study. The maxillary and mandibular arches were each divided into right and left halves to allow comparative evaluation of the two depigmentation techniques. In the maxillary arch, one half was allocated for gingival depigmentation using the scalpel technique, whereas the contralateral half was assigned for depigmentation using the bur technique. Following completion of the surgical procedure, topical vitamin E was applied over the depigmented maxillary sites. Similarly, in the mandibular arch, one half was designated for depigmentation using the scalpel technique, and the other half for depigmentation using the bur technique. After the completion of depigmentation, topical vitamin C was applied to the treated mandibular sites.

Local anesthesia (lignocaine with adrenaline 1:100000) was administered in the maxillary anterior area from the premolar to the premolar. The phenotype of the gingiva was assessed via a periodontal probe. The phenotype was recorded to be 1 mm (Figure 2).

Using a No. 15 blade with a Bard-Parker grip, the pigmented epithelial layer of the gingiva was removed along with a thin layer of connective tissue while ensuring that the orientation of the blade was parallel to the long axis of the teeth. The minimum level of pressure and force was applied to prevent gingival pitting after surgery (Figure 3).

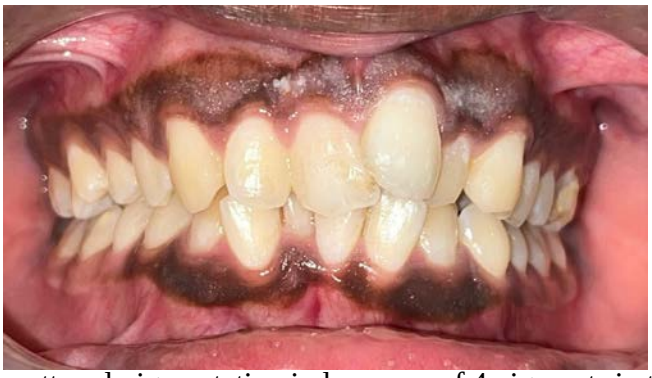

Figure 1. Dummett oral pigmentation index score of 4 pigments in the maxilla and mandible

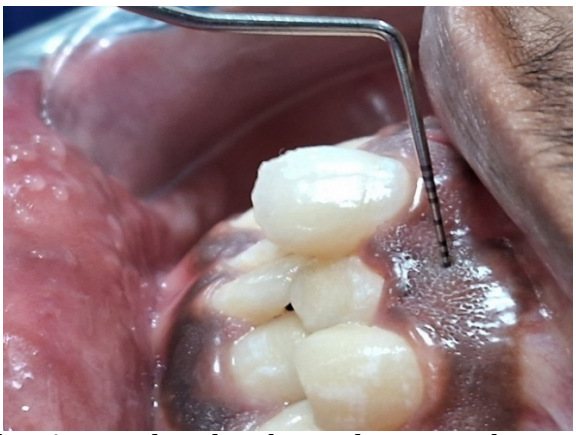

Figure 2. William's periodontal probe used to assess the gingival thickness

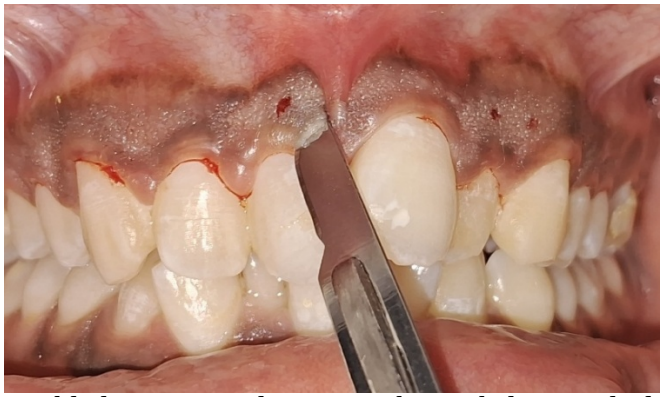

Figure 3. The No. 15 blades were used to excise the epithelium and a layer of connective tissue

The gingival tissue was mechanically stripped or abraded via a diamond bur, and similar tissues were excised using the scalpel technique (Figure 4). After the tissue was removed, vitamin E (400 mg of capsule extraction containing tocopheryl acetate) was applied to the surface of the maxilla[5] (Figure 5), and vitamin C (topical ascorbic acid powder (1000 mg/ml) mixed with saline to form a mixed slurry paste for 10 minutes) was applied to the surface of the mandible (Figure 6)[4]. The bur was maneuvered with minimal pressure possible, employing feather-light sweeping strokes and ensuring that prolonged contact with the bur at one site was avoided.

All possible precautions were taken to eliminate the residual pigmented layers. A periodontal dressing was placed over the surgical area, as displayed in Figure 7.

After surgery, the patient was instructed to rinse with chlorhexidine mouthwash every 12 hours. The surgical site was reviewed after the periodontal pack was removed. Different indices were used to assess pain, itching, and tissue healing, as well as repigmentation (Tables 1, 2 and 3). Pigmentation was evaluated after 15 days, 1 month, 3 months, and 6 months (Figures 8, 9 and 10).

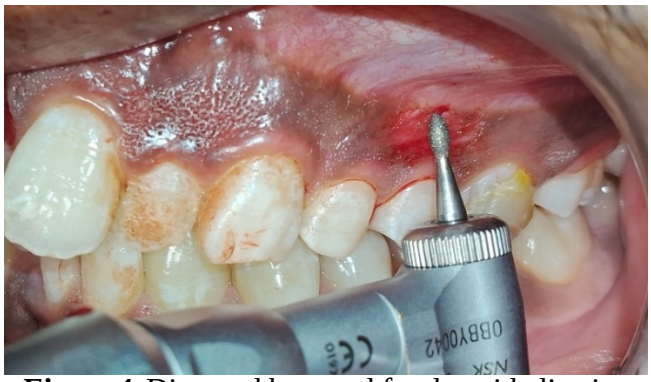

Figure 4. Diamond bur used for de-epithelization

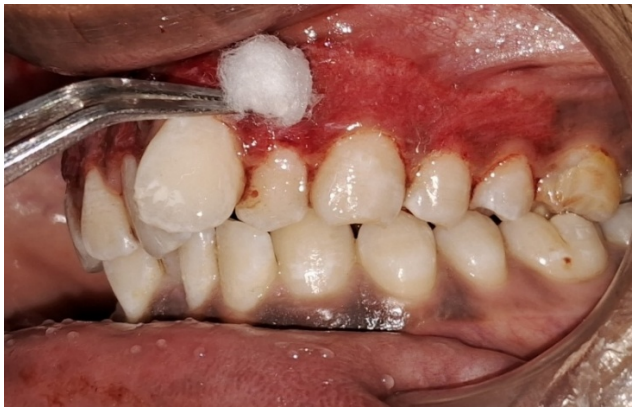

Figure 5. Topical application of vitamin E

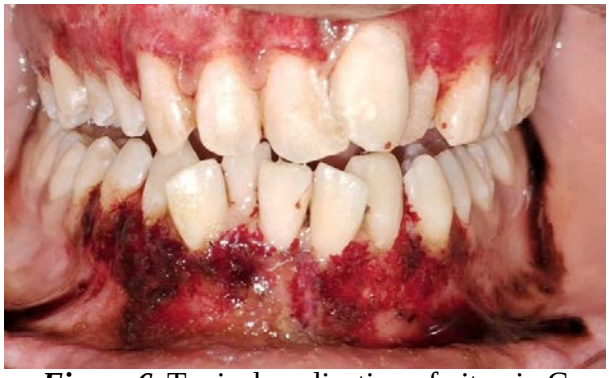

Figure 6. Topical application of vitamin C

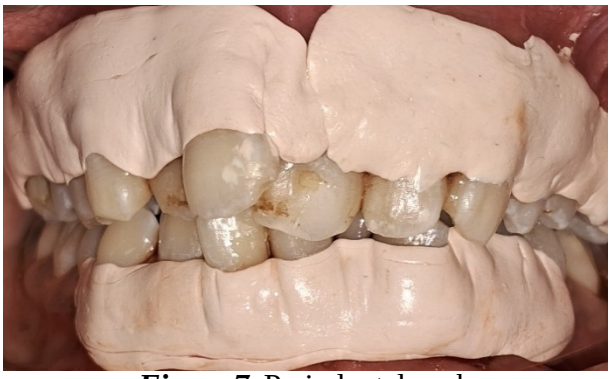

Figure 7. Periodontal pack

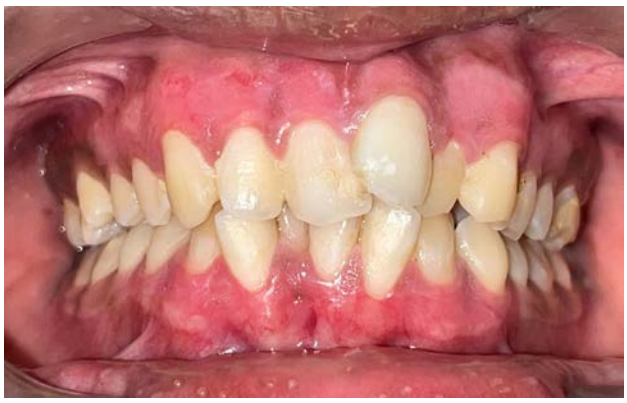

Figure 8. Fifteen-day follow-up

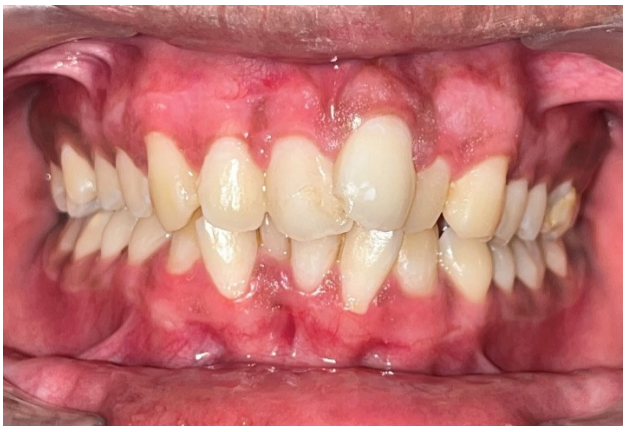

Figure 9. Three-month follow-up

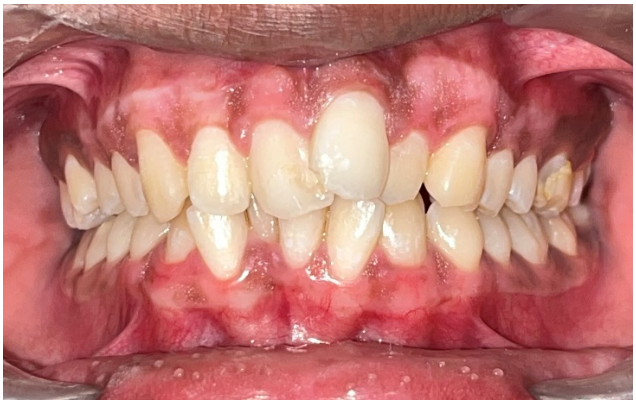

Figure 10. Six-month follow-up

Table 1. Visual analog scale (VAS) score for pain after 24 hours, 7 days, 15 days, and 1 month[6]

Points	Assessment
0	Pain is absent
1–3	Presence of mild pain
4–6	Presence of moderate pain
7–10	Presence of severe pain

Table 2. Verbal scales for the itching of wounds after 24 hours, 7 days, 15 days, and 1 month[3]

Points	Assessment
0	No itching
1	Mild itching present
2	Moderate itching present
3	Severe itching present
4	Extremely severe itching present

Table 3. Wound Healing Index (WHI) of Huang et al. (2005) after 15 days, 1 month, 3 months, and 6 months[7]

Points	Assessment
1	Uneventful healing with absence of gingival edema, erythema, suppuration, patient discomfort, or flap dehiscence
2	Uneventful healing with slight gingival edema, erythema, patient discomfort, or flap dehiscence observed, but no suppuration
3	Poor wound healing with marked gingival edema, erythema, patient discomfort, flap dehiscence, or any suppuration

Results

The patient reported no pain, with a score of 0, in either arch at any of the follow-up intervals. No itching was reported in any of the regions. Additionally, in both arches, healing occurred uneventfully, with mild erythema observed in the maxillary arch only on the 15th day of follow-up on the side of the scalpel technique. Furthermore, no erythema was noted during the later follow-up periods. No suppuration, dehiscence, or patient discomfort was reported. Pigmentation started appearing in both arches at 6 months after the procedure, more prominently in the maxillary arch, where vitamin E topical application was carried out. The results indicated that both vitamin C and vitamin E applications were efficient at managing gingival hyper-pigmentation, with no postoperative complications and adequate maintenance of patient esthetics and comfort.

Discussion

Gingival depigmentation is governed by certain factors such as patient needs, patient acceptance, and aesthetic outcomes. Furthermore, the choice of techniques is also dependent on the gingival biotype and severity of pigmentation[8]. One frequent approach to manage gingival hyperpigmentation is the conventional scalpel surgical technique. This therapeutic approach requires resection of the gingival epithelium along with some connective tissue, followed by healing of the surgical site through secondary intention[5]. The scalpel method is a widely used, affordable approach associated with mild to moderate postoperative discomfort. An evaluation of the scalpel method versus bur abrasion revealed equally favorable outcomes, with no significant differences in tissue healing, gingival repigmentation, or severity. Mild bleeding or discomfort was associated with both procedures. Both scalpels and microabrasions have been proven to be effective in reestablishing the original shade of the gingiva[8].

In this case report, both the scalpel technique and bur abrasion resulted in satisfactory outcomes, with no postoperative complications or discomfort. Healing was adequate for both techniques; mild erythema was observed on the 15th day of treatment in the maxillary arch on the side of the scalpel technique, which later healed with no further complications during the other follow-up periods.

Furthermore, vitamin C functions as a powerful antioxidant and minimally invasive depigmenting modality agent that has been effectively utilized[3]. Vitamin C binds to copper ions at the catalytic site of tyrosinase and blocks its ability to convert tyrosine into dopaquinone, resulting in diminished melanin formation. Additionally, ascorbic acid aids in postsurgical tissue recovery by enhancing collagen biosynthesis and the differentiation and maturation of fibroblasts. Sheel et al. reported satisfactory aesthetic results after gingival depigmentation with a scalpel along with local applications of ascorbic acid, resulting in lower pain scores and no repigmentation areas at the nine-month follow-up[9]. In this case report, vitamin C positively contributed as a depigmentation agent, with enhanced healing and no other complications.

Additionally, vitamin E is known for its antioxidant properties, protecting cell membranes from oxidative stress and interfering with the lipid peroxidation of melanocyte membranes. The depigmentation effect can further be triggered by an increase in the level of intracellular glutathione and the attenuation of tyrosinase[10]. Vitamin E can also exert its antinociceptive effects by interacting with nitric oxide (a free radical that is employed in central pain processing) because of its antioxidant properties and ability to promote wound healing[5,11].

A split-mouth clinical comparative study evaluating the therapeutic effectiveness of topical application of vitamins E and C for enhancing healing following gingival depigmentation suggested that the use of vitamin E and vitamin C topically yields beneficial effects; nonetheless, the application of vitamin E leads to accelerated wound repair and less postoperative discomfort. In this case report, vitamin E had beneficial effects on hyperpigmented gingiva, with no pain or discomfort and adequate wound healing[5].

Moreover, gingival repigmentation is recognized as a clinical recurrence of melanin pigmentation following a phase of therapeutic depigmentation therapy[12]. Gingival repigmentation is thought to occur because of the movement of nearby melanocytes into the surgical site[13]. Relapse can be influenced by the periodontal phenotype, nicotine use, ultraviolet exposure, genetic makeup of the skin, and employed procedure[3]. In this case, the application of vitamins C and E along with the scalpel technique and bur abrasion resulted in the initiation of repigmentation after 6 months. Hence, these interventions are effective and well-tolerated for managing gingival hyperpigmentation.

Limitations

The present case report has certain limitations that should be acknowledged. Given that this study was conducted on a single patient, the findings cannot be generalized to a larger population. The simultaneous use of two depigmentation techniques along with the topical application of vitamins C and E made it difficult to independently evaluate the exact contribution of each intervention to wound healing and repigmentation control. In addition, a follow-up period of six months may not be adequate to assess the long-term recurrence of gingival pigmentation. Therefore, further randomized controlled clinical studies with larger sample sizes and longer follow-up durations are needed to validate these findings.

Conclusion

The technique for successful management of gingival hyperpigmentation can depend on the gingival biotype, degree of gingival hyperpigmentation, case selection, and patient cooperation. In this case report, satisfactory outcomes were achieved with respect to pain, discomfort, and wound healing following the application of both vitamins C and E. The results were esthetically acceptable to patients with no further complications. Like vitamin C, vitamin E can also be used as a depigmentation agent, which provides new perspectives and alternatives for managing gingival hyperpigmentation. However, these findings can be further supported by upcoming randomized clinical studies with larger populations and longer observation periods, which may provide better insight into this outcome.

Ethical Approval and Consent to Participate

The clinical procedure described in this case report was conducted in accordance with institutional ethical standards and the principles of the Declaration of Helsinki. In accordance with the institutional guidelines of the Institutional Ethics Committee, KMC Manipal, ethical approval is waived for case reports and case series involving fewer than ten participants. Written informed consent was obtained from the participants prior to the procedure, after providing a complete explanation of the treatment and its documentation for publication purposes.

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Disclosure Statement

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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APSTRAKT I KLJUČNE REČI

Originalni radovi moraju da sadrže strukturni apstrakt od 250 reči, podeljenih na sledeća 4 paragrafa:

Uvod: opisuje problem o kome se radi u radu

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Rezultati: opisuje primarno rezultate

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Strukturni apstrakti nisu potrebni kod uvodnika i pisma. Ispod apstrakta stoje ključne reci i to tri do pet. Ključne reči mogu biti uzete samo iz Medical Subjects Headings (MeSH).

Apstrakt treba da bude preveden i na engleski jezik (US style), sa naslovom, imenima autora, institucija i ključnim recima.

Za pisanje radova u formi prikaza slučaja, treba uraditi strukturirani apstrakt, na sledeći način:

Osnova problema: (opisati problem ili pojavu u nekoliko rečenica),

Metode rada: (opisati kako je obrađen i dijagnostikovan pacijent i koja bolest ili poremećaj je u pitanju),

Rezultati: (opisati rezultate rada i krajnji ishod),

Zaključak: (1-3 rečenice koja može da služi i kao opis celog postupka koji je rađen i napisan u radu).

TABELE I FIGURE

Svaka tabela sa jasnim naslovom na srpskom i engleskom treba da bude otkucana sa duplim proredom na odvojenom papiru. Obeležiti brojevima tabele jednu za drugom kako nailaze posle prvog navođenja u tekstu (obeležavaju se arapskim brojevima). Dati svakoj kolumni kratko ili skraćeno zaglavlje. Staviti objašnjenja u legendama svih nestandardnih skraćenica korišćenih u tabeli. Za jedinice i merenja vidi odeljak niže. Ne koristiti unutrašnje horizontalne i vertikalne linije. Staviti sve tabele na kraju vaseg fajla. Uvek odvojiti posebne kolumne upotrebom tabulatora, a ne upotrebom razmaknice, tabele moraju biti u tekst formatu.

Linijski prikazani dijagrami i ilustracije (fotografije, fotomikrografije itd.), trebaju biti osmišljene kao figure. Oni takođe treba da budu smešteni na odvojenom listu papira i numerisani jedan za drugim arapskim brojevima u saglasnosti sa prvim koji je citiran u tekstu. Figure treba da budu profesionalno nacrtane i fotografisane. Svaka figura treba da bude etiketirana pozadi ukazujući broj figure, prezime i prvo slovo imena autora, i vrh figure. Fotografije treba da se daju u dva primerka. Kolor fotografije ce se štampati samo u dogovoru sa urednikom ili ako autor sam snosi troškove. Fotomikrografije moraju imati obeleženu unutrasnju razmeru, i simbole, i strelice ili slova treba da su u kontrastu sa pozadinom. Na fotografijama pacijenata mora se sakriti identitet, osim ako se pacijenti u pismenoj formi slože sa objavljivanjem njihovih fotografija sa identitetom. Ukoliko ste pozajmili ili već publikovali negde fotografije priložite i pismenu dozvolu za reprodukovanje. Naslovi i detaljna objašnjenja fotografija treba da budu data u legendama. Ako su korišćeni simboli, strelice, brojevi ili slova za identifikaciju delova slike objasniti svaku jasno u legendi.

ZAHVALNOSTI

Priznanja i zahvalnosti prethode literaturi specificirajući generalnu podršku kao i odeljenje i ime šefa odeljenja, priznanja tehničkoj pomoći i konačno finansijskoj i materijalnoj pomoći. Navesti naziv i broj projekta, odnosno naziv programa u okviru koga je nastao članak i naziv institucije koja je finansirala projekat, u posebnoj napomeni pri dnu prve strane članka.

LITERATURA

Autori su odgovorni za tačnost literaturnih podataka. Reference treba da budu na posebnom listu i delu odmah iza teksta. Samo reference bitne za studiju mogu biti citirane. Kada je citiranje literature neophodno primeniti Vankuver stil. Na posebnom listu se navode citati referenci koji su označeni rednim brojevima po redosledu u kome se pojavljuju u tekstu i svaki citat odgovara brojevima koji sadrži navedenu referencu. Primeri tačnih oblika referenci :

RADOVI U ČASOPISIMA

1. Standardni članak u časopisu (lista svih autora, ali ako je broj veći od šest citirati tri i dodati et al): Glass DA, Mellonig JT, Towle HJ. Histologic evaluation of bone inductive proteins complexed with coralline hydroxyapatite in an extraskeletal site of the rat. J Periodontol 1989; 60:121-125.

2. Organizacija kao autor: Federation Dentaire Internationale. Technical Report No. 28. Guidelines for antibiotic prophylaxis of infective endocarditis for dental patients with cardiovascular disease. Int Dent J 1987;37:235.

3. Nije dat autor: Coffee drinking and cancer of the pancreas (editorial).BMJ 1981;283:628.

4. Volumen sa suplementom: Magni R, Rossoni G, Berti R, BN52021 protect guinea pig from heart anaohylaxis. Pharmacol Res Commun 1988; 20 Suppl 5:75-8.

Knjige ili druge monografije

5. Lični autor (i): Tullman JJ, Redding SW. Systemic Disease in Dental Treatment. St.Louis: The CV Mosby Company;1983:1-5.

6. Poglavlje u knjizi: Rees TD. Dental management of the medically compromised patient. In: McDonald RE, Hurt WC,Gilmore HW, Middleton RA, eds.Current Therapy in Dentistry, vol.7. St. Louis: The CV Mosby Company; 1980:3-7.

7. Disertacije i teze: Teerakapong A. Langerhans Cells in human periodontally healthy and diseased gingiva. (Thesis). Houston, TX: University of Texas; 1987.92 p.

Ostali publikovani materijal

8. Novinski članak: Shaffer RA.Advances in chemistry are starting to unlock mysteries of the brain. The Washington Post 1989 Ang 7; Sect. A:2 (col. 5).

Reference-elektronski citati

9. On line časopisi bez podataka o volumenu i strani. Berlin JA , Antman EM. Advantages and limitations of metaanalytic regressions of clinical trials data. Online J Curr Clin Trials (serial online). June 4;doc 134. Accessed July 20, 2000.

10. Online časopisi sa podacima o volumenu i strani. Fowler EB, Breault LG. Ridge augmentation with a folded acellular dermal matrix allograft: A case Report. J Contemp Dent Pract (serial online). 2001;2(3):31-40. Available from: Procter&Gamble Company, Cincinnat, OH. Accessed December 15, 2001.

11. World Wide Web.Centers for Disease Control and Prevention. Preventing emerging infectious diseases: Addressing the problem of antimicrobial resistance. Available at: http://www.cdc.gov/ncidod/emergplan/antiresist/. Accessed November 5, 2001.

JEDINICE MERE

Sva merenja treba da budu izražena u terminima Internacionalnog Sistema Jedinica (Si).

SKRAĆENICE I SIMBOLI

Ako se koriste nestandardne skraćenice potrebno je prilikom prvog korišćenja celog izraza u tekstu dati njegov puni naziv, a zatim u daljem tekstu koristiti skraćenicu. Nazivi simptoma, znakova i bolesti, kao i anatomski i histološki detalji ne mogu se skraćivati.

OFFPRINTS

Korespondirajući autori svih tipova radova izuzev pisama, novosti i pregleda knjiga primiće 1 broj časopisa oslobođena plaćanja.

SIMBOLI ZA OZNAČAVANJE (FUSNOTE)

Mogu se koristiti samo za identifikaciju zapošljenja autora, za objašnjenje simbola u tabelama i ilustracijama itd. Koristite sledeće fusnote: *,&, #,**, itd.

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Poslati 3 kopije rada i elektronsku verziju (CD-ROM, E-mail). Kopije rada i sav sadržaj treba spakovati u tvrdu kovertu kako bi se sprecilo oštećenje za vreme poštanskog saobraćaja. Radovi moraju biti potkrepljeni sa zatvorenim pismom potpisanim od svih autora. Ono mora da sadrži: a) izjavu da je rad pročitan i odobren od svih autora; b) informaciju o prethodnoj ili dupliciranoj publikaciji ili davanju rada na drugom mestu ili nekog njenog dela ranije; c) izjavu o finansijskim ili drugim vezama koje mogu dovesti do sukoba interesa; d) ime, adresu i broj telefona autora za korespondenciju koji je odgovoran za komunikaciju i korespondenciju; e) izjavu da su klinička i eksperimentalna istraživanja sprovedena u skladu sa institucijskim etičkim komitetom ili sa Helsinskom deklaracijom. Sem ovoga, pismo treba da sadrži i obaveštenje o vrsti rada i da li autori plaćaju ekstra cenu za kolor reprodukcije.

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Sekretari: Asist. Simona Stojanović, Mr. sci dr Miloš Tijanić

Klinika za Stomatologiju

Bul. Zorana Đinđića 52

18000 Niš, Srbija

E-mail: tarana.simona@gmail.com, tijanicm@yahoo.com

Predavanje materijala direktno uredniku ili bilo kom članu uređivačkog odbora otežaće i odužiće proces recenzije i prijema rada za štampanje.

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Skladištenje informacije: CD-ROM u Windows XP ili veći format. Software: radovi na disku treba da budu u Word-u za Windows. Etiketa: Napišite prvo ime autora na nalepnici CD-a, zajedno sa imenom i verzijom korišćenog word procesora. Oznaciti sve CD sadržajem figura, dijagrama itd, sa imenom prvog autora, imenom fajla, formatom i sabijenim šemama ako su korišćeni. Fajlovi: priložiti tekst i tabele svakog rada kao pojedinacni fajl, ali stavite sve figure, grafikone itd., u odvojenim fajlovima. Dozvoljeni grafički formati su EPS i TIF. Veličina figura treba da bude 8,5 cm ili 18,0 cm u rezoluciji od minimalno 300 dpi. Molimo Vas da pošaljete originalne fotografije, ne šaljite foto-kopije. Format: unosite svoj tekst besprekidno, samo umetnuti hard return na kraju paragrafa ili poglavlja, podnaslova, lista itd. Ne upotrebljavajte softwareski plan stranica. Molimo Vas da koristite Times New Roman 12 font za Word za Windows. Neku reč ili frazu u tekstu koju želite da izdvojite označite kroz rad u italic pismu. Boldirajte ono što se koristi uzastopno u tekstu za određene matematicke simbole, na primer, vektori. Molimo da proverite disk na virus i verifikujte da on sadrži ispravan fajl.

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Autori mogu predati svoje revidirane radove uključujući tabele i figure na CD-u sa PC ili Mac fajlom. Vratiti revidirane radove sa celokupnim materijalom na istu adresu sekretarijat.

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Acta Stomatologica Naissi is a scientific journal of the University of Niš, Faculty of Medicine and Clinic of Dental Medicine, which publishes articles relevant to the science and practice of Dentistry in general and related areas.

Please read carefully the following instructions to authors prior to manuscript preparation and submission. Papers which are not prepared according to the propositions and instructions will be returned to authors for corrections before forwarding them to reviewers. In case of unacceptable articles only illustrations will be returned.

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Acta Stomatologica Naissi publishes editorials, original scientific or clinical articles, review articles, preliminary reports, case reports, technical innovations, letters to the editor, articles from up-to-date literature, book reviews, reports and presentations from national and international congresses and symposiums which have not been previously submitted for publication elsewhere. All submitted articles will be reviewed by at least 2 reviewers, and when appropriate, by a statistical reviewer. Authors will be notified of acceptance, rejection, or need for revision within 6 weeks of submission. Articles are not paid for.

LANGUAGE

All submitted articles should be written in bilingual (Serbian and English) language. Abstracts should be written in Serbian and precise and grammatically correct English language, preferably US English. Avoid using Latin terms; however if necessary, put them in parentheses.

ETHICS

When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) or with the Helsinki Declaration (1964, amended in 1975 and 1983) ofthe World Medical Association.

GENERAL INSTRUCTIONS

PREPARATION

Articles should be written on A4 white bond paper size (21x29.5cm) on one side of the paper only, and double-spaced (including illustration legends and references) providing 25 mm ample margins all around. Only one copy of the manuscript should contain the surname and the author's first name initial in the upper right corner. Manuscripts should be organized as follows: Title Page, Abstract and Key words, Introduction, Patients/Materials and Methods, Results, Discussion, Conclusions, Acknowledgments, References, Figure Legends, Tables, Figures. Title page is numbered as page 1, and all other pages should be numbered conse-quently.

TITLE PAGE

The title page should contain: a) the full title of the article (in upper case); b) first name, middle initial, and last name of each author without the academic degree; c) name of department and institutional affiliation for each author; d) running title of no more than 10 characters. At the bottom of the page, please indicate the name, academic degree and address (including E-mail, telephone and fax number) of the author responsible for correspondence.

It is recommendable to use the words appropriate for indexing and searching. If there are not such words in the title, then subtitle should be added.

If the article in the previous version has been orally exposed (under the same or similar title), such information should be separately noted at the bottom of the first page ofthe article.

Abstract and Key words

All original abstracts should be submitted with a structured abstract, consisting of no more than 250 words, and the following 4 paragraphs:

Background: Describes the problem being addressed.

Material and Methods: Describes how the study was performed.

Results: Describes the primary results.

Conclusion: Reports what authors have concluded from these results, and notes their clinical implications.

Key words: A maximum of 5 key words drawn from MeSH documentation. Abstract should be translated into English (US style), with the title, name(s) of author(s), institutional affiliation and key words.

To write papers in the form of a case report, a structured abstract should be done, as follows:

Basis of the problem: (describe the problem or occurrence in a few sentences),

Methods of work: (describe how the patient was treated and diagnosed and which disease or disorder is in question),

Results: (describe the results of the work and the final outcome),

Conclusion: (1-3 sentences that can also serve as a description of the whole procedure that was done and written in the paper).

To write papers in the form of a case report, a structured abstract should be done, as follows:

Basis of the problem: (describe the problem or occurrence in a few sentences),

Methods of work: (describe how the patient was treated and diagnosed and which disease or disorder is in question),

Results: (describe the results of the work and the final outcome),

Conclusion: (1-3 sentences that can also serve as a description of the whole procedure that was done and written in the paper).

TABLES AND FIGURES

Each table with a brief title (on Serbian and English) should be typed double-spaced on a separate sheet of paper. Number tables consecutively (with Arabic numbers) in the order of their first citation in the text. Give each column a short or abbreviated heading. Place explanations in legends of all nonstandard abbreviations which are used in table. For units and measurements see paragraph below. Do not use internal horizontal and vertical rules. Place all tables at the end of your file. Always separate the individual columns using tabulators, not using space bar, i.e. tables must be in text format. Line drawings diagrams and halftone illustrations (photographs, photomicrographs, etc.) should be designated as figures. They should be listed on separate sheet and numbered consecutively with Arabic numerals according to the order in which they have been first cited in the text. Figures should be professionally drawn (not simply typewritten) and photographed. Each figure should be labeled on its back indicated the number of the figure, last name and the first letter of the author, and the topside of the figure. Photographs should be supplied in two copies. Color photographs are published only in case if author himself bears expenses. Photomicrographs must have internal scale markers, and symbols, arrows or letters should contrast with the background. Photographs of patients must conceal their identity unless patients approve the publishing of the photograph in written form. If you borrow or use already published photographs please submit a written permission for reproduction. Permission is not required for the documents in the public domain. Figures will not be returned unless requested. Captions and detailed explanations of the figures should be given in the legends. If symbols, arrows, numbers, or letters are used to identify parts of the figure identity and explain each one clearly in the legend.

ACKNOWLEDGEMENTS

Acknowledgements are positioned before the reference list specifying general support by department chairman, acknowledgements of technical as well as financial and material support. Acknowledgement includes the title and number of the project, i.e. the title of the programme within which the article was composed and the title of the institution funding the project; it should be written as a separate notification at the bottom ofthe first page of the article.

REFERENCES

Authors are responsible for accuracy of literature data. References should be listed in a separate section immediately following the text. Only references important for the study should be cited. It is necessary to apply Vancouver style. Citations are numbered consecutively in the order in which they appear in the text and each citation corresponds to a numbered reference containing publication information about the source cited in the reference list at the end of the publication. Examples of references are given bellow:

Journals:

1. Standard journal reference. (Note: list all authors if six or less; when seven or more, list only first three and add et al): Glass DA, Mellonig JT, Towle HJ. Histologic evaluation of bone inductive proteins complexed with coralline hydroxyapatite in an extraskeletal site of the rat. J Periodontol 1989;60:121-125.

2. Corporate author: Federation Dentaire Internationale. Technical Report No.28. Guidelines for antibiotic prophylaxis of infective endocarditis for dental patients with cardiovascular disease. Int Dent J 1987;37:235.

3. No author given: Coffee drinking and cancer of the pancreas (editorial). BMJ 1981;283:628.

4. Volume with supplement: Magni R, Rossoni G, Berti R, BN52021 protect guinea pig from heart anaphylaxis. Pharmacol Res Commun 1988; 20 Suppl 5:75-8.

Books or other monographs:

5. Personal author(s): Tullman JJ, Redding SW. Systemic Disease in Dental Treatment. St. Louis: The CV Mosby Company; 1983:1-5.

6. Chapter in a book: Rees TD. Dental management of the medically compromised patient. In: McDonald RE, Hurt WC, Gilmore HW, Middleton RA, eds. Current Therapy in Dentistry, vol. 7. St. Louis: The CV Mosby Company; 1980:3-7.

7. Dissertations and thesis: Teerakapong A. Langerhans Cells in human periodontally healthy and diseased gingiva. (Thesis). Houston, TX: University of Texas; 1987.92 p.

Other published material:

8. Newspaper article: Shaffer RA.Advances in chemistry are starting to unlock mysteries ofthe brain. The Washington Post 1989Ang 7; Sect.A:2 (col. 5).

References - electronic quotations:

9. Online journals without volume and page information. Berlin JA, Antman EM. Advantages and limitations of metaanalytic regressions of clinical trials data. Online J Curr Clin Trials (serial online). June 4; doc 134. Accessed July 20, 2000.

10. Online journals with volume and page information. Fowler EB, Breault LG. Ridge augmentation with a folded acellular dermal matrix allograft: A case Report. J Contemp Dent Pract (serial online). 2001;2(3):31-40. Available from: Procter&Gamble Company, Cincinnat, OH. Accessed December 15, 2001.

11. World Wide Web.Centers for Disease Control and Prevention. Preventing emerging infectious diseases: Addressing the problem of antimicrobial resistance. Available at: http://www.cdc.gov/ncidod/emergplan/antiresist/. Accessed November 5, 2001.

UNITS OF MEASUREMENTS

All measurements should be reported in terms of the International System of Units (SI)

ABBREVIATIONS AND SYMBOLS

Avoid abbreviations in the text but whenever possible use standard abbreviations. However, if nonstandard abbreviations are used, the full term of which and abbreviation stands for should precede its first use in text. Names of symptoms, signs and diseases, as well as anatomic and histologic characteristics cannot be abbreviated.

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