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### Reporting Guidelines in Health Research

#### Dr. Sujita Shrestha

Chief Editor

Guidelines for reporting health research are important tool which improves accuracy and transparency of publications. Well developed health research reporting guidelines provide structured format for authors to present research methods, results and conclusions. Additionally, it gives valuable information on research publications, enhancing reproducibility, clarity and reliability of study findings. The efficient use of reporting guidelines help researchers to publish high quality health research. A research group consisting; academicians, clinicians, medical journal editors, statisticians, systematic reviewers and content experts had developed reporting guidelines and flow diagrams. A reporting guideline is a checklist and flow diagram which facilitates to improve quality of health research articles and guides authors in reporting specific type of research. Reporting guidelines is accessible depending upon the research/study design which includes detailed checklist of 20 to 40 items. The main purpose of reporting guidelines was to determine the minimum set of information that gives complete description of the specific type of study.

The EQUATOR (Enhancing the QUAlity and Transparency Of health Research; Network's Library for Health Research Reporting) is an international initiative that aims to improve the quality of research publications. It also provides comprehensive list of reporting guidelines and other material which helps to improve reporting. Reporting guidelines enables readers to assess and repeat research and also helps to ensure research work done precisely. Research studies should be; scientific, have good methodological quality, and accurate. Detailed reliable guidelines and checklists developed by experts are available for different types of research studies.<sup>1</sup>

Some of the commonly used guidelines in health research reporting are:

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) is a guidelines for reporting systematic reviews and meta-analyses, published in 2009, comprises of 27-item checklist and four-phase flow diagram. The checklist includes; title, abstract, introduction, methods, results, discussion, and source of funding or finance. The flow chart maps different features such as screening, eligibility, and inclusion/exclusion criteria for the report.<sup>2</sup> CONSORT (Consolidated Standards of Reporting Trials) is a useful set of guidelines for complete and transparent reporting of randomized controlled trials developed in 2010. It includes 25-item checklist and flow diagram. The checklist guides; trial design, analyze and interpretation of findings and flow diagram depicts different phases of the trial.3 STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) includes 22-item checklist, specifically designed to address observational studies; cohort, case-control, and cross-sectional studies introduced in 2007. STREGA (STrengthening the REporting of Genetic Association studies), extension of STROBE for genetic association studies.<sup>4</sup> MOOSE (Meta-analysis Of Observational Studies in Epidemiology) is a set of guidelines for reporting and enhancing epidemiological meta-analysis published in 2000. It consists of 35-item checklist, further divided into subcategories: title and abstract, introduction, sources and study selection, results, and discussion.<sup>5</sup> STARD (STAndards for the Reporting of Diagnostic accuracy studies) commenced in 2003 for reporting studies on diagnostic or prognostic accuracy. It includes 25-point checklist that comprises; heading, title, abstract, keywords, introduction, methods, and discussion. The flow diagram helps in reporting recruitment protocol and order in which trials and tests have been conducted. SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials), developed in 2007; one of the most recent sets of guidelines for reporting scientific trial protocols with 33-item checklist. It had five major domains; administrative information, introduction, methodologies, ethical concerns, and appendices. SAMPL (Statistical Analyses and Methods in the Published Literature) developed in 2013, used in reporting basic statistical analysis published in biomedical journals.8 CARE (Case Report), 10-item checklist developed in 2013.9 COREQ (COnsolidated criteria for REporting Qualitative research) introduced in 2007, 32-item checklist for interviews and focus groups.<sup>10</sup> SRQR (Standards for Reporting Qualitative Research) was published in 2014, consists of 21 items.<sup>11</sup>

Reporting guidelines are potent tools which help to improve transparency, completeness and accuracy of health research and increase value of published research. Most of the medical and biomedical journals believe in evidence based practice thus, reporting guidelines helps to improve the quality of health research. These guidelines help researcher in preparing or reviewing a specific type of research which includes minimum set of items to be reported in the form of checklist and flow diagram. Guidelines enable another researcher to replicate studies accurately and improve reproducibility. It also provide framework for peer reviewers to assess research manuscript effectively and helps to publish quality articles. Reporting guidelines serve as a roadmap for researchers/authors to include all the essential information required for publication.

Most of the medical journals of Nepal have not endorsed reporting guidelines thus; editors and researchers may have difficulty in assessing quality and valid research. Therefore, implementing reporting research guidelines in publication adds value to manuscript and plays vital role in maintaining the integrity, quality and credibility of research.

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## Prevalence of Helicobacter pylori infection among the patients undergone for endoscopic biopsy

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#### **ABSTRACT**

**Introduction:** Helicobacter pylori (H.pylori) is well known organism which colonizes in the gastric mucosa. It can cause variety of upper gastrointestinal disorder such as; chronic gastritis, peptic ulcer, gastric mucosa associated lymphoid tissue (MALT) lymphoma and gastric cancer.

**Objective:** To identify the prevalence of H.pylori based on histopathology among the patients undergoing for endoscopic biopsy.

Materials and Method: Cross sectional study was conducted after the ethical approval from KDC-IRC. A total of 84 endoscopic biopsies with histological examination from Baishak 2077 to Baishak 2079 were included in the study. Statistical analysis was done using percentage, mean, sensitivity, specificity, positive and negative predictive value. Chi –square test was used to analyze the relation between the various variables. The p value of <0.05 was taken as statistically significant.

**Result:** The prevalent of H.pylori infection was 42.9% (36) among them 64.3% (54) were male and 35.7% (30) were females. The most frequent type of gastritis was chronic erosive gastritis 35.7%(30,), followed by chronic active HP associated gastritis 28.6%(24), chronic inactive HP associated gastritis 10.7%(9), chronic gastritis 4.8%(4), chronic follicular gastritis 3.6%(3), suppurative gastritis2.4%(2), superficial gastritis 2.4%(2), eosinophillic gastritis 1.2% (1) and reactive gastropathy 1.2%(1).

**Conclusion:** The prevalence of H.pylori infection among the gastritis patients was high therefore; examination for H.pylori infection in patient undergoing for the endoscopic examination was suggested.

Keywords: Gastritis; Endoscopy; Helicobacter pylori

#### **INTRODUCTION**

Helicobactor pylori (H.pylori) is a spiral, gram negative rod shaped bacilli which has strong association with chronic active gastritis, gastric adenocarcinoma and mucosa associated lymphatic tissue lymphoma (MALToma).¹ Presence of in gastric mucosa was discovered in 1983 by Warren and Marshall. The prevalence of H.pylori infection is 50% worldwide and 90% in developing countries.² In Nepal the prevalence of H.pylori infection is 30% to 87%.².³ The present study aims to determine the prevalence of H.pylori infection among the patient undergone for endoscopic biopsy in Kantipur Dental College and Grande City Hospital.

#### MATERIALS AND METHOD

A cross sectional study was conducted among the patient with endoscopic biopsy after ethical approval from KDC-IRC. A total of 84 gastric biopsies with histological examination from Baishak 2077 to Baishak 2079 entered in hospital record were included in the study studied. The biopsy specimens were processed, embedded in paraffin, sliced, and stained

with hematoxylin and eosin (H and E) after being fixed overnight in 10% buffered formalin. Geimsa stain was done for each biopsy to demonstrate the H.pylori. Histological reporting; gastric mucosa inflammation, atrophy, intestinal metaplasia, and neoplasia and H. pylori colonization were included. Gastritis was classified into: chronic active HP associated follicular gastritis, chronic active HP associated gastritis, chronic erosive gastritis, superficial gastritis and chronic inactive HP associated gastritis. Statistical analysis was done using SPSS 20. Mean, sensitivity, specificity, positive and negative predictive value was calculated. Chi—square test was used to analyze the association between the various independent variables. The p value of <0.05 was taken as statistically significant.

#### **RESULT**

Among 84 cases of endoscopic biopsy, 54 (64.3%) were males and 30 (35.7%) females and H.pylori infection prevalent was 42.9%. The association between the gender and H.pylori infection was statistically insignificant with p value of 0.189 as shown in Table 1.

Table 1: Association between gender and H.pylori infection

H.pylori	Male (%)	Female(%)	Chi square	P
Present	26(48.1)	10(33.3)		
Absent	28(51.9)	20(66.7)	1.728	0.189
Total	54	30		

The age of patients undergone for the procedure ranged from 24 to 70 yrs with mean age of 45.15 (SD  $\pm$  13.42) years. The association between age and H.pylori infection was also statistically insignificant with p value of 0.99 as shown in Table 2. The frequency of H.pylori infection was more among age 50-59 years.

Table 2: Association between age and H.pylori infection

H. pylori	H. pylori Age					Chi square	P
	< 30	30-39	40-49	50-59	>=60		
Present	7(46.7)	8(44.4)	7(43.8)	8(42.1)	6(37.5)		
Absent	8(53.3)	10(55.6)	9(56.2)	11(57.9)	10(37.5)	0.305	0.99
Total	15(17.8)	18(21.4)	16(19.1)	19(22.6)	16(19.1)		

The most frequent type of gastritis was chronic erosive gastritis 30 (35.7%), followed by chronic active HP associated gastritis 24 (28.6%) and chronic inactive HP associated gastritis 9 (10.7%) Table 3.

Table 3: Frequency of different histological type of gastritis

Types of Gastritis	N	%
Chronic erosive gastritis	30	35.7
Chronic active HP associated gastritis	24	28.6
Chronic inactive HP associated gastritis	9	10.7
Chronic active HP associated follicular gastritis	7	8.3
Chronic gastritis	4	4.8
Chronic HP associated follicular gastritis	3	3.6
Superficial gastritis	2	2.4
Suppurative gastritis	2	2.4
Eosinophillic gastritis	1	1.2
Reactive gastropathy	1	1.2
Total	84	100

In the present study, the histopathological finding of atrophy of mucosa associated with H. pylori associated gastritis was 25%, the finding of intestinal metaplasia in H.pylori associated gastritis was 30.6% with significant p value of 0.015 and 0.042 respectively Table 4.

Table 4: Frequency of atrophy and metaplasia associated with gastritis

	HP associated gastritis (N=36)	HP not associated gastritis (N=48)	p value	
Atrophy				
Absent	27 (75%)	45 (93.8%)	0.015	
Present	9(25%)	3(6.2%)	0.015	
Metaplasia				
Absent	25(69.4%)	42(87.5%)	0.042	
Present	11(30.6%)	6(12.5%)	0.042	

#### **DISCUSSION**

H.pylori is one of the most common chronic bacterial infections. The prevalence of H.pylori infection in developing country varies widely from 11% to 70% whereas in developed country rate of infection is low 20 to 40%. The reason for variation in prevalence is due to differences in socioeconomic status of the population.

The transmission of H.pylori is mainly by oral to oral or faecooral route. The lack of safe drinking water, sanitation, poor diet, and overcrowding plays role in determining the prevalence of the infection. <sup>2, 4, 5</sup>

In the present study the prevalence of H.pylori infection was 42.9% which was comparable with other studies; 30%

to 87%.<sup>3,4,6,7</sup> The prevalence of H.pylori infection was high among 50 to 59 years old population which was similar in Shakya RP *et. al* study and Shrestha UK *et. al* study.<sup>6,7</sup> But, Ansari S *et. al* study reported that the prevalence was more in the young people.<sup>8</sup>

In the present study, the association between H.pylori infection and age and with gender was statistically insignificant with p value of 0.99 and 0.189 respectively. Similar to present study; Shakya RP et. al and Shokrzadeh et. al reported no significant difference according to gender. In contrast, a study by Kaore et. al found higher prevalence in female. The study of Graham et. al mentioned that the prevalence of H.pylori infection was twice high in blacks than in white.

In the present study according to the histopathological feature, the most frequent type of gastritis was chronic erosive gastritis (35.7%), followed by chronic active HP associated gastritis (28.6%). Similarly, in KC Shiva Raj *et al* study the most common pathology in histopathology was chronic active gastritis (42.1%) followed by chronic persistent gastritis (38.2%) and chronic follicular gastritis (19.1%). Chronic active gastritis (85.3%) was the most common finding followed by chronic persistent gastritis (83.3%) and chronic follicular gastritis (41.4%).<sup>3,11</sup>

The histopathological finding of atrophy of mucosa associated with H. pylori associated gastritis was 25%, the finding of intestinal metaplasia in H.pylori associated gastritis was 30.6% with significant p value of 0.015 and 0.042 respectively. The incidence of atrophy and intestinal

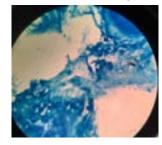
metaplasia was high in the present study as compared to the other study. In Shrestha UK *et. al*, the atrophic changes and intestinal metaplasia were detected in very low number of cases. In Shrestha R *et. al* study the intestinal metaplasia was detected in 5% cases and KC Shiva Raj *et. al* study revealed intestinal metaplasia in 3% and atrophy in 2.35% cases. <sup>3,7,11</sup>

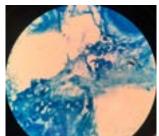
#### **CONCLUSION**

The prevalence of H.pylori infections detected by histopathology method was 42.9%. The infection was more in male than in female. The frequency of the H.pylori was more common in age of 50-59 years. The most common histopathology finding was chronic erosive gastritis followed by chronic active gastritis. Therefore, patient undergoing endoscopic procedure should always be examined for H.pylori infection and appropriate management of the infection should be advised.



#### Photograph No.1 and No.2





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# Assessment of Hounsfield unit in the maxillary and mandibular ridges using CBCT

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#### **ABSTRACT**

**Introduction:** The cone beam computed tomography (CBCT), was developed for the evaluation of dentomaxillofacial structures, and more frequently it is used in dentistry because it is a less complex device that produces images with satisfactory resolution, with little artifact incidence and lower dose of radiation. Multislice and cone beam CT images are frequently used to determine mineral density of craniofacial bone structures.

**Objective:** To examine the bone densities of edentulous in each sextant by CBCT.

Materials and Method: Based on CBCT images, the voxel values representing bone density in each sextant of 112 sites were calculated in the range from -20 to 2,000 Hounsfield units (HU). The bone densities of these regions were categorized according to Misch's classification and compared among individuals and between sexes and 2 different age groups.

**Result:** Our study also compared gender-based differences in Hounsfield unit in each sextant and showed no statistically significant difference between them. Similarly, no statistically difference in different age group. The maximum average mean HU in point A of mandibular A is 1059.

**Conclusion:** In total alveolar bone density after extraction shows decrease in bone density. Considering gray scale in CBCT is the criteria in measurement of bone density before implant placement, it is recommended because of the lower dose and cost of CBCT in comparison to CT scan.

Keywords: Bone density; CBCT value; dental implant; Misch's classification.

#### INTRODUCTION

Among various radiographic techniques used, Cone beam computed tomography (CBCT), provides the details on the anatomical structures, associated pathologies and also helps to determine the bone density. The density of a tissue is represented using the Hounsfield scale, with water having a value of zero Hounsfield units (HU), tissues denser than water having positive values, and tissues less dense than water having negative values. The Hounsfield unit was created by Sir Godfrey Hounsfield and was obtained from a linear transformation of the measured attenuation coefficient of water and air.

Low-density tissues are assigned darker (blacker) colors and high-density structures are assigned brighter (whiter) colors. HUs have been correlated with bone density and treatment planning for dental implant. Misch bone density classification may be evaluated on the CT images by correlation to a range of HUs. Many CBCT software programs are now available that allow for preoperative

determination of bone density in the implant site. To every change in form and function of bone certain definitive change in internal and external architecture of bone occurs. With regards to the importance of the clinical application of gray scale, in determining the bone quality for dental implant placement and the increasing use of CBCT for dental application the present study was undertaken to use edentulous alveolar ridge after months of tooth extraction. But grey value is arbitrary and variable, so the derived density provides less than meaningful data and the ability to access the density or quality of bone is limited.

The first purpose of the study is to assess Hounsfield unit in the edentulous site using CBCT and compare the values with Misch bone density classification scheme in Nepalese population. The other aim of the study is to compare the Hounsfield unit with age of the patient, to compare the Hounsfield unit with sex of the patient and to compare the Hounsfield unit of alveolar crest of maxilla with alveolar crest of mandible.

#### MATERIALS AND METHOD

The study was conducted in 20<sup>th</sup> September 2021 to 20<sup>th</sup> April 2022 in the Department of Periodontology and Oral Implantology, Kantipur Dental College Teaching Hospital and Research Centre, Basundhara, Kathmandu after ethical approval taken from the Institutional Review Committee (KDC-IRC ref. no. 26/021).

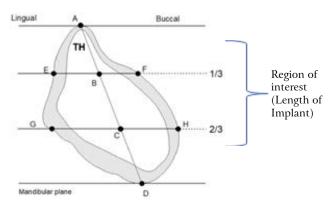
Non-probability convenience sampling method was used and the sample size was calculated using the formula:  $n = z2 \sigma 2/d2$ ; where n = required sample size, z = 1.96 at 95% confidence interval. Hence, n = 112. Therefore, a total of 112 subjects were included in the study.

Both male and female patients with chronological age between 20-65 years, who were indicated to undergo dental implant placement as dictated by his/her treatment plan were considered in the study. The CBCT scans of the subjects fulfilling the inclusion and exclusion criteria were selected for the study. The inclusion criteria included patients in the age range of 20-65 years with missing teeth in the maxillary or mandibular arches with CBCT images obtained using the same machine and general adjustment settings. The exclusion criteria included insufficient bone for implant placement, and edentulous ridge before 6 months of extraction, diseases that alter the radiodensity of bone such as Osteoporosis, Paget's disease, Fibrous dysplasia etc. and the presence of CBCT artifacts such as beam hardening, ring artefact and motion artefact. The CBCT images were obtained from the subjects who had taken the scans, not particularly for the study but as per the indication for the diagnosis or treatment planning. All CBCT images were studied to allow measurement of HUs in the Posterior maxilla (PMx), Posterior mandible (PMn), Anterior maxilla (AMx) and Anterior mandible (AMn). The maximum values were recorded and measured. The increasing HU values denoted denser bone. CBCT values were recorded in Hounsfield units and the obtained values were compared between both sexes using the independent sample t-test and Pearson correlation coefficient was used to evaluate whether age was correlated with the HU values.

DICOM images were loaded in the Carestream software and virtual implant was positioned in each implantation site. Thereafter, the density measurement scale HU tool was

used to measure the bone density. The bone density at the implantation site was determined by the mean of voxels grey values for the trabecular bone in the cervical (crest), body  $(1/3^{rd}$  the length) and apical regions  $(2/3^{rd}$  the length).

Fig. 1 Schematic representation of edentulous ridge with 3 different points



Point A to point D: line from crest to base of the alveolar ridge Point A to point B: 1/3rd the length of implant Point A to Point C: 2/3rd the length of implant

Data were entered and analyzed in SPSS version 20. Descriptive statistics were calculated. For qualitative variable like gender, frequency and percentage were calculated. For quantitative variables like bone density and age, mean  $\pm$  standard deviation was calculated. Independent t-test was used to determine the differences in the bone density values (HU) between mandible and maxilla.

In edentulous patients or partially dentate patients with multiple sites, a mean value was taken to assign for that region. All the information was recorded in a specifically designed proforma. In order to address any bias, all readings were taken by one investigator and CBCT from only one system was included in the study.

#### **RESULT**

The majority of the bone in Posterior maxilla (PMx), Anterior maxilla (AMx), Posterior mandible (PMn) and Anterior mandible (AMn) was D3 falling in the range of 350-850 HUs with mean HU of 642, 816, 486 and 697 respectively.

Table 1. Descriptive analysis of Posterior Maxilla

HFU_TOTAL	N	Minimum	Maximum	Mean	Std. Deviation
Posterior maxilla (PMx)	22	153.67	1528.00	642.7879	339.44203
Anterior maxilla (AMx)	23	345.00	1753.00	816.0435	320.18598
Posterior mandible (PMn)	40	93.00	892.00	486.5000	214.16043
Anterior mandible (AMn)	26	271.67	1157.00	697.6923	236.46534

In our study, the highest mean value for the bone density of 816 HU was shown by the anterior maxillary sextant with the maximum value of 1753.00 HU and minimum value of 345.00 HU. Whereas, the minimum mean Hounsfield unit was shown by the posterior mandible as 486.5000 HU.

Table 2. Hounsfield unit in each point in maxilla

Maxilla	A point	B point	C point
Mean	1059.19	572.71	556.24
Std. Deviation	261.800	310.367	348.417
Minimum	580	255	15
Maximum	1559	1214	1216

Table 3. Hounsfield unit in each point in mandible

Mandible	A point	B point	C point
Mean	597.76	366.60	445.81
Std. Deviation	327.477	218.446	409.596
Minimum	10	-89	-219
Maximum	1247	921	1600

The mean HU of 1059.19 is present in point A of lower anterior and point A of posterior maxilla shows 597.76. Our study also compared gender-based differences in Hounsfield unit in each sextant and showed no statistically significant difference between them.

#### DISCUSSION

The highly satisfactory success rate obtained with dental implants in the treatment of various edentulous cases depends on the volume and quality of the bone. The initial stability of the implant is, in effect, one of the fundamental criteria for obtaining osseointegration. In our study, we proposed to modify Misch's classification to create subcategories within D3 as D3a (850-600) HU and D3b (601 to 150) HU as most of the sextant showed D3 bone as Sogo in 2012. Although HU has a potential role in bone quality assessment, its relevance has been questioned due to recent evolutions in implant dentistry which have gone beyond the evaluation of bone density.

Most grading scales are based on the characterization of cross-sectional trabecular morphology and cortical bone thickness. Yet, there is no single universally accepted system for classifying bone quality in the maxilla and mandible. The most traditional method applied during preoperative implant assessment is that of Lekholm and Zarb, categorizing bone quality into four groups according to the degree of cortication and the trabecular bone morphology.

In the present study, gender was not equally distributed for each sextant. In the assessment of anterior maxilla, number of males were 8 and number of females were 14 whereas in other sextants, the difference in numbers of male and female was not significant. Unequal distribution of sample in each sextant is the major limitation of study. In terms of bone density, no significant difference was seen between the two genders which is in contrary to the

study by Khaled A. Alswat (2017) which has presented the tendency of males to have higher bone density than females. Moreover, the study showed that the difference in density could be influenced by gene difference, level of physical activities, nutrition level etc.

Though Misch classified anterior mandible as D1 bone and D4 bone in posterior, but our study shows D3 bone in lower anterior, which could be due to decrease in bone density after tooth loss. Acute disuse window loses mineral in bone which ultimately loses bone density. Studies shows 40% and 12% decrease in cortical and trabecular bone density repectively in disuse atrophy.

The advantage in using CBCT, decrease in radiation dose than the use of CT in implant dentistry and CBCT measure both the bone quality and quantity but measurement of bone density is higher in CBCT than CT in implant dentistry.

Various other issues are also associated with the use of Hounsfield unit values in CBCT. These issues relate to the limited-field CBCT geometry, variability in axial plane, variability between axial slices, high image noise.

In CT scan, Hounsfield unit is proportional to the degree of x-ray attenuation and it is allocated to each pixel to show the image that represents the density of the tissue. In CBCT, the degree of x-ray attenuation is shown by gray scale i.e., Voxel value (VV). But lower radiation dose and reduced costs of CBCT make this a useful substitute for CT. The influence of bone density on the crest of alveolar bone determines the speed and torque of first drill. This study measures the mean bone density in each point of each sextant. The result of study also helps to size, type and number of implants and its placement.

In addition, Hounsfield unit above 160 demonstrates normal bone mineral density which can be alternative to DEXA measurement. However, in order to be more accurately define the bone density with CBCT, a conversion ratio needs to be applied to the Volex Value (VV).

#### **CONCLUSION**

The study concluded that alveolar bone density after extraction shows decrease in bone density. Considering gray scale in CBCT is the criteria in measurement of bone density before implant treatments, it is recommended because of the lower dose and cost compared to CT scan.



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# Sexual Dimorphism in Permanent Maxillary Central Incisor in Nepali sample

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#### **ABSTRACT**

**Introduction:** The tooth crown size is a valuable tool and provides significant information on human evolution, biological alternations, in forensic evaluation and clinical odontology. The maxillary central incisors have contributed to sexual dimorphism, and several classifications of the form of maxillary central incisors are available in the literature.

**Objective:** To assess the degree of sexual dimorphism in maxillary central incisor in Nepalese population using crown linear diameters, crown module and crown index.

**Materials and Method:** This is a cross-sectional, comparative study which included 220 dental casts of patients seeking orthodontic treatment. This study was carried out at the Department of Oral Pathology, Kantipur Dental College and Hospital as per the inclusion criteria.

**Result:** Statistically significant sexual dimorphism was found in mesiodistal dimension and crown module of maxillary central incisor with males' central incisor measuring larger than females'. Left maxillary central incisor was found to be the most dimorphic in terms of mesiodistal dimension among the incisors.

**Conclusion:** Significant sexual dimorphism was observed in crown linear diameters and dental indices in the right and left maxillary central incisors in selected Nepali sample.

Keywords: Forensic dentistry, odontometry, sexual dimorphism

#### **INTRODUCTION**

The teeth are the hardest tissue in the human body, an excellent material in living and non-living populations for anthropological, genetic, odontologic, evolutionary and forensic investigation. Tooth size is affected by environmental, genetic, racial and cultural factors. Sex determination using dental features is primarily based upon the comparison of tooth dimensions in males and females, or upon the comparison of frequencies of nonmetric dental traits, like Carabelli's trait of upper molars, deflecting wrinkle of lower first molars, distal accessory ridge of the upper and lower canines or shovelling of the upper central incisors.

Various explanations for tooth-size dimorphism between males and females have been proposed such as differences in hormonal balance, the effect of the Y chromosome in increasing mitotic activity within the developing dental lamina, and involvement of the chromosome X in the enamel formation.<sup>3</sup> The differences in teeth dimensions are suggested either due to the amount of enamel or the amount of dentin.<sup>4</sup> Therefore, odontometrics are reasonably accurate predictors of sex and are good adjuncts for sex differentiation.<sup>5</sup> and can be an easy-to-use additional technique to determine sex in specific cases: in individual,

as well as in group (mass disasters, archaeological sites, etc.). $^6$ 

Gender discrimination is the important aspect of the human identification procedures that help in the establishment of biological profile from the skeletal and dental remains and also help in the facial reconstruction of unidentified bodies.<sup>7</sup> In forensic context, sex determination is an essential part of human identification. Predicting the sex simplifies identifications because missing persons of only the estimated sex need to be considered.<sup>8</sup> Although DNA profiling, finger prints, anthropometric data can be used as standard methods in the human identification procedures, odontometry can be an easier and cost effective option.<sup>2</sup>

Calculations of dental indexes, which derived from simple mathematical combinations of linear measurement could prove useful in sex determination.<sup>6</sup> Dental indexes include crown index (CI), crown module (CM) and crown area (CA).<sup>9</sup>

Gender determination is crucial for identification, as the number of possible matches is reduced by 50%. 10 Sexual dimorphism in tooth size has been explored focusing on the use of buccolingual (BL) and mesiodistal (MD) diameter – termed linear measurements. Premolars, first and second

molars as well maxillary incisors are also known to have significant differences.<sup>1</sup> As per our knowledge, there are no data for crown linear diameters and dental indexes of maxillary central incisor in Nepali population. Hence, this study aims to assess the degree of sexual dimorphism in maxillary central incisor in Nepalese population using crown linear diameters, crown module and crown index.

To assess the degree of sexual dimorphism in maxillary central incisor in Nepali sample population using crown linear diameters, crown module and crown index.

#### **MATERIALS AND METHOD**

This is a cross-sectional, observational, comparative study conducted at the Department of Oral Pathology of Kantipur dental College, Basundhara, Kathmandu. Dental casts of patients between the age of 13-49 years, visiting the Department of Orthodontics and Dentofacial Orthopaedics seeking orthodontic treatment at Kantipur Dental College and Hospital were taken.

Ethical clearance was obtained from the institutional review board of Kantipur Dental College (Ref. no: 24/022). The study was conducted between July-August, 2022. Non-probability, convenience sampling method was applied for sample collection.

From the study of Pillai *et al.*,<sup>11</sup> the mean standard deviation (SD) was 0.53 in the MD diameter of Maxillary central incisor (MCI).

Hence, using the formula 
$$n = \frac{(z^2SD^2)}{e^2}$$

Where z = 1.96 at confidence level 95%.

e = margin of error (0.07)

The calculated sample size was 220.

A total of 220 dental casts of the patients of Nepali origin with fully erupted complete set of morphologically well-formed, non-carious, non attrited, satisfactorily aligned maxillary teeth were included in the study. Teeth without history or clinical evidence of crown restoration, orthodontic treatment and trauma were included in the study. Dental casts of patients other than Nepali origin, incompletely erupted or morphologically altered maxillary teeth, carious or attrited, severely malaligned and teeth with history of crown restoration, orthodontic treatment and trauma were excluded from the study.

Data information sheet (Proforma) was developed. MD and BL crown diameters of both, right and left permanent MCI were taken in a dental casts using electronic digital caliper with an accuracy  $\pm$  0.01 mm. The mesiodistal crown diameter was defined as the greatest mesiodistal dimension, taken parallel to the occlusal and facial surface. The buccolingual crown diameter was defined as

the greatest distance between the buccal (or labial) and lingual (or palatal) surfaces of the crown, perpendicular to the mesiodistal diameter.<sup>12</sup> Measurements were done by a single examiner to eliminate interobserver error. Each measurement was taken three times and the average of the three values was noted to minimize the intra-observer error. Dental indices were calculated from MD and BL measurements of maxillary central incisor according to the formula given by Hillson.<sup>13</sup>

$$CM = \frac{(MD + BL)}{2}$$

$$CI = \frac{(100 \times BL)}{MD}$$

Percentage of sexual dimorphism in crown linear diameters and dental indices of maxillary central incisor was calculated according to the formula:<sup>14</sup>

Sexual dimorphism =  $[Xm/Xf] - 1 \times 100$ where Xm = mean value for malesXf = mean values for females.

Data was collected and statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version<sup>20</sup>. Independent t test was applied.

#### RESULT

The buccolingual and mesiodistal diameters were measured and compared on study cast (Fig. 1) for MD (a) and LP (b) dimensions as well as for CM and CI for right and left maxillary central incisors using independent t test and statistical analysis was done by using in Statistical Package for the Social Sciences (SPSS) 20 software.

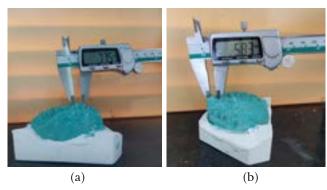


Fig 1: Greatest crown diameter of maxillary central incisor using digital caliper; (a) mesiodistal (b) labiopalatal

In the study we found that males showed greater MD, LP and CM on both right and left MCI as shown in Table 1. The mean difference value of both MD diameter, left LP diameter as well as right and left CM showed statistically significant difference between male and female with P<0.05. The mean differences between male and female crown linear diameters and dental indices are shown in

Fig. 2. Pearson chi square correlation-coefficient showed significant correlation (<0.01) between the right and left MCI using the odontometric parameters (Table 2).

The percentage (%) of sexual dimorphism between right and left maxillary central incisors using different odontometrics parameters are presented in Table 3. It was

observed that the most dimorphic odontometric parameter was left MD (3.37 %) followed by, left CM (3.11 %), left LP (2.77 %), right CM (2.12%), and right LP (1.67 %). CI showed negative value of sexual dimorph

Table 1: Assessment of sexual dimorphism using independent t test

Odontometric parameters	Gender	Mean	Standard deviation	Standard error of mean	P value
MD11	M	8.72	0.69	0.07	0.031*
	F	8.52	0.66	0.06	
LP 11	M	6.69	0.73	0.07	0.573 NS
	F	6.58	0.62	0.06	
CI 11	М	76.97	6.96	0.66	0.573 NS
	F	77.51	7.17	0.68	]
CM 11	М	7.71	0.63	0.06	0.048*
	F	7.55	0.55	0.05	
MD 21	М	8.59	0.65	0.06	0.01*
	F	8.31	0.61	0.06	
LP 21	М	6.67	0.67	0.06	0.046*
	F	6.49	0.64	0.06	
CI 21	М	77.83	6.98	0.67	0.605 NS
	F	78.83	7.48	0.71	1
CM 11	M	7.63	0.57	0.05	0.002*
	F	7.40	0.53	0.05	

<sup>\*</sup> Statistically significant MD- mesiodistal LP- labiopalatal NS not significant CI- crown index CM- crown module

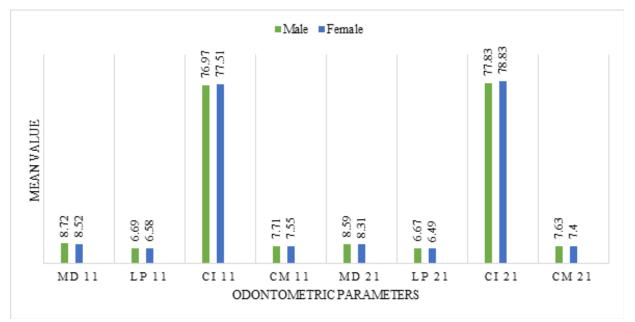


Fig 2: Bar graph showing sexual dimorphism in crown linear diameter and dental indices

Table 2: Pearson's chi square correlation-coefficient between right and left Maxillary Central Incisors using Odontometric Parameters

Odontometric parameters	Right MCI	Left MCI	Correlation coefficient
Mesiodistal	8.62	8.45	<0.01
Labiopalatal	6.64	6.58	<0.01
Crown index	77.24	78.33	<0.01
Crown module	7.63	7.52	<0.01

Correlation is significant at the 0.01 level (2 -tailed)

Table 3: Percentage of sexual dimorphism

Odontometric parameters	Right (%)	Left (%)
Mesiodistal	2.35	3.37
Labiopalatal	1.67	2.77
Crown index	-0.69	-0.64
Crown module	2.12	3.11

#### **DISCUSSION**

The general structure and morphology of the teeth are similar in both men and women, however, there are subtle differences, and such as variation in tooth dimensions can give many hints about differences between the sexes. Hence, teeth can be considered an important aspect for sex determination as they are resistant to postmortem destruction and fragmentation. The feasibility for measuring the dimensions of the teeth using morphometric devices could serve as a reliable method for solving medicolegal investigations and to identify victims of crime, natural disasters, and severe accidents. Measurements on dental cast are straight forward and reliable method for odontometrics.<sup>15</sup>

In our study, we analyzed the degree of the sexual dimorphism in fully erupted permanent right and left maxillary central incisors by measuring the maximum mesiodistal and buccolingual diameters, as well as calculating the CM and CI from study casts among Nepali population aged between 13-49 years seeking orthodontic treatment in the hospital.

The current study confirms the statistically significant difference in both right and left MD, CM and left LP (p<0.05) of maxillary central incisor where mean values of males were greater than that of females. Our findings were in agreement with the studies done by other authors, where they have observed that males had larger teeth than females in all dimensions. Mesiodistal dimension of MCI showed significant sexual dimorphism in our study which was in agreement with the findings of Khangaru et al., 2011. The Crown indices for maxillary central incisor were also greater in males than in females with significant differences for CM (p<0.05).

The percentage of sexual dimorphism was calculated for MD, BL, CI and CM. The difference between males and

females in the percentage of dental sexual dimorphism ranged from 3-9%.18 MD dimension showed highest sexual dimorphism (3.37%) followed by CM (3.11%) and LP (2.77%) dimension of the left MCI in our study. We found significant difference in terms of the MD, LP dimension as well as CM and CI of the right and left MCI similar to the finding of Shrestha B, 2019.19 Our finding suggested that one side of teeth is not representative in case of odontometric parameters between male and female. In studies by Staka et al.1 and da Costa et al.20 showed no significant differences between the right and left MCI as well as other teeth in the human dentition. MD dimension had more sex determination potential compared to BL dimension<sup>21</sup> which was in accordance with the result in our study where the MD dimension of the left MCI showed the highest sexual dimorphism. Iscan et al.<sup>22</sup> have shown the LP dimension to be better predictor of sex. Hence, for sex prediction, both linear dimensions MD and LP can be considered rather than choosing a single dimension. Also, CM showed a high rate of sexual dimorphism than CI in our study which was similar in the study by Staka et al., 2016.1

The different patterns of sexual dimorphism observed between different populations reflect genetic, epigenetic and environmental influences to dental development.<sup>10</sup> Odontometric features vary among the specific population and even within the same population in historical and evolutionary context.<sup>22</sup> Hence, it is of utmost importance to determine population specific values for identification on the basis of odontometrics.<sup>21</sup>

The present study confirmed that a significant sexual dimorphism existed in the MD and LP dimensions as well as CM of the maxillary right and left central incisors. Left maxillary central incisor was sexually more dimorphic compared to the right among the study samples.

Thus, further investigations based on genetic, ethnic,

and metabolic/hormonal reasons for sexual dimorphism in larger sample sizes may further clarify the etiology of sexual dimorphism as well as credibility of odontometrics in different study population.

#### **CONCLUSION**

This study demonstrated statistically significant sexual dental dimorphism in crown linear diameters and dental indexes in MCI in Nepalese population. MCI can be used in forensic investigations as an adjunct along with other accepted procedures for sex determination. The left MCI in terms of mesiodistal dimension was observed as the most dimorphic tooth among the MCI. Further study including larger representative sample of Nepalese population from various provinces has to be conducted to quantify and generalize the result among Nepalese population.

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# Three-dimensional evaluation of relationship between maxillary central incisor and nasopalatine canal

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#### **ABSTRACT**

**Introduction:** Primarily, orthodontic treatment is based on improving the occlusal relationship however, more attention is now laid to enhance the facial esthetics. Upright maxillary incisors are more preferable than the protruded ones. During the retraction of incisors there is a risk of contact with nasopalatine canal leading to apical root resorption which warrants the evaluation of the relationship between nasopalatine canal and maxillary central incisor root.

**Objective:** To evaluate the distance between maxillary central incisor and nasopalatine canal on Cone beam computed tomography.

**Materials and Method:** This is a cross-sectional observational study on the samples aged above 18 years. The study was conducted on CBCT records of 46 patients as per the inclusion criteria. The distance between maxillary central incisor root and nasopalatine canal was measured on the CBCT files.

**Result:** The mean anteroposterior distance between the most medial portion of maxillary central incisor root and nasopalatine canal was  $4.4\pm0.96$  mm at the mid-level (L2) and  $4.2\pm1.2$  mm at the oral opening of nasopalatine canal (L3). The mean anteroposterior distance between the most posterior portion of maxillary incisor root and the most lateral portion of nasopalatine canal was  $4.1\pm1.44$  mm at L2 and  $3.4\pm1.02$  mm at L3. Student t-test showed no significant gender variation.

**Conclusion:** The anteroposterior distance between the maxillary central incisor root and nasopalatine canal ranged from 3.4 to 4.4 mm. Proper CBCT evaluation is required for orthodontic movement of maxillary incisors.

Keywords: Cone beam computed tomography; Maxillary central incisor; Nasopalatine canal.

#### **INTRODUCTION**

The key factors of motivation for adults seeking orthodontic treatment are dissatisfaction with the appearance, desire to align the teeth and to improve the smile.<sup>1,2</sup> Primarily, orthodontic treatment is based on improving the occlusal relationship, but more attention is laid towards enhancing the facial esthetics.<sup>3</sup> The labiolingual inclination of maxillary incisors has an important role in the facial esthetics. An upright maxillary incisor is more preferable than the protruded ones.<sup>4</sup> Therefore, orthodontic treatment mainly focuses on retraction of protruded incisors in order to meet the patient's esthetic need.

During the retraction of incisors there is always a risk of contact with hard tissue structures, such as the labial, palatal or nasopalatine canal cortical plates. This may lead to apical root resorption and root deviation from the alveolar housing of dentition leading to dehiscence and fenestration.<sup>5</sup> Ackerman et al. have formulated "envelope of discrepancy" which has given limits for orthodontic tooth movement, according to which the limit for orthodontic

retraction of upper incisor is 7mm.<sup>6,7</sup>

The nasopalatine canal can act as a constraint for orthodontic tooth movement, mainly for retraction and intrusion of maxillary incisors. It is an anatomic structure present in the midline of the palatine process of maxilla, posterior to the roots of maxillary central incisors. It consist of nasopalatine vessels and nerves, branches of maxillary division of the trigeminal nerve and the maxillary artery within a thick cortical bone. Contact of root with nasopalatine canal during orthodontic tooth movement leads to root resorption and subsequently resulting in other unfavorable outcomes. Therefore, proper evaluation of the nasopalatine canal in relation to maxillary central incisor is one of the important requirements in orthodontic diagnosis and treatment planning.

Lateral and anteroposterior cephalograms are commonly used investigations in orthodontics. <sup>10</sup> Since all the anatomic structures cannot be properly evaluated using the two-dimensional radiographs, three-dimensional analysis

with cone-beam computed tomography (CBCT) is now popular. Likewise, precise evaluation of the nasopalatine canal and its surrounding structure is not possible with two-dimensional radiographs. This study aims to evaluate the distance between maxillary central incisor and nasopalatine canal using CBCT files.

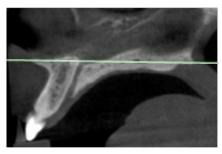
#### **MATERIALS AND METHOD**

This is an observational cross-sectional study done using secondary data in the Department of Orthodontics, Kantipur Dental College and Hospital, Kathmandu. The study was conducted in July 2022 after obtaining the ethical clearance from the Institutional Review Committee (Ref no. 16/022). A total number of 46 adults (20 female and 24 male) were selected meeting the inclusion criteria set for the study, which are (1) good quality pretreatment CBCT images; (2) age group of above 18 years; (3) presence of maxillary incisors and (4) angle between the long axis of

the maxillary central incisors and the palatal plane(U1-PP) within normal range (110.1 < U1-PP  $\leq$  121.50).<sup>11</sup>

CBCT reports from the Department of Oral Medicine and Radiology, were collected in a hard drive. Data information sheet was developed to gather the information from the samples. CBCT images were taken via Care Stream (CS) 9300, USA machine using standard protocol at 85 kV, 6.3 mA, 11.30 s, voxel size of 300  $\mu$ m and 17x13 cm field of view at lowest possible radiation using 'as low as reasonably achievable' concept.

Measurements were made as described by Cho *et al.*<sup>12</sup> The selected Digital Imaging and Communication in Medicine (DICOM) file was opened in CS imaging suite software and orthogonal slicing was selected. Prior to measurements, the three planes, sagittal, horizontal, and coronal were defined in each image and reference lines were drawn on each plane (Figure 1,2 and 3).



**Figure 1:** Palatal plane joining anterior nasal spine and posterior nasal spine in axial section

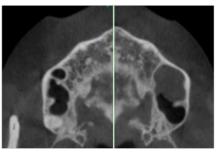


Figure 2: Line passing through the mid-palatal suture in sagittal section

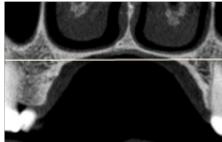
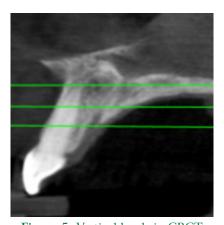


Figure 3: Line passing through the right and left greater palatine foramina in coronal section

Linear measurements were done in the axial section at three vertical levels (Figure 4,5): Root apex of maxillary central incisor (Level 1), mid root (Level 2) and oral opening of nasopalatine canal (Level 3).



Figure 4: Schematic diagram of vertical levels



**Figure 5:** Vertical levels in CBCT

The linear measurements done in axial section are shown in Figure 6,7,8 and 9. Inter-root distance was measured as a distance from Rm to Rm (Rm - most medial portion of maxillary central incisor root) and Rp to Rp (Rp - most posterior portion of maxillary central incisor root). Canal width was measured as a distance from Cl to Cl (Cl - most lateral portion of nasopalatine canal). Antero-posterior distance was measured from Rm and Rp to the canal respectively.

Angular measurements were done in the sagittal section. The angle formed by palatal plane (AB) with the long axis of maxillary central incisor (BE) and long axis of nasopalatine canal (CF) were measured (Figure 10 and 11).



**Figure 6:** Schematic diagram of landmarks for transverse measurements

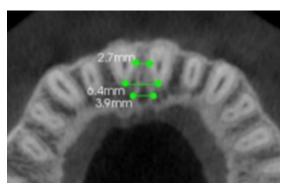


Figure 7: Transverse measurements in axial section of CBCT

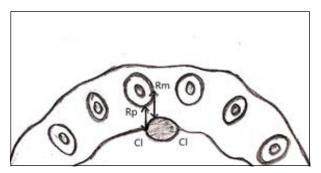


Figure 8: Schematic diagram of landmarks for anteroposterior measurements

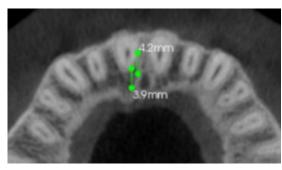


Figure 9: Antero-posterior measurements in axial section of  $\ensuremath{\mathsf{CBCT}}$ 

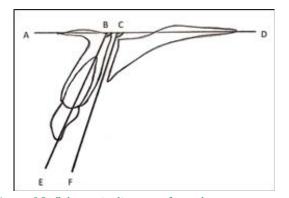


Figure 10: Schematic diagram of angular measurements

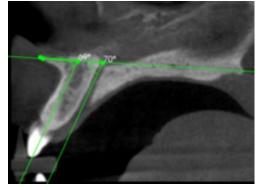


Figure 11: Angular measurements in sagittal section of CBCT

Data were collected and analyzed using SPSS V21.0. Descriptive statistics including mean and standard deviation were calculated for each parameter. Student's t-test was used to determine the gender variation. Pearson's correlation coefficient test was determined to evaluate the correlation between the angular parameters. The p-value <0.05 was considered statistically significant. Ten percentage of the sample size were re-evaluated after 4 weeks by the same investigator for intra-examiner reliability of the measurements.

#### **RESULT**

The sample comprised of CBCT reports of 46 subjects aged above 18 years. The descriptive statistics of canal width and inter-root distance of maxillary central incisors is presented in Table 1.

Table 1: Canal width and inter-root distance of maxillary central incisors

Vertical level						
Measurements	Root Apex (L1) Mid-Level (L2)		Opening (L3)			
Measurements	Mean ± SD	Mean ± SD	Mean ± SD			
Canal width(mm)	$4.7 \pm 1.34$	$5.0 \pm 1.20$	5.1 ± 1.11			
Rm-Rm	5.2 ± 1.56	4.7 ± 1.35	3.9 ± 1.26			
Rp-Rp	7.2 ± 1.56	$7.3 \pm 1.51$	7.1 ± 1.34			

The width of the canal was found to be greatest at Level 3 and least at Level 1 (Figure 11).

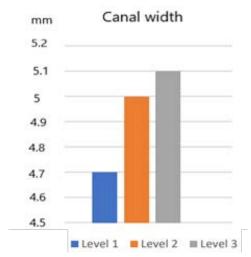


Fig No.12: Canal width at three levels

The descriptive statistics of antero-posterior distance of maxillary central incisor root and nasopalatine canal is presented in Table 2. Antero-posterior distance was not measurable at Level 1 because the root apex was farther away from the most lateral border of the incisive canal in all subjects. Student t-test showed no significant gender variation on antero-posterior measurements (p>0.05) as shown in Table 3.

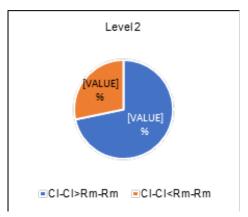
Table 2: Antero-posterior distance of maxillary central incisor root and nasopalatine canal

Vertical level						
Measurements	Root Apex   Mid-Level (L1) (L2)		Opening (L3)			
	Mean ± SD	Mean ± SD	Mean ± SD			
Canal width(mm)	4.7 ± 1.34	5.0 ± 1.20	5.1 ± 1.11			
Rm-Canal	N/A	$4.4 \pm 0.96$	$4.2 \pm 1.2$			
Canal-Rp	N/A	4.1 ± 1.44	$3.4 \pm 1.02$			

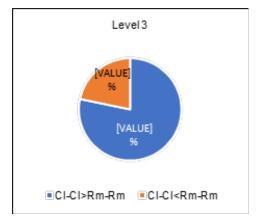
Table 3: Comparison of antero-posterior distance of maxillary central incisor root and nasopalatine canal between male and female

Vertical level												
M		Mid-Level (L2)			Oral opening of nasopalatine canal (L3)							
Measurements		Mean ± SD t-value p-value			Mean ± SD	t-value	p-value					
Rm-Canal	Male	$4.4 \pm 0.96$	1.37		1 27	1.27	1 27		0.89	4.4 ± 1.17	0.69	0.49
Kiii-Caliai	Female				0.69	4.1 ± 1.10	0.09	0.49				
Canal Dr	Male	$4.0 \pm 1.07$	-0.47	0.47	0.64	$3.4 \pm 1.00$	0.24	0.73				
Canal-Rp	Female	4.2 ± 1.76	-0.47	.7 0.64	$3.3 \pm 1.08$	0.34	0.73					

The percentage of subjects with nasopalatine canal width greater than the inter-root distance (Rm-Rm) was 71.73% and 78.26% at Level 2 and 3 respectively (Figure 12 and 13). At Level 1 nasopalatine canal width was smaller than the interroot distance. Similarly, nasopalatine canal width was smaller than inter-root distance (Rp-Rp) in all subjects at all vertical levels (Table 2).

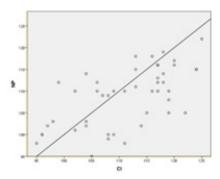


**Figure 13:** 28.27% and 71.73% of subjects with nasopalatine canal width greater than inter-root distance of the maxillary central incisors at Level 2



**Figure 14:** 21.74% and 78.26% of subjects with nasopalatine canal width greater than inter-root distance of the maxillary central incisors at Level 3

Pearson correlation coefficient showed moderate positive correlation (r=0.62) between angle formed by palatal plane with the long axis of maxillary central incisor and nasopalatine canal (Figure 14).



**Figure 15:** Pearson correlation coefficient between the angle formed by palatal plane with the long axis of maxillary central incisor and nasopalatine canal

#### **DISCUSSION**

The present study included samples with mixed growth patterns, as Costa *et al.*<sup>22</sup> reported little influence of growth pattern on the anteroposterior distance between maxillary central incisors and nasopalatine canal. Extraction of premolars followed by the retraction of anterior segment is a usual treatment modality in cases of convex facial profile with protrusive anterior teeth. The contact of maxillary central incisor root to the nasopalatine canal can cause root resorption. (13-17) It can also result in delayed tooth movement, with possible perforation and dehiscence. (15,18-20)

According to the 'envelope of discrepancy', the maximum amount of maxillary anterior retraction possible is 7 mm.<sup>5</sup> Moreover, the use of skeletal anchorage has broadened the limit of orthodontic tooth movement.<sup>6</sup> Contact of maxillary central incisor root to nasopalatine canal was fairly high after maximum anterior retraction.<sup>21</sup> The anteroposterior distance ranged from 3.4 to 4.4 mm in the present study, 5 to 6 mm in the study by Cho *et al.*<sup>12</sup> and Gull *et al.*<sup>14</sup> and 4.7 to 6 mm in the study by Al-Rokhami *et al.*<sup>24</sup> The differences in the measurements could be due to the variations in the craniometric measurements among different ethnic groups.<sup>25</sup>

The nasopalatine canal width increased from the level of root apex to the oral opening which is in concordance with the study by Cho *et al.*<sup>12</sup> and Khurana *et al.*<sup>26</sup> There was a positive correlation between the angle formed by palatal palate with long axis of maxillary central incisor and nasopalatine canal, which is similar to the study by Matsumara *et al.*<sup>27</sup> There was no gender variation in the anteroposterior distance which was in agreement to the study by Costa *et al.*<sup>22</sup>

Despite the measured anteroposterior distance, the risk of root contact is present only if the width of the nasopalatine canal is greater than the inter-root distance.<sup>22</sup> The present study showed more than 70% of the samples had canal width greater than the inter-root distance, indicating that about 3/4th of the cases might pose such a risk. Similarly,

Cho *et al.* reported the frequency to be 60%.<sup>13</sup> Based on the anteroposterior measurements, the possibility of contact to the canal by the mesio-palatal aspect of the root (Level 2 and 3) is greater than by the root apex (Level 1) during orthodontic retraction. Therefore, three-dimensional image plays a pivotal role in determining the amount of maxillary incisor retraction.

#### **CONCLUSION**

The anteroposterior distance between maxillary central incisor root and nasopalatine canal ranged from 3.4 to 4.4 mm. More than 70% of the samples possessed greater width of the nasopalatine canal than the interroot distance, indicating that a greater number of patients have contact of root with nasopalatine canal during incisor retraction. Envelope of discrepancy should be taken as a guideline for the determination of the amount of upper incisors retraction with proper CBCT evaluation for the precise orthodontic diagnosis and treatment planning.

#### **ACKNOWLEDGEMENT**

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### Morphological variations in Maxillary labial frenum: A cross-sectional study

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#### **ABSTRACT**

**Introduction:** Maxillary labial frenum is a dynamic structure that is subjected to variations during different stages of human growth and development. It can pose a significant problem if tension from lip movement pulls the gingival margin away from the tooth or if the tissue restrains the closure of a diastema during orthodontic treatment.

**Objective:** To assess the types of maxillary labial frenal attachments and the morphological variations among them.

**Materials and Method:** A cross-sectional observational study was conducted among 157 subjects within the age group of 19-29 years at Kantipur Dental College from June 2022 to July 2022. Non-probability convenience sampling method was utilized. Types of maxillary frenal attachments and the morphological variations were recorded according to Mirko and Sewerin classifications respectively. Data were compared and analyzed using SPSS 20.

**Result:** The mucosal type of frenal attachment was found in 51.6% of subjects followed by gingival in 25.5%, papillary in 19.1% and papilla penetrating in 3.8%. 66.2% presented with normal frenum, 22.9% with nodule, 10.2% with appendix and 0.6% with bifid frenum.

**Conclusion:** There are variations in the maxillary frenal attachments which can pose significant problems.

**Keywords:** Aberrant frena; maxillary labial frenum; frenal attachment; morphological variations.

#### INTRODUCTION

A frenum is a fold of mucous membrane composed of highly vascularized connective tissue covered with epithelium<sup>1</sup> with variable amount of dense collagen and elastic fibers, but no muscle fibers.<sup>2</sup>

Maxillary labial and mandibular labial and lingual frena are the most notable frena in the oral cavity.<sup>3</sup> Maxillary labial frenum extends from vestibular mucosa of the upper lip to the alveolar or gingival mucosa in the midline of maxillary arc. It is a dynamic structure that is subjected to variations during different stages of human growth and development.<sup>3,4</sup> Its primary function is to provide stability to the upper lip.<sup>3</sup>

It originates as a posteruptive remnant of the tectolabial bands, which are embryonic structures appearing about three months in utero as a continuous band of tissue and connect tubercle of upper lip and palatine papilla.<sup>2</sup> The frenum, also referred to as tectolabial frenum, at this stage, interestingly mimics the frenum that is considered abnormal in the postnatal life.<sup>5</sup> However, with time, the growth of alveolar process relocates the frenum in a more apical position. The failure of the attachment to

migrate results in a persistent band of tissue between the maxillary central incisors thereby resulting in several undesirable consequences.<sup>2</sup> However, one should always consider the fact that, like any other structure in the human body, the maxillary labial frenum also has a range of normality. It varies in bulk as well as in the height of attachment.<sup>5</sup> Therefore, it is important to distinguish normal from abnormal frenal attachments. Thus, this study aims to determine the prevalence of different types of frenal attachments and also the morphological variations encountered among Nepalese population.

Over time, several studies have been conducted and classifications have been proposed relating to the maxillary labial frenum. Sewerin I, in 1969, had reported eight morphological variations present in the upper labial frenum.<sup>6</sup> Later on in 1974, Mirko *et al.* suggested a classification on the basis of the site of frenal attachment.<sup>7</sup> Classification by Mirko *et al.* (1974)

- Mucosal: When the frenal fibers are attached up to mucogingival junction
- Gingival: When fibers are inserted within attached gingiva

- Papillary: When fibers are extending into interdental papilla
- Papilla penetrating: When the frenal fibers cross the alveolar process and extend up to palatine papilla

Classification by Sewerin I (1969)

- Normal frenum
- Normal frenum with nodule
- Normal frenum with appendix
- Normal frenum with nichum
- Bifid labial frenum
- Persistent tectolabial frenum
- Double frenum
- Wider frenum

#### MATERIALS AND METHOD

This was a cross-sectional observational study conducted in the Department of Periodontology and Oral Implantology, Kantipur Dental College Teaching Hospital and Research Centre, Basundhara, Kathmandu, Nepal from June to July, 2022. Ethical approval was taken from the Institutional Review Committee (KDC-IRC ref. no. 23/022).

The study population included the dental students enrolled in the college, and the patients visiting the Department of Periodontics, in the age range of 19 to 29 years. Subjects who had undergone surgery in the region of upper labial frenum, any trauma or injury to the mucosa at the site, any congenital or developmental abnormality in the upper frenum or premaxilla, missing one or both maxillary central incisors, past or ongoing orthodontic treatment and subjects taking any medication known to affect the gingiva, were excluded from the study.

Non-probability convenience sampling method was used and the sample size was calculated using the formula: n = z2pq/e2; where n = required sample size, z = 1.96 at 95% confidence interval, p = 0.776, q = 1-p (0.224), e = 0.07 (7% maximum permissible error). Hence, n = 136. Therefore, a total of 136 subjects were included in the study.

The examination of frenum was done in the clinical setting. The types of frenal attachment and the morphological variations among them were assessed on the basis of Mirko et al.7 and Sewerin6 classifications respectively.

#### RESULT

In the study, there were 109 females and 48 males as the study subjects. Out of the total of 157 subjects, when frenal attachments were studied on the basis of the classification given by Mirko et al.7, the most common type of frenal attachment was found to be the mucosal type which was present in 51.6% of subjects. This was followed by the gingival type constituting 25.5% of the subjects, then by the papillary type which was found in 19.1% and lastly by

the papilla penetrating type present in 3.8%. (Table 1) On the basis of the Sewerin classification6, 66.2% of subjects presented with normal frenum, 22.9% with nodule, 10.2% with appendix and 0.6% with bifid frenum. (Table 2)

Table 1 Prevalence of different types of attachment of maxillary labial frenum

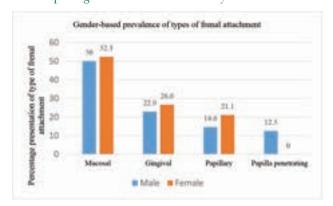
Type of frenal attachment	Frequency	Percentage
Mucosal	81	51.6
Gingival	40	25.5
Papillary	30	19.1
Papilla penetrating	6	3.8
Total	157	100

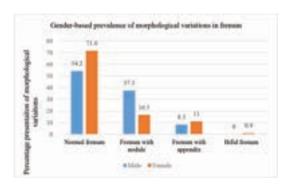
Table 2 Prevalence of morphological variations in maxillary labial frenum

Morphological variations	Frequency	Percentage
Normal frenum	104	66.2
Normal frenum with nodule	36	22.9
Normal frenum with appendix	16	10.2
Bifid frenum	1	0.6
Total	157	100

Likewise, when evaluating the prevalence of type of frenal attachment in each gender, out of 48 males, 50.0% had mucosal, 22.9% had gingival, 14.6% had papillary and 12.5% had papilla penetrating types of attachment. While, out of 109 females, 52.3% had mucosal, 26.6% had gingival and 21.1% had papillary types of frenal attachment. Similarly, evaluation of morphological variations revealed normal frenum in 54.2%, frenum with nodule in 37.5% and frenum with appendix in 8.3%, in males. Likewise, in females, 71.6% had normal frenum, 16.5% had frenum with nodule, 11% had frenum with appendix and 0.9% had bifid frenum. (Figure 1)

**Figure 1:** Gender-based prevalence of types of attachment and morphological variations of maxillary labial frenum





Comparing the presentation of frenal attachment between males and females, 29.6% of mucosal type was presented by males and 70.4% by females, 50% of gingival type by both males and females, 23.3% of papillary type by males and 76.7% by females, while 100% of the papilla penetrating type was seen in males. (Table 3) (Figure 2)

Likewise, 25% of normal frenum was presented by males and 75% by females, 50% of frenum with nodule was presented by both males and females, 25% of frenum with appendix by males and 75% by females, and bifid frenum by 100% females. (Table 4) (Figure 2)

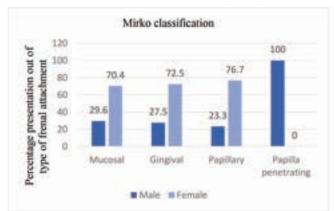
Table 3 Comparison of types of frenal attachment between genders

	Mirko classification	Ger	nder	Total
	Mirko classification	Male	Female	iotai
1	Count	24	57	81
	% within Mirko classification	29.6%	70.4%	100%
	% within gender	50.0%	52.3%	51.6%
	% of total	15.3%	36.3%	51.6%
2	Count	11	29	40
	% within Mirko classification	27.5%	72.5%	100%
	% within gender	22.9%	26.6%	25.5%
	% of total	7.0%	18.5%	25.5%
3	Count	7	23	30
	% within Mirko classification	23.3%	76.7%	100%
	% within gender	14.6%	21.1%	19.1%
	% of total	4.5%	14.6%	19.1%
4	Count	6	0	6
	% within Mirko classification	100%	0%	100%
	% within gender	12.5%	0%	3.8%
	% of total	3.8%	0%	3.8%
Total		48 (30.6%)	109 (69.4%)	157 (100%)

Table 4 Comparison of variations in maxillary labial frenum between genders

	Mirko classification	Ger	nder	Tr. c. 1	
	Mirko classification	Male	Female	Total	
1	Count	26	78	104	
	% within Sewerin classification	25.0%	75.0%	100%	
	% within gender	54.2%	71.6%	66.2%	
	% of total	16.6%	49.7%	66.2%	
2	Count	18	18	36	
	% within Sewerin classification	50.0%	50.0%	100%	
	% within gender	37.5%	16.5%	22.9%	
	% of total	11.5%	11.5%	22.9%	
3	Count	4	12	16	
	% within Sewerin classification	25.0%	75.0%	100%	
	% within gender	8.3%	11.0%	10.2%	
	% of total	2.5%	7.6%	10.2%	
4	Count	0	1	I	
	% within Sewerin classification	0%	100%	100%	
	% within gender	0%	0.9%	0.6%	
	% of total	0%	0.6%	0.6%	
Total		48 (30.6%)	109 (69.4%)	157 (100%)	

**Figure 2:** Comparison of percentage presentation of types of frenal attachment and morphological variations of maxillary labial frenum



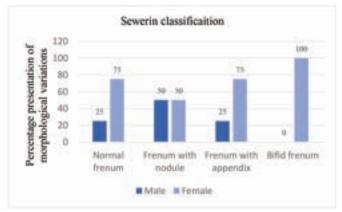
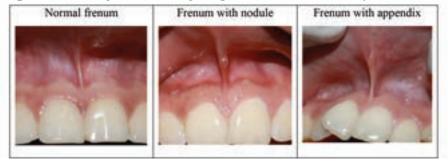


Figure 3: Clinical pictures of types of maxillary frenal attachment



Figure 4: Clinical pictures of morphological variations in maxillary labial frenum



#### **DISCUSSION**

Depending upon the extension of attachment of fibres, frena have been classified into mucosal, gingival, papillary and papilla penetrating types along with other anatomical variations. Papillary and papilla penetrating types are considered as pathological<sup>8</sup> and are associated with enhanced plaque accumulation, loss of papilla, recession, malalignment of teeth, diastema, relapse of diastema after orthodontic treatment, compromised denture fit or retention<sup>9</sup> and have also been linked as a feature of various syndromes.<sup>3</sup>

Back in the time, some studies described frenum to be abnormal simply because they were hypertrophied, while some suggested frena to be abnormal only in case they predisposed to midline diastema. Some suggested that hypertrophied frena usually diminished in size with increase in age and eruption of permanent teeth and also that a frenum should not to be described as aberrant simply because of the incidental presence of the space for which it might not be responsible. Therefore, frenum should always be considered as an associated structure rather than an isolated entity.<sup>5</sup>

A thick wide maxillary frenum attached close to the gingival margin is considered as a factor contributing to midline diastema. A thick maxillary frenum is considered normal during early developmental stage but eventually with the enlargement of alveolar process and eruption of maxillary anterior teeth, the frenal attachment thins out and relocates to a more apical position. Therefore, the papillary and papilla penetrating types of frenal attachment are considered pathogenic if it exists beyond the mixed dentition period. <sup>10</sup> High frenal attachment if not

intervened leads to recurrence of midline diastema even after successful orthodontic treatment.<sup>8</sup>

An adequate zone of attached gingiva is essential for maintaining the gingival health.<sup>11,12</sup> Hence, a frenum which is unusually large and wide with no apparent zone of attached gingiva in the permanent dentition and or when the interdental papilla shifts when the frenum is extended, is said to be pathogenic.<sup>10</sup>

A thick wide frenum is also considered to be a compromising factor for denture resistance and retention. To add to this, large notches that are required to accommodate such large frena are thought to be responsible for fracture of dentures. Therefore, early recognition and surgical removal of such frena is important for favourable treatment outcome with dentures. <sup>13</sup>

Moreover, frenal abnormalities reflect various underlying syndromes such as such as Turner syndrome, Ellis-van Creveld syndrome, Oral-facial-digital syndrome, Ehlers Danlos syndrome, Infantile hypertrophic pyloric stenosis, Holoprosencephaly etc. Various reports addressing these problems have been discussed.<sup>8,14–17</sup> Thus, the dentist needs to give due importance for frenum assessment during oral examination and prepare to correct any abnormalities whenever indicated.

There are very few studies that have been published pertaining to the different types of upper labial frena and their prevalence.

Jindal *et al.* in 2016 conducted a study on 500 subjects where 66% of patients presented with mucosal type of attachment followed by gingival type in 28.4%, papilla penetrating type in 3.2% and the least common being the papillary type found in only 2.4%. Moreover, normal frenum was the most common (77.6%) followed by frenum with nodule (12.4%) while frenum with appendix was found to be the least common (9%).<sup>3</sup> In a study by Rajani *et al.* in 2018 regarding the type of frenal attachment, mucosal type (42%) was found to be the most prevalent followed by gingival (34%), papillary (20%), and papillary penetrating type (4%).<sup>10</sup>

Similar studies have been conducted in the context of Nepal. In 2018, a study was conducted on 356 Nepalese individuals by Rajkarnikar et al.18 in which frequency of mucosal type of frenal attachment (70.5%) was seen to be the most common followed by gingival type (28.4%), then papillary type (0.8%) and the least common being the papilla penetrating type (0.3%).

Later on, Joshi *et al.* in 2021 conducted a similar study among 340 Newari children. They concluded that the mucosal type of attachment was observed in 60% of the

participants followed by gingival type seen in 29.7%. Regarding the morphological variations, more than 80% of the participants had simple frenum; frenum with nodule was present in 7.3% and frenum with appendix in 6.5% of study participants. Frenum with appendix was mostly present in males 10.1% than in females 2.5%.<sup>19</sup> The findings of all these studies are similar to the present study.

A study done back in 2012 by Upadhyay and Ghimire, who evaluated the frenal attachment among 198 children ranging from 1 to 14 years and concluded the gingival attachment as the most common type seen in 61.1%, as opposed to the finding of the present study, and papilla penetrating as the least common type of frenal attachment observed in only 8.1%, with no significant gender based difference.<sup>20</sup>

The present study reflects the prevalence of types of frenal attachment and also the frequency with which the morphological variations exists in the maxillary labial frenum within the study subjects. However, this study does not compare the findings among the different age groups and whether the tests to determine if the frenum is aberrant coincide with the types of attachments that are indeed considered to be the most pathogenic.

#### **CONCLUSION**

Maxillary labial frenum is a dynamic structure and has variations that can indeed have a variety of consequences. Correction of aberrant frenum is important. Therefore, it should never be overlooked and should always be considered as one of critical aspects of oral examination.

#### **ACKNOWLEDGEMENT**

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## Delayed reimplantation of avulsed tooth with 15-hours extra oral dry storage time

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#### **ABSTRACT**

A 27-year-old man reported to the department with a history of trauma. On examination it was noted that the patient had an avulsed tooth in relation to 21, Ellis Class I fracture in relation to 11 and 22. The avulsed tooth was brought by the patient wrapped in a paper. Although the tooth was in highly unfavorable storage condition, it was decided to reimplant the teeth, to relieve him from psychological, cosmetic and functional trauma. Now after 24 months, the tooth is, functional, firm and free of symptoms with minimal signs of resorption. Even though the long-term prognosis is uncertain, this treatment technique has proven to be an advantage for the patient by maintaining the height of alveolar bone and making the provision of an aesthetically acceptable permanent restoration.

Keywords: delayed reimplantation, avulsion, replacement resorption.

#### INTRODUCTION

Traumatic injuries to erupted permanent anterior teeth accounts for 0.5-16% of the cases.1 Avulsion is defined as a complete separation of a tooth from its alveolus following a traumatic injury which results in extensive damage to the pulp and periodontal tissues.<sup>1,2</sup> Reimplantation refers to the insertion and temporary fixation of completely or partially avulsed teeth that have resulted from traumatic injury.3 Amongst the various factors influencing the clinical success of reimplantation, duration of extra oral storage is identified as critical for functional healing. For best prognosis, reimplantation should be done within five minutes and according to Andersen, reimplantation of tooth beyond five minutes is delayed reimplantation. Reimplantation within 20-30 min after the injury or keeping the tooth in an appropriate storage media has also shown to produce good prognosis.<sup>1-3</sup> As the duration of extra oral time increases, the likelihood of root surface damage increases which leads to necrosis of the pulp tissue, cemental and periodontal ligament tissue leading to external root resorption and eventually loss of reimplanted teeth.<sup>4,5</sup> The aim of this case report is to present a case of delayed replantation of avulsed maxillary central incisor after an extended dry extra-alveolar period.

#### **CASE REPORT**

A 27 years old man reported to the out-patient department, with a chief complaint of fracture and loss of tooth in relation to upper front tooth region. Patient gave history of injury due to fall from bicycle 15hrs ago, he had brought the knocked out tooth rolled in a paper. Intraoral examination revealed Ellis Class I fracture in relation to

11, 22 and avulsed 21 (Figure 1). There was no fracture with respect to the nasomaxillary complex in radiographic examination (Figure 2).

Oral hygiene was fair, no other intraoral injury was detected clinically. Adjacent teeth elicited positive response to vitality test. The available treatment options were explained, and it was decided to reimplant the avulsed incisor after doing root canal treatment extra orally. Composite build-up was planned for 11 and 22. Patient was advised for tetanus prophylaxis; antibiotics and analgesics were prescribed.

Examination of the avulsed tooth revealed that the crown was intact and the root had closed apex (Figure 3), but the root surface was covered with dried remnants of periodontal tissue. Tooth was thoroughly washed in saline, and the root surface planned to remove necrotic periodontal tissues. Root canal treatment was completed with guttapercha points and MTA based sealer extra orally (Figure 4). Surface treatment of the root was done with citric acid and consecutively 2% sodium fluoride application was done on the root surface for five minutes and then rinsed with saline. Local anesthesia was administered, and socket was gently curetted to remove granulation tissue and irrigated with chlorhexidine gluconate and saline. Prior to reimplantation, doxycycline was coated over the root surface. Tooth was reimplanted into the socket using finger pressure. Once the tooth was properly seated, it was checked for alignment and occlusion and then splinted to the adjacent teeth with orthodontic wire and composite resin (Figures 5 and 6).

Patient was prescribed 0.12% chlorhexidine gluconate

mouth wash for maintaining the oral hygiene. Splint was removed after 14 days, and composite build-up done on 11 and 22 (figures 7 and 8). Patient was regularly kept under follow-up every six months. After 24 months, the tooth was asymptomatic, and no mobility noted. Periapical radiograph at the last visit showed no signs of resorption or ankylosis (figures 9 and 10).



**Fig.1:** Pre-operative photograph showing avulsion irt 21



Fig.2: Preoperative intraoral radiograph showing empty alveolar socket



Fig.3: Avulsed tooth



Fig.4: Extra-oral endodontic treatment



**Fig.5:** Splinting of the avulsed tooth with orthodontic wire and composite resin



Fig.6: Periapical radiograph after immediate replantation of avulsed tooth



Fig.7: Four weeks follow-up intraoral photograph of stabilized teeth



Fig.8: One year follow-up intraoral photograph



Fig.9: One year follow-up intraoral radiograph



Fig. 10: Follow-up radiograph at the end of 24 months.

#### **DISCUSSION**

Immediate reimplantation of the avulsed tooth is widely accepted as the most appropriate treatment. However, this may not always be possible due to various reasons, under such circumstances, even if the treatment is delayed, considering the benefits that may result from the therapy, reimplantation need to be attempted.<sup>3,6,7</sup> In this case, the avulsed incisor had been in dried state for a prolonged period. Hence it was anticipated that the chance of pulpal and periodontal healing would be extremely low. Treatment objective was to retain the avulsed tooth to maintain esthetics and occlusal function, to prevent inflammatory root resorption and to achieve periodontal healing.

When a Tooth has an extra-oral dry time of greater than 60 min, the periodontal ligament is not expected to survive. Pre-treatment of such a tooth, prior to its replantation, will render it more resistant to resorption. If excessive drying occurs before replantation, the damaged periodontal ligament cells will elicit a severe inflammatory response over a diffuse area on the root surface. The prognosis of reimplanted avulsed tooth appears directly related to the severity and surface area of inflammation on the root surface, and the resultant damaged surface must be repaired. Hence the tooth was thoroughly planned to remove all necrotic periodontal ligament cells.

As the extra oral time was prolonged and no immediate reimplantation was necessary, it was decided to perform endodontic treatment extra orally. MTA among other Bioactive cements demonstrated better performances, when applied to reimplanted teeth in preventing the progression of replacement resorption.<sup>8</sup>

Tooth was treated with citric acid so as to expose the collagen fibers on root cementum and promote a contact surface for re-attachment of periodontal ligament collagen fibers. The citric acid was used to demineralize the root surface and expose the collagenous matrix of the hard tissues of the root surface, which acts as a substrate for mesenchymal cells as well as inhibit bacterial adhesion.<sup>3,9</sup>

Before reimplantation, the tooth was soaked in sodium fluoride solution for five minutes. Andreasen and Andreasen recommends that the tooth should be soaked with 2.4% acidulated sodium fluoride solution before replantation.<sup>2</sup> Several authors have recommended the use of fluoride solutions in different forms and concentrations to treat the root surfaces in case of delayed tooth reimplantation assuming that demineralized dentin surface would be more prone to fluoride incorporation and might become more resistant to resorption. Fluoride directly acts on the cementum and dentin, by converting hydroxyapatite into fluorapatite.<sup>10</sup>

In an avulsed tooth, recommended procedure is to coat the

root surface with topical doxycycline before reimplantation. Doxycycline, a derivative of the tetracycline family, has an antibacterial effect and anti-inflammatory qualities, and is effective in reducing inflammatory and replacement resorption following dental trauma. Treating the root surface of an avulsed closed apex tooth is a rational and recommended procedure.<sup>11</sup>

It has been shown that the splinting technique which allows physiologic movement of teeth during healing, and which is in place for a minimal time period results in decreased incidence of ankylosis.<sup>3</sup> In this case, splinting was done with composite and wire as recommended in literatures. The advantage of this technique is that it is much less stressful to the injured area than other techniques.<sup>5</sup>

Both inflammatory resorption and replacement resorption may be diagnosed within 2-6 months of reimplantation. If resorption is not detected within 2 years, the risk of resorption is considerably reduced.<sup>3–5</sup> In the case presented here after two years the tooth was asymptomatic, firm and radiographically no signs of resorption or infection was observed.

#### **CONCLUSION**

Replantation of avulsed tooth is the treatment of choice in permanent dentition. Replantation can restore the patient's esthetic appearance and occlusal function and prevent physiological trauma, which may be associated with a missing anterior tooth. According to the presented case, replantation can be advised for avulsed tooth with prolonged extra-oral time, but the risk of resorption at long time should be considered.

Despite an extended extra-alveolar dry storage time, teeth with delayed replantation might be retained in a stable and functional position in the dental arch.



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### Histopathological variations in Pyogenic Granuloma: A Case Series

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#### ABSTRACT

Pyogenic granuloma is a reactive tumor-like lesion commonly affecting the oral cavity. These lesions usually appear as localized solitary nodule with predilection for gingiva. The natural course of this lesion can be categorized into three distinct phases; cellular phase, capillary/vascular phase and involutionary/healing phase. In this series, three cases of pyogenic granuloma showing varying histological phases has been described. Hence, knowledge of the various histopathological presentation of pyogenic granuloma is necessary for proper understanding of the lesion along with clinical correlation and also to rule out various soft tissue lesions with similar appearance.

Keywords: histopathology; oral cavity; pyogenic granuloma

#### INTRODUCTION

Pyogenic granuloma is benign, soft, solitary, non-neoplastic vascular proliferation of the skin and oral cavity.¹ The term "pyogenic granuloma" or "granuloma pyogenicum" was stated by Hartzell in 1904.¹ It occurs as a result of chronic irritation or trauma. The common clinical presentation is a smooth or lobulated mass that can be either sessile or peduculated.² Gingiva is most frequently involved site in the oral cavity and presents as a nodular growth which may be slow growing or rapid in nature.³ The natural course of this lesion can be categorized into three distinct phases namely; cellular phase, capillary/vascular phase and involutionary /healing phase.⁴ In this series, three cases of pyogenic granuloma showing varying histological phases has been described and the clinical descriptions are given in Table 1.

#### **CASE PRESENTATION**

Case 1: Microscopic evaluation showed parakeratinized stratified squamous epithelium. The underlying connective tissue was composed of loosely arranged collagen fibers with mixed inflammatory cells and few proliferating capillaries features resembled the features of Pyogenic Granuloma (cellular phase), Figure 1.

Case 2: Microscopic evaluation showed parakeratinized stratified squamous epithelium with the underlying

connective tissue showing loosely arranged collagen fibers. Large sinusoidal spaces with extravasated RBCs and areas of multiple proliferating small endothelial lined blood vessels with dense inflammatory infiltration. Histopathological features were suggestive of Pyogenic Granuloma (capillary phase), Figure 2.

Case 3: Microscopic evaluation showed parakeratinized stratified squamous epithelium. The underlying connective tissue revealed dense bundle of collagen fibers with plump fibroblasts arranged in streaming fascicles. Areas of large sinusoidal spaces with extravasated RBCs and areas of multiple proliferating small endothelial lined blood vessels with scanty inflammatory infiltration. The Histopathological features resembled the features of Pyogenic Granuloma (involutionary phase), Figure 3.



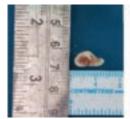


Figure 1:(a) Gross specimen (b) Parakeratinized epithelium and underlying cellular stroma with mixed inflammatory cells and few proliferating capillaries (10X)





Figure 2: (a) Gross specimen (b) Parakeratinized epithelium and underlying connective tissue shows large sinusoidal spaces with extravasated RBCs(10X)





**Figure 3:** (a) Gross specimen (b) Parakeratinized epithelium and underlying connective tissue shows dense bundles of collagen fibers (10x)

Table 1. Clinical Descriptions of the cases of Pyogenic Granuloma

S.N	Age	Gender	Site	Size	Histopathological Category
Case 1	33	Female	Gingiva	1cm×0.9cm	Cellular phase
Case 2	12	Female	Gingiva	1.3cm×1cm	Capillary phase
Case 3	28	Male	Gingiva	1.4cm×0.8cm	Involutionary phase

#### **DISCUSSION**

Pyogenic granuloma is also termed as lobular capillary hemangioma, telangiectatic granuloma, and "pregnancy tumor" as it has been frequently found in pregnant females.<sup>4</sup>

Bhaskar et al. reported that Pyogenic granuloma accounts for 1.85% of all oral biopsies.<sup>2</sup> Pyogenic granuloma is a type of reactive inflammatory lesion, which results in overgrowth of granulation tissue in response to various stimuli such as chronic low-grade local irritation, trauma, hormonal factors, or certain kind of drugs.5,6 Reported incidence of pyogenic granuloma has been found between 26.8% and 32% of all reactive lesions.4 Regezi et al. explained that foreign material is the known stimulant or injury as the etiological factor for pyogenic granuloma which results in exaggerated proliferation of connective tissue.<sup>4,7</sup> Ainamato et al. stated the cause of the lesion to be repetitive tooth brush trauma to the gingiva and trauma can cause release of various endogenous substances including angiogenic factors which may also lead to disturbances in the vascular system of the affected area.4,7 Elevated levels of estrogen and progesterone can modify the vascular response to local irritants which leads to the occurrence of this lesion during pregnancy.<sup>3,7</sup> Increased production of vascular endothelial growth factor, the basic fibroblast growth factor and decreased amounts of angiostatin, thrombopsondin-1, and the estrogen receptor are known to be involved during angiogenesis of the lesion.1,4,7

According to Sternberg *et al*, there are three phases of pyogenic granuloma described; namely, 1. Cellular phase 2. Capillary/vascular phase 3. Involuntary/healing phase.<sup>4</sup> The cellular phase consists of little lumen and compact cellular stroma.<sup>3</sup> The capillary phase has high vascularity

in the form of lobules and intraluminal RBC's.3 The involuntary/healing phase mainly represents healing of the lesion and intra and perilobular fibrosis.8 These phases can be correlated with the clinical presentation of the lesion. In the initial phase the mass appear as reddish blue whereas in late phase the lesion appears pale to pink.4 Depending upon the rate of proliferation and vascularity, there are two histopathological types of pyogenic granuloma; lobular capillary hemangioma (LCH type) and non-lobular capillary hemangioma (non- LCH type).9,10 According to Neychev et al. LCH type clinically presents as sessile lesion in 66.4% of overall cases and the non-LCH type presents as pedunculated lesion in 77% of all cases.<sup>2</sup> The lobular region of the LCH is characterized by proliferation of capillaries in a loose lobular configuration, imparting a glomerulus-like appearance whereas the non-LCH type shows high vascular proliferation resembling granulation tissue.<sup>2,7,11</sup> Diagnosis of pyogenic granuloma can often be challenging because of the similarities in the clinical presentation with many other intraoral hemangiomatous lesions.2 Usually, such lesions can be clinically diagnosed and confirmed following histopathology. The differential diagnosis of pyogenic granuloma includes irritation fibroma, hemangioma, benign salivary gland tumors and metastatic tumors of the oral soft tissues, Kaposi's sarcoma, fibroma, angiomatosis, angiosarcoma, non-Hodgkin's lymphoma, conventional granulation tissue, hyperplastic gingival inflammation, peripheral giant cell granuloma and peripheral ossifying fibroma.<sup>6,7,12</sup> Due to the presence of proliferating blood vessels, cavernous hemangioma can be differential diagnosis of pyogenic granuloma before histologically hemangioma shows endothelial cell proliferation without the infiltration of acute inflammatory cell.<sup>5</sup> Histopathologically, the absence of atypical cells and abundant vascular channels helps to differentiate pyogenic granuloma from Kaposi's sarcoma.<sup>3</sup> Bacillary angiomatosis consists of dense extracellular deposits of pale hematoxyphilic granules representing a bacterial material.<sup>8</sup> This features differentiates it from pyogenic granuloma. The late phase pyogenic granulomatous lesions can be mistaken for oral fibroma, peripheral giant cell granuloma, or peripheral ossifying fibroma due to the presence of extensive fibrosis in the connective tissue stroma.<sup>3</sup> However, the increased vascularity and the inflammatory components are suggestive of pyogenic granuloma which helps in differentiating it from these lesions.<sup>3</sup> Also, a prior history of bleeding from the granulomatous growth would be suggestive of the involutionary/healing phase.<sup>3</sup>

Conventional granulation is also considered as another differential diagonosis.<sup>8</sup> Despite the close association between pyogenic granuloma and conventional granulation tissue, pyogenic granuloma shows clinically different behaviour in comparison to conventional granulation tissue such as rapid growth and frequent recurrence.<sup>8</sup> Hyperplastic gingival inflammation can also be considered which on histopathology may be difficult to differentiate from pyogenic granuloma therefore in such cases pathologist must rely on the surgeons description of a distinct clinical mass to diagnose pyogenic granuloma.<sup>8</sup>

Peripheral giant cell granuloma(PGCG) clinically cannot easily be differentiated from pyogenic granuloma. PGCG

appears as a bluish to purple compared to red colored pyogenic granuloma. Radiographically PGCG is more likely to produce bone resorption compared to pyogenic granuloma. Histopathologically, PGCG differs from pyogenic granuloma in having multinucleated giant cells. Peripheral ossifying fibroma can be differentiated from pyogenic granuloma by its firm and light pinkish colour. Another characteristic feature of peripheral ossifying fibroma is alveolar bone involvement. Hence, a careful clinical and histopathological correlation is of utmost importance in identifying pyogenic granuloma.

#### **CONCLUSION**

Pyogenic granuloma is a benign inflammatory lesion resulting from an exaggerated response to chronic low-grade irritation. Despite various treatment options, recurrence rate is frequent. The patient follow-up is a must in preventing the recurrence of pyogenic granuloma. Hence, knowledge of the various histopathological presentation of pyogenic granuloma is necessary for proper understanding of the lesion in terms of diagnostic accuracy along with clinical correlation and also to rule out various soft tissue lesions with similar appearance.



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