OCCLUSAL CHARACTERISTICS AND SEX PREDICTION POTENTIAL OF MAXILLARY POSTERIOR TEETH IN MALAY POPULATION

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OCCLUSAL CHARACTERISTICS AND SEX PREDICTION POTENTIAL OF MAXILLARY POSTERIOR TEETH IN MALAY POPULATION

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF APPENDICES

LIST OF SYMBOLS

μ	Meu
±	Plus, or minus
℃	Degree centigrade
β	Beta
α	Alpha
%	Percentage

LIST OF ABBREVIATIONS

2D	Two dimensional
3D	Three dimensional
3D-DS	Three dimensional dental scans
ACS	Auto calibration select
AM	Antemortem
ANN	Artificial neural networks
ASUDAS	Arizona State University Dental Anthropology System
BH	Branched H
BL	Buccolingual
BPM1	Buccal cusp of first premolar
BPM2	Buccal cusp of second premolar
CBCT	Cone beam computed topography
CI	Confidence interval
CM1	Crown of first molar
CN	Cusp number
CPM1	Crown of first premolar
CPM2	Crown of second premolar
DA	Discriminant analysis
df	Degree of freedom
DNA	Deoxyribonucleic acid
DPR	Dental panaromic radiographs
DS	Dental scan

DVI	Disaster victim identification
GMA	Geometry morphometric analysis
GP	Groove pattern
PM1GP	Groove pattern of first premolar
PM2GP	Groove pattern of second premolar
M1GP	Groove pattern of first molar
HOX	Home box gene
HMD	Hausdorff mean distance
Hy	Hypocone
HyM1	Hypocone cusp of first molar
ICC	Intraclass correlation coefficient
ICP	Iterative closest points
IOS	Intraoral scanner
KS	Klinefelter's syndrome
LBPM1	Left sided first premolar buccal cusp
LBPM2	Left sided second premolar buccal cusp
LCM1	Left sided first molar crown
LCPM1	Left sided first premolar crown
LCPM2	Left sided second premolar crown
LGA	Logistic regression analysis
LHyM1	Left sided first molar hypocone
LMeM1	Left sided first molar metacone
LPaM1	Left sided first molar paracone
LPPM1	Left sided first premolar palatal cusp
LPPM2	Left sided second premolar palatal cusp

LPrM1	Left sided first molar protocone
M1	First molar
M2	Second molar
M3	Third molar
MD	Mesiodistal
Me	Metacone
MCI	Maxillary canine index
MeM1	Metacone cusp of first molar
MIOS	Medit i500 intraoral scanner
MnCI	Mandiibular canine index
Msx1	Msh home box gene 1
Msx2	Msh home box gene 2
OP	Occlusal pattern
OR	Odd ratio
OT	Occlusal table
Pa	Paracone
PaM1	Paracone cusp of first molar
Pax9	Paired box gene 9
PM	Postmortem
PM1	First premolar
PM2	Second premolar
PPM1	Palatal cusp of first premolar
PPM2	Palatal cusp of second premolar
Pr	Protocone
PrM1	Protocone cusp of maxillary molar

RBPM1	Right sided first premolar buccal cusp
RBPM2	Right sided second premolar buccal cusp
RCM1	Right sided first molar crown
RCPM1	Right sided first premolar crown
RCPM2	Right sided second premolar crown
RHyM1	Right sided first molar hypocone
RMeM1	Right sided first molar metacone
ROC	Receiver operating characteristic
RPaM1	Right sided first molar paracone
RPPM1	Right sided first premolar palatal cusp
RPPM2	Right sided second premolar palatal cusp
RPrM1	Right sided first molar protocone
rTEM	Relative technical error of measurement
SCWB	Semi cylinder wooden block
SD	Standard deviation
Shh	Sonic hedgehog
Sig.	Significance
STROBE	Strengthening the reporting of observational studies in epidemiology
SURF	Speeded up robust features
UD	Undefined
USM	Universiti Sains Malaysia
VC	Vernier caliper

CIRI-CIRI OKLUSI DAN POTENSI RAMALAN JANTINA GIGI POSTERIOR ATAS KEKAL DALAM POPULASI MELAYU

ABSTRAK

Pergigian forensik mempunyai dua aspek pengenalan manusia iaitu, pengenalan perbandingan dan pemprofilan pergigian (umur, jantina dan anggaran bangsa). Matlamat kajian ini adalah untuk menentukan simetri dua hala, dimorfisme seksual, dan ramalan jantina pada gigi posterior rahang atas populasi Melayu menggunakan kaedah dua dimensi dan tiga dimensi. Kajian ini juga menentukan keunikan corak alur dan permukaan oklusal gigi dalam pengecaman identiti manusia. Secara keseluruhan, 176 tuangan pergigian telah ditukar kepada imej digital dan imbasan pergigian menggunakan stereomikroskop digital Hirox KH7700 (Jepun) dan pengimbas intraoral Medit i500 (Medit Corp, Korea Selatan). Luas kuspa, luas korona, bilangan kuspa, corak alur, dan corak oklusal direkodkan menggunakan Hirox 2D, manakala keluasan kuspa dan keluasan permukaan oklusal diukur menggunakan perisian 3-Matic (Materialise NV, Belgium). Untuk keunikan, pengesanan digital GP ditindih menggunakan stereomikroskop Hirox 2D, manakala keunikan OT imbasan pergigian ditindih menggunakan perisian Cloudcompare (Cloudcompare, Paris, Perancis). Tiada perbezaan yang ketara antara gigi sebelah kiri dan kanan menggunakan kedua-dua kaedah 2D dan 3D ($p > 0.05$). Terdapat perbezaan yang signifikan secara statistik antara lelaki dan perempuan dengan kaedah 2D dan 3D ($p<0.01$). Ketepatan klasifikasi ramalan jantina ialah 80% untuk kaedah luas kuspa dan korona 2D. Tiada perbezaan yang ketara antara CN, GP, dan OP bagi gigi sebelah kiri dan kanan ($p > 0.05$), dan CN antara lelaki dan perempuan ($p > 0.05$). Terdapat perbezaan yang signifikan dalam prevalens GP dan OP premolar satu (PM1) dan

premolar dua (PM2) antara lelaki dan perempuan ($p < 0.05$). Walau bagaimanapun, tidak terdapat perbezaan yang signifikan dalam prevalens GP dan OP M1 antara lelaki dan perempuan ($p > 0.05$). Ketepatan klasifikasi jantina ialah 62.7% untuk kaedah 2D. GP menunjukkan 100% keunikan untuk PM1, PM2, dan M1. Ketepatan klasifikasi jantina ialah 82.7% untuk kaedah luas kuspa dan luas korona 3D. Topografi OT menunjukkan keunikan 100% untuk PM1, PM2, dan M1. Begitu juga, permukaan oklusal menunjukkan perbezaan yang signifikan secara xxiitatistic ($p<0.01$) antara nilai punca kuasa dua (RMS) bagi pasangan padan dan tidak padan. Kesimpulannya, gigi posterior atas dalam populasi Melayu menunjukkan dimorfisme seksual yang ketara dan berpotensi untuk anggaran jantina. GP dan OT gigi posterior maxillary menunjukkan 100% keunikan yang bagus untuk pengecaman identiti manusia

OCCLUSAL CHARACTERISTICS AND SEX PREDICTION POTENTIAL OF MAXILLARY POSTERIOR TEETH IN MALAY POPULATION

ABSTRACT

Forensic dentistry has two aspects of human identification i.e., comparative identification and dental profiling (age, sex, and race estimation). This study aimed to determine bilateral symmetry, sexual dimorphism, and sex prediction in the maxillary posterior teeth of Malay population using two-dimensional (2D) and three-dimensional (3D) methods. This study also determined the uniqueness of groove pattern (GP) and occlusal table (OT) of teeth for human identification. In total, 176 dental casts were converted to digital images and dental scans using Hirox KH7700 digital stereomicroscope (Japan) and Medit i500 intraoral scanner (Medit Corp, South Korea). The cusp area, crown area, cusp number (CN), and GP were recorded using 2D Hirox, while the cusp area and the surface OT area were measured using 3-Matic software (Materialise NV, Belgium). For uniqueness, digital tracings of GP were superimposed using 2D Hirox stereomicroscope, while OT of dental scans were superimposed using Cloudcompare software (Cloudcompare, Paris, France). There was no significant difference between the left- and right-sided teeth in both the 2D and 3D methods ($p > 0.05$) for all tested parameters. There was statistically significant difference between male and female in both 2D and 3D methods ($p < 0.01$) for all tested parameters of cusp and crown area. The sex prediction classification accuracy was 80% for the cusp and crown areas in the 2D method while the accuracy was 82.7% for the cusp and OT area in 3D method. There were no significant differences between the CN, GP, and OP of the left- and right-sided teeth ($p > 0.05$), and the CN between

Zmale and females ($p > 0.05$). There was a significant difference in the GP and OP of first premolar (PM1) and second premolar (PM2) between male and female ($p < 0.05$). However, there was no significant difference in the GP and OP of first molar (M1) between male and female ($p > 0.05$). Sex classification accuracy was 62.7% for the CN, GP, and OP of maxillary posterior teeth. The GP showed 100% uniqueness for PM1, PM2, and M1. The OT topography showed 100% uniqueness for PM1, PM2, and M1. Similarly, the OT showed statistically significant difference ($p<0.01$) between the root mean square (RMS) values of the matched and non-matched pairs. In conclusion, the maxillary posterior teeth in Malay population showed significant sexual dimorphism and sex determination potential. The GP and OT of maxillary posterior teeth show 100% uniqueness that indicated both parameters are useful for human identification.

CHAPTER 1

INTRODUCTION

Human identity has a significant legal and social impact on the deceased and living human beings. This identity is important for security purposes such as banking procedures, licences, visa, passport, immigration and etc for living people. For deceased persons, the importance of identification has frequently been addressed for multiple or single causalities (Pretty & Sweet, 2001). The discipline of identification always seeks long-lasting evidence that is tangible, that is not only straightforward and simple to collect but also sophisticated and detailed enough to establish its distinctiveness.

1.1 Dental identification

Dental identification is achieved by two means:

a) Dental profiling is performed in scenarios where there was no clue of antemortem (AM) dental records. Dental profiling or reconstructive identification was performed including information of sex, age at death and race.

b) Comparative identification is the second aspect of forensic odontology where the previous AM dental records of the deceased (suspected) are compared to postmortem (PM) dental characteristics of the deceased person.

1.1.1 Dental profiling

Dental profiling is performed when the tentative identity of a person is not available; therefore, AM records cannot be obtained. This situation arises when the remains are grossly decomposed and are found in unrelated locations. The aim of PM dental profiling is to narrow the search to small populations of individuals. The dental

profile constitutes a group of individual characteristics related to hard and soft tissues. They assist in the estimation of age, sex, ancestry, socioeconomic status, personal habits, systemic health, occupation, and dietary status.

1.1.1(a) Sex estimation

Sex estimation is an initial step in human identification by forensic investigators. The information collected on the dental and skull remains helps the forensic odontologist to assist in sex determination. Various tooth features are measured with linear measurements, such as crown size (Acharya & Mainali, 2007), cusp size (Eboh, 2019), and root length (Govindaram et al., 2018). Some researchers further analysed these linear data into the incisor index, mandibular canine index (MCI) (Rani, 2017), and crown index (Acharya & Mainali, 2008). From these studies, most findings showed males have larger values than females. Several studies highlighted that univariate and multivariate analyses may give different results when used for sex prediction (Khamis et al., 2014; Litha et al., 2017).

Other than tooth size, studies on non-metric features such as a distal accessory ridge (Noss et al., 1983), Carabelli's trait of the upper molars (Mosharraf, 2013), and shovelling of the upper central incisor (Khraisat et al., 2007) have shown significant differences between males and females but they were not verified for sex prediction model.

1.1.1(b) Age estimation

There are different methods for age estimation in children and young adults, and the adults.

According to Kurniawan et al. (2022), there are several methods that are commonly used for age estimation in children, adolescents and young adults:

1. Demirjian method [panoramic radiograph-based method using mandibular teeth from incisors up to second molar (M2)].

2. Willem's method (modified version of Demirjian method) (Fitri et al., 2022).

3. TCI Benindra method (tooth coronal index based on relationship between the tooth and its dental pulp chamber) (El Morsi et al., 2015).

4. AlQahtani method (atlas method utilising all the mandibular and maxillary teeth) (Al Qahtani et al., 2010).

5. Nolla's technique method (evaluation of 10 stages of tooth calcification from tooth seed formation until apical foramen closure) (Kurniawan et al., 2022).

6. Kvaal Method (based on the relationship between age and tooth to pulp ratio) (Kvaal et al., 1995).

7. Schour and Masslers method (development of the primary and permanent teeth, describing 21 chronological stages from 4 months to 21 years of age) (Jaquilin et al., 2018).

8. Moorer, Fanning and Hunt method (maturation stages for crown and root, which can vary accordingly whether the tooth is single or multirooted) (Sekhar et al., 2019)

Phulari and Dave, (2021) reported that there are four categories of age estimation in adults as follows:

1. Histological methods
    i) Combination of different regressive alterations of teeth [Gustafson's method (1950), Bang and Ramm method (1971), Johanson method (1971), Maples method (1979), Kashyap and Koteshwar method (1990), Lamendin method (1992), and Solheim method (1993)].

ii) Dentinal translucency—as a sole indicator

iii) Secondary dentine deposition—as a sole indicator

iv) Cementum—as a sole indicator (thickness of cementum, annulations, or rings of cementum)

v) Fluorescence from dentine and cementum

vi) Microscopic measurements by scanning electron microscope

2. Radiographical methods

i) Pulp-tooth dimension ratio (Kvaal: based on length, Cameriere: based on area and volume)

ii) Tooth coronal pulp cavity index—Ikeda method

iii) Age calculation using X-ray micro-focus computed tomographical scanning of teeth

iv) Age estimation using mental foramen and mandibular canal

3. Biochemical methods

i) Aspartic acid racemisation [Helfman and Bada method (1976), Ritz et al. (1995) method—(dentinal biopsy)]

ii) Enamel uptake of Radioactive carbon-14 (carbon dating) (Spalding et al., 2005).

iii) Miscellaneous biochemical methods

4. Genetic and epigenetic methods

i) Human telomere shortening

ii) Deoxyribonucleic acid (DNA) methylation

1.1.1(c) Ancestry determination using dental structures

A trait is a specific feature or characteristic of an individual that distinguishes it from others. The frequency of the occurrence of a trait can vary. The anomaly of one

population may be a trait of another (Dholia & Manjunatha, 2015). Caucasoid have high prevalence of the carabelli trait, while the same trait is uncommon in the Negroids (Yaacob et al., 1996). However, Negroids have a high prevalence of extra permanent teeth (Harris & Clark, 2008). Similarly, shovelling is characterised as the most distinctive diagnostic characteristic of Mongoloid dentition observed on the lingual surface of the central incisors (Yaacob et al., 1996). Mongoloid population is divided into two groups based on morphological characteristics of the teeth i.e., Sinodonty that exists in Northeast Asian groups and is associated with derived mass additive traits, and Sundadonty, which is documented to be in Pacific and Southeast Asian populations, characterised by its retained traits and a comparatively less complicated dentition (Scott & Turner, 1997). Sinodonty populations have been described by a high prevalence of maxillary central incisor shovellings and double scooping, single root in maxillary first premolar (PM1), maxillary first molar (M1) enamel extensions, pegged/reduced/missing maxillary third molar (M3), deflecting wrinkle presence and mandibular M1 with three roots. On the other hand, Sundadont populations have considerably low prevalence of these traits and a high prevalence of four-cusps in mandibular M2 (Turner, 1990).

Tooth dimensions also vary between different races. The population is divided into microdontic, microdontic and megadontic based on metric dental traits mesiodistal (MD) and buccolingual (BL) dimensions (Hanihara & Ishida, 2005). Microdontic populations included Native Americans, Philippine Negritos, Jomon, and Western Eurasians. The mesodontic population comprises of Polynesians and East-Southeast Asians, while megadotnic population included Australian Aborigines, Melanesians, Micronesians, and sub-Saharan Africans (Hanihara & Ishida, 2005).

Hence, based on the metric and non-metric traits, the profiling can be narrowed down to the ancestry determination in case of absence of AM records.

1.1.2 Comparative dental identification

This is a crucial step to detect any unique feature that may lead to confirm identification based on similarity at individual level e.g., whether it is Ali bin Ahmad. The fundamental idea is on the premise that no two unrelated people have identical tooth structures (Franco et al., 2015).

According to the American Board of Forensic Odontology (1986), outcome from comparative identification can be:

a. Positive identification - records match without any discrepancies. All the findings are attributed to the same individual. Even a single tooth with sufficient unique features can be employed for identification.

b. Possible identification – AM and PM records with consistent characteristics, however the uniqueness of the evidence is lacking.

c. Insufficient evidence - insufficient evidence to reach a conclusion

d. Exclusion – mismatched records that show clear demarcation.

If a positive match is made through dental identification, the Interpol disaster victim identification (DVI) guide states that it can be trusted as a standalone identifier (INTERPOL). Because details of the presence and distribution of teeth, as well as details of restored, non-restored, missing, and decayed surfaces of teeth, apparently occur in distinct patterns, the information stored within dental records can be distinguishing (Kiran et al., 2019).

1.2 Unique dental features

The AM and PM records can be in the form of plaster dental casts, radiographs, and photographs. Radiographic examination have high comparative values due to its uniqueness including biological variability, such as impacted teeth, root remnants, jawbone structure, sinus cavities, and dental treatments such as shape of restorations, root canal treatment, surgical procedures, and dental implants (Campobasso et al., 2007). Furthermore, in the current scenario, where there is improvement in oral health status, more cases are inconclusive because all teeth are sound without restoration or other treatments.

Teeth shape captured photographically on the smiling face also adds to the identification process of the study cast from skull-photo superimposition (Santoro et al., 2019). However, cosmetic dentistry has resulted in the loss of a potential identification tool, as an increasing number of people modify their anterior teeth, which typically form a smile pattern, in a way that may not match the original smiles in the AM database. Skull features like frontal sinuses are also considered a reliable tool for human identification (Andrade et al., 2022).

Apart from teeth and skull features, some other soft tissue structures, such as palatal rugae and lip prints, have been compared in the AM and PM records. Palatal rugae form an intrinsic and integral pattern for every individual and can play a vital role in human identification. The ease of reproducibility and lower level of variation make palatal rugae a potential tool in forensic odontology (Shetty et al., 2016). Furthermore, slow rugae loss at an average rate of one ruga in approximately 15 (±2) years after early adulthood (Suhartono et al., 2016) provides an advantage for the applicability of rugae in forensic odontology.

Cheiloscopy has also been used in forensic investigations to establish human identity. Lip-prints can be identified as far back as the second month of intrauterine existence. It is possible to compare lip prints and groove pattern (GP) discovered at a crime scene with a suspect's lip print (Herrera et al., 2018). Nevertheless, lip appearance appears to change with age, diminishing in length and broadening in width, leading to a thinner and longer appearance (Kim et al., 2019), which may lead to an incorrect diagnosis, particularly if the process of identification is performed after an extended period. The propensity for mobility of the lip crease pattern found on the lip vermilion border can generate lip impressions that vary according to the pressure, direction, and manner of making the impression (Dineshshankar et al., 2013).

Palatal rugae and cheiloscopy are soft tissue techniques that degrade because of the decomposition process and wound healing, therefore, limits the applicability in human identification.

Comparison of the GP in maxillary M1 and M2 also highlighted their uniqueness and pointed to the potential of this dental trait as a source of human identification (Roy et al., 2019). In terms of GP, previous studies focused mainly on the mandibular molars. Hence, the information regarding the GP in maxillary teeth is scarce. Since maxillary and mandibular teeth have different GP, it would be of immense advantage if further studies were conducted to explore the GP on other teeth as well such as the maxillary premolars and molars. Thus, this may in turn highlight the value of sound teeth for comparison between AM and PM records.

1.3 Admissibility of method by court of law

The methodology used in this study should be selected by focusing on expert opinion to determine whether it utilises valid scientific methods and procedures. The

Daubert rule (Daubert, 1993) suggests certain factors that aid in evaluating whether a particular scientific theory or study is reliable: (1) its empirical testability, (2) whether the theory or study has been published or subjected to peer review, (3) whether the known or potential rate of error is acceptable, and (4) whether the method is generally accepted in the scientific community.

Several scientific analyses and data collection methods have undergone significant transformations in forensic odontology to accommodate human identification. The conventional digital imaging method used for dental identification is a two-dimensional (2D) approach, such as analysing radiographs and plaster dental casts. Radiographs are usually used to compare dental treatments such as prostheses, and restorations in the AM and the PM records (Campobasso et al., 2007). In this modern era of digital technology, many 2D and three-dimensional (3D) imaging devices and software have been developed to achieve more robust forensic evidence for court and medicolegal purposes. Data collection methods are being carried out by using 2D digital images and 3D dental scans of the dental casts, for forensic investigations as these imaging systems have added to the future options to be used as forensic tools with promising accuracy and precision in their results (Roy et al., 2019; Bianchi et al., 2023). This is in addition to the ease of accessibility and storage of the data. Data analysis is performed using sophisticated software for comparative identification like superimposition of 3D dental scans (Gibelli et al., 2019), and palatal rugae (Gibelli et al., 2017) etc. Dental profiling is also performed using the 2D and 3D techniques for sex, race, and age estimation by capturing digital images using standardised tools that have shown high accuracy and precision (Shahid et al., 2015; Amornvit et al., 2021).

Higher accuracy also depends on the methodology used and the dental traits studied. Therefore, considering the values of biological evidence for forensic application, we propose to study the potential forensic applications in term of sex estimation based on cusp number (CN), GP, occlusal pattern (OP), and the cusp and crown area. We also propose to evaluate the uniqueness of GP and occlusal topography teeth among Malay population.

1.4 Problem Statement

DNA multiplex PCR (Osman et al., 2014), pelvic bone (Bytheway & Ross, 2010), and cranium (Yang et al., 2019) are regarded as gold standards for sex prediction However, there may be instances in which the DNA from the pelvis, cranium, or both is so degraded by PM changes that it cannot be used for sex determination. Hence, tooth sex prediction can be considered as an adjuvant in scenarios where the gold standards are not accessible.

With the awareness of oral hygiene, oral health status has improved resulting in less prevalence of caries. Hence, there are cases where the AM records bear no traces of any unique dental treatment to be matched for positive identification. Thus, the uniqueness in the dental records have to rely on other unique biological skull structures like sinuses, cheiloscopy, and palatal rugae. However, the sinuses may be affected from inflammation while cheiloscopy and palatal rugae could not survives decomposition.

To date, there has been no publication of sex prediction using occlusal traits (cusp area, crown area, GP, and CN) and uniqueness using the GP and occlusal topography among Malay population.

1.5 Justification of the study

In the due process of human identification, sex information also has merit in narrowing the search of AM dental records. Using these occlusal traits may increase the options for biological evidence of sex prediction. In a situation where the DNA is contaminated or degraded, and the non-availability of other biological evidence such as cranial and post-cranial traits due to trauma or incineration, tooth occlusal characteristics may be useful. Furthermore, the odontometric methods are prone to problems with caries (even initial caries at the proximal area) and trauma that affect their measurement landmarks.

When the subject is caries-free, the outcome of the comparison between AM and PM dental records may lead to a possible/inconclusive identity. To confirm identification in this case, forensic dentists rely on unique biological evidence on healthy teeth. Thus, occlusal trait assessment may be useful if the outcome of our study proves its uniqueness and the availability of occlusal traits in the AM records, in the form of dental casts or clinical photographs. Thus, by taking digital images or impressions from the deceased, odontologists can create postmortem 2D and 3D dental casts for comparison and superimposition. In conclusion, this thesis focused on sex prediction using 2D and 3D digital techniques of the occlusal features, and at the same time evaluate the uniqueness of occlusal table (OT) features for AM and PM comparisons.

1.6 Objectives

1.6.1 General:

To understand the association between the occlusal traits (CN, cusp area, total crown area, GP, OP, and occlusal topography) of occlusal surfaces of maxillary posterior permanent teeth and identification process in Malays.

1.6.2 Specific:

1. To determine and compare cusp and crown area of maxillary posterior teeth between right and left sides with 2D method.
2. To determine and compare the cusp and crown area of maxillary posterior teeth between males and females with 2D method.
3. To formulate sex prediction model using cusp and crown area with 2D method.
4. To determine and compare prevalence of CN, GP, and OP of maxillary posterior teeth between right and left sides with 2D method.
5. To determine and compare prevalence of CN, GP, and OP of maxillary posterior teeth between males and females with 2D method.
6. To determine sex prediction model using CN, GP, and OP of maxillary posterior teeth with 2D method.
7. To determine the uniqueness of GP of maxillary posterior teeth with 2D method.
8. To determine and compare cusp and crown OT area of maxillary posterior teeth between right and left sides with 3D method.
9. To determine and compare the cusp and crown OT area of maxillary posterior teeth between males and females with 3D method.

10. To formulate sex prediction model using cusp and crown OT area with 3D method.

11. To determine the uniqueness of occlusal topography of maxillary posterior teeth with 3D method.

1.7 Research Question(s)

1. Is there any difference in cusp and crown area of maxillary posterior teeth between right and left sides with 2D method?
2. Is there any difference in the cusp and crown area of maxillary posterior teeth between males and females with 2D method?
3. Can sex prediction model be formulated using cusp and crown area of maxillary posterior teeth with 2D method?
4. Is there any difference in the prevalence of CN, GP, and OP of the maxillary posterior teeth between the right and left sides using the 2D method?
5. Is there any difference in the prevalence of CN, GP, and OP of maxillary posterior teeth between males and females using the 2D method?
6. Can the sex prediction model be determined using the CN, GP, and OP of the maxillary posterior teeth using the 2D method?
7. Is there any uniqueness in the GP of maxillary posterior teeth using the 2D method?
8. Is there any difference in cusp and crown OT area of maxillary posterior teeth between right and left sides with 3D method?
9. Is there any difference in the cusp and crown OT area of maxillary posterior teeth between males and females with 3D method?

10. Can sex prediction model be formulated using cusp and crown OT area of maxillary posterior teeth with 3D method?
11. Is there any uniqueness in the occlusal topography of maxillary posterior teeth using the 3D method?

1.8 Null Hypothesis

1. There was no difference in cusp and crown area of maxillary posterior teeth between right and left sides with 2D method.
2. There was no difference in the cusp and crown area of maxillary posterior teeth between males and females with 2D method.
3. There was no association between cusp and crown area and sex in maxillary posterior teeth using 2D method.
4. There was no difference in prevalence of CN, GP, and OP of maxillary posterior teeth between right and left sides using 2D method.
5. There was no difference in prevalence of CN, GP, and OP of maxillary posterior teeth between males and females with 2D method.
6. There was no association between CN, GP, and OP, and sex in maxillary posterior teeth using 2D method.
7. There was no difference in cusp and crown OT area of maxillary posterior teeth between right and left sides with 3D method.
8. There was no difference in the cusp and crown OT area of maxillary posterior teeth between males and females with 3D method.
9. There was no association between cusp and crown OT area and sex of maxillary posterior teeth using 3D method.

CHAPTER 2

LITERATURE REVIEW

2.1 Tooth Development

Development of human tooth tissue, also known as odontogenesis, begins in the maxillary and mandibular processes as a thickened band of odontogenic epithelium, known as the primary epithelium band during 6th week of embryonic life from ectoderm and mesoderm tissues (Nanci, 2018). Tooth development can be divided into distinct phases to facilitate observation of various histological, molecular, and morphological alterations at each stage. (**Figure 2.1**).

2.1.1 Bud stage

The bud stage initiates in the 8th week of intrauterine life when the cells inside the dental lamina begin to proliferate and invaginate in locations corresponding to future tooth positions (Kwon & Jiang, 2018) (**Figure 2.1a**). The transition from the bud to cap stage marks the first phase of morphologic differentiation between tooth germs, which results in different types of teeth.

2.1.2 Cap stage

During this stage, enamel knots begin to appear at the locations of future cusps and tips in the enamel organ (**Figure 2.1b**). As many essential growth factors are expressed by the cells of the enamel knot, they serve as signalling centers and play a significant role in determining tooth shape (Jukka Jernvall & Thesleff, 2000). The primary enamel knot determines the arrangement of cusps. In multicusped teeth, such as premolars and molars, the primary enamel knot gives rise to a secondary enamel knot, which acts as a signalling centre, similar to the primary knots for tooth shape determination (Thesleff, 2003). At this point, the expression of signalling molecules

precedes the folding and growth of the dental epithelium. The Slit1 gene is expressed in primary and secondary enamel knots during the formation of molar cusps (Matalova et al., 2005; Nanci, 2018). As a result of spatial differences in cell proliferation, the formation of secondary enamel knots causes the folding of the inner enamel epithelium (Kwon & Jiang, 2018). The order in which secondary enamel knots and cusps appear corresponds closely with their relative height and the order in which they begin to mineralise (Jernvall et al., 1994). These enamel knots also stimulate the differentiation of odontoblasts in the bell stage, which then starts dentine deposition (Kawashima & Okiji, 2016).

2.1.3 Bell stage

In bell stage, the tooth germ continues growing with deepening of epithelial layer to resemble a bell that determines the crown shape. The bell stage is further divided into an early bell stage and the advanced bell stage (also known as maturation stage). In the early bell stage, four cell layers are identifiable (de Sousa-Romero, 2016) (**Figure 2.1c**). First is the outer enamel epithelium made up of cuboidal cells at the periphery of the enamel organ. Second is the stratum intermedium which is a layer of cells between inner enamel epithelium and stratum reticulum. Third is the inner enamel epithelium comprising of the columnar cells next to the dental papilla. Fourth is the cervical loop that is a junction where inner and outer enamel epithelium meet at the periphery of the enamel organ forming a rim (de Sousa-Romero, 2016). The cervical loop is the site of proliferation that guides the cervical part of the crown enamel formation. The early bell stage is followed by the advanced bell stage that is final stage of histodifferentiation and morpho differentiation of tooth crown (**Figure 2.1d**). Different rates of mitosis and differences in the duration of cell differentiation are shown to be responsible for the various crown shapes caused by the folding of enamel

organ (Jernvall et al., 1994). The mitotic activity stops during this stage at the location of the final cusp. The shape of the tooth crown is determined at the bell stage when the locations and heights of the tooth cusps are determined in the 18th week of embryonic life (Nanci, 2018; Rathee & Jain, 2021).

Odontoblast and ameloblast differentiation from dental papilla and inner dental epithelium, respectively, are important processes at this stage (Phulari, 2016). Inner epithelial cells induce the peripheral cells of dental papilla to form odontoblasts (Nanci, 2018). During odontoblast differentiation, odontoblasts move out from basement membrane to the dental papillae while maintaining a connection to the basement membrane, resulting in the formation of the odontoblastic process. At the initiation of dentine mineralisation, the basal membrane becomes discontinuous and disappears. Odontoblasts secrete unmineralised dentine matrix known as pre-dentine. This is followed by mineralisation at random points to form mineralised dentine (Kawashima & Okiji, 2016).

Odontoblastic deposition of the first dentinal layer is followed by the enamel formation initiation. The inner epithelial cells differentiate into ameloblasts which then secrete enamel matrix at the amelodentinal junction. This is followed by the mineralisation and formation of hydroxyapatite crystals. The crystals enlarge with enamel maturation on enamel epithelium (Nanci, 2018). The dentinogenesis and amelogenesis are followed by the root formation, cementogenesis and periodontal ligament formation.

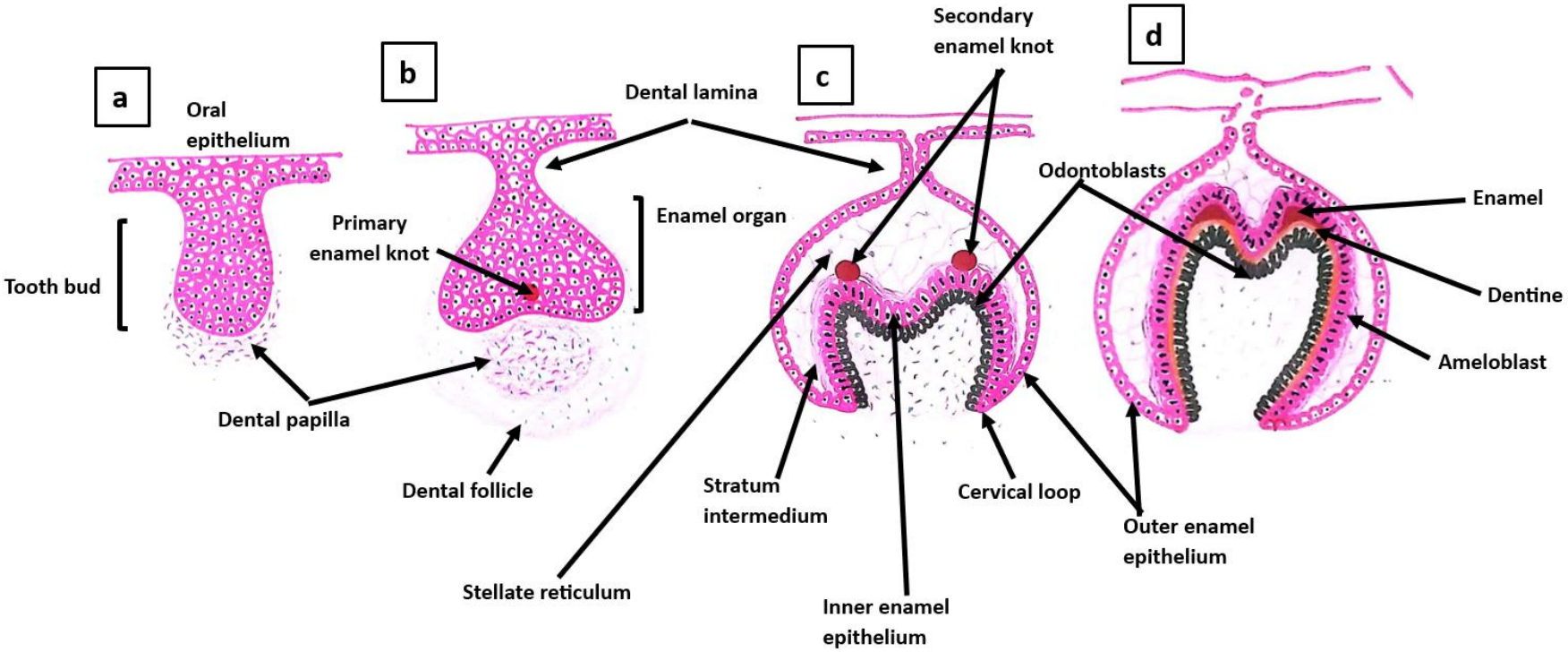

Figure 2.1 Tooth development stages. a) bud stage b) cap stage c) bell shape d) late bell stage. Adapted from Nanci (2018)

2.2 Molecular and genetic factors in tooth development

Multiple proteins and genes are exhibited and play a crucial role in determination of the type of tooth that will develop (Nanci, 2018). Tooth development initiation is controlled by various paracrine signalling molecules that initiate interaction between the ectoderm and ectomesenchyme (Yu & Klein, 2020). On the ninth to eleventh embryonic days, signalling mechanisms of organogenesis, which start the development of tooth epithelium, involve tumour necrosis factor, fibroblast growth factor, bone morphogenic protein, sonic hedgehog (Shh), and Wingless-related integration site pathways (Cakan et al., 2013; Yu & Klein, 2020).

Additionally, tooth development is also controlled by homeobox (HOX) genes, a number of different mesenchymal regulatory molecules and their receptors (Rathee & Jain, 2021). HOX genes are further categorised as muscle segment [Msh homebox1 (Msx1) and msh homebox 2 (Msx2)], distal-less, goosecoid, orthodontical, Shh and paired box gene 9 (Pax9) (Rahayu, 2016). Pax9 is an important transcription factor in tooth morphogenesis and serves a role in establishing the inductive capability of the tooth mesenchyme as it is required for the mesenchymal expression of Msx1, and bone morphogenetic protein 4 genes (Abu-Siniyeh et al., 2018). Moreover, the growth of all the molars is also regulated by Pax9 (Thesleff, 1995). The Msx1 and Msx2 are the genes that determine the developmental position and progression of tooth buds, respectively (Chen et al., 1996; de Sousa-Romero, 2016).

Individual characteristics result from the interaction of genetic, environmental, and epigenetic factors. (Williams et al., 2014). Epigenetic factors are the behavioural and environmental factors that change the way of genetic expression without altering the DNA sequence (Al Aboud & Jialal, 2022). If genetic factors are unable to account

for a phenotypic change, then environmental factors could possibly be accountable (Dempsey & Townsend, 2001).

According to Alvesalo et al. (1987), the X and Y chromosomes play a significant part in the development of enamel and dentine. Amelogene is a gene for enamel deposition located on the X chromosome. The X chromosome influence is restricted to enamel deposition only (Alvesalo, 1997). On the other hand, the Y chromosome, which is found in males only, influences both the enamel and the dentine (through cell proliferation) to promote the dental growth (Alvesalo et al., 1987). Thus, the mitotic potential is increased through amelogenesis and dentinogenesis, which, at various phases of development, leads to an increase in cell division (Alvesalo et al., 1975).

Sexual dimorphism is caused by the different implications of the sex chromosome on amelogenesis and dentinogenesis (Alvesalo et al., 1975). This is related to a study that demonstrated thick enamel as well as large crown proportions in females with an extra X chromosome, while males on average had larger crown proportions than females (Alvesalo & Kari, 1977). Studies have demonstrated that there was no significant difference in the enamel thickness between the two genders but the dentine was thicker in males as compared to females concluding that the sexual dimorphism in teeth was based on dentine thickness and not the enamel thickness (García-Campos et al., 2018; Stroud et al., 1994).

Studies have shown that the genetic factors play a vital role in the development of the numerous morphologic characteristics like the cusps, ridges, and GP of the teeth (Kangas et al., 2004; Palomino et al., 1977). Kangas et al. (2004) also reported that dental characters appear to be non-independent and a small variation in expression of a single gene can lead to change in CN, position, and shape. Thus, a variation in a gene

can lead to variation in the tooth morphological characteristics in different populations. Important information on the phylogeny of man and differences among races as well as sub-races can be uncovered by careful description and investigation of these qualities.

2.3 Formation of the occlusal traits

At least four growth centers contribute to the development of each tooth. These centers are known as developmental lobes, and they originate from the tooth germ (Brand & Isselhard, 2013; Nelson & Ash, 2019). The lobes mature and expand until they fuse within their bony crypt. This lobe fusion is known as coalescence. The connection between these lobes is defined by lines on the tooth known as developmental grooves, which become visible after tooth formation (Manjunatha, 2013). Supplementary grooves also form because of the wrinkling at the fusion of the lobes.

The number of developmental lobes required for tooth formation is dependent upon the kind of tooth and the number of cusps it has. Three labial and one lingual lobe (forms cingulum) combine to form the anterior teeth (Brand & Isselhard, 2013). The mamelons of the anterior teeth are the incisal ridges of these three labial developmental lobes, which are divided by developmental fissures. The same developmental lobe pattern is seen in the premolars with three labial and one lingual lobe (Nelson & Ash, 2019). In contrast to anterior teeth, the three labial lobes in premolars fuse to form one buccal cusp while the lingual lobe develops into lingual cusp (Brand & Isselhard, 2013). In the case of mandibular premolars with two lingual cusps, the fusion is between two lingual lobes namely mesiolingual and distolingual lobes. Presence of two lingual cusps in a premolar also alters the position of the

developmental groove depending on the location of the labial and the lingual lobes (Nelson & Ash, 2019).

Maxillary molar teeth develop from two facial and two labial lobes. An exception is the maxillary M1 that usually has an additional lobe called rudimentary lobe (Brand & Isselhard, 2013). The cusp of carabelli may also exist in case of presence of the rudimentary 5^{th} lobe (Manjunatha, 2013). Mandibular molars also develop from 4-5 lobes (Phulari, 2016). The number of lobes in molar teeth correspond to the number of cusps. However, there is a possibility for a tooth to have only three cusps or more than four cusps (Nayak et al., 2013). The presence or absence of a cusp as well as the size of the cusps alters the position of the developmental and the supplementary grooves (Nelson & Ash, 2019). The presence of an accessory tubercle like Carabelli trait may also effect the size of the adjacent cusp (Kondo & Townsend, 2006). Takahashi et al. (2007) reported that variability in the phenotype of maxillary molar crowns is the result of dynamic interactions among the cusps that develop at different intervals and grow at different rates and durations. Hence, this results in the variation of the OT morphology of the teeth varies from person to person.

2.4 Factors affecting tooth development

Disturbances during the tooth development stages may lead to various abnormalities in regard to number, size, position, form, shape, structure, eruption time or even a structural variation (Cakan et al., 2013; Klein et al., 2013; Nelson & Ash, 2019). These disturbances can be due to genetic, environmental or both factors (Brook, 2009; Brook et al., 2014).

2.4.1 Local and systemic factors affecting tooth development

During the prenatal, perinatal, and postnatal stages of development, environmental factors affect the human dentition. By analysing the tooth type affected and the location of the impacts generated, one can estimate the timing of insults. For instance,calcification of deciduous tooth crown begins at 12.5 weeks gestation period for deciduous incisors, M1 and M2 up to 20 weeks in gestation, for deciduous canines. All the crown growth culminates at 11 months postnatal (Kraus & Jordan 1965). Any kind of insults occurring at specific periods during the development of a tooth may be recorded as the lesions of the tooth's structure upon locations (Seow, 2014; Taji et al., 2000). This lesion, which may be systemic or localized, will be captured on a group of teeth or an individual tooth. It could be either bilateral or unilateral. Various types of local and systemic factors can cause alterations in the micromorphology as well as ultrastructure of tooth crowns.

2.4.1(a) Local factors

Trauma to deciduous teeth may disturb the development of their successor permanent teeth. The severity of traumatic injuries to the primary teeth was found to be correlated with the severity of developmental disorders of the permanent teeth, and the younger the child was at the time of the trauma to the deciduous teeth, the more severe the developmental disorders of the permanent teeth (Lenzi et al., 2015). Injury to the developing tooth can lead to peg-shaped lateral incisor (Nelson & Ash, 2019), tooth germination or fusion (Shrivastava et al., 2011), talon cusp (Anggraini et al., 2019) and dense evaginated odontome etc (Arora et al., 2020).

Developmental dental defects can be localised to one part of a tooth or they may have a generalized effect on the tooth structure (Chadwick et al., 1997). A direct trauma to the ameloblasts during matrix formation, due to a blow to the tooth or fall

history, may result in localized defects in a single tooth or a few adjacent teeth. The defect's appearance is determined by the developmental stage at which the event happened. Trauma during the later maturation phase of amelogenesis results in opaque enamel (Wong, 2014), whereas, hypoplastic defects will develop if trauma occurs during matrix formation. Examples of local factors leading to hypoplastic enamel consist of microorganisms from an infected deciduous tooth (Garg et al., 2015; McCormick , 1967). These infections might extend to the tooth germ of the permanent dentition. The results have varied enamel defects ranging from opacities to hypoplasia of the replacing permanent teeth.

Another local factor could be the trauma due to surgical procedure. This is quite common in patients with cleft lips and palate which require extraction of the premolars or other surgical repairs. These defects are predominantly found in areas adjacent to the site of the surgical repair of the lip or the palate (Rothhammer, 1968). Local birth trauma as a result of laryngoscopy and endotracheal intubation, a prerequisite in preterm children to cope the respiratory distress further increase the dangers of damage to the developing enamel in deciduous maxillary incisor teeth (Seow, 2014).

2.4.1(b) Systemic factors

Multiple anomalies arise during the dental developmental stage as a result of complex interaction of genetic, epigenetic and environmental factors (Altan et al., 2019). The action of several developmental regulatory genes are active in odontogenesis in different tissues which, on disruption can result in dental anomalies (Brook, 2009). The clinical association of abnormalities of number, size, and shape reflects the repetitive signalling patterns that occur during the sequential process of initiation and morphogenesis (Brook, 2009). The most common abnormality is a tooth