3D MORPHOMETRIC EVALUATION OF PALATAL RUGAE AMONG MALAYSIAN MALAY POPULATION

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by

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LIST OF SYMBOLS

mm = millimetre

DSC= Dice Similarity Coefficient.

HD= Hausdorff Distance.

LIST OF ABBREVIATIONS

3D	Three-dimensional
USM	Universiti Sains Malaysia
JEPeM	USM human ethics committee
UTHSCSA	University of Texas Health Science Center in San Antonio

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PENILAIAN MORFOMETRIK 3D RUGA LELANGIT DALAM KALANGAN POPULASI MELAYU MALAYSIA

ABSTRAK

Ruga lelangit atas mempunyai masa depan yang menarik dan menyakinkan untuk tujuan mengenalpasti identiti manusia. Kajian terdahulu menunjukkan ruga lelangit atas mempunyai banyak ciri morfologi dan dimensi yang pelbagai daripada satu individu ke individu lain mahupun dalam kalangan kembar. Tambahan pula ruga lelangit terletak di kawasan anatomi yang selamat terlindung, belakang '*boons',* pipi dan bibir yang memberi pilihan alternatif yang baik untuk mengenalpasti identiti manusia dalam bencana besar dan kemalangan. Kajian ini bertujuan untuk menilai perbezaan dan keunikan ruga lelangit atas dalam kalangan Melayu Malaysia menggunakan model gigi 3D. Pengimbas 3D Next Engine Laser (USA) digunakan untuk mendigitalisasi 130 model gigi plaster pesakit Melayu Malaysia (65 lelaki dan 65 perempuan) yang diperolehi daripada arkib Klinik Ortodontik Hospital Universiti Sains Malaysia. Model 3D yang terhasil diperjelaskan menggunakan perkakas lembut Paint 3D (USA) untuk penilaian bentuk ruga. Kategori saiz ruga dan arah dinilai menggunakan perkakas lembut penyelidikan 3Matic (Belgium). Metod klasifikasi Thomas and Kotze (1983) yang diterangkan oleh Kapali *et al*. (1997) telah digunakan. Keunikan ruga lelangit atas dibuat dengan menindih 42 pasang model

digital (21 model dipendua daripada pesakit sama dan 21 model daripada pilihan rawak dalam kumpulan). 42 set tindihan diperiksa untuk pemuatan permukaan lelangit atas oleh satu penilai buta. Prevalens ruga lelangit dibentangkan dalam bentuk peratusan manakala purata dan sisihan piawai untuk bilangan ruga. Analisis *Chi-square* menilai hubungkait antara jantina dan variabel prevalens ruga lelangit. Ujian t bebas dijalankan untuk membezakan perbezaan jantina dalam bilangan ruga. Keputusan betul/salah tindihan dipersembah dalam bentuk peratus. Nilai $P<0.05$ dianggap signifikan secara statistik.

Jumlah bilangan ruga adalah 1359 (673 lelaki dan 686 perempuan). Bentuk gelombang adalah bentuk yang predominan dalam kalangan sampel, diikuti oleh bentuk lengkung dan tegak. Ruga predominan dalam kategori saiz ruga adalah saiz perdana. Ruga arah kedepan adalah paling prevalen dalam kalangan sampel. Tiada perbezaan signifikan antara lelaki dan perempuan dalam ciri ruga kecuali arah ruga di lelangit kiri yang ruga kebelakang adalah paling prevalen dalam perempuan ($p=0.001$). Ruga lelangit adalah unik. Tiada dua individu yang mempunyai corak ruga yang sama. Bentuk gelombang, saiz perdana dan arah kedepan adalah paling prevalen dalam Melayu Malaysia. Ruga lelangit adalah unik yang mana boleh digunakan dalam proses mengenalpasti identiti manusia dalam kes yang mana rekod pre-mortem dan post-mortem diperolehi.

3D MORPHOMETRIC EVALUATION OF PALATAL RUGAE AMONG MALAYSIAN MALAY POPULATION

ABSTRACT

Palatal rugae pattern may have a promising and interesting future for human identification purpose. Previous studies showed that palatal rugae pattern has a lot of morphological and dimensional characteristics that are different from a person to another even among the identical twins. In fact, palatal rugae are anatomically located in a well protected place, behind boons, teeth, cheeks and lips, which provides a good alternative option for human identification in mass disasters and accidents. This study aimed to evaluate the variability and uniqueness of palatal rugae among Malaysian Malays using 3D dental models. A 3D Next Engine Laser scanner (USA) was used to digitize plaster dental casts of 130 Malaysian Malay patients (65 males and 65 females) obtained from the archive of Orthodontic Clinic, Hospital Universiti Sains Malaysia. The resulting 3D models were highlighted using Paint 3D software (USA) for the purpose of rugae shape assessment. Rugae size category and direction were assessed using 3Matic research software (Belgium). The classification method of Thomas and Kotze (1983) as described by Kapali *et al.* (1997) was adopted. Palatal rugae uniqueness was performed by superimposing 42 pairs of digital models (21 models were duplicated from the same patients and 21 models were randomly selected from the group). The 42 superimposition sets were examined for palatal rugae surface fitting by a single blinded evaluator. Prevalence of palatal rugae was presented in percentages while mean and standard deviation were presented for number of rugae. Chi-square analyses were used to evaluate the association between sex and prevalence of palatal rugae variables. Independent t-test was used to compare sex difference in number of palatal rugae. Correct/wrong decision of superimposition was presented in percentages. P-value <0.05 was considered statistically significant. Total number of rugae was 1359 (673 in males and 686 in females). Wavy shape was the predominant rugae among the sample, followed by curve shape and straight shape respectively. The predominant rugae size category was the primary size. Forwardly directed rugae were the most prevalent rugae among the sample. There was no significant difference regarding rugae features between males and females except for the rugae direction in the left side of the palate, as the backward rugae direction was more prevalent among the females (p=0.001). Palatal rugae pattern was unique. No two individuals have the same pattern. Wavy shape, Primary size and forward direction were the most prevalent among Malaysian Malays. Palatal rugae is unique thus could be used for human identification in the case where pre-mortem and post-mortem records are available.

CHAPTER 1
INTRODUCTION

1.1 Palatal Rugae

Palatal rugae refers to the irregular and asymmetric mucosal folds that are located behind the incisive papilla in the anterior third of the hard palate, and laterally extended on both sides of the median palatine raphe (Figure 1.1). Each single palatal ruga is confined to one side of the palate and does not extend to the other side. The rugae are surrounded by the buccal cheeks, lips teeth and bones, which explains their resistance to the thermal, traumatic, and other factors (Hauser *et al.*, 1989; Muthusubramanian *et al.*, 2005). Thus, from our point of view, the use of palatal rugae for human identification purposes may be of great importance, especially in violent accidents in which the body maybe exposed to major deformities as they may maintain their integrity more than other parts of the body.

The difference of palatal rugae pattern from one person to another made it a subject of interest for many researchers in the field of forensic. Many studies were conducted on specific populations proved the uniqueness of the palatal rugae patterns and their differences from one person to another among their study samples (Bansode and Kulkarni, 2009; Gibelli *et al.*, 2018; Indira *et al.*, 2012). Several other studies have been conducted to investigate the ability of palatal rugae patterns to predict the gender and/or race and concluded that palatal rugae can be used for human identification in combination with other identifiers by predicting race and/or gender (Hosmani *et al.*, 2018; Malekzadeh *et al.*, 2018; Nayak *et al.*, 2007; Saraf *et al.*, 2011).

Figure1.1 A maxillary dental cast showing the palatal rugae

1.2 Human identification

Owing to the frequency of the wars, heinous acts of terrorism, train accidents, earthquakes, floods and other types of mass disasters in which the victims are usually unknown, the human identification becomes a major challenge to the workers in the medico-legal field, and the need for additional resources becomes urgent for victims identification (Prajapati *et al.*, 2018).

Human identification is of great importance legally as it preserves the people's rights and allows them to have their duties. The fact that the family of a deceased person does not recognize the body of their relative is unfortunate and has many negative effects that may affect them emotionally for a long period of time, in addition to the difficult social situation they face as they need to bury the body and receive the condolences. Moreover, the failure to establish the identification of a missing person may result in a state of confusion about his legal rights and duties, especially financial ones, for example, the missing person who has heirs, they need to confirm the death, so they can dispose of his assets and property (Filho *et al.*, 2009; Hinchliffe, 2011).

1.3 Forensic odontology and human identification

The primary identifiers in mass disasters according to International Criminal Police Organization (INTERPOL) are fingerprints, forensic odontology and deoxyribonucleic acid analyses (DNA) (INTERPOL, 2018).

Forensic odontology plays a significant role in comparative and reconstructive human identification. Based on the hypothesis that two individuals do not have the same dental features, the ante-mortem dental records of a deceased person such as dental charts radiographic images, digital images, dental casts, prostheses could be compared to the post-mortem records to establish the identity (INTERPOL, 2018). From post-mortem dental autopsy, a lot of information about the race, sex, age, social status or geographic region could also be obtained by assessing the dental and oral profile of the unknown dead person (Ata-Ali and Ata-Ali, 2014; Ehtisham *et al.*, 2016; Hinchliffe, 2011; Pretty and Sweet, 2001). Reconstructive human identification may also be employed to establish the race, gender, age, or occupation of a deceased person which would narrow down the search for ante-mortem dental record (Edgar, 2005; Poongodi *et al.*, 2015).

Rugoscopy is a part of forensic odontology that refers to the method of palatal rugae assessment for human identification purpose (Caldas *et al.*, 2007). Palatal rugae pattern, due to its uniqueness for everyone, can be used for human identification by comparing the records of the deceased person (Bansode and Kulkarni, 2009; Dawasaz and Dinkar, 2013; Gibelli *et al.*, 2018). The predictive ability of the rugae between gender and between ethnic groups can also help narrow the search and give results in conjunction with the other methods when the ante-mortem records are not available (Hosmani *et al.*, 2018; Kapali *et al.*, 1997; Saraf *et al.*, 2011).

1.4 Problem statement and study rationale

The significant role of forensic odontology in human identification can be supported by employment of rugoscopy as an aid when the quality of dental records was compromised and other methods are not applicable. Sex prediction, as a part of reconstructive human identification process, are deemed necessary to facilitate the search of ante-mortem records. In another scenario, when the ante-mortem information of palatal rugae is available (such as clinical photographs, prostheses, or dental casts), the comparative identification can be applied.

Only few studies have assessed palatal rugae morphometric features on 3D models. No previous study has used the combination of Next Engine Laser Scanner and 3-matic software to assess the palatal rugae morphometric features. All the studies that assessed the palatal rugae size on 3D models have measured the linear measurement of rugae from the most medial to the most lateral point of each rugae. In our study we measured the true rugae length following the curvatures of each single ruga.

No previous study has evaluated palatal rugae morphometric features of a Malaysian Malay population. So far, only two published palatal rugae studies have been conducted on Malaysian population (Chong *et al.*, 2020; Sherif *et al.*, 2018). Both studies did not evaluate the uniqueness of palatal rugae among the Malaysian population. Sherif *et al.* (2018) published data pertaining to palatal rugae shape, direction and unification for Malays. This pilot study utilised a wide age range which included 6 years old children and small sample size. The variables included were also limited to the qualitative features of palatal rugae. They studied the palatal rugae characteristics on conventional plaster models (Sherif *et al.*, 2018).

Another study was conducted by Chong *et al.* (2020) to assess the genetic influence on palatal rugae pattern. Their sample consisted of 81 pairs of Malaysian siblings. Their ages ranged between 15 and 30 years old, which means that a part of the participants was growing patients. They did not focus on a specific ethnic group and did not compare the rugae pattern between males and females (Chong *et al.*, 2020).

1.5 Justification

Although fingerprints is the preferred approach used for human identification in the case of mass disasters when the victims cannot be visually recognised, nevertheless, in situation of fragmented or burned bodies, this method might not be applicable (Prajapati *et al.*, 2018). Fingerprints are dependable on the integrity of skin epidermis.

DNA analysis also demonstrates its own limitations even though its high accuracy. Some of limitations are due to degeneration of DNA material, prolonged time and expensive cost (Sweet and Dizinno, 1996).

Dental records are another important source for human identification in situation of mass disasters by analysing the teeth characteristics, dental occlusion, dental restorations, and oral diseases (INTERPOL, 2018; Petju *et al.*, 2007). This method is limited in the absence of good quality dental records as well as edentulous victims, damaged or loss of teeth structures, and also the changes due to the dental treatment after recording (Ehtisham *et al.*, 2016; Hinchliffe, 2011).

The limitations of these three primary identifiers necessitate the utilization of another alternative or complementary methods for human identification (Prajapati *et al.*, 2018). A lot of studies have been conducted to verify the uniqueness of palatal rugae among random samples. They concluded that palatal rugae pattern is different from person to another including evidences among identical twins (Dawasaz and Dinkar, 2013; Gibelli *et al.*, 2018; Taneva *et al.*, 2017).

Many studies also have proven the stability of rugae pattern throughout the life and its resistance to the changes that may occur due to burning and violent accidents during mass disasters (Dawasaz and Dinkar, 2013; Muthusubramanian *et al.*, 2005; Shukla *et al.*, 2011).

Palatal rugae as a mucous membrane tissue may undergo decomposition and deformation after death, which necessitate taking the post-mortem records from the cadaver as quick as possible. However, Muthusubramanian *et al.* (2005) founded that palatal rugae still intact within first week after death under ideal mortuary storage conditions (Muthusubramanian *et al.*, 2005).

1.6 Objectives of the study

1.6.1 General

To evaluate the applicability of palatal rugae as an aid for human identification.

1.6.2 Specific

1.6.2(a) To determine the mean and the prevalence of rugae pattern among Malaysian Malays population.

1.6.2(b) To compare the total number of rugae between Malaysian Malays males and females.

1.6.2(c) To compare the rugae shape between Malaysian Malays males and females.

1.6.2(d) To compare the rugae size category between Malaysian Malays males and females.

1.6.2(e) To compare the rugae direction between Malaysian Malays males and females.

1.6.2(f) To assess the uniqueness of palatal rugae.

1.7 Research Questions

1.7.1 What is the mean and the prevalence of rugae pattern in Malaysian Malays population?

1.7.2 What is the difference in total rugae number between Malaysian Malays males and females?

1.7.3 What is the difference in rugae shape between Malaysian Malays males and females?

1.7.4 What is the difference in rugae size between Malaysian Malays males and females?

1.7.5 What is the difference in rugae direction between Malaysian Malays males and females?

1.7.6 Are the palatal rugae unique for each individual?

1.8 Hypothesis

1.8.1 Curve rugae shape is the dominant, primary rugae size is the dominant rugae size category and the forward direction is the dominant rugae direction.

1.8.2 Total number of rugae between the males and females is different significantly.

1.8.3 Rugae shape are significantly different between the males and females.

1.8.4 Rugae size category are significantly different between the males and females.

1.8.5 Rugae direction are significantly different between the males and females.

1.8.6 Palatal rugae are unique among the Malaysian Malay population.

CHAPTER 2
LITERATURE REVIEW

2.1 Embryology of palatal rugae

The palatal rugae begins to form during 12th-14th prenatal week (Peavy and Kendrick, 1967). They acts as struts which help in keeping the palatine shelves horizontal when they have been elevated (Motabagani, 2006). Pourtois (1972) suggested that palatal rugae participate in stiffening of the oral epithelium during the development of the secondary palate in the prenatal period by thickening of lateral palatal processes (Buchtova *et al.*, 2003).

A full description of development of palatal rugae is described by Peterkova *et al.* (1987). Firstly, the epithelial cells are thickened and protruded into mesenchyme forming rugal anlage. The epithelium continues thickening and protruding over the surface, the basal membrane levels within the thickened epithelium, and mesenchymal cells condense near the oral epithelium forming the primitive ruga. The thickened epithelium with adjacent mesenchymal cells arranged in a manner resembling magnetic lines of power. The primitive rugae surface delimitation becomes distinct forming what is called rugal core, which is located beneath the ruga and consists of a fibrous stroma and condensed mesenchymal cells. Finally, the definitive rugae is formed due to the protrusion of the rugal mesenchyme into the palatal (inter-rugal) level. Definitive ruga contains an extensive ridge of connective tissue covered with epithelium, and a well-formed rugal core that markedly bulg into the oral cavity (Peterkova *et al.*, 1987).

The palatal rugae appears as linear elevated ridges due to their composition of cuboidal epithelium radiated to form a rounded projection, these epithelial cells are multiplied and pushed outwards by the underlying mesenchyme (Thomas and

Rossouw, 1991). Glycosaminoglycans is believed to be the main element of palatal rugae structure which helps the rugae to maintain its shape throughout the life (Thomas and Van Wyk, 1987).

2.2 Physiology (functions) of palatal rugae

The palatal rugae play a role in mastication process by facilitating food transportation and crushing. Palatal rugae also provide position for the tongue and contribute in perception of food taste and texture because of the gustatory and tactile receptors on their surfaces (Buchtova *et al.*, 2003; Studdert *et al.*, 2011). Palatal rugae also participate in breastfeeding, as they stabilize the breast and prevent its slipping out of its position during the sucking process (Walker, 2014).

Based on the observation of speech improvement achieved by reproduction of palatal rugae in complete denture which has confirmed by many researchers (Adaki *et al.*, 2013; Zaki Mahross and Baroudi, 2015), palatal rugae may also play an important role in producing the correct sound of some letters, especially those sounds that need contact between the palate and tongue tip. Palatal rugae also play a role in phonetics as they disperse the sound waves in many directions (Barbo *et al.*, 2018).

2.3 Applications of palatal rugae in dentistry

There are several clinical and forensic potential applications in dentistry.

2.3.1 Clinical applications

2.3.1(a) Reference points in orthodontic treatment

Simmons *et al.* (1987) used a longitudinal data of growing subjects consisted of 20 males and 21 females to evaluate the stability of the medial rugal region anteroposteriorly. Each subject had four dental casts taken at intervals and they found that the anteroposterior length of the medial rugal region increases significantly but not consistently between males and females. They suggested that palatal rugae region responds to the normal growth process of the bone beneath it. This means that medial rugal reference points are not stable and cannot relied upon for tooth migration assessment .

Abdel-Aziz and Sabet (2001) evaluated the palatal rugae stability during the orthodontic treatment and the possibility of using the palatal rugae in superimposition to analyse the orthodontic treatment changes. The authors superimposed the scanned photos of pre and post orthodontic casts of 50 patients aged 17-25 years who underwent symmetrical extraction of premolars followed by retraction of canine and anterior segment using headgear as anchorage. The patients were treated with straight wire technique. They found that orthodontic treatment and associated teeth movement have no effect on palatal rugae position. They also found that the most reliable points to be used as reference points for cast superimposition are the lateral third rugae points.

Hoggan and Sadowsky (2001) evaluated the possibility of using the palatal rugae for tooth movement measuring. They assessed the anteroposterior movement of the maxillary first molars and central incisors using cephalometric and dental casts variables, and found that there was no statistically significant difference. They concluded that palatal rugae can be reliably used as reference point to assess tooth movements anteroposteriorly. Similar results have been achieved by Shetty *et al.* (2017) as they evaluated the validity of using palatal rugae as reference points on dental casts to assess the degree of tooth movement pre and post-orthodontically. They found no statistically significant difference between lateral cephalometry and dental casts methods. Both of studies agreed that medial end of the third palatal rugae are the most suitable reference points for teeth movement assessment (Hoggan and Sadowsky, 2001; Shetty *et al.*, 2017).

Jang *et al*. (2009) investigated the stability of palatal rugae and possibility of using them as landmarks for maxillary dental casts superimposition. The sample of their study consisted of 10 maxillary dental casts obtained from 10 orthodontically treated patients. The treatment included right and left premolars extraction and anchorage of three palatal mini screws as anchorage for the purpose of anterior teeth retraction. The casts were measured using a 3D laser scanning system. The casts were superimposed once based on the mini screws and again based on the palatal rugae. The central incisors displacement was measured using both methods. It was founded that there were no significant differences between measurements of these two methods. They concluded that superimposition of maxillary dental casts based on the medial points of the third palatal rugae and on the palatal vault as reference ponts is a reliable method for orthodontic tooth movement assessment (Jang *et al.*, 2009).

Thiruvenkatachari *et al.* (2009) had assessed teeth displacement post-orthodontically. They compared the results with those on cephalometric analysis. They concluded that their method is accurate and reliable and similar to the results that have been achieved from cephalometric analysis.

Shailaja *et al.* (2018) conducted a study to compare the shape and positional changes of palatal rugae before and after rapid maxillary expansion. They found that palatal rugae were stable in shape and number, but their position significantly changed. They concluded that palatal rugae can be used clinically by the orthodontist to assess the palatal separation after palatal expansion using the inter-rugal distance.

From the previous studies, we can conclude that palatal rugae stability after orthodontic treatment is related to the specific type of the applied orthodontic treatment, as the palatal rugae respond to the positional changes of the palatal bone.

If the palatal bone was stable, the palatal rugae will be stable and thus can be used for assessment of orthodontic teeth movement by measuring the positional changes of teeth in relation to palatal rugae reference point, especially the medial point of third rugae. If the palatal bone was not stable, such as in case of growing patients or maxillary expansion orthodontic appliances, the palatal rugae position will change according to the underneath palatal bone changes, which explains the possibility of using the palatal rugae for assessing the maxillary expansion appliances results by measuring the inter-rugal distance (Lanteri *et al.*, 2020; Saadeh *et al.*, 2017c).

However, none of those studies found rugae shape change during orthodontic treatment. Only slight dimensional changes were reported, which may not affect manual forensic identification based on palatal rugae.

2.3.1(b) Palatal rugae in prosthodontics

Research as early as Silverman (1967) has explained the relationship between some phonetic problems and improper denture thickness in the palatal rugae area. The importance of duplicating the palatal rugae was proven by Zaki Mahross and Baroudi (2015). They investigated the influence of palatal rugae reproduction on complete dentures in speech. Three edentulous patients were selected and four dentures with different materials and different thickness in the palatal area were constructed for each patient. Each patient wore each denture and were asked to read a paragraph with lingo-palatal sounds /s/z/sh/t/d/and/l/. The speech was recorded, and the sound was analysed using Computerized Speech Lab (CSL) (spectrogram). The authors found that quality of the sounds in the complete denture with reproduced palatal rugae was improved in comparison to the conventional denture. The increased thickness in the anterior region of the denture could cause faulty lingo-palatal sounds (Zaki Mahross and Baroudi, 2015).

Adaki *et al.* (2013) revealed the importance of customizing the rugae on complete dentures to enhance the speech. In their study the anterior palatal surface of each participant's denture has been modified, and the impact of this modification on phonetics has been evaluated. The speech was recorded without dentures, with conventional dentures, with arbitrary rugae dentures and with customized rugae dentures. According to the acoustic analysis that has been applied, it was found that the pronunciation of sounds like 's', 'sh', 't', 'd' was clearer with rugae incorporated dentures than conventional dentures. It was also found that customized rugae dentures produced clearer sounds than arbitrary rugae dentures. Many speech errors were shown with conventional dentures according to intelligibility reports (Adaki *et al.*, 2013).

Based on the previous studies and similar studies, Prosthodontists should be encouraged to customize rugae on dentures, which may be as a source of palatal rugae ante-mortem record for forensic purposes, especially in edentulous persons or in case of missing teeth during the disasters.

2.3.1(c) Palatine rugae in cleft palate

Park *et al.* (1994) studied the palatal rugae pattern among cleft palate patients to investigate their diagnostic significance. The samples included the maxillary dental casts of 16 submucous cleft palate patients, 17 isolated clefts patients (their clefts were in the secondary palate), and 10 non-cleft controls. The authors found that 14 of the 16 submucous cleft palate patients had palatal rugae curving towards the bony notch in the posterior border of the hard palate. This special rugae pattern can be used to early diagnose the submucous cleft palate (Park *et al.*, 1994).

2.3.2 Applications of palatal rugae in forensic dentistry

2.3.2(a) Why is the palatal rugae valid for human identification?

The **uniqueness** of palatal rugae pattern, its **stability** throughout the life and its **resistance** to external factors make it a subject of interest and study for many forensic researchers as an alternative method for human identification when the fingerprints, dental record comparisons and DNA methods are not applicable.

A lot of studies have investigated the uniqueness of palatal rugae. Indira *et al.* (2012) compared palatal rugae pattern among 100 subjects in Bengaluru city, India. Their sample contained 70 males and females equally distributed, five families (each family contained father, mother and two children) and five dizygous twin pairs. They found that no two palatal rugae patterns are alike (Indira *et al.*, 2012). Taneva *et al.* (2017) assessed the similarity and differences of the palatal rugae pattern in a female pair of identical twins that are around 15 years old. Three-dimensional digital models of the upper jaws of the pair were obtained with an intraoral digital scanner, iTero HD 2.9 (Align Technology, Inc., San Jose, CA). The digital models were manipulated and superimposed using Geomagic Control 14, Geomagic (Research Triangle Park, NC, USA) software. They found that the palatal rugae of the identical twins contains a similar pattern but not identical features (Taneva *et al.*, 2017).

Some of the studies that evaluated the uniqueness of palatal rugae pattern have compared two or more sets of dental casts to verify the similarities or matching among their samples (Bansode and Kulkarni, 2009; Jadoon *et al.*, 2018). They have demonstrated that palatal rugae pattern is unique among their samples. Some other studies superimposed the digital dental casts based on palatal rugae reference points to evaluate palatal rugae uniqueness, they also confirmed palatal rugae individuality (Gibelli *et al.*, 2018; Hemanth *et al.*, 2010; Mohammed *et al.*, 2013).

According to the previously mentioned studies, we can conclude that palatal rugae pattern has the same power as fingerprint, DNA and dental profile for human identification.

Palatal rugae dimensional changes may occur because of normal growth, but their number and shape remain stable throughout the life. Dawasaz and Dinka (2013) evaluated the stability of palatal rugae pattern over a period of one year. One hundred and twenty patients of Goan origin (60 men and 60 women) between 18 and 23 years old were selected for the study. It was also found that no statistically significant difference in the rugae pattern collected after 12 months of the same individuals which confirms the validity of utilizing rugae pattern for deceased human identification by comparing pre- and post-mortem records (Dawasaz and Dinkar, 2013).

Stability of palatal rugae throughout orthodontic treatment was also investigated by many researchers. It was proven that palatal rugae can be slightly affected dimensionally but not morphologically by the orthodontic treatment and thus they are valid for human identification with the cases that underwent orthodontic treatment. Bansode and Kulkarni (2009) investigated the stability of the palatal rugae in patients treated with fixed orthodontic treatment. They have also investigated the accuracy of identification by comparing pre and post-orthodontic rugae patterns. Thirty preoperative and thirty postoperative dental casts were obtained, and another thirty casts were also randomly selected for their study. The rugae pattern was assessed and classified according to the method modified by Kapali *et al.* (1997). All the areas in the postoperative casts and the randomly selected casts were trimmed except the rugae areas. Thirteen examiners were selected to match the postoperative casts and randomly selected casts to the preoperative casts. Although dental and sometimes bony changes occurred during the fixed orthodontic treatment, there were no changes in rugae pattern. 90% correct matches have been achieved by 13 examiners. The result showed that the palatal rugae pattern was stable and valid for human identification (Bansode and Kulkarni, 2009).

Shukla *et al*. (2011) validated the palatal rugae pattern stability in the course of orthodontic treatment. Fifty orthodontic cases ranging from 18 to 27 years were selected with pre- and post-orthodontic treatment casts of maxillary arches. The treatment period varied from 8-30 months. The medial and lateral ends of first, second and third rugae in addition to the median palatal raphe were marked. The casts then were photographed and the measurements were taken using UTHSCSA Image Tool version 2.0 computer program to assess the changes in distance perpendicularly and transversely. Thirty blinded examiners compared 50 trimmed pre orthodontic casts to similarly prepared one hundred casts (50 post orthodontic casts and 50 another selected dental casts) for possible matches based on the pattern of rugae. Significant dimensional changes between the pre-orthodontic and post-orthodontic images were found. The percentages of correct matches for examiners had a median of 90%. These results demonstrated that although palatal rugae change dimensionally during orthodontic treatment, their morphological pattern remains stable throughout life (Shukla *et al.*, 2011).

Palatal rugae anatomical position provides them a protection during mass disasters, fires and violent accidents, and make them less susceptible to traumatic, thermal and decomposition conditions. Muthusubramanian *et al.* (2005) evaluated the resistance of palatal rugae morphology toward conditions simulating those found in mass disasters. They assessed the palatal rugae in pan facial third degree burn victims and human cadavers in storage for about 12 hours. Palatal rugae in 93% of the assessed burn victims were normal and can be easily identified, and 77% of the assessed cadavers had no changes in the palatal rugae when reassessed after seven days. The changes that have been observed in palatal rugae were less pronounced than in the generalised body (Muthusubramanian *et al.*, 2005).

2.3.2(b) How can the palatal rugae be used for forensic purposes?

Palatal rugae can be used for human identification by comparing the post-mortem record to the ante-mortem record or by reconstructive human identification as the palatal rugae has discriminant power for gender and/or race dimorphism which can help narrow the search.

2.3.2(b)(i) Comparative approach

Palatal rugae ante-mortem record can be achieved from the dental casts used for orthodontic or prosthodontic treatment, or from the dentures of edentulous patients. In case the ante-mortem record of palatal rugae is available, it can be compared to the post-mortem record to determine either a match or mismatch result, the comparison might be performed manually using the visual constructive comparison or by superimposing the digital images.

In a case report, Thomas and Van Wyk (1988) were able to distinguish the identity of a burnt body after they compared the palatal rugae on the denture found in the victim’s mouth with that in an old denture found in his house (Thomas and van Wyk, 1988).

Gibelli *et al.* (2018) conducted a study to verify the uniqueness of 3D models of the palate. 26 orthodontic patients aged between 15 and 20 years were selected for their study. At least two casts were obtained from each patient, the time period between fabricating each two casts ranged from 6 months to 48 months. Laser scanner (iSeries, Dental Wings©, Montreal, Canada) was used to obtain the 3D digital model of each cast. Identification and selecting of the palatal surface were manually performed using a 3D image elaboration software (VAM®, Canfield Scientific Inc.), which also was used to biometrically superimpose the 3D models 250 times. Sixty-three matches were achieved in which the models belonged to the same subjects, and 214 mismatches in which the models belonged to different subjects.

The root mean square of point-to-point distance was found to be significantly different ($p < 0.0001$) between the matches and mismatches cases, (mean 0.26 mm; SD 0.12) for matches cases and (mean 1.30; SD 0.44) for mismatches cases (Gibelli *et al.*, 2018). From our point of view, any mistake might be committed during determining the reference points of palatal rugae may lead to negative mismatching of those models belonging to the same person. This may necessitate strict rules for palatal rugae reference points determination.

Mohammed *et al.* (2013) evaluated the efficacy of palatal rugae 2D images superimposition for personal identification purpose. They took alginate impressions for one hundred participants and fabricated plaster dental casts. All teeth were trimmed from the casts to prevent identification relying on teeth features. Only the incisor teeth were not trimmed that they would be used as references during the matching process. The one hundred casts were photographed with a standardized orientation and camera position. After one month interval, a second set of impressions were taken from the same participants. Same standardized photographing process was also performed to get another set of photographs. The two sets of photographs (200 photographs) were given codes and sent to three blinded evaluators who superimposed them using (Adobe Photoshop 8.0.) software in order to manually identify the pairs. They got 100% correct matching results (Mohammed *et al.*, 2013).

Hemanth *et al.* (2010) evaluated the ability of a computerised software to identify the individuals based on photographic images of the palatal rugae. A digital camera was used to obtain 100 standardised intra oral images. Using a software, they manually plotted the initiation and termination points on the rugae. The photographs were transferred to their software which was called the Palatal Rugae Comparison Software (PRCS Version 2.0). Later, same photographs were marked again and transferred to the same software (PRCS Version 2.0) one by one to be matched. Five evaluators have automatically matched the rugae pattern in the software. Three of them achieved 100% correct results and two evaluators achieved 99% correct results. These results support the individuality of the rugae and emphasise the efficacy of using computerised software in the identification of individual by using palatal rugae images. The authors did not elaborate the mechanism of matching used in their own software (PRCS Version 2.0). However, their method also relied on manual determining of the reference points which make the mistake possible (Hemanth *et al.*, 2010).

Unlike 3D models matching, matching of palatal rugae pattern on 2D models needs a careful standardization of the orientation during the digitizing process, as the z axis of the rugae which represents the height of rugae may affect the matching process in case of different orientation.

In our study, no reference point was manually plotted for matching of 3D models. The matching was performed according to the whole palatal rugae zone including all rugae curvatures.

2.3.2(b)(ii) Reconstructive approach

This section reviewed the applicability of palatal rugae to predict race and gender. This two information may be helpful in narrowing down the search of ante-mortem dental records.

Gender prediction

The study of palatal rugae for the purpose of gender prediction was the subject of study by many researchers. Saadeh *et al.* (2017) validated the palatal rugae ability for sex dimorphism. They recruited 252 maxillary dental casts of adults (119 males, 130 females). A Perceptron ScanWorks® V5 3D scanner was used to scan the casts. The authors have recorded the linear and angular measurements relating to the first three rugae. They applied independent sample t-tests and paired samples t-tests to test the sex dimorphism. They also applied a multiple logistic regression was to model sex using associated palatal rugae measurements. The authors found general tendency for larger dimensions in males as they achieved significantly larger values for 9 out of 28 parameters. 71.4% of the subjects were correctly classified based on four linear rugae measurements and one angular measurement together. They concluded that palatal rugae may be applicable for identification in some circumstances where the other methods are not applicable (Saadeh *et al.*, 2017b).

Saraf *et al.* (2011) compared the rugae pattern in Indian males and females. They used 120 subjects (60 males and 60 females) of Indian origin and between the age group of 22-26 years. The rugae pattern were recorded according to the classification of Tomas & Kotze (1983) and Kapali *et al.* (1997) including rugae number, length, shape, orientation and unification. According to their study, the differences in morphological characteristics between males and females were significant, while no significant difference in number and length between the two sexes as shown by the chi-square test. They suggested that palatal rugae morphological pattern can be used as an adjunct for sex differentiation among Indian population (Saraf *et al.*, 2011).

Malekzadeh *et al.* (2018) compared the palatal rugae patterns among males and females and its usefulness in sex identification. One hundred and thirty pre-orthodontic casts equally distributed between males and females were evaluated. The age of the subjects ranged between 17 and 25 years. The authors utilised Thomas *et al.* (1983) classification system. There was no significant difference between the two genders in term of total number of rugae. A significant difference regarding the rugae size and shape were observed, while the sex was determined with a precision of 70% according to the discriminant function analysis (Malekzadeh *et al.*, 2018). Other studies showed no significant differences regarding palatal rugae pattern between males and females in their population (Ahmed and Hamid, 2015; Kolude *et al.*, 2016; Pillai *et al.*, 2016). This might be due to the small sample in their studies which were 50 males and 50 females in all these studies.

Race prediction

Many studies have assessed the rugae pattern on geographical area population or on specific ethnic groups (Ahmed and Hamid, 2015; Santos and Caldas, 2012; Suhartono *et al.*, 2016). Some of those studies compared between two or more populations (Hosmani *et al.*, 2018; Nayak *et al.*, 2007; Saini and Garg, 2018).

Nayak *et al.* (2007) compared the palatal rugae pattern in two different linguistic Indian groups from different geographic regions in India. Thirty dental casts of each group (equally distributed between males and females) were analysed to assess the palatal rugae shape differences between the two populations and to evaluate the correlation between the rugae pattern and ethnicity. The rugae pattern was classified according to the method modified by Kapali *et al.* (1997). They modified the classification method regarding the unification type which was not further differentiated into diverge and converge types. They reported that wavy and curved rugae shape were dominant in both groups, followed by straight shape, while the least common rugae shape was the unified shape. The circular shape was not observed, but few rugae of non-specific shape were noticed. Chi-square analysis confirmed that the number of the straight forms was statistically more in the southern group while the curved forms were more in the western group (Nayak *et al.*, 2007).

A total of 940 subjects (466 South Indians and 474 North Indians) at the age of 18-23 years were included in a study conducted by Shanmugam *et al.* (2012). The sample included. The rugae were classified into five shapes, straight, curved, wavy, circular, and unification. The rugae length and total number were not considered in this study. All rugae shapes were higher in number among the South Indian population except non-specific rugae. A significant difference in the two genders was found in the straight, unification and circular rugae patterns. Based on rugae shapes, a discriminant function equation was created. This equation allowed for distinguishment of the Southern and Northern Indian population with an accuracy of 87.8% (Shanmugam *et al.*, 2012).

Ibeachu *et al.* (2014) compared the rugae shape between two Nigerian tribes (Igbo and Ikwerre). Their study was conducted on a sample of 140 subjects divided equally between the two ethnic groups. They found a significant difference regarding the dominant shape as the Igbos showed the domination of wavy shape while the curve shape was the dominant among the Ikwerres (Ibeachu *et al.*, 2014).

2.4 Classification of palatal rugae

There are a lot of classification methods of palatal rugae morphological and dimensional characteristics. Many of these methods are similar where they have classified the rugae according to the shape into five main types:

Straight (line), Curve, Wavy (serpentine or sinuous) Circular (annular) and Nonspecific (polymorphic, composite, compound, or anomaly). Some methods have described another shapes, such as point, angle, bifurcated, trifurcated.

The differences between these methods were mostly in the variables they adopted. Some classification methods relied on one variable and some others combine more than a variable. The most common variables are the rugae shape, size and direction. Other variables were also used such as rugae strength, unification and location. These classification methods differ also in the name they have given to the specific shapes or directions, they might name the straight shape as line, the anterior direction as forward and so on.

The variation in assessment are as follows

2.4.1 Goria classification method

This classification method is considered the first classification method as it was developed in 1911. The rugae in this system were classified into simple and developed. Rugae were also specified according to their number and their zone in relation to the teeth.

2.4.2 Torbo classification

In 1932, a Spanish researcher called Torbo Hermosa classified the palatal rugae according to their shapes into two main categories; Simple and composed. The simple category was subclassified into six shapes and each shape was symbolized by a Latin letter as illustrated in table 2.1 composed