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Ameloglyphics why Not with Stains?- An Observational Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: In forensic Odontology, the term ameloglyphics refers to the study of enamel rod end patterns. These enamel rod end patterns are specific and are different for each individual. Enamel is the hardest tissue and it is resistant to several environmental factors like fire, acid exposure and microbial decomposition.
Aims: This study was done to evaluate various stains and staining methods in tooth prints under stereomicroscope. The purpose of the study is to find out the utility of several stains for studying the enamel rod end patterns in forensic Odontology.
Methods: Twenty seven freshly extracted maxillary premolars were collected for the study from the Department of Oral & maxillofacial Surgery. All the teeth collected were irrespective of age to avoid the bias related to different enamel characteristics of deciduous teeth.
Results: In our study, hematoxylin and toluidine blue showed the surface score of 7 out of 9 which was the highest when compared to remaining stains. On comparing 3 different staining methods

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using hematoxylin and toluidine blue stains in soak method showed superior score when compared to other two methods.

Conclusions: To conclude, Ameloglyphics patterns can be studied by staining the tooth. Hematoxylin and toluidine blue stains can be used for studying the Ameloglyphics pattern. Soak method of staining the tooth can be done to study the pattern. Ameloglyphics patterns can play an important role in personal identification using staining methods and shows promising future in field of forensics.

Keywords: Ameloglyphics; enamel patterns; staining; in vitro study.

1. BACKGROUND

The term Forensic in Latin means "before the forum". The term forensic science is defined as "areas of endeavour that can be used in the judicial system and which can be adopted by the court and the forensic community to separate truthfulness from falsehood" [1]. Keiser defined Forensic Odontology "as that branch of Odontology which in the interest of justice deals with the proper handling and examination of dental evidence with proper handling and examination of dental evidence and with the proper evaluation and presentation of dental findings" [2,3]. Sex identification and age estimation using dental findings can play a major role in investigation. Bite mark analysis, rugoscopy, cheiloscopy, radiographic & photographic study, genetic material analysis, PCR for pulpal & periodontal tissue DNA analysis etc are the most commonly accepted [4,5]. Microscopically the enamel rod pattern forms a pattern on the crown of the tooth surface known as perikymata [6]. These patterns of perikymata are unique and different from individuals and it follows the course of ameloblasts and enamel forming patterns. The direction of enamel rods is always a controversy whether they run in unidirectional or multidirectional. Evidence suggests that the multidirectional enamel rod pattern makes it resistant to environmental factors and it differs from the other tooth in dentition and from person to person. There are eight distinct sub patterns observed, of which wavy- branched, wavy- u branches and linear branched are quite common and linear unbranched, whorl-open, whorl-closed, loop and stem like are less common [7,8]. These enamel rod end patterns can be studied using cellulose acetate peel technique and view under compound microscope [5,7]. The drawbacks of these methods are the tooth needs to be etched in case of acetate peel technique and gives only the negative replica of the tooth surface pattern [7]. These enamel rod end patterns are found resistant to high temperatures and acid exposure [6]. The rationale is to study the enamel rod end pattern on the tooth itself without replication. The null hypothesis stated is that enamel rod end patterns cannot be studied using stains and staining methods. This study was aimed to find out the utility of several stains for studying the enamel rod end patterns in forensic Odontology. The objectives of the study are to compare different methods of staining on the middle third of the labial and buccal surface of the tooth. To study the ability of stains to study the tooth prints on the labial surface of the tooth.

2. METHODS

Twenty seven freshly extracted maxillary premolars were collected for the study from the Department of Oral & maxillofacial Surgery.

All the teeth collected were irrespective of age to avoid the bias related to different enamel characteristics of deciduous teeth. Both male and female tooth samples were collected based on the availability. The following method was done the tooth was first cleaned and the buccal surface was selected as the representative area. Enamel rods are oriented almost perpendicular to the external surface of the tooth and therefore this area was selected as a representative. Teeth with any decay, restoration or any other regressive alterations like attrition, abrasion, erosion, hypoplasia were excluded from the selection. Tooth with complete intact buccal surface which is extracted due to periodontal and orthodontic reasons were included in the study.

2.1 Inclusion Criteria

- Tooth of both male and female
- Permanent dentition- only maxillary premolars
- Clinically normal tooth

2.2 Exclusion Criteria

- Tooth with wear faucets
- Developmental anomalies
- Tooth with dental caries

- Tooth with restoration

All the extracted teeth were scaled and air dried. Initially the first tooth subjected to acid etching on the buccal surface of the tooth was etched with 37% orthophosphoric acid for 1 min and rinsed water, dried and soaked in hematoxylin stain for 2 min and viewed under stereo microscope. Due to the poor uptake stains the remaining teeth were not etched and they were directly stained. The stained tooth was positioned in a wax over a glass slide and placed under a stereo microscope. Under the microscope it showed irregular areas of staining patches of stain. To avoid this the acid etching was excluded and directly the tooth was scaled and subjected to stain for 1 min, air dried and viewed under a stereo microscope. This direct staining technique was better and stained the whole surface. Likewise all the teeth were scaled and staining was done using soak method for 1 min and air dried. Totally 15 stains were included in this study. The studied stains include Hematoxylin, Toluidine blue, Eosin, Methyl green, Methyl violet, methyl blue, gram stain, acidcarbolfuschin, acid schiff stain, van giesonstain, alcian blue, aniline blue, leishman stain, Giemsa stain. The pattern was studied by taking a photo micrograph using a digital camera. The same surface scoring based on division of labial surface into thirds was done and analysed. The stereo microscope surface score of each tooth was given based on the division of the labial surface into thirds.

To study the better method of staining the tooth was scaled and subjected to 3 techniques.

1) soak technique
2) Cotton swab technique
3) Micro tip applicator technique

First was the soak technique: the tooth was soaked in the hematoxylin stain for 1 min, air dried, blotted on tissue paper and viewed under a stereo microscope. Second was the cotton swab technique: the stain was topically applied on the tooth for 1 min, air dried and viewed under a stereo microscope. Third was the micro tip applicator technique: the tooth was scaled and a micro tip applicator was used to stain the tooth, air dried and viewed under a stereo microscope. Each technique was studied by using 2 premolar teeth and stain used was hematoxylin. A trained oral and maxillofacial pathologist was considered as an investigator. The investigator was blinded from the technique and stain used to avoid bias.

Stains studied- 15 (n=15)
Method of staining - 2 stains
Total number of tooth - (n=12)
Hematoxylin and toluidine blue
Soak method (n=2)
Application with cotton (n=2)
Application with micro tip applicator (n=2)

3. RESULTS

In our study on comparing the technique of staining to be done. 15 different stains were studied on maxillary premolars using the soak method. All the 15 teeth were subjected to staining and viewed under a stereomicroscope. The surface score was given by the blinded investigator and compared. The results of our study showed that hematoxylin and toluidine blue were superior and can be used for studying Ameloglyphics patterns. The results of our study authentically showed that Ameloglyphics pattern is superior in soak method when compared to cotton application and microtop application both in hematoxylin and toluidine blue. On comparing the ability of various stains to study the tooth surface. In our study the soak method showed high scores of 8 and 7 using Hematoxylin& 6 and 7 when using toluidine blue.

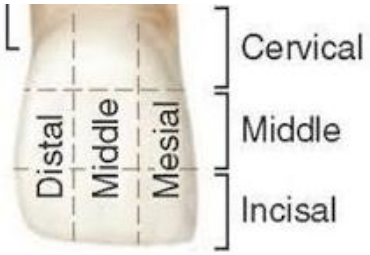

Fig. 1. Represents the scoring of the labial surface of the tooth in thirds

4. DISCUSSION

Age estimation and sex identification due to natural or man-made disasters is essential in dealing with social and medico legal purposes [9]. Skeletal and dental remains presents a great problem for forensic experts in personal identification especially when only fragments of the body are recovered. Various anthropological studies and features of teeth like morphology, crown size, dental index and odontometric differences were used for personal identification.

Decomposed or burnt bodies are difficult or impossible to identify. Each [5]. tooth has millions of enamel rods. These rods run in oblique

direction and wavy arrangements of rods since the length of rods are greater than the thickness [6,10]. The formation of enamel is a dynamic process and follows the course of ameloblasts and is reflected on the outer surface of the tooth unidirectional or multidirectional. Recording enamel rod patterns are simple, rapid and inexpensive and can be done even by a dental auxiliary. But periodic recording must be done since wear and tear of enamel can alter the patterns [6].

Table 1. Table represents the stains used for staining and surface score and surface staining characteristics on examination of labial surface of maxillary premolars under stereo microscope

Stains studied premolar	Surface Score (Out of 9)	Surface staining characteristics
Hematoxylin	7	smooth & diffuse
Toluidine blue	7	diffuse with patch areas of hyper staining
Eosin	0	smooth & diffuse-pattern was not visible
Methyl green	0	poor stain uptake, pattern not visible
Methyl blue	0	good stain uptake, pattern not visible
Methyl violet	4	areas of hyper staining, pattern was visible
Gram stain	0	No stain uptake, pattern was not visible
Acid CarbolFuschin	3	good stain uptake, poor pattern visibility
Acid Schiff stain	2	good stain uptake, Pattern was not visible
Vangieson stain	0	poor stain uptake, pattern was not visible
Alcian blue	1	good stain uptake, pattern was not visible
Aniline blue	0	No stain uptake, no pattern visible
Leishmann stain	0	patchy areas of staining, pattern was not visible
Giemsa stain	2	good stain uptake, poor pattern visibility

Table 2. Table represents 3 different techniques used for staining using hematoxylin and toluidine blue stain further surface scores were given on studying them under stereo microscope

Technique (For 2 minutes)	Surface score (out of 9) Hematoxylin		Surface score (out of 9) Toluidine blue	
soak method	8	7	6	7
cotton application	2	3	1	3
micro tip application	1	0	0	1

Fig. 2. Picture represents the Ameloglyphics patterns on staining the labial surface of maxillary premolars using 15 different stains by soak method and studied under a stereo microscope

Fig. 3. Picture represents Ameloglyphics patterns of labial surface of maxillary premolars staining of tooth using hematoxylin(3c,3d) and toluidine blue(3a,3b) by soak method and studied under stereo microscope

Enamel patterns are studied by the peel method. The Peel method is done using acetate film on the acid etched surface of the tooth. These were studied on a light microscope and photographs were analysed. Verifinger SDK v5.0 software was used for the analysis of ameloglyphics patterns for personal identification. Manjunath et al checked the reliability of this software by recording ameloglyphics patterns and subjected them to biometric analysis and found results to be the same in all the three recordings [5].

Rakesh et al in his study analysed the reliability of ameloglyphics for person identification. The teeth to be studied were exposed at various temperatures (80°C, 400°C,600°C and 750°C). The tooth prints obtained after such environmental insult showed high similarity with the original tooth print. Juneja et al in her study exposed the tooth to acid exposure 36.46% hydrochloric acid at various intervals (5min, 10min and 20min) and 10 other tooth were exposed to various temperatures (80°C, 400°C,600°C and 750°C) [6]. An in vitro experimental study by Gupta et al compared different individuals and 4 different classes of tooth and results showed variability in patterns intra and inter observer [11]. Haut et al studied enamel print pattern in deuteromalay subrace on female permanent maxillary central incisors and found linear branched type of pattern to be most common. All the above mentioned studies were done based on acid etch acetone peel technique where the acid etch can be done only in extracted teeth and doing it in patients will be unethical. Hence there was a need for a technique for studying the ameloglyphics pattern in live individuals. We in our study analysed the staining method for studying the ameloglyphics pattern of the labial surface of the maxillary premolar tooth under stereomicroscope using 15 different stains and found hematoxylin and toluidine blue superior over other stains (Table 1). In our study 3 different methods were compared and studied under a stereomicroscope soak method of staining (Table 2).

Limitations of this study were only extracted maxillary premolars were included in the study. Only 15 stains were studied and many other stains remain unexplored. Our study was done only for staining and removal capacity of the stain was not considered. Future scope of research includeinvivo study and stain study in all different classes of tooth including incisors, canine, and molars. Primary dentition also can be studied. Long term follow up study can be done on subjects to compare the permanence of patterns.

5. CONCLUSION

Amelyphics pattern shows a promising area in the field of forensic Odontology. These ameloglyphics patterns and resistance to environmental factors shows a promising role and can be used as a successful identification tool in forensic Odontology. The value of using these Ameloglyphics patterns as a tool in forensic Odontology lies in ability to replicate and permanency. This type of record must be updated periodically to overcome the changes that may be due to wear and tear of enamel.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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