Ameloglyphics patterns of primary and permanent dentition using staining method- an observational study

ABSTRACT

BACKGROUND

Forensic Odontology is a 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. Various applications of forensic dentistry include age estimation, bite mark analysis, ameloglyphics, rugoscopy, cheiloscopy, photographic study, genetic material analysis, and PCR for pulp DNA analysisThe aim of this study is to find out the utility of Hematoxylin and Toluidine blue stains in studying enamel rod end patterns of various dentitions in forensic odontology.

RESULTS

In our study, Mean and standard deviation value of Hematoxylin and Toluidine blue showed the surface score of 6.4±1.782 and 6.6±1.979 respectively. Both maxillary and mandibular Permanent central Incisors showed lowest scores when compared to remaining dentition included in the study.

CONCLUSION

To conclude, Ameloglyphics patterns can be studied by staining the tooth. The Ameloglyphics pattern can be analyzed using hematoxylin and toluidine blue stains. Ameloglyphics patterns can be used as a valuable tool in personal identification when stained correctly, and they have a bright future in forensic dentistry.

Keywords: Ameloglyphics, enamel rod end patterns, staining, in-vitro study.

INTRODUCTION

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). According to Keiser, Forensic Odontology is defined “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). Various applications of forensic dentistry include age estimation, bite mark analysis, ameloglyphics, rugoscopy, cheiloscopy, photographic study, genetic material analysis, and PCR for pulp DNA analysis(4,5).

Perikymata is a pattern formed by the enamel rod pattern on the crown of the tooth surface when viewed under a microscope(6). Due to the undulating path of ameloblasts during formative patterns, these perikymata patterns are peculiar. Microscopically, groups of enamel rods run in a different direction than the rest of the dentition and from person to person. Each tooth in an individual's mouth has its own Ameloglyphics pattern. Each tooth in an individual's mouth has its own Ameloglyphics pattern. Wavy-branched, wavy-unbranched, linear branched, linear unbranched, whorl-open, whorl-closed, Loop, stem-like are eight distinct sub patterns observed in tooth prints(7). Various techniques, such as the cellulose acetate peel technique and the rubber base sensation, may be used to investigate these patterns (5,8) The disadvantages of these approaches are that the acetate peel technique requires the tooth to be etched and only provides a negative replica of the tooth surface pattern(7,8). These enamel rod end patterns have been found to be heat and acid resistant (6).

The rationale of the study is to study the enamel rod end pattern on the tooth without having to replicate it. The null hypothesis states that stains and staining techniques cannot be used to study enamel rod end patterns. The aim of this study is to find out the utility of Hematoxylin and Toluidine blue stains in studying enamel rod end patterns of various dentitions in forensic odontology. The objective of the study is to compare hematoxylin and toluidine blue in analysing Ameloglyphics patterns in labial surface of both primary and permanent dentition.

MATERIALS AND METHODS

50 freshly extracted teeth were collected for the study from the Department of Oral & Maxillofacial Surgery. To prevent prejudice related to different enamel characteristics of deciduous teeth, all of the teeth were collected regardless of age. Depending on the availability, both male and female tooth samples were obtained. The procedure was as follows: the tooth was cleaned first, and the buccal surface was chosen as the representative area. Since enamel rods are almost perpendicular to the tooth's external surface, this region was chosen as a representative. Teeth with deterioration, restorations, or other regressive changes such as attrition, abrasion, corrosion, or hypoplasia were not included in the study. The research included teeth with a fully intact buccal surface that were removed for periodontal or orthodontic purposes.

Inclusion criteria:

- Tooth of both primary and permanent dentition
- Tooth of both male and female
- Clinically normal tooth

Exclusion criteria:

- Teeth with wear faucets
- Developmental anomalies
- Tooth with Dental caries
- Tooth with restoration

Tooth stained with hematoxylin and toluidine blue 25 each.

5 primary maxillary central incisor; 6 permanent maxillary central incisor; 4 permanent mandibular central Incisor; 6 permanent maxillary canine; 4 permanent mandibular canine; 10 maxillary premolars; 10 mandibular premolars.

Under a stereo microscope, the stained tooth was placed in wax over a glass slide. It was found under a microscope that there were irregular areas of staining spots. To prevent this, the tooth was scaled, stained for 1 minute, air dried, and examined under a stereo microscope. This approach was superior because it stained the entire surface. Similarly, all of the teeth were scaled and stained for 1 minute before being air dried. This research looked at a total of 15 stains. A picture micrograph was taken with a digital camera to study the pattern. The labial surface scoring was performed and analyzed based on the division of the labial surface into thirds. Every tooth's stereo microscope surface score was determined by dividing the labial surface into thirds.

RESULTS

In our study on comparing the hematoxylin and toluidine blue stains on staining both primary and permanent dentition using soak method and studied under stereo microscope. The surface score was given by the blinded investigator and compared (Tab3). The results of our study showed Hematoxylin and Toluidine blue with the overall mean and standard deviation of 6.4±1.782 and 6.6±1.979(Tab4). Both maxillary and mandibular permanent central Incisors showed lowest scores when compared to remaining dentition included in the study. Statistical analysis was done using SPSS version 25. Paired sample t test showed no statistically significant difference was found between hematoxylin and toluidine blue with the confidence interval of 95%

DISCUSSION

Forensic Odontology is crucial in identifying remains, particularly those that are dead or rotting. Identification of a person is important in circumstances where there is a loss of life due to natural or man-made disasters, as well as for social and medical purposes (9). Forensic experts face a significant challenge in identifying skeletal and dental remains, particularly when only fragments of the body are retrieved. For personal identification, anthropological studies and features of teeth such as morphology, crown size, dental index, and odontometric variations were used. Decomposed or burned remains are difficult to locate, if not impossible. Teeth aid detection in these conditions because they are resistant to destruction and can

tolerate temperature and acid exposure. When dental evidence is decomposed or mutilated, it is called a preference of evidence. The toughest material that covers the tooth's crown is enamel. There are millions of enamel rods in each of the five teeth. Since the length of the rods is greater than the thickness, they run in an oblique direction and in wavy arrangements(6,10) . Enamel formation is a complex, well-organized process that results in a series of enamel rod patterns on the outer surface of the tooth. This procedure is simple, convenient, and inexpensive, and it can even be performed by a dental assistant. However, since enamel wear and tear can change the patterns, periodic recording is necessary(6).

We in our study analysed the ability of staining method using the hematoxylin and toluidine blue stain for studying Ameloglyphics pattern under stereo microscope. For this labial surface of various dentition both primary and permanent teeth were stained using soak method. No statistically significant difference between hematoxylin and toluidine blue were obtained with a confidence interval of 95%(Tab2). The results of our study showed Hematoxylin and Toluidine blue with the overall mean and standard deviation of 6.4±1.782 and 6.6±1.979(Tab3). Both maxillary and mandibular permanent central Incisors showed lowest scores when compared to remaining dentition included in the study.

Ameloglyphics patterns were previously studied by the cellulose acetate film peel method. This peel method is used to analyze enamel patterns. An acid etched surface on an acetate film is reproduced using the peel process. Various materials, such as cellulose acetate strips, cellophane tape, and rubber base impression material, may be used for the peel process. A light microscope was used to examine them, and images were analyzed. The program Verifinger SDK v5.0 was used to evaluate ameloglyphics patterns for personal recognition. Manjunath et al tested the software's reliability by capturing ameloglyphics patterns and subjecting them to biometric analysis. The findings were consistent across all three recordings(5). Gupta et al. attempted to compare tooth prints from different individuals, the same individuals, and four different groups of teeth in their experimental sample. None of the trends showed intra- and inter-observer similarity, nor did any specific class of tooth display similarity, according to the findings (11,12) .

In their research, Rakesh et al looked into the reliability of ameloglyphics for individual identification. The teeth were heated to different temperatures (80°C, 400°C, 600°C, and 750°C). After such an environmental insult, the tooth prints collected displayed a high degree of resemblance to the original tooth print. Juneja et al. exposed the tooth to 36.46 percent hydrochloric acid at different intervals (5, 10, and 20 minutes) and 10 other teeth to various temperatures (80°C, 400°C, 600°C, and 750°C) in their research (6). Haut et al investigated enamel print patterns on female permanent maxillary central incisors in the deuteromalay subrace and discovered that linear branched patterns were the most widespread. Many of the research described above used the acid etch acetone peel procedure, which can only be used on extracted teeth and is immoral to use on patients. As a result, a method for studying the ameloglyphics pattern in living people was needed.
Limitations of this study were only extracted teeth were included in the study. Only 2 stains were studied and many other stains remain unexplored. Our study was done only for staining

the labial surface of the tooth to study Ameloglyphics patterns and removal capacity of the stain was not considered. Future scope of research includeinvivo study and stains study in different classes of tooth in primary dentition and permanent molars. Long term cohort studies can be done on subjects to compare the permanence of patterns.

CONCLUSION

In the field of forensic Odontology, the amelyphics pattern is a new concept. The uniqueness of these Ameloglyphics patterns, as well as their tolerance to environmental influences, suggest that they have a promising future as a forensic Odontology identification tool. The ability to reproduce and permanency of these Ameloglyphics patterns as a tool in forensic Odontology is what makes them valuable. Even a dental auxiliary can perform this forensic evidence technique because it is simple, inexpensive, and fast. This procedure can be used to supplement antemortem dental records of troops, firefighters, and other personnel.This form of record must be revised on a regular basis to account for changes in enamel wear and tear.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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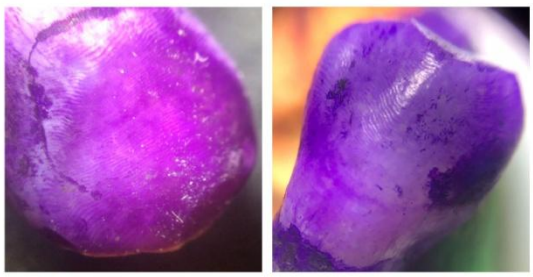

Fig1a represents the labial surface of maxillary premolar stained using hematoxylin by soak method and viewed under a stereo microscope.
Fig1b represents the labial surface of maxillary premolar stained using toluidine blue by soak method and viewed under a stereo microscope.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
HEMATOXYLIN STAINING	25	2	9	6.48	1.782
TOLUIDINE BLUE STAINING	25	2	9	6.60	1.979
Valid N (listwise)	25				

Tab1 represents descriptive statistics of Mean and standard deviation of the scores.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	HEMATOXYLIN STAINING - TOLUIDINE BLUE STAINING	-.120	1.269	.254	-.644	.404	-.473	24	.641

Tab2 table represents the independent sample t test which showed no significant difference on the scores between hematoxylin and toluidine blue stain on the labial surface of the tooth under stereo microscope

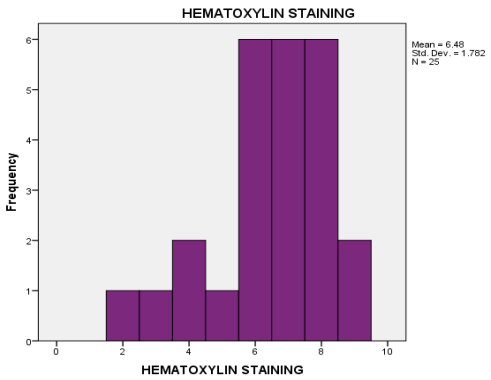

Fig2 bar chart represents hematoxylin scores on the labial surface of tooth under stereo microscope

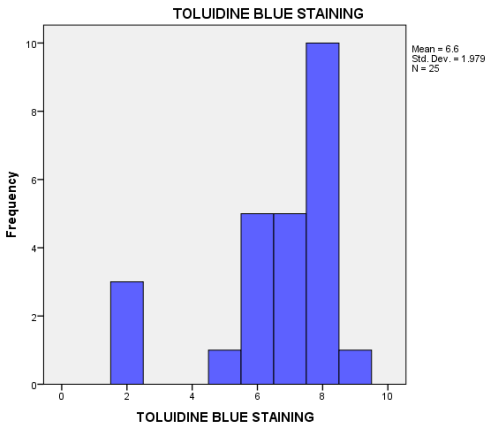

Fig3 Bar chart represents toluidine blue scores on the labial surface of tooth under stereo microscope.

Tooth stained	Hematoxylin	Toluidine blue
primary central incisor	9	8
primary central incisor	8	8
primary central Incisor	8	8
primary central Incisor	8	8
primary central Incisor	8	9
permanent Maxillary central incisor	4	5
permanent Maxillary central incisor	3	2
permanent Maxillary central incisor	2	3
permanent mandibular central incisor	2	2
permanent mandibular central incisor	4	2
permanent Maxillary canine	7	6
permanent Maxillary canine	7	6
permanent Maxillary canine	6	6
permanent Mandibular canine	5	6
permanent mandibular canine	7	6
permanent maxillary Premolar	8	7
permanent maxillary Premolar	7	8
permanent maxillary Premolar	6	8
permanent maxillary Premolar	8	7
permanent maxillary Premolar	8	8
Permanent mandibular Premolar	8	8
Permanent mandibular Premolar	6	9
Permanent mandibular Premolar	6	8
Permanent mandibular Premolar	7	8
Permanent mandibular Premolar	6	8

Tab3- table represents the scores of the labial surface of the tooth on staining by soak method using hematoxylin and toluidine blue under stereo microscope.

TOOTH	HEMATOXYLIN	TOLUIDINE BLUE
5 PRIMARY MAXILLARY CI	8 ±0.554	7.2 ±0.656
3 PERMANENT MAXILLARY CI	3 ±0.924	3.5 ±2.079
2 PERMANENT MANDIBULAR CI	3 ±1.386	2
3 PERMANENT MAXILLARY CANINE	6.6667 ±0.533	6
2 PERMANENT MANDIBULAR CANINE	6 ±1.386	6
5 PERMANENT MAXILLARY PREMOLAR	7.4 ±0.701	7.6 ±0.429
5 PERMANENT MANDIBULAR PREMOLAR	6.6 ±0.701	8.2 ±0.351
TOTAL(Mean ±S.D)	**6.4±1.782**	**6.6±1.979**

Tab4- table represents the mean and standard deviation of scores of labial surface of various dentitions stained using hematoxylin and toluidine blue and examining them under stereo microscope.