ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 01 | January - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

AGE DETERMINATION AMONG DIFFERENT AGE GROUPS USING ENAMEL ETCHING PATTERN: A SCANNING ELECTRON MICROSCOPIC STUDY

Forensic Dentistry

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ABSTRACT

Background- The determination of age and sex is among the important aspects of forensic odontology and vital in medicolegal investigations. In Forensic Odontology, teeth play a major role in age estimation due to the presence of the two major mineralised structures of teeth that are enamel and dentine which are comparatively more resistant to changes produced in mass disasters. Though enamel is the hardest substance of the human body, but with advancing age it becomes more mineralized due to exposure with the oral environment which results in hypermineralization of surface layer of enamel which can impact its etching pattern. **Aim-** The aim of the study was to evaluate if the enamel surface can be used as a parameter to determine the age **Materials And Methods-** here hundred freshly extracted teeth from individuals with known age group were collected and etching procedure was done and then it was subjected to scanning electron microscopy. **Results And Conclusion:** The predominant etching pattern seen in 18-28, 29-39, 40-50 and 62-72 year age group after acid etching is Type I pattern with frequency of 80%, 75%, 60% and 50% respectively. While in 51-61 year age group, it is Type II pattern (60%). A significant difference was observed in the respective age groups among the type of etching pattern. This technique can be a very useful adjunct for age determination in the field of forensic odontology.

KEYWORDS

Acid etching, enamel etching, forensic odontology

INTRODUCTION:

Aging is an inevitable process affecting all living beings. It is characterised by an imbalance of homeostasis, heightened vulnerability to infections and diminished capability for adaptation in response to stimulus-induced adaptation. Teeth alterations associated with ageing start in utero and last till senility. The primary determinants of age-related changes are molecular composition and morphological contrast. Aging is governed by physiological as well as pathological changes brought on by environmental and functional factors. [1]

The two mainstays of forensic anthropology are the determination of age and sex of an individual. Both these factors play a key role in medico-legal investigations. Forensic investigations rely on various age estimation indicators that influence an individual's age. [2]

In Forensic Odontology, teeth play a major role in age estimation because enamel and dentine, its two main mineralised components, are considerably more resistant to alterations inflicted by mass disasters. [3] Though enamel is the hardest substance of the human body, with advancing age it becomes more mineralized due to exposure to the oral environment which results in hypermineralization of the surface layer of enamel which can impact its surface microscopic characteristics and irregularities (etching pattern).[2] The etching patterns observed are as follows:

- Type 1 (honeycomb pattern)- enamel prism cores are selectively removed
- Type 2 (cobblestone pattern)- peripheral regions of the prisms are removed leaving a relatively unaffected prism core[4]
- Type 3- areas corresponding to both Types I and II etching patterns were seen
- Type 4- pattern is pitted and is seen mostly in cervical areas. It was demonstrated that etched prismless enamel displays no rod or prism patterns[5]
- Type 5- pattern is flat and smooth, lacking microirregularities; often seen in fluoride-treated teeth or patients residing in high-fluoride areas.[6]

Aim: The present study aims to assess if the enamel surface can be used as a parameter to determine the age.

MATERIALS AND METHODS:

A hundred freshly extracted human premolars and molars with no caries on the buccal aspect with age group ranging between 18-72 years were collected from the Department of Oral and Maxillofacial Surgery, Panjab University, Chandigarh. The extracted teeth were divided into five groups of age groups, i.e., 18-28 years, 29-39 years, 40-50 years, 51-61 years and 62-72 years. Informed consent was obtained from the patients for the use of teeth for the study. Ethical clearance was obtained from the Ethical Committee of Panjab University, Chandigarh. Teeth were sectioned at the cementoenamel junction using a diamond disc, and the buccal surface of the crown was retained for experiments

The buccal surfaces of the hundred tooth crowns were ground with 600-grit silicon carbide paper. The buccal surface of the tooth was etched with 37% orthophosphoric acid (N-etch Ivaclor Vivadent, Switzerland) for 15 s. It was rinsed for 10 s and air-dried. These samples were then again sealed in disposable envelopes and taken for scanning electron microscopy (SEM) analysis. The etching effect of enamel surfaces was assessed by using SEM. The sample was mounted on labelled aluminum stubs using a double-sided adhesive tape and placed in the vacuum chamber of a gold coating unit (JEOL JFC-1100 Fine Coat Ion Sputter). The specimens were sputtered at 100-120 mA gold under a vacuum and visualised under a scanning electron microscope (SEM-JEOL JSM-6100 Scanning microscope, Tokyo, Japan) SEM analysis was carried out at Sophisticated Analytical Instrument Facility - SAIF, Panjab University, Chandigarh. The blinding protocol was followed. After recording the values, statistical analysis was done

STATISTICAL ANALYSIS AND RESULTS:

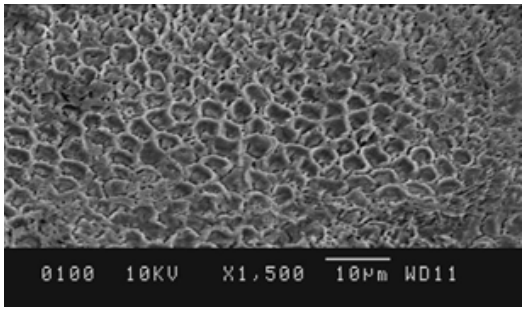
The test was done to evaluate the percentage of various etching patterns (Type 1,2,3,and 4) prevalent in age groups 18-28, 29-39, 40-50, 51-61 and 62-72 years. It was seen that in the age group 1 (18-28 years)- type 1 enamel etching pattern is seen in 80%, type 2 pattern in 10%, type 3 pattern in 5%, type 4 in 10% teeth. In group 2 (29-39 years)- type 1 enamel etching pattern is seen in 75%, type 2 in 15%, type 3 in 10% and no type 4 etching pattern is seen. In group 3 (40-50 years)- type 1 etching pattern is seen in 60%, type 2 in none, type 3 in 25% and type 4 in 15% teeth. In group 4 (51-61 years)- type 1 etching pattern is seen in 30%, type 2 in 60%, type 3 in 10% and type 4 in none. In case of group 5 (62-72 years)- type 1 etching pattern is seen in 50%, type 2 in 40%, type 3 in 10% and none showed type 4 pattern. The

predominant etching pattern seen in 18-28, 29-39, 40-50 and 62-72 year age group after acid etching is Type I pattern with frequency of 80%, 75%, 60% and 50% respectively. While in 51-61 year age group, it is Type II pattern (60%). A significant difference was observed in the respective age groups among the type of etching pattern. This technique can be a very useful adjunct for age determination in the field of forensic odontology.

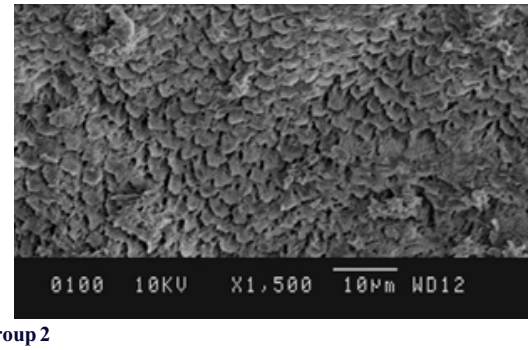
Table 1: Enamel Etching Patterns

Group	Types	Number	Percentage
1	1	16	80.0
	2	2	10.0
	3	1	5.0
	4	2	10.0
2	1	15	75.0
	2	3	15.0
	3	2	10.0
	4	0	0.0
3	1	12	60.0
	2	0	0.0
	3	5	25.0
	4	3	15.0
4	1	6	30.0
	2	12	60.0
	3	2	10.0
	4	0	0.0
5	1	10	50.0
	2	8	40.0
	3	2	10.0
	4	0	0.0

Chi Square- 36.606
P value- <0.001
Significance- HS



Group 1



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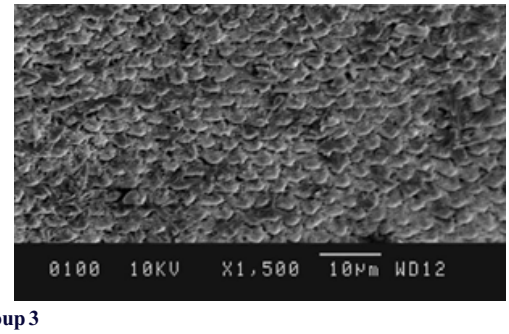

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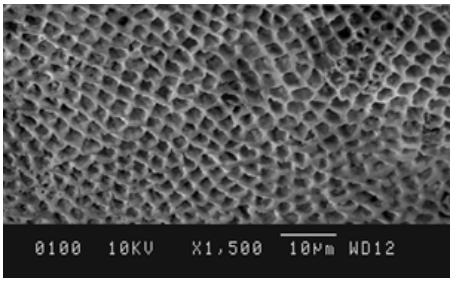
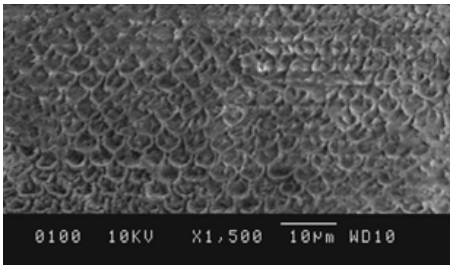


Group 4



Group 5

DISCUSSION:

The process of identifying an individual serves as a cornerstone of forensic investigations with accurate age estimation holding particular significance for administrative, ethical and medico-legal purposes. Among the array of parameters employed for identification of a person, forensic odontologists have increasingly turned to teeth as reliable indicators of chronological age. This relation stems from the limited variance of teeth from environmental, dietary or endocrine influences as well as their remarkable resilience to post mortem degradation, including mechanical, chemical and heat related damages. [7] an intriguing aspect of teeth is their exceptional durability as they are the most indestructible part of the human body, exhibiting minimal turnover of its natural structure. Consequently, teeth not only persist after death but also remain relatively unchanged for millennia thereafter. [8]

Enamel renowned as the body's toughest substance, assumes particular importance for forensic odontologists, especially during mass disasters owing to its ability to withstand various mechanical and thermal traumas. The highly organised arrangement of hydroxyapatite crystals in the outer layer of enamel contributes to its hardness, shielding the underlying dentine and pulp. However, enamel undergoes subtle changes over time, manifesting as unique microscopic characteristics and irregularities on the surface of the crown known as enamel etching pattern. [EEP] These patterns, like fingerprints are distinct for each individual and serve a highly reliable method for identification. EEP change with age and these alterations could be attributed to the ionic exchange that occurs with the environment. [2]

Age estimation has found an invaluable application in the analysis of EEP, which is made possible by scanning electron microscopic evaluation after prism cores and boundaries are etched with 37% phosphoric acid. Enamel's topography is enhanced by acid etching. 10 micrometre of the surface layer is removed, leaving behind a 5-50 micrometre morphologically porous enamel. As a result of the morphological structure of enamel prisms, selective demineralisation occurs. At pertinent microregions, such as prism heads and boundaries, the acid has a higher demineralisation potential owing to variations in the hydroxyapatite crystal angulation in these areas. [14]

Poole and Johnson (1967) discovered that acid etching of teeth surfaces results in a distinct honeycomb pattern along with significant loss of prism cores. They also observed another pattern characterised by preferential breakdown of prism peripheries, previously documented by Muller and Schait (1957) using etching. According to Poole and Johnson, variations in the etching patterns caused by acids or chelating agents may stem from differences in chemical composition of enamel prisms at their cores versus peripheries, and changes in crystal orientation relative to the direction of attack. [3,9]

Silverstone et al identified an additional pattern where the etching

displayed a combination of types 1 (honeycomb) and 2 (cobblestone) resulting in widespread roughening without distinct prism morphology. They attributed the diverse etching effects to orientation of enamel crystals. [4]

Galil et al further described two additional EEP. Types 1,2 and 3 were consistent with earlier findings with type 3 predominantly in middle third of teeth and types 1 and 2 mostly observed in the coronal third. Type 4 exhibited a pitted enamel surface with random depressions, showing no preferential destruction of cores or peripheries, possibly due to underlying variations in enamel structure. Pattern 5 displays a smooth, uniform surface without discernible prism outlines. Both type 4 and 5 patterns were less characterised and primarily found in the cervical areas. [10]

This etch pattern classification was modified by Robson RH et al where they consolidated types 1 and 2 into a single pattern (type A) as it is often difficult to differentiate between the two. Type B corresponds to etch type 3, type C to type 4, type D to etch type 5 of Galil and Wright classification. [13]

Extensive research has been conducted on dental materials to improve bond strength since the advent of acid etching to facilitate adherence to enamel, [11,12] but a research void exists in the analysis of surface topography of etched enamel, especially with regards to age estimation. Our is the second study in this context.

In the present study, significant variations in EEP across different age groups (18-72 years) were observed, with highest prevalence (80%) of type 1 pattern in 18-28 years old (group 1), 60% of group 4 (51-61 years old) displayed type 2 pattern. These findings resonate with those of James D and Sindhu R.[2] It was noticed that type 1 EEP decreases with age. This is attributable to the ionic exchange that occurs leading to continuous enamel maturation and resulting in hypermineralised enamel with age. Type 4 pattern was the least prevalent pattern of enamel etching in this study and type 5 was not reported in any study group. These findings underscore the utility of EEP in age estimation and highlight their potential as adjuncts in individual identification.

In conclusion, the analysis of EEP represents a promising avenue for age estimation in forensic odontology, offering valuable insights into the association between enamel morphology and chronological age. Further research is this domain is warranted to enhance the precision and reliability of age estimation methods, thereby contributing to the advancement of forensic science.

Acknowledgments: The authors would like to sincerely thank Mr. Ravinder Singh, Statistician, Mr. Tarsem Raj, Lab Technician and Mr. Harkamal Singh, SAIF-Panjab University, Chandigarh, for the SEM analysis.

Limitations: Evaluation of enamel etching patterns is not suitable for individuals who have experienced significant tooth wear or dental restorations. Additionally, this pattern may be affected by factors such as nutrition, genetics, and environmental conditions.

Financial Support And Sponsorship: Nil.
Conflicts Of Interest: There are no conflicts of interest.

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