Original Article

Are Teeth an Adjunctive for Age Estimation in Acid Environment?

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Background: Human identification in mass disasters, homicide, and acid attacks is possible because teeth are the only durable structures that remain. In chemical accidents such as acid attacks or uneventful accident in chemical industries, the acids mask the victim's identification when skeletal structures are destroyed and soft tissues cannot provide reliable information.

Aims and Objectives: To observe the morphological changes of teeth exposed to acid environment and to aid in identification and age estimation of an individual.

Materials and Methods: Sixty noncarious teeth were taken for the study. Ten teeth each were immersed in concentrated (Conc.) HCl, Conc. HNO_3 , Conc. H_2SO_4 , aqua regia, Conc. acetic acid, and Conc. formic acid. Teeth were retrieved, washed in distilled water, dried, photographed, and radiographed at intervals of time and again placed in their corresponding acids. Age estimation was done using Kvaal method.

Statistical Analysis: statistical analysis was done using Student's *t*-test.

Results and Conclusion: The exact age of the victim is not possible in all cases. We can get an approximate age range with certain acids such as H_2SO_4 , acetic acid, and formic acid, whereas with Conc. HCl, Conc. HNO₃, and aqua regia, age estimation is possible for a certain period of time.

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KEY WORDS: Acid environment, age estimation, Kvaal method, radiographs

INTRODUCTION

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Forensic odontology is one of the most standouts among the most unexplored and intriguing branches of forensic sciences. Age estimation constitutes a vital calculate, the recognizable proof of a person is measurable, and look for ideal age estimation strategies has proceeded throughout the years until the present day.^[11]

Human identification in mass disasters, homicide, and acid attacks is possible because teeth are the only durable structures that remain. In chemical accidents such as acid attacks or if any uneventful accident takes place in chemical industries, the skeletal structures are destroyed completely and soft tissues cannot provide reliable information, hence thereby rendering identification of the individual difficult.^[2]

Various dental age calculation methods were described in the literature, but most of the methods offered include extraction of teeth and preparation of microscopic sections of teeth, which may not be acceptable for ethical, religious, or scientific reasons. In such circumstances, a radiographic approach is used that offers a relatively nondestructive method and eliminates the need for extraction of teeth.^[3] Any method used for age estimation in forensic sciences should clarify issues with significant legal and social consequences for individuals as well as for the community.^[4]

The dental pulp is a delicate soft tissue that is enclosed within the confines of dentin and enamel and is well protected from the

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external tooth environment. The regressive changes in the pulp have also been related to age. The chronological age effects the developmental and regressive changes to the tooth.^[5] With the increase in age, the size of the pulp decreases because of secondary dentin deposition, which is a continuous process throughout the life.^[6] Hence, dental pulp can be used as a parameter to survey the age of an individual notwithstanding amid later times of life, when different strategies cannot be utilized. Kvaal method is one such technique, which was at first connected to intraoral periapical radiographs and as of late on computerized orthopantomography and digital radiovisiography (RVG) to assess the age of an individual even during later periods of life when other methods cannot be employed.

AIM OF THE STUDY

The aim of this study was to observe the morphological changes of teeth exposed to organic and inorganic acid environment and to obtain dimensions that can aid in identification and age estimation of individual.

MATERIALS AND METHODS

Sixty noncarious, nonattrited, unabraded anterior teeth or premolars were obtained for the study. The teeth were

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distributed in six groups, each group containing 10 teeth. 37% hydrochloric acid (Conc. HCl), 65% nitric acid (Conc. HNO₃), 96% sulfuric acid (Conc. H_2SO_4), aqua regia (hydrochloric acid:nitric acid 1:3), Conc. acetic acid, and Conc. formic acid are of concern in the study. Conc. HCl, Conc. H_2SO_4 , Conc. HNO₃, and aqua regia are inorganic acids whereas acetic acid and formic acid are organic acids. Ten teeth samples were immersed in 25 ml of each acid solution and observed for 8 h. Teeth were retrieved from acid environment, washed in distilled water, dried, photographed, and radiographed at intervals of 15 min, 30 min, 1 h, 3 h, 5 h, and 8 h and again placed in their corresponding acids. Age estimation was done using a method by Kvaal *et al.* [Figures 1-7].

KVAAL METHOD OF AGE ESTIMATION

Kvaal method for age estimation uses radiographs as an indicator for age assessment. In this method, pulp-to-tooth ratio was calculated. The age is derived using pulp-to-tooth ratios in the formula for age estimation by Kvaal *et al.* Pulp-root length (R), pulp-tooth length (P), tooth-root length (T), pulp-root width at cementoenamel junction (A), pulp-root width at midroot level (C), and pulp-root width at midpoint between A and C (B) were measured. Finally, mean value of all ratios excluding T (M), mean value of width ratio B and C (W), and mean value of length ratio *P* and R (L) were substituted in formula. Age = 129.8-316.4(M)-66.8(W-L).^[3]

STATISTICAL ANALYSIS

Statistical evaluation was done using Student's *t*-test. Mean and standard deviation were taken and comparison was done. SPSS Software (IBM, Newyork, USA) was used for the analysis. Test is statistically significant when *T* value >2.18 for P < 0.05. Test is not significant when *T* value <2.18 for P < 0.05. Mean estimated age error was calculated.

Regression formula for age in years using dental radiographs (RVG) is shown below.

Equation	ľ ²	SEE (years)
Age=129.8-16.4(M)-66.8(W-L)	0.76	±5 years

 r^2 : Coefficient of determination, SEE: Standard error of estimate in years

RESULTS

Results of morphological observations:

- For tooth placed in Conc. HCl, the enamel portion of tooth started dissolving within 15 min. Progression of dissipation was seen in 30 min, and by the end of an hour, enamel portion was completely lost. By the end of 8th h, tooth morphology altered completely
- Teeth placed in Conc. HNO₃ showed mild yellowish discoloration in the root in 15 min. Dissipation of enamel was clearly seen by the end of an hour. By 8th h, tooth morphology altered completely like the previous acid
- 3. No obvious change was noticed in 30 min for tooth placed in Conc. H₂SO₄. Chalky white appearance with dissolution of enamel was observed within 3 h. Morphology of tooth was retained after 8 h
- Teeth placed in glacial acetic acid showed no change at all in 30 min. By 5th h, slight enamel dissolution was seen, but not much alteration was seen in the morphology of tooth.
- 5. No obvious change was noticed in 30 min for tooth placed in Conc. formic acid. Chalky white appearance with dissolution of enamel was observed within 3 h. Morphology of tooth was retained after 8 h
- For tooth placed in aqua regia (HCL:HNO₃ 1:3), mild crack was observed within 15 min. Progression of crack was seen in 30 min, and by end of 1 h, morphology of tooth was altered, and by 8th h, morphology got dissipated.



Figure 1: Teeth before immersion in acid



Figure 2: Effect on teeth after 15 min of immersion in acids. Order of acids is Conc. HCl, Conc. HNO₄, Conc. H₂SO₄, Conc. acetic acid, Conc. formic acid, and aqua regia

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Figure 3: Effect on teeth after 30 min of immersion in acids



Figure 5: Effect of acids after 3 h on teeth

Radiographical interpretation of teeth with various acids:

- a. Specimen in Conc. HCL, Conc. HNO₃, and aqua regia showed vast change in dimensions for every time interval. By the end of 8th h, it was impossible to estimate the age of victim
- b. Tooth placed in Conc. H₂SO₄, glacial acetic acid, Conc. formic acid showed very less change in dimensions, thus assisting in approximate age estimation.

Results after age estimation:

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a. With Conc. HCl and Conc. HNO₃, the estimated age error was found to be \pm 3.4 years and 3.9 years, respectively.

Figure 4: Effect after 1 h of immersion in acid

Figure 6: Effect on teeth after 5 h of immersion in acids

After 3 h of acid contact, age estimation was not possible since the morphology of tooth was altered completely

- b. With Conc. H_2SO_4 , Conc. acetic acid, and Conc. formic acid, the estimated age error was found to be \pm 3.7 years, \pm 2.6 years, and \pm 3.8 years, respectively
- c. With aqua regia, age estimation is possible till 30 min. By 1st h, pulp obliterated. Only the pulp space was left. By 1st h, the morphology of tooth altered making age estimation difficult. By 8th h, the tooth length, root length, pulp length, and pulp width diminished completely make age estimation impossible.

Figure 7: Effect of acids on teeth after 8 h of immersion in acid

DISCUSSION

Acids are known to erase a person's identification. In such cases, victim's tissues provide reliable information. Human dentition serves as an indicator for individual's identification and age determination.^[7] The teeth selected are anteriors and premolars because they are less complicated to measure and withstand the acid trauma. Hence, they are best chosen for morphological assessment and radiographic age estimation. Teeth do not react in the same way in all acids.^[8]

Before we started our study, we hypothesized that all acids may dissolve the teeth irrespective of the type of acids used making age estimation impossible. However, on the contrary, we found that teeth immersed in Conc. H_2SO_4 , Conc. acetic acid, and Conc. formic acid retained their morphology even after 8 h.

From the above experimental observation, we can conclude that teeth do not react in the same way in all acids.

Teeth placed in Conc. HCL, Conc. HNO₃, and aqua regia form soluble salts of calcium chloride and calcium nitrate making them dissolve.

Teeth placed in Conc. H_2SO_4 form insoluble calcium sulfate failing to dissolve forming an insoluble precipitate.

Acetic acid reacts with calcium carbonate of teeth to form carbon dioxide, calcium acetate, giving a chalky white appearance to the enamel.

Formic acid reacts with calcium to form insoluble calcium oxalate. The crown gives chalky white appearance.

From the above study, we can substantiate that human dentition can serve as evidence when a person is affected by Conc. H_2SO_4 , acetic acid, and formic acid who aim at destructing the identification of a person. There is clear

evidence in the damaging limits of various acids utilized as a part of this review. The data inferred in this review on the morphological changes observed in teeth after specific times of drenching in a corrosive arrangement can be used for recognizable proof of the corrosive utilized. The observed morphological changes may fluctuate when the centralization of the corrosive utilized is unique in relation to that utilized as a part of the present review; notwithstanding, it must be noticed that the concentrations that we have utilized are the ones that are most ordinarily accessible economically.

A similar study was done by Raj *et al*. Our study is in accordance with the study done by them who concluded that teeth can serve as a tool in identification, i.e., age estimation and sex determination of a victim when contacted with H_2SO_4 but not when attacked by HCl and HNO_3 .^[2]

Our study is also in accordance with the study done by Jadhav *et al.*, who concluded that HCl, HNO_3 , and H_2SO_4 cause changes in the teeth and it is possible to estimate the approximate age and deduce the approximate duration, for which a body has been immersed in acid based on the changes observed.^[9]

CONCLUSION

Age estimation of a victim can be done, but it has its own limitations. The estimation of approximate age of the victim is possible. We can get in an approximate age range with certain acids such as H_2SO_4 , acetic acid, and formic acid, whereas with Conc. HCl, Conc. HNO₃ and aqua regia, age estimation is possible for a certain period of time after that it is not possible for age estimation. Although number of samples is small, the teeth still serve as evidence in victim identification and approximate age estimation but with few acids and up to a particular time. Further studies with large sample size are required for determining its usefulness in this role.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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