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Original Research

A comparative analysis of different decalcification techniques & their morphological effects over teeth: Relevance in Forensic Dentistry

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ABSTRACT:

Post-mortem identification using teeth destroyed by effects of acid

Background: Teeth are the hardest tissues of the human body. Every individual has a unique dental pattern. Natural teeth are most resistant to destruction and can persist long after the skeletal structures have been destroyed by physical or chemical agents. New methods of destroying life, for example, using acids as a mode of destroying the body after a murder has been in use for quite some time, as the nature of crime is changing day by day. This makes the job of forensic odontologists difficult for personal identification. It is of great interest for forensic experts to know whether the human body can be destroyed using acid, if yes then, can teeth be the best evidence to identify the body in such cases. **Aim**: The aim was to identify agents (acids) most commonly used in such crimes and morphological changes in teeth which can be used to deduce the approximate duration of time elapsed after immersion into acid to destroy the body.**Materials and methods**: Teeth were immersed in 25 ml aqueous solutions of three different acids (65% HNO_3, 96% H_2SO_4, 37%HCl) periodically for morphological changes. They were assessed for changes until completely dissolved.**Results:** In Group I (37%HCl), complete dissolution of teeth was observed at 15hr and remaining bits at 20hr. In Group II (65%HNO_3), complete dissolution of teeth was observed at 15hr. In Group III (96%H_2SO_4), teeth completely disintegrated into white precipitate at 144hr.**Conclusion:** Group III (96%H_2SO_4) takes the longest time to disintegrate into white precipitate, indicating the formation of insoluble calcium salts. Therefore, this information could be of great help to forensic experts as an aid in human identification.

Keywords: Acids, Decalcification of teeth, Forensic dentistry, DNA, Human identification.

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INTRODUCTION

Nowadays, the magnitude of crime is changingday by day. Acids are being used to get rid of the mortal remains and destroy subsequent pieces of evidence after the murder for quite some time now. Thus,investigation and identification ofa deceased individual from the residual remains become very difficult for the forensic odontologist. It is of great interest for forensic experts to know how much time it takes for the tooth to bedestroyed using acids.With this knowledge, teeth canbecome the best evidence to identify the body in such casesof acid usage.[1]

A tooth consists offour layers namely, enamel, dentin, pulp, and cementum along with a mineralized extracellular matrix component that regulates tooth morphogenesis.[2]Natural teeth are most resistant to destruction and can persist long after the other skeletal structures have been obliterated by physical or chemical agents.[3]Human teeth canthus, play a vital role for both legal and humanitarian purposes by acting as an excellent source of identification of an individual where routine forensic procedures fail.

Past literary insights have revealed extremely few studies dealing with the destruction of humantissues especially the teeth after acid immersion.Teeth are an exemplary source of identification as they canwithstand the criminal's attempts to mask the offences and the evidence held within the crime scene.[4]Thus, morphological changes in teeth due to acids cansurely help forensic odontologists in assisting the identification process & also provide a cost-efficient alternative to solving a crime.

AIM OF THE STUDY

This study aimed to observe the morphological changes in natural teeth after complete immersion in acid to find out the type of acid used as well as the total time of immersion into it.

MATERIALS AND METHODS

A total of 36 extracted teeth were obtained from the extractionsin orthodontic procedures, from periodontally compromised patients, and in other routine extraction procedures. All the teeth were sound and devoid of caries, periodontal diseases,or any form of pigmentation. Before the experimental procedures, teeth were stored in adry environment at room temperature.

Three different types of acids (25 ml)each were used in this study with specific concentrations as following:

1. An aqueous solution of HCl 25 ml (14.6ml of 37% HCL + 10.4ml of water)
2. An aqueous solution of HNO_3 25 ml (16.25ml of 65% HNO_3+ 8.75ml of water)
3. An aqueous solution of H_2SO_4 25 ml (24ml of 96% H_2S0_4+ 1ml of water)

All extracted teeth to be immersedwere divided equally into three individual groupscontaining the respective acids i.e.

Group 1 - 12 extracted teeth in HCL

Group 2 - 12 extracted teeth in HNO_3

Group 3 - 12 extracted teeth in H_2S0_4

Teeth were examined for any morphological changes at the following time intervals: 30 mins, 1hr, 2hr, 4hr,8hr,15hr, 20hr,24hr, 48 hr, 72 hr, 96 hr, 120 hr, and 144 hr. Specimens were followed up until the complete dissolution or complete precipitation took place.

Table 1 – Various morphological changes occurring in the teeth after acid immersion.

Duration	37% HCL	65% HNO_3	96% H_2S0_4
0.5 hr	Effervescence, translucency at root tip in 10/12	Effervescence in 12/12	No change
1 hr	Translucency in 12/12	Teeth turned yellow, and translucent in 10/12	No change
2 hrs.	Disintegration of apical third root in 9/12	Disintegration of the apical third of root in 11/12	No change
4 hrs.	Disintegration up to middle third of root in 10/12	Disintegration up to middle third in 10/12	No change
8 hrs.	Splitting of teeth in 10/12. dissolution	Nearly complete dissolution in 10/12	White precipitate in 12/12
15 hrs.	Nearly complete dissolution in 10/12	Complete dissolution in 9/12	Increase in white precipitate in 12/12
20 hrs.	Complete dissolution in 10/12		Increase in white precipitate in 12/12
24 hrs.			White precipitate increased; teeth can be recognized 12/12
48 hrs.			White precipitate increased; teeth can be recognized 12/12
72 hrs.			White precipitate increased, tooth gets split & fragmented 10/12
96 hrs.			White precipitate increased, teeth can still be recognized 12/12
120 hrs.			White precipitate increases, 12/12. The tooth is unrecognizable; Partial dissolution 10/12
144 hrs.			Complete dissolution of tooth into a white precipitate 10/12

Fig 1 – Morphological changes after immersion in 37% HCL

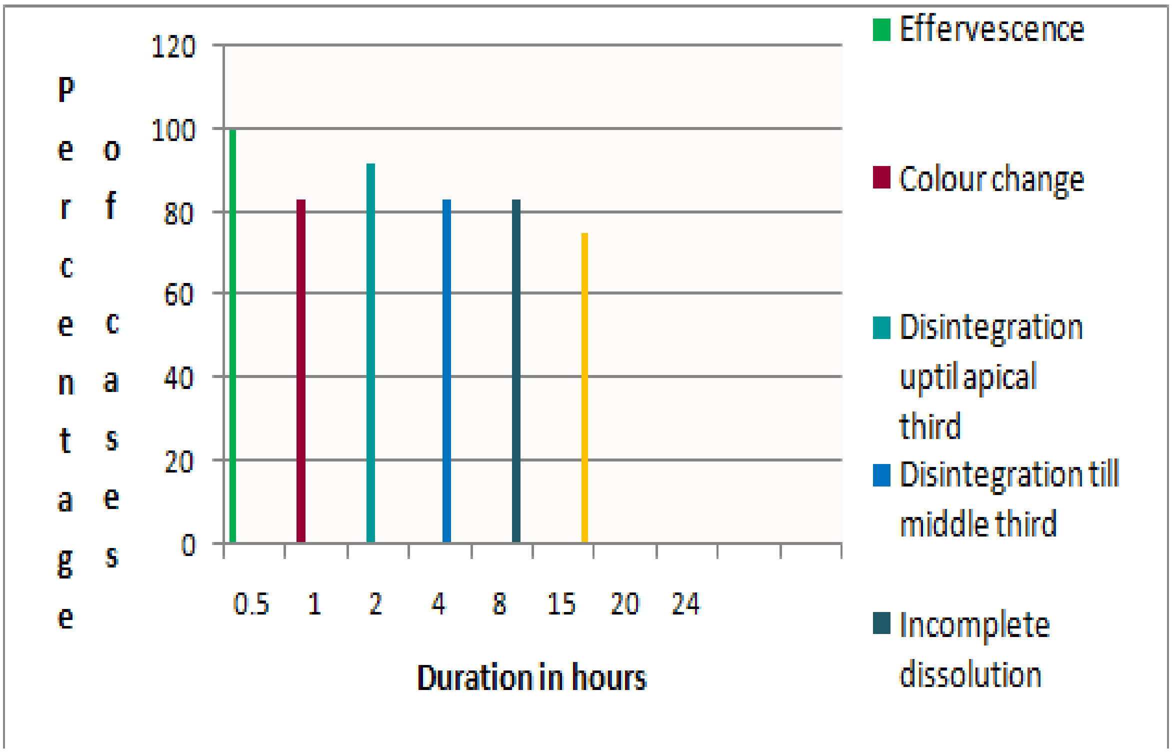


Fig 2 – Morphological changes after immersion in 65% HNO_3

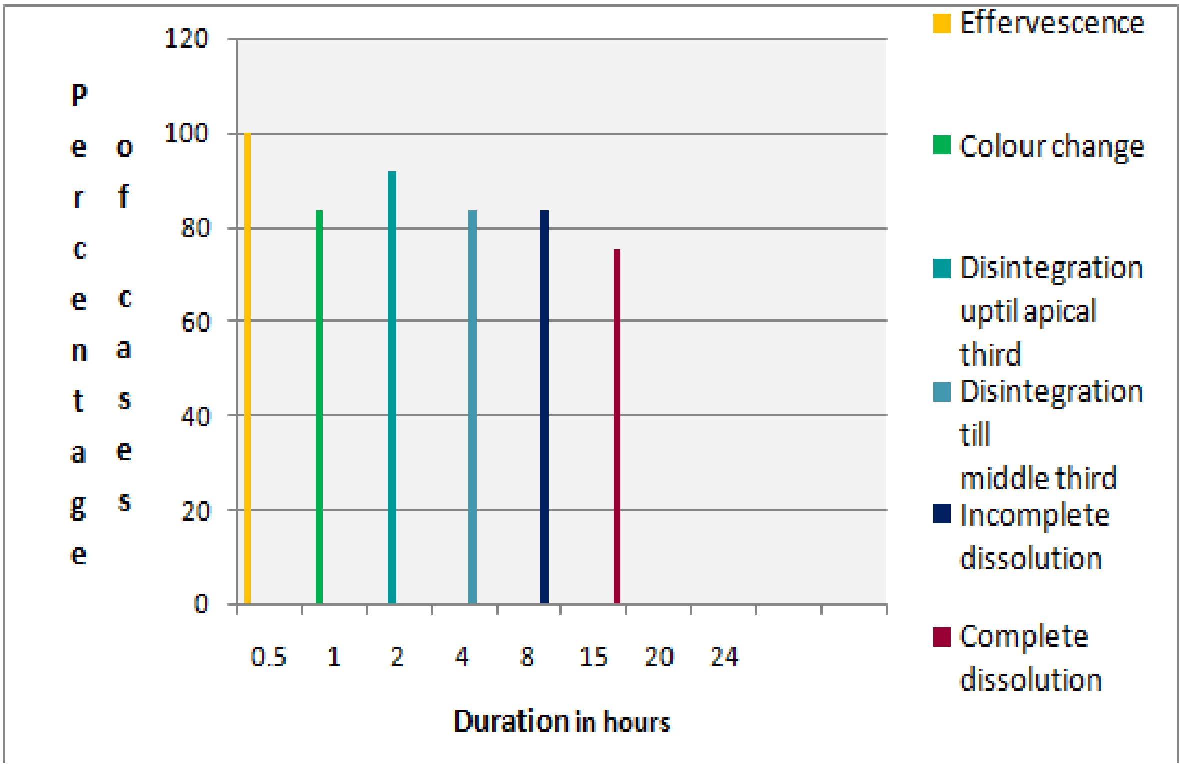
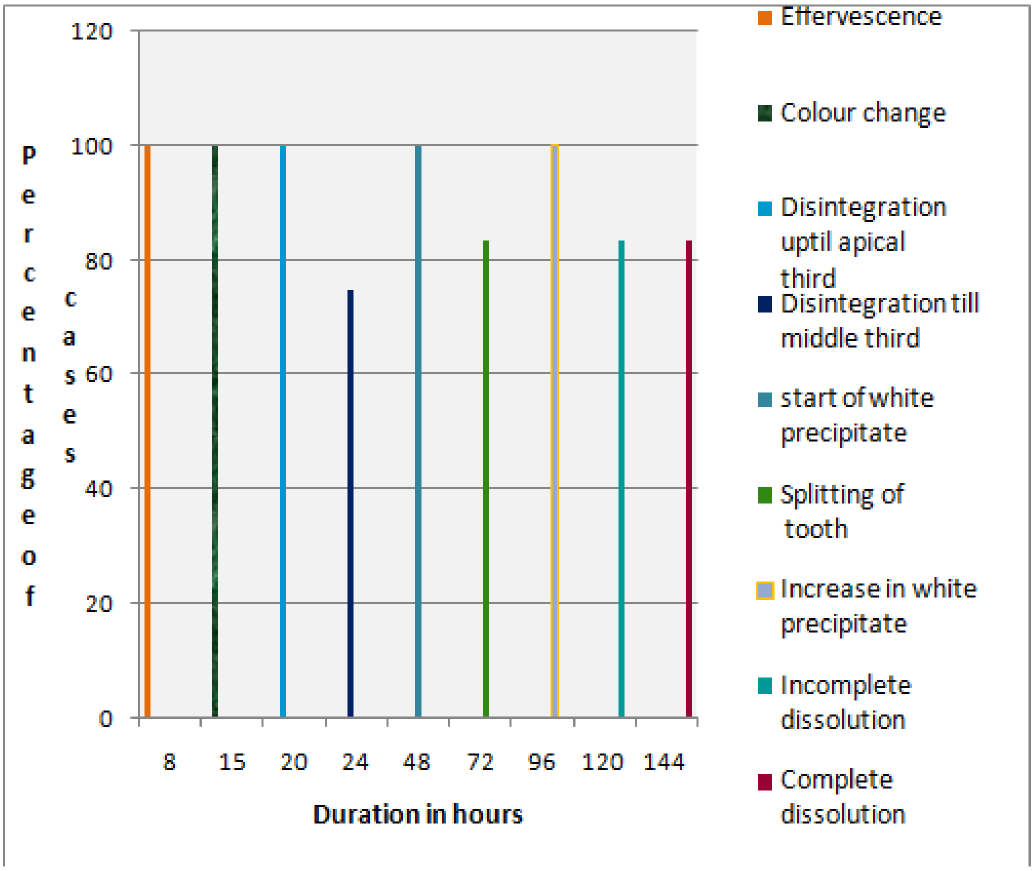


Fig 3 – Morphological changes after immersion in 96% H_2S0_4

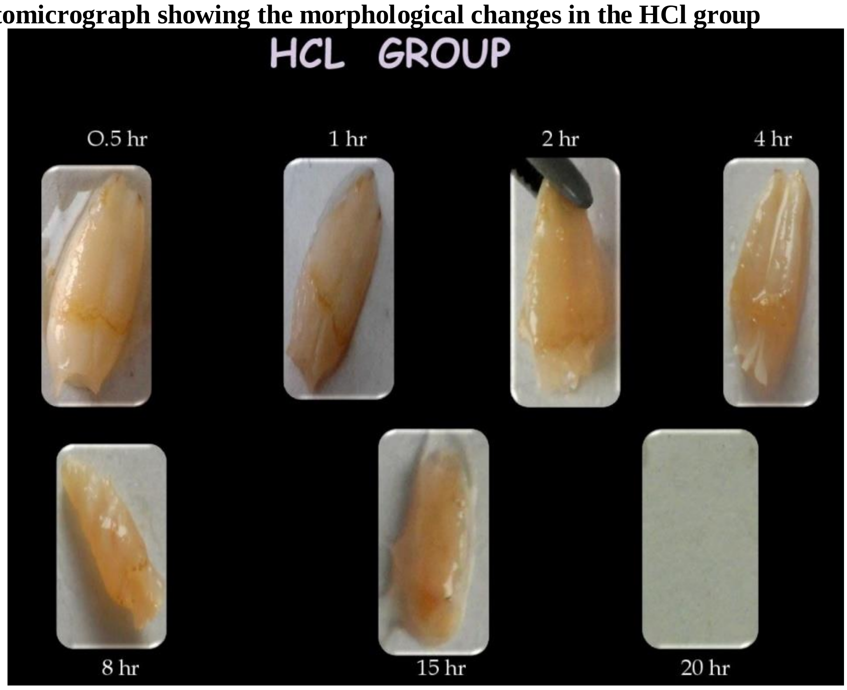


RESULTS

Various morphological changes in respective acid groups have been depicted in the above-mentioned table (Table1).

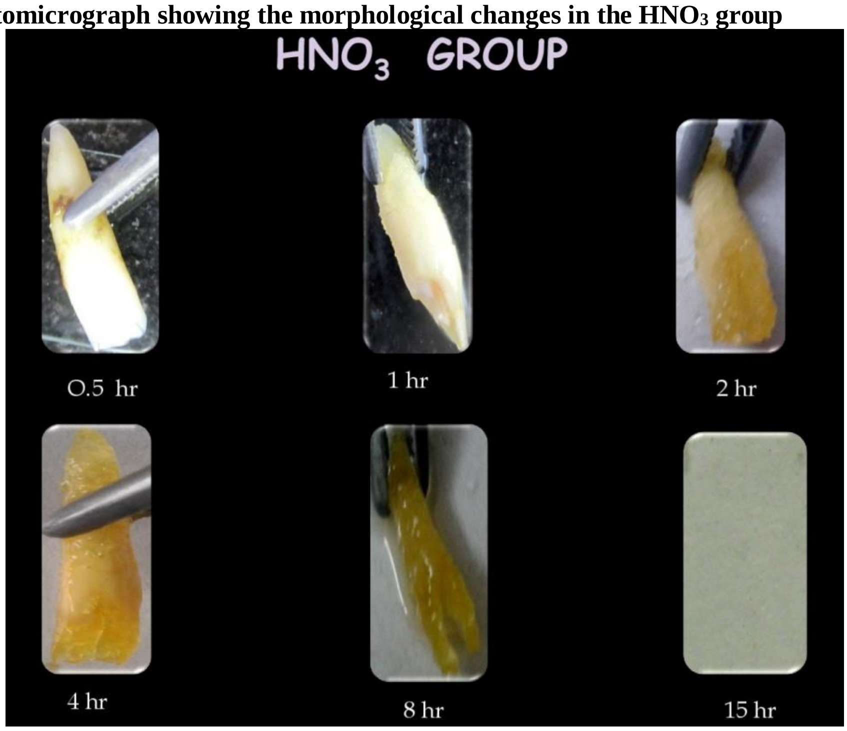
In Group I (37% HCL), effervescence was first noticed after 30 minutes leading to translucency which later on increased at 1 hr.Disintegration of the apical third of the root was evident at 2 hr which proceeded up to the middle third in 4 hr. After 8 hr of immersion, splitting was noticedwhich led to incomplete dissolution of teeth at 15 hr.Complete dissolution of few remaining bits was seen at 20 hr. (Fig 1 & 4)

Figure - 4: Photomicrograph showing the morphological changes in the HCl group



Group II (65% HNO_3)results revealed the start of effervescence at 30 minutes leading to translucency and yellowish discoloration of teeth at 1 hr. At 2 hr, the disintegration of the apical third of the root was evident which further involved the middle third part of the teeth at 4 hr.Nearly complete dissolution was seen at 8 hr and later at 15 hr, complete dissolution of all teeth was evident. (Fig 2 & 5)

Figure - 5: Photomicrograph showing the morphological changes in the HNO_3 group



In Group III (96% H_2S0_4) no change was visible until 4 hr. At 8 hr, the formation of white precipitate was noticed at the bottom of the container which progressively increased until l20 hr of immersion. White precipitate increased withtime and the teeth remained recognizable until 48 hr. Teeth got split & fragmentedat 72 hr but were still recognizable at 96 hr. At 120 hr, teeth were unrecognizable and partial dissolution was observed. By 144 hr, teeth had completely disintegrated into a white precipitate in the container. (Fig 3, 6,7)

Figure - 6: Photomicrograph showing the morphological changes in the H_2S0_4 group. (0.5 hr -15 hr)

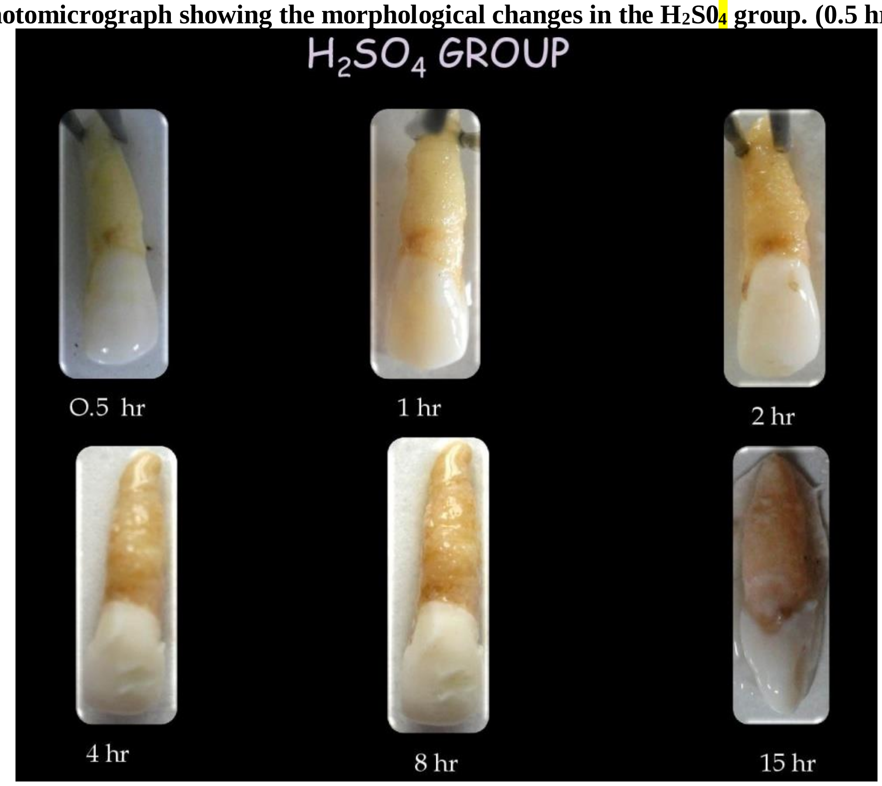
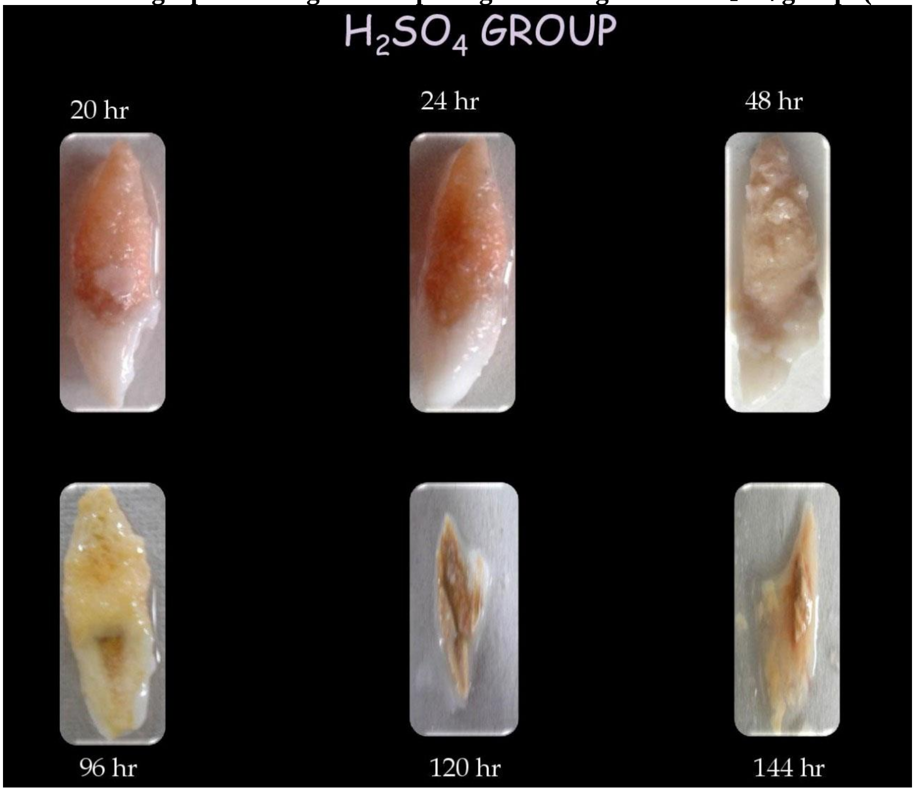


Figure - 7: Photomicrograph showing the morphological changes in the H_2S0_4 group. (20 hr-144 hr)



DISCUSSION

Forensic dentistry symbolizes theoverlap between the dental sciences and the legal profession. Through the specialty of forensicodontology, dentistry plays a small yet significantrole in assisting those involved in crime investigations by identifyingthe crime victims as well as lethal assaults throughdental records.[5]Forensic dentistry has evolved a long wayovermanydecadesin contributing to the indispensablerole as the final counsel of defense, by helping to ascertain the circumstances of death by making use of teeth which are physical characteristics that sustain throughout the decomposition process and are recognizable even after violent assaults. The theme of this study was important becauseit has been contemplated that criminal syndicates will often use acids or caustic materials to destroy human bodies to evadethe personal identification process.The concentrations of the acids used were the ones most commercially available.[3]The resultant morphological changes in the immersed teeth depended upon the individual chemical reactions of all the three acids namely HCl, HNO_3, and H_2S0_4.

In the case of 37% HCl-treatedteeth, the sole reason forthe complete dissolution of teeth was due to the formation of soluble salt by a chemical reaction.Similarly, a soluble calcium nitrate salt was obtained after complete dissociation which imparted a yellowdiscoloration to all teeth inthe 65% HNO_3 group.Lastly, in thecase ofthe 96% H_2S0_4 group, incomplete dissolution of teeth led to the appearance of white precipitatethus, indicatingthe formation of insoluble calcium salt.

Henceforth, it wasevident that all the three acids had a different working mechanism of tooth disintegration as was displayed by varied morphological changes which were taken into consideration.An analysis of commercial procurement of various acids showed that out of all the three above-mentioned acids, HCl and H_2S0_4 can be easily availed than HNO_3. But when it comes to cost-effectiveness, HCl is certainly more affordable than the two.[3]

Hence, based on our morphological findings, it is most likely that37% HCl would be used in such heinous mutilating crimes. Moreover, certainbiochemical alternativesshould also be availed to explore which acid has been utilized for such grave assaults.The destruction of the teeth obtained as a progressivephenomenon was achieved with HCL in 20 hr, with HNO_3 in 15 hr,and formation of insoluble precipitate with H_2S0_4in 144 hr. Such morphological aspects allow us to identify the tooth as of human origin, having a single root or multi-rooted, deciduous or permanent,and restored or unrestored teeth.

When it is no longer possible to identify dental structures thathave been dissolved in acid, other types of investigations are available:chemical or histological analysis of the residues, the possibilityof an eventual DNA analysis, and the chemical analysis offinal obtained residues.[1,6]

Accurate DNA quantification methods are a prerequisite for precise analysis of the obtained mortal remains. One of the few approaches employed for DNA extraction is the organic method whichis composed of phenol-chloroform and is indicatedfor high molecular weight DNA. Since this process makes use of multiple tubes, the probability of more errors is expected. The second mechanism employed is the usage of isopropyl alcohol which is composed of ammonium and isopropanol. Being affordable, it can be utilized as a substitute for the organic method. The third technique which can be used for DNA extraction is Chelex 100 which is not cost-effective yet it is extremely quick in action with the lowest rate of contamination.lastly, the FTA Paper is composed of absorbent cellulose paper with several chemical agentsthus speeding up its usage.[7]

Apart from human teeth being used in decalcification techniques for personal identification, some other major uses of teeth in human identification have been documented like the absence of some teeth, tooth restorations, carious lesions, and prosthesis involving the whole dentition form the concrete basis of dental identification. Considering various dental anomalies in association with the periapical area, surrounding soft tissue as well as bone, it can be duly ascertained that all dentitions are extremely different from each other and can be considered crucial evidence during forensic investigations.[8]

Human teeth can also play a pivotal role in determining the gender of an individual at times when all routine procedures fail. There are several dental features like crown morphology and size, length of the roots, etc. which are typically characteristic of males and females. Usually, in these procedures, the mesiodistallength, as well as the buccolingual dimensions, are examined as this carries special importance in young individuals where secondary sexual characters have not yet been developed. If all the teeth in dentition are compared, then the mandibular canines show the greatest dimensional variability amongst them i.e. they are larger in males than in females.[9]Therefore, these parameters can also possibly help forensic odontologists in making the personal identification process easier.

It would be appropriatefor us to mention thatfurther investigations aboutthe corrosive effects of acids on restored teeth as well as teeth adjacent to artificial prostheses could elicit important data to substantiate our findings. Additional studies that delve into the dual effects of acid-inducedand thermal changes to teeth and their restorative materials need to be taken up for a proper, precise, and more accurate outcome which could be of utmost significance to the investigating authorities.

CONCLUSION

The morphological behavior of natural teeth after complete immersion in acid could reveal important information regarding the type of acid used as well as the total time of immersion which could be of great help to forensic experts as an aid to human identification.It also seems viable to reach a good approximation in determining the interrelationship between destruction rate and variable time of exposure to such corrosive agents as well as comparison of the residues of dissolution with the antemortemrecords.

ACCOMPANYING SHEET

I. What is already known about this topic?

Very few studies have been conducted to study the effect of various acids on teeth.

(ii) What this study adds?

Our study reemphasizes the fact different acids decalcify the teeth in a different pattern and by studying the subsequent decalcification patterns in teeth, we can trace the exact acid used to create the crime which could be another useful tool in forensic dentistry.

(iii) Suggestions for further development

Experiments can be further conducted by altering the conditions liketemperature etc. to mimic variable real-life conditions and adding other variables like gender or carious teeth. This will provide a broader picture of the topic.

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