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ORIGINAL RESEARCH

Reliability of Teeth for Identification after Exposure to varying Degrees of Temperature

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ABSTRACT

Introduction: Violence and crime in human lives from bomb explosions, fire accidents, wars, plane crashes, and natural disasters make identification of victims difficult. The carbonized bodies, advanced stage of decomposition among other circumstances, highlight the need to employ faster, more accurate methods during identification of victims. This study was planned to evaluate the changes in teeth after exposure to varying levels of temperature simulating real-life fire disasters for forensic identification.

Materials and methods: A total of 128 freshly extracted molars and premolars were collected from patients of age group between 12 and 70 years. Samples of 128 teeth were divided into three groups and were subjected to varying degrees of temperatures of 100°C, 200°C, 500°C, 600°C, 700°C, and 800°C in an electric furnace. After subjecting the teeth for each range of temperature, they were analyzed for morphological changes under stereomicroscope. Pulp available was processed for normal histological procedures for observation of the tissue under light microscope, and blood grouping of the pulp was done by adsorption and elution technique. Deoxyribonucleic acid (DNA) quantification of the heat-treated teeth was done by ultraviolet spectrophotometry. The analysis of variance test and Tukey's test were used for multiple variables.

Results: There were statistically significant results in DNA obtained from each temperature. The blood grouping from pulp was not available above 500°C. Furthermore, there was a progressive increase in the weight loss of teeth analyzed by the thermogravimetric method.

Conclusion: Heat-treated teeth with detectable amounts of DNA suggested it is useful to pursue further analysis, such as restriction enzyme digestion, polymerase chain reaction, and Southern blotting.

Keywords: Blood grouping, Deoxyribonucleic acid quantification, High temperatures, Histopathology, Identification, Tooth pulp.

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INTRODUCTION

Identification in forensic dentistry is the capacity to recognize with minimum sources of tissues remaining as in fires, explosions, and decomposing or charred body. Solving crime with the help of teeth identification dates back to more than 2000 years. The first recorded case was made between 49 and 66 AD: Lollia Paulina, a rich Roman woman, was identified after death from the unique arrangement of her teeth. Dr Paul Revere, a dentist, identified a bridge of silver and ivory he had constructed 2 years earlier on the brutally murdered corpse of Dr Warren. Individual identification has achieved a place in legal process by testing the blood group for the first time in the German courts in 1920.[1-3] The work of identification is easier if the bodies are well preserved and other personal belongings remain for forensic identification. However, if the victims are carbonized, or in advanced degree of tissue destruction, the process of establishing identity is difficult or impossible. Dental identification is one of the most reliable and frequently applied methods of identification, predominantly by the comparisons of antemortem and postmortem records.[4] When it is impossible to visualize the identity of the tissues, dental identification too becomes a huge challenge.[5-7] Since teeth are the most calcified structures of the human body, invariably they are found to be one of the remains in most of the fire disasters. The forensic odontologists are often called upon to undertake an examination of dental remains to come to a conclusion on an identification.[8]

Identification from blood group of the deceased person is recognized as one of the reliable techniques in forensic dentistry since 1900. ABO blood group substances are found in blood and body fluids, such as saliva, semen, liver bile, and other fluids. Tooth pulp blood vessels contain abundant antigens, so the blood grouping is a possibility. A study done by Suzuki found the origin of blood group substance in hard dental tissue by infusion sedimentation. The presence of ABO blood group antigens in soft and hard dental tissues assists in identification, when teeth and bone are the only tissues remaining. Concentration of blood group antigens in

tooth material depends on oral and environmental bacterial contamination and also on the high temperatures. In forensic science, the ABO system is a boon, since antigens in erythrocytes are also associated with other cells, and tissues in the body are stable to the violent conditions of heating or drying to a certain degree. Since pulp tissue is protected by the hard tissues of the teeth and has numerous blood vessels, blood group antigens are expected to be present.[9-11]

Under experimental conditions and from actual forensic cases, it was observed that dental pulp tissue could be extracted in most cases. Dental pulp can be a prime source in identification by means of recovering deoxyribonucleic acid (DNA), as evidentiary tool for victim identification and verification in forensic science. It is widely accepted that DNA preservation in bone and teeth is superior to soft tissues, as the soft tissues are not preserved in most of the disasters involving fire.[12-14]

Hence, this study was done to evaluate the changes in the tooth after exposure to varying levels of temperature, simulating fire disasters to find out the reliability of the teeth in forensic identification.

MATERIALS AND METHODS

A total of 128 freshly extracted molars and premolars were collected with detailed clinical data from patients attending the Department of Oral Surgery from 2012 to 2013. They were divided into three groups: Group I with 60 teeth for studying the morphology and histopathology, group II with 20 teeth for blood grouping, and group III with 48 teeth for DNA quantification. Samples collected for blood grouping from the pulp were tested for their blood group first using blood-soaked gauze from the socket. Selected teeth were molars and premolars of age group 12 to 70 years. Teeth advised extraction for orthodontic treatment or periodontally involved teeth without pulp pathology were included. Teeth with any pathology, such as caries or periapical lesions (nonvital pulp), morphological/developmental anomalies, molars and premolars with any prosthetic fitting and teeth with fractures/trauma, severe attrition, abrasion, erosion were excluded.

Teeth were collected immediately after extraction, cleaned with running water and with normal saline. The collected teeth were subjected to temperatures of 100°C, 200°C, 500°C, 600°C, 700°C, and 800°C for 20 minutes of duration. Examination of the unsectioned tooth was done to analyze the changes in the tooth morphology with the help of stereomicroscope at each range of temperature. Pulp was removed by sectioning the teeth longitudinally using a rotating disk with a micromotor and water spray, and the tooth was supported with a wax block. After 500°C, the fragile teeth were crushed with a mortar and pestle.

Tissue processing for pulp histopathology was done after removal of the pulp, fixed in 10% of formalin for a minimum of 24 hours. After fixation, the pulp was processed and the tissue that was embedded in paraffin blocks were made into L-shaped molds. A thickness of 5 µm was obtained using a microtome. Hematoxylin and eosin-stained sections were examined under light microscope.

For determination of blood group, adsorption-elution method was used. The pulp was placed in a test tube, and a drop of saline with sterile cotton thread was added. The test tubes were placed in the incubator at 56°C for 30 minutes, so the blood group antigens of dental pulp get absorbed by the sterile cotton thread. Blood-stained threads of 1.5 to 2 mm length were cut and placed in a drop of anti-A serum in a slide cavity. Similar piece was placed in anti-B serum. The slides were then kept in refrigerator at 4°C for 2 hours for complete adsorption; later antiserum was pipetted off and washed three to four times in saline. Slides were then placed in an incubator at 56°C for 30 minutes. A drop of 0.5% suspension of known red blood cell blood group was added and the samples were incubated at 56°C for 15 minutes and observed under microscope for agglutination.

The DNA quantification was done by UV spectrophotometry. After the collection of lysis sample, it was prepared for binding. Wide bore pipette tip is used to reduce shearing of the DNA when transferring contents into the column. The column is centrifuged to remove the traces of residual ethanol. The column is then placed in a new 2 mL collection tube. Eluted DNA was noted down at 260 nm range of wavelength and recorded in the computer software.

RESULTS

At 100°C, 80% of the samples showed no change or alteration in the morphological appearance of the enamel; 20% of the teeth samples appeared dark in color; at 200°C, there was a loss of translucency in 90% of the samples; at 500°C, 70% were carbonated coal black in color; 30% showed variations from brown to black, cracks, small spicules, and globules. Changes in the dentin by the heat since the deep furrows were present in the enamel, exposing the dentin directly to heat. At 600°C, 20% appeared dark brown in color; 80% showed fragments, with colors ranging from dark brown to dark gray; at 700°C, 100% of the samples appeared dark gray with the long deep grooves, fragmented teeth bits; at 800°C, ash contents were increasing, with samples showing fragments of grayish white (Table 1). Histopathology of the pulp at 100°C showed intact tissue, at 200°C it showed degeneration, at 500°C pulp tissue was lost with carbonated remains (Table 2).

The thermal and weight loss changes in the tooth were recorded by the thermogravimetric method, and the DNA

Table 1: Macroscopic observation of the heat-treated teeth from stereomicroscope

Temperature	*Enamel*	*Dentin*	*Cementum*
100°C	No changes but few appeared dark	No changes observed as dentin was not visible	No changes
200°C	Changes observed enamel appeared dark in all the teeth	Dentin was not visible	No changes observed
500°C	Three-fourth of the teeth sample appeared coal black in color	Dentin was visible, enamel had cracks with deep grooves	Cementum at CEJ appeared darker
600°C	The color of the enamel was dark grayish. Furrows, cracks, fragments, grains were visible	Dentin appeared dark brown	Cementum was dark gray
700°C	Color was gray with more fragments	Spicules of dentin were visible	Cementum appeared white with grains
800°C	White, cracked mud appearance with fragments and grains	Dentin could not be differentiated	Cementum could not be differentiated

Table 2: Histopathology of the pulp

Temperature	*Histopathology of the pulp*
100°C	Pulp was intact, displayed fibroblasts, blood vessels and collagen fibers, tissue destruction was seen to some extent
200°C	Pulp was in multiple bits, most of the cells undergoing degeneration
500°C	Carbonated tissue remains were seen

Table 4: Statistical analysis of the tooth remaining by thermogravimetric analysis

		Tooth remaining			
Temperature	*N*	*Mean ± SD*		*Minimum*	*Maximum*
100°C	10	97.8240	0.23477	97.55	98.35
200°C	10	92.8710	0.50415	92.32	93.71
500°C	10	66.4270	1.02655	65.34	68.14
600°C	10	64.7740	0.36543	64.27	65.35
700°C	10	63.8630	0.36621	63.32	64.58
800°C	10	62.9980	0.22720	62.54	63.26

F = 9147.298; p < 0.001 vhs; SD: Standard deviation

quantification by spectrophotometer was analyzed by analysis of variance test and found to be statistically significant. Weight loss analyzed by the thermogravimetric method showed gradual increase in the mean values to be very highly significant ($p < 0.001$; Tables 3 to 5; Graphs 1 and 2). Multiple variables of the tooth loss were compared by Tukey's honest significant difference. The mean difference showed increasing values drastically till 500°C.

Blood grouping was done by adsorption-elution technique (Table 6 and Graph 3). At 100°C, 60% of the sample matched the blood groups of the patient. At 200°C, the results matched 100% with the patient's blood group.

The DNA quantification at 100°C, 200°C, and 500°C showed DNA in 90 to 100% of the samples; at 600°C, 20% showed negligible amount of DNA; at 700°C, 30% of the samples showed negligible amount of DNA and 10% showed no DNA in the sample; at 800°C, 30% showed no DNA and 20% showed negligible amounts of DNA (Table 6, Graph 4).

Table 3: Statistical analysis of the weight loss by thermogravimetric analysis

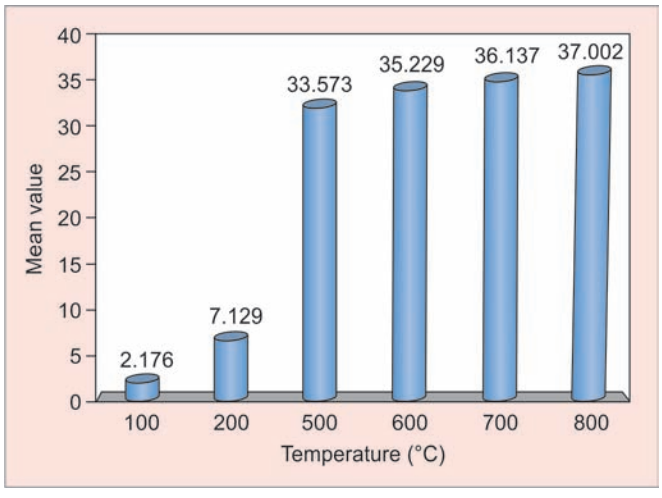
Weight loss	*n*	*Mean ± SD*	*Minimum*	*Maximum*
100°	10	2.1760 ± 0.23477	1.65	2.45
200°	10	7.1290 ± 0.50415	6.29	7.68
500°	10	33.5730 ± 1.02655	31.86	34.66
600°	10	35.2290 ± 0.37012	34.65	35.76
700°	10	36.1370 ± 0.36621	35.42	36.68
800°	10	37.0020 ± 0.22720	36.74	37.46

F = 9155.95; p < 0.001 vhs; SD: Standard deviation

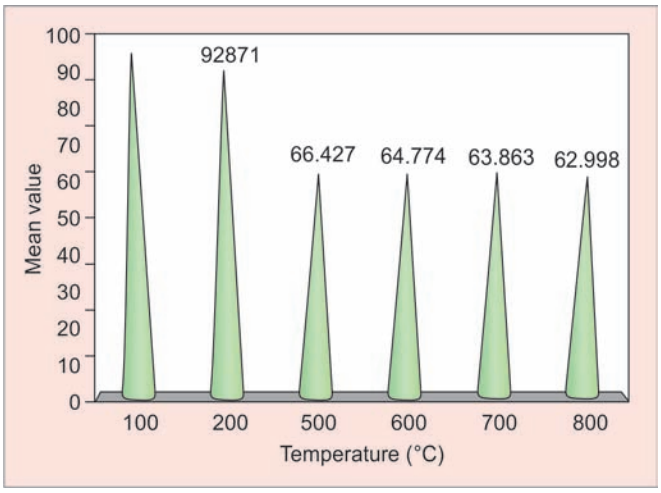
Table 5: Statistical analysis of the blood grouping done by adsorption-elution technique

	Group		
	100°	*200°*	*Total*
O positive			
Count (%)	4 (40.0)	5 (50.0)	9 (45.0)
A positive			
Count (%)	4 (40.0)	2 (20.0)	6 (30.0)
B positive			
Count (%)	1 (10.0)	3 (30.0)	4 (20.0)
AB positive			
Count (%)	1 (10.0)	0 (0.0)	1 (5.0)
Total			
Count (%)	10 (100.0)	10 (100.0)	2 (100.0)

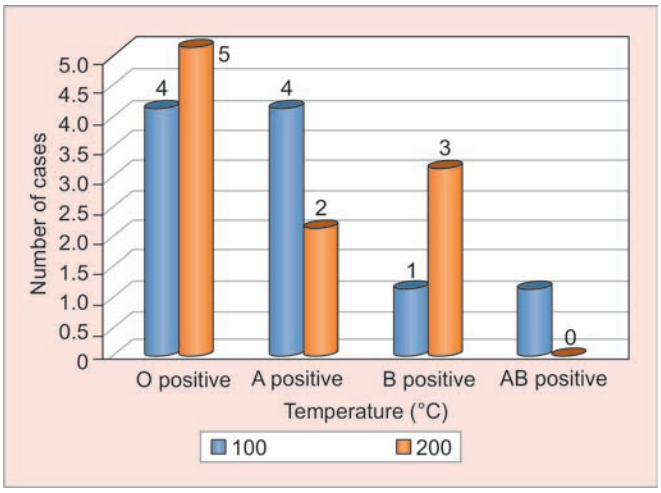
$\chi^2 = 2.778$; p = 0.427



Graph 1: The gradual weight loss by thermogravimetric analysis



Graph 2: The tooth remaining by thermogravimetric analysis

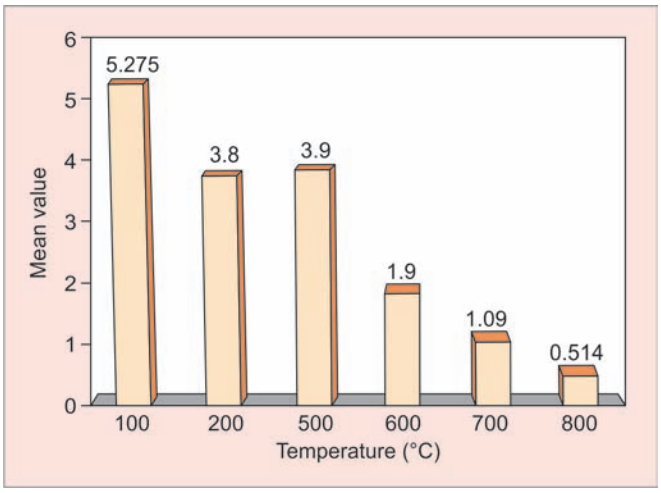


Graph 3: The blood grouping done by adsorption-elution technique

Table 6: Spectrophotometric quantification of DNA

Temperature	*n*	*Mean ± SD*	*Minimum*	*Maximum*
100°C	8	5.2750 ± 0.87137	3.70	6.10
200°C	8	3.8000 ± 1.96323	0	5.60
500°C	8	3.9000 ± 1.35013	2.20	5.70
600°C	8	1.9000 ± 1.30274	0.30	3.80
700°C	8	1.0875 ± 1.47400	−1.60	2.70
800°C	8	0.5143 ± 1.73439	−1.60	2.80

F = 12.1; p < 0.001 vhs; SD: Standard deviation

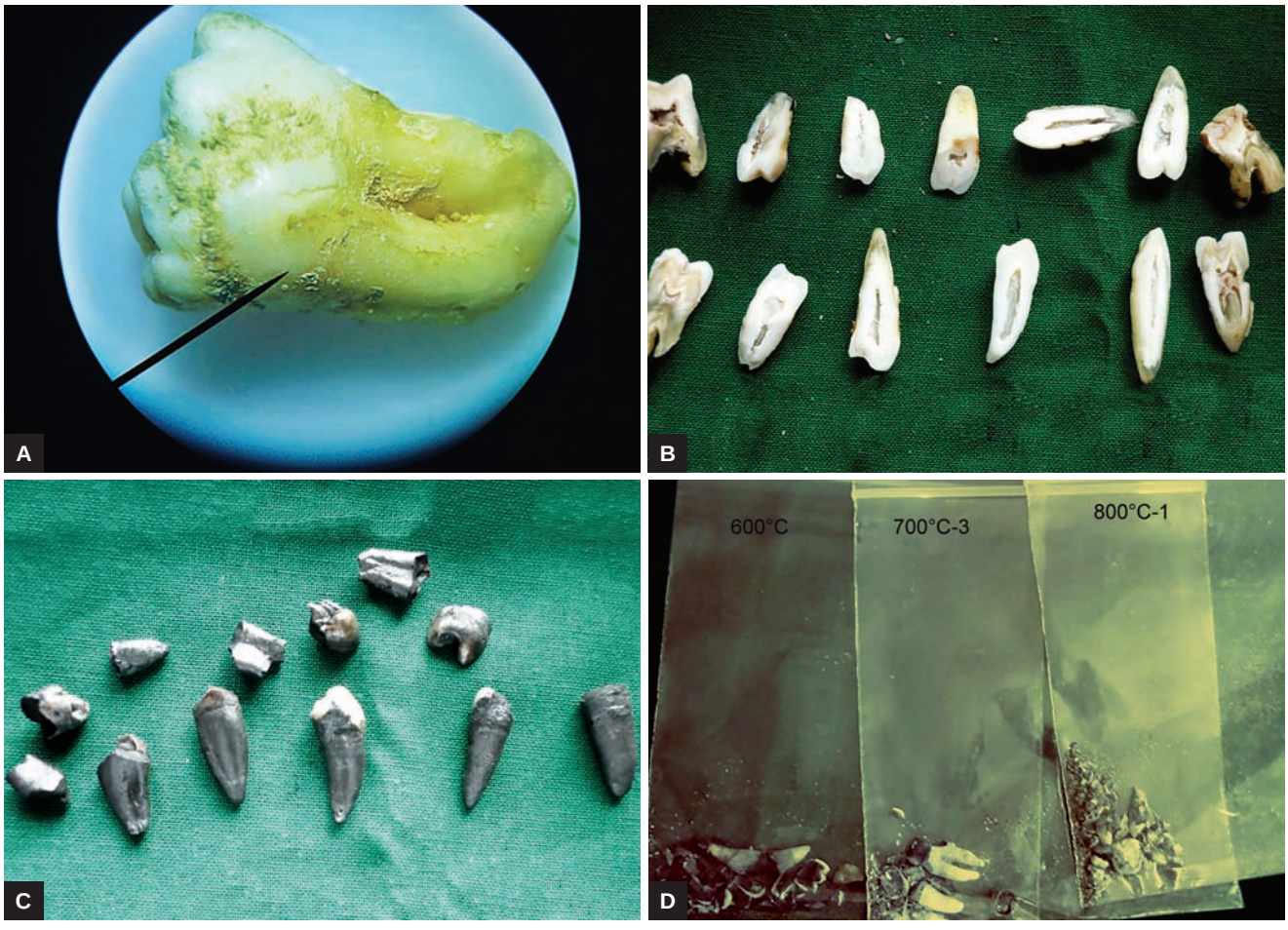


Graph 4: The DNA spectrometry

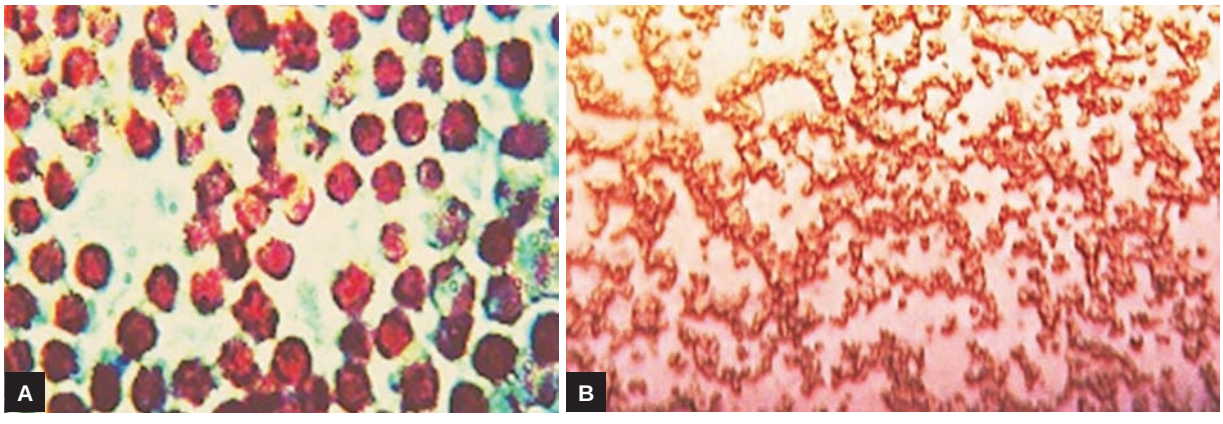
DISCUSSION

Forensic investigations have become more demanding, as the challenges are rising especially if minimal tissues are remaining. This can be due to any mass disasters, such as fire accidents producing high temperatures as in domestic fires, vehicle accidents, and suicidal self-induced fires or also in crimes done to conceal it. Teeth serve as one of the prime source for identification in fire disasters because enamel being the hardest mineralized substance of all forms a protective layer around the dentin and pulp.

It is important to know the different degrees of temperatures the body tissues react. The color of the burnt tissues gives an indication of how approximately the temperature rise would have happened to cause the destruction. In our study, after heating the teeth samples at 100°C, the enamel appeared a little darker with loss of natural hue in less than 20% of the teeth samples. Enamel has 95 to 96% crystalline apatite constituting for its hardness, with maximum thickness attained in molars and premolars being 2 to 2.5 mm. Thus, the major portion of inorganic substance renders enamel, withstand the mechanical forces making it brittle, therefore, underlying more resilient dentin is required to its integrity. The color of an enamel can range from yellowish white to darker hue as it is dependent on the translucency of enamel, yellowish teeth having a thin translucent enamel through the yellow color of the dentin is visible and grayish teeth having more opaque enamel, the translucency is due to the calcification, dehydration decreases the enamel translucency. The larger size of the crystals in enamel makes it more stable for heat. The orientation of the hydroxyapatite crystals also plays an important role in its reaction to heat stress.[15] This study showed differences in each tooth at 100°C, which are mainly due to the variation in the thickness of each tooth. At 200°C the color change was observed in 90% of the teeth samples, but they were all structurally intact. Thus, it is the chemical reaction to fire taking place at 500°C where the color of the enamel ranged from gray to black. A study done by Harsanyi found that the enamel goes through a series of color changes ranging from dark grayish to brown at 300°C, porcelain to white at 1000°C; structurally, the enamel begins to crack and eventually break into fragments separating from the underlying dentin.[8] Similar results were recorded in a study done by Shipman et al.[16] In this study,



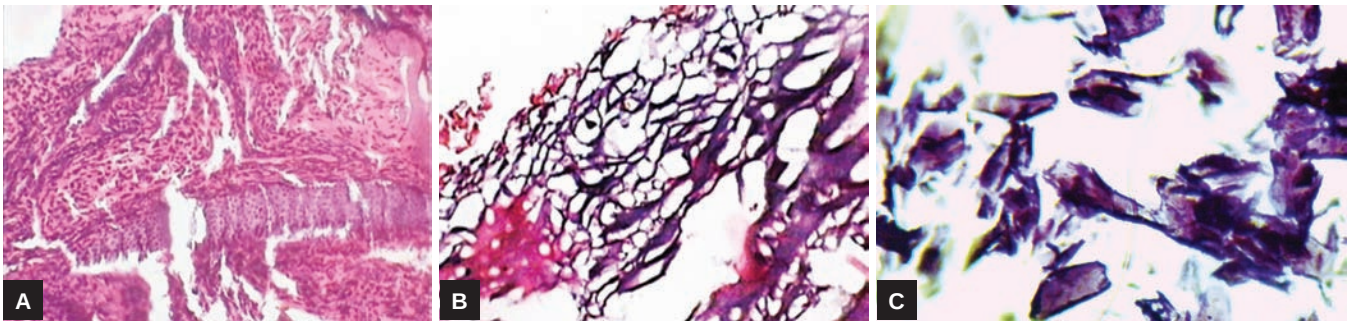
Figs 1A to D: Teeth morphology after heat treatment. (A) Teeth at 100°C; (B) teeth at 200°C; (C) teeth at 500°C, and (D) teeth at 600°C, 700°C, and 800°C



Figs 2A and B: Microphotographs (40×) of ABO blood grouping by adsorption and elution. (A) No reaction of red blood cells observed, and (B) agglutination of red blood cells

changes were noted to that of above studies till 200°C, and at 500°C, the enamel color was almost black. At 600°C and 700°C, there was change in color from black to dark gray and at 800°C, the tooth was entirely grayish white in color. In a study done by Bachmann et al,[17] it was shown that enamel whitening occurs due to water elimination. Before heat treatment, the enamel is transparent because of the presence of water in its liquid state.

In real-life disasters, cementum is exposed to the fire at cementoenamel junction (CEJ), directly as in enamel, but rest of it is protected by the gingival, periodontal ligaments, maxilla or mandible, and alveolar bone. Hence, speculating that, here in our study, the teeth were subjected directly to heat without the cementum being protected by the alveolus, the changes observed are difficult to assess as compared with the enamel heat



Figs 3A to C: Hematoxylin and eosin-stained microphotographs of pulp histopathology after heat treatment: (A) At 100°C (10×) intact pulp; (B) at 200°C (10×) pulp undergone tissue degeneration, and (C) at 500°C (40×) carbonated tissue remains

exposure. Heat first affects CEJ as it is the exposed portion, so observations are made based on CEJ in this study. Depending on the changes at CEJ, the following were observed: At 100°C, no color changes were observed under stereomicroscope; at 200°C, light brown spots were seen; as studies mentioned before, they have also done on cremated teeth which were not simulating the situation of fire disasters since they used the teeth which were already isolated from the alveolus and did match to the present scenario.[8] Freshly extracted teeth were used here, with almost closer to real-life accidents except by the fact that teeth are exposed to higher temperature compared with the actual true fire disasters.

Observation from the CEJ is done at this study. At 500°C, the color changed from brown to black; at 600°C, the color change was from black to dark gray but could not differentiate from the color of the enamel at this temperature; at 700°C, the color of the CEJ was not appreciated much than the previous temperature. At 800°C, cementum was fragmented and appeared white. Once the enamel is exposed to certain level of heat, the dentin starts getting affected by the fire, though it might get affected indirectly through enamel. Color changes in the dentin were observed only after 600°C. After the enamel starts getting long furrows, it was seen as gray in color. At 700°C, it appeared gray white and at 800°C it appeared white along with fragments. Comparing with the previous studies done, as mentioned earlier, the results were similar, but they could observe the changes as early as at 300°C since the exposure time was longer than in this study. In our study, weight loss of the specimens began at 100°C with maximum weight loss occurring between 200°C and 600°C. The weight loss was evident from 200°C; the entire tooth was subjected to the temperature and weight loss and ash content of the tooth (inorganic content) were observed. Statistical analysis done on this was found to be highly significant. The Tukey's test done on multiple variables of weight loss showed the mean values from 100°C to 200°C was 4.95, 100°C to 500°C was 31.4, 100°C to 600°C was 33.05. There was a drastic increase in the values from 100°C to 500°C. Reyes-Gasga et al[18] performed a chemical analysis with X-ray characteristic energy-dispersive spectroscopy, and the results indicated a strong correlation between the removal of the OH^- groups from the hydroxyapatite unit cell and the expulsion of absorbed water and lattice water registered during heating, thus indicating weight loss and color change as water is lost mainly from the organic content of the tooth. In this study, histopathology of the hematoxylin and eosin-stained sections of heat-treated pulp was able to resist heat till 100°C and 200°C. At 500°C, there were very minimal carbonated pulpal tissue with one or two visible cells with distorted morphology. It indicated the enamel and dentin start to react after 200°C, and in this study, till 200°C there is a possibility for retrieval of the cells from the pulp chamber for further investigations. Myers et al[19] have done a study on effects of extreme heat in teeth with implications for histological processing found in microscopic examination following decalcification. Histological processing revealed changes, such as severe tissue fragmentation, vapor bubbles within dentinal tubules, altered staining, charring, and tissue shrinkage. Dentin appeared to be the most reliable microscopic identifier of incinerated dental tissues.

The blood grouping is one of the methods of identification from the tooth pulp and dentin in forensic odontology. In our study till 200°C, we were able to find out the blood group. As the temperature went higher, it was not possible to retrieve the antigens from the pulp because the lysis of red blood cells takes place in high temperatures. Group II consisted of 20 teeth samples to study the blood grouping in the teeth samples after freshly extracted and subjected to heat in a furnace. Bregt Smeets et al[20] in their study found tooth pulp with lot of blood vessels and showed blood group antigens are bound to be present in pulp. This was confirmed in many studies. As for enamel and dentin, however, Kemp et al[21] referred that the enamel contains very less inorganic content and dentin up to 28%. Both, therefore, especially dentin, would be able to contain blood group antigens, located in the dentin tubule. In this study, it was possible to retrieve the ABO

antigens from the pulp after subjecting it to varying degrees of temperatures. At 100°C, the results were 60% efficient and 40% of the samples did not match with the blood group of the patient. Among the pulp samples at 200°C, the results were 100% matching the patient's blood group. It is possible to depend on the results of ABO blood grouping after fire accidents to certain levels of temperatures, since this study did not show any significance of antigen present in the dentin or the pulp after subjecting the tooth for 20 minutes at 500°C. The results were not conclusive as none of them matched the respective blood groups of the patients. Aswath et al[22] conducted a study to find the blood grouping and found 57 teeth out of 60, with positive results. Blood group elicited from capillary blood done by slide-agglutination method matched with that of the pulpal blood group elicited by absorption-elution method. Three showed negative results. Similar study was done by Prabhawati et al[23] to determine the ABO blood group from the dental pulp of extracted teeth at various time intervals by absorption-elution technique. They found ABO blood groups could be identified from 88% of permanent teeth and 44% of deciduous teeth. Determination of the ABO grouping from the body is sometimes difficult due to hemolytic erythrocytes and due to various reasons of long time putrefaction, mummification, or skeletonization of the body. The ABO blood grouping methods utilized in the forensic autopsies are hemagglutination, absorption-elution, and histochemical techniques and ABO genotyping method.[10] In this study, it was proved that the blood grouping can be done from pulp. The results at 100°C were 60% efficient, and shows that the amount of blood group antigen present is dependent on condition of the pulp present.

In a study conducted by Pötsch et al,[13] the genomic DNA obtained from a dental sample ranged from 6 to 50 µg; DNA did not depend on the type of tooth, age, or sex of the donor. They found no influence of storage conditions or time periods for retrieval of the DNA. In addition to genomic DNA, cells contain mitochondrial DNA (mtDNA) that also aid in identification. In cases of genomic DNA, it cannot be analyzed because it is too degraded, and mtDNA can be present in sufficient quantity. Polymerase chain reaction (PCR) method enables differentiation of one individual from another with level of reliability and with about 1 ng of the target DNA. The quality DNA extracted from the tooth is necessary in DNA analysis.[6] Malaver and Yunis[24] found 20 teeth from unidentified bodies buried in 1995 and exhumed in 2000, and provided 45 DNA samples: 5 from the pulp, 20 from dentin, and 20 from cementum. The pulp produced the strongest PCR amplification signals, while dentin and cementum signals were very similar to each other. Hanaoka et al[25] extracted DNA from 50 teeth (pulpal and hard tissues). The DNA obtained from the dental pulps ranged from 3 to 40 µg, and no correlation was found between the storage period and the amount of DNA. The high molecular weight DNA obtained from the dental pulp was able to be further analyzed by multilocus probes or PCR. In this study, DNA quantification was done on 48 teeth samples using nano-drop spectrophotometer after heat treatment under different ranges of temperatures. It showed amplifiable DNA present at the temperature range of 100°C, 200°C, 500°C, 600°C, 700°C, and 800°C, with variations after 500°C. Teeth can be a reliable source for the DNA extraction at high temperatures, till 800°C can still be a possibility. Hence, it is important to know that the DNA is possible to be obtained after teeth are subjected to very high temperatures, as the purified DNA obtained is compatible with downstream applications, such as restriction enzyme digestion, PCR, and Southern blotting.

CONCLUSION

Simulating the actual scene from the fire disasters, this study intended to bring out the possible ways to get evidentiary support for identification based on the teeth. Morphological study of the teeth after heat treatment was done to see the changes taking place in each temperature, thus the teeth when collected from the actual fire incidents can be deduced to the range of temperature the victim has been burnt. Since the type of fire disasters as would have happened in household fires, bomb blasts, or traffic accidents can be speculated by looking at the color of the teeth, the forensic odontologist can give valuable information. Histopathology of the teeth after heat treatment gives information of temperature, the tissues in the teeth still viable, as an evidentiary tool. Since teeth can withstand high temperatures, it holds a good chance of getting ample amount of information for further aid in age, sex determination, blood grouping, as well as molecular basis of identification procedure. Blood grouping from the pulp gives information as to whether it can be relied upon as a sole identifier. The DNA identification process has proved to be a mainstay for the identification in fire disasters. However, to a lesser extent, the effects of the reactions can be considered in real fire disasters, since teeth are not directly exposed to fire. Henceforth, the results observed in this study can be anticipated to give more positive and better results in actual fire disasters.

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