Original Article

Isolation of DNA from human teeth exposed to different decalcifying solutions for forensic identification: A study

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Abstract

Background: Teeth are selected as source of DNA material mainly due to the high durability found in its structures (enamel, cementum, and dentin), which often succeeds in preserving the integrity of genetic material. Attempts of perpetrators of crime in the destruction of evidence including dead bodies are on the rise. Hence, the use of advanced techniques such as DNA analysis in such a scenario has been the choice.

Aim: The purpose of this study was to evaluate the extraction and amplification of DNA from human teeth exposed to different chemicals Nitric Acid at 25%, Formaldehyde at 25 % , and Acetic Acid at 25 %.

Objective: The objective of the study was to determine the intactness of DNA using polymerase chain reaction.

Materials and Methods: The experimental study was performed with a sample of 15 subjects who underwent tooth extraction from which 5 samples of oral mucosal cells were taken as controls (reference population). The experimental population was divided into three equal parts, which were exposed to different chemical solutions, namely Nitric Acid 25 %, Formaldehyde at 25 %, and Acetic Acid at 25 % compared with the control group (oral mucosal cells [5 samples]). The silica method was used for the extraction of DNA from teeth and the organic method was used for the extraction of DNA from oral mucosa cells.

Results: Estimation of DNA quantity and size distribution was done on an Agrose Gel Electrophorosis. From our study we could observe that the teeth that were immersed in 25% Formaldehyde & 25% Acetic Acid were having intact DNA, which we were able to isolate & amplify. There was degradation of DNA tooth which were immersed in 25% Nitric Acid, thus the identification & amplification was not possible.

Conclusion: The present study demonstrated that DNA extraction may be limited under exposure to chemical solutions or bodies that undergo intentional postmortem alterations, such as carbonization and dissolution, hamper the degradation of DNA due to high temperature and acid pH. Therefore, testing the collection of DNA from the human teeth in such conditions could contribute significantly to the field of forensic genetics.

Key Words: *Decalcifying agents, DNA extraction, DNA fingerprinting, forensic genetics, forensic odontology*

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Introduction

Forensic odontology has proven for many times its value for human identification in forensic sciences.[1,2] Forensic science relies mainly on three scientific pathways for human identification: fingerprints, teeth, and DNA analyses.[3] Fingerprint analysis is a quick and low-cost procedure compared to the other pathways. While on the contrary, it may not be feasible when the soft tissues are damaged by external factors,[2] such as in charred and putrefied bodies.[4] Teeth which act as a major source of DNA because of their ability to withstand to undergo changes. Some authors suggest that teeth are better sources of DNA than skeleton bones.[5] Dental analysis is also a quick and low-cost procedure, but it also has the advantage of relying upon the integrity of the teeth – which are the most durable part of the human body.[6] However, this analysis depends on the availability of dental records for a comparative procedure, which may not exist in populations without access to dental treatment.[7] Over the last decade, the DNA analysis approach has become the gold standard for human identification.[8] The DNA analysis is performed by screening the genetic profile of the victim and matching it with genetic profiles of a potential

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candidate. Bodies that undergo intentional postmortem alterations, such as carbonization and dissolution, hamper the degradation of DNA due to high temperature and acid pH.[9]

Therefore, testing the collection of DNA from the human teeth in such conditions could contribute significantly to the field of forensic genetics.

The present research was conducted to evaluate the extraction and amplification of DNA from human teeth immersed in Nitric Acid at 25 %, Formaldehyde at 25 %, and Acetic Acid at 25 %.

Materials and Methods

Sample description

This experimental study was performed with a sample of 15 subjects, who underwent the extraction of a single tooth (experimental population, n = 15) [Figure 1]. The dental extractions were performed in a university environment by a dental surgeon following therapeutic indications. The inclusion criteria consisted of the absence of caries or restorative material clinically detectable in the teeth. The exclusion criteria consisted of teeth surgically sectioned during the extraction. A control group was created by collecting samples of oral mucosal cells from five subjects (control population, n = 05).

After cleaning, the teeth of the experimental population were divided into three equal groups. Group 1 was immersed in Nitric Acid at 25% (n = 05); Group 2 was immersed in Acetic Acid 25% Formaldehyde n = (05); and Group 3 was immersed in 25% formaldehyde (n = 05). These teeth remained immersed in the chemical solutions for 4 days complying with the protocol of previous studies.[10,11]

Materials

Decalcifying solutions Nitric Acid (25%), Acetic Acid, (25%) Formaldehyde (25%) were used. Extraction solution used were 0.45 M EDTA and 0.25 mg ml^{-1} proteinase K, pH 8.0 a volume of 10 ml is required/500 mg of sample. Binding buffer: 5 M GuSCN, 25 mM NaCl, and 50 mM Tris per sample 40 ml are required. Washing buffer: 50% v/v ethanol, 125 mM NaCl, 10 mM Tris, and 1 mM EDTA, pH 8.0, are required.

In our study, we used two methods: for DNA extraction, the silica method was used and for the isolation of DNA from oral mucosal cells, we used the organic method.

Method for DNA extraction from teeth: Silica method

Preparation of the tooth sample

The dirt was removed from the surface of the specimen with a tissue. Specimens were immersed into decalcifying reagents for (4 days). Samples were ground with a mortar and pestle until a fine-grained powder was obtained. Five hundred milligram of decalcifying sample was transferred into a 15 ml tube. DNA release was done; 10 ml extraction solution was added to each 500 mg sample powder. Tubes were sealed with Parafilm and incubated with gentle agitation for (2 h). DNA purification: 200µl isoamyl alcohol was added the sample was allowed to pass through spin column. Then, the sample was centrifuged for 2 min at 5000g, then added 100 µl silica suspension. Then, the silica column was dried at room temperature for 15 min with open lids. 50 µl TE buffer was added to the dried silica column, it was then incubated with closed lids for 10 min; this was again centrifuged for 2 min at 16,000 g and was collected in a 50 ml tube. After this procedure, the DNA quality and size distribution were estimated on an agarose gel.

Method for DNA extraction from oral mucosal cells: Organic method

The collection of oral mucosal cells was done using Cytobrush using a vial. 500 µL of EDTA was added and incubated at 37°C for 1 h. After incubation, 1ml of TRIS NaCl EDTA (TAE) was added with sodium dodecyl sulfate (SDC) and 10 of proteinase K was also added, it was mixed for 15 s. The sample was again incubated at 56°C for 1 h. The entire sample was transferred into the 2 ml Eppendorf tube. 1 ml of lysate was added with 1 ml of phenol–chloroform isoamyl alcohol and mixing was done for about 15 min. The samples were again incubated for 5 min at room temperature and were centrifuged at 10,000 rpm for 10 min. The supernatant was collected into the 1.5 ml fresh tube. It was added by an equal amount of isopropanol and was incubated for 30 minutes and centrifuged at 10,000 rpm for 15 minutes. After this the supernatant was discarded. The DNA pellet was washed in 20 µL Nuclease Free water (NFW) and stored at - 40. Estimation of DNA quantity and size distribution was done on an Agrose Gel Electrophorosis. After DNA quantification, amplification of Actin gene (reference gene) was done through PCR by using forward and reverse primers. Forward Primer :CCCAAGGCGAACAGAGAAAAG, Reverse Primer: TGTACGACCACTGGCATAAAG. Primer sequence and Temperature of Actin gene is given in [Table 1]. All the samples were amplified through polymerase chain reaction (PCR), using the Identifiler kit (Applied Biosystems¤, Waltham, USA) with the following PCR solution given in [Table 2]. The Standard PCR conditions for Actin gene are given in [Figure 2].

Results

From our study, we could observe that the teeth that were immersed in Formaldehyde & Acetic Acid had intact DNA, which is visible in 0.8% electrophoresis gel [Figure 3 and 4] group 1, group 2 and group 3 from 1 to 15 bands. On the contrary, there was degradation of DNA in tooth which were

Table 1: Primer sequence and temperature of actin gene

Actin gene	Primer sequence	Amplification size (bp)	Temperature in °C
Forward primer	CCCAAGGCGAACAGAGAAAAG	110 bp	55
Reverse primer	TGTACGACCACTGGCATAAAG		55

immersed in 25% nitric acid; thus, the identification was not possible [Figure 5] from 16 to 20 bands.

DNA quantification was done by using Nanodrop Spectrophotometer (ND-1000, Thermo Fisher Scientific), along with determining the absorbance ratio at 260/280 nm for evaluating the quality of obtained viable DNA. The DNA purity was determined by 260/280 ratio. The quality of DNA in Group 1 (control group), that is in oral mucosal cells, was of fine quality which is visible in 0.8% gel electrophoresis and confirmed by 1.8 ratios of 260/280 DNA-to-protein ratio. The quality of DNA in Group 2 and Group 3 teeth that were immersed in formaldehyde and acetic acid, respectively, was of good quality as compared with the control group. In Group 4, teeth that were immersed in the nitric acid the quality of DNA were poor as compared with the other three groups [Table 3 and Figure 6].

Discussion

Teeth are selected as the source of DNA material mainly due to the high durability found in its structures (enamel, cementum, and dentin), which often succeeds in preserving the integrity of genetic material. The durability of the human teeth plays an important part resisting to natural and PM alterations induced by men such as putrefaction and carbonization, respectively.[12,13] Cadaveric alterations evolved following the current changes in criminality, which include more complex mechanisms compared to decades ago. Criminals are more aware of crime scene investigations.

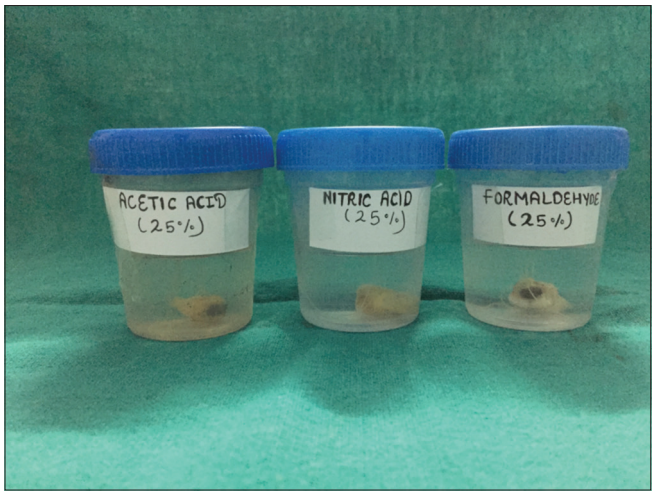

Figure 1: Human teeth exposed to different chemicals such as nitric acid 25%, formaldehyde at 25%, and acetic acid at 25%

Table 2: Reaction mixture composition for polymerase chain reaction

Reaction component	Volume	Final concentration
5X taqs buffer	4 µL	1×
Tag polymerase	0.4 ul	2 units
dNTP mix	2 µL	1×
Actin forward and reverse primer each	1 µL	1×
Template DNA	1-5 µL	100 ng
Water, nuclease free	Up to 20 µL	
Total volume	20 µL	

Standard PCR conditions for Actin gene

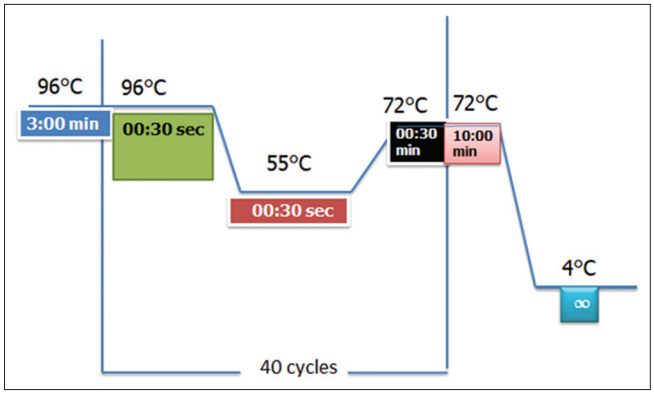

Figure 2: Standard PCR conditions for Actin gene

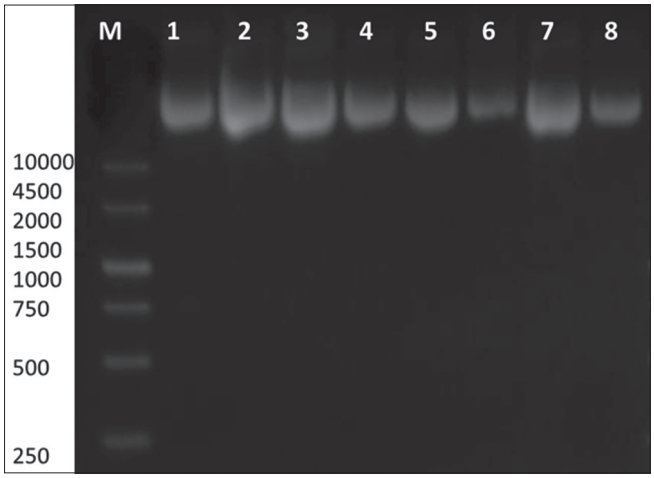

Figure 3: M=ladder.: 1-5 Number =Control (Oral mucosal cells), 6-10 Number =Formaldehyde ,11-15 Number =Acetic Acid ,16-20 Number =Nitric Acid

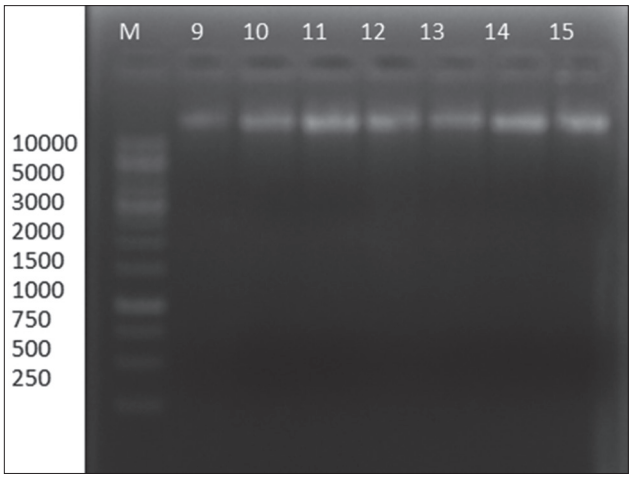

Figure 4: 6-10=Formaldehyde, 11-15=Acetic Acid 16-20=Nitric Acid 1-5 =Control

Table 3: Concentration of DNA quantified by Nanadrop and 260/280 DNA to protein ratio

Sample	DNA concentration in ng	A260/A280 ratio
Oral mucosal cells	700	1.82
Oral mucosal cells	690	1.86
Oral mucosal cells	624	1.88
Oral mucosal cells	458	1.8
Oral mucosal cells	489	1.87
Formaldehyde	400	1.80
Formaldehyde	550	1.82
Formaldehyde	560	1.81
Formaldehyde	600	1.7
Formaldehyde	450	1.89
Acetic acid	300	1.8
Acetic acid	430	2.0
Acetic acid	500	1.9
Acetic acid	410	1.82
Acetic acid	310	1.81
Nitric acid	310	1.6
Nitric acid	490	1.7
Nitric acid	380	1.5
Nitric acid	420	1.53
Nitric acid	317	1.5

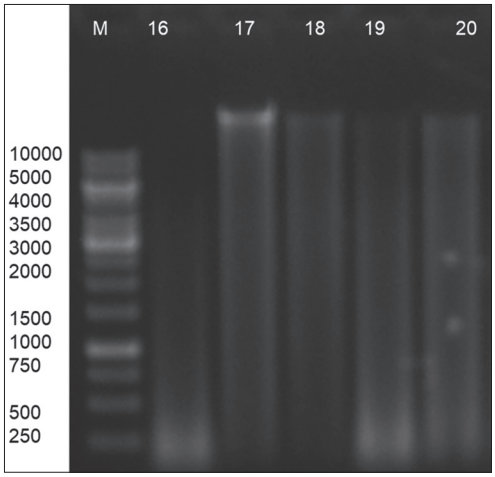

Figure 5: 16-20=Nitric Acid

Consequently, they became more concerned of vanishing forensic evidence. The present study simulated a cadaveric alteration induced by men. In the simulation used in this research, human teeth were immersed in formaldehyde at 25% (Group 1) which is used as a preservative in the medical laboratory, acetic acid at 25% (Group 2), and nitric Acid at 25% (Group 3) which are chemical acid solutions used to hamper the identification process. In our study, we found that the samples which were immersed in 25% Formaldehyde [Group 1] the DNA was intact thus the isolation of DNA and amplification of actin gene reference gene) was possible [Figure 3, 4 and 7]. In (Group 2 and 3) DNA isolation and amplification was optimal. On the Contrary in (Group 4) teeth immersed in 25% Nitric acid the isolation and amplification was not possible. [Figure 5 and 8].

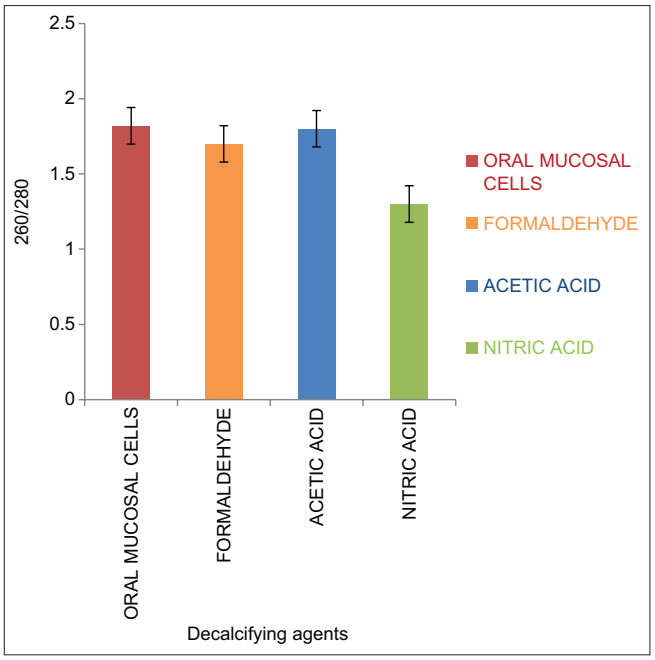

Figure 6: 260/280 ratio of DNA/Protein in different decalcifying solutions

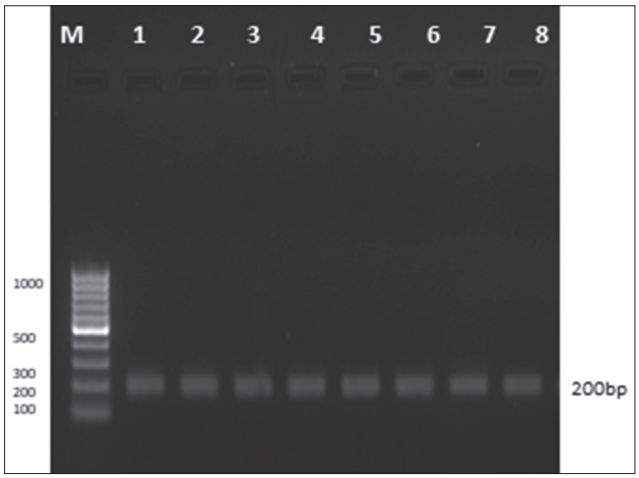

Figure 7: 1-5 Number =Control ,6-10 Number =Formaldehyde, 11-15 Number =Acetic Acid ,16-20 Number =Nitric Acid. Amplification of actin gene was possible in Group 1 ,2 and 3. Whereas in group 4 Nitric acid, the DNA amplification was not possible

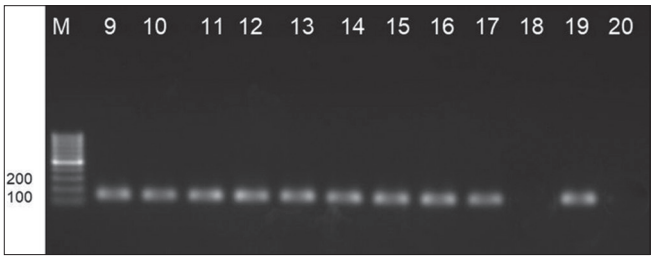

Figure 8: 1-5 Number =Control ,6-10 Number =Formaldehyde, 11-15 Number =Acetic Acid ,16-20 Number =Nitric Acid

Conclusion

Despite simulating a problem-based situation in forensic sciences, our research did not include the outlook found in real scenarios, such as the oral soft and hard tissues

adjacent to the teeth that protect them against the chemical substances. Furthermore, the time set for the experiment was fixed in 4 days. More studies are necessary for the field to test the influence of (1) protection tissues adjacent to the teeth (e.g. performing animal studies) and (2) time variations (e.g. setting longitudinal studies). In general terms, the present research provided a technical observation on the isolation and amplification of DNA from teeth found in complex circumstances, mimicking the new trend of evolved criminal mechanisms against the forensic evidence.

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