Original Article

Dental Tissue as an Imperative Marker for Human Identification in Mass Disaster

Anshika Khare, Vrinda Saxena, Manish Jain, Vidhatri Tiwari, Binu Santha, Vijayta Sharva

Department of Public Health Dentistry, People's Dental Academy, Affiliated by People's University, Bhopal, Madhya Pradesh, India

Abstract

Background: Mass disaster events allied with astounding damage of humanity, consequently leading with complexity to recognize human eccentricity. Forensic science has an enormous role in human identification after disaster. As human teeth are admirable source of DNA due to their relatively higher degree of physical and chemical resistance; thus, revival of genetic material is promising with teeth; in case of disasters. Therefore, human teeth can be used as an imperative resource for human identification in mass disasters.

Aim and Objectives: This study aims to compare DNA quantity and purity, of extracted human teeth buried in soil and establishes the result that dental tissue can be used as an imperative marker in human identification.

Material and Methods: An *in vitro* experimental study conducted with 30 extracted human teeth. All teeth divided into two groups depending on the time of buried in soil – (i) Group 1 (old group): It was comprised of 14 teeth, buried for 12 months and (ii) Group 2 (new group): It was comprised of 16 teeth, buried for 6 months. Then, DNA isolation, quantification, and purity assessment was and results analyzed by SPSS version 20 using paired and unpaired Student's *t*-test.

Results: This study illustrates that the entire samples were amplifiable in polymerase chain reaction and showing reverently high-quality results. DNA purity was not significantly affected by the storage period of teeth in soil.

Conclusion: The study concluded that DNA isolation and assessment of quantity and purity can be successfully done from extracted teeth buried in soil. The quantity and purity of DNA retrieved from those teeth who buried for 6 months was high. The quantity of DNA was significantly affected by the storage period of teeth but the purity or quality of DNA was not. Thus, it established the fact the dental tissue can be used as an imperative marker for human identification.

Key Words: *Dental pulp, DNA, polymerase chain reaction technique*

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Introduction

Mass disasters are events dealing with astonishing trash of human kind. Millions of people are dying in mass disasters in all over the world; resulting in damages, disability even fatality of large number of people. It is extremely difficult to recognize human identity following such events; according to Rajshekhar *et al*., disaster response and management has always been one of the biggest challenges to a community.[1]

Forensic sciences have a huge role in human and victim identification in mass disasters and criminal cases as well. Human teeth are marvelous source of DNA due to their relatively higher degree of physical and chemical resistance thus revival of genetic material is promising with teeth in case of disasters.

Teeth – due to their composition – are the most resistant tissue of the human body; dental enamel provides high resistance against adverse conditions that can degrade DNA as well as whole dental structure.[2] Teeth location within the jawbones are largely protected from the environmental and physical conditions, jawbone provides additional protection to DNA compared to bones and making them a preferred source of DNA in many cases.[3,4] Therefore, DNA extracted from teeth is often of higher quality[5,6] and is less prone to contamination than DNA extracted from bones.[7]

Polymerase chain reaction (PCR) technique has achieved increased importance for postmortem DNA analysis in forensic cases because of the millions of copies amplified from one specific sequence of DNA.[8] After PCR, DNA profiling systems can reveal the exact identity of a person.

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Address for correspondence:
Dr. Anshika Khare, E-mail: khare.anshika58@gmail.com

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The currently performed DNA profile tests are very reliable and give details about an individual's identity, sex, physical characteristics, ethnicity, and place of origin.[9]

Few studies have been carried out using teeth as sources in victim identification, but the evidence and literature was Scarce. Hence, this study is being conducted to scrutinize the use of teeth in identification of human in mass disasters utilizing Hi PurA™ Forensic Sample Genomic DNA Purification kit, under standardized protocol and quantification and purity assessment by PCR technique and spectrophotometric analysis.

Materials and Methods

To ascertain the fact that dental tissue can be used as an essential marker for human identification; an *in vitro* experimental study, carried out on extracted human teeth, collected from different patients reported in the Department of Public Health Dentistry, People's Dental Academy, Bhopal, for their extraction within 1 week in June 2016. Tooth extraction was performed with the help of standard GDC extraction (forceps and elevator) kit. Informed consent obtained from all the patients ahead of their tooth extraction and attaining their demographic details. A total of 30 teeth collected for *in vitro* experimental procedure.

Ethical clearance obtained from the Institutional Ethical committee of People's Dental Academy, Bhopal, and prior acquiescence to perform the study obtained from the Director, Centre for Scientific Research and Development (CSRD) People's University, Bhopal.

To make sure the viability of proposed methodology, pilot test was performed on five teeth at CSRD, People's University, Bhopal.

Inclusion criteria and exclusion criteria

Teeth with vital pulp and intact teeth were included in the study whereas teeth, which are nonvital and carious teeth, excluded from the study.

Methodology

Sample (teeth) collection

A total of 30 teeth were collected from the different patients. Then, all teeth were divided into two groups (named as group 1/old group and group 2/new group) depending on the time of buried in soil.

- Group 1(old group):- It was comprised of 14 teeth, buried for 12 months
- Group 2 (new group):- It was comprised of 16 teeth, buried for 6 months.

Immediately after the completion of buried time in soil DNA isolation, quantification, and purity assessment was perform by following steps:

1. DNA extraction
2. DNA purification
3. Determination of the Quantity and Quality of isolated DNA
4. Agarose Gel electrophoresis
5. Primer dilution
6. PCR analysis.

Figure 1: Picture of amplifiable tooth samples

DNA extraction

Wash the teeth (commercial bleach and 100% ethanol for 5 min than distilled water)

↓

Dry at room temperature

↓

Cut the teeth in longitudinal section (with high-speed hand piece and bur in the presence of cool sterile water)

↓

Pulp was removed with sterile spoon excavator and placed into a 1.5 ml collection tube

Then DNA isolation was done with the help of isolation kit (Hi PurA™Forensic Sample Genomic DNA Purification Kit).

DNA purification

It was done according to method Maniatis *et al.*, 1982.[10]

i. A solution of bovine pancreatic ribonuclease A (1 mg/ml) was prepared in 0.3M sodium acetate, pH 5.0
ii. To each DNA preparation, RNase A was added to a final concentration of 20 µg/ml and incubated for 1 h at 37°C
iii. The mixture was deproteinized once with Phenol : Chloroform : Isoamyl alcohol (25:24:1, v/v) followed by twice with Chloroform : Isoamyl alcohol (24:1, v/v)
iv. The aqueous phase was recovered and one-tenth the volume of 3 M sodium acetate (pH 5.0) was added to the supernatant. The DNA was precipitated with double the volume of chilled absolute ethanol
v. The precipitated DNA was recovered by centrifugation at 10,000 rpm for 10 min at 20°C and washed with 70% ethanol
vi. The DNA pellet was lyophilized and re-hydrated with TE buffer (10 mM Tris HCl, pH 7.5, 1 mM ethylenediaminetetraacetic acid, pH 8.0).

Determination of the quantity and quality of isolated DNA

The purity of DNA was tested by spectrophotometric method at 260 nm and 280 nm followed by qualitative checking on 0.8% agarose gel (Maniatis 1982).[10]

The ratio of absorbance at 260–280 nm indicates the purity of DNA samples.

A_{260}/A_{280}=	1.7–1.8	→	Pure DNA
	>1.9	→	RNA contamination

<1.8 → Protein contamination

Agarose gel electrophoresis

Gel electrophoresis of the genomic DNA was carried out for qualitative analysis of samples prepared. A good DNA preparation appears as a sharp single band. A submarine horizontal agarose slab gel apparatus as described by Maniatis *et al.* 1982[10] was used [Figure 1].

Primer dilution

In the laminar flow hood, reconstitute the dried oligos/polymer in molecular biology grade water to make a 100 µM stock solution. From the nano moles in the synthesis-: e.g., 46.6 nmoles. To make the 100 µM stock, multiply this by 10 and add that many µl of water. Make a small amount of working solution by diluting aliquoted 100 µM stock with molecular biology grade water. 1:10 giving a 10 µM solution for genomic PCR (1 µl of this stock in a 20 µl PCR mix gives 0.4 µM of primer in the final mix).

Polymerase chain reaction analysis

Amplification of DNA was done by PCR analysis carried out as per the standardized protocol.

The amplified fragments were separated on 1.5% agarose gel/1X TBE buffer and subjected to electrophoresis at a constant voltage of 70 for 2–3 h. Finally, the gels stained with ethidium bromide (0.5 µg/ml), visualized under ultraviolet rays, and documented in Bio-Rad Gel Doc System. Each PCR was conducted as an experiment, with controls (distilled water instead of template DNA) to test the purity and viability of reagents.[11] The analysis was performing for all the samples at least three times with each selected primers to check the reproducibility.

Results

After performing PCR and spectrophotometric assay in both group of samples the quantity and purity of DNA was compare by SPSS version 20 using paired and unpaired Student's '*t*'-test.

Table 1 shows the mean value of DNA concentration before PCR in Group 1 and 2 were 56.67 ± 25.14 and 155.20 ± 34.11 respectively and the result was statistically significant (*P* = 0.001). Whereas the mean value of DNA concentration after PCR in Group 1 and 2 was 307.99 ± 62.68 and 337.84 ± 30.17, respectively, and the result was not statistically significant (*P* = 0.101) [Table 1].

As shown in Table 2, purity of DNA concentration in Group 1 and 2. The mean values of purity in Group 1 and 2 were 1.64 ± 0.07 and 1.66 ± 0.06, respectively, and the result was not statistically significant (*P* = 0.392) [Table 2].

This study illustrates that the entire samples were amplifiable in PCR and showing reverently high-quality result, but the protein contamination in the older sample was higher as compared to that newer even after nucleic acid purification. DNA purity was not significantly affect by the storage period of teeth in soil.

Table 1: Mean and standard deviation of DNA concentration in Groups 1 and 2 before and after polymerase chain reaction

	Mean±SD	
	DNA concentration before PCR	DNA concentration after PCR
Group 1	56.67±25.14	307.99±62.68
Group 2	155.20±34.11	337.84±30.17
Total	109.22±58.17	323.91±49.61
Minimum	12.09	201.46
Maximum	88.02	397.26
Unpaired *t*	8.891	1.696
P	0.001 (HS)	0.101 (NS)

PCR: Polymerase chain reaction, SD: Standard deviation, HS: Highly significant, NS: Not significant

Table 2: Mean and standard deviation of DNA purity between Groups 1 and 2 (OD 260/280 ratio)

	DNA purity (mean±SD)
Group 1	1.64±0.07
Group 2	1.66±0.06
Total	1.65±0.06
Student's *t*	0.870
P	0.392

SD: Standard deviation

Discussion

This study conducted among 30 extracted teeth buried in soil for 12 and 6 months (to achieve the cause of mass disaster). This was a pioneer attempt to establish the results that dental tissue can be used as an imperative marker in human identification after mass disaster. The main focal point of this study was DNA isolation, DNA quantification, and purity assessment of extracted teeth conditioned for disaster environment by burying in soil.

This study gives strong evidence that teeth are richest source of DNA in mass disasters because DNA isolation was possible from both groups of teeth buried for 12 and 6 months. Our results are in synchronization with study conducted by Devaraju *et al*.,[12] in this study, teeth were incinerated from 100°C to 800°C, genomic DNA was obtained only between 100°C and 300°C whereas it was not obtained above this temperature. When the teeth incinerated from 300°C to 800°C mtDNA extracted from 300°C to 700°C, but no DNA obtained above 700°C and concluded that teeth are the richest source of DNA even in cases where the specimens are highly decomposed.

In our study, DNA concentration was significantly different in Group 1 and 2 before PCR. Teeth those buried for 6 months had a higher DNA concentration as compare to those buried for 12 months. However, it can be compensate by PCR because million number of amplified DNA can form by PCR. It shows that DNA concentration was dependent on storage period of teeth. A comparable result was found in the study conducted by Rubio *et al*.,[13] where teeth were stored at room temperature for time ranging from 1 to 18 months. They observed a 50% decline in nuclear DNA quantity in the 1st month, followed by a period of stability until a further reduction at 18 months. The average DNA yields from the

18-month group were only 10% of those obtained from the fresh teeth.

According to this study, teeth those buried for 6 months had a slight higher DNA purity as compare to those buried for 12 months, but it was not statistically significantly. Therefore, it confirms that DNA purity is not considerably dependent upon duration buried in soil.

The current study wrap up by substantiating, DNA isolated from dental pulp is ample in amount by which identification of human is very much possible in the mass disasters. Our result is in concord with the Malaver and Yunis[5] study, conducted in 2003. In this study, 20 teeth obtained from unidentified bodies buried in 1995 and exhumed in 2000, providing 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*.[14] investigated the efficiency of DNA extraction from hard dental tissues at different concentrations of a decalcifying solution. The DNA obtained from the dental pulp was of high molecular weight, which allowed analysis by multilocus probes or PCR. On the other hand, the material obtained from the hard dental tissues showed satisfactory analysis only by the PCR technique.

Last of all explains that DNA obtained from dental tissue had unique in quality, purity, and effortlessly used for forensic purposes.

Conclusion

The study concluded that DNA isolation, amplification, and purity assessment made possible from buried extracted human teeth. The entire samples were amplifiable in PCR and showing reverently high-quality result but the protein contamination in the older sample was higher as compared to that newer even after nucleic acid purification. DNA purity was not significantly affected by the storage period of teeth in soil. Further, to identify exact identity of a person STR analysis can also be perform from these sample volumes. It established the result that dental tissue can be used as an imperative marker to recognize human identity in disaster management.

Limitations

Since the sample, volume was less; the recovery procedure was difficult to conduct and STR analysis not performed. Thus, only DNA isolation, amplification, and purity assessment was done.

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Conflicts of interest

There are no conflicts of interest.

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