

Sex determination from tooth pulp deoxyribonucleic acid using polymerase chain reaction

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Introduction

Identity is the valuable embellishment for each human throughout his entire life. Multiple incidents occur varying from natural calamities to human-made calamities, including earthquakes, volcanic eruptions, floods, limnic eruptions, tsunami, cyclones, and tornadoes. From the birth to death, human body undergoes various changes, which

Abstract

Introduction: In this fast era of numerous unwanted disasters and because of the severely devastated and degenerated body remains, personal identification of unknown remains has become the most difficult and challenging task. In such instances, dental pulp plays a vital role in identification through deoxyribonucleic acid (DNA). **Aim:** The aim of the study is to determine sex from tooth pulp tissues by DNA analysis using polymerase chain reaction amplification method under different environmental conditions. **Materials and Methods:** The human extracted teeth were exposed to different conditions such as heat, soil, and open environment. The DNA was extracted from all these teeth including freshly extracted teeth, then quantified, and further amplified with male and female primers. **Results:** Quantity of DNA content achieved ranged from 5.21 to 62.87 ng/ μ l. The accuracy in determining sex from pulp DNA ranged from 92% to 100% in the study groups, except from the teeth exposed to uncontrolled heat, as the pulp tissue was burnt completely. The intergroup analysis was statistically highly significant ($P < 0.001$). Gender determination using the quantity of DNA was found to be nonsignificant ($P > 0.05$). **Conclusion:** The dental pulp is the reliable source for sex determination in the humid or dry environment compared to uncontrolled heat.


Key words: Dental pulp, DNA, DNA amplification, forensic odontology, forensic science, sex identification

include degeneration and aging.^[1-3] People, places, and things involved in criminal activities or any natural disaster are very well depicted by forensic science. This incredible science lends hand in investigating and delivering judgment for criminal or civil cases and naming an unknown individual during these proceedings. These days, forensic odontology is considered reliable and accurate, for personal

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How to cite this article: Pawar RK, More CB. Sex determination from tooth pulp deoxyribonucleic acid using polymerase chain reaction. J Forensic Dent Sci 2018;10:107-10.

Access this article online	
Website: www.jfds.org	Quick Response Code 
DOI: 10.4103/jfo.jfds_67_16	

identification, particularly in several fatality incidents and full devastation of body tissues.^[1,4-7]

Various hard and soft tissue structure of the human body undergoes disintegration when exposed to environmental conditions. However, teeth structure remains unaffected, and hence, each structure is used in forensic science for personal identification. Deoxyribonucleic acid (DNA) of tooth pulp plays a vital role in sex determination.^[5,6] The modern progress in molecular biology has transfigured each and every aspects of forensic dentistry. DNA is the expression of life, which capitulates information outside our imagination. DNA fingerprinting is an important tool in forensic odontology for personal identification and detection of mysterious human remains.^[3-8]

Teeth are considered as an exceptional resource of genomic DNA due to their resistance against environmental battering such as ignition, acid immersion, trauma, mutilation, and disintegration. Since natural teeth are the most durable of all tissues, they can persist definitely much longer than other skeletal structures, which have been destroyed by physical agents.^[3-5] DNA amplification by polymerase chain reaction (PCR) method is uncommon due to high cost and lack of forensic laboratories. To date, very few studies of minimal sample size are conducted in India on this subject. After searching various databases till date, we did not come across any study related to the use of tooth from ashes of the burnt body, soiled tooth, and tooth exposed to the environment for human identification. Hence, the aim of the study was to determine sex from tooth pulp tissue by DNA analysis using PCR amplification method under different environmental conditions.

Materials and Methods

The present study was conducted after obtaining approval from the Institutional Ethics Committee bearing number SVIEC/ON/DENT/BMPG13/D14222. The total of 200 teeth was equally divided into four groups – Group I: freshly extracted [Figure 1a], Group II: collected from crematorium [Figure 1b], Group III: teeth kept in open environment for 6 months, without any preservative, in open petri dish [Figure 1c], and Group IV: teeth buried in underground soil at 30 cm depth, for 6 months. During the teeth burial period, the atmospheric temperature ranged from 15°C to 25°C. The temperature below the ground may be approximately 5°–7° less than the atmospheric temperature. The underground temperature was impossible to regulate and monitor [Figure 1d].

Each tooth was subjected to pulp retrieval procedure with necessary precautions. DNA was extracted through DNA extraction buffer solution. The extracted sample was washed with 100% and 70% ethanol subsequently. The DNA was then quantified by nanospectrophotometer. The sample

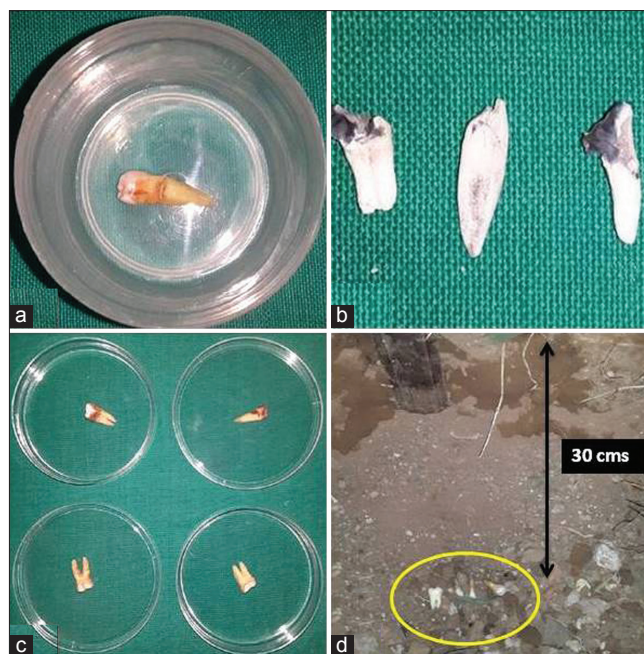


Figure 1: (a) Shows freshly extracted tooth, (b) teeth collected from crematorium, (c) teeth kept in open environment for 6 months, (d) teeth buried in underground soil, at depth of 30 cm for 6 months

was then amplified through PCR with male (AMELY) and female (AMELX) primers. Further, the samples were then subjected to gel electrophoresis (0.8% agarose gel) and then observed under ultraviolet light transilluminator for analysis. The PCR amplicon if reached up to 977 base pairs (bp) or 788 bp of DNA ladder, then the sample was concluded to be of female [Figure 2a] or male [Figure 2b], respectively. The statistical analysis was conducted with Statistical Package for Social Sciences (SPSS) Version 16, (International Business Machines (IBM) Corporation, New York, USA) using the ANOVA test, *post hoc* Tukey's test, and discriminant function analysis.

Results

The total of 200 participants was equally divided into four groups – Group I (50), Group II (50), Group III (50), and Group IV (50). The study participant's age ranged from 21 years to 84 years with a mean of 53.36 + 11.08 years. It was distinctly observed that the obtained quantity of DNA in Group I ranged from 41.84 to 62.87 ng/μl; in Group III, 12.12–49.16 ng/μl; and in Group IV, 5.21–41.98 ng/μl. The overall DNA quantity ranged from 5.21 ng/μl to 62.87 ng/μl. Unfortunately, the DNA quantity could not be obtained in Group II. These findings were statistically highly significant ($P < 0.001$) [Table 1].

It is significant to note that there was no difference in DNA quantity of male and female participants ($P > 0.05$) [Table 2]. The accuracy rate in determining sex from pulp DNA in Group I, III, and IV was 100%, 94%, and 92%, respectively, and was highly significant ($P < 0.001$) [Table 3].

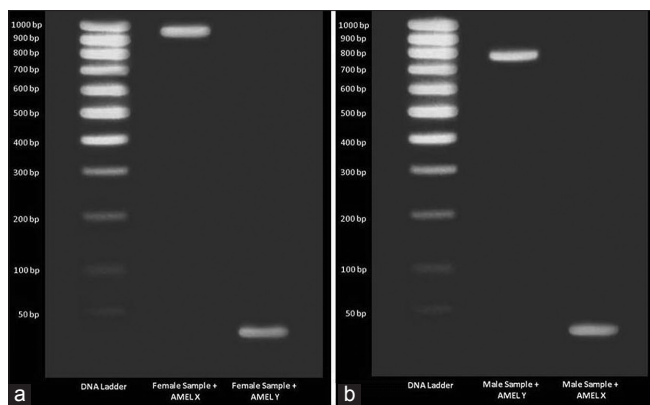


Figure 2: Shows gel electrophoresis results: (a) for female sample, (b) for male sample

Discussion

The anatomical position of the dental pulp protects it from various stimuli such as temperature, microbes, or oral fluid. Pulp offers the best source of DNA for reliable genetic analysis in forensic science. DNA is an identical unit of each individual. The smallest amount of DNA can divulge and decipher the biggest mystery. The quality and quantity of pulp tissue will depend on the environmental insult.^[5-8]

In the present study, we were able to retrieve pulp tissue only in three groups except in Group II. It is for the first time that such types of groups have been studied globally. The mean DNA quantity yielded from all the freshly extracted teeth 54.64 ng/μl, which was sufficient to amplify the DNA. Naik *et al.*^[9] were able to retrieve the mean DNA quantity of 26.41 ng/μl, which on correlation with our study was significantly low. In our study, the freshly extracted teeth showed a wide range of DNA quantity. The reason for the same may be the varying size of the pulp cavity, which is directly proportional to the age of the participant.

Similarly, the mean DNA quantity yielded from teeth buried in the soil at the depth of 30 cm for 6 months (Group IV) was 28.56 ng/μl and was sufficient for amplification, which resulted in 92% positive results. Battepati and Shodan^[10] in their study achieved 100% positive results in amplifying the DNA quantity extracted from 30 teeth buried in the soil for 2 months. This discrepancy may be due to varying duration and temperature gradient of the soil. Malaver and Yunis^[11] were successful in quantifying DNA contents from the teeth collected from the bodies, which were buried for 5 years. From this, it can be concluded that pulp DNA can be quantified up to 5 years of burial.

In our study, the teeth collected from crematorium did not yield pulp tissue. The reason for this may be that the teeth were exposed to an uncontrolled high temperature which must have led to total burning out of pulp. It is

Table 1: Quantification of pulp deoxyribonucleic acid through nanospectrophotometer

Group	DNA quantity (ng/μl)			Significance
	Minimum	Maximum	Mean ± SD	
I	41.84	62.87	54.64 ± 4.97	<0.001 (HS)
III	12.12	49.16	35.90 ± 5.66	
IV	5.21	41.98	28.56 ± 8.55	

HS: Highly significant, ng/dl: Nanogram/deciliter, SD: Standard deviation, DNA: Deoxyribonucleic acid

Table 2: Discriminant analysis of pulp deoxyribonucleic acid quantity for sex determination

Group	Sex	Mean of DNA quantity (ng/μl)	Obtained P	Significance
I	Male	54.61 ± 5.50	0.965	P > 0.05 (NS)
	Female	54.68 ± 4.19		
III	Male	35.58 ± 5.77	0.514	
	Female	36.79 ± 5.43		
IV	Male	27.05 ± 8.86	0.111	
	Female	31.03 ± 7.60		

ng/dl: Nanogram/deciliter, NS: Not significant, DNA: Deoxyribonucleic acid

Table 3: Polymerase chain reaction amplification of pulp deoxyribonucleic acid in study groups

Group	Positive conclusion, n (%)	Negative conclusion, n (%)	Significance
I	50 (100)	0	<0.001
II	0	50 (100)	(HS)
III	47 (94)	3 (6)	
IV	46 (92)	4 (8)	

HS: Highly significant

significant to note here that even the teeth at the time of collection were morphologically altered and the parts were deformed.

The studies conducted by Silva *et al.* and Vemuri *et al.* on controlled heat gave contrary results to our study. Silva *et al.*^[12] had exposed the teeth to controlled heat at 600°C, 800°C, and 1000°C. They concluded that at 600°C, they could quantify pulp tissue and the DNA content in less quantity, but beyond the pulp tissue was unable to retrieve. Similarly, Vemuri *et al.*^[13] exposed the teeth samples at 100°C, 200°C, 300°C, and 400°C. They could quantify sufficient amount of pulp tissue up to 200°C but could not beyond it.

Conclusion

The experimental groups in our study were unique. The accuracy in determining sex from dental pulp ranged from 0% to 100%, depending on the study group. We could not achieve the sex determination from the teeth exposed to uncontrolled heat. The less amount of DNA content was yielded in teeth samples buried under soil. The quantity of DNA did not show any significant difference in teeth of male and female participants.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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