

Quantitative evaluation of DNA from the tooth pulp exposed to varying temperatures

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Received : 16-12-2015
 Review Completed : 06-04-2016
 Accepted : 28-06-2016

Keywords : DNA, temperatures, forensic dentistry, pulp, tooth

Abstract :

Context : During natural calamities like fire accidents, many times the human body gets charred beyond recognition. Tooth, being the hardest structure in the human body protects the pulp within from such accidents. Forensic identification through the pulpal DNA could be a very useful tool in such situations.

Aim : This study was designed to evaluate the quantity of DNA obtained from the pulp when exposed to varying temperatures.

Methods and Material : Extracted teeth were subjected to the following temperatures:-80°C, 37°C, 100°C, 200°C, 300°C, 500°C and 1000°C. Pulp from these teeth were retrieved by horizontally sectioning the teeth using a fine needle diamond point in a high speed air rotor, followed by extraction of DNA with the HipuraA™ Forensic Genomic DNA purification spin kit. The samples were quantified using personal computer(PC) based double beam spectro photo meter. Kruskal Wallis test and Mann Whitney U test were used for statistical analysis.

Results : Statistically significant difference ($p < 0.005$) was seen in the quantity of DNA obtained from pulp subjected to higher temperatures as compared to oral temperature. However, there was no significant difference ($p > 0.05$) in the quantity of DNA obtained from teeth subjected to -80°C

Conclusions : Increase in temperature decreases the amount of DNA from the tooth pulp where as a decrease in temperature does not cause any change in the quantity of DNA.

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Introduction

Teeth are the hardest structures in the human body.¹ During a disaster even after the other tissues are destroyed, teeth have been found to survive.² The hardness of the tooth is due to the extremely mineralised and highly resistant outer tissue, the enamel³. The pulp, located in the central area of the tooth is a loose connective tissue and it consists of cells, fibres, ground substance and nerves. It is also a very rich source of DNA.⁴ The DNA contains each individual's unique genetic information. The double stranded molecule carries genomic information from one strand to the other. The nucleus present in the cells of the body contains genomic or nuclear DNA that contain the genetic material from both parents, where as, the mitochondria contains mitochondrial DNA and is representative of maternal

inheritance⁵. Teeth are excellent sources of genomic and mitochondrial DNA.⁵ Because of its location, the dental pulp, being surrounded by hard tissues, is preserved from extreme environmental conditions. Hence, the DNA which is present abundantly in the pulp also remains preserved. With the advent of Polymerase Chain Reaction (PCR) technique, even small amounts of DNA recovered from the tissues can be characterized and be used for DNA fingerprinting.⁴ This technique can be valuable in identification of victims of crime or disasters when conventional methods fail.

The objective of this research was to evaluate the effect of temperatures on the quantity of pulpal DNA. This knowledge would help the forensic odontologist to

determine whether the DNA obtained from such teeth be sufficient for further processing for identification or sex determination.

Methods

Collection of Teeth

After obtaining the ethical clearance from the University Ethical Committee, forty eight freshly extracted maxillary second premolar teeth were collected in sterile vials containing 20ml of saline. Each tooth was kept in separate bottle to avoid contamination.

Conditions of Exposure to various temperatures

The teeth were divided into eight groups of six teeth each.

Group 1 was the control group – oral temperature (37°C)

Group 2 was subjected to -80°C in a deep freezer (REMI) for five days

Group 3, 4, 5, 6 and 7 were subjected to 100°C, 200°C, 300°C, 500°C and 1000°C in a dental burnout furnace (Ivoclarvivadent, Programat P300) for 10 minutes each.

Group 8 was subjected to direct flame for 2 minutes.

Tooth Sectioning

The teeth in each group were subjected to their respective temperatures and were then sectioned at the cement-enamel junction using a fine needle diamond point in a high speed air rotor.^{vi} Constant cooling of the area being sectioned was ensured with a copious amount of water spray from the air rotor. Sectioning was done from all around the tooth towards the centre where the pulp was present. This was done to prevent injury and loss of the pulp. Once the sectioning cuts were made all around the tooth the final separation of the crown from the root was carried out by breaking it gently with fingers. This ensured that the entire pulp was retained intact without any loss.

Retrieval of the Pulp

Using a number 15 endodontic broach, the entire pulp was extirpated from the root. The pulp horn within the sectioned crown was probed with the broach to retrieve any fragment of the pulp within. The pulp thus recovered

was immediately placed in sterile vials containing 2 ml of saline. They were stored in -20°C till all samples were retrieved for further processing of DNA extraction.

DNA Extraction and Quantification

Genomic DNA was extracted according to instructions of the HipurA™ Forensic Genomic

DNA Purification Spin Kit. DNA quantification was carried out using a Systronics P.C based double beam UV spectrophotometer at 260 and 280 nm (The wavelengths for which DNA and protein absorb). The elution buffer was used to dilute the samples and to calibrate the spectrophotometer. Concentration of DNA was calculated using the formula,

$$\text{Concentration of DNA sample } (\mu\text{g/ml}) = 50 \times A_{260} \times \text{dilution factor}$$

The data was tabulated (Table 1) and was analysed using Kruskal Wallis test and Mann Whitney U test (Table 2). The P value was set as 0.005.

Table 1 : DNA recovered from each sample

TEMPERATURE(°C)	DNA (µg/ml)					
	1	2	3	4	5	6
37	3.09	2.965	3	2.877	3.3.057	3.025
-80	3	2.99	3.037	2.925	3.07	3.065
100	2.61	2.74	2.655	2.635	2.75	2.697
200	2.53	1.78	2.145	2.315	2.5	1.951
300	1.53	0.72	0.805	1.095	0.93	1.227
Direct heat	0.62	0.37	0.35	0.426	0.33	0.485
500*	-	-	-	-	-	-
1000*	-	-	-	-	-	-

*No results for DNA quantity as the teeth were completely charred at these temperatures.

Table 2 : Statistical analysis of the data

TEMPE-RATURE	MEAN (SD)	MEAN DIFFERENCE (95%CI) FROM 37°C	P-VALUE**	% REDU- CTION OF DNA
37°C	3.00(0.075)	-	-	-
(-)80°C	3.01(0.054)	-0.01(-0.09-0.07)	0.810(NS)	-
100°C	2.68(0.057)	0.32(0.23-0.40)	0.004*	0.11
200°C	2.20(0.301)	0.79(0.51-1.08)	0.004*	0.26
300°C	1.05(0.299)	1.95(1.67-2.23)	0.004*	0.65
Direct heat	0.43(0.108)	2.57(2.45-2.69)	0.004*	0.86
H value [†]	33.092(5)			
p-value	<0.005*			

*Kruskal Wallis test
 **Mann Whitney U Test
 * $p < 0.005$ statistically significant
 SD - Standard Deviation
 CI - Confidence Interval
 $p > 0.05$ Non-significant, NS

Discussion

In the present study, extracted maxillary second premolars were subjected to various temperatures, viz., oral, increasing and decreasing (-80°C) temperatures. The pulp from the teeth was extirpated and the amount of DNA present in each tooth was assessed. From this study it was observed that there is a significant decrease ($p < 0.005$) in the quantity of DNA that could be retrieved after subjecting the tooth to higher temperatures (100°C , 200°C , 300°C , 500°C , 1000°C and direct heat) from 37°C . The decrease in the amount of DNA that was observed in our study could be due to autolysis and degradation and/ or fragmentation of DNA as the temperature increased. Lessig et al⁷ conducted a similar study where they concluded that DNA typing is difficult at increased temperatures and was more successful at room temperature. In another study carried out by Vemuri et al⁸, it was also observed that an increase in temperature decreases the quantity of DNA that is retrievable. The results observed in our study are in concurrence with the above.

On assessing the quantum loss of the DNA between each change in temperature, it was observed that there was a steady increase in the percentage loss. This indicates that, as the temperature increased more amount of DNA is lost from the pulp. This could be the reason for another observation from our study that at 500°C and 1000°C there was a total loss of the pulp (Table 1). In a study conducted by da Silva et al⁹, where the teeth were subjected to 6000°C , 8000°C and 10000°C for 10 minutes each yielded $0.188\text{-}0.3\mu\text{g/ml}$, $0.104\text{-}0.418\mu\text{g/ml}$ and $0.04\text{-}0.372\mu\text{g/ml}$ respectively. However, this yield was obtained from the pulverised root portion of the tooth. In our study, we employed the technique of obtaining DNA from the pulp rather than the root. Crushing of the root has disadvantages like contamination by bacterial endonucleases and PCR inhibitors on the surface of the tooth⁶. On the other hand, relying on the pulp for DNA analysis also has its own disadvantages in forensic science. The tooth may not contain pulp at all due to previous endodontic therapy or it may be infected, contaminating the DNA samples⁵. Dentine has also been found to be a potential source of mitochondrial DNA when the quantity and quality of available DNA is limited^{5,6}.

In our study, one group of teeth were exposed to the heat directly to mimic the actual fire disasters. It was observed that the DNA

was able to be retrieved from this group even though to a lesser amount ($0.43\mu\text{g}$). And the percentage of reduction of DNA (0.86%) was higher as compared to the other groups (Group I, II, III, 0.11% , 0.26% & 0.65%). However, in our study the duration of exposure of teeth to direct heat was only for 2 minutes. In reality the teeth are covered and protected by other tissues around them. Hence during a calamity, they are the last structures to be affected by heat. The actual amount of damage to the pulp could be less and the amount of retrievable DNA could be higher. In a case report published by Sweet and Sweet¹⁰, DNA from the pulp of the tooth of a homicide victim was used to identify the victim who was burnt at approximately 1093°C for 30-40 minutes.

Thus, increase in temperature decreases the amount of DNA that can be retrieved, although identification is possible at fairly high temperatures. In addition to studying the effect of higher temperatures, we also assessed the effect of decrease in temperature on the quantity of DNA. The DNA obtained from the teeth stored at -800°C was compared with the DNA at oral temperatures (370°C). It was found that there was no significant difference ($p > 0.05$) in the amount of DNA (3.01 and $3.00\mu\text{g/ml}$ respectively) present in both. This indicates that preserving the human tooth at a decreased temperature does not alter the quantity of DNA present. Burger et al carried¹⁰ out a study on the effect of environmental factors on the preservation of DNA in teeth and concluded that teeth which are preserved at -200°C yield enormous amount of amplifiable DNA. We observed in our study, that storing teeth at lesser temperatures (-800°C) also yielded a significant quantity of DNA.

Our study indicates that during analysis of the tooth, the forensic odontologist could expect the entire amount of pulp DNA for identification of the victim in cases where the teeth were preserved at sub-zero temperatures yet as the temperature increases the quantity of DNA decreases. However, even small traces of DNA that is extracted could be used for sex determination or DNA fingerprinting. In a study conducted by Potsch et al¹², genomic dot blot hybridisation was performed for sex determination from DNA from the dental pulp with only $50\text{-}100\text{ ng}$ ($0.05\text{-}0.1\mu\text{g}$). Furthermore, STR-based systems for PCR techniques provide excellent sensitivity as less as 0.20 ng of DNA.¹³

Conclusion

Within the limitations of the present study, it could be concluded that in case of fire disasters where bodies get charred, by knowing the temperature and also the duration of exposure, it is possible to estimate the quantity of DNA that can be obtained from dental

pulp. This can determine whether amplification could be carried out with the amount of DNA retrieved because insufficient DNA may provide only a partial profile or no profile at all.

References

1. Cameron JM, Sims BG. Forensic dentistry. Churchill Livingstone 1973;23-25
2. Rothwell BR. Principles of dental identification. Dent Clin North Am 2001;45:2253-70
3. Malaver PC, Yunis JJ. Different dental tissues as source of DNA for human identification in forensic cases. Croatian medical journal 2003;44:306-309
4. Girish KL, Rahman FS, Tippu SR. Dental DNA fingerprinting in identification of human remains. Journal of forensic dental sciences 2010;2:63-68
5. Muruganandhan J, Sivakumar G. Practical aspects of DNA-based forensic studies in dentistry. Journal of forensic dental sciences 2011;3:38-45
6. Smith BC, Fisher DL, Weedn VW, Warnock GR, Holland MM. A systematic approach to the sampling of dental DNA. Journal of forensic sciences 1993; 38:1194-1209.
7. Lessig, Edelmann J. Individualisation of dental tissue--an aid for odontological identification? The Journal of forensic odontology 1995;13:1-3
8. Vemuri S, Ramya R, Rajkumar K, Rajashree P. Influence of various environmental conditions on DNA isolation from dental pulp for sex determination using polymerase chain reaction. SRM Journal of Research in Dental Sciences 2012;3:231-235
9. Alves da Silva RH, Queizi R, Pereira Bertolacini CD, MacielCarvalho SP, da Silva Gasque KC, de Almeida-e-Silva CT, RibeiroBicudo LA. Human identification analysis using PCR from the root portion of dental elements under different conditions of temperature and exposure time. RSBO RevistaSul-Brasileira de Odontologia 2012;967-73
10. Sweet DJ, Sweet CH. DNA analysis of dental pulp to link incinerated remains of homicide victim to crime scene. Journal of forensic sciences 1995; 40:310-314.
11. Burger J, Hummel S, Herrmann B, Henke W. DNA preservation: a microsatellite-DNA study on ancient skeletal remains. Electrophoresis 1999; 20:1722-1728.
12. Pötsch, L., et al. Application of DNA techniques for identification using human dental pulp as a source of DNA. International journal of legal medicine 1992; 105.3:139-143.
13. Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM, Smerick JB, Budowle BJ. Validation of STRs for forensic usage: Performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. Forensic Sci. 2001; 46:647-60.