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Original Article

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

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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 avery 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 spink it. The samples were quantified using personal computer(PC) based double beam spectro photo meter. Kruskal Wallis test and Mann WhitneyU 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.

Introduction

Teeth are the hardest structures in the human body.[1]During adisaster 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 enamel[3]. 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 inheritance[5]. 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 finger printing.[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,

Concentration of DNA sample (µg/ml) = 50 x A_{260} x 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

TEMPERATURE	MEAN (SD)	MEAN DIFFERENCE (95%CI) FROM 37°C	P-VALUE##	% REDUCTION 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 al [7] 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 al[8], 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 al , where the teeth were subjected to 6000°C, 8000°C and 10000°C for 10 minutes each yielded 0.188-0.3µg/ml, 0.104-0.418µg/ml and 0.04-0.372µg/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 tooth[6]. 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 samples[5]. 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 (0.43µg). 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 Sweet[10], 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 3.00µg/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 carried[10] 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 al[12], genomic dot blot hybridisation was performed for sex determination from DNA from the dental pulp with only 50-100 ng (0.05-0.1µg). 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.

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