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Quantity of DNA Isolated from the Pulp Tissue of Primary Teeth Exposed to Various Environmental Conditions

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Original Research Article

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Abstract: The isolation of human genomic DNA is an important step in forensic analysis. The pulp tissue of teeth is a rich source of human genomic DNA which is encased in the hard tissue casing of enamel and dentin. The present study was done to analyze the quantity of DNA from the pulp tissue of deciduous teeth after exposure to various environmental conditions.120 deciduous teeth were divided into six groups of 20 each. First group of 20 teeth were kept at room temperature for 3 months . Second group of 60 teeth were divided into 20 each & kept in well water for 3 months, 9 months & 15 months. Third group of 40 teeth were divided into 20 each and kept at sea water for 9 months & 15 months. The DNA isolation was done from all the samples and quantification was done by using Biospectrometer. Mann-Whitney U Test was used for comparison. There was no statistical difference between group I and group II- 3 months as well as group II -9 months & group III – 9 months. There was a statistical difference between group II- 15 months with group III-15 months. Intra group comparison within group II and in group III showed a statistical difference in group III & no difference in group II for 9 and 15 months.
Keywords: Forensic Odontology, DNA analysis, Gender Determination, Polymerase Chain Reaction, Deciduous pulp tissue.

INTRODUCTION

In India as well as over the world, crimes of different nature are on the increase. Both criminals and educated elite of the society, who make use of sophisticated technical measures, while committing their crimes socially, put the forensic scientist, police and the public off the scene. So, crimes challenge society in detection, diagnosis and identification of criminals. Role of forensic scientist, therefore with the scientific diagnostico-forensic aids in detection, and treatment; can never be overemphasized in civilized modern world, if justice is to be sought in solving medico legal problems[1].

Gender determination is an important element in the analysis of biological evidence submitted to forensic laboratories[2]. Sex determination from teeth can provide an important means of personal identification in the event of a mass disaster such as an air plane crash or fire[3]. The accurate identification of human remains is a public duty justified by social, legal and insurance considerations. Fingerprints and dental means represent the most scientifically reliable modes of identification. In general, the greater the degree of tissue destruction, the greater the importance of dental characteristics in effecting proper identification[4].

The enamel and dentin of human teeth act like an armour coating to protect the DNA rich inner aspect of the tooth from various environmental conditions.

Despite exposure of the body to burial, mutilation, explosion or incineration, it is usually possible to extract DNA from pulp tissue of tooth with sufficient quality and quantity to conduct a Polymerase Chain Reaction (PCR) based analysis.

MATERIALS & METHODS

Aim of the study

The present study was done to Isolate & quantify genomic DNA from the pulp tissue of deciduous teeth after exposing the teeth to various environmental conditions

Sample selection

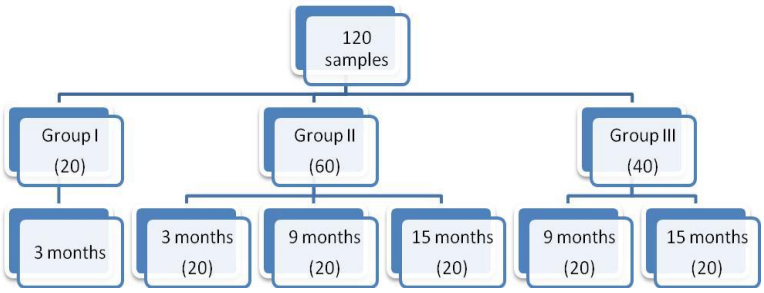
120 deciduous teeth collected from children who reported to the Department of Pedodontics & Preventive Dentistry were selected for the study. Ethical clearance was obtained from the Central Ethics Committee, Nitte (Deemed to be University). Informed consent was obtained from the parents of all children from whom the teeth were collected.

The collected teeth samples were divided into 3 groups

Group I- Deciduous teeth kept at room temperature for 3 months

Group II- Deciduous teeth kept at well water for 3 months,9 months & 15 months

Group III- Deciduous teeth kept at sea water for 9 months & 15 months



Study Design

Inclusion criteria

- Teeth samples were collected from children of both sexes.
- Healthy teeth

Exclusion criteria

- Children with oral or systemic infections
- Teeth with carious lesions
- Parents who have not given consent

Coding of all samples was done. Longitudinal sectioning of teeth was done using a hard tissue microtome and pulp tissue was collected and stored in phosphate buffer saline (PBS) in labelled bottles and refrigerate at -80°C. The equipments used for DNA isolation were: AccuBlock (Digital dry bath) Fig 1, Vortex mixer(Fig 2) and centrifuge(Fig 3).

Fig 1:

Fig 2:

Fig 3:

DNA isolation from the pulp tissue was done using the Nucleospin TissueKit as per the manufacturer's instructions. The isolated DNA samples were labelled and stored at -20°C (Fig 4)

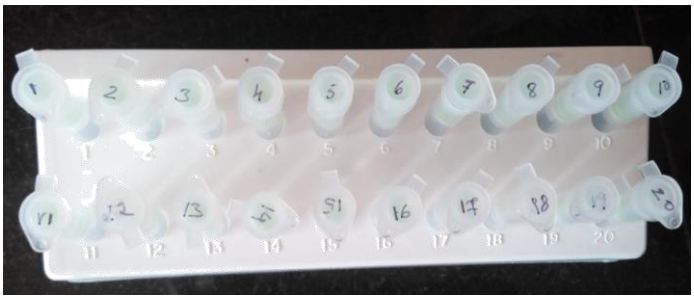


Fig 4:

Fig 5:

SERIAL NUMBER	DESCRIPTION
Fig 1	Accublock (Digital Dry bath) – for DNA isolation
Fig 2	Vortex mixer – for DNA isolation
Fig 3	Centrifuge- for DNA isolation
Fig 4	Labelled DNA samples stored in Eppendorf Tubes
Fig 5	NanodropTM100 Spectrophotometer used for DNA quantification

DNA quantification was done by NanoDrop™ 100 (Thermo Scientific, USA) spectrophotometer (Fig 5)

RESULTS

The following comparison between the groups for DNA quantification has been done.

- Comparative evaluation of group 1 -3 months with group II 3 months
- Comparative evaluation of group II 9 months with group III 9 months
- Comparative evaluation of group II, 15 months with group III ,15 months
- Intra- group comparison in group II
- Intra- group comparison in group III

Table-1: Comparative evaluation of group I-3 months with group II- 3 months

	Median	I Q R	Mann- whitney U
			Test
Group 1B	8.35	4.58 to 14.98	0.185
Group 11	5.75	4.3 to 9.18aa	
3 months			

There is no difference between the groups

Table-2: Comparative evaluation of group II 9 months with group III 9 months

		Median	I Q R	Mann- whitney U
				Test
	Group II	16.5	12.08 to18.48	< 0.133
	9 months			
	Group III	17.55	15.2 to 21.18	
	9 months			
	No significant difference			

3. Comparative evaluation of group II- 15 months with group III-15 months

		Median	I Q R	Mann- whitney U Test
				< 0.001
	Group II	17.55	15.2 to21.18	
	15 months			
	Group III	16.9	11.38 to 19.48	
	15 months			

Since the P value is <0.05 there was a difference in median quantification of DNA between the two groups at 5% level of significance

Table-3: Intra- group comparison in group II -9 months with group II -15 months

	Median	I Q R	Mann- whitney U
			Test
Group II	16.5	12.08 to18.48	< 0.203
9 months			
Group II	17.55	15.2 to 21.18	
15 months			

No Significant difference

Table-4: Intra- group comparison in group III -9 months with group III -15 months

	Median	I Q R	Mann- whitney U
			Test
Group III	16.9	11.38 to19.48	< 0.001
9 months			
Group III	17.7	14.1 to 22.03	
15 months			

Since the P value is <0.05 there was a difference in median quantification of DNA between the two groups at 5% level of significance

DISCUSSION

DNA isolation from the pulp tissue of primary teeth by using Nucleospin kit was successful in all the samples studied. Silica based method was used for the isolation of DNA which has been shown to be a viable alternative to the commonly used organic method 6. In addition to improving the yield of DNA extraction; it also decreases the contamination of proteins and potential chemical injury associated with phenol/chloroform method.

DNA quantification was done by using Biospectrometer. We could quantify DNA from the pulp tissue of all primary teeth irrespective of the type of the tooth, age or sex of the donor. The comparative evaluation of the quantity of DNA gave significant results between the groups.

- There was no statistical difference in the quantity of DNA between group 1 samples kept at room

temperature for 3 months and group II samples kept at well water for 3 months.

- There was no statistical difference in the median quantity of DNA between group II 9 months (well water) with group III 9 months (salt water). From this inference we might assume that the storage medium does not have much influence on the quantity of DNA upto 9 months even though there was a slight increase in quantity of mean DNA in group III which could be due to the type of the teeth stored in group III. Studies have shown that quantity of DNA depends on the type of the tooth. Study by Sara etal found that the quantity of was less in incisor teeth compared to molar teeth.6
- There was a statistical difference in the quantity of DNA between group II- 15 months with group III-15 months. It showed the mean quantity of DNA in group II was more compared to group III after 15 months of exposure. So prolonged exposure in sea water can cause degradation of DNA that could be the reason for the less quantity in group III after 15 months.
- There was no statistical difference in the intra group comparison between group II-9 months & group II 15 months which shows there is no change in the quantity of DNA on exposure of teeth in well water for prolonged period upto 15 months
- There was a statistical difference in the intra group comparison between group III -9 months &group III – 15 months which shows an increase in mean quantity of DNA in group III -15 months compared to group III -9 months. This could be due to the type of the teeth stored in this group.

CONCLUSION

All the teeth samples were selected randomly and stored in different media without taking into consideration the type of the teeth stored. This could be the reason for the varying quantity of DNA in different groups. The integrity and quality of DNA will be assessed during the PCR amplification technique which will give us more information regarding the role of various storage media used in this study for sex determination.

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