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Effect of extreme heat on dna obtained from tooth of burnt corpses

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ABSTRACT

Introduction: Identification of sex has a crucial role in personal identification. This study aims to determine the sex of an individual from burnt teeth samples exposed to a burning temperature of 250^0C for 15minutes and 45 minutes respectively through RT-PCR on amelogenin sex marker.

A total of 60 tooth samples were subjected to the burning temperature at 250^0C for 15minutes and 45 minutes respectively. The tooth was grinded with tissue lyzer and DNA extraction was done by the phenol-chloroform method. All tooth samples were quantified for DNA concentration and then analyzed with RT-PCR

Although there are various methods of DNA isolation and extraction from teeth, yet, comparison across these methods to measure the effectiveness of the specific method is not clearly established. Thus, the primary objective of this study was to measure the effectiveness across the grinding and broaching methods of DNA extraction in freshly extracted tooth pulp.Real Time-Polymerase Chain Reaction (RT-PCR) and subsequent multiplex STR typing.

Method: A total of 40 freshly extracted normal teethwere randomly collected.Isolation and extraction of DNA was doneby organic extraction method. Precipitation of samples was done using 100% chilled ethyl alcohol followed by concentration and washing of DNA via column based techniqueusing DNA binding buffer and DNA wash buffer. Agarose gel electrophoresis was done to roughly estimate the DNA content while exact quantity of DNA was estimated by RT-PCR technique.

Result: Independent sample t test analysis revealed that the mean quantity of DNA (in µg/l) was significantly higher in broaching method (M=29.91, SD=0.65) than grinding (M=9.71, SD=0.45), $t(38)=114.19$, $p<0.000$. Similarly, quality of DNA was analyzed using smear quality and it was found that the quality of DNA for broaching (M=4.55, SD=0.51) was significantly higher that the grinding method (M=2.55, SD=0.6), $t(38)=11.3$, $p<0.000$.

Conclusion: The significant quantitative and qualitative loss of DNAwas observed intooth samples exposed to processed via grinding method compared to broaching method which was further supported by the fact that DNA profiles generated from the DNA extracted using broaching method provided adequate resolution of the autosomal markers andsex identification marker (amelogenin marker) in the present study which isvaluable for human identification and the gender identification finallyleading to individualization.

Keywords: Amelogenin, DNA, Hydroxyapetite, Gender Identification, Electrophoresis,Forensic Investigation

INTRODUCTION

The earthquakes, floods, tornados, wildfires, aircraft crashes may be considered as natural calamities. Mishap or apartment fire is also an additional man-made disaster. These increased mass disasters result in the increasing rate of fatality which poses strain in the identification of the deceased.Forensic Odontology is a field of study and research in forensic science which establishes the personal identification with tooth samples. The Watson & Crick model of DNA in 1953 revolutionized the field of of molecular genetics. In 1985 Jefferey et al has given radiolabeled molecular probes which consist of variable regions of DNA and recognize specific patterns of DNA in individual.This technique of human identification is called DNA fingerprinting.This new technique now became as an adjunct to for judicial delivery system like establishing paternity test and investigations. Although fingerprints have been utilizing for recognition from the specimen exposed to fire, flames, heat and explosions as very rudimentary specimens can be recovered.Cremation by burning of dead bodies is widely used funeral procedure performed in our culture.Mostly cremation takes place at the temperature of 1000^0C for 70 minutes leaving severly burned tooth and fragmented longer bones which may serve as primary ource of evidence.(11) Owing to physical and chemical resistance to extremely unfavourable environmental conditions and incineration tooth has its crucial role in identification where conventional methods can be not used. Owing to highly mineralized and highly resistant to heat and decomposition DNA analysis for sex determination and subsequent identification can easily be done with tooth samples even from fragmented, decomposed and burnt corpses. The DNA in the tooth is principally present in the pulp that can be collected by crushing, sectioning, and trepanation. Anatomically tooth comprises of coronal and radicular sections in which the crown or coronal section is covered by enamel and root or radicular portion is covered by cementum. The crown portion is emerging into the oral cavity while the radicular portion remains embedded into the alveolar ridge.

Based on the morphological characteristics, the tooth can serve as an identification tool. Teeth can serve as a source of human tissue in the form of pulp and human cells in the form of embedded cells in the calciferous material of the teeth for DNA extraction. Tooth pulp is assumed to be the potential source for DNA extraction and validated for forensic identification purpose[5].

Several methods are used to extract DNA from the tooth which facilitates forensic identification. It includes aspartic acid racemization for age estimation, racial origin estimation by analysis of the occlusal surface of a tooth or by the presence of accessory cusp or supernumerary cusp, the cusp of carrebelli in the posterior tooth or shovel-shaped incisors is one of the examples.

The pulp containing central cavity of tooth constitutes the coronal portion called as pulp chamber and pulp canals is a radicular portion of the tooth. The pulp is soft connective tissue & has nutritive, formative, sensory, and defense functions. The coronal and radicular pulp comprises of odontoblasts, fibroblasts, endothelial cells, peripheral nerve cells, undifferentiated mesenchymal cells, and nucleated blood cells. The highly developed vascular network enters through apical orifice present at the tip of root with ramifications into the sub-odontoblastic region.

The objective of our study is to compare the quantity and yield of DNA by agarose gel electrophoresis and real-time polymerase chain reaction and subsequent multiplexing with amelogenin marker for sex determination in Delhi NCR region.

MATERIALS AND METHODS

Participant/sample

A total of 60 teeth were randomly selected from various dental clinics of the Delhi-NCR region as a sample for this study. Consent from the person who was scheduled for dental extraction was obtained. The extracted teeth collected from the patients had a history of some periodontal pathology/wasting disease/moderately carious tooth. The samples were collected based on the criteria of mild to moderate carious tooth, tooth undergoing orthodontic extraction, extraction performed by the registered dental practitioner, participants of the age range of 20 to 60 years, and permanent mandibular premolar and molar. Since premolar and molar teeth are voluminous thus they were assumed to be a rich source of DNA compared to the incisors and canines. However, a grossly carious tooth, an anterior tooth-like central incisor, lateral incisor, canines& primary teeth as well as permanent tooth under root canal treatment was excluded from the study. Each tooth of the collected sample is considered as an exhibit in this study. Sample preparation was done by washing the extracted tooth with the solution of ethyl alcohol before grinding. Tissue lyzer machine was used for grinding the tooth. All the laboratory work was performed in the Central Forensic Science Laboratory, CBI, New Delhi, a NABL accredited laboratory strictly as per the DNA standards.

Materials

The chemicals used for Phenol-Chloroform-Isoamyl Alcohol (PCI)based organic extraction method was-

i. Proteinase K (20mg/ml),EDTA(0.5 M; pH 8.0),
ii. Sodium Dodecyl Sulfate (SDS),
iii. Sodium Chloride (NaCl),
iv. Tris Saturated Phenol: Chloroform: Isoamyl alcohol i.e. PCI (25:24:1 v/v),
v. Absolute Ethyl alcohol,
vi. 70% Ethyl Alcohol,

DNAClean & Concentrator Kit (M/S Zymo Research) was used to clean &concentrate the DNA for further downstream processing. The chemicals used for Quantitation of DNA done using both Agarose Gel Electrophoresis method as well as RT-PCR method were:

i. Bromophenol blue loading dye,
ii. Interchelating Ethidium bromide solution
iii. Tris-Acetic acid-EDTA (TAE) running buffer.
iv. Quantifiler Human DNA quantification kit

The outcome of the study was achieved by using the following seven-step procedure-

S**tep 1:**Eachtooth sample was pulverized tissue Lyzermachine after washing it with ethyl alcohol as well as MilliQ water thoroughly and collected in a 1.5 ml Eppendorf

tube. After each step of the crushing of the tooth, the jar was washed with warm water to avoid contamination.

Step 2: 300 µl Stain Extraction Buffer + DTT (0.006gm/ml of SEB) was added to each Eppendorf tube containing the tooth sample. Each sample was vortexed for 15 seconds to facilitate homogenization.20 µl Proteinase-K was added to each sample and they were incubated for 48hours at 56°C in Hot Water Bath Shaker. The samples were vortexed followed by centrifugation for 5min at 5000rpm. The supernatant was scooped out carefully using micropipette into fresh Eppendorf tubes.

Step 3:300µl PCI was added to each tube and centrifugation was done at 15000 rpm for 15 minutes. The top layer was scooped out into fresh Eppendorf tubes.

Step 4: Then precipitation was done with 100% chilled ethyl alcohol. The tubes were in Deep Freezer for 20 minutes at -20°C. It is followed by centrifugation at 10,000 rpm for 10 min. The supernatant was removed from each tube. Washing of the pellet was done with 70% Ethyl alcohol.

Step 5: DNA purification and concentration were achieved by DNA binding buffer and DNA wash buffer (supplied with DNA Clean & Concentrator Kit, M/S ZymoResearch).

Step 6: Basic quality and quantity of DNA was obtained by Agarose gel electrophoresis technique and accurate Quantitation of DNA was evaluated by Real-time PCR technique. The gel electrophoresis technique is a semi-quantitative/qualitative assay that allows for the estimation of the concentration and quality of DNA present in a specimen. The method consists of the electrophoresis of DNA in an agarose gel matrix by incorporating a fluorescent intercalating dye such as Ethidium bromide (EtBr). The agarose gel-based quantitation has two step procedures. It comprises of preparation of gel followed by Electrophoresis. The same samples were analyzed by Quantitative PCR (Real-Time PCR). It is one of the most accurate, precise, and efficient methods currently available for human DNA quantitation. The rate of progression of the amplification process is detected and measured by the accumulation of fluorescent dyes. The initial quantity of DNA in the sample was detected by monitoring the exponential growth phase of the reaction. It is also measured by the cycle number at which the fluorescent intensity of the sample overcomes the background noise or threshold. This cycle number is directly proportional to the quantity of DNA in the reaction. Analysis of the quantity of DNA in the sample is performed using software that compares the unknowns with the best fit regression line constructed from the standards. The principle behind the method is that as the PCR amplification process progresses, there is an increase in fluorescence from SYBR Green dye. As the SYBR Green dye binds to double-stranded amplicon, it undergoes a conformational change and emitsfluorescence at a greater intensity. The TaqMan probe principle relies on the 5´–3´ nuclease activity of Taqpolymerase to cleave a dual-labeled probe during hybridization to the complementary target sequence and fluorophores-based detection. As in real-time PCR methods, the resulting fluorescence signal permits quantitative measurements of the accumulation of the product during the exponential stages of the PCR.

Step 7 After quantitation of DNA, STR profilingwas done for determining the sex of an individual.Commercially available kits, STR kits supply allelic ladders containing common STR alleles that have been previously characterized for the number of repeat units via DNA sequencing.

RESULTS

The mean quantity of DNA (in µg/l) wascompared acrossheating duration for 15 minutes and 45 minutes at 250^0C. This temperature range imitates the burning temperature for corpses at the scene of the crime. It was assumed that qualitatively and quantitatively, both way, the obtained DNA extracted at extreme temperaturefor 15 minutes yields better outcome as compared to burning for 45 minutes.It was assumed that qualitatively and quantitatively DNA obtained from heating at 250^0C for 15 minutestooth will be lesser compared to freshly extracted tooth. Level of significance was tested using independent sample t test at $p<0.05$ using one-tailed assumptions. A significant difference was observed between freshly extracted ($M=29.91$, $SD=0.65$) and heating at 250^0C ($M=5.51$, $SD=0.31$), $t(38)=150.89$, $p<0.000$. The outcome of the result suggested that heating of tooth at 250^0C for 15 minutes significantly destroys the quantity of DNA. Furthermore, heating of tooth at 250°C for 45 minutes was performed and it was observed that mean quantity of DNA was zero for the entire 20 samples. It suggests that heating of tooth at 250°C for 45 minutes completely destroys the DNA. Refer figure 1.

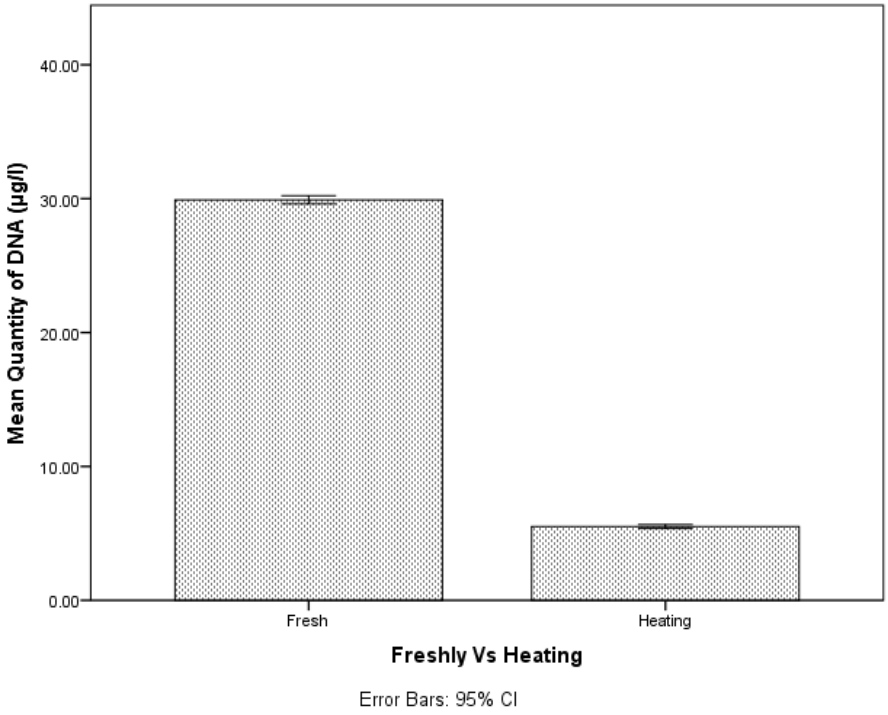

Fig 1: Mean quantity of DNA of heating at 250^0 for 15 and 45 minutes

Quality of DNA was also examined for tooth burnt at 250^0C for 15 and 45 minutes of durations and it was compared with the freshly extracted tooth obtained from agarose gel electrophoresis. The smear quality was compared across two burnt durations of 15 and 45 minutes and freshly extracted tooth. The quality of DNA based on the smear quality was categorized into five subcategories, extremely good, good, average, below average, and poor qualities. It was assumed that qualitatively the DNA obtained from mild to moderate carious tooth will be lesser compared to the freshly extracted teeth. The level of significance was tested using independent sample t-test at $p<0.05$ using two-tailed assumptions. A significant difference was observed between freshly extracted ($M=4.55$, $SD=0.51$) and burnt for 15 minutes duration ($M=2.17$, $SD=0.8$), $t(38)=9.4$, $p<0.000$. The outcome of the result suggested that the median quality of DNA obtained from the burnt condition for 15 minutes duration was significantly lesser than the freshly extracted tooth. Besides no DNA was obtained for 45 minutes of burnt conditions. Refer to Fig 2.

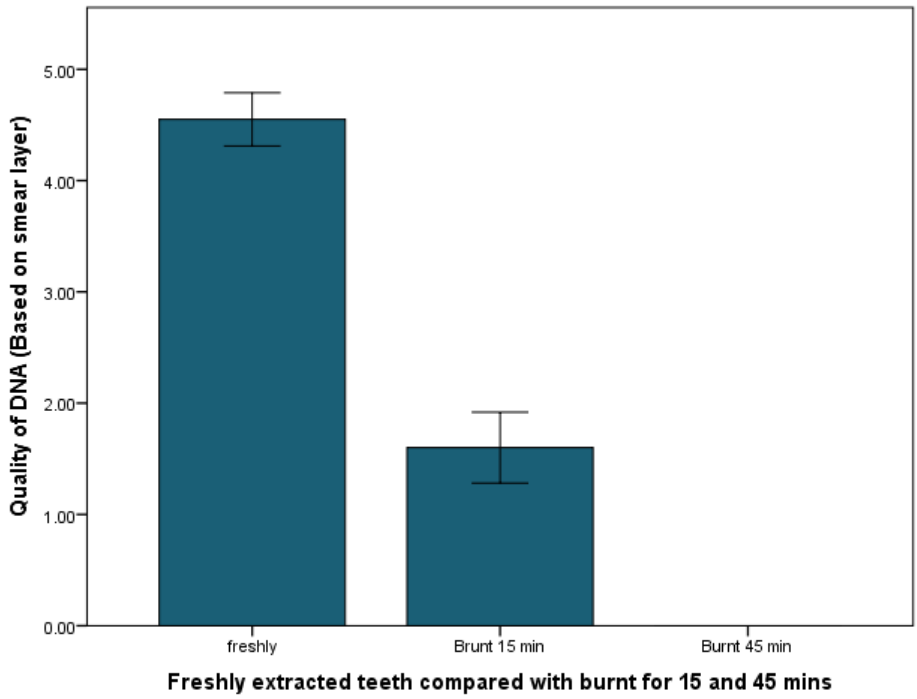

Fig 2: Quality of DNA obtained based on the smear quality by Agarose Gel electrophoresis across freshly extracted and heating at 250^0C for 15 and 45 minutes

DNA Profile generated using Promega powerplex fusion DNA amplification kit which includes 23 Autosomal STR markers and Amelogenin marker from grinding method of the tooth. The STR image for the Amelogenin marker obtained from tooth exposed for 15minutes at 250^0C is depicted below. Refer to figure 3. Similarly, figure 4 denotes the STR image obtained from tooth exposed for 45minutes at 250^0C.

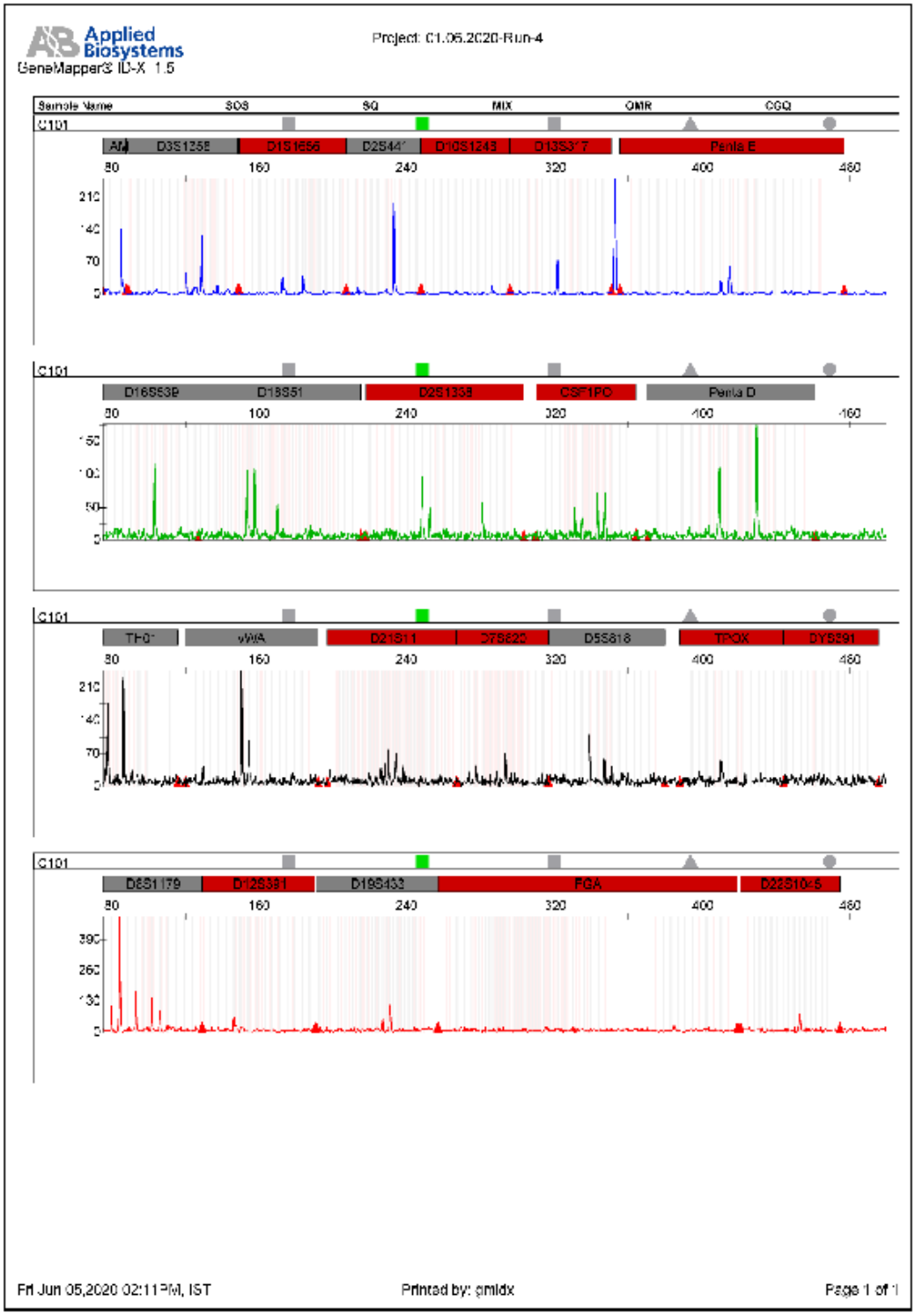

Fig 3: STR Profile generated using Promega powerplex fusion DNA amplification kit which includes 23 Autosomal STR markers and Amelogenin marker from with the burning temperature of 250^0C for 15minutes

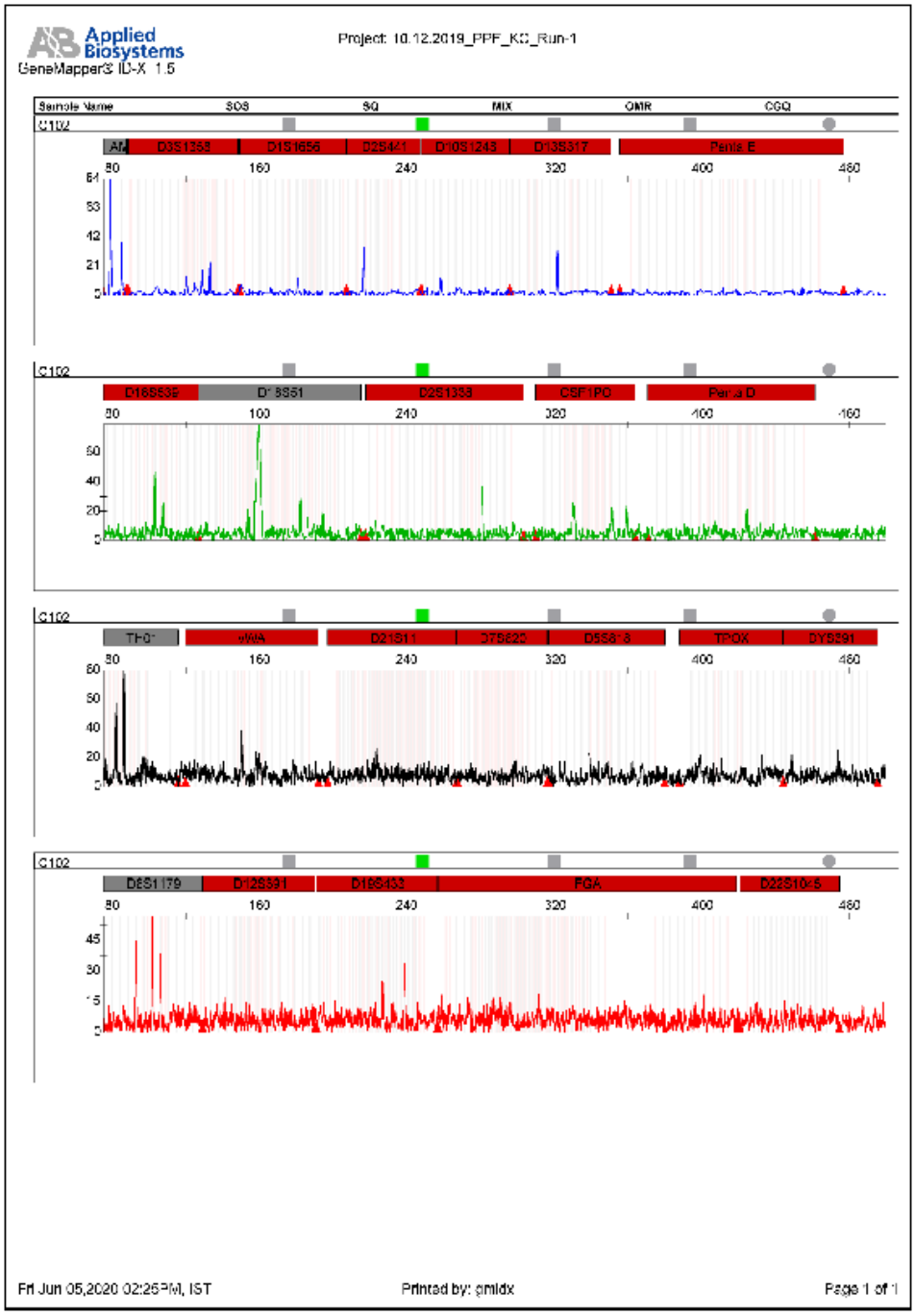

Fig 4: DNA Profile generated using Promega powerplex fusion DNA amplification kit which includes 23 Autosomal STR markers and Amelogenin marker with the burning temperature of 250^{0}C for 45 minutes.

DISCUSSION

In the present study, extracted permanent mandibular molars were subjected to a burning temperature of 250^{0}C for 15 minutes and 45 minutes. The DNA is obtained from pulverized tooth samples subjected to extreme heat at different time durations was assessed. It had been observed that there is a significant decrease in the quantity of DNA when the tooth is exposed to the temperature of 250^{0}C for 15 minutes. This may be due to fragmentation in strands of DNA when it is exposed to a higher temperature and there is a steady drop in the amount of retrieval DNA with prolonged exposure of exhibit/tooth sample. The outcome of our study is concurrent with the study carries out by Vemuri et al. Our study also reveals that DNA extraction is more effective with the phenol-chloroform method of extraction in stressful conditions imitating forensic crime scenes only barring prolonged exposure where no DNA can be extracted. These findings were in correlation with Sivagami et al, Murakami et al, Corte Real and Vemuri, et al. To rule out any ambiguity between the quantity of DNA obtained from various tooth types, permanent mandibular molars is chosen for the study. Our study also in accordance with the study conducted by Urbani et al. where the efficacy of retrievable DNA from the dental pulp of a permanent tooth which had been exposed to a higher temperature for prolonged duration to assess the gender by PCR. It has been evaluated in their study that polymerase chain reaction is considered to be reliable in sex determination when teeth are subjected to higher

temperatures but less reliable when subjected to a higher temperature for a longer duration. However, in our present study we have used RT-PCR technique of DNA quantitation to get exact quantity of DNA. Among homologous sex chromosomes i. e X and Y, the amelogenin gene is considered to be most suitable for sex determination because it shows a variation in size and pattern of the nucleotide sequence in male and female. Hence it is used as a sensitive marker for sex determination because the amel gene present on X and Y chromosome are 106 and 112 base pairs(bp) in length. When we compared the DNA profile generated from a tooth exposed for 15 minutes to a profile generated from tooth exposed for 45 minutes at the temperature of 250^0C the visible differences can be concluded in the following basic points. First, the peaks are sharp which shows a steady decrease at all the loci throughout the plot. But the profile generated from a tooth exposed for 45 minutes not shows any peak. This is mainly because the quantity of DNA extracted from the tooth is scanty while no amount is retrievable from the tooth exposed for 45 minutes. Secondly, the profile generated in the case of a tooth exposed for 15minutes is noisy, stutter, and extra peaks. This is because of the number of contaminants present in the extracted DNA which sometimes competes for binding of the primers in the reaction mix during the amplification process. Besides the above differences, it can be opinioned that the amplifiable DNA that can generate a complete DNA profile for subsequent use in human identification and comparison can be obtained. However, the careful procedure of optimized DNA extraction and its purification is to be employed for its success in the generation of the readable genetic profile,

The present study shall be further explored using a tooth sample under various demanding environmental conditions, as in this study only freshly extracted normal tooth samples were considered. Considering all the factors together, DNA integrity decreases considerably due to the effect of putrefaction. In this study, the pulp material was taken into consideration for DNA extraction, although other parts of the tooth, mainly cementum may be accountable as the other source of DNA. Several methodologies have been validated for tooth sampling and DNA extraction because of the method of processing a key determinant of the quality of the yield of DNA. As stated, all subjects included in this study belong to the same geographical area, and differences between gender and age were matched with their dental pathology.

CONCLUSION

In cases such as an explosion, fire, decomposed corpses or skeletonized bodies identification of human bodies with scanty remaining material is always a challenging task. However, with the advent of molecular technology tooth is considered to be a very good source even in severely decomposed or burnt conditions. DNA extraction from a tooth is also very cost-effective as compared to DNA extraction from bone tissues.

Abbreviations: Phenol CholroformIsoamylalcohol (PCI), Short Tandem Repeat(STR), Real Time Polymerase chain reaction (RT-PCR).

Conflict of Interest: There is no conflict of interest associated with this research.

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Ethical approval: Hence extracted tooth were the sample for this study, no institutional ethical approval was required.

Contributions: Conceptualization of research idea: LK, TD, RD; Hypothesis generation: LK, TD RD; Review of literature: LK, VK, KC, BM; Sample collection and analysis: LK, KC, BM, RD; Statistical analysis: LK, VK, KC, BM, Result writing: LK, VK, KC, BM, Discussion: LK, TD, RD, VK, KC, BM; Manuscript preparation: LK, TD, VK, KC, BM Final proofreading and editing: LK, TD,RD, VK, KC, BM; Citation and references: LK, VK, KC, BM; Journal selection and manuscript submission: LK, VK.

REFERENCES

1. Gunn A. Essential forensic biology. John Wiley & Sons; 2019.
2. Higgins D, Austin JJ. Teeth as a source of DNA for forensic identification of human remains: a review. Sci Justice. 2013;53(4):433-41. doi: 10.1016/j.scijus.2013.06.001, PMID 24188345.
3. Manjunath BC, Chandrashekar BR, Mahesh M, Vatchala Rani RM. DNA profiling and forensic dentistry–A review of the recent concepts and trends. J Forensic Leg Med. 2011;18(5):191-7. doi: 10.1016/j.jflm.2011.02.005, PMID 21663865.
4. Datta P, Datta SS. Role of deoxyribonucleic acid technology in forensic dentistry. J Forensic Dent Sci. 2012;4(1):42-6. doi: 10.4103/0975-1475.99165, PMID 23087582.
5. Hervella M, Iñiguez MG, Izagirre N, Anta A, de-la-Rúa C. Nondestructive methods for recovery of biological material from human teeth for DNA extraction. J Forensic Sci. 2015;60(1):136-41. doi: 10.1111/1556-4029.12568, PMID 25047360.
6. Nelson SJ. Wheeler's dental anatomy, physiology and occlusion-e-book. Elsevier Health Sciences; 2014.
7. Jeffreys A, Wilson V, Thein S. Hypervariable'minisatellite'regions in human DNA. Biotechnology (Reading, MA). 1985; 467: 1992;24.
8. Butler JM. Short tandem repeat typing technologies used in human identity testing. BioTechniques. 2007;43(4):Sii-Sv:ii-v. doi: 10.2144/000112582, PMID 18019344.
9. Alia-García E, Parra-Pecharromán D, Sánchez-Díaz A, Mendez S, Royuela A, Gil-Alberdi L, et al. Forensic identification in teeth with caries. Forensic Sci Int. 2015;257:236-41. doi: 10.1016/j.forsciint.2015.08.021, PMID 26386340.
10. Devaraju, Rama Raju; Gantala, Ramlal; Ambati *et al.* DNA detection in tooth exposed to different temperatures: an in vitro study. Journal of Indian Academy of Oral Medicine and Radiology 26(4):p 393-397, Oct–Dec 2014. doi: 10.4103/0972-1363.155681

11. Zagga AD Zagga AD. Forensic study for genetic sex determination of burnt powdered skeletal fragments from Sokoto, Northwestern Nigeria. Journal of Dental and Medical Sciences. 2013; 7(5):47-54 doi:10.9790/0853-0754754