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# Using PCR amplification for dental that exposer to different temperatures

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**Abstract :** Identification of human remains, terrorism, and disaster and fire victims is one of the fields of forensic evidence, and in the spaces to be determined by the remains of burnt bones or teeth **.**

The current study included the extraction of DNA from samples of female human teeth (third molar) in two ways, after exposing the teeth to different temperatures and different times. As it has been appointed to collect thirty teeth, three of them are not exposed to extreme temperatures and 27 sample teeth divided into three groups: Group A (100°C) for (60,30, and 10) minutes. B group exposed to (500°C) for two (60,30, and 10) minutes, and Group C (1000°C) for (60,30, and 10) minutes. It was extracted the DNA by is phenol chloroform isoamylalcohol. Genomic DNA was obtained under100C° and 500 °C,for both methods but no DNA was obtained under 1000C°.

Present study show high mean of concentration of DNA at 100°C 24±2.5for 10 minutes with purity1.6±0.5 , while high concentration of DNA at 500°Cwas 19.4±2 with purity. PCR amplification after extract DNA was used

**Keywords:** DNA; PCR; Forensic Destiny.

## Introduction

In science and technology natural calamities and crimes continue to persist in human life. Identification of human remains is essential for different reasons including, criminal, legal , humanitarian and social grounds. The human body becomes distorted to a great extent in case of burns, accidents and mass disasters like earthquake so much, so that identification of the individual becomes a challenge.

However dental remains can be used for identification as using them is cost effective, reliable and fast. Forensic Odontology or forensic dentistry was defined in 1970 by Keiser-Neilson as: "that branch of forensic medicine which, in the interest of justice deals with the proper handling and examination of dental evidence and with the proper evaluation and presentation of the dental findings".[1]

In mass disaster or forensic identification , where dead body or its remains are highly decomposed or severely burnt ,bone and tooth are of the only accessible source of DNA. Due to their unique composition and structure ,DNA molecules in bone and teeth are largely protected from environmental challenges and biological attack[2]. Bone and tooth samples were subjected to one of five harsh environmental conditions to produce skeletal samples of varying levels of preservation. these included surface exposure, burial, immersion in saltwater or freshwater, and extreme heat[3].

Now a days, use different method of running DNA fingerprint such as STR, SNP, VNTRS, in forensic odontology[4]. The main external factors that may limit the recovery of information from body residue and determines the procedures of human identification are the elements present or connected with fire, such as ,burnt, flames, heat and explosions, the teeth play an important role in identification and criminology, due to the high matchless of dental features in addition to the relatively high degree of physical and chemical resistance of the dental structure. Due to their capacity of the enduring environmental changes, the teeth represent a good source of DNA because this biological material may provide the necessary relation for identification of an individual in case of failure of traditional methods for dental identification[5].

## Material and method

### Subject

Samples ;30 human teeth samples were collected from Dental Center/ Diwaniya Branch in Qadisiyah. the type of teeth sample that collect is molar. the study was conducted during period from 7/8-11/10/2016, 27 teeth were divided in three groups, samples burn by using furnace ,group A1 burned at 100°C,group B1 burned at 500°Cand group C1 burned at 1000°C for 10,30,60 minutes for each group and 3 fresh control teeth.

### Procedures

DNA extraction;DNA was extract from sampling according to phenol chlorophom t method adopted by the protocol which mentioned by [6] isolation DNAfrom pulp samples of the teeth, amples grand by used liquid nitrogen ,it crushed by pestle, used bio nano drop for measurement the concentration and purity.PCR primer; the Exon -7 CYP1A1 was detected by PCR as fallowing:

Forward:GAACTGCCACTTCAGCTGTCT, reverse:AGCTGCATTTGGAAGTGCTC.PCR was performed as fallowing; 94Cfor 5 min(94 for 30 sec,85Cfor 1 min,72C for 50 sec)35 cycles, then final extension 72C for10 sec. The co-amplified product Exon-7 CYP1A1:(213 bp) were analyzed by electrophoresis on 1.5%agarose gel.

## Result

The result of present study clarified showed the DNA was obtained from burn teeth at 100°Cand 500, whereas it was not obtained at 1000°C.The mean concentration of amount of DNA at 100°C for 10,3,60 minutes was24.9±2.5,16.7±7.6,20.1±4.0 respectively, the purity 1.6±0.5,1.2±0.05,1.3±0.2 for 10, 30, 60minutes(table1)At 500°Cthe mean concentration of amount of DNA for 10,30,60 minuteswas19.4±2.9, 16.7±7,11.6±10.1respectivly ,the purity was1.6±0.3,1.3±0.11,1.2±0.3respectively table(2). The concentration and purity for control sample68.9±4.3, 1.3±0.05respectively.To amplified the DNA from the all samples that extraction from burn teeth, exon -7 CYP1A1were employ, the size fragment (213bp). Electrophoresis pcr product in figure(1)of groupA all band appear except lane 7,8 were thin band . Group B non-specific band appear in lane 5, 6.lane11no band appear .Group C,no band appear at all except lane2(control)

**Table (1) purification and concentration of extracted DNA from burn human teeth sample at100C° for (10,30,60) minutes by using phenol :chloroform :iso-amyle alcohol method**

| | | Concentration | Purity |
|---|---|---|---|
| Time at 100C° | Control | 68.9±4.3a | 1.3±0.05a |
| | 10 | 24.9±2.5a | 1.6±0.5a |
| | 30 | 16.7±7.6a | 1.2±0.05ab |
| | 60 | 20.1±4.0b | 1.3±0.2b |

Data are reported as means ± SD

**Table (2) mean of purification and concentration of extracted DNA from burn human teeth samples at 500C° for ( 10,30,60) minutes by using phenol :chloroform :isoamyle alcohol**

| | | Concentration | Purity |
|---|---|---|---|
| | Control | 68.9±4.3a | 1.3±0.05a |
| Time at 500 C° | 10 | 19.4±2.9a | 1.6±0.32a |
| | 30 | 7.6±4.4 a | 1.3±0.11a |
| | 60 | 11.6±10.1b | 1.2±0.3a |

Data are reported as means ± SD

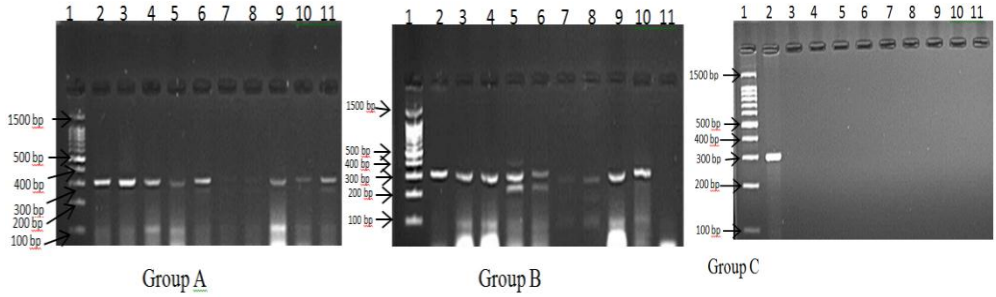


**Figure (1) : Electrophoresis of PCR products of Exon -7 CYP1A1 on 1.5% agarose at 70 volt for one and half hour, lane 1: 100bp DNA marker, sample of human burn teeth extraction by phenol chloroform isoamyl alcohol, visualization with Ethidium Bromide UV light.Group A –lane 2 control ,100°C for 10 minutes lane3,4,5. 30 minutes lane 6,7,8,60 minutes lane 9,10,11.Group B–lane2 control, 500°C for 10 minutes lane3,4,5. 30 minutes lane4,5,6.60 minuteslane9,10,11Group C-1000°C–2 control , for 10 minutes lane 3,4,5.30 minutes lane6,7,8,60 minuteslane9,10,11.**

## Discussion

In issues of forensic cases and random events (arsons, fires, aviation incident) and mainly in terrorist attacks ,high- temperatures causes thermic degradation of biological material[7].The unique composition and structure of both teeth and bone and their location on the body made provide extra production of DNA. Many factor limited the DNA content dental diseases negative impact of human DNA content,advance age lead to decrease in DNA. A Previous studies for researchers *Maciejewska etal*,(2015) and Devaraju etal,(2015)that dna can be found in burn teeth sample under 100°C to 500 °C by using organic method and also no presence of DNA in 1000°C, Girish et al,(2010)used phenol:chrolofom:isoamyl alcohol method of extraction DNA from burn teeth. Devaraju (2014)DNA genomic could not be found above 300°C,the teeth subject were evaluated for mtDNA.

Shrishaileta,l(2011) success of the extracted DNA from the bones of burnt manner phenol chlorofom above 700°C.phenol: chrolofom :isoamyle with n-butanol a benefit way of extraction DNA from bone skeletal remains. The Previous studies did not mention the amount of DNA extracted and purity except Devarajuetal,(2015) The high amount of DNA in may belong the many factors , healthy teeth, age,Damage to teeth . the advancing age of human leads to a decrease in DNA content that give the low concentration of DNA add to the high heat that exposed the teeth in present study[8].Tsuchimochi etal,(2002)use chelex 100resin in order to extract DNAfrom dental pulp under 100°C,200°C,300°C,400°C,500°C for 2 minute,all sample all gave a DNA content but no PCRproduct was obtained from those above 400°C.

The success of amplification pcr depend on many factors such as DNA purification the size of primer. PCR techniques include nuclear DNA on the other hand mtDNA rich and can be identify . Exon -7(hose keeping gene) was concentrative gene in human that not effected by experimental factor[9]. In present study nonspecific band appear (addition band) related to dilation second allele , the reason for occurrence such product annealing temperatures, low concentration of DNA gave of thin band on the product of PCR, [10].Previous studies have shown that the amount of DNA extracted from the pulp, given more signals when

amplification[11].teeth transform partial or total incineration that lead to failure mtDNA extraction and amplification[12].

Previous studied show the possibility of amplification of DNA isolated from teeth subjected to 200°C, 400°C, 500°C and 600°C for 60 minutes. They found that amplification of the autosomal loci of 50% of the samples exposed to 200°C and 400°C was successful they applied organic method of DNA isolation), whereas after exposure to higher temperatures (500°C and 600°C), only mtDNA could be successfully amplified[13].PCR is the best trustworthy method in markedly decayed or preadolescent bodies or burn bodiesVemuri etal,(2012). Remualdo etal(2004) assessed the PCR amplification of DNA obtained from teeth under (200°C,400°C,500°C,600°C)during 60 minutes testing (organic ,ammomium isopropyl/acetate ,and silica) for PCR amplification organic method were amplified[14].Teeth are prefect source of genomic DNA and mtDNA because PCR analysis allow comparing the collected postmortem sample to know antemortem samples or parental[15].The exposure of the DNA molecule to height temperatures possibly led it to fragmentation in to a smaller particles that why not possible to obtain PCR product from the teeth submitted ho high temperature. An important issue of amplification was the size of primer used ,312bp which large fragment for degraded material this pointing to the relevance showing the size of the fragment to be amplified, as well as the safety of the sample, which can contribute to the success of the analysis[16,17].

## Conclusion

Forensic density in case (explosions, mass disaster.fire) plays main roles to identify human remains. Several organic compounds such as phenol, chloroform, and isoamyl alcohol for extraction of DNA, and amplification for human remaining. Teeth exposer for 1000°C doesn't allow obtain DNA.

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