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Dental Sex Determination by Multiplex PCR in Iraqi Samples.

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ABSTRACT

The Present study aims to investigate determination in dental Iraqi samples that buried in wet soil for 160 days,54 teeth samples were collected from dental clinic then by using multiplex PCR technique human sex was identified, the results show that 61.6% of samples were males while 36,2% were female , 0.8% nonspecific bands and 0.4% was no amplification results , thus the buried sample have efficiency in sex determination in a high percentage.

Keywords: sex determination, wet soil, multiplex PCR.

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INTRODUCTION

Odontology or forensic dentistry in forensic medicine was defined by Keiser-Neilson in (1970) is a branch of forensic medicine deal with dental as source of nucleic acid for used it in fingerprinting (Neilsen, 1980). The Forensic Odontology become important component in modern day investigations to identified people mass disasters, accidents, and if the victim's bodies cannot be recognized by visual methods. The natural teeth are the most durable organs in the bodies of vertebrates, and humankind's understanding of their own past and evolution relies heavily upon remnant dental evidence found as fossils (Tobias, 1990).

Teeth and bones are important sources of DNA available for identification of degraded or fragmented human remains. The unique composition of teeth and their location in the jawbone provide additional protection to DNA compared to bones making them a preferred source of DNA in many cases. Despite this, post-mortem changes in the structure and composition of teeth, and the location and diagnosis of DNA within them are poorly understood(Higgins and Austin, 2013)

Different types of DNA fingerprinting now used in different applications such as short tandem repeat (STR),single nucleotide polymorphism (SNP) and insertion , deletion (Alonso *et al*., 2005) in the cases where the DNA is highly degraded and also provide ancestry and/or phenotypic information when there is no presumptive identification or comparative ante-mortem sample available and the analyzing samples containing little or no nuclear DNA, mitochondrial DNA (mtDNA) analysis may be useful (Melton., 2001 ;Budowle., 2003;Butler, 2007) mtDNA present in much higher copy numbers and is more robust due to its cellular location than nuclear DNA it is more likely to be preserved in highly degraded tissues (Foran, 2006).The use of mtDNA analysis has been shown to be especially valuable in missing persons cases (Budowle *et al* ., 2005) as it can be compared to more distant relatives than is possible with nuclear STRs (Ginther,1994).

Nuclear and mtDNA have good recovered from human skeletal remains for long time like hundreds or thousands years after death (Rohl and Hofreiter, 2007) Teeth are a favorite skeletal source of DNA because their unique composition and location within the jawbones they are protected from the environmental and physical conditions which accelerate the processes of postmortem decomposition and DNA degradation (Schwartz, and Schwartz,1991; Alvarez *et al* 1996). DNA extracted from teeth is often of higher quality sometimes and the contamination with other DNA was less than DNA extracted from bones (Ricaut *et al* 2003; Gilbert *et al* .,2005) A number of studies support the trusty of teeth DNA in genetic analysis of human identification (Baker *et al* 2001).

MATERIALS AND METHODS

Samples; samples were collected from dental center, 27 teeth were used fresh as DNA source, 27 teeth were buried in wet soil for 160 dyes.

DNA extraction; DNA was extract from samples according to GeneMATRIX UROX Bone DNA Purification Kit as fallowing:

- Teeth were grinded by liquid nitrogen,400 mg of this was put in score tube then 800 µl Lyse BN buffer. Suspend the sample thoroughly.
- 40 µl of Proteinase K was added , it Mixed by vortexing the tube then incubated at 56Cº with agitation.
- 40 µl of activation Buffer BN was added onto the spin-column and kept it at room temperature then it transferred to the spin-column.
- 800 µl of Sol BN buffer was added and. Mixed thoroughly by vortex in g tube then it incubated for 10 min at 56oC.
- Centrifuged the lysate in a micro centrifuge for 3 min at 14 000 rpm.
- Transferred 1200µl of the supernatant to a new 2 ml micro centrifuge tube .And Add 600 µl of 96 % ethanol it mixed thoroughly by vortexing the tube.
- 600 µl of the lysate was transferred to the spin-column placed in a collection tube.Centrifuge for 30 seconds at 12000 rpm.
- The flow-through was discarded and placed back the spin-column in the collection tube.

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- the remaining supernatant was transferred to the spin-column placed in a collection tube. Centrifuge for 1 min at 12000 rpm to filtrate the remains of the lysate through the resin.
- Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.
- 500 µl of Wash BNX1 buffer was added to the spin-column and centrifuged for 1 min at 12000 rpm.Discarded the flow-through and placed back the spin-column in the collection tube.
- 500 µl of Wash BNX2 buffer was added to the spin-column and centrifuged for 2 min at 12000 rpm.Placed the spin-column in a new collection tube (1.5-2 ml) and added 30-100 µl of Elution buffer that preheated to 70Cº to elute the bound DNA.
- The spin-column/collection tube assembly was incubated for 5 min at room temperature.Centrifuged the spin-column for 30 seconds at 12000 rpm.
- DNA was stored at -20Cº.

PCR and primer; primers used for multiplex PCR as fallowing : F-CAT GAA CGCATT CAT CGT GTG GTC'; and R- CTG CGGGAA GCA AAC TGC AAT TCT T' for SRY , and F- CCC TGA TGA AGA ACT TGT ATC TC and R-GAA ATT ACA CAC ATA GGT GGC ACT' for ATL1 , PCR was performed as fallowing ; 94 C for 5 min, (95 for 30 sec, 62C for 30 sec, 72 for 30 Sec) 30 cycles then final extension 72 for 10 min. Electrophoresis of PCR product was visualize using 1% agarose, 70 V 20 mA, for 40 mints.

RESULTS

The results of present study clarified that used fresh and buried dental samples have high efficiency for human sex determination as show in DNA extraction and PCR products, the electrophoresis of DNA that extract from fresh samples were high concentration and no lysis in it but it show different lysis level in samples that buried in wet soil , 98% of DNA samples were low lysis level while 1.2% had DNA bands with smear, 0.6% had smear only and 0.2% had complete lysis as in figure (1).

PCR products were 61.6% of samples show two bands thus it males while 36,2% show one bands, 0.8% show three bands and 0.4% show no bands as show in figure (2) The second round of PCR show that 90% of the Samples questionable have one band as show in figure(3)

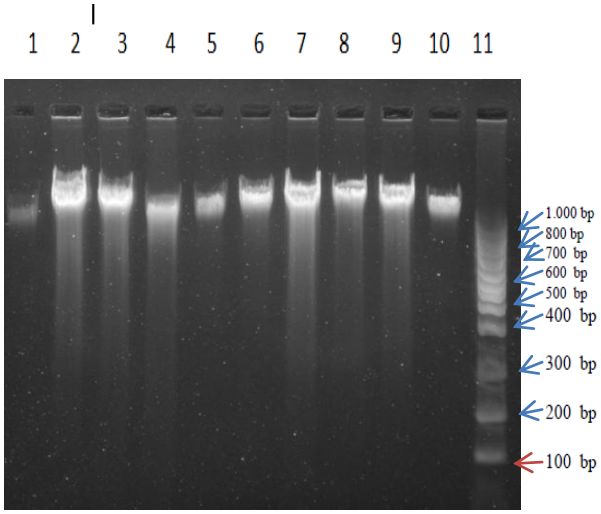

Figure (1) Electrophoresis pattern of DNA extraction from buried dental samples ,Lane 1; complete lysis, Lane 2,3,4,7bands with high lysis Lane 5,10 bands ,no lysis; Lane 6 band with low lysis. Lane 8,9 bands with midrate lyses; lane 11 DNA marker (100bp).

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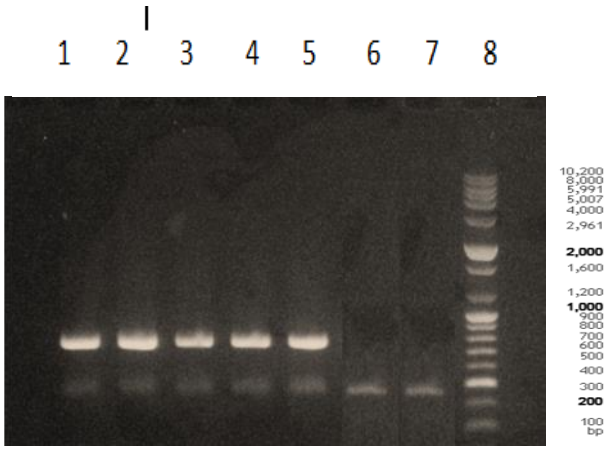

Figure (2) Electrophoresis pattern of PCR products for sex determination in DNA sample lane 1,2,3,4,5 male samples; lane 6,7 female sample; lane 8 DNA marker (100 plus) .

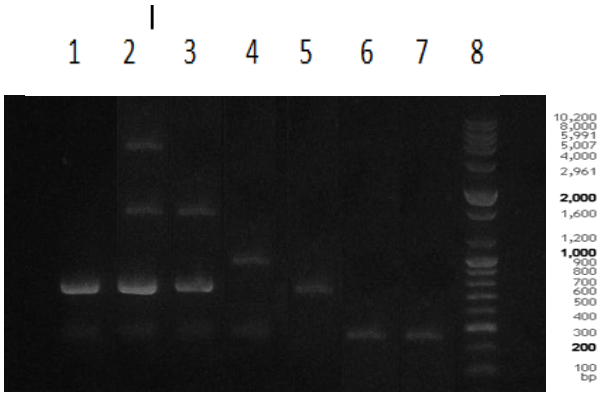

Figure (3) Nonspecific amplification of PCR products for sex determination in DNA samples only lane 1 , 6 and 7 specific Amplification , lane 2,3,4,5 ambiguous products , lane 8 DNA marker(100bp).

DISCUSSION

The different lyses level in DNA may be because the effect of external environment factors that cause break in DNA strands and these breakings happened in one strand or in double strands, the level of lyses depending on the breaking in DNA. Researchers improver that no standardized forensic protocols directing the handling, sampling and DNA extraction of teeth, Protocols used for sampling and extraction of tooth tissues predominantly mirror those used for bone despite the fact that these two tissues are morphologically and biochemically different (Dobberstein *et al*., 2008). like the protocols for teeth used by the International Commission of Missing Persons (ICMP), suggested by Parsons *et al*(2007) are same with that used for bone but it different in the outer surface which removed during bone preparations only. It aims to extract DNA from the rich tissue and avoid minerals in teeth tissue that interact with post handling DNA.The outcome of genetic analysis of teeth is dependent on the quantity and quality of DNA, the level of degradation and the efficiency of DNA sampling and extraction methods.

It is unclear as to whether the full potential of teeth as a source of DNA has yet to be realized. By drawing together the current knowledge of tooth structure and post-mortem diagnosis of DNA in tooth tissues, this paper aims to investigate efficiency of PCR in Iraqi dental sample which buried in wet soil for months in forensic application especially it has been important forForced displacement and mass graves, DNA degradation in teeth has been shown to be time dependent but the relationship between time and degradation is complex andis greatly affected by environmental factors [Rubio et al 2007; Rubio et al., 2012]. It is initiated in the post-mortem period by the release of endogenous intracellular enzymes (e.g. lipases,

nucleases, and proteases), and continued by exogenous enzymes produced by invading microorganisms and environmental invertebrates (Alaeddini et al 2010).

The limited porosity of teeth and their protected location physically restricts the actions of exogenous organisms on cells in teeth. However, the actions of endogenous enzymes and the processes of spontaneous hydrolysis and oxidation still occur, although these proceed at a slower pace than the actions of exogenous organisms (Caviedes-Bucheli*et al* 2006).

The PCR-based sex determination identified by the presence (male) or absence (female) of the SRY gene has already been described in present study it improved its efficiency in Iraqi dental samples for used in forensic applications, the false positive amplification may be because the lyses in DNA or nonspecific amplification of primer thus it must use high fidelity DNA polymerase or second round PCR.

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