CukurovaMedicalJournal

Araştırma Makalesi / ResearchArticle

An Efficient Method of Extracting PCR Amplifiable DNA from Human Skeletal Fragments and Teeth Specimens

Diş Örneklerinden ve İnsan İskelet Parçalarından DNA'nın PCR ile Çoğaltılmasında Etkili Bir Yöntem

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CukurovaMedicalJournal 2014;39(4):712-721.

ABSTRACT

Purpose: Extraction and successful PCR amplification of DNA from humanremains in historical and forensic cases have great importance. The purpose of this study is to demonstrate the efficiency of a method of DNA extraction and PCR amplification of embalmed dried human cadaveric skeletal fragments and teeth specimens from Sokoto, Northwestern Nigeria.

Materials and Methods: The efficiency of a method of DNA extraction and PCR amplification was tested on thirteen (13) embalmed dried human cadaveric skeletal fragments and nine (9) teeth specimens from Sokoto, Northwestern Nigeria were used in the study.

Results: Of the 13 embalmed dried human cadaveric skeletal fragments, 12 (92.3%) samples amplified with apparent bands. For the 9 embalmed dried human teeth specimens, 7 (78%) samples amplified with apparent bands.

Conclusion: The study has shown that our method of DNA extraction and PCR amplification was efficient on embalmed dried human cadaveric skeletal fragments and teeth specimens, from Sokoto, Northwestern Nigeria.

Key words: Extraction, PCR amplifiable DNA, embalmed, dried human cadaveric bones and teeth.

ÖZET

Amaç: Tarihsel ve adl ivakalard ainsandan DNA'nın izolasyonu ve başarılı PCR amplifikasyonu büyük öneme sahiptir. Bu çalışmanın amacı; Kuzeybatı Nijerya, Sokoto'dan diş örnekleri ve mumyalanmış kurutulmuş insane kadavra iskelet parçalarının DNA ayıklanması ve PCR amplifikasyonu için etkili bir yöntemi göstermektir.

Materyal ve Metod: Çalışmaya dahil edilen Kuzeybatı Nijerya, Sokoto'dan 9 diş örneğinin ve 13 mumyalanmış kurutulmuş insane kadavra iskelet parçalarının DNA izolasyonu ve PCR amplifikasyonu için etkil ibir metot ile test edildi.

Bulgular: 13 mumyalanmış kurutulmuş insane kadavra iskelet parçalarının olduğu örneklerin 12'sinde bantlar çok iyi gözlendi. 9 diş örneğin 7'sinde ise yine bantlar barizdi.

Sonuç: Bu çalışma, Kuzeybatı Nijerya,Sokoto'dan mumyalanmış kurutulmuş insane kadavra iskelet parçalarının ve diş örneklerinin DNA izolasyonuve PCR amplifikasyonu için kullandığımız yöntemin verimli olduğunu gösterdi.

AnahtarKelimeler: İzolasyon, kurutulmuş insan kadavra kemikleri ve dişleri, mumyalamak, PCR

INTRODUCTION

Extraction and successful PCR amplification of DNA from humanremains in historical and forensic cases have great importance,but is particularly difficult because the methods employed atpresent are not always satisfactory. Previous studies have shownthat DNA can persist in ancient remains and the best subjectsfor such investigations are bone and tooth samples since theyare much more abundant than soft tissue remains and generallybetter preserved[1].

The factors that commonly prevent PCR amplification of DNAfrom ancient remains may vary between burial sites. They mayoriginate either from the environment of the remains in theform of humic acid, fulvic acid, hidroxi-apatite, tannin andcontaminating DNA, or from degradation in the biological sample[2]. Ancient DNA is heavily modified and these modifications,which are mainly attributed to oxidative processes, are responsiblefor the low recovery rate of undamaged DNA from archaeologicalspecimens[3]. In the case of bone, collagen type I[4]and Maillard products[5] are the main inhibitory factorsof successful PCR amplifications.

The first steps of DNA extraction in the majority of the previouslypublished methods were the powdering of bone material and incubationin various extraction buffers[6]. Classically,in the next step the DNA was extracted with phenol-chloroform or the extract was dialysed against EDTAand Tris–HCl buffered solution[7]. After extraction,the aqueous phase was concentrated by means of ethanol or isopropanolprecipitation[8], or microconcentrators[9].Alternatively, after the incubation step, DNA could be separatedwith glass-milk or silica suspension and could be eluted fromsilica pellet[10]. The Chelex-based method involvedboiling the bone powder in Chelex suspension, followed by PCRamplification of the supernatant[11].

Experiments that compared the phenol-chloroform and Chelextechniques concluded that although the Chelex method was simpleand fast, inhibitory substances had not been eliminated in mostof the cases[12]. Another study showed that sodium-acetate-isopropanolextraction was possibly better than the phenol-chloroform methodand resulted in about three times the quantity of extractedDNA than the glass-milk method[13].

An efficient method for extraction of PCR amplifiableDNA from cadaveric human skeletal fragments and teeth specimens is described here. The efficiencyof this protocol was demonstrated on 13 dried cadaveric human skeletal fragments and 9 dried teeth specimens, from Sokoto, North-western Nigeria.

MATERIAL and METHODS

A total of twenty two[22] samples were used in this study. They comprised of thirteen[13] dried human skeletal fragments and nine[9] dried human teeth specimens.

The skeletal fragments and teeth specimens were obtained from the remains of dissected cadavers in the Department of Anatomy, College of Health Sciences, UsmanuDanfodiyo University, Sokoto, Nigeria.

The skeletal fragments comprised of (1) Skull (2) Clavicle (3) Scapula (4) Radius (5) Ulna (6) Rib (7) Lumbar vertebra (8) Shaft of femur (9) Hip bone (10) Sacrum (11) Tibia (12) Fibula (13) Calcaneum. Each skeletal fragment measured about 0.5cm x 0.5cm.

The nine[9] dried human teeth specimens comprised of 3 incisors, 1 canine, 2 premolars and 3 molars. Each specimen consisted of a whole tooth.

Sample Collection

Samples of cadaveric skeletal fragments and teeth specimens were collected from the remains of the dissected cadavers in the Department of Anatomy, College of Health Sciences, UsmanuDanfodiyo University, Sokoto, Nigeria,

using sterile bone cutter and forceps, sterile hand gloves, disposable masks, ruler before being transferred into glass vials.

The laboratory experiment was carried out at the Department of Medical Molecular Genetics, Division of Human Genetics and Genome Research, National Research Centre, Cairo, Egypt.

Laboratory Procedures

Precaution Against Contamination While Handling Skeletal Fragments and Teeth Specimens for DNA Extraction and Amplification

Samples of dry skeletal fragments and teeth specimens were initially placed in a freezer at -20°C (for minimum of 72 hours) to eliminate surface contamination from the depositional environment and post depositional handling. In addition to the specific and stringent precautions against contamination recommended by Cooper and Poinar[14]while handling skeletal tissues for molecular analysis, other peculiar precautions were undertaken to handle the dry teeth samples before grinding.

With gloved hands, each tooth was held with a sterile forceps (CE Stainless Pakistan) and washed under running tap. The surfaces of the tooth were brushed with an abrasive paper. The tooth was then cleaned with 4% hypochlorate bleach (4ml chlorex + 96ml distilled water). The surfaces of the tooth were further brushed with a tooth brush. The tooth was rinsed with distilled water, then placed on a piece of a sterile aluminium foil in a hood and exposed to UV light for 15-30 minutes, before grinding.

Protocol for Grinding of Skeletal Fragments and Teeth Specimen

The equipment and surface of the hood for grinding skeletal fragments and teeth specimens were cleaned with distilled water, sterilised with 70% ethanol(Art-Nr. K928.3 ROTH. Ethanol vergällt.≥99.8%, mit Ca. 1% MEK.Carl Roth GmbH + Co. KG.2.5L. Schoemperslenstr. 3-5, 76 185 Karlsruhe) and UV irradiated before and after grinding each sample. A sheet of aluminium foil (HelwanAluminium Foil. 15M x 40CM. Made in Egypt) sterilised with 70% ethanol(Art-Nr. K928.3 ROTH. Ethanol vergällt.≥99.8%, mit Ca. 1% MEK.Carl Roth GmbH + Co. KG.2.5L. Schoemperslenstr. 3-5, 76 185 Karlsruhe) wasplaced on the surface of the hood.

About 0.5cm x 0.5cm fragment from each cadaveric skeletal fragment and whole of each tooth respectively, was fragmented using sterile bone cutter and forceps (CE Stainless Pakistan). Samples were further sterilised with 70% ethanol (Art-Nr. K928.3 ROTH. Ethanol vergällt.≥99.8%, mit Ca. 1% MEK.Carl Roth GmbH + Co. KG.2.5L. Schoemperslenstr. 3-5, 76 185 Karlsruhe) and a sterile soft tissue was used to absorb excess ethanol from the sample (to dry the sample and minimise the PCR inhibitory effect of alcohol) before grinding. Each sample (one sample at a time) was then placed in a sterile mortar and pestle (MN 100cl), for pulverisation. Pulverisation continued until the bone fragment or tooth specimen turned into powder form. Aliquot of the ground bone or tooth powder, respectively, was then transferred into 1.5ml microtubes (1.5ml microcentrifuge tubes (Bio Basic Inc. (BBI). Cat. No. BT620NS – 100. Sterilized 1.5ml mmicrocentrifuge certified RNaseDNase and pyrogen – free. Lot. No. 08112), already labelled (sample number, name of sample and date) on the flat white cap writing surfaces and on the sides with a permanent marker (STAEDTLER permanent Lumocolor Art. Nr.313-3. EAN 40 07817 308677), placed in a microtube rack (LP ITALIANA SPA – Milano/made in Italy) and stored in a refrigerator (Elite. Air Multi=flow. Freezer and Refrigerator. No frost) at -80°C, before DNA extraction.

DNA Extraction

The extraction of DNA from all the samples was done by standard phenol-chloroform method established by the Department of Medical Molecular Genetics, Division of Human Genetics and Genome Research, National Research Centre, Cairo, Egypt[15]. The samples consisted of about 0.3mg and 0.5mg aliquot of the ground bone and tooth powder, respectively. The equipment

and surface of the DNA extraction hood were cleaned with distilled water, sterilised with 70% ethanol (Art-Nr. K928.3 ROTH. Ethanol vergällt.≥99.8%, mit Ca. 1% MEK.Carl Roth GmbH + Co. KG.2.5L. Schoemperslenstr. 3-5, 76 185 Karlsruhe) and UV irradiated. A sheet of aluminium foil (HelwanAluminium Foil. 15M x 40CM. Made in Egypt) sterilised with 70% ethanol (Art-Nr. K928.3 ROTH. Ethanol vergällt.≥99.8%, mit Ca. 1% MEK.Carl Roth GmbH + Co. KG.2.5L. Schoemperslenstr. 3-5, 76 185 Karlsruhe) was placed on the surface of the hood.

To each 0.3mg and 0.5mg of bone and tooth powder, respectively, contained in 1.5ml microtubes (Bio Basic Inc. (BBI). Cat. No. BT620NS – 100. Sterilized 1.5ml microcentrifuge certified RNaseDNase and pyrogen – free. Lot. No. 08112), 600 µl extraction buffer (8% D sucrose, 50mM EDTA, pH 8, 50mM Tris HCL, 5mM sodium acetate, 5mM ammonium acetate, 5.5% triton X-100) was added, using sterile pipettes and disposable filtered tips (BioSTC. High Performance Micro volume Pipettors.Calibration Confirming.DN 12650 & ENISO 8655.Standards – Germany. 100-1000µl (Sr No: 712689). The tubes were shaken and vortexed using Retsch Mixer. 220V 25W, to ensure thorough mixing of the contents.To the above mixture, 600 µl phenol (BioFlux.Biozol-RNA/DNA extraction reagents for phenol, equilibrated, stabilized. Volume: 100ml. Storage: 2-8^{o}C. Lot. No: 20090701. Cat. No: BSA02M1), taken from the lower layer was added, using sterile pipettes and disposable filtered tips (BioSTC. High Performance Micro volume Pipettors.Calibration Confirming.DN 12650 & ENISO 8655.Standards – Germany. 100-1000µl (Sr No: 712689). The tubes were shaken, vortexed using Retsch Mixer, then sealed with Para film foil (PARRAFILM “M.” 4 W x 125 FT. ROLL. RECHINEY PLASTIC PACKAGING. MENASHA, WI 54952. CHICAGO, IL. 60631), to avoid spillage of the contents, before mounted on a mixer (Rotator. Model No.:Simi/Rotator. S. No.:1003. Lot No.:800 Rot. 1H. Volt: 220. Wat: - 30. Made in Egypt), at its maximum speed, for 72 hours. The tubes were fixed on the rotator with a solutape (Nova premium quality. Made in UAE). The tubes were removed from the rotator and the Para film seal removed from the tubes. The tubes were then centrifuged in a microcentrifuge (SiGMALaborzentrifugen, D -37520 Osterode am Harz, Germany. Model =1K15. Serial No =69422. Year =1999. Max. Speed (dependent on rotor) rpm =5300. Max. Capacity (density of material max 1.2kg/dm^2 =53ml. Kinetic energy Nm =4980. Total power consumption W =450. Electrical supply V/HZ =230/50 1Ph. Input fuse A =6, 3 AT. Interference suppression acc to EN 55011 cl. B. Made in Germany), at 14,000 rounds per minute (rpm) for 15 minutes. About 500 µl from the supernatant of each sample was transferred into a new 1.5ml microtube (Bio Basic Inc. (BBI). Cat. No. BT620NS – 100. Sterilized 1.5ml microcentrifuge certified RNaseDNase and pyrogen – free. Lot.No. 08112), using sterile pipettes and disposable filtered tips (BioSTC.High Performance Micro volume Pipettors.Calibration Confirming.DN 12650 & ENISO 8655.Standards – Germany. 100-1000µl (Sr No: 712689). To each supernatant, 500 µl chloroform (HPLC grade.Rankem Chloroform. M. W. 119.38. Product code: CO580. Pack: 1 Litre. Batch no: R155C06. Ranbaxy, Fine Chemicals Limited, New Delhi, India) was added, using sterile pipettes and disposable filtered tips (BioSTC. High Performance Micro volume Pipettors.Calibration Confirming.DN 12650 & ENISO 8655.Standards – Germany. 100-1000µl (Sr No: 712689). The tubes were vortexed and centrifuged again as done previously. About 500 µl from the supernatant of each sample was transferred into a new 1.5 ml microtube (Bio Basic Inc. (BBI). Cat. No. BT620NS – 100. Sterilized 1.5ml microcentrifuge certified RNaseDNase and pyrogen – free. Lot.No. 08112), using sterile pipettes and disposable filtered tips (BioSTC.High Performance Micro volume Pipettors.Calibration Confirming.DN 12650 & ENISO 8655.Standards – Germany. 100-1000µl (Sr No: 712689). To each supernatant, 500 µl

Isopropanol (LAB-SCAN analytical sciences.PROPAN-2-OL HPLC.2.5L. Code No. C19C11X. Batch No. 0731/9. Manufacturing Date: Feb 2009. Expiration Date: Feb 2012. POCH SA. 44-101 Gliwice, ul.SowinsKiego 11, Poland) was added, using sterile pipettes and disposable filtered tips (BioSTC. High Performance Micro volume Pipettors.Calibration Confirming.DN 12650 & ENISO 8655.Standards – Germany. 100-1000µl (Sr No: 712689). The tubes were shaken and vortexed again, using Retsch Mixer, before being placed, in a freezer (Elite. Air Multi=flow. Freezer and Refrigerator. No frost) at -20°C for 24 hours. The tubes were then centrifuged in a microcentrifuge (SiGMALaborzentrifugen, D - 37520 Osterode am Harz, Germany. Model =1K15. Serial No =69422. Year =1999. Max. Speed (dependent on rotor) rpm =5300. Max. Capacity (density of material max 1.2kg/dm^2 =53ml. Kinetic energy Nm =4980. Total power consumption W =450. Electrical supply V/HZ =230/50 1Ph. Input fuse A =6, 3 AT. Interference suppression acc to EN 55011 cl. B. Made in Germany), at 14,000 rpm for 15 minutes. The supernatant was discarded by gently raising the bottom of the tube slightly higher than the mouth, and the pellets left at the side of the tube. The pellets were washed with 500 µl 70% ethanol (Art-Nr. K928.3 ROTH. Ethanol vergällt.≥99.8%, mit Ca. 1% MEK.Carl Roth GmbH + Co. KG.2.5L. Schoemperslenstr. 3-5, 76 185 Karlsruhe), taken using sterile pipettes and disposable filtered tips (BioSTC. High Performance Micro volume Pipettors.Calibration Confirming.DN 12650 & ENISO 8655.Standards – Germany. 100-1000 µl (Sr No: 712689) and centrifuged in a microcentrifuge (SiGMALaborzentrifugen, D - 37520 Osterode am Harz, Germany. Model =1K15. Serial No =69422. Year =1999. Max. Speed (dependent on rotor) rpm =5300. Max. Capacity (density of material max 1.2kg/dm^2 =53ml. Kinetic energy Nm =4980. Total power consumption W =450. Electrical supply V/HZ =230/50 1Ph. Input fuse A =6, 3 AT. Interference suppression acc to EN 55011 cl. B. Made in Germany), at 14,000 rpm for 15 minutes. The supernatant was discarded, and the pellets in the tubes were left to dry at room temperature. The opened tubes were then sealed with parafilm foil (PARRAFILM “M.” 4 W x 125 FT. ROLL. RECHINEY PLASTIC PACKAGING. MENASHA, WI 54952. CHICAGO, IL. 60631) to minimize contamination. Separate sterile needles (Omeco 10ml 21G x 11/2”, single use syringe, sterilised, non-toxic, non pyrogenic, Lot 22543 Made in Egypt) were used to perforate the top of the seal of each tube, to enhance dryness. The pellets were dissolved in 40-50 µl ddH_2O (DEPC water) (Art. T143.1. ROTH.Wasserfur die. Molekularbiologie.DEPC- bhandelt water.M 18.01.250ml. Carl Roth GmbH + Co. KG. 76 185 Karlsruhe), by pipetting up and down with sterile pipettes and disposable filtered tips (BioSTC. High Performance Micro volume Pipettors.Calibration Confirming.DN 12650 & ENISO 8655.Standards – Germany. 10-100 µl (Sr No: 712689). The eluted DNA was stored in a refrigerator (Elite. Air Multi=flow. Freezer and Refrigerator. No frost) at -80°C before used for amplification.

PCR Amplification

PCR amplification of the extracted DNA from skeletal fragments and teeth specimens used previously prescribed *alphoid* r*epeats* primers by Witt and Erickson[16] as shown in Table 1.

Table 1: *AlphoidRepeats* Primers and Their Sequences Used for PCR Amplification of the Extracted DNA in the Study[16].

NAME OF PRIMER	SEQUENCE OF PRIMER
Forward primer for X =X1	5`- AAT CAT CAA ATG GAG ATT TG-3’
Reverse primer for X = X2	5’-GTT CAG CTC TGT GAG TGA AA-3’
Forward primer for Y = Y11	5-’ATG ATA GAA ACG GAA ATA TG-3’
Reverse primer for Y = Y22	5’-AGT AGA ATG CAA AGG GCT C-3

For the 13 skeletal fragments, a PCR master mix for 14 samples/tubes (one extra tube for correction of pipettion errors) was prepared for X (X1 and X2) primers, in a 0.5ml PCR tube. The master mix was constituted from: 2.5 µl of 10x buffer, 2.5 µl of dNTPs, 2.5 µl of forward primer for X = X1, 2.5 µl of reverse primer for X = X2, 0.5 µl of Taq DNA polymerase and 17.5 µl of DEPC water. A total volume of 28 µl from the master mix was transferred into 13 tubes, and 1 µl Paraffin oil added to seal and avoid evaporation of the reaction mixture. A volume of 2 µl DNA from the respective 13 skeletal fragments was finally added to the 13 tubes (containing PCR reagents for X), to accomplish a reaction volume of 30 µl for each tube.

For the 9 teeth specimens, a PCR master mix for 10 samples/tubes (one extra tube for correction of pipettion errors) was prepared for Y (Y11 and Y22) primers, in a 0.5ml PCR tube. The master mix was constituted from: 2.5 µl of 10x buffer, 2.5 µl of dNTPs, 2.5 µl of forward primer for Y = Y11, 2.5 µl of reverse primer for Y = Y22, 0.5 µl of Taq DNA polymerase and 17.5 µl of DEPC water. A total volume of 28 µl from the master mix was transferred into 9 tubes and 1 µl Paraffin oil added to seal and avoid evaporation of the reaction mixture. A volume of 2 µl DNA from the respective 9 teeth samples was finally added to the nine tubes (containing PCR reagents for Y), to accomplish a reaction volume of 30 µl for each tube.

Normal PCR was accomplished in a thermocycler (Minicycler^{TM}MJ RESARCH) in a 30 µl reaction volume, separately, for skeletal fragments and teeth specimens, to amplify selected sequences of the *alphoidrepeats*, as follows; Initialization step at 95ºC for 5 minutes. Denaturation step at 94ºC for 40 seconds.Annealing step at 55ºC for 40 seconds.Extension/elongation step at 72ºC for 40 seconds. Steps 2-4 were repeated for 35 cycles. Final extension/elongation step 72ºC for 40 seconds. Cooling of reaction process was at 4ºC.

The expected amplification products of*alphoid repeats*sequences were visualised by electrophoresis in 1.5% agarose gel containing 4 µl ethidiumbromide.

ΦX174 DNA/BsuRI (HaeIII) was used as molecular weight marker and was included in the first lane for skeletal fragments (Fig1) and in the 5th lane for teeth specimens (Fig 2).

X-specific primer of the *alphoid* r*epeats* amplified at 130 base pair (bp) bands and Y-specific primer amplified at 170 base pair (bp) bands respectively.

RESULTS

A total of 13 dried human cadaveric skeletal fragments and 9 teeth specimens were used in the study.

Of the 13 skeletal fragments, 12 (92.3%) samples (sample 1-12) showed apparent bands. Of all the samples that amplified, sample 6 appeared with a band with the highest signal/density.

For the teeth specimens, 7 (78%) samples amplified with apparent bands. Of these, sample 2 produced a band with the highest signal/density, while sample 6 showed a band with lowest signal/density.

DISCUSSION

The amplification of X primers with *alphoid repeats* for genomic DNA from human cadaveric skeletal fragments is shown in Fig. 1. Skeletal fragments 1-12 amplified with sharp, apparent and clear bands. This signifies the good quality of the genomic DNA in these samples. Of course, it is apparent that all the amplifications were with apparent, clear and visible bands, they were not of the same quality. The highest signal/density was seen in sample 6, followed by sample 3. However, the bands from the remaining samples that amplified were with low signal or density, but still apparent and visible. Although the X bands of all the samples that amplified were with a primer-dimer, yet the bands were very apparent, sharp,

clear and with good density/signal. However, there was no visible band for sample 13. Thus, there was no amplification for this sample. This signifies the poor quality/severe degradation of genomic DNA in the sample or presence of PCR inhibitors (Fig. 1).

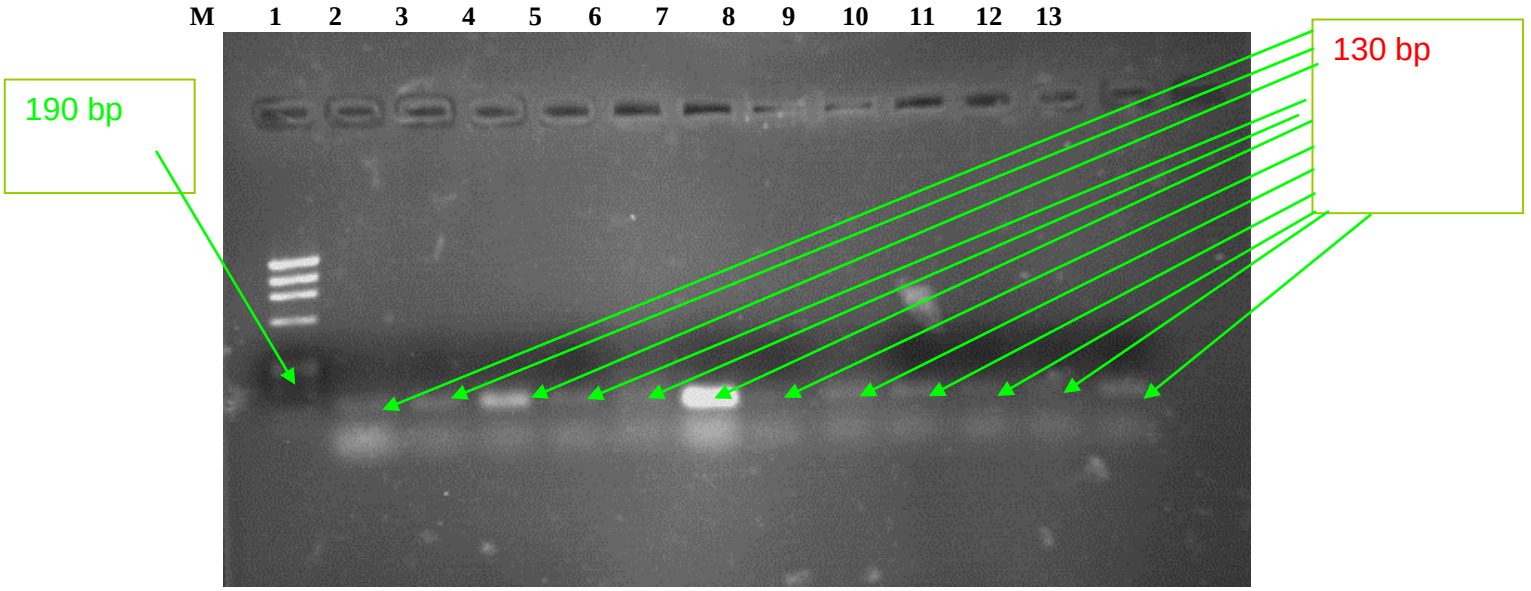

Figure 1. Amplification of the *alphoidrepeats* X primers from the DNA of the human skeletal fragments samples electrophoretically separated on 1.5% agarose gel (Reassay: 005/14).
Lanes: M = 1000 bp DNA ladder (Lot: 73020G3. Toyobo); 1 = Skull; 2 = Clavicle; 3 = Scapula; 4 = Radius; 5 = Ulna; 6 = Rib, 7 = Lumbar vertebra; 8 = Shaft of femur; 9 = Hip bone; 10 = Sacrum; 11 = Tibia; 12 = Fibula; 13 = Calcaneum.

The amplification of Y chromosome for the 9 teeth specimens is shown in Fig. 2. Teeth specimens 1, 2, 4, 5, 6, 7 and 8 showed apparent amplifications. Sample 2 amplified with a band with the highest signal/density, signifying the good quality of the genomic DNA in this sample. However, tooth specimen 6 appeared with faintest band, therefore, having the least density/signal. Teeth specimens 4, 5, 7 and 8 amplified with visible bands but showed primer-dimers (the lower signal of each band). Presence of primer-dimers explains the low signal of Y bands resulting from poor quality of the genomic DNA in these samples. There were no visible bands for teeth specimens 3 and 9. Thus, there was no amplification for these samples. This signifies the poor quality and or severe degradation of genomic DNA in these samples or presence of PCR inhibitors (Fig. 2).

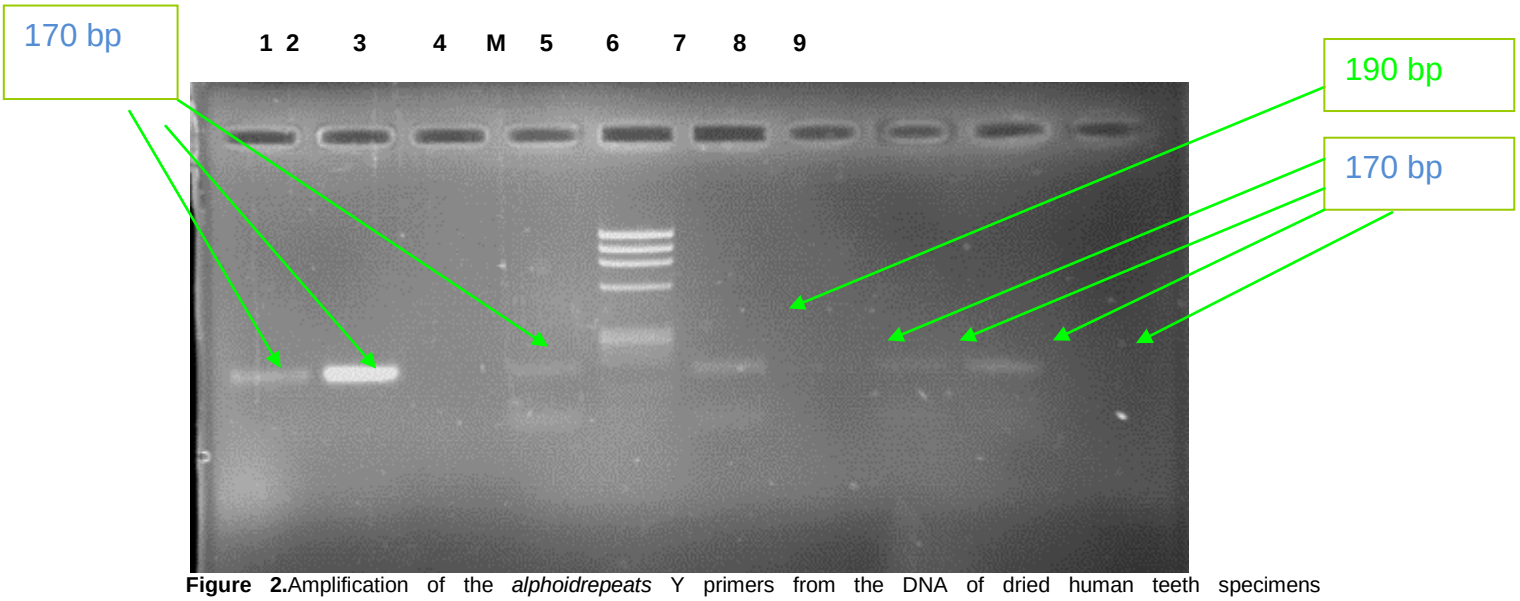

Figure 2. Amplification of the *alphoidrepeats* Y primers from the DNA of dried human teeth specimens electrophoretically separated on 1.5% agarose gel (Reassay: 005/14).
Lanes: 1-3 Incisors; 4 = Canine; M = 1000 bp DNA ladder (Lot: 73020G3. Toyobo); 5 and 6 = Premolars; 7-9 = Molars.

Faint bands or failure of amplification is an indication that the DNA in such samples was degraded or there were PCR inhibitors. The PCR inhibitors could be from the fixative and other environmental agents to which the samples were exposed. For the fact that these samples were from embalmed cadavers, the fixative agents might have negative effects on the PCR of the samples. Thus, fixative agents might be the source of PCR inhibition in these samples.

When an organism dies, its DNA normally becomes degraded by endogenous nucleases. Under certain circumstances, such as rapid desiccation, low temperatures or high salt concentrations, nucleases can themselves become destroyed or inactivated before all nucleic acids are reduced to mononucleotides. If this is the case, slower but still relentless processes start affecting the DNA[17]. Furthermore, deamination, depurination and other hydrolytic processes will lead to destabilization and breaks in DNA molecules. All these processes create problems for the retrieval of DNA sequences. For example, a high proportion of cytosine and thymine residues in extracts of ancient tissues are oxidatively modified to hydantoins oxidation products of the pyrimidine bases (cytosine and thymine), which block DNA polymerases and thus the PCR[4].In cadavers, DNA degrades very quickly, even in early post-mortem periods. The degradation of soft tissues is particularly evident after short intervals of time, a consequence of the rapid bacterial increase that is natural in decomposing corpses, especially in those that are exposed to hot temperatures in tropical countries[18], like Nigeria. Secondly, high molecular weight DNA, that is, that which can be analyzed, in human remains or in recent post-mortem material, is very scarce due to the degradation of genetic material. Exogenous agents, like microorganisms, humidity and many organic compounds, to which the corpses were exposed, also reduce the amount of informative DNA available[19].

Aldehyde fixing agents, such as formaldehyde and paraformaldehyde, are potent cross-linking agents. Formaldehyde fixation has a number of drawbacks which are familiar to histologists but which are not often appreciated by most PCR investigators. First, it is difficult to remove totally from tissues, even with extensive washing for

several days[20]. The residual formaldehyde groups left in tissue can continue forming cross-links with protein or nucleic acids long after the agent has been removed[21]. It is therefore quite possible that formaldehyde-fixed tissue can also react with PCR reagents after they have been added[22]. A second and probably more important property of formaldehyde is its ability to cross-link the histones which normally coat DNA[23]. Cross-linked histones are likely to be a major obstacle to the progression of *Taq* polymerase along the target DNA and they also impose constraints on the size of the PCR products that can be generated. Teo and Shaunak[22] demonstrated that cross-linking of histones on plasmid DNA by formaldehyde severely reduces its ability to be amplified by PCR.

Other types of non–cross-linking damage to DNA are also likely. Formaldehyde treatment causes single-strand breaks in treated cells[24]. It is worth noting that the recovery of intact high-molecular-weight DNA from formaldehyde-fixed tissues is very poor [25], and that this fragmented DNA amplifies poorly in PCR reactions. Teo and Shaunak[22] suggest that part of the failure to amplify DNA from fixed cells may be due to the presence of large numbers of single-stranded breaks in the target DNA.

CONCLUSION

The study has shown that our method of DNA extraction and PCR amplification was efficient on embalmed dried human cadaveric skeletal fragments and embalmmeddried human teeth specimens, from Sokoto, Northwestern Nigeria.

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Geliş tarihi/Received on :06.01.2014
Kabul tarihi/Accepted on: 14.02.2014