IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 7, Issue 2 (Jul. – Aug. 2013), PP 01-08
www.iosrjournals.org

Polymerase Chain Reaction (PCR)-Based Sex Determination Using Unembalmed Human Cadaveric Skeletal Fragments From Sokoto, Northwestern Nigeria.

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Abstract: *The strategy developed for sex determination in skeletal remains is to amplify the highly degraded DNA, by use of primers that span short DNA fragments. To determine sex of unembalmed human cadaveric skeletal fragments from Sokoto, North-western Nigeria, using Polymerase Chain Reaction (PCR). A single blind study of Polymerase Chain Reaction (PCR)-based sex determination using amelogenin gene and alphoid repeats primers on unembalmed human cadaveric skeletal fragments from Sokoto, North-western Nigeria, was undertaken. With amelogenin gene, genetic sex identification was achieved in four samples only. PCR Sensitivity = 40%, Specificity = 100%, Predictive value of positive test = 100%, Predictive value of negative test = 25%, False positive rate = 0%, False negative rate = 150%, Efficiency of test = 50%. Fisher's exact probability test* ***P = 1****. Z-test: z-value = -1.0955,* ***p>0.05; not statistically significant.*** *With alphoid repeats primers, correct genetic sex identification was achieved in all the samples. PCR Sensitivity = 100%, Specificity = 0%, Predictive value of positive test = 100%, Predictive value of negative test = 0%, False positive rate = 0%, False negative rate = 0%, Efficiency of test = 100%. Fisher's exact probability test* ***P = 1.*** *Z-test: z- and* ***p values were invalid****. The study, has demonstrated the applicability of PCR method of sex determination in unembalmed human skeletal fragments from Sokoto, Northwestern Nigeria. With amelogenin gene primers, correct genetic sex identification was achieved in four samples only. With alphoid repeats primers, correct genetic sex identification was achieved in all the samples. Therefore, alphoid repeats is more efficient and more reliable than amelogenin gene, in sex determination from unembalmed human skeletal fragments. This is the first known study determining the sex of unembalmed human skeletal fragments by means of PCR in Nigeria. There is need for further studies in Nigeria to complement the findings of this study.*

Key words: *PCR, sexing, bones, Sokoto, Nigeria.*

I. Introduction

Bones are an important source to obtain genetic material since they are inner the body and because their mineral condition, which cortical protects the medullar part from external factors and micro organisms that may degrade the DNA [1]. In mass disaster situations, Andelinovic *et al* (2005) [2], presented that DNA analysis from bone and tooth material allowed identify 109 victims of 12 year war in the former Yugoslavia. Traditional identification methods would not give such good results [3]. DNA analysis from bones and teeth would also guide anthropologic studies to identify population migration origin in ancient times [4,5]. Even though sample integrity is a fundamental factor in DNA extraction, studies with degraded biological material showed to be possible to analyze genetic material when it is fragmented (200 to 1200 pair bases) [6-8].

PCR is an *in vitro* method for the enzymatic amplification of specific DNA sequences, using two oligonucleotide primers that hybridize two opposite strands of the DNA, and flank the region of interest in the target DNA. A repetitive series of cycles involving template denaturation, primer annealing, and the extension of the annealed primers using DNA polymerase, results in the exponential accumulation of the specific fragment that is being sought. Assay time is reasonably short [9]. PCR analysis for forensic purposes has been applied to parentage testing, sex determination and species identification [10]. As DNA is often only present in forensic specimen in trace amounts, this method is of great value as it can theoretically be used to detect genetic material from as little as a single cell.(9) Because of the margin of error in the known metrical and non-metrical methods, it is necessary to use more sensitive and accurate methods to identify sex from tissue [11].

The general objective of this study was therefore, to use PCR-based method to identify sex of unembalmed human cadaveric skeletal fragments, from Sokoto, Northwestern Nigeria.

II. Materials and Methods

A total of twelve (12) unembalmed human (cadaveric) skeletal fragments from the carpus and phalanges of the hand and foot were used in this study. They comprised of: (1) Little finger (2) Capitate (3) Lunate (4) Hamate (5) Trapezium (6) Scaphoid (7) Middle finger (8) Small toe (9) Triquatrum (10) Pisiform (11) Trapezoid (12) Index finger.

This study was a single blind type. Information about the morphological sex of the samples was withheld from me by my supervisors and my colleagues. Samples were collected by my colleagues from pre-embalmed cadavers for dissection in the Department of Anatomy, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria, and handed over to me (the investigator).

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.

The protocol for precaution against contamination was based on the method employed by Cooper and Poinar, (2000),[(12)] with minimal modification. While Cooper and Poinar, (2000) [12] used separate rooms for DNA extraction and PCR amplification, we used same room for these, but under two different hoods. Specific and stringent precautions were undertaken to handle specimen during DNA extraction and amplification. Specimen were handled (using disposable masks, gloves and laboratory coats) in an area where no other DNA studies were simultaneously carried out. The DNA extraction and the setting up of PCR reactions of DNA templates were carried out under two different hoods, daily irradiated with UV rays (254nm). Only disposable sterile tubes, filtered tips, sterile reagents and solutions, exclusively dedicated for DNA studies, were used. A set of pipettes, once used was not used again for DNA extraction, PCR amplification and analysis of the PCR products.

Samples of unembalmed cadaveric skeletal fragments were initially placed in a freezer (Elite. Air Multi=flow. Freezer and Refrigerator. No frost) at -20°C (for minimum of 72 hours) to eliminate surface contamination from the depositional environment and post depositional handling. The equipment and surface of the 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 before and after grinding each sample. A sheet of aluminium foil (Helwan Aluminium Foil. 15M x 40CM. Made in Egypt) sterilized 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.

About 0.5cm x 0.5cm from each of the 12 bone samples was fragmented using sterile bone cutter and forceps (CE Stainless Pakistan). Samples were further sterilised with 70% ethanol and a sterile soft tissue was used to absorb excess ethanol from the sample (to dry the sample and minimize 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 pulverization. Pulverization continued until the bone fragment turned into powder form. Aliquot of the ground bone powder was then transferred into 1.5ml microtubes (1.5ml microcentrifuge tubes (Bio Basic Inc. (BBI). Cat. No. BT620NS – 100. Sterilized 1.5ml microcentrifuge certified RNase DNase 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.

The extraction of DNA from all the samples was done by standard phenol-chloroform method for bones, established by the Department of Medical Molecular Genetics, Division of Human Genetics and Genome Research, National Research Centre, Cairo, Egypt [13]. The samples consisted of about 0.3mg aliquot of the ground bone powder.

PCR amplification of the extracted DNA for sex determination from samples used previously prescribed *amelogenin* gene multiplex primers by Faerman *et al.*, 1995 [14] and Matheson and Loy, 2001 [15] and *alphoid repeats primers* by Witt and Erickson, 1989 [16] and Murakami *et al.*, 2000 [17].

A PCR master mix was prepared in a single 0.5ml PCR tube from: **2.5 µl of 10x Buffer**, **2.5 µl of dNTPs**, **2.5 µl of $MgCl_2$**, **4 µl of forward primer M4**, **2 µl of X reverse primer M5** and **2 µl of Y reverse primer M6**, **1.25 µl of Taq polymerase** and **6.25 µl of DEPC water.** A total volume of **23 µl** from the master mix was transferred into 12 0.25ml microtubes. **1 µl Paraffin oil** was added to seal and avoid evaporation of the reaction mixture. **2 µl DNA** (template) from the respective 12 samples was finally added to accomplish a reaction volume of **25 µl** for each tube.

Normal PCR was accomplished in a thermocycler (Minicycler™ MJ RESARCH), in a **25 µl** reaction volume, to amplify selected sequences of the *amelogenin* gene, as follows; Denaturation step: was at 94°C for 1 minute. Annealing step: was at 55°C for 2 minutes. Extension/elongation step: was at 72°C for 2 minutes. The above first three steps were repeated for 40 cycles. Final extension/elongation: was at 72°C for 15 minutes. Cooling of the reaction process: was at 4 °C for 48hours.

Amplification with *alphoid repeats* primers was prepared separately each for X (X1 and X2) primers, and Y (Y11 and Y22) primers, respectively, in separate 0.5ml PCR tubes. The master mix for each of X and Y chromosomes was separately 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, **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 **33 µl** from the master mix was transferred into 12 tubes, for X and Y, respectively and **1 µl Paraffin oil** added to seal and avoid evaporation of the reaction mixture. A volume of **2 µl** DNA from the respective 12 samples was finally added to the 12 tubes (containing PCR reagents for X and Y respectively), to accomplish a reaction volume of **35 µl** for each tube.

Normal PCR was accomplished in a thermocycler (Minicycler^{TM} MJ RESARCH in a **35 µl** reaction volume, to amplify selected sequences of the *alphoid repeats*, as follows; Initialization step at 95 ºC for 5 minutes. Denaturation step at 94 ºC for 40 seconds. Annealing step at 55 ºC 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 at step 4ºC for 48 hours.

The protocol employed by Maniatis, *et al.*, (1982) [18] for preparation of Agarose gel electrophoresis was adopted**.**

Amplification with *amelogenin* primers was at 330 base pair (bp) bands for X chromosome, and 218 base pair (bp) bands for Y chromosome, respectively.

With *alphoid* repeats primers, X-specific primer amplified at 130 base pair (bp) bands and Y-specific primer amplified at 170 base pair (bp) bands respectively.

The expected amplification products of *amelogenin* gene and *alphoid repeats* sequences were visualised by electrophoresis in 1.5% agarose gel containing 4 µl ethidium bromide.

For *amelogenin* gene, a molecular weight marker was included in the middle and last lanes (Figures: 1a and 1b), respectively.

For *alphoid repeats,* a molecular weight marker was included in the first lanes (Figures: 2a and 2b).

Fisher's exact test (probability) and Z-test were employed for comparison of values. The sensitivity, specificity, efficiency, predictive value of positive tests, predictive value of negative tests, false positive rates and false negative rates of the PCR were determined according to the arithmetic definitions of these terms.

III. Results

Table 1 shows the result of PCR sex identification of the samples used in this study, with *amelogenin* gene. Amplification and genetic sex identification were achieved in four samples only (samples 4, 8, 11 and 12). Of these, only sample 8 amplified with both X and Y chromosomes. The others (samples 4, 11 and 12) amplified with only Y chromosome, thus, the X chromosome was negative. Amplification of Y chromosome for sample 11 was a band in a smear (smash band). Samples 1-3, 7, 9 and 10, produced smear only. Samples 5 and 6 were negative for both X and Y chromosomes. Sex identification was not achieved in samples that produced smear only (samples 1-3, 7, 9 and 10) and those that were negative (5 and 6).

Table 1: Results of Genetic Sex Identification of Unembalmed Bone (UEB) Samples Using *Amelogenin* Gene Primers.

Bone fragment serial number	Bone sampled	Morphological sex	PCR results with *amelogenin* gene primers		Genetic sex
			X chromosome amplification (330 bp)	Y chromosome amplification (218 bp)	
UEB 1	Little finger	Male	Smear	Smear	Unidentified
UEB 2	Capitate	Male	Smear	Smear	Unidentified
UEB 3	Lunate	Male	Smear	Smear	Unidentified
UEB 4	Hamate	Male	Negative	Positive	Male
UEB 5	Trapezium	Male	Negative	Negative	Unidentified
UEB 6	Scaphoid	Male	Negative	Negative	Unidentified
UEB 7	Middle finger	Male	Smear	Smear	Unidentified
UEB 8	Small toe	Male	Positive	Positive	Male
UEB 9	Triquatrum	Male	Smear	Smear	Unidentified
UEB 10	Pisiform	Male	Smear	Smear	Unidentified
UEB 11	Trapezoid	Male	Negative	Smash band at 218 bp	Male
UEB 12	Index finger	Male	Negative	Positive	Male

Genetic sex identification was achieved in four samples (4, 8, 11 and 12) only (see Figures 1a and 1b). PCR Sensitivity = 40%, Specificity = 100%, Predictive value of positive test = 100%, Predictive value of negative test = 25%, False positive rate = 0%, False negative rate = 150%, Efficiency of test = 50%. Fisher's exact probability test **P = 1**. Z-test: *z-value = -1.0955,* ***p>0.05;*** **not statistically significant.**

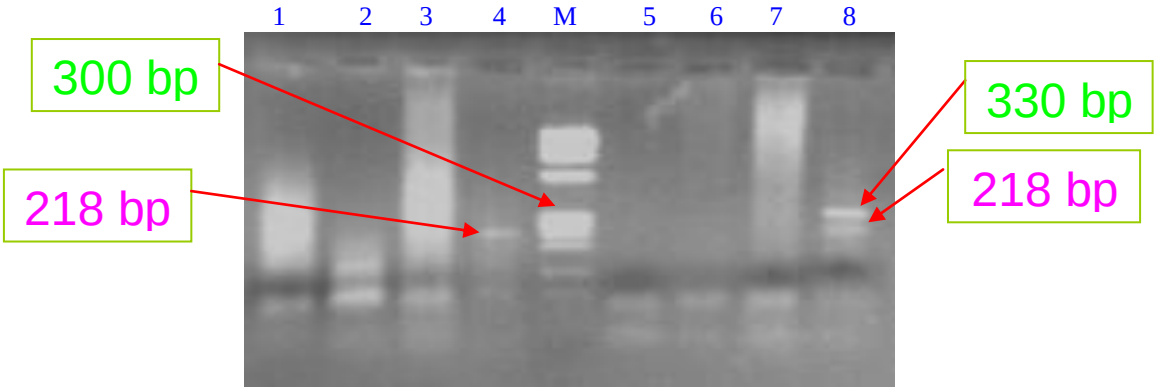

Figure 1a: Amplification of the *amelogenin* gene X and Y primers from the DNA of the unembalmed human bone fragments 1-8, electrophoretically separated on 1.5% agarose gel (Reassay: 005/14).

Lanes: 1 = Little finger; 2 = Capitate; 3 = Lunate; 4= Hamate; M = 1000 bp DNA ladder (Lot: 73020G3. Toyobo); 5 = Trapezium; 6 = Scaphoid; 7= Middle finger; 8= Small toe.

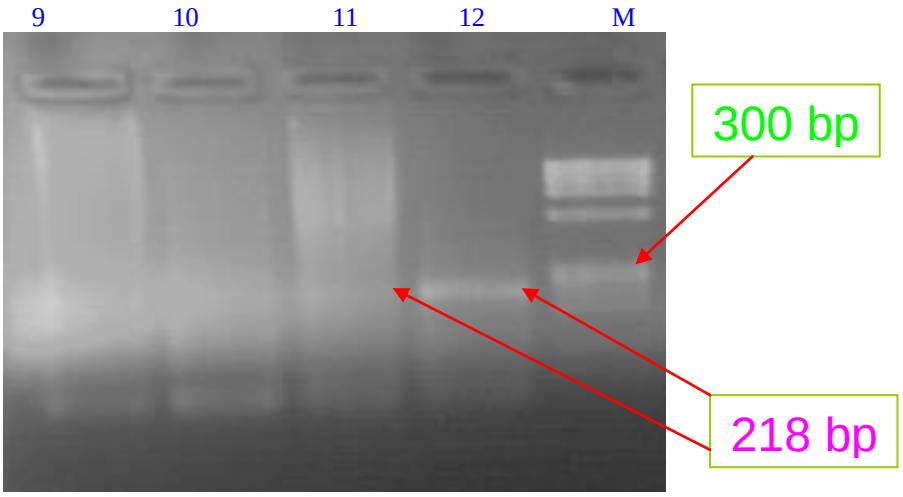

Figure 1b: Amplification of the *amelogenin* gene X and Y primers from the DNA of the unembalmed human fragments 9-12, electrophoretically separated on 1.5% agarose gel (Reassay: 005/14).

Lanes: 9 = Triquatrum; 10 = Pisiform; 11= Trapezoid; 12 = Index finger; M = 1000 bp DNA ladder (Lot: 73020G3. Toyobo).

Details of the genetic sex identification for the samples using *alphoid repeats* primers are shown in Table 2. Amplifications for both X and Y chromosomes were achieved in all the samples. Correct genetic sex identification was achieved in all the samples. Thus, all the samples were genetically identified as males which agreed with their morphological sexes (males).

Table 2: Results of Genetic Sex Identification of Unembalmed Bone (UEB) Samples Using *Alphoid Repeats* Primers.

Bone fragment serial number	Bone sampled	Morphological sex	PCR results with *alphoid repeats* primer		Genetic sex
			X chromosome amplification(130bp)	Y chromosome amplification (170 bp)	
UEB 1	Little finger	Male	Positive	Positive	Male
UEB 2	Capitate	Male	Positive	Positive	Male
UEB 3	Lunate	Male	Positive	Positive	Male
UEB 4	Hamate	Male	Positive	Positive	Male
UEB 5	Trapezium	Male	Positive	Positive	Male
UEB 6	Scaphoid	Male	Positive	Positive	Male
UEB 7	Mid finger	Male	Positive	Positive	Male
UEB 8	Small toe	Male	Positive	Positive	Male
UEB 9	Triquatrum	Male	Positive	Positive	Male
UEB 10	Pisiform	Male	Positive	Positive	Male
UEB 11	Trapezoid	Male	Positive	Positive	Male
UEB 12	Index finger	Male	Positive	Positive	Male

Correct genetic sex identification was achieved in all the samples. All the samples were males morphologically, and were genetically identified as males (see Table 2; Figures 2a and 2b). PCR Sensitivity = 100%, Specificity = 0%, Predictive value of positive test = 100%, Predictive value of negative test = 0%, False positive rate = 0%, False negative rate = 0%, Efficiency of test = 100%. Fisher's exact probability test **P = 1.** Z-test: *z*- and ***p values were invalid***.

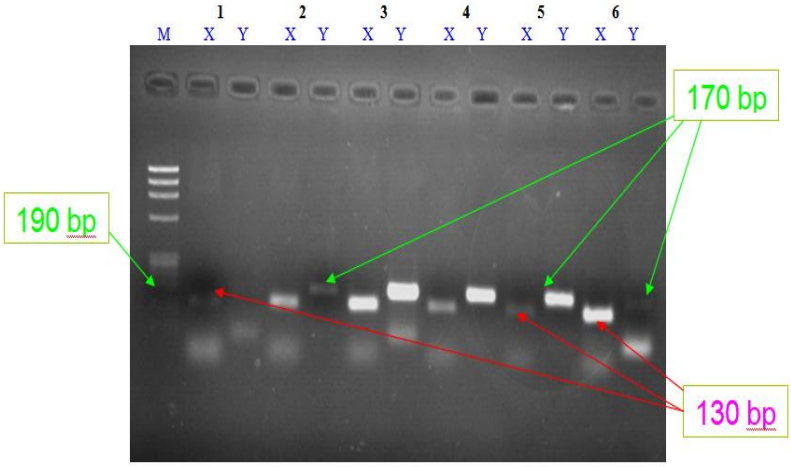

Figure 2a: Amplification of the *alphoid repeats* X and Y primers from the DNA of the unembalmed human bone fragments 1-6, electrophoretically separated on 1.5% agarose gel (Reassay: 005/14).

Lanes: M = 1000 bp DNA ladder (Lot: 73020G3. Toyobo); 1 = Little finger; 2 = Capitate; 3 = Lunate; 4= Hamate; 5 = Trapezium; 6 = Scaphoid.

Figure 2b: Amplification of the *alphoid repeats* X and Y primers from the DNA of the unembalmed human bone fragment 7-12, electrophoretically separated on 1.5% agarose gel (Reassay: 005/14).

Lanes: M = 1000 bp DNA ladder (Lot: 73020G3. Toyobo); 7= Middle finger; 8= Small toe; 9 = Triquatrum; 10 = Pisiform; 11= Trapezoid; 12 = Index finger.

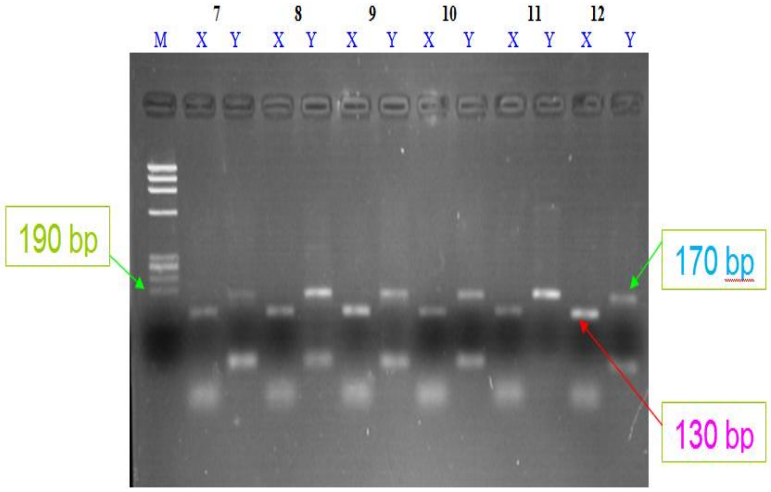

Figure 2b: Amplification of the *alphoid repeats* X and Y primers from the DNA of the unembalmed human bone fragment 7-12, electrophoretically separated on 1.5% agarose gel (Reassay: 005/14).

Lanes: M = 1000 bp DNA ladder (Lot: 73020G3. Toyobo); 7= Middle finger; 8= Small toe; 9 = Triquatrum; 10 = Pisiform; 11= Trapezoid; 12 = Index finger.

Table 3 compares the results of genetic sex identification of the samples between *amelogenin* gene and *alphoid repeats* primers. Amplification of both chromosomes with *amelogenin* gene was achieved in sample 8 only. Amplification of Y chromosome only, with *amelogenin* gene, was achieved in samples 4, 11 (with smash band at the right size) and 12. With *amelogenin* gene, both X and Y chromosomes showed either smear only (samples 1-3, 7, 9 and 10) or no amplification at all (X chromosome of samples 4 and 11 and both X and Y

chromosomes of samples 5 and 6). Consequently, the genetic sexes of only samples 4, 8, 11 and 12 were correctly identified. Thus, genetic sex identification was not achieved with *amelogenin* gene, for the remaining samples. With *alphoid repeats*, amplification of both X and Y chromosomes was achieved in all the samples. Therefore, correct genetic sex identification for all the samples was achieved with *alphoid repeats.*

Table 3: Results of Comparison of PCR Amplification of Unembalmed Bone (UEB) Samples Between *Amelogenin* Gene and *Alphoid Repeats* Primers.

Bone fragment serial number	Bone sampled	Morpho-logical sex	*Amelogenin* gene			*Alphoid* repeats		
			X (330 bp)	Y (218 bp)	Genetic sex	X (130 bp)	Y (170 bp)	Genetic Sex
UEB 1	Little finger	Male	Smear	Smear	Uniden-tified	Positive	Positive	Male
UEB 2	Capitate	Male	Smear	Smear	Uniden-tified	Positive	Positive	Male
UEB 3	Lunate	Male	Smear	Smear	Uniden-tified	Positive	Positive	Male
UEB 4	Hamate	Male	Negative	Positive	Male	Positive	Positive	Male
UEB 5	Trapezium	Male	Negative	Negative	Uniden-tified	Positive	Positive	Male
UEB 6	Scaphoid	Male	Negative	Negative	Uniden-tified	Positive	Positive	Male
UEB 7	Middle finger	Male	Smear	Smear	Uniden-tified	Positive	Positive	Male
UEB 8	Small toe	Male	Positive	Positive	Male	Positive	Positive	Male
UEB 9	Triquatrum	Male	Smear	Smear	Uniden-tified	Positive	Positive	Male
UEB 10	Pisiform	Male	Smear	Smear	Uniden-tified	Positive	Positive	Male
UEB 11	Trapezoid	Male	Negative	Smash band at 218 bp	Male	Positive	Positive	Male
UEB 12	Index finger	Male	Negative	Positive	Male	Positive	Positive	Male

With *amelogenin* gene, the genetic sexes of only samples 4, 8, 11 and 12 were identified. Genetic sex identification for all the samples was achieved with *alphoid repeats.*

IV. Discussion

Qualitative work on medical genetics is problematic and scientific progress in this very important and topical field is very slow in Nigeria. This slow progress is attributable to paucity of the necessary, basic investigatory facilities [19]. DNA-based sex determination of skeletal remains was first performed by amplification of Y chromosome-specific sequences [20], a method that this study employed. In this study, primers that span short DNA fragments from the *amelogenin*-encoding gene and *alphoid repeats* were used for sex determination from unembalmed human skeletal fragments.

With the use of *amelogenin* gene in the twelve (12) unembalmed human skeletal fragments used in this study (Table 1; Figures 1a and 1b), amplification and genetic sex identification were achieved in four samples (samples 4, 8, 11 and 12) only. Of these, only sample 8 amplified with both X and Y chromosomes. The others (samples 4, 11 and 12) amplified with only Y chromosome, thus, the X chromosome was negative.

Amplification of Y chromosome in sample 11 was a band in a smear (smash band) at 218 bp (right size). Samples 1-3, 7, 9 and 10, produced smear only. Samples 5 and 6 were completely negative (no band, no smear) for both X and Y chromosomes. Genetic sex identification was not achieved in samples that produced only smear (samples 1-3, 7, 9 and 10), and in those that were completely negative (no band, no smear) for both X and Y chromosomes (samples 5 and 6). The genetic sexes of the samples that were identified as males (samples 4, 8, 11 and 12), agreed with their respective morphological sexes (males).

Presence of smear is an indication of presence of DNA in a sample. However, such DNA could not produce a definite and specific band to allow accurate genetic sex identification. This usually results from inefficient priming or inefficient extension [21]. A series of PCRs containing different concentrations of the primers was set to find the optimal concentration. A series of touchdown PCRs containing different concentrations of Mg^{2+} to find the optimal concentration was also employed to improve the amplification. Again, a minimum possible temperature for annealing step was used. Finally, adjuvants such as bovine serum albumin (BSA) and dimethyl sulfoxide (DMSO) were separately added to the reaction mixture, as suggested by Sambrook and Russell, 2001 [21]. However, all these remedies did not yield better result than the ones presented or shown here.

Negative amplification of either X or Y chromosome is indicated by empty gel wells, without smear or visible band on the gel (samples 5 and 6). Failure of amplification could result from errors in PCR buffer formulation, failure to load sample DNA and errors in loading sample into gel wells. The remedy lies in demonstrating the presence of DNA in the sample using for example the X-specific primers, and repeatedly amplifying the putative female sample with the Y-specific primers and obtaining negative results (no band observed)[14]. Nevertheless, a positive result following amplification with both X and Y primers is sufficient to identify the sample as male [22]. Negative amplification could mean that the DNA was not retrievable, i.e. there was no amplifiable DNA for the primers employed by this study, in such samples. Thus, the DNA could have been severely degraded by multiple factors, to single strands or the base sequences were too short for the

primers employed to amplify this DNA. As well, failure to amplify a DNA may result from the presence of PCR inhibitors which interfere with the PCR reaction [23,24].

Sample eleven (11) that amplified with only Y chromosome with a smash band at 218 base pairs (bp), as shown in Figure 1b, could not be dependable in conclusive decision making on the gender identification of the sample.

A preferential amplification of Y chromosome was noticed in the samples. This was similarly reported by Faerman *et al.,* 1995 [14] and Matheson and Loy, 2001 [15]. The higher sensitivity of detection of the Y chromosome might be due to the shorter amplification product or to the nature of the allele-specific primers. Regardless of the reason, this suggests that this method (sex identification with *amelogenin* gene) is more prone to miss a female specimen than a male. On the other hand, the more efficient amplification of the Y allele suggests that presence of a single 330 bp product indicates a female [14].

The success rate for gender identification with *amelogenin* gene in this study was 33.3% only. However, this is favourably comparable with reports by earlier workers who conducted similar studies on bone fragments using *amelogenin* gene. In 1995, Faerman *et al.,* 1995 [14], reported 41% success rate, Faerman *et al.,* 1998[25] reported 44% success, Mays and Faerman, 2001[26] reported 42% and Matheson and Loy, 2001 [15] reported 40%. *Amelogenin* PCR applied to larger number of Etruscan samples shows a low success rate (Vernessi *et al.,* 1999 [27].

PCR sex identification for the samples using *alphoid repeats* primers recorded 100% success rate. Amplification was achieved for both X and Y chromosomes in all the samples (Table 2, Figures 2a and 2b). There was correct genetic sex identification for all the samples. Thus, there was no discrepancy between the genetic and morphological male sexes of the samples. The bands produced were sharp and of the right molecular weight; 130 bp for X and 170 bp for Y chromosomes, respectively.

Comparing the results of genetic sex identification of the samples between *amelogenin* gene and *alphoid repeats* primers (Table 3), this study has found that with *amelogenin* gene, although 4 samples amplified (samples 4, 8, 11 and 12), amplification of both X and Y chromosomes was achieved in only one sample (sample 8). The others (samples 4, 11 and 12), amplified with Y chromosome only, thus, the X chromosome did not amplify. The remaining samples in this group showed either smear only (samples 1-3, 7, 9 and 10), or no amplification (negative) at all (samples 5 and 6). Hence, the success rate for genetic identification was 33.3% only. However, with *alphoid repeats,* amplification of both X and Y chromosomes was achieved in all the samples. There was 100% success rate of genetic sex identification for the samples, with *alphoid repeats.* The genetic sexes of these samples confirmed their morphological sexes (males), and this was based on the amplification of both X and Y chromosomes of the samples, with *alphoid repeats.* Although, with *amelogenin* gene, only four samples (4, 8, 11 and 12), amplified, their genetic sexes were correctly identified as males, and agreed with the identification (males) by the *alphoid repeats* primers, as indicated above. However, the genetic sex identification of these four samples was based on the amplification of only the Y chromosome in samples 4, 11 (with smash band at 218) and 12, and amplification of both X and Y chromosomes in sample 8, as found with *alphoid repeats* in all the samples. Failure of amplification and subsequent inability to genetically identify the sexes of samples 1-3, 5-7, 9 and 10, with *amelogenin* gene, but having achieved it (amplification and correct genetic sex identification) with *alphoid repeats,* could be due to the fact that the base sequence of *amelogenin* gene primers are larger (330 bp and 218 bp, respectively, for X and Y chromosomes) than their counter parts (130 bp and 170 bp, respectively, for X and Y chromosomes) in *alphoid repeats.*

V. Conclusions

The study, has demonstrated the applicability of PCR method of sex determination in unembalmed human skeletal fragments from Sokoto, Northwestern Nigeria. With *amelogenin* gene primers, correct genetic sex identification was achieved in four samples only. With *alphoid repeats* primers, correct genetic sex identification was achieved in all the samples. Therefore, *alphoid repeats* is more efficient and more reliable than *amelogenin* gene, in sex determination from unembalmed human skeletal fragments. This is the first known study determining the sex of unembalmed human skeletal fragments by means of PCR in Nigeria. There is need for further studies in Nigeria to complement the findings of this study.

Acknowledgements

We wish to thank the Education Trust Fund, Usmanu Danfodiyo University, Sokoto, Nigeria for the grant to undertake the bench work of this study at the Department of Medical Molecular Genetics, Division of Human Genetics and Genome Research, National Research Centre, Cairo, Egypt.

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