Molecular sex identification of dry human teeth specimens from Sokoto, Northwestern Nigeria

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Abstract

Background: The advent of molecular techniques has revolutionized the ability of scientists to estimate the sex of individuals. Forensic odontology plays an important role in establishing the sex of victims with bodies mutilated beyond recognition due to major disaster. The genetic difference between males and females is defined by the presence or absence of the Y-chromosome. The use of *alphoid*-repeat primers in sex estimation was first applied on dried blood. Generally, the X, Y *alphoid* repeats blind test attest to the accuracy of genetic testing, and also point the potential for occasional error in morphometric sexing. **Aim:** To estimate genetic sex of dry human teeth specimens from Sokoto, Northwestern Nigeria, using polymerase chain reaction (PCR). **Materials and Methods:** A single-blind study of DNA analysis for sex estimation of nine dry human teeth specimens from Sokoto, Northwestern Nigeria, through PCR, using *alphoid* repeats primers, was undertaken. **Results:** The genetic sex of each group of the teeth samples were accurately (100%) identified. For each group of teeth, 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*. **Conclusion:** This study has demonstrated the successful use of *alphoid-repeat* primers in genetic sex identification of human dry teeth samples from Sokoto, Northwestern Nigeria. This is the first known study estimating the sex of human dry teeth specimens by means of PCR in Nigeria. There is need for further studies in Nigeria to complement the findings of this study.

Key words: Nigeria, sexing, Sokoto, teeth

Introduction

Teeth are the ideal organ to estimate sex from fragmented, decomposed and burnt bodies as they are highly mineralized and are the most resistant to heat and decomposition; hence, they are considered the most durable of animal tissues.[1] Teeth also are a good source to obtain genetic material. This is true due to their great tissue resistance (enamel, dentin, cementum and pulp) against external injurers.[2] Tooth can be considered as a reliable source of DNA for repeating genetic analysis even months after death, devoid of any special procedures for preservation.[3] Malaver and Yunis (2003)[4] extracted DNA obtained from dentin and cementum of 20 corpses that had been buried for at least 5 years. Teeth are able to withstand temperatures between 150-450°C;[5] therefore making them important where human remains were subjected to fire.[6]

Unlike skeletal remains, the human origin of dental material is rarely in doubt. However, discrimination between male and female teeth is extremely difficult. Male teeth are usually larger than female teeth but there is conflicting evidence as to the reliability of determination from tooth size.[7] Lunt (1974)[8] is of the opinion that such linear measurements are unreliable in determining the sex of an individual. In 1991, Schwart *et al*.,[9] found that dental pulp, which had been exposed to a variety of different environmental conditions contained sufficient DNA for restriction fragment length polymorphism (RFLP) analysis. Similarly in 1993, Smith *et al*.,[10] demonstrated that sufficient DNA could be obtained from either crushed teeth or teeth that had been sectioned conservatively and that the method of sampling did not affect the ability to perform DNA-typing analysis.

Sex determination is the first step of personal reconstruction phase of identification in forensic medicine.[11] While the establishment of identity from intact fresh corpses is often obvious, the correct estimation of sex may be difficult after catastrophic events such as fires, high impact crashes and explosions and in criminal cases where highly decomposed bodies are found.[1] Problems of sex identification arise in cases where skeletal remains are fragmentary or are those of juveniles, infants[12] or persons with alterations in genetic code; such as women with Y chromosome.

Despite its importance in cell biology and evolution, the centromere has remained the final frontier in genome assembly and annotation due to its complex repeat structure. However, isolation and characterization of the centromeric repeats from newly sequenced species are necessary for a complete understanding of genome evolution and function.[13] Alpha satellite DNA covers the centromeric region of all human chromosomes over distances often as large as several megabases. It is composed of a basic 171-bp long unit that is organized into tandemly arranged higher order repeats (HORs); HORs contain variable numbers of basic repeats, from 4 (chromosome 2) to as many as 34 (chromosome Y). The HORs of a given chromosome are highly homogeneous, with sequence identity often exceeding 99% along the blocks they constitute. The length of the *alphoid* blocks also varies substantially.[14]

Probes specific for repeat sequences derived from the heterochromatic region of the long arm of the Y chromosome were used to develop the first simple screening test for sex diagnosis through dot blot hybridization in small samples of unrestricted genomic DNA.[15,16] These probes have also been used for *in situ* hybridization.[17] However, the use of a probe specific to the heterochromatic region of the Y chromosome may be of limited reliability because of its inability to detect Y sequences in individuals lacking this region of the Y chromosome, as demonstrated in some normal, fertile males.[18] Conversely, this region of the

Y may be carried as a translocation in 1 of 3,000 apparently normal females.[19] Fragments of Yq may also be absent in Turner syndrome mosaics, whose marker chromosome is a Y derivative. Thus, probes for the heterochromatic region of the long arm of the Y chromosome have an increased risk of an incorrect diagnosis of sex.[20]

The only repetitive DNA family likely to exhibit the properties of significant chromosome specificity is the *alphoid* (alpha) satellite family located in peri-centromeric regions of all human chromosomes.[21] Alphoid satellite sequences on the Y have a higher repeat organization detected with restriction enzymes such as *Eco*RI:[22] A 5.5-kb fragment was found to be characteristic for these Y *alphoid* repeats. Dosage experiments have demonstrated that there are about 100 copies of this fragment on the Y chromosome.[22]

The use of *alphoid* repeat primers in sex estimation is expected to allow more accurate result since the X chromosome-specific *alphoid* repeat sequence can be detected along the Y chromosome-specific repeat sequence.[11]

The general objective of this study was therefore, to use PCR-based method to conduct DNA analysis with *alphoid*-repeat primers for sex estimation of dry teeth specimens from Sokoto, Northwestern Nigeria.

Materials and Methods

A pilot study of nine dried human teeth specimens were grouped into three as follows:
- Three dry teeth samples from embalmed cadavers. They included; 1 canine and 2 molars that are dark brown. Dry, and mildly brittle. Although mildly eroded, contained a crown, root and closed pulp cavity
- Three dry teeth specimens (for disposal) from adult patients. They included; 1 white molar and 2 white premolars. Dry and hard. Contained a full crown and root with a closed pulp cavity
- Three deciduous teeth specimens. All were incisors and white, dry and hard. Comprised of only the crown. No root. Empty, very shallow, dried pulp cavity.

This study was a single-blind type. Information about the morphological sex of the teeth samples was withheld from me by my supervisors. Samples of embalmed cadaveric teeth specimens were collected by my colleagues from the remains of the dissected cadavers in the Department of Anatomy, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. Teeth samples extracted (for disposal) from patients were collected by my colleagues from the Dental Unit, Usmanu Danfodiyo University, Teaching Hospital, Sokoto, Nigeria. Deciduous teeth were provided by my supervisors, in Nigeria, and was 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.

Laboratory procedures

In addition to the specific and stringent precautions against contamination as recommended by Cooper and Poinar, (2000)[23] was followed while handling ancient bones for molecular analysis, other peculiar precautions were also undertaken to handle the dry teeth samples before grinding. Samples of the dry teeth were initially placed in a freezer at −20°C (for minimum of 72 hours) before the following cleaning procedures were applied to eliminate surface contamination from the depositional environment and post-depositional handling:

With gloved hands, each tooth was held with a sterile forceps (CE Stainless, Pakistan) and washed under running tap water. The surfaces of the tooth were brushed with an abrasive paper. The tooth was then cleaned with 4% hypochlorate bleach (4 ml chlorex + 96 ml distilled water). The surface of the tooth was 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 ultraviolet (UV) light for 15-30 minutes, before grinding.

The equipment and surface of the hood were cleaned with distilled water, sterilized with 70% ethanol, and the UV irradiation was done, before and after grinding each sample. A sheet of aluminium foil was sterilized with 70% ethanol and placed on the surface of the hood. A whole tooth was grinded as a specimen at a time. Each tooth sample was first fragmented using sterile bone cutter and forceps (CE Stainless, Pakistan). Samples were further sterilized 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 effects of alcohol) before grinding. Each sample (one sample at a time) was then placed in a sterile mortar and pestle, for pulverization. Pulverization continued until the tooth turned into powder form. Aliquot of the ground tooth powder was then transferred into 1.5 ml microtubes and stored in a refrigerator at −80°C, before DNA extraction.

The extraction of DNA from all the samples was done by standard phenol-chloroform method for teeth established by the Department of Medical Molecular Genetics, Division of Human Genetics and Genome Research, National Research Centre, Cairo, Egypt.[24] About 0.5 mg aliquot of the ground tooth powder from each sample was transferred into 1.5 ml microtube. About 600 μl of 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 and vortexed. To this, 600 μl of phenol was added and left on a rotator for 72 hours. The contents were centrifuged at 14,000 revolutions per minute (rpm),

for 10 minutes. From the supernatant about 500 μl was transferred into a new 1.5 ml microtube. To this, 500 μl chloroform was added and centrifuged as above. About 500 μl was transferred from the supernatant into a new 1.5 ml microtube. About 500 μl isopropanol was added and left overnight in a freezer at −20°C. The contents were centrifuged and the supernatant was discarded gently. To the contents, 500 μl of 70% ethanol was added, centrifuged and discarded gently. Open-lid was left to dry before elution with 40 μl of diethylpyrocarbonate (DEPC) water.

PCR amplification of the extracted DNA for sex determination used previously prescribed *alphoid*-repeat primers by Witt and Erickson, 1989[25] as shown in Table 1. A PCR master mix was prepared separately, each for X (X1 and X2) primers, and Y (Y11 and Y22) primers, respectively, in two separate 0.5 ml 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 nine tubes, for X and Y, respectively and 1 μl of paraffin oil added to seal and avoid evaporation of the reaction mixture. A volume of 2 μl of DNA from the respective nine teeth samples was finally added to the nine 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™ MJ RESARCH) in a 35 μl reaction volume, to amplify selected sequences of the *alphoid repeats*, as follows: (i) Initialization step at 95ºC for 5 minutes. (ii) Denaturation step at 94ºC for 40 seconds. (iii) Annealing step at 55ºC for 40 seconds. (iv) Extension/elongation step at 72ºC for 40 seconds. Steps 2-4 were repeated for 35 cycles. (v) Final extension/elongation step 72ºC for 40 seconds. (vi) Cooling of reaction process was at 4ºC.

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

The ΦX174 DNA/BsuRI (HaeIII) was used as molecular weight marker and was always included in the first lanes [Figures 1-3].

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

Statistical methods

Data was initially sorted out manually and then tabulated and entered into the computer using Microsoft Excel and Minitab 15.1 statistical package. Statistical tests were
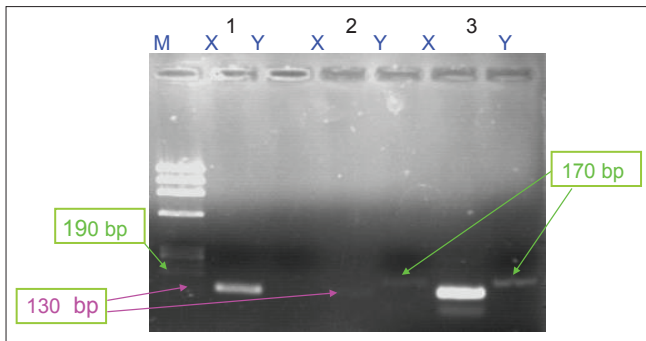
Figure 1: Amplification of the alphoid repeats X and Y primers from the DNA of the embalmed cadaveric teeth specimens electrophoretically separated on 1.5% agarose gel (Reassay: 005/14). Lanes: M = 1000 bp DNA ladder (Lot: 73020G3. Toyobo; 1 = canine; 2 = molar; 3 = molar
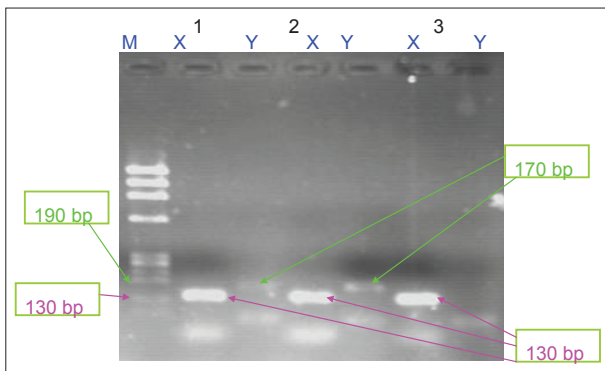
Figure 2: Amplification of the alphoid repeats X and Y primers from the DNA of the adult unembalmed (from patients) teeth samples electrophoretically separated on 1.5% agarose gel (Reassay: 005/14) Lanes: M = 1000 bp DNA ladder (Lot: 73020G3. Toyobo); 1 = tooth sample1; 2 = tooth sample 2; 3 tooth sample 3
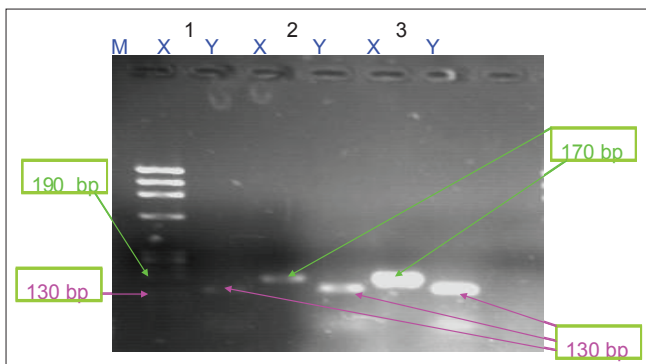
Figure 3: Amplification of the alphoid repeats X and Y primers from the DNA of the deciduous teeth samples electrophoretically separated on 1.5% agarose gel (Reassay: 005/14). Lanes: M = 1000 bp DNA ladder (Lot: 73020G3. Toyobo); 1 = Incisor; 2 = 1 = Incisor; 2 = Incisor; 3 = Incisor 3

employed for data analysis. For the fact that the samples were small, Fisher's exact test (probability) and Z-test were employed for comparison of values.[26] 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.

Results

Table 2 shows the details of the PCR sex determination of the cadaveric teeth, using *alphoid-* repeat primers. Amplification of X chromosome was achieved in all the samples. Amplification of Y chromosome was achieved in the samples 2 and 3 only. No amplification of Y chromosome was achieved for sample 1. Correct genetic sex identification was achieved for all the samples. Thus, genetically, sample 1 was correctly identified as female, while samples 2 and 3 were correctly identified as males.

In Table 3, the details of the genetic sex identification of the adult unembalmed (from patients) teeth with *alphoid repeats* are shown. Amplification of X chromosome was achieved in all the samples. Amplification of Y chromosome was achieved in samples 1 and 2 only. No amplification of Y chromosome was observed for sample 3. Correct genetic sex identification was achieved for all the samples. Thus, both samples 1 and 2 were correctly identified as males, while sample 3 was correctly identified as female, genetically.

In Table 4, the details of the genetic sex identification of the deciduous teeth with *alphoid repeats* are shown. Amplification of X chromosome was achieved in all the samples. Amplification of Y chromosome was achieved for samples 1 and 2 only. No amplification of Y chromosome was achieved for sample 3. Correct genetic sex identification was achieved for all the samples. Thus, both samples 1 and 2 were correctly identified as males, while sample 3 was correctly identified as female genetically.

Discussion

Sex estimation using teeth is important in markedly decayed or skeletonized bodies. In forensic medicine, it is necessary to develop a method for reliable determination of sex in a single tooth regardless of whether it is permanent or deciduous or whether it is an incisor, canine or molar.[11] In the present study, PCR-based sex determination was carried out on dry human teeth specimens using *alphoid repeats* primers and chose to characterize the DNA extracted from the samples studied by agarose gel electrophoresis only.

PCR sex determination of cadaveric teeth, using *alphoid*-repeat primers [Table 2 and Figure 1] has indicated that amplification of X chromosome was achieved in all the samples. Amplification of Y chromosome was achieved in samples 2 and 3 only. No amplification of Y chromosome was observed for sample 1. The amplification of X in the first tooth is with a sharp, apparent and clear band. This signifies the good quality of the genomic DNA in this sample. However, the second tooth had a very low signal of both X and Y bands and the third had a low signal of Y band. This signifies the poor quality of DNA in these samples. Although the X band of the third tooth was with

a primer-dimer, yet the band was very apparent, sharp, clear and with good density [Figure 1]. Correct genetic sex identification was achieved for all the samples. The genetic sex identification of samples 2 and 3 was based on the amplification of both X and Y chromosomes in both the samples. The genetic sexes of these samples were identified as males, which confirmed the morphological sexes of the samples. The genetic sex identification of sample 1 was based on the amplification of the X chromosome alone, as the Y chromosome did not amplify. The genetic sex of this sample (female) agreed with its morphological sex. There was no discrepancy between the respective genetic and morphological sexes of all the samples in this group.

Details of the genetic sex identification of group of teeth from patients have shown that amplification of X chromosome was achieved in all the samples [Table 3 and Figure 2]. Amplification of Y chromosome was achieved for samples 1 and 2 only [Table 3 and Figure 2]. No amplification of Y chromosome was observed for sample 3 [Table 3 and Figure 2]. It is apparent in this figure [Figure 2] that first and second teeth amplified with very low signals of Y chromosome and in both samples, there are double bands for both X and Y chromosomes. The upper signals are the real expected bands, while the bands below are primer-dimers. Presence of primer-dimers explains the low signal of Y bands resulting from poor quality of the genomic DNA in these samples. The X band in the third tooth is with a good signal. The band is sharp and apparent signifying the good quality of the genomic DNA in this tooth

sample [Figure 2]. Correct genetic sex identification was achieved for all the samples. The genetic sex identification of samples 1 and 2 was based on the amplification of both X and Y chromosomes [Table 3 and Figure 2]. The genetic sexes of these samples were identified as males, which confirmed the morphological sexes of the samples. The genetic sex identification of sample 3 was based on the amplification of X chromosome only, the Y chromosome was negative [Table 3 and Figure 2]. The genetic sex of this sample (female) tallies with its morphological sex. There was no discrepancy between the respective genetic and morphological sexes of all the samples.

The findings in deciduous teeth [Table 4 and Figure 3] for genetic sex identification have shown that amplification of X chromosome was achieved in all the samples. Amplification of Y chromosome was achieved for samples 1 and 2 only [Table 4 and Figure 3]. No amplification of Y chromosome was achieved for sample 3 in both groups [Table 4 and Figure 3]. The amplification of both X and Y *alphoid*-repeat primers in the first tooth showed faint bands, and this is more pronounced in the X chromosome.

Table 1: *Alphoid* repeats primers and their sequences used for PCR amplification of the extracted DNA in the study[25]

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´

PCR: Polymerase chain reaction

Table 2: Results of genetic sex identification of embalmed cadaveric teeth specimens using *alphoid-repeat* primers

Tooth serial number	Tooth sampled	Morphological Sex	PCR results with *alphoid- repeat* primers		Genetic sex
			X chromosome amplification (130 bp)	Y chromosome amplification (170 bp)	
1	Molar	Female	Positive	Negative	Female
2	Premolar	Male	Positive	Positive	Male
3	Premolar	Male	Positive	Positive	Male

Correct genetic sex identification was achieved for all the samples. Thus, genetically, sample 1 was identified as female, while samples 2 and 3 were correctly identified as males [Table 2 and Figure 2]. 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, PCR: Polymerase chain reaction

Table 3: Results of genetic sex identification of adult unembalmed (from patients) teeth using *alphoid-repeat* primers

Tooth serial number	Tooth sampled	Morphological Sex	PCR results with *alphoid-repeat* primers		Genetic sex
			X chromosome amplification (130 bp)	Y chromosome amplification (170 bp)	
1	Molar	Male	Positive	Positive	Male
2	Premolar	Male	Positive	Positive	Male
3	Premolar	Female	Positive	Negative	Female

Correct genetic sex identification was achieved for all the samples. Thus, both samples 1 and 2 were correctly identified as males, while sample 3 was correctly identified as female genetically [Table 2 and Figure 2]. 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, PCR: Polymerase chain reaction

Table 4: Results of genetic sex identification of deciduous teeth using *alphoid-repeat* primers

Tooth serial number	Tooth sampled	Morphological Sex	PCR results with *alphoid-repeat* primers		Genetic sex
			X chromosome amplification (130 bp)	Y chromosome amplification (170 bp)	
1	Incisor	Male	Positive	Positive	Male
2	Incisor	Male	Positive	Positive	Male
3	Incisor	Female	Positive	Negative	Female

Correct genetic sex identification was achieved for all the samples. Thus, both samples 1 and 2 were correctly identified as males, while sample 3 was correctly identified as female genetically [Table 2 and Figure 2]. 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, PCR: Polymerase chain reaction

This indicates poor quality of the genomic DNA. In the second tooth both X and Y bands are sharp, clear and with good density, signifying the good quality of DNA in this tooth. The amplified band (X) of the third tooth is of high density signifying the good quality of genomic DNA in this sample [Figure 3]. Although the bands for X chromosome of second and third teeth samples are apparent and sharp, they still showed primer-dimers [Figure 3]. Correct genetic sex identification was achieved for all the samples. The genetic sex identification of samples 1 and 2, was based on the amplification of both X and Y chromosomes [Table 4 and Figure 3]. The genetic sexes of these samples were identified as males, which confirmed their morphological sexes [Table 4]. The genetic sex identification of sample 3 was based on the amplification of the X chromosome only, the Y chromosome was negative [Table 4 and Figure 3]. The genetic sex of this sample (female) tallies with its morphological gender. There was no discrepancy between the respective genetic and morphological sexes of all the samples [Table 4].

Generally, the success rates of our findings in the three groups of teeth samples, base on the use of *alphoid* repeats in sex estimation, are favorably comparable with the report of Lin *et al.,* (1995)[27] from Dalian Medical University, China, despite the fact that our samples vary. They reported that with *alphoid*-repeat primers sex determination was possible even with very ancient human bone samples >1300 years. In two adult cases where sex estimation was externally impossible, the sex was determined to be male because both X-specific and Y-specific bands were clearly detected. Amplification of rib samples from a child mummy showed that it was definitely male.[27]

Similarly, Cipollaro *et al.,* (1999)[28] reported from the Universita` degli Studi di Napoli ''Federico II,'' Italy, on the successful amplification of ancient (79 A. D) DNA and gender determination of 8 femuri, 4 tibia and 1 humerus from Pompeii Archeological Site, using Y-specific *alphoid* repeats. This is comparable with our findings in the teeth samples. However, contrary to our series in which we were able to successfully estimate the sexes of all our samples (despite the fact that some chromosomes; Y of first tooth in cadaveric teeth, Y of third teeth of both adult unembalmed and deciduous groups did not amplify), Cipollaro *et al.,* (1999)[28] could not achieve amplification; and therefore, could not determine the sex of 1 tibia due to very poor preservation of the specimen.[28]

From the Okayama University Medical School, Japan, Murakami *et al.,* (2000),[11] reported on the sensitivity of PCR for detection of the Y chromosome-specific *alphoid* repeat and the X chromosome-specific *alphoid* repeat sequences. Sex could be determined by PCR of DNA extracted from the pulp of 16 freshly extracted permanent teeth and dentine including the surface of the pulp cavity of 6 freshly extracted milk teeth. Sex could be determined using the pulp in all 20 teeth (10 males 10 females) preserved at room temperature for 22 years. For the pulp of teeth stored in sea water, the sex could be determined in all 8 teeth immersed for 1 week and in 5 of 6 teeth immersed for 4 weeks. In the remaining 1 tooth, in which sex determination based on the pulp failed, the sex could be determined correctly when DNA extracted from the hard tissue was examined. For teeth stored in soil, the sex could be determined accurately in all 8 teeth buried for 1 week, 7 of 8 teeth buried for 4 weeks, and in all 6 teeth buried for 8 weeks. The sex of a mummified body estimated to have been discovered half-a-year to 1 year after death could be determined readily by examination of the dental pulp. In the skeletons of 2 bodies placed under water for approximately 1 year and approximately 11 years 7 months, pulp tissues had been dissolved and lost, but sex determination was possible using DNA extracted from hard dental tissue.[11] These results are comparable and favorable with our findings in terms of the tissues and or organs used in the research works and in terms of success rates recorded by either study.

Our findings are similar to the report of Matheson and Loy (2001),[29] from the University of Queensland, Brisbane, Australia, on the *alphoid* repeats for the genetic sex identification of 9400-year-old, ten human skull samples from Çayönü Tepesi, Turkey, despite the fact that the tissues/organs are different. They reported that the X, Y *alphoid* repeats method produced a male identification in three of the 10 samples of the Çayönü Tepesi skull samples, similar to samples 2 and 3 from cadaveric teeth, samples 1 and 2 from both adult unembalmed teeth and deciduous teeth groups of the present study. The six other samples amplified with the X *alphoid* repeat primers but not with the Y *alphoid* repeat primers.[29] This is comparable with Y *alphoid* repeats of sample 1 from cadaveric teeth and Y *alphoid* repeats of samples 3 from both adult unembalmed and deciduous teeth of our samples. Hence, the success rates of accurate sex estimation that we recorded from all the three groups of our samples are favorably comparable with the report of Matheson and Loy (2001).[29]

Conclusion

This study has demonstrated the successful use of *alphoid repeats* primers in genetic sex identification of human dry teeth samples from Sokoto, Northwestern Nigeria. This is the first known study estimating the sex of human dry teeth specimens by means of PCR in Nigeria. There is need for further studies in Nigeria to complement the findings of this study.

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