Revista Odonto Ciência
Journal of Dental Science

ORIGINAL ARTICLE

Rev Odonto Cienc 2017;32(1):1-9
http://revistaseletronicas.pucrs.br/ojs/index.php/fo
http://dx.doi.org/10.15448/1980-6523.2017.1.21133

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Sex estimation study in skulls from Brazil's southeastern population using Physical Anthropology and DNA

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ABSTRACT

OBJECTIVE: This study aimed to determine the sex in human craniums using methodologies of Physical Anthropology, quantitative (Forensic Data Anthropology Bank, FDB, 1986) and one qualitative (Walker, 2008) and genetic analysis by amelogenin.

METHODS: The sample was composed of 66 skulls (34 males and 32 females) from the Center for Study and Research in Forensic Science, Guarulhos, SP. The methodologies were applied by two researchers who were unaware of the cranium's sexes. For the statistical analysis, there were performed descriptive analysis, average, standard deviation, linear discriminant analysis and logistic regression.

RESULTS: The qualitative methodology presented an accuracy of 89.52%. For the DNA, it was possible to determine the sex in 86.15% of the sample. Analyzing the results for each skull in three different methodologies, we reached 100% correct.

CONCLUSION: As a result of this study, it is recommended that physical anthropology be the chosen method if it presents good accuracy when applied to different populations or if it is validated for the analyzed population. Otherwise, genetic analysis should be used for the determination of the sex.

Key words: Physical Anthropology; DNA; Molecular Biology; Sex characteristics; Sex differentiation

Estudo estimativo do sexo em crânios oriundos da região metropolitana de Guarulhos utilizando Antropologia Física e DNA

RESUMO

OBJETIVO: O presente estudo propõe determinar o sexo de crânios humanos, a partir de metodologias quantitativa (Forensic Data Anthropology Bank, FDB, 1986) e qualitativa (Walker, 2008) da antropologia física além de análise genética da amelogenina.

MÉTODOS: A amostra foi composta de 66 esqueletos (34 masculinos e 32 femininos) do Centro de Estudos e Pesquisa Forense de Guarulhos, SP. As metodologias de avaliação foram aplicadas por dois pesquisadores que desconheciam o sexo dos crânios. Para análise estatísitica, foi realizada análise descritiva (média e desvio-padrão).

RESULTADOS: A análise qualitativa alcançou uma acurácia de 89.52%. A partir da análise do DNA, foi possível determinar o sexo em 86.15% das amostras. Ao avaliar, cada crânio, seguindo as 3 metodologias, foi possível alcançar 100% de acurácia.

CONCLUSÃO: A partir dos resultados do presente estudo, fica recomendado o uso da antropologia física para a determinaçnao do sexo, desde que esteja validada para a população em análise. Além disso, a análise genética deve ser utilizada para a determinação do sexo de crânios humanos.

Palavras-chave: Antropologia física; DNA; Biologia molecular; Características sexuais; Determinação sexual

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Received: June 24, 2015
Accepted: March 14, 2017

Conflict of Interests: The authors state that there are no financial and personal conflicts of interest that could have inappropriately influenced their work.

INTRODUCTION

In human identification, an accurate estimation of sex reduces of the total number of subjects during the identification process of unknown subjects. Sex estimation of skeletal remains may be done through the anthropologic evaluation and through the molecular biology. Forensic skeletal examination of remains offers several advantages: it is relatively easy to apply, results are rapidly available, costs are low, and only basic osteometric instruments are required. Disadvantages are that they cannot normally be applied to children's skeletons, and they must be population specific, as has been pointed out by many studies [1-8].

DNA is often regarded as providing infallible evidence of sex using amelogenin. It has some distinct advantages; it does not require population adjustments, it can provide reliable genetic sex identification under the right circumstances and can be used on remains of any age. However, it also has limitations. It is subject to degradation, it is expensive and results are not immediately available. Morphological sexing may be based on measurements or observations, quantitative or qualitative. Both approaches rely on comparing an unidentified skull to criteria established from documented samples. Qualitative methods are based on visual scoring of specific traits using standardized definitions [2]. Quantitative traits are measurements between specifically defined craniometric points [16]. Both the somatoscopic and the somatometric methods show that normally the male skulls are larger, and have more robust muscle attachment area, as shown in several studies [4, 5, 7, 10-14].

From the technical and criminalist point of view, DNA can be collected from any biologic specimen, however, the biological material recovered in forensic scenes may suffer environmental changes (temperature, soil pH, humidity), that may cause breaks and changes in the chain of nucleotides and, consequently, modify the composition and the normal structure of the DNA, making the analysis impossible [3]. In DNA analysis for sex determination, traditionally, amelogenin has been used and became a standardized method in the kits used in the human identification [8]. Amelogenin or AMEL is a protein secreted by the ameloblasts, found on the enamel. The gene that encodes the female amelogenin is located on the X chromosome and has 106 pairs of bases, while the one found on the Y chromosome contains 112 pairs of bases. Females have homozygous alleles and identical genes and males are heterozygous [1].

This paper will utilize the morphoscopic traits of Walker (2008) [14] and metric traits as defined by Moore-Jansen et al. (1986) [9]. This will allow a direct comparison of the two methods to ascertain how well they agree with each other and with documented sex. Sex was also determined from amelogenin commonly present in the identification kits, called multiplex, containing several STRs. This will allow a determination of degradation dna undergoes under the conditions of burial.

METHODS

The project was submitted to the Committee for Ethics in Faculty of Dentistry of São Paulo at the University of São Paulo (FO-USP), approved under no. FR-365970.

The sample consisted of 66 skulls, 32 females and 34 males, all documented as to age, sex and origin, from the collection of the Center of Study and Research in Forensic Sciences, of city of Guarulhos, SP. Only intact skulls with mandibles of individuals above 20 years old were included. The skeletons were excavated from a local graveyard of Guarulhos. After five years of burial, the skeletons are unearthed and families are called to remove them. The skeletons that are not removed by the family in a specific legal period of time, become part of the collection of the Laboratory of Forensic Anthropology and are used in research. There is an obituary, in which are recorded the features of these skeletons. They came from multiracial origins from different places in Brazil. The causes of death vary, ranging from diseases to violent death.

For the DNA analysis two teeth or two vertebrae (from the edentulous skeletons) were extracted for the genetic analysis. One skull (002/10) was excluded because it had been submitted to the physiochemical cleaning process before the collection of the material. This research was submitted to the Ethics Committee in Research of the Faculty of Dentistry of São Paulo and was approved under protocol number 138/2010.

Method of the Forensic Data Bank

The method of the FDB (Moore-Jansen et al. 1984) [9] describes 34 skull measurements shown in **Table 2**.

Method of Walker

Walker's method [14] evaluates the following morphological criteria: Mental Eminence, Supraorbital Margin, Supraorbital Ridge/glabella, Nuchal Crest and the Mastoid process. The traits are assigned scores from 1 to 5 by visual assessment using the criteria presented in Buikstra and Ubelaker (1994) [2]. Criteria 1 and 2 indicate the most delicate, thinnest and smallest structures, more common in women, while 4 and 5 describe widest, roughest and most angular structures typically found in males. Category 3 is intermediate between the two sexes.

Means and standard deviations were computed for the measurements, and frequencies for the ordinal traits. Linear discriminant analysis was done for the measurements and logistic regression was done on both. Walker's formula, as given below valited:

$$\text{Logito} = (\text{glabella} \times -1.375) + (\text{Mastoid process} \times -1.185) + (\text{Eminence Menutal} \times -1.150) + 9.128$$

The probability of being of the female sex (PF) and of the male sex (PM) was calculated according to the equations: $PF = 1/(1+e^{-\text{logito}})$ and $PM = 1 - PF$.

Calibration of the researchers

All the measurements were done by the researchers in thirty two skulls. To evaluate the intra-observer error, just the researcher who applied the methodologies repeated the same measurements in 14 skulls in a period of approximately 1 month. For the measurements the paired "t" Test was applied and for the Walker, the Kappa index was used, with an accuracy between 50 and 100%. In the inter-examiner evaluation, such index was lower for some variables, however when the classification in the scores from 1 to 5 done by the researchers was analyzed, the difference was in just one number in the scale, there not being the possibility of error in the final classification of the sex.

Analysis and tabulation of the results

The measurements, all of them in millimeters, were noted on a specific form created with the objective of registering measurements. The data obtained in the anthropologic analysis was tabulated using the program Excel (Microsoft Office®). The statistical procedures were executed in the Program STATA 12, with a significance level of 5%.

Analysis by DNA

Preparation of the teeth and of the vertebrae

Sweet and Hildebrand's (1998) [15] method was used for preparation of the teeth. Preparation consisted of physicochemical cleaning process with scaling using periodontal curettes and 70% alcohol wash. Then they were sectioned in their coronary portion with a sterilized carborundum disc, substituted to each unit and sprayed from 3 to 4 immersions in liquid nitrogen maintaining the rise and fall movement of the pestle constant. From 1 to 5 g of each pulverized dental sample was used. The preparation of the vertebrae followed the protocol described by Kemp and Smith (2005) [16]. The vertebrae were aggressively cleaned with sandpaper to remove the exterior layer and contamination. They were cleaned with alcohol and bleach diluted for 20 minutes and rinsed with sterile distilled water. A manual bandsaw was used to fragment them into very small pieces. The pieces of bone were air dried. From 1 to 5 g of the pulverized bone sample was used.

Extraction of the DNA

The extraction of the DNA was done according to the instructions of the QIAamp DNA Investigator Kit (Quiagen™,Venlo, Limburgo, Países Baixos). After the extraction of the DNA, electrophoresis was run on 1% agarose gel with a molecular weight marker of 100 pairs of bases. The image was visualized to confirm the presence of genetic material. DNA was quantified using the Spectrophotometer NanoDrop 2000 (Thermo Scientific™, Wilmington, Estados Unidos da América, EUA).

Reactions of PCR and primers used

The chosen region for the amplification through the PCR technique was the amelogenin (Forward Sequence: GTTTCTTCCCTGGGCTCTGTAAAGAATAGTG; Reverse Sequence: TCAGAGCTTAAACTGGGAAGCTG [6-FAM]). The primers were synthesized by the company *Life Technologies Brasil, Commerce and Industry of Biotechonology Products LTDA* (*Carlsbad, Califórnia, EUA*), being that one of them was marked with 6FAM fluorescence while the other one was not marked.

For the PCR reaction, the Thermal Cycler Veriti 96 well was used (Applied Biosystems™, *Carlsbad, Califórnia, EUA*). A mixture with a total volume of 15µL was obtained, containing water, buffer (2X), $MgCl_2$ (50mM), dNTPs (10Mm), Primer F and R (1µM of each), DNA polymerase (5U) and DNA mold. Human DNA from blood was used as a positive control. The negative control consisted in replacing the DNA with water, this way it was possible to detect possible contaminations of any of the reagents. Forty cycles of the following program were run: Hot-start, 95°C for 10 minutes; Denaturation, 94°C for 30 seconds; Hybridization, 54°C for 1 minute; Extension, 72°C for 1 minute; concluding with the final Extension at 72°C for 60 minutes.

Analysis of fragment

After the PCR, a quantity of 1µL of the product of each sample had an addition of 9µL of HI-DI (highly deionized) formamide and of 0.3µL of LIZ 600, and the fragment was analyzed in the ABI 3500 (Applied Biosystems™) sequencer with the GeneMapper (Applied Biosystems™) software. The data obtained through the use of the Molecular Biology for the amelogenin was statistically studied through a descriptive analysis.

RESULTS

Method of Walker

Table 1 presents the Walker scores for the sample of 66 crania. Both the logistic and linear discriminant analysis, showed the mastoid process to be the single best sex discriminator, achieving 82.35% accuracy for males sex and 90.63% for females. Combining the mastoid process with the supraorbital ridge/glabella, the accuracy was 85.29% and 93.75% for males and females respectively. No further improvement was obtained by adding the remaining three variables.

For the logistic regression, the equations that offer the best results for both sexes (accuracy of 89.52%) were the following ones:

$$\text{Logito} = -7.74 + \text{Mastoid process} \times 1.45 + \text{Glabella} \times 1.51$$

$$\text{Logito} = -7.15 + \text{Mastoid process} \times 1.28 + \text{Supraorbital Margin} \times 1.29 + \text{Mental Eminence} \times 0.31$$

$$\text{Logito} = -7.87 + \text{Mastoid process} \times 1.35 + \text{Supraorbital Margin} \times 0.12 + \text{Glabella} \times 1.39 + \text{Mentual Eminence} \times 0.15$$

The probability of being of the female sex (PF) and of the male sex (PM) was calculated according to the equations: $PF = 1 / (1 + e^{-logito})$ and $PM = 1 - PF$.

Table 1. Distribution of qualitative variables according with categories

Variables	Categorie	Female		Male		Total	
		N	%	N	%	N	%
Nuchal Crist (p<0,001*)	1	7	21.88	1	2.94	8	12.12
	2	15	46.88	5	14.71	20	30.30
	3	7	21.88	8	23.53	15	22.73
	4	3	9.38	13	38.24	16	24.24
	5	0	0.00	7	20.59	7	10.61
Mastoid Process (p<0,001*)	1	9	28.13	2	5.88	11	16.67
	2	20	62.50	4	11.76	24	36.36
	3	2	6.25	15	44.12	17	25.76
	4	1	3.13	8	23.53	9	13.64
	5	0	0.00	5	14.71	5	7.58
Supraorbital Margin (p<0,001*)	1	8	25.00	2	5.88	10	15.15
	2	19	59.38	3	8.82	22	33.33
	3	5	15.63	18	52.94	23	34.85
	4	0	0.00	10	29.41	10	15.15
	5	0	0.00	1	2.94	1	1.52
Glabela (p=<0,001*)	1	10	31.25	1	2.94	11	16.67
	2	15	46.88	2	5.88	17	25.76
	3	6	18.75	13	38.24	19	28.79
	4	1	3.13	11	32.35	12	18.18
	5	0	0.00	7	20.59	7	10.61
Mentual Eminence (p=<0,001*)	1	3	9.38	7	20.59	3	4.55
	2	15	46.88	12	35.29	22	33.33
	3	12	37.50	11	32.35	24	36.36
	4	2	6.25	4	11.76	13	19.70
	5	0	0.00	0	0.00	4	6.06

Method of the FDB

In the **Table 2**, it is presented the data of the measurements in the 66 skulls, showing the Mean and Standard deviation and the Confidence interval for each measurement, divided by sex. With the exception of the Nasal Breadth (al-al), Interorbital breadth (d-d) and Mandibular Angle measurements, all the other measurements presented the highest mean for the male sex. The majority of the measurements resulted in a statistically significant difference between the sexes confirmed by the value of $p<0.05$; except the Nasal Breadth (al-al), Maximum Alveolar Length (pr-alv), Upper Facial Height (n-pr), Upper Facial breadth (fmt-fmt), Orbital height (OBH), Orbital Breadth, Biorbital breadth (ec-ec), and Body Thickness at M. For. Right measurements.

Through the logistic and linear discriminant analysis, the highest accuracy percentage occurred in the combination of the Cranial Base Length (ba-n), Maximum Length (g-op), Bizygomatic Breadth and Nasal Height (n-ns) measurements. Linear discriminant analysis yielded an accuracy rate of 80.77% for males and 96.15% for females, 92.31% in the logistic analysis for both sexes.

For the logistic regression, the equation that better discriminated the sex (92.31%) is related to a combination of the best Cranial Base Length (ba-n), Maximum Length (g-op), Bizygomatic Breadth and Nasal Height (n-ns) measurements:

$$\text{Logito} = -135.89 + \text{Nasal Height (n-ns)} \times 0.29 + \text{Cranial Base Length (ba-n)} \times 0.56 + \text{Maximum Length (g-op)} \times 0.13 + \text{Bizygomatic Breadth (zy-zy)} \times 0.01$$

The probability of being of the female sex (PF) and of the male one (PM) was calculated according to the equations: $PF = 1/(1+e^{-\text{logito}})$ and $PM = 1 - PF$.

Genetic Analysis through amelogenin

In the analysis by DNA, from the total of 65 elements evaluated, 30 were teeth and 35 vertebrae. Just 23% (n=15) presented satisfactory conditions for the analysis by DNA, all the teeth. All the vertebrae were in a bad situation, with changes, excavated and whitened, and with a fungal aspect. The determination of was possible in 86.15% (n=56) of the elements. In the males (n=33), sex identification was

possible in 93.94% (n=31) of the sample. For females (n=32), sex determination was possible in 78.12% (n=25) of the elements. Among the elements in which the sexual determination was not possible (n= 9), were 6 vertebrae and 3 teeth, only 1 of which (1 tooth) was in a satisfactory situation for the genetic study.

Table 2. Mean, Standard deviation and Confidence interval of quantitative variables according to categories

Variables	Male			Female			Difference	
	Mean (mm)	SD	CI (95%)	Mean (mm)	SD	CI (95%)	Mean	P
g-op	181.91	7.28	180.47-183.35	171.48	6.30	170.23-172.73	-10.43	0.000
eu-eu	141.62	6.22	140.39-142. 85	136.26	7.17	134.84-137.68	-5.36	0.002
zy-zy	129.16	5.70	128.13-130.29	121.10	4.35	120.23-121.95	-8.07	0.000
ba-b	135.56	7.21	134.12-136.98	127.72	6.95	126.35-129.11	-7.82	0.000
ba-n	102.40	5.12	101.91-104.57	95.70	4.34	94.81-96.54	-6.71	0.000
ba-pr	99.22	8.55	97.52-100.90	93.53	5.32	92.47-94.59	-5.68	0.115
ecm-ecm	58.38	7.13	56.97-59.79	54.29	5.90	53.13-55.47	-4.08	0.022
pr- alv.	56.14	4.80	56.51-61.74	53.10	4.91	52.12-54.08	-3.03	0.205
AUB	102.1	6.34	100.67-103.21	97.03	4.16	96.20-97.85	-5.10	0.000
n-pr	68.19	5.39	68.07-71.79	64.08	3.02	63.49-68.69	-4.10	0.070
ft-ft	98.75	4.48	98.86-100.64	95.99	5.35	94.93-97.05	-3.76	0.003
fmt-fmt	98.74	4.41	95.45-99	96.54	5.33	95.48-97.60	-2.20	0.781
n-ns	51.17	3.13	49.05-51.28	46.25	3.04	45.65-46.85	-4.92	0.000
al-al	24.94	2.29	24.66-26	25.96	2.01	24.56-25.36	0.02	0.975
d-ec R	40.21	1.46	39.91-40.49	38.93	2.02	37.80-39.08	-1.28	0.005
d-ec L	40.15	1.56	39.59-40.37	38.85	2.03	38.45-39.25	-1.30	0.007
OBH R	34.12	2.24	33.76-37.84	34.10	2.53	33.60-34.60	-0.03	0.962
OBH L	34.63	2.27	33.74-38.49	33.90	2.41	33.42-34.38	-0.72	0.225
ec-ec	95.32	4.96	90.28-95.68	94.11	4.32	89.67-94.53	-1.21	0.309
d-d	20.38	3.05	19.86-26.69	21.69	2.52	21.19-22.19	1.30	0.076
n-b	111.70	5.43	110.79-117.10	107.75	4.64	106.82-108.67	-3.95	0.002
b-l	113.21	7.55	111.69-114.73	108.31	5.57	107.19-109.40	-4.91	0.007
l-o	97.44	6.25	96.20-98.68	92.22	5.54	91.11-93.31	-5.23	0.001
ba-o	35.36	2.46	34.87-35.85	33.61	2.57	33.10-34.12	-1.75	0.006
FOB	30.64	2.43	30.16-31.12	28.91	2.24	28.45-29.35	-1.74	0.004
MDH R	28.47	3.49	27.78-29.16	25.54	3.11	24.92-26.16	-2.93	0.001
MDH L	28.98	2.91	28.40-29.08	25.89	2.65	25.36-26.41	-3.09	0.001
gn-id	27.82	6.33	26.56-29.08	22.35	6.68	21.02-23.67	-5.47	0.001
BHMR	26.81	5.76	25.67-27.95	22.09	5.99	20.91-23.29	-4.71	0.002
BHML	26.09	5.91	24.92-27.26	21.53	5.79	20.38-22.68	-4.56	0.002
BTMR	13.20	2.42	12.72-13.86	12.06	1.78	11.71-12.41	-1.15	0.032
BTML	12.88	2.12	12.46-13.30	12.05	1.64	11.72-12.38	-0.83	0.083
cdl-cdl	117.42	6.86	116.06-118.78	111.36	6.86	110.03-112.69	-6.06	0.001
MRBD	31.66	3.56	31.15-32.65	29.74	2.67	29.21-30.27	-1.92	0.017
MRBE	32.53	3.92	31.75-33.31	30.38	2.65	29.85-30.90	-2.15	0.012
Ang. MD	123.09	7.93	121.51-124.66	127.62	8.57	125.93-129.33	4.54	0.029
Ang. ME	123.23	7.55	121.73-124.73	127.61	6.18	126.39-128.84	4.39	0.013
go-go	96.91	5.34	88.82-90.94	89.88	6.09	95.70-98.12	-7.03	0.000
MRBD	29.88	4.06	27.56-28.66	28.11	2.78	28.96-30.57	-1.76	0.045
MRBE	30.48	3.99	28.04-29.06	28.55	2.56	29.68-31.65	-1.92	0.023
MRHD	51.47	5.97	46.31-48.01	47.16	4.30	50.28-52.65	-4.31	0.001
MRHE	51.41	6.22	46.01-47.31	46.66	3.27	50.18-52.64	-4.75	0.000
Angle M.	107.86	6.45	102.90-105.72	104.31	7.09	106.58-109.14	-3.55	0.037

Maximum Length (g-op), Maximum Breadth (eu-eu), Bizygomatic Breadth (zy-zy), Basion-Bregma (ba-b), Cranial Base Length (ba-b), Basion- Prosthion (ba-pr); Maximum Alveolar Breadth (ecm-ecm), Max. Alveolar Length (pr-alv); Biauricular Breadth (AUB), Orbital height (OBH); Upper Facial Height (n-pr); Upper Facial breadth (fmt-fmt); Min. Frontal Breadth (ft-ft), Nasal Height (n-ns), Nasal Breadth (al-al); Orbital Breadth Left and Right (d-ec), Biorbital breadth (ec-ec); Interorbtial breadth (d-d); Frontal Chord (n-b), Parietal Chord (b-l), Occipital Chord (l-o), Foramen Magnum Length (ba-o), Foramen Magnum Breadth (FOB), Mastoid Length Left and Right (MDH), Chin Height (gn-id), Body Height at Mental For. Left and Right (BHM), Body Thickness at M. For. (BTM), Bicondylar Breadth (cdl-cdl), Min. Ramus Breadth Left and Right (MRB), Max. Ramus Breadth (MRB), Bigonial Diameter Left and Right (go-go), Max. Ramus Height Left and Right (MRH), Mand. Length (Mand.L), Mand. Angle Left and Right (Angle M). R- Rigth, L- Left.

Table 3. Distribution of analyzed element in each skull, classification in tooth or vertebrae, registered sex in the obituary, found condition and the results of the genetic analysis

Skulls	Sex	Tooth	Vertebrae	Condition	Analysis	Skulls	Sex	Tooth	Vertebrae	Condition	Analysis
001/11	Male		X	Bad	Positive	034/10	Female		X	Bad	Positive
002/10					Excluded	035/10	Male	X		Bad	Positive
003/09	Female		X	Bad	Positive	036/10	Female	X		Bad	Negative
004/09	Male	X		Bad	Positive	037/10	Male		X	Bad	Positive
005/09	Male	X		Good	Positive	038/10	Female	X		Good	Positive
006/10	Male	X		Bad	Positive	039/10	Female	X		Good	Positive
007/10	Male	X		Bad	Positive	040/10	Male	X		Good	Positive
008/10	Female		X	Bad	Positive	041/10	Female	X		Good	Negative
009/10	Female		X	Bad	Positive	042/10	Male	X		Good	Positive
010/10	Male	X		Boa	Positive	043/11	Male	X		Bad	Positive
011/10	Male	X		Bad	Positive	044/11	Female		X	Bad	Negative
012/10	Female		X	Bad	Positive	045/11	Female	X		Bad	Positive
013/10	Female		X	Bad	Positive	046/11	Male	X		Good	Positive
014/10	Male		X	Bad	Positive	047/11	Female	X		Good	Positive
015/10	Male		X	Bad	Positive	048/11	Male	X		Good	Positive
016/10	Female		X	Bad	Positive	049/11	Female	X		Good	Positive
017/10	Female		X	Bad	Positive	050/11	Male	X		Good	Positive
018/10	Male	X		Bad	Positive	051/11	Male	X		Good	Positive
019/10	Female		X	Bad	Positive	052/11	Female		X	Bad	Positive
020/10	Female		X	Bad	Positive	053/11	Male		X	Bad	Positive
021/10	Male	X		Bad	Positive	054/11	Female		X	Bad	Negative
022/10	Male	X		Bad	Negative	055/11	Male		X	Bad	Positive
023/10	Female		X	Bad	Positive	056/11	Female		X	Bad	Negative
024/10	Female	X		Good	Positive	057/11	Female		X	Bad	Positive
025/10	Male	X		Bad	Positive	058/11	Male		X	Bad	Negative
026/10	Female	X		Bad	Positive	059/11	Male		X	Bad	Positive
027/10	Male		X	Bad	Positive	060/11	Male		X	Bad	Positive
028/10	Female		X	Bad	Positive	061/11	Female		X	Bad	Positive
029/10	Male	X		Bad	Positive	062/11	Female		X	Bad	Positive
030/10	Male		X	Bad	Positive	063/11	Female		X	Bad	Negative
031/10	Male		X	Bad	Positive	064/11	Female		X	Bad	Positive
032/10	Male	X		Good	Positive	065/11	Female		X	Bad	Positive
033/10	Male	X		Good	Positive	066/11	Female		X	Bad	Negative

Comparation of three methodologies

We examined whether the sex was correctly estimated by the different methods for each skull studied using the formulas that provide the best outcome for the methodologies. Comparisons were cross tabulated in table. There was obtained 100% of accuracy, or when a skull may not have sex estimated by a method responded positively to another methodology (**Table 3**).

DISCUSSION

The results of this research showed that in the males, the great majority of the measurements presented a mean value superior to the one found in females, proving that the determination of the sex can be done by skull analysis, as described by several authors such as Gilles and Elliot, 1963 [7]; Konigsberg and Hens, 1998 [10]; Rogers, 2005 [13]; Dayal et al., 2008 [4].

It is known that sexual dimorphism may be influenced by ethnic factors. Because of this, the same methodology may present different results when applied to distinct communities for sex estimation. This way, there is the need of the validation of methods whose results were promising in distinct racial groups. Especially in Brazil, such analysis becomes extremely necessary for it being a country with a multiracial general characteristic, with a mixture among white, black and indian people and with distinct regions in which there are specific and local miscegenation, such as in the South and Northeast [17].

Table 4. Sex correctly estimated for the three methods evaluated

Skull	DNA	Walker	FDB	Skull	DNA	Walker	FDB
1	Correct	Correct	Correct	34	Correct	Correct	Correct
2	Excluded	Correct	Correct	35	Correct	Correct	Correct
3	Correct	Correct	Incorrect	36	Incorrect	Incorrect	correct
4	Correct	Correct	Correct	37	Correct	Correct	correct
5	Correct	Correct	Correct	38	Correct	Correct	correct
6	Correct	Incorrect	Correct	39	Correct	Correct	correct
7	Correct	Correct	Correct	40	Correct	Correct	correct
8	Correct	Correct	Incorrect	41	Incorrect	Correct	correct
9	Correct	Correct	Correct	42	Correct	Correct	correct
10	Correct	Correct	Correct	43	Correct	Correct	correct
11	Correct	Correct	Correct	44	Incorrect	Correct	correct
12	Correct	Correct	Correct	45	Correct	Incorrect	correct
13	Correct	Correct	Correct	46	Correct	Correct	Incorrect
14	Correct	Incorrect	Correct	47	Correct	Correct	Correct
15	Correct	Incorrect	Correct	48	Correct	Correct	Correct
16	Correct	Correct	Correct	49	Correct	Correct	Correct
17	Correct	Correct	Correct	50	Correct	Incorrect	Correct
18	Correct	Correct	Correct	51	Correct	Correct	Correct
19	Correct	Correct	Correct	52	Correct	Correct	Correct
20	Correct	Correct	Correct	53	Correct	Correct	Correct
21	Correct	Correct	Correct	54	Incorrect	Correct	Correct
22	Incorrect	Correct	Correct	55	Correct	Correct	Correct
23	Correct	Correct	Correct	56	Incorrect	Correct	Correct
24	Correct	Correct	Correct	57	Correct	Correct	Correct
25	Correct	Correct	Correct	58	Incorrect	Correct	Incorrect
26	Correct	Correct	Correct	59	Correct	Correct	Correct
27	Correct	Correct	Correct	60	Correct	Correct	Correct
28	Correct	Correct	Correct	61	Correct	Correct	Correct
29	Correct	Incorrect	Correct	62	Correct	Correct	Correct
30	Correct	Correct	Correct	63	Incorrect	Correct	Correct
31	Correct	Correct	Correct	64	Correct	Correct	Correct
32	Correct	Correct	Correct	65	Correct	Correct	Correct
33	Correct	Correct	Correct	66	Incorrect	Correct	Correct

Because of this, the anthropologic methodologies used in this study were selected according to the results of researches published in other countries, involving different populations such as the method of the Forensic Anthropology Data Bank (FDB) (1986) [9] and the method of Walker (2008) [14], with accuracy above 80% [18].

The accuracy rate obtained in this study with the application of the anthropology was equal or superior to the one found in other similar studies, pointing that the methods analyzed present a high discrimination efficiency between the sexes [4,7,10-13,18-20].

We also attempted to validate Walker (2008) [14] by applying his formula to the Brazilian data (S = Glabella * -1.37 + Mastoid. * -1.18 + Mental * -1.15 + 9.128). It achieved 88.24% accuracy for males and 81.25% for females in our sample, a result very close to his (88.24% for males and 86.4% for females). Despite similar results of this study (89.52%) and that of Walker (88%), the distribution of the scores in the numerical categories were somewhat different from Walker's. In our sample female skulls were classified mainly in categories 2 and 3, while the majority of the male skulls fell into categories 3 and 4, except the mental eminence element that classified these skulls between the scores 2 and 3. In Walker's (2008) study, the majority of the male skulls fit in the scores 2, 3 and 4, while the female skulls fit in the scores 1 and 2. The difference may be attributed to population variation, emphasizing again the necessity of population specific sexing criteria. This variation can be explained by the ethnic (genetic), nutritional and cultural differences among the groups, factors that influence the craniofacial growth, as argued by (Walker, 2008) [14].

This study demonstrates the effectiveness of both visual and metric sexing. It agrees with other studies that find nasal height and bizygomatic breadth are the most dimorphic dimensions. It agrees with Walker's results that the mastoid and glabella region are the most dimorphic visually assessed traits.

The genetic assessment of sex relied on samples of teeth and vertebrae. The majority of the sample (77%) presented itself in a very unsatisfactory condition; in other words, with dirt, humidity and enveloped in leaves and earth. The failure to recover DNA was non-randomly distributed in the cemetery and exposure to sewage may be responsible for the degradation. In particular, all the vertebrae had a whitened aspect compatible with the presence of fungus, and portions were fragmentary or easily disintegrated touching, a fact that can probably explain the worst result of these elements in the genetic study (from the 9 that were not amplified, 6 were vertebrae).

In the analysis applying the amelogenin gene for the determination of the sex, it was possible to determine the biologic sex in 86.15% (n=56) of the elements, results that are in accordance with the literature [21, 22]. Decreasing proportions were found by Tschentscher et al., 2008 [23], with 50% and by Ganswindt et al. in 2003 [6] and Ricaut et al., in 2005 [24], with 43%.

It is believed that the rate of 13.5% of failure (n=9) in the sex classification had occurred mainly due to the precarious condition of the sample, culminating in genetic material of inferior quality and very degraded, as observed in the quantification of the samples supported by the literature [24].

In relation to the conformity between the sex found and the real sex of the individual, sex determination was possible in 93.94% of the male sample (n=31) and in 78.12% (n=25) of the female sample. None of the male sample presented reading error, as being homozygous (XX). Therefore, in this study, the "deleted-amelogenin males" or DAM issue did not occur, as reported by some authors [3, 25]. Therefore, in all cases in which it was not possible to determine sex, it occurred due to the absence of the amplification of the gene, without the peak formation in the image. The results obtained with the male sample were very relevant since the great difficulty in the use of the amelogenin is present only in the referred sex, according to the literature.

Finally, comparing the best results found in the application of the Physical Anthropology, whose qualitative methodology presented around 89% and the quantitative one, 92.31% with the data obtained in the DNA analysis by the amelogenin gene (86.15%). And, when applying three methods, at least one of them was always correct.

CONCLUSION

The choice of the method for the estimation of the sex should be guided by the analysis of the state and the presentation of the forensic remains, as well as in the analysis of the context of the forensic service, taking into consideration the applicability, the reproducibility, the cost of the technique, of the material and of the professionals, and the demand of trained people. In these aspects, Anthropology emerges with advantages in relation to DNA, presenting as the only inconvenient the necessity of adjusting the chosen methodology due to the ethnic and cultural differences among the population groups. In the case of the molecular biology, such standardization is not necessary. Thus, as a result of this study, it is recommended that physical anthropology be the chosen method if it presents good accuracy when applied to different populations or if it is validated for the analyzed population. Otherwise, genetic analysis should be used for the determination of the sex.

ACKNOWLEDGEMENTS

We thank to Edgard Michel Crosato (University of São Paulo, School of Dentistry, Social Dentistry Department), Dayse Oliveira de Alencar (DLE – Specialized Diagnostic Laboratory), Chiara de Campos Legnaro and Rodrigo Gonçalves Quiezi for cooperating with this study.

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