Morphometric Analysis of Tongue in Individuals of European and African Ancestry

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Abstract

The maxillary dental arch is generally preserved due to its relatively protected location, as well as due to the strength of the teeth because of cadaveric spasm. However, when the victim is edentulous, or when there are not enough comparative data to effectively analyze the dental arches, human identification information based on forensic dentistry methods is very limited. Therefore, since the tongue is an organ of easy access and presents resistance to decomposition and carbonization because it is located in a humid closed cavity, it has become an important organ in post-mortem evaluation. The objective of study was to compare the human tongue dimensions and collagen percentage according to ancestry, gender and age groups. Forty-five tongues were removed during autopsy performed between 1 and 8 hours after death and immediately fixed in formaldehyde in order to avoid decomposition. The tongue length, width and thickness of autopsied individuals were analyzed. Collagen percentage was determined using AxioVision software on slides stained with Picrosirius. The subjects were classified according to gender, age, and ancestry. The tongues length of African ancestry individuals were significantly longer than European ancestry tongues (p = 0.02). The European descendants showed significantly higher collagen percentage than the African descendants (p = 0.01). There was no significant difference regarding collagen percentage between gender groups or age groups. There were also no significant differences in tongue thickness and width according to the ancestry, gender or age groups. This study is the first to compare the size and collagen percentage of the human tongue between different gender, ethnic and age groups.

In conclusion the African ancestry individuals were significantly longer and the European ancestry tongues had a higher collagen percentage. Therefore we suggested that tongue length and collagen percentage analysis could be used as additional parameter for human identification, especially in cases of quartering or even in high-impact accidents where isolated parts of the human body can be found far from the site of the murder or accident. However, further research are needed to confirm these findings.

Introduction

Human identification is the process of determining the identity of a person, or a set of measures with the purpose of disclosing an identity [1].

The evaluation of bones and teeth is extremely common for the identification of bodies that are burned, decomposed, skeletonized, mutilated or fragmented for any reason. Human identification using bone samples can raise evidence regarding gender, ancestry, and estimated age [2]. There are several gender determination procedures in forensic anthropology that analyze morphological characteristics of the skull, jaw and pelvis [3]. For example, closure of the cranial sutures and dental-alveolar wear provide valuable information about the estimated age of adults [4]. Although these evaluations help determine the gender and age of the individuals, the validity of this approach depends on the condition of the bones and bone fragments [5].

Identification through examination of the dental arches requires analysis of previous dental records of the person suspected, so that the ante-mortem and post-mortem examinations may be contrasted, resulting in a positive or negative identification [2]. The maxillary dental arch is generally preserved due to its relatively protected location, as well as due to the strength of the teeth because of cadaveric spasm [5]. However, when the victim is edentulous, or when there are not enough comparative data to effectively analyze the dental arches, human identification information based on forensic dentistry methods is very limited [6].

Therefore, since the tongue is an organ of easy access and presents resistance to decomposition and carbonization because it is located in a humid closed cavity, it has become an important organ in post-mortem evaluation [7,8].

Studies have shown that the organs of the human body are generally larger in male individuals and in African descendants and also that they decrease in size with aging [9-21]. It is also known that individuals of African ancestry generally have higher collagen percentage in skin when compared to individuals of European ancestry [22]. Furthermore, several studies have shown that collagen decreases in several organs with aging [23]. However, we did not find any studies assessing collagen percentage and sizes of the human tongue, or associating it with different age, gender or ethnic groups in the literature to date.

Therefore, the tongue evaluation for the recognition of corpses is important especially in cases of quartering or even in high-impact

accidents where isolated parts of the human body can be found far from the site of the murder or accident.

Thus, the aim of this study was to compare the human tongue dimensions and collagen percentage according to ancestry, gender and age groups.

Materials and Methods

Selection of research subjects

After approval by the Ethics Committee on Human Research of the Federal University of Triangulo Mineiro (UFTM), Uberaba, MG, Brazil (protocol number 2635), 44 autopsy protocols were selected at the Clinical Hospital of UFTM. The tongues were removed during an autopsy performed between 1 and 8 hours after death and immediately fixed in formaldehyde in order to avoid decomposition. The individuals were classified according to the following criteria:

a) Gender: Male (n = 29), Female (n = 15);

b) Ethnicity: European ancestry (n = 33), African ancestry (n = 11); and

c) Age: First the subjects were divided into five groups: 20 - 29 years, 30 - 39 years, 40 - 49 years, 50 - 59 years, and 60 - 79 years.

Posteriorly the individuals were divided into three groups: 20 - 39 years, 40 - 59 years, and 60 - 79 years. This grouping in only three groups allowed an increase in the number of cases per group. All individuals of African ancestry were pure.

Morphometric evaluation of the tongues

Morphometric evaluations of the tongues were evaluated length, width and thickness of 44 tongues of necropsied individuals adults previously stored in a 3.7% formaldehyde solution.

In order to evaluate the tongue length, a flexible millimeter ruler was placed along the median lingual sulcus on the tongues lingual dorsum, from the epiglottis to the apex. A digital caliper was used to analyze the width and thickness of the tongues. Ten consecutive measurements of width and thickness were performed from the base to apex of each tongue, and the higher values of width and thickness were used for the statistical analyses. The measurements were expressed in millimeters. During the tongue handling all personal protective equipment like masks, aprons, protective goggles and gloves were used. A single trained, calibrated examiner blindly analyzed the tongues.

Morphometric analysis of collagen

For collagen analysis, a section from the middle region of each tongue was collected, measuring about 1.0 x 1.0 cm wide and 0.5 cm thick. This section was histologically processed and the slides were stained by Picrosirius. For collagen analysis, was used a light biological microscope, Axio 4.1 (ZEISS, Berlin, Germany) and AxioCam camera (ZEISS, Berlin, Germany) for capturing images, a computer and AxioVision 4.8 software (ZEISS, Berlin, Germany) with 40X objective and a polarizing filter. The images shown in the microscope were transmitted to the computer monitor. In the polarized images, collagen birefringence was shown in reddish yellow, and it was

automatically quantified (Figures 1A and 1B). All the fields of each fragment, approximately 20 fields per fragment, were analyzed.

Statistical analyses

GraphPad Prism 4.0 statistical software (GRAPHPAD SOFTWARE, San Diego, USA) was used for the statistical analyses. The Shapiro-Wilk test was used to evaluate the normality. In order to compare the length, width, thickness and collagen percentage of the tongues between European and African ancestry groups and between male and female groups, Student's t-test was used when quantitative variables showed normal distribution, and the Mann-Whitney test was used when quantitative variables showed non-normal distribution. The Kruskal-Wallis test was used to compare the age groups with non-normal distribution. Spearman correlation test was used to evaluate the correlation between the ages and collagen percentage. The significance level was 5% ($p < 0.05$).

Results

The African ancestry individuals showed significantly longer tongues than the European ancestry individuals ($p = 0.02$) (Table 1).

There was no significant difference when comparing the tongue length between males and females (Table 2), or between age groups (Tables 3 and 4). There were also no significant differences in thickness or width of tongues according to the ancestry (Table 1), between males and females (Table 2), and between age groups (Tables 3 and 4).

The European ancestry showed significantly higher collagen percentage than the African ancestry ($p = 0.30$) (Table 1). There was no significant difference regarding collagen percentage between gender groups (Table 2) or age groups (Tables 3 and 4). There was no significant correlation between collagen percentage and age, and between collagen percentage and tongue width, length and thickness (data not showed).
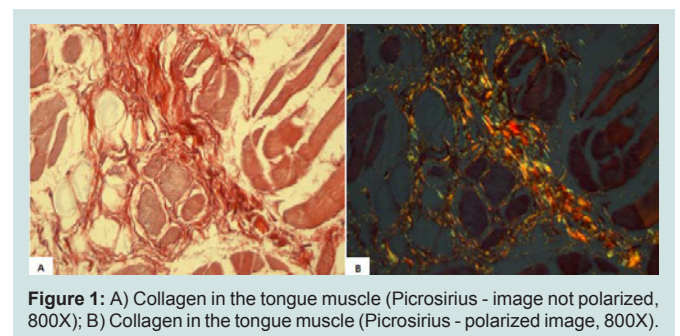

Figure 1: A) Collagen in the tongue muscle (Picrosirius - image not polarized, 800X); B) Collagen in the tongue muscle (Picrosirius - polarized image, 800X).

Table 1: Length, width, thickness and collagen percentage of tongue of European ancestry and African ancestry subjects.

	European ancestry (n = 33)	African ancestry (n = 11)
Length (mm)[a]* Mean ± SD	88.64 ± 9.33	96.00 ± 9.11
Width (mm)[b] Mean ± SD	43.49 ± 5.58	44.08 ± 4.09
Thickness (mm)[c] Mean ± SD	28.32 ± 3.13	29.33 ± 6.29
Collagen Percentage (%)[d]* Median (Max - Min)	4.36 (1578.00 - 0.02)	3.39 (3210.00 - 0.01)

Table 2: Length, width, thickness and collagen percentage of tongue according to sex.

	Male (n = 29)	Female (n = 15)
Length (mm)[a] Mean ± SD	90.31 ± 8.56	90.80 ± 11.98
Width (mm)[b] Mean ± SD	44.18 ± 5.27	42.60 ± 5.10
Thickness (mm)[c] Median (Max - Min)	29.10 (43.10 - 21.90)	28.40 (36.90 - 20.60)
Collagen Percentage (%)[d] Median (Max - Min)	3.95 (1019.00 - 0.02)	4.80 (10578.00 - 0.01)

[a]Student t, t = 0.15; p = 0.87; [b]Student t, t = 0.95; p = 0.34; [c]Mann-Whitney, U = 183.5; p = 0.40; [d]Mann-Whitney, U = 1563.00; p = 0.30

Table 3: Length, width, thickness and collagen percentage of tongue according to five age groups.

	20 - 29 years (n = 9)	30 - 39 years (n = 11)	40 - 49 years (n = 13)	50 - 59 years (n = 6)	60 - 79 years (n = 5)
Length (mm)[a] Median (Max - Min)	93.00 (110.00 - 80.00)	86.00 (105.00 - 75.00)	90.00 (105.00 - 80.00)	89.00 (100.00 - 70.00)	91.00 (115.00 - 70.00)
Width (mm)[b] Median (Max - Min)	40.97 (52.00 - 35.90)	47.30 (50.90 - 35.90)	43.00 (32.80 - 52.00)	43.40 (50.80 - 40.70)	43.70 (49.70 - 31.70)
Thickness (mm)[c] Median (Max - Min)	29.50 (34.40 - 22.10)	22.70 (43.10 - 20.60)	27.40 (34.30 - 23.90)	29.55 (31.90 - 25.60)	29.10 (36.90 - 21.90)
Collagen Percentage (%)[d] Median (Max - Min)	4.43 (22.67 - 0.09)	4.42 (0.34 - 0.01)	4.55 (26.23 - 0.03)	3.15 (25.02 - 0.02)	4.82 (1019.00 - 0.04)

[a]Kruskal Wallis, K = 1.39; p = 0.34; [b]Kruskal Wallis, K = 1.98; p = 0.73; [c]Kruskal Wallis, K = 1.61; p = 0.80; [d]Kruskal Wallis, K = 7.83; p = 0.09

Table 4: Length, width, thickness and collagen percentage of tongue according to three age groups.

	20 - 39 years (n = 20)	40 - 59 years (n = 19)	60 - 79 years (n = 5)
Length (mm)[a] Median (Max - Min)	89.00 (110.00 - 75.00)	90.00 (105.00 - 70.00)	91.00 (115.00 - 70.00)
Width (mm)[b] Median (Max - Min)	42.35 (52.00 - 35.90)	43.00 (52.00 - 32.80)	43.70 (49.70 - 31.70)
Thickness (mm)[c] Median (Max - Min))	28.50 (43.10 - 20.60)	27.70 (34.30 - 23.90)	29.10 (36.90 - 21.90)
Collagen Percentage (%)[d] Median (Max - Min)	4.42 (32.10 - 0.01)	42.15 (37.28 - 0.02)	3.98 (1019.00 - 0.04)

[a]Kruskal Wallis, K = 0.12; p = 0.93; [b]Kruskal Wallis, K = 0.44; p = 0.80; [c]Kruskal Wallis, K = 0.12; p = 0.93; [d]Kruskal Wallis, K = 2.25; p = 0.32

Discussion

Anthropological patterns have been investigated in order to contribute to anatomical data collection from corpses and skeletons indifferent parts of the world, hence facilitating human identification. These studies are aimed at establishing protocols for determining the gender, age, height, ancestry and individualization factors [24,25]. A preliminary assessment during post-mortem examination allows forensic anthropologists to gather the necessary information for a rough estimate of the actual anatomical parameters of an individual [26,27]. Some studies have demonstrated that African ancestry individuals have a larger bone density [20], larger palatal area [28],

increased hip flexion strength [29], greater muscle mass [30,31], higher total-body calcium, phosphorus, sodium, chlorine, and potassium [32], higher lean body mass [33], higher density of dermal cells [22], and numerous superficial vessels on the skin compared to European descendants [34]. Although morphometric studies have not been conducted comparing the dimensions of the human tongue among ethnic groups, we believe that in the present study, the tongues of African ancestry individuals were longer than those of the European ancestry, perhaps because the African ancestry individuals had greater tongue muscle mass as demonstrated in the appendicular muscle in previous studies [30,31]. The greater lingual length in individuals of African ancestry would be justified by the higher levels of testosterone in these individuals, which is a hormone directly associated with muscle hypertrophy.

There was no significant difference in tongue length, width or thickness between men and women. Although studies comparing tongue length, width or thickness between the sexes have not been found, studies have been carried out comparing muscle strength and tongue mobility between female and male individuals [13,31]. However, the difference in lingual muscle strength between genders is still controversial as it has already been reported greater strength in the male tongue, although no differences were found in another study [35,36]. It is also known that women have a higher percentage of muscle fibers in the middle region of the tongue, which gives greater muscle mobility [37]. Since the previous studies evaluated only the muscular strength and mobility of the tongue, the present study was the first to compare tongue length, width and thickness between male and female individuals.

It is known that the axial force of the tongue increases during childhood and adolescence, and stabilizes in adulthood [38]. However, some studies in living individuals showed that the muscle strength of the tongue reduces after 60 years due to decreased muscle mass and motor units and due to decreased in the density of the muscle fibers [36,38-42]. Only one study that evaluated the thickness of the human tongue according to age groups was found in the literature. In this study, the authors observed *in vivo* that elderly individuals had lesser tongue thickness compared to young individuals [38]. However in the present study there was no significant difference in the length, width and thickness of the tongues when comparing different age.

Although it has been described in the literature that individuals of African ancestry have a greater amount of collagen in several tissues, in the present study, the tongues of African ancestry individuals had a lower percentage of collagen than the tongues of European ancestry individuals [22]. It is known that in the tissues of African ancestry individuals the bundles of collagen are smaller, there is a greater amount of glycoproteins in the dermal interstitium, and the macrophages are larger and more numerous than in European ancestry individuals [22,43]. Therefore, in African ancestry individuals the collagen bundles would be more spaced, being intercalated by the other components of the extracellular matrix, which could justify the fact that we found a lower percentage of collagen in this group, since this percentage of collagen, was evaluated by area. The present study is the first study to compare the size and collagen percentage of the human tongue between different gender, ethnic and age groups.

In conclusion the African ancestry individuals were significantly longer and the European ancestry tongues had a higher collagen percentage. Therefore we suggested that tongue length and collagen

percentage analysis could be used as additional parameter for human identification, especially in cases of quartering or even in high-impact accidents where isolated parts of the human body can be found far from the site of the murder or accident. However, furthers research are needed to confirm these findings.

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