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Sofia Fernandes Franco

Dissertação de Mestrado em Ciências e Técnicas Laboratoriais Forenses

Gandra, outubro de 2019

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Trabalho realizado sob a orientação de:
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DECLARAÇÃO DE INTEGRIDADE

Sofia Fernandes Franco, estudante do Mestrado em Ciências e Técnicas Laboratoriais Forenses do Instituto Universitário de Ciências da Saúde, declaro ter atuado com absoluta integridade na elaboração desta Dissertação.

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Agradecimentos

Em primeiro lugar, tenho de agradecer à minha orientadora, Professora Doutora Alexandra Teixeira, pela sua ajuda fundamental na concretização deste trabalho. A sua enorme disponibilidade, simpatia e dedicação foram imprescindíveis para a realização deste projeto.

Ao meu coorientador, Professor Doutor Daniel Mongiovi, pela disponibilidade e atenção desde o início, o que me levou à concretização deste trabalho. Obrigada também pelos comentários e opiniões durante o decorrer do trabalho.

Ao meu coorientador, Professor Doutor Vítor Matos, que me permitiu desenvolver este projeto utilizando material pertencente à Universidade de Coimbra. Sem esta "ponte" entre as duas universidades, ser-me-ia impossível realizar um trabalho com este tipo de amostra.

Ao Professor Doutor Álvaro Azevedo, pela sua enorme ajuda no que respeita ao estudo estatístico deste projeto. A sua clareza e simplicidade de comunicação foram igualmente fundamentais para o sucesso deste trabalho.

Aos meus colegas que me acompanharam neste percurso. Obrigada pelo apoio e incentivo necessário para enfrentar os desafios que nos eram propostos. A vocês, desejo-vos um futuro brilhante.

A todos os professores deste mestrado por todo o apoio e conhecimento transmitido durante este percurso, o que me permitiu imergir no mundo das Ciências Forenses.

À Micaela, por todos os momentos de amizade e confiança que me proporcionou ao longo destes dois anos. Uma amiga para a vida, independentemente do tempo e da distância que nos separa. O Porto, sem ti, não teria sido a casa que foi.

À Carolina, pela companhia que me fez durante o desenvolver deste projeto, por todos os desabafos e conselhos e pela partilha de momentos inesquecíveis. Foste, certamente, dos melhores reencontros que já tive.

À Rita e à Débora, por me terem acolhido tão carinhosamente no querido 88. Obrigada por todos os serões de cantorias, danças e filmes, mas, acima de tudo, por estarem sempre prontas para me ouvirem e me incentivarem. Obrigada por me terem feito sentir em casa, logo desde o início. Com vocês, o Porto tornou-se muito mais bonito.

Ao João, por me ter apoiado em todos os momentos de insegurança e me ter feito perceber que basta ter vontade para podermos seguir os nossos sonhos. Obrigada pelo incentivo, antes e durante este percurso.

À minha avó, por me acolher em sua casa sempre de braços abertos e por me ter tratado como uma filha desde o momento em que me mudei para Portugal. Ao meu avô, por me desejar sempre o melhor e me apoiar em todas as decisões que tomo.

Aos meus irmãos que, estando muito longe, relembram-me sempre onde assentam as minhas origens. As vossas ambições e conquistas orgulham-me e dão-me força para alcançar as minhas.

Ao meu pai, por, desde sempre, me ter apoiado e incentivado a seguir o caminho que quero. Obrigada pelas lições e conselhos que me fizeram crescer e tornar-me na pessoa que sou hoje. Obrigada por me transmitires a vontade de querer alcançar sempre mais e melhor.

À minha mãe, por me apoiar incansavelmente em todas as decisões que tomei. Obrigada por estares sempre ao meu lado, nos bons e maus momentos, por me transmitires a força do universo sempre que me senti mais em baixo e por celebrares as minhas vitórias, mesmo que pequenas, como se fossem tuas. Obrigada por seres o meu exemplo de garra, força e coragem.

A todos os que, de uma maneira ou de outra, me foram incentivando através de um pequeno "força" e "tu consegues". Foram esses momentos que me permitiram ultrapassar as dificuldades do dia-a-dia e que me levaram à conclusão deste trabalho.

A todos, o meu Muito Obrigada!

Abstract

When unidentified human skeletal remains from forensic contexts are analysed, one of the primary goals is to retrieve their identity. If fundamental osteological elements for the reconstruction of the biological profile are missing or fragmented, then teeth must be employed as they resist various adverse factors.

Odontometric parameters, which have been applied worldwide in order to understand sexual dimorphism within and between human populations, show that the canine is the most dimorphic tooth. However, because single-rooted teeth, such as the canines, are more prone to *postmortem* loss than multiradicular ones, the molars may be considered an excellent alternative for sexual diagnosis in forensic contexts.

The aims of this research are to evaluate the existence of sexual dimorphism in the diagonal dimensions of the mandibular first and second molars, as well as the development of a simple method to estimate the sex of an individual using the dentition.

With the aim of determining the applicability of diagonal crown dimensions for sexual diagnosis, the first and second left mandibular molars belonging to 135 individuals from the Coimbra Identified Skeletal Collection (20^{th} Century), housed at the Department of Life Sciences, University of Coimbra, were studied. Independent t-tests ascertained the relationship between tooth size and sex, and a ROC analysis established cut-off points for each dimension. Additionally, the mesiodistal crown dimensions of the molars and the right canine were taken in 59 individuals with the purpose of comparing the diagonal crown dimensions' results with the most commonly assessed measurements. The latter dimensions were also subject to a logistic regression to evaluate their predictability.

A correct sex classification of up to 65,2% for the first molar and 67,7% for the second molar was calculated for the diagonal dimensions, while for the mesiodistal dimensions an accuracy of 69,5% for the first molar and of 74,6% for the canine was obtained. These results suggest that although diagonal dimensions of posterior teeth present moderate sexual dimorphism and might constitute a corroborating method for sexual diagnosis, the canine presents better results and should be considered whenever present.

Keywords: Sexual Dimorphism; Odontometry; Molars; Mandible; Forensic Odontology.

Resumo

Um dos principais objetivos quando são encontrados restos humanos esqueléticos não identificados é a recuperação da sua identidade. No caso de elementos osteológicos fundamentais à reconstrução do perfil biológico que estejam em falta ou fragmentados, os dentes devem ser empregues, pois resistem a vários fatores adversos.

Parâmetros odontométricos, utilizados mundialmente de forma a se compreender o dimorfismo sexual intra e inter-populações humanas, indicam que o canino é o dente mais dimórfico. Contudo, uma vez que os dentes monorradiculares, como os caninos, são mais propensos à perda *postmortem* que os multirradiculares, os molares podem ser considerados uma alternativa excelente para a diagnose sexual em contextos forenses.

Os objetivos deste estudo são avaliar a existência de dimorfismo sexual nas dimensões diagonais dos primeiro e segundo molares mandibulares, bem como o desenvolvimento de um método simples para a estimativa do sexo de um indivíduo utilizando a dentição.

Com o objetivo de determinar a aplicabilidade das dimensões diagonais das coroas para a diagnose sexual, foram estudados os primeiro e segundo molares mandibulares esquerdos pertencentes a 135 indivíduos da Coleção de Esqueletos Identificados de Coimbra (Século XX), do Departamento de Ciências da Vida, Universidade de Coimbra. A relação entre o tamanho dos dentes e o sexo foi verificada por testes-t independentes e uma análise ROC determinou pontos de corte para cada dimensão. Adicionalmente, foram medidas as dimensões mesiodistais das coroas dos molares e do canino direito de 59 indivíduos, com o objetivo de comparar os resultados das dimensões diagonais das coroas com as medidas mais estudadas. As últimas dimensões foram também sujeitas a uma regressão logística para avaliar a sua capacidade de previsão.

Para as dimensões diagonais foi calculada uma classificação correta do sexo de até 65,2% para o primeiro molar e 67,7% para o segundo, enquanto as dimensões mesiodistais revelaram uma classificação de 69,5% para o primeiro molar e 74,6% para o canino. Estes resultados sugerem que, embora as dimensões diagonais dos dentes posteriores possuam um dimorfismo sexual moderado e possam constituir um método corroborativo na diagnose sexual, o canino apresenta melhores resultados e deve ser considerado sempre que se encontre presente.

Palavras-chave: Dimorfismo Sexual; Odontometria; Molares, Mandíbula; Odontologia Forense.

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List of Abbreviations

DNA – Deoxyribonucleic acid

MD – Mesiodistal

BL – Buccolingual

MLDB – Mesiolingual-distobuccal

MBDL – Mesiobuccal-distolingual

mm – Millimeters

ROC – Receiver Operating Characteristic

AUC – Area Under Curve

1. Introduction

Forensic Science is defined by the Oxford English Dictionary as «the application of scientific methods and techniques to matters under investigation by a court of law» (Oxford dictionaries, n.d.). It is comprised of the knowledge of several scientific fields, along with their distinctive methods, to serve the Law and more specifically the Criminal Law (Magalhães, Dinis-Oliveira 2016). Therefore, we can refer to Forensic Science as multidisciplinary, in which every subdiscipline, such as Forensic Anthropology and Forensic Odontology, has the same purpose: to examine evidence and provide conclusions that are able to answer the legal system's questions (Plourd 2010; Magalhães, Dinis-Oliveira 2016).

Of all the questions that Forensic Science may help answer, the most frequently asked are related to human identification and information regarding body-associated items (Plourd 2010). In the early days of this scientific field, the most common method of investigation was the mere observation of evidence (Eckert 1997; Magalhães, Dinis-Oliveira 2016). However, in recent years new methods and techniques have been developed, increasing the validity of each step of the investigation and, consequently, the formulation of better and more specific conclusions (Eckert 1997). In this sense, the number of possible approaches from different fields that can aid in human identification has increased (Magalhães, Dinis-Oliveira 2016). Nonetheless, the first expert to evaluate a human body in a forensic context is the Forensic Pathologist, often accompanied by a Forensic Anthropologist depending on the degree of decomposition, as their prevalent knowledge in human osteology vastly contributes to the positive identification of human remains.

1.1. Forensic Anthropology

Many attempts have been made to define Forensic Anthropology. One of the earliest was proposed by anthropologist Stewart in 1979, who defined the field as «the branch of physical anthropology, which for forensic purposes, deals with the identification of more-or-less skeletonised remains known to be, or suspected of being human». This description might have been accurate at the time but is very restricted and incomplete in view of today's broader Forensic Anthropology. In 1988, İşcan defined it as «a multidisciplinary field combining physical anthropology, archaeology and other fields, including forensic dentistry, pathology and criminalistics»; while a better definition, it lacks recent fields of activity in Forensic Anthropology such as age estimation and facial identification of living individuals (İşcan, Steyn 2013).

The importance of Forensic Anthropology has been growing in the Forensic Science's world and although each country has its own practices and procedures, this field's goals are the discovery and recovery of skeletonised human remains, the identification of these human remains and, most recently, the assessment of the age of undocumented living people. It also aids in the estimation of the *postmortem* interval and the determination of cause of death, and the need for its activity has been growing in mass disasters, where a large number of fatalities call for an efficient and accurate identification (Cattaneo *et al.* 2006; İşcan, Steyn 2013; Cardoso, Marinho 2016).

1.1.1. History

The beginning of Forensic Anthropology dates to the late eighteenth and nineteenth centuries. At this time, the skeletal analysis was considered an applied area of Anatomy and Anthropology (Ubelaker 2006; Ubelaker 2008).

In Europe, it is believed that the work of French Jean-Joseph Sue, in 1755, and its improvement with the work of Mathieu Orfila in the following years, launched the communities' interest for Forensic Anthropology. In 1859, Paul Broca founded the Société d'Anthropologie de Paris, the first official organization of Physical Anthropology. Since then, Anthropologists started contributing with their input in the resolution of legal cases (Ubelaker 2006; Ubelaker 2008).

Meanwhile, in the United States, it was Thomas Dwight that began to research cases related to Forensic Anthropology and understand the importance of this field. Along with the work of Wilton Krogman, new methods were explored, and Forensic Anthropology has since then been a better-recognised field, involved in a higher number of cases than before, which led to the foundation of the Physical Anthropology section of the American Academy of Forensic Sciences, in 1972, and the American Board of Forensic Anthropology, in 1977 (Ubelaker 2006; Ubelaker 2008). Similarly, in Europe, the Forensic Anthropology Society of Europe as a section of the International Academy of Legal Medicine was formed in 2004, offering training and promoting the study of this field. Thus, with the increased number of Forensic Anthropology organizations worldwide, investment in the area has been made with the research and publication of novel subjects, which greatly improves the knowledge that Forensic Anthropology has to offer (Ubelaker 2006; Ubelaker 2008).

In Portugal, the Legal Medicine Institute was founded in 1918 and has since evolved into the National Legal Medicine and Forensic Sciences Institute. It currently holds four scientific departments: Forensic Chemistry and Toxicology Service, Forensic Genetics and Biology Service, Forensic Technologies and Criminalistics Service and Forensic Clinical and Pathology Service. This last service includes cases that require Forensic Anthropology and Dental Medicine expertise (Vieira 2012; INMLCF, n.d.).

1.1.2. The Forensic Anthropologist

The background of a forensic anthropologist may vary according to their country. In North America, the majority of forensic anthropologists come from a Physical Anthropology and Archaeology background, while in most European countries, many have a medical degree. Exceptions to this norm include the United Kingdom, where forensic anthropologists have a tight relationship with Archaeology, and Portugal, as well as German-speaking countries, where their background education resembles the North-American one (Cunha, Cattaneo 2006; İşcan, Steyn 2013).

At the beginning of this field, the anthropologist was called to the laboratory solely to assist in the identification of human bones in a forensic investigation. Nowadays, the forensic anthropologist's role is much larger and allows both field and laboratory intervention (Cardoso, Marinho 2016).

In order to achieve the best results, the forensic anthropologist should participate in the recovery of the human remains in the field, as the chance of serious mistakes such as leaving small bones behind and improper care of the remains until they arrive at the laboratory is greatly reduced (Cunha, Cattaneo 2006; Cattaneo 2007; Pickering, Bachman 2009; Cunha 2014; Cardoso, Marinho 2016). In this sense, at the recovery site, the anthropologist should be able to:

- Distinguish and recover human skeletal remains (Cardoso, Marinho 2016);
- Recreate the chain of events that took place immediately prior to the death of the individual, considering the remains' position, in collaboration with other forensic specialists (Pickering Bachman 2009; Cardoso, Marinho 2016);
- Make a taphonomic assessment and estimate the time that passed since death, known as the *Postmortem* Interval (PMI); the success of this step is greatly improved when working alongside forensic entomologists and botanists (Cunha, Cattaneo 2006; Cattaneo 2007; Cardoso, Marinho 2016).

In the laboratory, with the skeletal remains safely recovered and cleaned, the forensic anthropologist may then proceed with the attempt of identifying the deceased. In this stage, the more skeletonised the remains are, the better. The goals of the forensic anthropologist in the laboratory are:

- To provide an identification through the skeleton by establishing a biological profile (sex, age, ancestry, and stature) and identifying individualizing factors present in the bones (Cunha, Cattaneo 2006; Cattaneo 2007; Pickering, Bachman 2009; Stanojevich 2012; Cunha 2014; Cardoso, Marinho 2016);
- Further investigate the events prior to the death by aiding in the determination of cause and manner of death. This could be achieved by studying the traumatic lesions of the bones (Cunha, Cattaneo 2006; Cattaneo 2007; Cunha 2014; Cardoso, Marinho 2016).

The forensic anthropologist is the better-equipped specialist to determine when, in a person's life, a traumatic lesion took place since they hold extensive osteological knowledge, so it is their role to distinguish between *antemortem* (before death), *perimortem* (during death) and *postmortem* (after death) lesions whenever these exist (Cunha, Cattaneo 2006; Cattaneo 2007; Cunha 2014; Cardoso, Marinho 2016). The forensic anthropologist should also be able to distinguish the type of instrument that caused the lesion since these have different classifications. Should a *perimortem* lesion be represented in the skeletal remains in question, the forensic anthropologist is capable of identifying a violent death (Stanojevich 2012; Cunha 2014).

The forensic anthropologist's contributions should always be accompanied by other forensic specialists' findings, as only with the combination of different fields of knowledge it is possible to acquire the most information. With this multidisciplinary approach kept in mind for every forensic scenario, the anthropologist can be considered a great asset in every case (İşcan, Steyn 2013; Cunha 2014; Cardoso, Marinho 2016).

1.2. Human Identification

One of the main purposes of a death investigation is to identify the deceased. It is a necessary step since social, moral, financial and legal issues are involved and it should be done quickly and successfully (Keiser-Nielsen 1963; Thompson, Black 2007; Molina 2010). When the body has suffered almost no modifications, it might be easily identifiable by its facial appearance and the identity can be confirmed by a friend or family member. Even with a disfigured face, body features and distinctive marks can still prove useful in the recognition (Thompson, Black 2007; Jackson, Jackson 2008). However, when the deceased is no longer recognisable, either by the action of the decomposition process or by a catastrophic event, other methods must be employed (Jackson, Jackson 2008; Molina 2010; Stavrianos *et al*. 2010).

There are two types of methods used when identifying a human body: primary and secondary identification methods. Comprising the primary or comparative methods are the fingerprints, odontology, and DNA; these are only applied when both *antemortem* and *postmortem* information exist since it is the comparison between these two that enables an identification (Keiser-Nielsen 1963; Avon 2004). In these circumstances, one of the following occurs: positive identification, possible identification or exclusion. When a positive identification is achieved, the *antemortem* and the *postmortem* sets of data carry the same information, with absolutely no irreconcilable discrepancies and with sufficiently unique features that allow for an assurance of the identity. In a possible identification, the two sets match the information, but this is not enough to establish an identity as there are no distinctive traits. It is the outcome when neither a positive identification or exclusion is reached. When it comes to an exclusion, it is possible to confirm that the presumed identity does not belong to the deceased because the *postmortem* information has irreconcilable discrepancies when compared with the *antemortem* data (Avon 2004; Thompson, Black 2007).

The use of fingerprints to identify a person has prevailed for over one hundred years as it usually presents great results, but it is restricted to the integrity of soft tissues. With the process of decomposition, the soft tissue becomes gradually scarce and this method becomes problematic. The same can be said when dealing with charred or water submerged remains (Galloway, Charlton 2007; Jackson, Jackson 2008; Tabor, Schrader 2010; Uhle 2010). The dentition, however, is known to be composed of the hardest and most durable tissue in the human body, surviving decomposition and various drastic events such as a fire. This quality, combined with its highly discriminative traits, makes it an excellent method for identification (Keiser-Nielsen 1963; Pretty, Sweet 2001; Avon 2004; Hardy 2007; Tabor, Schrader 2010; Caldas, Pérez-Mongiovi 2016). On the other hand, the analysis of DNA has been growing in the last decades as scientific advances have been made in this field. Very little biological material is needed for its analysis and this is possible to achieve even after decomposition has taken place, by using the bones and teeth. It is considered a method of choice when very efficient and specific results are needed, although it entails high costs and is time-consuming. In this sense, it is wise to use this method as the last resource (Goodwin, Hadi 2007; Ubelaker 2008; Smith, Sweet 2010; İşcan, Steyn 2013).

The secondary or reconstructive methods include the biological profile, distinctive marks such as scars and tattoos, photographs, clothing and jewellery, among others; a reconstructive technique is usually adopted when there is no *antemortem* data to compare with, as there are no clues about the person's identity. In such cases, all of que acquired *postmortem* information is applied in order to find a presumptive identity (Rutty 2007).

A reconstructive technique consists of a full examination of the remains to acquire the maximum amount of information concerning the deceased, as it will narrow the number of possible identities, simplifying the search process (Sassouni 1963; Jackson, Jackson 2008; Cardoso 2013). The first step in achieving an identity when dealing with skeletal remains is to assure that these belong, in fact, to a former living human being (Cattaneo 2007; Jackson, Jackson 2008; Molina 2010; Cunha 2014). Afterwards, the anthropologist proceeds with the determination of the biological profile, followed by the individualising factors. These methods will then, hopefully, lead to a possible identity which could subsequently be confirmed by the comparative methods mentioned above (Jackson, Jackson 2008; Cardoso 2013).

1.3. The Biological Profile

The biological profile consists of four general parameters that are shared by every person: sex, age, ancestry, and stature. The diagnosis of these traits through skeletal remains truly helps in the discovery of the identity, as it develops a preliminary portrait of the deceased (Scheuer, Black 2007). Once the biological profile is established, the anthropologist will focus on the individualising factors, including anatomical variants, such as the sternal foramen, bone trauma, bone pathology and surgical implants (İşcan 2001; Jackson, Jackson 2008; Cardoso 2013; Cunha 2014).

To accurately determine the biological profile, the presence and integrity of key skeletal elements is ideal. When this isn't the case, the forensic anthropologist's work is hampered and they might not be able to achieve a full biological profile (Scheuer 2002; Scheuer, Black 2007; Gill-King 2010). It should also be taken into consideration whether the remains belong to an adult or subadult individual, seeing as some characteristics, such as sex, are impossible to diagnose in a juvenile skeleton and, therefore, might compromise the results (Scheuer 2002; Gill-King 2010).

1.3.1. Age

When evaluating age at the time of death, the forensic anthropologist should always bear in mind that two types of ages exist, the biological age and the chronological age. The skeleton and dentition are able to provide the biological age, which corresponds to the age the person appears to have had, while the chronological age is the actual number of years that the person lived. The biological age is influenced by the growth and maturation of subadults and the degeneration of the body in adults. Since these are highly affected by health, nutrition and external factors such as the person's occupation, the correlation between biological and chronological age isn't constant throughout the person's life. Thus, when assessing the age of skeletal remains, the anthropologist isn't able to give a precise number of years, but rather an age range (Scheuer 2002; Cardoso 2013; İşcan, Steyn 2013; Cunha 2014).

The dentition provides very useful information when it comes to the aging of subadult remains. Since the mineralization and eruption of the teeth occur in many phases until early adulthood, narrow age ranges are possible. The same can be said about the growth of the immature skeleton; developmental stages of ossification centres, length of the long bones and fusion of the epiphyses are associated with age in a predictable manner (Scheuer 2002; Scheuer, Black 2007; Jackson, Jackson 2008; Gill-King 2010; Cardoso 2013; İşcan, Steyn 2013; Cunha 2014).

When dealing with adult skeletal remains, however, the determination of the age will result in wider ranges since the degeneration associated with aging occurs at a much slower pace compared to the maturation process, and it is associated with a higher variability, differing greatly from individual to individual. Methods are usually based on the fusion of the cranial sutures and morphological changes in the pubic symphysis, sacroiliac joint and sternal end of the fourth rib. The accuracy in this estimation can be improved if two or more methods are used simultaneously, though it should be kept in mind that the older the skeletal remains, the more difficult it is to determine the age at the time of death (İşcan 2001; Scheuer 2002; Scheuer, Black 2007; Cardoso 2013; İşcan, Steyn 2013; Cunha 2014).

1.3.2. Ancestry

The assignment of skeletal remains to a certain population is the hardest step in the construction of the biological profile. Small differences between populations, allied to the high variability between individuals, are responsible for the low accuracy in the estimation of this biological profile's category. However, when examining the remains, the forensic anthropologist should always try to match them to a Caucasian, Asian or an African population (Scheuer, Black 2007; Gill-King 2010; Cunha 2014).

For the ancestry, the examiner should focus on the skull, more specifically on the face, since the majority of the differences are held on the facial features. Ancestry diagnosis can be made through morphological and metric methods or a combination of the two. Amongst the morphological methods, there are two different types of traits: anthroposcopic and non-metric or discrete. The anthroposcopic traits are the features that alter in shape, such as the orbits or the palate, while the discrete traits are minor dental and skeletal variants that are found in higher percentages in certain populations. These include supernumerary bones in the skull such as the Inca bone, and different dental features such as the shovel-shaped incisors and the Carabelli's cusp (Albanese, Saunders 2006; Gill-King 2010; İşcan, Steyn 2013; Cunha 2014).

Computer software programmes such as FORDISC and AncesTrees rely on craniometry and are a great tool for anthropologists to determine the ancestry of unknown human remains (Navega *et al*. 2015). It was also found that a metric assessment increases the accuracy of ancestry estimation, according to a study that evaluated the accuracy of ancestry estimation based in 99 forensic cases, obtaining an overall accuracy of 90,9% of correct ancestry classification (Thomas *et al*. 2017).

1.3.3. Stature

The living stature of an individual is possibly the simplest parameter to estimate in the biological profile. Should the entire skeleton be present, this estimation can be accomplished by the sum of the height of the skeletal elements that contribute to the person's stature, such as the long bones (Jackson, Jackson 2008; Cardoso 2013; İşcan, Steyn 2013). However, a complete and preserved skeleton is hardly found in forensic scenarios so alternative methods should be exercised.

It is possible to estimate stature by the length of the long bones with regression equations since these exhibit a correlation with a person's height. The most accurate results are reached when the longest bones, such as the femur and tibia, are measured, and even more when multiple bones are considered. When those are absent or fragmented, other bones such as the humerus, metatarsals or metacarpals should be applied, though the accuracy slightly declines. Similarly to the age determination, it is impossible to provide a precise height for the skeletal remains, so results are given in a range (Scheuer 2002; Gill-King 2010; Stanojevich 2012; Cardoso 2013; Cunha 2014).

If the remains belong to a subadult, the same regression equations can't be used since the long bones haven't reached maturity. In this case, diaphyseal length is to be considered (İşcan, Steyn 2013).

The establishment of the height should be saved for last when determining the biological profile, as the equations used are sex and ancestry-dependent (Scheuer 2002; Scheuer, Black 2007).

1.3.4. Sexual Diagnosis

The sex of the skeletal remains is usually one of the first parameters that is established when determining the biological profile. As there can only be two outcomes, male or female, it is helpful to diagnose the sex at the beginning of the examination as it will lower the number of persons that could possibly match the remains to about one half. On the other hand, the estimation of age and stature differs whether the remains belong to a male or female person, so these parameters should only be evaluated after sexual diagnosis (Scheuer 2002; Scheuer, Black 2007).

While in living persons and fresh cadavers the anatomical differences allow for an instant sexual diagnosis, in the skeleton these differences are much subtler and frequently overlap (White, Folkens 2005; İşcan, Steyn 2013). Nonetheless, depending on the skeletal element examined, a high accuracy may be achieved.

The pelvis is commonly accepted as the most dimorphic skeletal element in the body as it takes part in the female reproductive system. The male pelvis is described as high and narrow, as opposed to the broad and shallow female pelvis, whose area should be big enough to allow childbirth. The second most dimorphic element is considered to be the skull. Combined with the hip bones, accuracy rates of up to 98% have been reported (İşcan, Steyn 2013). When these are absent or deteriorated, a metric assessment of the long bones could be made as, generally, the male bones are more robust and larger than the female's (Scheuer 2002; Bruzek, Murail 2006; Black 2007; Ubelaker 2008; Scheuer, Pickering, Bachman 2009; Cardoso 2013).

The sexual diagnosis through the skeleton is merely possible when it comes to adult remains since the differences between sexes are only developed after puberty. Therefore, when faced with subadult remains, the only reasonable method to adopt is the metric assessment of several bones, even if accuracy is reduced. When greater precision is required, DNA extraction and analysis from teeth or bones should be considered. (White, Folkens 2005; Scheuer, Black 2007; Ubelaker 2008; Pickering, Bachman 2009; Cardoso 2013; Cunha 2014).

1.4. Recovery of incomplete or fragmented remains

Often are the cases where the fundamental skeletal elements to ascertain the biological profile are absent or fragmented. Key events affecting bone structure and preservation include taphonomic conditions and mass disasters.

Forensic Taphonomy is considered the study of all the events that took place from death to the discovery of the remains (Stanojevich 2012; Cardoso 2013; İşcan, Steyn 2013), so while doing the taphonomic evaluation, the anthropologist must be able to distinguish the natural taphonomic process, such as the sequence of decomposition of a cadaver, from any intentional changes that the body might have suffered (Cattaneo 2007; Stanojevich 2012; Cunha 2014).

The decomposition of a body is the process of losing the soft tissue in a fresh cadaver, which ends when the remains are completely skeletonised. This process is influenced by different taphonomic factors, so the decomposition diverges greatly from one cadaver to another (Pinheiro 2006; Cardoza 2011; İşcan, Steyn 2013). There are three types of taphonomic factors that affect the cadaver:

- Environmental factors: these are related to nature and can be abiotic, such as the temperature, sunlight and rainfall, or biotic, such as plants and animals. These often produce a great influence on human remains, particularly fauna activity since the scattering of bones, as well as the biting executed by some animals, might be enough to crush the remains (Stodder 2008; Gill-King 2010; İşcan, Steyn 2013);
- Individual factors: aspects associated with the body, such as its weight and age at death (İşcan, Steyn 2013);

- Cultural or behavioural factors: these result from the influence of other human beings, such as embalming, inhumation or attempts at destruction of evidence (İşcan, Steyn 2013).

Depending on the absence or presence of these taphonomic factors, the decomposition of the human remains will be affected, to a higher or lower extent, therefore the forensic anthropologist must be able to distinguish them, as it will aid in the recreation of the sequence of events immediately prior to and after the death, and in the estimation of the *postmortem* interval (İşcan, Steyn 2013).

Besides the decomposition, mass disasters are equally or even more accountable for the lack of integrity found in human remains. Two types of classification can be made regarding mass disasters: natural, accidental or criminal, when it comes to the event's origin, and open, closed or open and closed disasters, when it comes to the information about the number of victims and their identity. When a closed disaster takes place, such as an air crash, the exact number of victims and their identities are known; in an open and closed disaster, which is the example of a train collision, only a few of the victims are known; finally, in an open mass disaster, there is no previous information regarding the number and identity of victims and it is the case of earthquakes or tsunamis – it presents as the more challenging scenario (Cattaneo *et al*. 2006; İşcan, Steyn 2013; Prajapati *et al*. 2018).

A consequence of the decomposition and mass disasters is the disarticulation or fracture of the bones. Regarding the decomposition, with the degradation of soft tissue in the joints, the bones are no longer connected and become disarticulated. Because of the lower amount of soft tissue in the skull, this is usually the first element to skeletonise and disarticulate with the vertebrae. With the skull separated from the rest of the body, the possibility of finding incomplete skeletal remains is much higher as this element might roll away due to gravity. The chance of finding the mandible detached from the skull is also high, as the temporomandibular joint is equally one of the first to disarticulate. This explains the cases where only the skull or mandible are found (Roksandic 2002; Pinheiro 2006; Cardoza 2011; Cunha 2014). On the other hand, due to the severe damage implicit in a mass disaster, the majority of the remains encountered will be fragmented and essential bones for the establishment of the biological profile, like the pelvis for the determination of the sex, might be rendered useless for identification (Stodder 2008; Gill-King 2010; Pereira *et al*. 2010; Acharya *et al*. 2011; İşcan, Steyn 2013).

In the presence of highly fragmented or burnt human remains, preserved elements, such as teeth, should be used to help in the identification process. The teeth, as already mentioned earlier, are composed of enamel, the hardest and most stable tissue in the body. They are often preserved through decomposition, fire, and severe trauma, making them an excellent alternative to fragmented remains (Avon 2004; Stavrianos *et al*. 2010; Tabor, Schrader 2010; Cardoza 2011; Vishwakarma, Guha 2011). Similarly to the bones, the teeth may be able to provide useful information for the biological profile when no *antemortem* records are found. In this sense, besides the age and ancestry determination where the dentition plays an important role, teeth can also be used for sex estimation (Rao *et al*. 1989; Hardy 2007; Pereira *et al*. 2010; Acharya *et al*. 2011; Zorba *et al*. 2012).

1.5. Odontologic methods for Sexual Diagnosis

In scenarios where the optimal bones for the estimation of the skeletal remains' sex are absent or fragmented, teeth may be a way of achieving that information. However, this procedure should be made with caution as sexual diagnosis by the teeth alone might not deliver satisfactory results and should be considered a corroborating method (İşcan, Steyn 2013).

The sexual differences that might be observed in teeth are based on the divergence of body size between males and females. This principle is applied to the size of the teeth, with male teeth being generally larger than female's, and the most used and straightforward measures are the mesiodistal and buccolingual crown diameter (Scott, Turner 1988; Ramakrishnan *et al*. 2015; Kondo, Manabe 2016). The mesiodistal crown diameter (MD) is defined as the maximum crown length, which is the distance between the most mesial (closest to the midline of the dental arch) and most distal (closest to the lateral) points of the tooth crown, taken parallel to the occlusal plane. This measure might also be described as the distance between both contact points of the tooth, although these might not match the furthest points when the dentition is crooked, as well as when it comes to premolars and molars. The buccolingual crown diameter (BL), on the other hand, is described as the maximum crown breadth, matching the greatest distance between the buccal/labial (facing the mouth/lips) and lingual (facing the tongue) crown surfaces and perpendicular to the mesiodistal diameter (Aftandilian 1994; White, Folkens 2005; White *et al*. 2011; İşcan, Steyn 2013; Kondo, Manabe 2016).

Several studies have already applied these linear measures and recorded the existence of sexual dimorphism in teeth. Although some reported better accuracies with one measurement over the other, the best results were achieved when both were considered (Garn *et al*. 1966; Hattab *et al*. 1996; Ateş *et al*. 2006; Acharya, Mainali 2008; Ashwini 2015). In other studies, however, the authors decided to verify the sexual dimorphism in the dentition by testing crown indexes that were derived from the former two linear variables (Scott, Turner 1988), with the most common one being the Mandibular Canine Index.

1.5.1. Mandibular Canine Index

The canine tooth is believed to be one of the most valuable teeth in the dentition due to its ability to survive trauma, being less affected by periodontal diseases and being the less extracted tooth (Anderson, Thompson 1973; Sherfudhin 1996; Kaushal *et al*. 2003), thus the number of studies that focus on this particular tooth is high. Amongst them, most identify the canine to be the most sexually dimorphic tooth of the dentition (Moorrees *et al*. 1957; Garn *et al*. 1967; Lysell, Myrberg 1982; Hattab *et al*. 1996; Yuen *et al*. 1997; İşcan, Kedici 2003; White, Folkens 2005; Ateş *et al*. 2006; Karaman 2006; Acharya, Mainali 2007; Acharya, Mainali 2008; Cardoso 2008; Zorba *et al*. 2011; Angadi *et al*. 2013; Viciano *et al*. 2013; Khamis *et al*. 2014).

In 1989, Rao and colleagues developed the Mandibular Canine Index (MCI) as a simple, quick and reportedly accurate method, reaching an overall 85.9% of accuracy in sexual diagnosis. Because of its simplicity, the MCI is one of the most explored methods and while some agree with its practicability (Sherfudhin 1996; Kaushal *et al*. 2004), others disagree with its reliability, as low accuracies have been achieved, particularly when these studies were conducted in different populations (Sherfudhin *et* al. 1996; Acharya, Mainali 2009; Srivastava 2010; Acharya *et al*. 2011; Vishwakarma, Guha 2011; Silva *et al*. 2016; Azevedo *et al*. 2019). Therefore, in an attempt to raise the accuracy levels on dental sexual dimorphism methods, studies focusing on other teeth were developed (Zorba *et al*. 2012; Narang *et al*. 2015).

1.5.2. Posterior teeth and alternative measures

The permanent dentition is classified into two categories: anterior and posterior teeth. The anterior dentition includes the single-rooted incisors and canines, while the posterior teeth are comprised of the premolars and molars. The latter are characterized by generally having more than one root, with the exception of the second premolars, and sometimes first premolars and third molars, which can be single-rooted (White, Folkens 2005; Scott 2008; İşcan, Steyn 2013).

As a consequence of multiple roots, the posterior teeth are more strongly attached to the dental arch and, therefore, less likely to suffer *postmortem* loss, as opposed to the anterior teeth. Therefore, some researchers consider the molars a better choice over the canine for the study of sexual dimorphism since they are more frequently found in fragmentary remains (Flower 1885; Beyer-Olsen, Alexandersen 1995; İşcan, Steyn 2013; Zorba *et al*. 2013; Narang *et al*. 2015). Additionally, some studies reported the molars as the most dimorphic teeth in the dentition, as opposed to the canine, so the focus on posterior teeth for sexual diagnosis has been rising (Garn *et al*. 1966; Beyer-Olsen, Alexandersen 1995; Prabhu, Acharya 2009).

While evaluating sexual diagnosis using molar teeth, some difficulties might arise during the measurement of the traditional mesiodistal diameter, especially when these are fixed in the jaw. In addition, the suffering of wear and attrition of teeth will compromise the mesiodistal dimension, even at earlier stages, since it decreases the tooth's length. Even though the attrition produces a worse impact on incisors and canines, it cannot be ignored in posterior teeth and it represents an issue, particularly when dealing with severely worn out teeth, so alternative measures were explored (Hillson *et al*. 2005).

Hillson and collaborators (2005) defined six innovative measures that are comprised in diagonal diameters for molars, and cervical dimensions. The two diagonal diameters coincide with the maximum diagonal dimensions of the crown which go from the most mesiolingual to distobuccal (MLDB) and mesiobuccal to distolingual (MBDL) points. These measurements prove advantageous, as the diagonal axes do not include contact points, and, therefore, do not suffer from mild attrition. The remaining four measures consist in cervical diameters of the linear and diagonal dimensions previously mentioned. In this way, the cervical diameters are the maximum mesiodistal, buccolingual and diagonal dimensions measured at the base of the crown, along the cement-enamel junction. The benefits of cervical measurements are great since they are only affected by attrition when most of the crown has been lost, but gingival modifications in life may alter the cement-enamel line and compromise these dimensions. These six alternative measures proved to have a correlation with the traditional ones and to be as good and as reliable, thus enabling various studies to use them (Karaman 2006; Pereira *et al*. 2010;

Hasset 2011; Zorba *et al*. 2012; Viciano *et al*. 2013; Zorba *et al*. 2013; Ashwini 2015; Manchanda *et al*. 2015; Tabasum *el at*. 2017).

In comparison with mesiodistal and buccolingual crown diameters, studies have reported higher levels of sexual dimorphism in diagonal dimensions, with a diagnosis accuracy reaching up to 85.1% (Pereira *et al*. 2010; Zorba *et al*. 2013). When cervical dimensions are adopted, however, accuracy levels of up to 85.3% in linear and up to 93% in diagonal cervical measures are achieved (Hasset 2011; Zorba *et al*. 2011; Zorba *et al*. 2012; Zorba *et al*. 2013; Viciano *et al*. 2013). Consequently, these results suggest that alternative dental measurements are considered promising for sex determination and should, sometimes, be used over the traditional dimensions as they accomplish better results.

Parallel to Hillson's alternative measures, the sexual dimorphism in molar cusps has been explored (Kondo *et al*. 2005; Peiris *et al*. 2006), and relatively good results have been reported, especially in the second molar (Kondo *et al* 2005). However, more research is necessary to validate this method.

1.5.3. Population variation

Sexual differences in the skeleton obey to patterns that are distinct in different populations. As there is variation between individuals, the same happens when it comes to entire populations in which every one of them has a tendency for certain morphological or metrical traits. Therefore, the degree of sexual dimorphism may vary between different populations, as well as the accuracy of the sexual diagnosis within a specific method (Aftandilian 1994; Scheuer 2002; White, Folkens 2005; Pickering, Bachman 2009; İşcan, Steyn 2013).

Regarding the dentition, the same pattern seems to follow, with crown size differences among various populations. These differences are believed to be due to genetic and environmental factors (Zorba *et al*. 2012) and due to secular trends when dealing with archaeological remains (İşcan, Steyn 2013), implying that sexual dimorphism in the dentition is population-specific as well. This fact is shown across numerous studies and explains why some methods for sexual diagnosis are not reliable for every population (Flower 1885; Lavelle 1972; Beyer-Olsen, Alexandersen 1995; Otuyemi, Noar 1996; Yuen *et al*. 1997; İşcan, Kedici 2003; Peiris *et al*. 2006; Acharya, Mainali 2007; Narang *et al*. 2015; Silva *et al*. 2016; Azevedo *et al*. 2019).

With this information taken into consideration and in order to establish accurate sex determination methods, population-specific standards should be developed and not be used interchangeably. It is also of absolute interest that these methods be simple and inexpensive since some investigations are held in countries with few resources and low technology; it is the case when dealing with some mass disaster events (Vodanović *et al*. 2007; Pereira *et al*. 2010; İşcan, Steyn 2013; Azevedo *et al*. 2019).

In the case of Portugal, studies on sexual dimorphism in teeth are scarce (Cardoso 2008; Pereira *et al*. 2010; Gonçalves *et al*. 2014; Silva *et al*. 2016; Gouveia *et al*. 2017; Azevedo *et al*. 2019) and none of them have a special focus on diagonal dimensions of the molars.

2. Objectives

The aims of this study are:

1) To evaluate the existence of teeth sexual dimorphism in alternative diagonal dimensions of both first and second mandibular molars from an early 20^{th} century Portuguese collection of identified skeletons;
2) To determine the mandibular canine's mesiodistal dimension capacity to evaluate sexual dimorphism in comparison to the first and second mandibular molar dimensions;
3) To develop a simple and straightforward method using the dentition for sex estimation in forensic contexts.

3. Materials and methods

3.1 Sample

The study sample comprised 135 human mandibles from individuals belonging to the Coimbra Identified Skeletal Collection (20th Century), housed at the Department of Life Sciences, University of Coimbra.

The Coimbra Identified Skeletal Collection (20th Century) holds 505 human skeletons (266 males and 239 females) belonging to individuals that were born between 1822-1921 and died between 1904-1936. Age at death falls between 7 and 96 years old. This collection was most likely assembled between 1915 and 1942 and is made up of unclaimed human remains from the Coimbra Municipal Cemetery (Cemitério Municipal da Conchada). Detailed biographical information was transcribed from the cemetery records for each individual and it is known that the majority of individuals had a Portuguese nationality, and both lived and died in Coimbra (Marques 2018).

Sample selection consisted of all Portuguese individuals from both sexes, aged between 18 and 59 years old, presenting the first and/or second mandibular molars, and taking into consideration the following exclusion criteria:

- Poorly preserved first and second molars;
- Noticeable signs of teeth attrition and cusp wear.

Of the analysed 135 mandibles, 78 belonged to male and 57 to female individuals. Regarding the age at death range, 128 were between 19-44 years old and 7 were between 45-59 years old.

3.2. Measurements

Diagonal dimensions were measured using a *Mitutoyo Digimatic* caliper (*Figure 1*) with a calibration of 0,01 mm on the left first molar of each mandible, as well as on the left second molar when present. When left molars were absent, teeth from the right side of the mandible were considered. The diagonal dimensions correspond to the mesiolingual-distobuccal and mesiobuccal-distolingual diameters. Additionally, mesiodistal crown diameters of the left molars and right mandibular canine were determined (Hillson *et al*. 2005).

Each dimension was measured by the same observer in triplicate and in a non-consecutive manner, in order to avoid bias. The first measurement was discarded and the latter two were averaged to obtain the final value.

Figure 1 – Mitutoyo Digimatic caliper.

3.2.1. Diagonal crown dimensions

The mesiolingual-distobuccal (MLDB) and mesiobuccal-distolingual (MBDL) dimensions were measured with the caliper beaks perpendicular to the occlusal plane, matching the distance between the most mesiolingual and most distobuccal points, and the distance between the most mesiobuccal and most distolingual points of the molars, respectively (*Figure 2)*.

Regarding the sample, 135 first molars were measured with 78 belonging to male and 57 to female individuals. Due to their absence in some mandibles, only 127 second molars were examined. For the second molar, 77 mandibles belonged to male and 50 to female individuals.

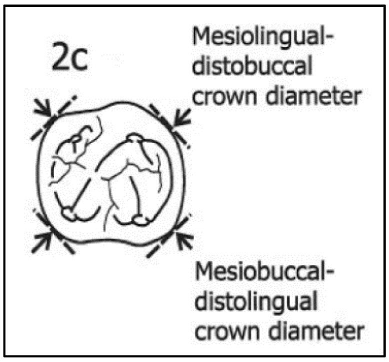

Figure 2 – Diagonal crown dimensions of molar (Hillson *et al*. 2005).

3.2.2. Mesiodistal crown diameters

The mesiodistal (MD) crown diameters were measured with the caliper beaks parallel to the occlusal plane, matching the distance between the most mesial and most distal points of the teeth, as shown in *Figure 3* and *Figure 4*.

Since only 59 out of the 135 studied mandibles presented canines, a subgroup of 59 mandibles – 33 males and 26 females – was formed in which the mesiodistal diameter was taken from the first and second molars and canines.

Figure 3 – Mesiodistal crown dimension of first molar.

Figure 4 – Mesiodistal crown dimension of canine.

3.3. Statistical analysis

The statistical analysis was performed using the 25.0 version of SPSS (Statistical Package for Social Sciences) software.

In order to assess the reliability of the measurements, the intraobserver error was evaluated by Bland-Altman analyses for each dimension. Since the first taken measure was previously eliminated, the difference between the second and third enabled the error estimation.

After validation of the intraobserver error, independent t-tests were applied to all the dimensions to evaluate the correlation between tooth size and sex, followed by ROC analysis to determine a cut-off point for each dimension. Additionally, logistic regression was tested on the mesiodistal dimensions of the molars and canines. Variance between the sexes was calculated by Nagelkerke R Square and the usefulness of the model was certified through the Hosmer-Lemeshow test.

The established level of significance was of 5%.

3.3.1. Reliability analysis

Diagonal dimensions – first molar

For the first molar, mean and standard deviation of the difference were, respectively, 0,0097 mm and 0,13544 mm for MLDB and -0,0119 mm and 0,07602 mm for MBDL (*Table 1*).

Table 1 – Data regarding the difference between the second and third measurements of diagonal dimensions on the first molar.

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
dif_ML_DB (mm)	135	0,0097	0,13544	0,01166
dif_MB_DL (mm)	135	-0,0119	0,07602	0,00654

A one-sample test was performed to evaluate the statistical significance of the intraobserver error, with a significance level of 5%. Since *p*>0,05 for both MLDB and MBDL (*Table 2*), the error was not statistically significant in these dimensions. In a 95% confidence interval, the error fell between -0,25576 and 0,275162 mm for MLDB and between -0,1609 and 0,137099 mm for MBDL, as shown in *Figures 5* and *6*.

Table 2 – Statistical significance of the intraobserver error of diagonal dimensions on the first molar.

One-Sample Test						
	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed) (*p*)	Mean Difference	Lower	Upper
dif_ML_DB (mm)	0,832	134	0,407	0,00970	-0,0134	0,0328
dif_MB_DL (mm)	-1,811	134	0,072	-0,01185	-0,0248	0,0011

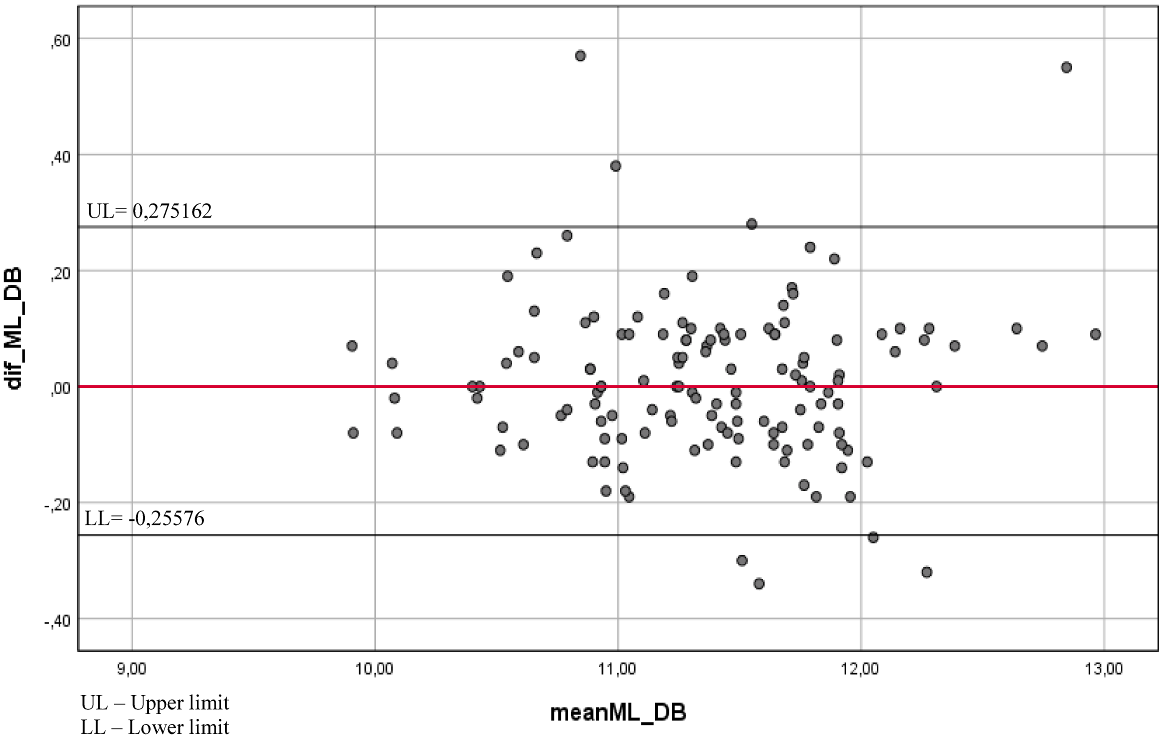

Figure 5 – Graph depicting the error for MLDB dimension on the first molar, within a 95% confidence interval.

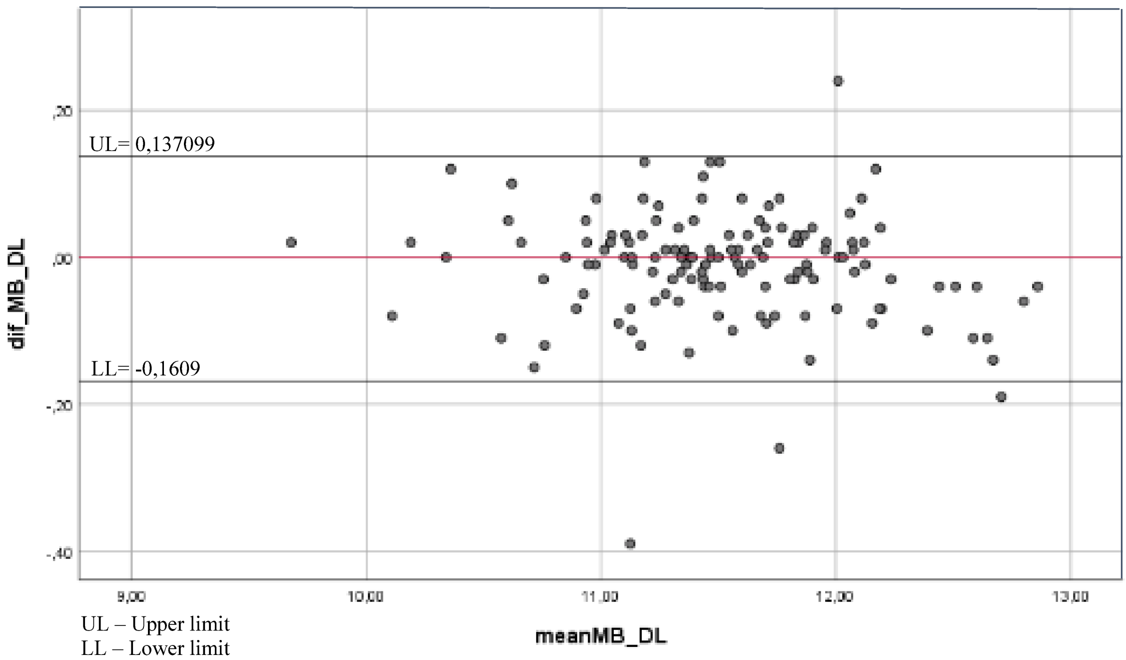

Figure 6 – Graph depicting the error for MBDL dimension on the first molar, within a 95% confidence interval.

Lastly, and according to *Tables 3* and *4*, it was certified through regression that the error was not proportional to the magnitude of the measurement since the independent variable (B) was really close to 0 in both dimensions.

Due to the results achieved by the Bland-Altman analysis, further statistical analysis of these dimensions was allowed.

Table 3 – Regression verifying absence of proportionality between error and magnitude of measurement in MLDB dimension on the first molar.

Coefficients[a]								
		Unstandardized Coefficients					95,0% Confidence Interval for B	
Model		B	Std. Error	Standardized Coefficients Beta	t	Sig. (*p*)	Lower Bound	Upper Bound
1	(Constant)	-0,055	0,231		-0,240	0,811	-0,512	0,401
	meanML_DB	0,006	0,020	0,024	0,282	0,778	-0,034	0,046

a. Dependent Variable: dif_ML_DB

Table 4 – Regression verifying absence of proportionality between error and magnitude of measurement in MBDL dimension on the first molar.

Coefficients[a]								
		Unstandardized Coefficients					95,0% Confidence Interval for B	
Model		B	Std. Error	Standardized Coefficients Beta	t	Sig. (*p*)	Lower Bound	Upper Bound
1	(Constant)	0,174	0,135		1,292	0,198	-0,092	0,441
	meanMB_DL	-0,016	0,012	-0,119	-1,382	0,169	-0,039	0,007

a. Dependent Variable: dif_MB_DL

Diagonal dimensions – second molar

The mean and standard deviation of the difference between the second and third measurements of the diagonal dimensions in the second molar were -0,0068 mm and 0,11583 mm for MLDB and -0,0063 mm and 0,09773 mm for MBDL, respectively.

The intraobserver error was found to be not statistically significant for the diagonal dimensions in this tooth, as $p>0,05$ for both MLDB and MBDL. Error fell, with 95% of confidence, between -0,233827 and 0,220227 mm for MLDB and between -0,197851 and 0,185251 mm for MBDL.

Regression was made to verify that the error was not proportional to the magnitude of the measurement, with an independent variable (B) of 0,005 for MLDB and -0,012 for MBDL.

Figures and tables regarding Bland-Altman analysis of the second molar can be found in Appendix A.

Mesiodistal diameter – molars and canine

The mean and standard deviation of the difference between the second and third measurements of the mesiodistal diameter were, respectively, -0,0125 mm and 0,10469 mm for the first molar, -0,0271 mm and 0,14294 mm for the second molar and 0,0063 mm and 0,05502 mm for the canine.

The intraobserver error was not statistically significant for these dimensions since $p>0,05$ for all teeth. In a 95% confidence interval, error fell between -0,21774 and 0,192656 mm for the first molar, -0,30728 and 0,253039 mm for the second molar and -0,10157 and 0,114111 mm for the canine.

It was equally verified through regression that the error was not proportional to the magnitude of measurement since the independent variable (B) was of 0,005 for all dimensions.

Figures and tables regarding Bland-Altman analysis of the mesiodistal diameters can be found in Appendix B.

4. Results

A summarized table with the mean and standard deviation of the studied measurements is presented below.

Table 5 – Data regarding the number of individuals per sex, mean, standard deviation and standard error mean of the measured dimensions.

			Sex	N	Mean	Std. Deviation	Std. Error Mean
Diagonal dimensions	First molar	ML_DB dimension	Male	78	11,5109	0,56037	0,06345
			Female	57	11,1785	0,55320	0,07327
		MB_DL dimension	Male	78	11,6970	0,52485	0,05943
			Female	57	11,2866	0,52271	0,06923
	Second molar	ML_DB dimension	Male	77	11,3307	0,66864	0,07620
			Female	50	11,0705	0,65658	0,09285
		MB_DL dimension	Male	77	11,2241	0,62303	0,07100
			Female	50	10,8295	0,58611	0,08289
Mesiodistal dimension		1st molar (mm)	Male	33	11,1698	0,56530	0,09841
			Female	26	10,7744	0,57089	0,11196
		2nd molar (mm)	Male	33	10,5374	0,67789	0,11800
			Female	26	10,3217	0,60690	0,11902
		Canine (mm)	Male	33	6,8320	0,47072	0,08194
			Female	26	6,3865	0,35408	0,06944

Overall, it is possible to observe that the mean size of the first and second molars is greater in males than in females.

4.1 Diagonal dimensions

In order to evaluate the statistical differences between male and female molars, independent t-tests were performed.

In *Table 6* is represented all data regarding the independent t-test for the diagonal dimensions measured on the first molar. Statistically significant differences were found between male and female dentition size since $p \leq 0{,}05$ for both MLDB and MBDL (respectively *p*=0,001 and *p*=0,0005). Similar results were obtained for the diagonal dimensions on the second molar, with *p*=0,033 for MLDB and *p*=0,001 for MBDL.

Table 6 – Independent t-test for diagonal dimensions on the first molar.

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig. (*p*)	t	df	Sig. (2-tailed) (*p*)	Mean Difference	Std. Error Difference	Lower	Upper
ML_DB	Equal variances assumed	0,032	0,858	3,422	133	0,001	0,33239	0,09712	0,14029	0,52449
	Equal variances not assumed			3,429	121,702	0,001	0,33239	0,09693	0,14051	0,52427
MB_DL	Equal variances assumed	0,219	0,641	4,495	133	0,000	0,41041	0,09130	0,22982	0,59100
	Equal variances not assumed			4,498	121,106	0,000	0,41041	0,09124	0,22977	0,59104

With a statistically significant difference between the sexes confirmed, a Receiver Operating Characteristics (ROC) analysis followed for all dimensions (*Table 7* and *Figure 7*). With the ROC analysis, it was possible to determine the statistical significance of the area under the curve (AUC) and to achieve the optimal cut-off values for each measurement.

Table 7 – Data regarding ROC analysis for diagonal dimensions on the first molar.

Case Processing Summary	
Sex	Valid N (listwise)
Positive[a]	78
Negative	57
Larger values of the test result variable(s) indicate stronger evidence for a positive actual state.	
a. The positive actual state is Male.	

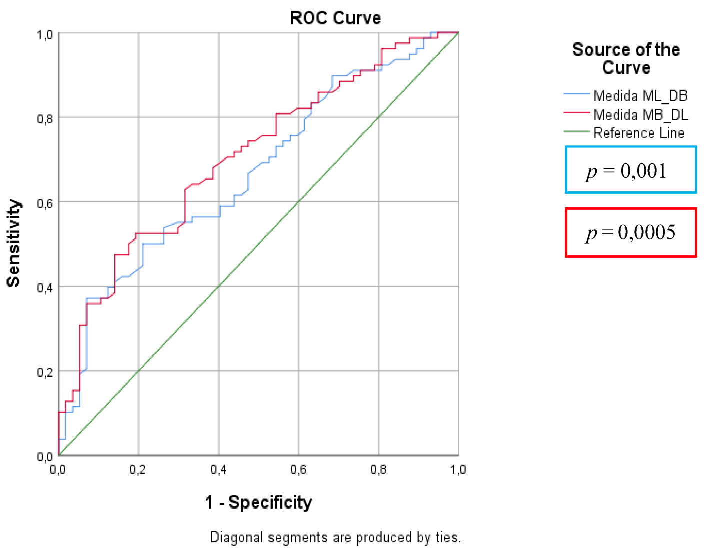

Figure 7 – ROC curve for diagonal dimensions on the first molar.

For the diagonal dimensions on the first molar, it was found that the AUC for both diagonal dimensions was statistically significant, with areas corresponding to 66,8% and 70,5% of the graph for MLDB and MBDL, respectively (*Table 8*). The cut-off point for MLDB was 11,2725 mm, thus individuals with a MLDB dimension up to this value were considered female and individuals measuring equal or above were considered male. This cut-off value is associated with a sensitivity of 66,7% and a specificity of 52,6%. Regarding the MDBL dimension, the cut-off value was found to be at 11,4375 mm with a sensitivity of 67,9% and a specificity of 61,4%.

Table 8 – Statistical significance of the Area Under the Curve for diagonal dimensions on the first molar.

Area Under the Curve					
Test Result Variable(s)	Area (%)	Std. Error[a]	Asymptotic Sig.[b] (*p*)	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
ML_DB dimension	0,668	0,046	0,001	0,577	0,759
MB_DL dimension	0,705	0,044	0,000	0,617	0,792

The test result variable(s): ML_DB dimension, MB_DL dimension has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

The ROC curve analysis done for the diagonal dimensions on the second molar revealed that both MLDB and MBDL were statistically significant in sex prediction since AUC was of 61,2% for MLDB and 69,2% for MBDL. For MLDB, the cut-off point was 11,1900 mm with a sensitivity of 57,1% and specificity of 64%. Concerning the MBDL dimension, the cut-off point was 10,9125 mm, associated with a sensitivity of 70,1% and a specificity of 64%.

Tables and figures related to the independent t-test and ROC analysis of the diagonal dimensions on the second molar are found in Appendix C.

4.2 Mesiodistal dimensions

The independent t-test for the mesiodistal dimension on the molars and canine revealed that the differences observed between sexes were statistically significant, with the exception of the second molar MD (p=0,01 for first molar and p=0,0005 for canine). Since p=0,209 for the second molar, the mesiodistal size differences between males and females in this tooth proved to be not statistically significant, and this dimension was, consequently, not considered for the ROC analysis and logistic regression.

The AUC was statistically significant and was registered at 73,5% for the first molar and 80,1% for the canine, with cut-off points of 10,8875 mm and 6,5400 mm, respectively. According to these cut-off points, the first molar revealed to be able to correctly diagnose males in 72,7% of cases and 65,4% in females, while the canine had a sensitivity of 72,7% and a specificity of 76,9%.

Tables and figures related to the independent t-tests and ROC analysis of the mesiodistal dimension on the first molar and canine are found in Appendix D.

4.3 Logistic regression

A multivariate analysis of the mesiodistal dimensions of the first molar and canine was performed using logistic regression, in order to evaluate the method's predictability.

A Pearson correlation between the two dimensions was first determined, and a value of 0,656 was achieved (*Table 9*).

Table 9 – Correlation between the mesiodistal dimension of the first molar and the canine.

Correlations			
		MD dimension of 1st molar (mm)	MD dimension of canine (mm)
MD dimension of 1st molar (mm)	Pearson Correlation	1	0,656**
	Sig. (2-tailed) (p)		0,000
	N	59	59
MD dimension of canine (mm)	Pearson Correlation	0,656**	1
	Sig. (2-tailed) (p)	0,000	
	N	59	59
**. Correlation is significant at the 0.01 level (2-tailed).			

The first step in the logistic regression analysis was to idealise a model in which the mesiodistal dimensions were not considered as variables, thus identifying the correct percentage of classification when these are not present, as well as their statistical significance. A correct percentage of 55,9% was achieved when considering all male individuals, since this is the sex of the majority of the individuals (*Table 10*).

On the other hand, it was found that the variables included in this idealised model were not statistically significant (p=0,363) (*Table 11*), while the mesiodistal dimensions were (p=0,011 for first molar and p=0,0005 for canine) (*Table 12*).

Table 10 – Correct percentage of sexual diagnosis when teeth dimensions are not considered.

Block 0: Beginning Block

Classification Table[a,b]					
			Predicted		
			Sex		
Observed			Male	Female	Percentage Correct
Step 0	Sex	Male	33	0	100,0
		Female	26	0	0,0
	Overall Percentage				55,9

a. Constant is included in the model.
b. The cut value is 0,500.

Table 11 – Statistical significance of included variables in the model (constant B).

Variables in the Equation							
		B	S.E.	Wald	df	Sig. (p)	Exp(B)
Step 0	Constant	-0,238	0,262	0,827	1	0,363	0,788

Table 12 – Statistical significance of excluded variables in the model (mesiodistal dimension of first molar and canine).

Variables not in the Equation					
			Score	df	Sig. (p)
Step 0	Variables	MD dimension of 1st molar (mm)	6,498	1	0,011
		MD dimension of canine (mm)	12,985	1	0,000
	Overall Statistics		13,044	2	0,001

After assessing the variable's significance, it was determined through Nagelkerke R Square that the variance between the sexes is explained by these mesiodistal dimensions in 31% (*Table 13*) and certified the usefulness of the model by goodness of fit

Hosmer-Lemeshow test. Since $p>0,05$, it was proved that the model is not useless and that it presents a good method for diagnosing the sex (*Table 14*).

Table 13 – Percentage of differences between males and females explained by the mesiodistal dimension of first molar and canine.

Block 1: Method = Enter			
Model Summary			
Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	65,457[a]	0,231	0,310
a. Estimation terminated at iteration number 5 because parameter estimates changed by less than 0,001.			

Table 14 – Goodness of fit of the logistic regression model.

Hosmer and Lemeshow Test			
Step	Chi-square	df	Sig. (*p*)
1	11,812	8	0,160

Afterwards, and since MD of first molar and canine were statistically significant in the model, it was evaluated the percentage of correctly classified individuals when both variables were considered (*Table 15*). Values of 69,7% for male and 69,2% for female were obtained, with an overall 69,5%.

Table 15 - Correct percentage of sexual diagnosis when teeth dimensions are considered.

Classification Table[a]					
			Predicted		
			Sex		
Observed			Male	Female	Percentage Correct
Step 1	Sex	Male	23	10	69,7
		Female	8	18	69,2
	Overall Percentage				69,5
a. The cut value is 0,500.					

Lastly, it was verified the statistical significance of the individual dimensions when these are subject to a multivariate analysis. It was found that when both first molar and canine MDs are considered together, the first molar is no longer statistically significant (*p*=0,644), and the canine is much more important for the diagnosis of sex (*p*=0,008) (*Table 16*).

Table 16 – Statistical significance of individual dimensions in a multivariate analysis.

Variables in the Equation							
		B	S.E.	Wald	df	Sig. (*p*)	Exp(B)
Step 1[a]	MD dimension of 1st molar (mm)	-0,303	0,654	0,214	1	0,644	0,739
	MD of canine (mm)	-2,606	0,988	6,957	1	0,008	0,074
	Constant	20,198	7,463	7,325	1	0,007	591566361,210

a. Variable(s) entered on step 1: Comprimento MD do 1º molar (mm), Comprimento MD do canino (mm).

5. Discussion

The first established goal when dealing with unknown skeletal human remains is the achievement of the identity; without it, it is impossible to make progress in a forensic investigation. It is of good practice to start with the determination of the biological profile – particularly the sex of the individual – since it immediately lowers the number of possible candidates to whom the remains belong to (Keiser-Nielsen 1963; Scheuer 2002; Scheuer, Black 2007; Molina 2010).

The most reliable element to diagnose the sex of a skeleton is the pelvis, followed by the skull (Scheuer 2002; Bruzek, Murail 2006; Scheuer, Black 2007; Ubelaker 2008; Pickering, Bachman 2009; Cardoso 2013; İşcan, Steyn 2013). While the combination of these elements can reach very high percentages of correct sex classification, in forensic contexts they are often absent or damaged and unable to be used. In this sense, stronger alternative body elements should be examined (Roksandic 2002; Cardoza 2011; Cunha 2014).

The dentition presents itself as the most durable element in the human body due to its composition. The enamel is the hardest tissue found in the body, providing teeth the necessary resistance to endure fire, trauma, and decomposition, and these often remain preserved after death. On the other hand, it has been proved that tooth size differs between male and female individuals, which in turn allows the dentition to be used as secondary elements in the sex determination process (Avon 2004; White, Folkens 2005; Pereira *et al*. 2010; Acharya *et al*. 2011; Cardoza 2011; Vishwakarma, Guha 2011; Tabor, Schrader 2012; Zorba *et al*. 2012; İşcan, Steyn 2013).

The first studies considering the presence of sexual dimorphism in tooth size focused on the canine since this is reported to be the sturdiest tooth of the dentition, maintaining its integrity when subject to diseases and trauma (Anderson, Thompson 1973; Sherfudhin 1996; Kaushal *et al*. 2003; White, Folkens 2005). It is also considered to be the most dimorphic tooth when the dentition is evaluated in its entirety, hence a great number of studies focusing on the canine (Moorrees *et al*. 1957; Garn *et al*. 1967; Lysell, Myrberg 1982; Hattab *et al*. 1996; Yuen *et al*. 1997; İşcan, Kedici 2003; Ateş *et al*. 2006; Acharya, Mainali 2007; Cardoso 2008; Zorba *et al*. 2011; Angandi *et al*. 2013; Viciano *et al*. 2013; Khamis *et al*. 2014). However, the canine, like all anterior teeth, is single-rooted. This means that, due to *postmortem* conditions, anterior teeth are more likely to be lost compared to the posterior dentition, which normally presents multiple roots. As posterior teeth are more commonly found in fragmentary remains, some researchers consider the study of this type of dentition more promising, in order to obtain more information about the remains' identity (Flower 1885; White, Folkens 2005; Scott 2008; İşcan, Steyn 2013; Narang *et al*. 2015).

The majority of research explores the dentition's sexual dimorphism by using the tooth's width and breadth. These are known as the mesiodistal and the buccolingual crown diameters (Scott, Turner 1988; Ramakrishnan *et al*. 2015; Kondo, Manabe 2016). However, as these might be difficult to measure when it comes to posterior teeth, Hillson and colleagues (2005) defined alternative dimensions, in which the diagonal crown diameters for molars are included. These dimensions started, then, to be included in various studies (Karaman 2006; Pereira *et al*. 2010; Hasset 2011; Zorba *et al*. 2012; Viciano *et al*. 2013; Ashwini 2015; Manchanda *et al*. 2015) with some of them achieving better results than the linear crown dimensions (Zorba *et al*. 2013; Tabasum *el at*. 2017).

When analysing the different studies that focus on the sex determination of individuals by using odontometric methods, divergent results are found, which leads to one conclusion: sexual dimorphism varies according to the population. An ideal method of sexual diagnosis for one population might not deliver the same results in another, thus the lack of consensus regarding the most dimorphic dimension (Flower 1885; Lavelle 1972; Beyer-Olsen, Alexandersen 1995; Otuyemi, Noar 1996; Yuen *et al*. 1997; İşcan, Kedici 2003; Peiris *et al*. 2006; Acharya, Mainali 2007; Narang *et al*. 2015; Silva *et al*. 2016; Azevedo *et al*. 2019).

The Portuguese population has received little attention when it comes to the dentition's sexual dimorphism (Cardoso 2008; Pereira *et al*. 2010; Gonçalves *et al*. 2014; Silva *et al*. 2016; Gouveia *et al*. 2017; Azevedo *et al*. 2019), with none of the studies evaluating the dimorphism of diagonal dimensions of the molars. Therefore, the present study pursued to determine the difference of diagonal molar dimensions between male and female individuals of an early 20th Century Portuguese skeletal collection. Most studies focus only on the first molar but since this tooth is subject to more attrition and more likely to suffer *antemortem* loss, the second molar was also considered in this study.

In order to validate the diagonal dimensions of the first and second left molars, these were subjected to a reliability analysis. This first step consisted of a Bland-Altman analysis, characterized by a one sample t-test in which the null hypothesis was that the mean of the difference between the second and third measures of the dimension was equal to zero. Since the null hypothesis was not rejected (p>0,05) for both diagonal dimensions on both first and second molars, the intraobserver error was considered insignificant, thus allowing the statistical analysis of these dimensions (Whitley, Ball 2002; Myles, Cui 2007).

An independent t-test followed for each dimension, evaluating the diagonal size differences between male and female molars. In this case, the null hypothesis was that the difference of the means of each population (male and female) was equal to zero or, in other words, that the mean of the measures in males was the same as the mean of females. The null hypothesis was rejected for all four dimensions since a *p* value under 0,05 was obtained, indicating that statistically significant differences exist between the sexes, with male individuals presenting larger teeth. This is especially true for the MBDL dimension on the first molar; a *p* value of 0,0005 emphasizes this sex difference and it suggests, with certainty, that the rest of the population behaves in the same pattern regarding this dimension (Whitley, Ball 2002).

One of the aims of the present study was to elaborate a simple method for sex determination; after confirmation of differences between the sexes, it was necessary to understand which values indicated that an individual belonged to the male or female sex. Since size classifies as a continuous rather than a discrete variable, it was ascertained a value in which measures that go up to that point were considered to belong to females, and equal or higher values belonging to males. In order to determine this key size value, ROC analysis took place.

The ROC analysis is a method used in situations when two outcomes are achieved, which are characterised as positive and negative state (Bewick *et al*. 2004). For the present study, each diagonal dimension had an associated ROC curve that fell on the upper left triangle of the graph, implying their prediction capacity. It was also confirmed that there were statistically significant differences between the AUCs and the graph's diagonal for all dimensions. Out of the four, MBDL of the first molar was the better classifier, with an AUC of 70,5%.

Associated with the ROC curve are the concepts of sensitivity and specificity; sensitivity translates in the method's ability to detect the positive outcome in the sample population, that is, the proportion of positives that is able to correctly classify, while specificity is the ability to detect the negative outcome. Applied to this study, and since the positive state was considered as male, the sensitivity of a variable is its capacity to detect male individuals, while specificity corresponds to the capacity to detect female individuals. The ideal diagnosing method would have a sensitivity and specificity of 1; however, as these concepts are interrelated, a very high sensitivity would entail a low specificity and vice versa. To achieve the previously mentioned size value that discriminates male individuals from females, also known as a cut-off point, a compromise between sensitivity and specificity should be made. The best cut-off value is the one that correctly diagnoses the maximum number of both males and females, and it corresponds to the point in the ROC curve closest to the upper left corner of the graph – where sensitivity and specificity equal 1 (Metz 1978; Bewick *et al*. 2004). Regarding the four diagonal dimensions, the one presenting a higher sensitivity associated with its cut-off point was MBDL of the second molar – 70,1% – while the highest specificity was obtained by both MLDB and MBDL of the second molar – 64%.

The correct sex classifications for the diagonal dimensions on the first and second left molars are described in *Table 17*. Overall accuracies ranged between 59,8% and 67,7%, with MBDL of the second molar being the most dimorphic dimension. Studies on other populations using the same dimensions are summarized in *Table 18* and overall accuracies ranged between 58,3% and 76,6%.

Table 17 – Correct classification of sexual diagnosis for each diagonal dimension on the first and second left molars.

				Correct classification (%)		
Dimension			Cut-off point (mm)	Male	Female	Overall
Diagonal	First molar	MLDB	11,2725	66,7	52,6	60,7
		MBDL	11,4375	67,9	61,4	65,2
	Second molar	MLDB	11,1900	57,1	64	59,8
		MBDL	10,9125	70,1	64	67,7

Across the various studies focusing on sexual dimorphism on the teeth, two recurring ways to introduce results are found: present a percentage of correctly identified individuals, which is the case in this study, and calculate the percentage of sexual dimorphism that a tooth conveys. This last method was adopted by Zorba and colleagues (2012) and is based on the formula given by Garn and collaborators (1967).

Table 18 – Sexual dimorphism results obtained for diagonal dimensions on the molars across populations.

Study	Population	Tooth	Dimension	Accuracy (%)
Karaman 2006	Turkish	Mandibular and maxillary molars	MLDB and MBDL	73,3
Manchanda *et al.* 2015	Indian	First molar	MLDB	72,5
			MBDL	67,5
		Second molar	MLDB	62,3
			MBDL	70
Tabasum *et al.* 2017	Indian	First and second molars	MLDB	68,1-69,4
			MBDL	58,3-62,5
Zorba *et al.* 2013	Greek	First and second molars	MLDB and MBDL	76,6
Study	**Population**	**Tooth**	**Dimension**	**Sexual dimorphism (%)**
Zorba *et al.* 2012	Greek	First molar	MLDB	3,36
			MBDL	3,04
		Second molar	MLDB	4,19
			MBDL	4,43

The results of the present study are in accordance with those obtained in studies from other populations. However, 67,7% of correct sex classification does not yield a high accuracy: this proves a lack of very strong sexual dimorphism in diagonal dimensions of molars. On the other hand, difficulties were found while measuring these dimensions in very crooked teeth placed in the mandible, which might also influence the moderate accuracy achieved. In this sense, in a smaller sample, we ventured to evaluate the sexual dimorphism of the mesiodistal dimension of molars and of the widely studied canine.

The mesiodistal dimensions of the left first and second molars and right canine were subjected to the same reliability and statistical analysis previously described for the diagonal dimensions. The intraobserver error was not significant for all dimensions, but the results of the independent t-tests revealed that there were statistically significant differences between the sexes only on the MD of first molar and canine. Since the value of p did not reject the null hypothesis of the difference of means equalling zero for the second molar MD, this variable was discarded from the rest of the study. Regarding the ROC analysis, the canine declared the best performance out of the two dimensions, with an AUC of 80,1%, sensitivity of 72,7% and specificity of 76,9%.

The correct male, female and overall sex classifications for the mesiodistal dimensions on the first molar and canine are described in *Table 19*. The variable that presented the best sexual diagnosis was the mesiodistal dimension of the canine, with an overall accuracy of 74,6%. Studies on other populations using the same dimensions are summarized in *Table 20* and overall accuracies ranged between 63,9% and 75% for the first molar, and between 65,7% and 85,8% for the canine.

Table 19 – Correct classification of sexual diagnosis for each mesiodistal dimension on the first molar and canine.

Dimension		Cut-off point (mm)	Correct classification (%) Male	Female	Overall
Mesiodistal	First molar	10,8875	72,7	65,4	69,5
	Canine	6,5400	72,7	76,9	74,6

Table 20 – Sexual dimorphism results obtained for mesiodistal dimensions on the canine and first molar across populations.

Study	**Population**	**Tooth**	**Dimension**	**Accuracy (%)**
Anderson, Thompson 1973	Canadian	Canine	MD	74,3
Beyer-Olsen, Alezandersen 1995	Medieval Norwegian	Canine	MD	70,7
Acharya, Mainali 2009	Nepalese	Canine	MD	69,1
Acharya *et al*. 2011	Indian	Canine	MD	65,7
Azevedo *et al*. 2019	Portuguese	Canine	MD	85,8
Beyer-Olsen, Alezandersen 1995	Medieval Norwegian	First molar	MD	75
Narang *et al*. 2015	Indian	First molar	MD	70,5
Tabasum *et al*. 2017	Indian	First and second molars	MD	72,2-63,9

Study	Population	Tooth	Dimension	Sexual dimorphism (%)
Garn *et al*. 1966	American	Canine	MD	6,2
Garn *et al*. 1967	North-western Europe	Canine	MD	6,4
Lysell, Myrberg 1982	Swedish	Canine	MD	5,7
Hattab *et al*. 1996	Jordanian	Canine	MD	5,2
Yuen *et al*. 1997	Chinese	Canine	MD	5,27
Kaushal *et al*. 2003	Indian	Canine	MD	7,96
Kaushal *et al*. 2004	Indian	Canine	MD	7,954
Vishwakarma, Guha 2011	Indian	Canine	MD	12,51
Zorba *et al*. 2011	Greek	Canine	MD	5,81
Khamis *et al*. 2014	Malays	Canine	MD	6,3
	Chinese	Canine	MD	5,4
	Tamils	Canine	MD	5,5
Garn *et al*. 1966	American	First molar	MD	7
Garn *et al*. 1967	North-western Europe	First molar	MD	4,8
Lysell, Myrberg 1982	Swedish	First molar	MD	3,1
Strond *et al*. 1994	Caucasian	First molar	MD	5
Hattab *et al*. 1996	Jordanian	First molar	MD	4,7
Yuen *et al*. 1997	Chinese	First molar	MD	1,4
Zorba *et al*. 2011	Greek	First molar	MD	2,51
Khamis *et al*. 2014	Malays	First molar	MD	2,5
	Chinese	First molar	MD	4,2
	Tamils	First molar	MD	3,1

The mesiodistal results of the first molar and canine achieved in the present study are in conformity with those obtained in studies from other populations. Additionally, and although in a smaller sample, the mesiodistal dimensions presented higher sexual diagnosis results when compared with the diagonals, so further statistical analysis of MD dimensions was pursued in order to evaluate the method's ability to assess the sex when both variables are considered together.

Logistic regression is a useful statistical method for the analysis of cases that respond with two outcomes, assessing the probability of prediction (Bewick *et al*. 2005). In the present study, logistic regression was used for a multivariate analysis with two variables, so the correlation between these had to be primarily acknowledged. For the mesiodistal dimensions, a Pearson correlation was determined, and a 0,656 value was attained, which corresponds to a high moderate positive correlation (Bewick *et al*. 2003; Mukaka 2012).

Following the correlation assessment, it was determined the statistical significance of each dimension. If these were not considered in the model, a low accuracy level of 55,9% would be achieved because the model would predict every individual as male, since no other discriminatory factor would exist. This is corroborated by the statistically insignificant constant included in a no variable model. On the other hand, both first molar and canine MDs were found to have a statistically significant influence on the model, which could largely benefit from the inclusion of the variables, particularly MD of canine since p=0,0005. With this in mind, a better performing model was sought.

The model's goodness of fit was evaluated with a Hosmer-Lemeshow test. A Hosmer-Lemeshow test detects the uselessness of a model by comparing the predicted outcomes with the real ones. When the p value rejects the null hypothesis, it means that the particular model has a good prediction (Bewick *et al*. 2005). In the present study, the null hypothesis for the Hosmer-Lemeshow test was that the predicted number of individuals of one sex was different from the actual number of individuals from that same sex. This hypothesis was rejected since p=0,16, implying that the model was a good fit for sex determination.

Additionally, a number given by Nagelkerke R Square meant that the mesiodistal dimensions of the first molar and canine, when considered together, explained the sexual differences between human individuals in 31%. This is a very interesting result and further indicates that sexual dimorphism is present in the human dentition, since only two small size dimensions are responsible for such variance between individuals.

When both first molar and canine MDs were included in the model, the percentage of correctly classified individuals rose to 69,5%. Verifying the statistical significance of the individual dimensions when combined for a multivariate analysis, only the mesiodistal dimension of the canine played a significant role, while the first molar is no longer statistically significant (p=0,644). This can be explained by the fact that the two variables are correlated to some extent, as was previously observed by the Pearson correlation value. It is also interesting to observe that, when comparing the multivariate result with the individual mesiodistal dimension accuracy, MD of canine revealed a higher percentage of correct sex classification of 74,6%.

The present study confirms the existence of sexual dimorphism in teeth; the results achieved corroborate the numerous studies that claim the canine as the most dimorphic tooth since its mesiodistal dimension reached the highest accuracy of all the addressed dimensions. Nonetheless, it should be taken into consideration that the small canine sample size of 59 individuals is less than half of the molars' sample used for the diagonal dimensions study. A larger canine sample ought to be studied, in order to perform a more reliable direct accuracy comparison between molars and canines, when taking into consideration all measurements evaluated.

In forensic contexts, there are many circumstances which prevent genetic analyses so a simple, quick and inexpensive method such as the metric assessment of teeth might be the only option to diagnose the sex (Vodanović *et al*. 2007; Pereira *et al*. 2010; İşcan, Steyn 2013). Reviewing the results of the present study, the canine was the most dimorphic tooth, indicating that it should be evaluated whenever present. A high accuracy of 85,8% was achieved in a contemporary Portuguese population study (Azevedo *et al*. 2019), further encouraging that this tooth should always be considered and that molar diagonals should not be used instead of the canine. However, when the canine is absent, the diagonal dimensions of the molars are considered an alternative since they have moderate sexual dimorphism, are not altered by light attrition and can be easier to measure (Hillson *et al*. 2005). Nevertheless, they should always be used with caution. An accuracy of up to 67,7% in the present sample and of up to 76,6% in Indians, Turks and Greeks provide a solid foundation for the study of sexual dimorphism in diagonal teeth dimensions. Yet, it should always be kept in mind that teeth alone are not enough to establish, with certainty, the sex of an individual and these should only be used as a way to achieve a presumptive identity when there is no suspicion, or as a confirmatory method (İşcan, Steyn 2013).

Sexual dimorphism is conditioned not only by the population's origin, but also due to the passage of time. This means that samples consisting of individuals from medieval and past centuries will have different values of sexual dimorphism when compared with modern populations. Therefore, it would also be important to compare the results from the present study with those from a recent Portuguese sample, as it would provide a great contribution to the research of the dentition's sexual dimorphism in the Portuguese population and enable future studies to investigate secular trends in this specific nationality.

One of the most researched methods is Rao's Mandibular Canine Index (1989) but few studies have obtained good results, so the mesiodistal dimension of the canines is preferred. It should also be noted that for the MCI, both canines and all incisors need to be present, which might not be frequently found in forensic contexts (Azevedo *et al*. 2019). In this sense, it is wise to invest future research into the sexual dimorphism of the less *postmortem* lost posterior dentition. However, even if the current observed differences in molar teeth were statistically significant, accuracy for correct sex classification was not very high, implying that to further validate the usefulness of posterior teeth in forensic scenarios, more research concerning additional novel alternative methods should be explored. It is the case of various molar cusp dimensions and ratios (Kondo *et al*. 2005; Kondo, Manabe 2016), which are also suspected of bearing significant sexual dimorphism and could, possibly, deliver more satisfactory results when compared to linear and diagonal crown dimensions.

6. Conclusion

In the present study, statistically significant differences were found between sexes regarding teeth size in an early 20th century Portuguese sample, confirming higher values for male individuals when compared with females.

The best diagonal dimension evaluated was the second molar MBDL, which presented a correct sex classification of 67,7%. Nonetheless, linear dimensions, more specifically the mesiodistal dimension, provided better results when applied to the first molar and the canine. The latter was evaluated as the most dimorphic dimension, with a correct sex classification of 74,6%. Therefore, whenever present, the focus should fall on this tooth.

Since the present sample was formed by individuals belonging to the early 20th century, it would be very enriching to compare the results achieved with those from a modern Portuguese sample to further explore if secular trends on sexual dimorphism are present on the diagonal molar dimensions.

Additional research regarding diagonal dimensions in molar teeth and concerning additional novel alternative methods is needed to further validate the usefulness of posterior teeth in forensic scenarios.

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Appendices

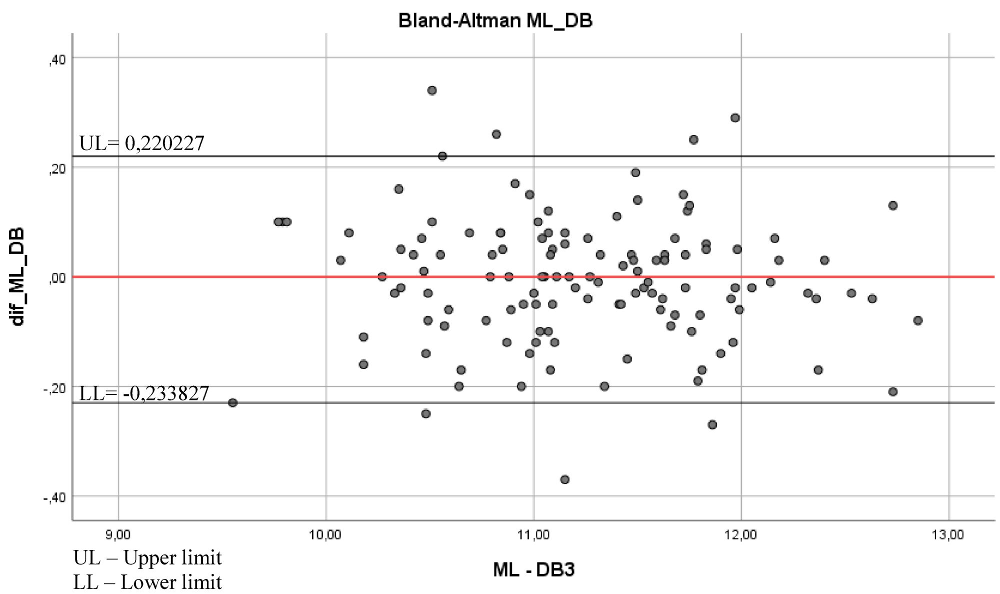
Appendix A

Table A1 – Data regarding the difference between the second and third measurements of diagonal dimensions on the second molar.

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
dif_ML_DB (mm)	127	-0,0068	0,11583	0,01028
dif_MB_DL (mm)	127	-0,0063	0,09773	0,00867

Table A2 – Statistical significance of the intraobserver error of diagonal dimensions on the second molar.

One-Sample Test						
	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed) (*p*)	Mean Difference	Lower	Upper
dif_ML_DB (mm)	-0,659	126	0,511	-0,00677	-0,0271	0,0136
dif_MB_DL (mm)	-0,726	126	0,469	-0,00630	-0,0235	0,0109



UL – Upper limit
LL – Lower limit

Figure A1 – Graph depicting the error for MLDB dimension on the second molar, within a 95% confidence interval.

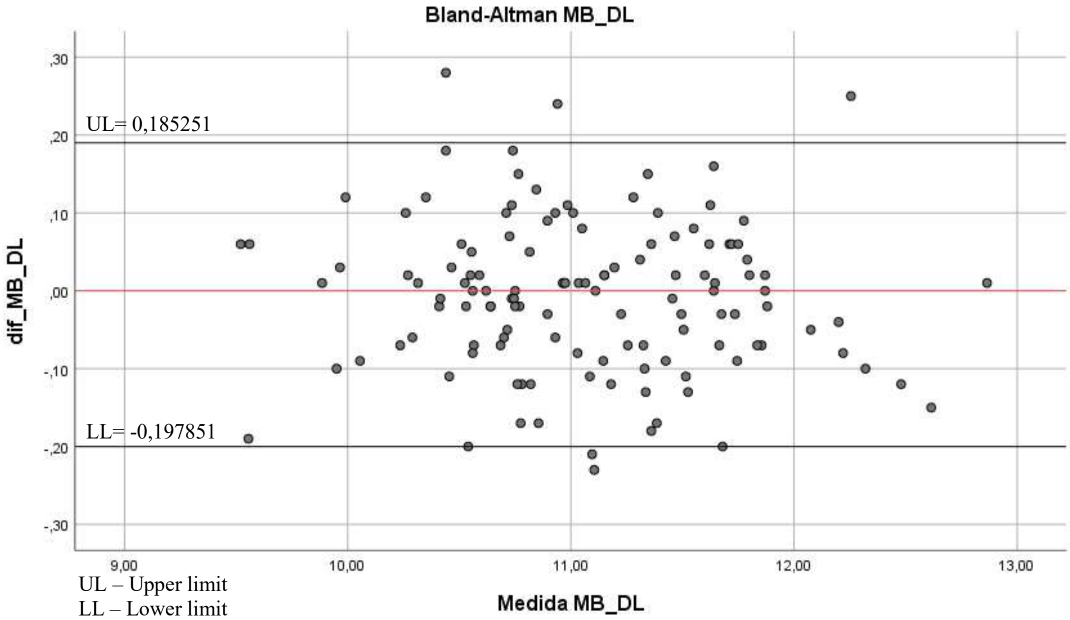


Figure A2 – Graph depicting the error for MBDL dimension on the second molar, within a 95% confidence interval.

Table A3 – Regression verifying absence of proportionality between error and magnitude of measurement in MLDB dimension on the second molar.

Coefficients[a]								
		Unstandardized Coefficients					95,0% Confidence Interval for B	
Model		B	Std. Error	Standardized Coefficients Beta	t	Sig. (*p*)	Lower Bound	Upper Bound
1	(Constant)	-0,059	0,173		-0,342	0,733	-0,401	0,283
	ML_DB dimension	0,005	0,015	0,027	0,303	0,762	-0,026	0,035

a. Dependent Variable: dif_ML_DB

Table A4 – Regression verifying absence of proportionality between error and magnitude of measurement in MBDL dimension on the second molar.

Coefficients[a]								
		Unstandardized Coefficients					95,0% Confidence Interval for B	
Model		B	Std. Error	Standardized Coefficients Beta	t	Sig. (*p*)	Lower Bound	Upper Bound
1	(Constant)	0,131	0,152		0,866	0,388	-0,169	0,432
	MB_DL dimension	-0,012	0,014	-0,081	-0,909	0,365	-0,040	0,015

a. Dependent Variable: dif_MB_DL

Appendix B

Table B1 - Data regarding the difference between the second and third measurements of mesiodistal diameter on molars and canine.

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
MD difference of 1st molar (mm)	59	-0,0125	0,10469	0,01363
MD difference of 2nd molar (mm)	59	-0,0271	0,14294	0,01861
MD difference of canine (mm)	59	0,0063	0,05502	0,00716

Table B2 – Statistical significance of the intraobserver error of mesiodistal diameter on molars and canine.

One-Sample Test						
	Test Value = 0					
	t	df	Sig. (2-tailed) (*p*)	Mean Difference	95% Confidence Interval of the Difference Lower	Upper
MD difference of 1st molar (mm)	-0,920	58	0,361	-0,01254	-0,0398	0,0147
MD difference of 2nd molar (mm)	-1,457	58	0,150	-0,02712	-0,0644	0,0101
MD difference of canine (mm)	0,875	58	0,385	0,00627	-0,0081	0,0206

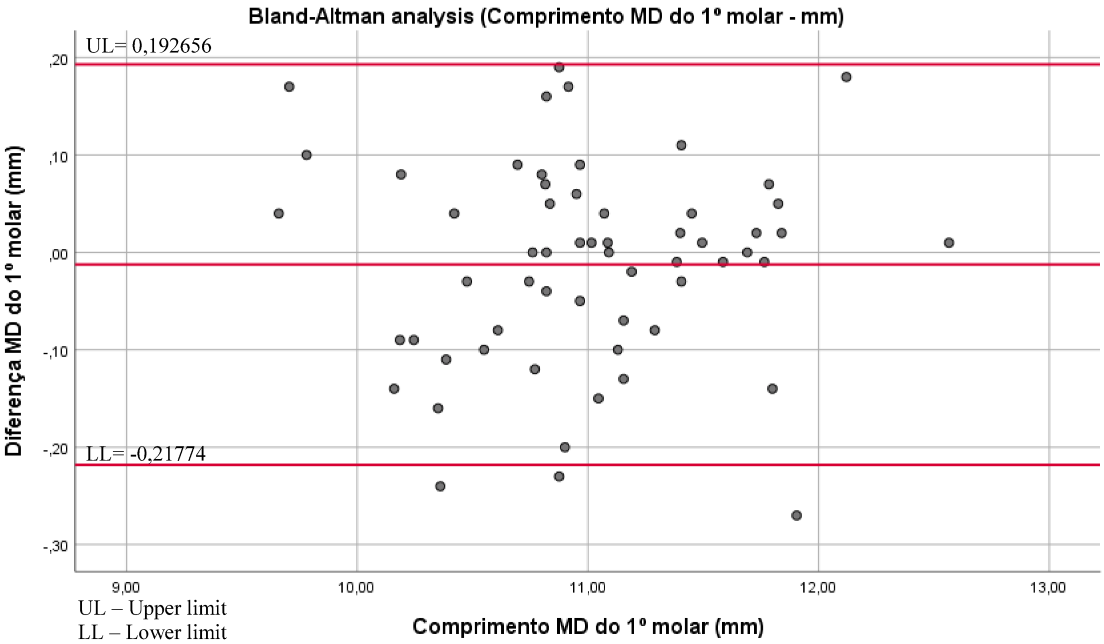


Figure B1 – Graph depicting the error for mesiodistal diameter on the first molar, within a 95% confidence interval.

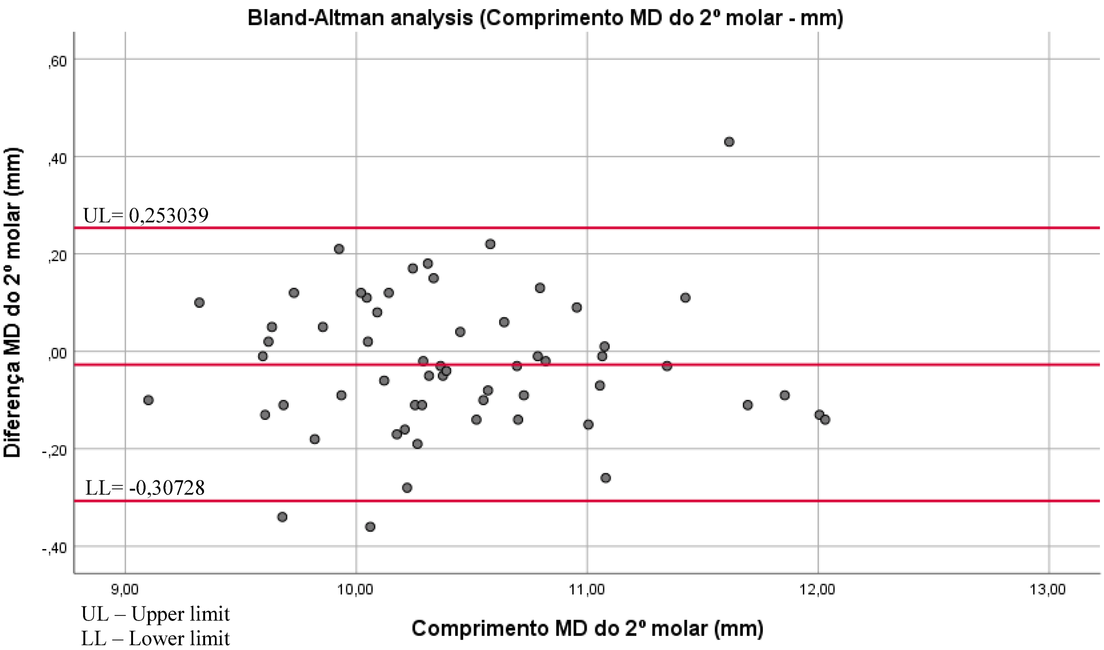


Figure B2 – Graph depicting the error for mesiodistal diameter on the second molar, within a 95% confidence interval.

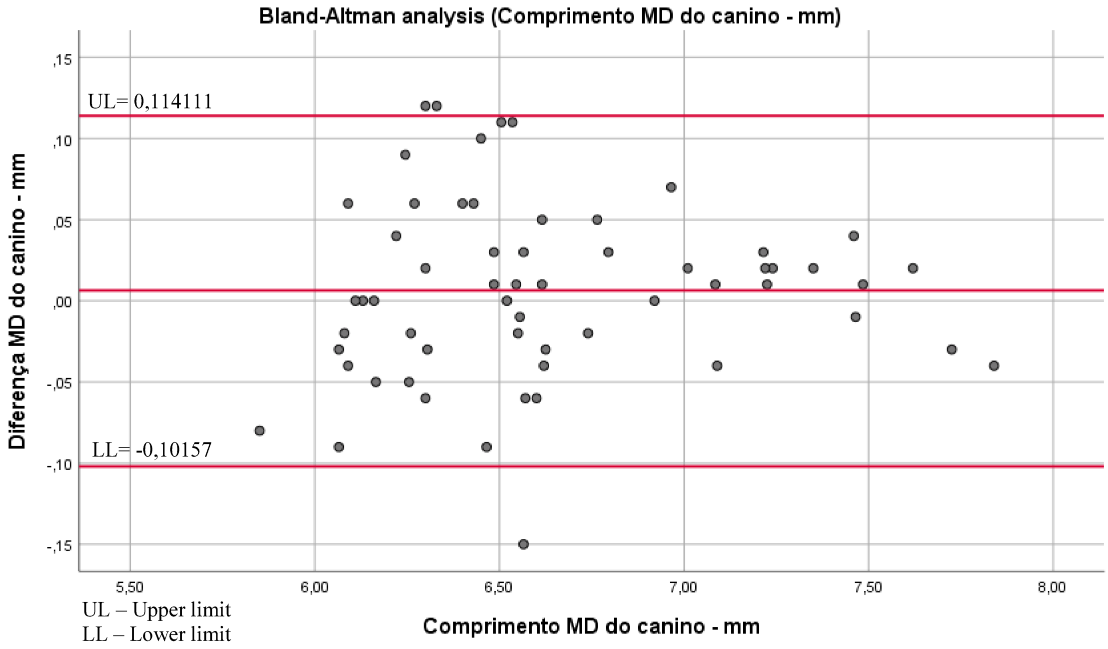


Figure B3 – Graph depicting the error for mesiodistal diameter on the canine, within a 95% confidence interval.

Table B3 – Regression verifying absence of proportionality between error and magnitude of measurement in mesiodistal dimension on the first molar.

Coefficients[a]								
Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig. (*p*)	95,0% Confidence Interval for B Lower Bound	95,0% Confidence Interval for B Upper Bound
1	(Constant)	-0,068	0,256		-0,266	0,791	-0,580	0,444
	Mean_MD of 1st molar (mm)	0,005	0,023	0,029	0,218	0,828	-0,041	0,052

a. Dependent Variable: Diferença MD do 1º molar (mm)

Table B4 – Regression verifying absence of proportionality between error and magnitude of measurement in mesiodistal dimension on the second molar.

Coefficients[a]							
Model	Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig. (*p*)	95,0% Confidence Interval for B Lower Bound	95,0% Confidence Interval for B Upper Bound
1 (Constant)	-0,084	0,304		-0,277	0,783	-0,693	0,525
Mean_MD of 2nd molar (mm)	0,005	0,029	0,025	0,188	0,852	-0,053	0,064

a. Dependent Variable: Diferença MD do 2º molar (mm)

Table B5 – Regression verifying absence of proportionality between error and magnitude of measurement in mesiodistal dimension on the canine.

Coefficients[a]							
Model	Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig. (*p*)	95,0% Confidence Interval for B Lower Bound	95,0% Confidence Interval for B Upper Bound
1 (Constant)	-0,028	0,102		-0,278	0,782	-0,232	0,176
Mean_MD of canine (mm)	0,005	0,015	0,045	0,340	0,735	-0,025	0,036

a. Dependent Variable: Diferença MD do canino (mm)

Appendix C

Table C1 – Independent t-test for diagonal dimensions on the second molar.

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig. (*p*)	t	df	Sig. (2-tailed) (*p*)	Mean Difference	Std. Error Difference	Lower	Upper
ML_DB measure	Equal variances assumed	0,007	0,935	2,158	125	0,033	0,26021	0,12059	0,02156	0,49887
	Equal variances not assumed			2,166	106,174	0,033	0,26021	0,12012	0,02208	0,49835
MB_DL measure	Equal variances assumed	0,331	0,566	3,568	125	0,001	0,39459	0,11058	0,17575	0,61344
	Equal variances not assumed			3,615	109,335	0,000	0,39459	0,10914	0,17829	0,61090

Table C2 – Data regarding ROC curve analysis for diagonal dimensions on the second molar.

Case Processing Summary	
Sex	Valid N (listwise)
Positive[a]	77
Negative	50

Larger values of the test result variable(s) indicate stronger evidence for a positive actual state.
a. The positive actual state is Male.

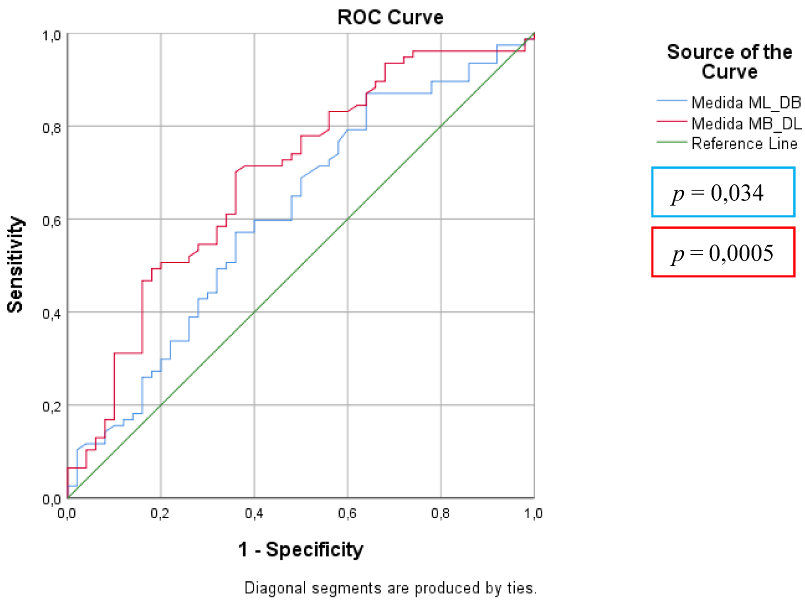


Figure C1 – ROC curve for diagonal dimensions on the second molar.

Table C3 – Statistical significance of the Area Under the Curve for diagonal dimensions on the second molar.

Area Under the Curve					
				Asymptotic 95% Confidence Interval	
Test Result Variable(s)	Area (%)	Std. Error[a]	Asymptotic Sig.[b] (*p*)	Lower Bound	Upper Bound
ML_DB dimension	0,612	0,052	0,034	0,510	0,713
MB_DL dimension	0,692	0,049	0,000	0,597	0,787

The test result variable(s): ML_DB dimension, MB_DL dimension has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Appendix D

Table D1 – Independent t-test for mesiodistal dimension on the molars and canine.

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig. (*p*)	t	df	Sig. (2-tailed) (*p*)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
1st molar (mm)	Equal variances assumed	0,021	0,886	2,656	57	0,010	0,39543	0,14888	0,09729	0,69356
	Equal variances not assumed			2,653	53,569	0,010	0,39543	0,14906	0,09652	0,69433
2nd molar (mm)	Equal variances assumed	0,245	0,623	1,270	57	0,209	0,21569	0,16985	-0,12442	0,55581
	Equal variances not assumed			1,287	56,018	0,203	0,21569	0,16760	-0,12006	0,55144
Canine (mm)	Equal variances assumed	6,512	0,013	4,011	57	0,000	0,44543	0,11106	0,22303	0,66783
	Equal variances not assumed			4,147	56,902	0,000	0,44543	0,10741	0,23034	0,66052

Table D2 – Data regarding ROC curve analysis for mesiodistal dimension on the first molar and canine.

Case Processing Summary	
Sex	Valid N (listwise)
Positive[a]	33
Negative	26

Larger values of the test result variable(s) indicate stronger evidence for a positive actual state.

a. The positive actual state is Male.

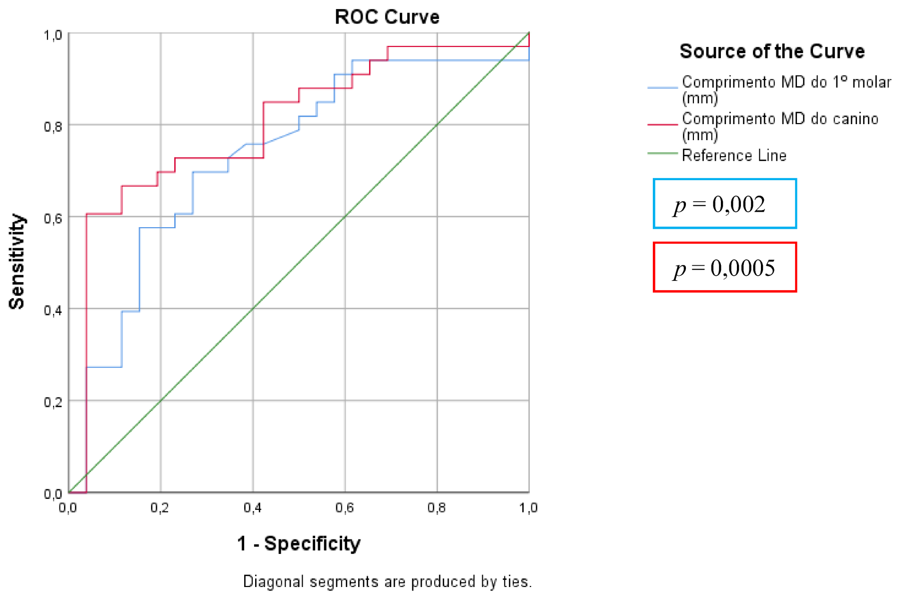


Figure D1 – ROC curve for mesiodistal dimension on the first molar and canine.

Table D3 – Statistical significance of the Area Under the Curve for mesiodistal dimension on the first molar and canine.

Area Under the Curve					
Test Result Variable(s)	Area (%)	Std. Error[a]	Asymptotic Sig.[b] (*p*)	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
MD of 1st molar (mm)	0,735	0,067	0,002	0,603	0,866
MD of canine (mm)	0,801	0,060	0,000	0,684	0,918

The test result variable(s): MD of 1st molar (mm) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5