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### The educational value, both past and present, of an ancient scientific collection: the collection of anatomical preparations illustrating the various phases of bone development, from the second month of intrauterine life to adulthood

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#### Abstract

Italy's museums possess an enormous patrimony of historical scientific artefacts. This raises important questions regarding the conservation and safeguard of such materials and prompts reflection as to the utility of current modalities of popularising science. The collections housed in scientific museums were created in order to promote scientific education by making science more accessible and more comprehensible. The authors ask whether this heritage can still be used for educational purposes today, and examine a collection of preparations on the ossification of human bones in the Anatomical Museum of the University of Siena. They conclude that such materials can still be of educational value if they are made part of exhibitions that meet the needs of the public and of students in training. Indeed, it is essential to bear witness to the long pathway of the development of scientific knowledge and, in particular, to the value of the research on which this knowledge is based. Through the implementation of ad hoc exhibitions, this precious historical scientific patrimony can continue to play an important role in presenting medical/healthcare issues of topical interest without losing sight of the relevance of past experience to basic teaching.

#### Keywords

educational value, ethics, ancient scientific collection, medical education, anatomical preparations, Museum of Siena, Italy.

#### Introduction

The evolution of knowledge and its diversification into distinct fields have led the most ancient universities and research institutes to become the custodians of an extraordinary cultural heritage. Built up or acquired over the centuries for the purposes of research or teaching, this rich patrimony, which bears witness to the evolution of knowledge, has not yet been sufficiently mined and deserves to be valued more highly.

Back in 1924, the Florentine physician and science historian, Andrea Corsini, in his essay "Per il patrimonio storico-scientifico italiano", which appeared in the journal

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"Archivio di Storia della Scienza", drew attention to that enormous scientific patrimony that was "destined to decay and to be lost", as it was "neglected and unsupervised" [Corsini, 1924]. He therefore made the truly revolutionary proposal that science and its instruments should be regarded as a "cultural heritage" in the modern sense, i.e. that it should be safeguarded and preserved in the collective memory.

Italy, which had been unified only a few decades earlier, had inherited a considerable and heterogeneous scientific patrimony, in some cases from private collections dating back to the Renaissance period – a patrimony that was scattered throughout the country and was often used for teaching or research purposes. What worried Corsini and his colleagues who constituted the group for the safeguard of the national scientific heritage was that this wealth of material would continue to be dilapidated and lost and that the original collections might be further fragmented, not least as a result of the rapid development of technology and of the scientific disciplines themselves.

Throughout the 1920s, an ample debate raged as to the fate of historical scientific material in Italy. Despite the support of the Minister of Education Giovanni Gentile, however, the need to safeguard the national scientific heritage remained unrecognised. Indeed, Law N.1089 of 1 June 1939, "Safeguard of Material of Artistic or Historical Interest" focused on material that presented "artistic, historical, archaeological or ethnographic interest", including "palaeontology, prehistory and primitive civilisations"; most scientific material was therefore excluded.

Only in 2004 did such material come under protection, when the Code of Cultural Heritage (Legislative Decree n. 42 of 22 January 2004) recognised "materials and instruments more than 50 years old of interest to the history of science and technology" among "objects of specific dispositions of safeguard" (article 11). In addition to normative issues, it must also be borne in mind that one of the greatest difficulties in protecting this scientific heritage lay in the very nature of the materials themselves: often, they were objects of everyday use, which were thrown away once their utility had ceased; sometimes they were too specialistic to be of interest to the ordinary citizen; in other cases, their scientific purpose was deemed too important for them to become "museum exhibits".

The aim of the present article is to highlight the value of this rich heritage. This value is not merely economic; indeed, this patrimony is far more important, in that it bears witness to the history and evolution of science, while at the same time maintaining the memory of ancient institutions. And it is precisely this latter aspect that carries a specific meaning: the museum is the "repository of the memory and identity of a community".

First of all, it is important to examine the dynamics that led to the formation of these museum collections and to try to determine what the function of an antique scientific collection should be in the 21<sup>st</sup> century.

#### A collection of preparations regarding the ossification of human bones

Here, by way of example, we present a collection of preparations kept in the "Leonetto Comparini" Anatomical Museum of the University of Siena. In the 1990s, the university took the decision to safeguard and conserve its own scientific materials that were no longer in use, to study them and to make them available in the museum setting [Vannozzi, 2017]. This decision was regarded as a "rescue operation" in a context of surprising abandonment and, at the end of the 20<sup>th</sup> century, constituted a change of mentality that marked the beginning of the systematic recovery of scientific historical materials. Having been studied and catalogued, these are now on display in the eight museums of the Siena University Museum System - SIMUS.

The Anatomical Museum of the University of Siena can be traced back to 1850, when the director of the Institute of Anatomy, Giovanni Battista Vaselli, was given the title of "Praefectus of the Anatomical Museum". Subsequently, in 1883, Guglielmo Romiti (1850-1936), a lecturer in anatomy, rearranged the collections with the help of the dissector Pilade Lachi, starting from Paolo Mascagni's valuable preparations. On that occasion, the catalogue of the Anatomical Museum of the Royal University of Siena [Romiti, 1883] was published. Among the exhibits listed in the catalogue (numbers 50-84) was the collection of preparations regarding human bone development, which had been created by Pilade Lachi.

Lachi had the idea of putting together this collection of anatomical preparations after seeing a similar collection at the Orfila Museum in Paris, subsequently named the Delmas-Orfila-Rouviere Museum of Anatomy. Housed in the building of the Faculty of Medicine of the René Descartes University, this museum boasted almost 6000 exhibits, including numerous preparations of brain, skeletons, craniums and a fabulous collection of anatomical wax models.

On 2011 the collections were donated to the University of Montpellier and exhibited in the medical faculties.

At the Orfila Museum, Lachi saw what he did not hesitate to call "the finest of collections concerning osteology. [...] It is made up of all the bones of our body, but each is presented in various periods of bone development: from the first months of intrauterine life up to adulthood. Thus, the mode of growth of each bone can be studied, the nuclei of ossification and the age at which these nuclei combine to constitute a single piece of bone as we see it in manhood" [Lachi, 1880].

On returning to Siena, Lachi decided to put together a similar collection for the Anatomical Museum of the University. This was the origin of the osteological collection illustrating the various phases of bone development, from the second month of intrauterine life up to adulthood, which is the subject of the present article.

The realisation of the collection was no simple matter; not only was it necessary to "supervise the maceration of the tiny skeletons, so as not to miss any of the bony centres", Lachi also needed to "have the subjects necessary in order to catch ossification in its various phases" [8]. Indeed, procuring the necessary cadavers was certainly the greatest difficulty and resulted in lengthy delays in the creation of the preparations. In order to highlight the passage from one stage of ossification to the next, each bone was presented in its development at 65 days of intrauterine life and – according to the case – at 3, 4, 4.5, 5, 6 and 9 months of gestation, and then at the ages of 1, 2, 4, 7, 13, 15, 18, 21 and 30 years, "this latter being the period in which the bones constantly display their complete development" [Lachi, 1880].

Even today, these preparations are presented in this way: "On as many panels as there are bones in our body (he arranged) in rows the various stages of each bone, such that, on picking up each panel, you can take in at a glance the changes that each bone undergoes before reaching its final state. Next to each phase, the age of the bone is reported, so that, as well as recognising the above-mentioned changes, you can realise the age at which they occur" [Lachi, 1880].

## The preparation that is the "true and clear demonstration" of the fruits of research

A preparation of this kind is "nothing less than the exposition and true and clear demonstration of observations that have come under our senses" [Lachi, 1880]; that is to say, it is the result of the research carried out at the Anatomical Institute of the University of Siena, set up as a teaching aid. And it was precisely on such pieces that the anatomists of the time based their ideas concerning the process of ossification, while at the same time finding in them their founding proof.

Indeed, Lachi and his colleagues admitted primary and secondary points of ossification. These latter, "not being constant in terms of either existence or period of development" [Lachi, 1883], had given rise to considerable dispute among scholars. And it was for this reason that, in the introduction to the published brochure that presented the collection, Lachi saw fit to dwell on the meaning of "point of ossification" and "centre of ossification".

Today, the most recent studies in embryology have shown that the formation of the skeleton during embryonic life, and also the remodelling that takes place during the postnatal period, occur through the interaction of various factors. Indeed, environmental signals, intracellular signalling pathways, transcription factors and co-regulators, such as vitamins, are able to support the differentiation of the mesenchymal cells towards the mature osteocyte of mineralised bone. During the third week of embryonic life, gastrulation takes place, a fundamental developmental stage in which three pri-



**Figure 1.** P. Lachi, *Anatomical preparation on the development of the bones of the hand* – Anatomical Museum L. Comparini Università di Siena, Images Archives SIMUS.



Figure 2. P. Lachi, Anatomical preparation on the development of the humereus – Anatomical Museum L. Comparini University of Siena, Images Archives SIMUS.

mary germ layers are formed: ectoderm, mesoderm and entoderm, from which various apparatuses will derive. The skeletal apparatus develops from the mesoderm (paraxial and somatic) and from the neural crest. The paraxial component of the mesoderm is arranged in a series of "small pieces" of tissue, known as somites, located alongside the neural tube, and from which, at the end of the fourth week of embryonic life, the mesenchymal cells will derive; these latter have the characteristic of migrating and differentiating into various cell lines, such as fibroblasts, chondroblasts and osteoblasts.

At that time, however, Lachi, who also integrated his knowledge of anatomy with that of histology, embryology, comparative anatomy and physiology, obviously did not have all this information. He could not therefore know that there are two modalities of skeletal accretion: direct or membranous ossification and indirect or endochondral ossification; each of these modalities is proper to different bones. Direct ossification begins from the mesenchymal cells; these transform into osteoblasts, which in turn produce non-lamellar bony tissue. After mineralisation, this will be replaced by



**Figure 3.** P. Lachi, Anatomical preparation on the development of the ethmoid – Anatomical Museum L. Comparini Università di Siena, Images Archives SIMUS.

bony tissue. This process is typical of the bones of the cranial vault, the face and the clavicle. By contrast, indirect ossification, which regards all the other sites, derives from mesenchymal cells that transform into chondroblasts; these, in turn, produce cartilaginous tissue. In this way, "bone drafts" are formed; these are made up of cartilage and will be replaced by bony tissue. This type of ossification occurs from several centres of ossification and takes place over a fairly long period. Indeed, ossification of the long bones begins around the eighth week of embryonic life and is complete by the age of 18-20 years.

However, the knowledge available to the anatomists of the middle of the 19th century was obviously much more limited, and the debate revolved around the above-mentioned concepts of "point of ossification" and "centre of ossification". By "point of ossification" - Lachi wrote - "we should mean the most limited and smallest part in which ossification is deployed to a given portion of membranous or cartilaginous skeleton, and whence it radiates to constitute a clearly visible piece of bone. Now, one of two things may happen: either more than one of these parts contribute to the formation of a single bone, uniting and fusing together very early, such that we do not have enough time to recognise their primitive separation; or, by contrast, these points remain separate for a more or less long period of time. [...], in which case, as the term has a very restricted meaning, it should be replaced by the expression "centre of ossification" [...], as this suggests a more or less large area of tissue in which the first traces of ossification are manifested, and from which it radiates in all directions until it unites, more or less late, with similar areas, giving rise to the complete bone" [Lachi, 1883].



Figure 4. P. Lachi, Anatomical preparation on the development of the tibia – Anatomical Museum L. Comparini Università di Siena, Images Archives SIMUS.

On the basis of these presuppositions, each panel displaying Lachi's bone preparations in their various phases of accretion was accompanied by a thorough description of the features of each phase, the principal modifications to be noted between one phase and the next, and the most innovative studies conducted on each specific issue; for this purpose, he reviewed the works of the leading scholars of anatomy and embryology of his day, from Alexis Boyer to Luigi Calori, and from Theodor Karl Gustav von Leber to Rudolf Albert von Kölliker.

This collection is therefore the result of attentive observations and in-depth studies by this teacher, who created a useful aid for the teaching and the study of anatomy. It is, to all intents and purposes, a set of preparations for educational use, responding in excellent fashion to the medical student's need to "see" how the human body is made up. Indeed, anatomy is the art of sectioning the human body so that it can be seen. And it is in this way that the student observes and learns. It is no coincidence that the main place of anatomical dissection is the anatomy theatre, the etymology of which harks back to the root of the Greek verb  $\vartheta \varepsilon \dot{\alpha} \circ \mu \alpha_i$ , meaning "I look".

#### Conclusions

Drawings, preparations, models and contrivances have always been used to do scientific work and to communicate scientific knowledge. Over the centuries, these materials have accumulated in the laboratories and lecture theatres of universities and other institutions, in some cases constituting valuable collections that are now exhibited in science museums. Moreover, it should be pointed out that similar devices are still used today in scientific communication, even though the pencil has been replaced by the digital drawing, and technological means of communication have replaced the observation of anatomical preparations. We might therefore be led to believe that the antique collections of educational tables, preparations and models that constitute the patrimony of many museums of anatomy are destined for oblivion or, at best, to be regarded as simple scientific curiosities from a past era.

In reality, this is not the case. At least, it will not be the case if we construct around this heritage projects of scientific communication aimed at explaining science to young people and to all those who are interested, in order to make them aware of how our current ample knowledge has been achieved – projects that explain this arduous pathway, sometimes strewn with error, and which can promote debate and dialogue among scholars.

Moreover, if we endorse the concept enunciated by Giulio Carlo Argan back in November 1951 at the UNESCO-ICOM meeting in Paris: "the foundation of the museum constitutes the positive recognition of its educational capacity" [Argan, 1949], we can well assert that collections of historical scientific material, such as that presented in this paper, constitute indispensable aids to the dissemination of science. For all these reasons, such collections should not be looked upon as dusty relics; rather, they represent a fundamental tessera in the mosaic of the history of science.

Thus, museums are not inert containers for antiquities that are surrounded by a sacred aura and accessible only to a cultural elite. Instead, in addition to being places of scientific research and safeguard of our cultural heritage, they become places of scientific research and dissemination – just like the Greek *museion* – places where moments of informal education and socialisation foster esteem for knowledge. In this way, this extraordinary "mine" of knowledge, which has been so assiduously safeguarded, can take on fresh life through the direct involvement of citizens, thereby contributing to the construction of a democratic society, fostering individual and social sustainability, creating new professional skills and promoting widespread well-being.

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#### **Conflict of interest statement**

The authors declare no conflict of interest.

#### **Authors' contributions**

MM and DO designed the study. MM and DO conceived the study; MM, DO and MA drafted the manuscript; MM, DO and MA revised the manuscript. MM, DO, MA

performed a search of the literature. All authors critically revised the manuscript. All authors have read and approved the latest version of the paper for publication.

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## Elongated styloid process: literature review and morphometric data on a collection of dried skulls

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#### Abstract

The styloid process is a subtle process of variable length that originates from the petrous part of temporal bone . From the process originate three muscles: the stylopharyngeus, the styloglossus, the stylohyoideus and two ligaments :stylohyoid and stylomandibular . The styled process of the temporal bone has a variable length as demonstrated for studies conducted in threedimensional computed tomography or dental panoramic three-dimensional scanning, and in dried skulls The normal length of the styloid process is particularly important to establish for the relations of closeness with vascular and nervous structures and their possible compression by an elongated styloid process. Several studies have focused on the effects of changes in length and course of the styloid process, highlighting the relations of the carotid artery and the glossopharyngeal nerve to explain cerebrovascular symptoms or Eagle's syndrome characterized by neck pain and dysphagia. However the association for the abnormal length of styloid process and Eagle's syndrome is not always present and many cases are asymptomatic. In the present study we propose a review of the studies performed with different in vivo radiological techniques and on dried skull collections on the normal and pathological length of the stiloyd process and on its association with the Eagle's syndrome. We also present a morphometric study carried out in dried skulls of our museum collection. The results are discussed in light of the possible variations in the muscular and ligamentous structures consequent to the elongation of the styloid process.

#### Keywords

Elongated styloid process, Eagle's syndrome.

#### Introduction

The styloid process (SP) is a subtle pointed process of variable length that originates from the petrous portion of temporal bone and is localized anteriorly to the stylomastoid foramen.

From the process originate three muscles: the stylopharyngeus, the styloglossus, the stylohyoideus and two ligaments :stylohyoid and stylomandibular . The SP projects inferiorly and anteriorly into the parapharyngeal space and it is in relationship with the internal carotid artery medially and with various encephalic nerves as glossopharyngeal, vagus, accessory, hypoglossal .The SP of the temporal bone has a variable length as demonstrated for studies conducted in: three-dimensional computed tomography (3dct) or dental panoramic three-dimensional scanning.Sökler

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and Sandev (2001) reported an average value of about 22 mms and a limit of 30 mms for normality.Gozil et al.(2001) considered elongated a process of 40 mms . Sudhakara et al.(2013) on 600 digital panoramic radiographs found that the mean average length of elongated styloid processes was 3.67±0.62 cms. Jung et al.(2004) with the study of panoramic radiographs had found an average value of 28 mms in length and suggested that the SP should be considered to be elongated, when its length exceeds 45 mms. Keur et al.(2001) stated that, if the length of the process or the mineralized part of ligaments which appeared in radiography was 30 mms or more, this could be considered an elongated SP. Basekim et al.(2005) examined the length of the styloid process of 138 subjects with 3dct finding an average length of 28.3 mms and considered the maximum normal length of the process 40 mms. De Andrade et al.(2012) measured the styloid length in cephalometric radiography finding a mean value of 32.39 mms. Data onto the osteometric values of the SP is scanty with significant length differences existing on the studies attributed to different races or ethnicities (De Paz et al. ,2012;Rathva et al., 2013; Natsis et al. ,2014). Natsis et al.(2014) in a study carried out on an one hundred and forty-nine adult dried skull, classified the SP in three types according to their lengths. The processes were classified as short <18 mms, normal 18-33 mms and elongated 33 mms. The majority of the processes were normal (45.8 %), 27.5 % were short and 26.7 % elongated. In another study by Custodio et al.(2016) on a population of 15 dried skulls, the authors found an average of 19.25 or 24.98 mms depending on the measurement made with a lateral or posterior view of the styloid process. Vadgaonkar et al.(2015) reported in a morphometric study on a collection of 110 dried skulls a length of 17.8+-9.3 mms for the right process and 18.2+-5.6 mms for the left process. Several studies have been limited to reports on isolated cases of excessively long processes (Prabhu et al . ,2007; Magotra et al. ,2008; Paraskevas et al.,2009; Akhaddar et al., 2010; Lins et al. , 2010) which curve medially and anteriorly with variable length up to 140 mm. ( Kubíkova and Varga, 2009 ). An excessive length of the styloid process may be due to calcification - ossification of the styloid ligament which may be bilateral (Jagadish et al.2010) or more rarely unilateral(Guarna and Aglianò,2018), less than 0.9 % according to (Vougiouklakis ,2006). In a review of 1215 autopsies ,Langlais et al. (1986) recognizes three types of elongated and ossified styloid processes: the first two are interrupted and have pseudo-articulations, the third is an uninterrupted segment formed by the ossified SL. .Campos et al. (2011) reported that the etiology of styloid bone elongation can be explained by a genetic alteration or according to several theories. The ossification of the SL may be the consequence of a pharyngeal trauma which causes metaplastic change . According to the anatomic variance theory ,the elongated SP and the ossified SL are anatomical variations that occur without any trauma.

The abnormal length can be in some cases accompanied by a set of symptoms, characterized by neck pain , dysphagia, headache, sore throat, ear pain , mandibular dysfunction clinically framed in Eagle's syndrome(Eagle 1937,1948). Yavuz et al. (2008) stressed that length in isolation is not a risk factor, but that its combination with increased acuity in deviation from the norm, both anteriorly and medially, makes the elongated styloid processes the cause of the syndrome. Stylocarotid syndrome, the less common vascular variant of the Eagle syndrome, can present as headache, transient ischemic attack (TIA), or stroke (Chang et al., 2007). Stylocarotid syndrome results from the styloid process compressing the internal carotid artery

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(ICA) or external carotid artery.(David et al., 2014) .The abnormal length of styloid process raises questions about the anatomical changes that can come to be established for a change of length and insertion of the stylohyoid and stylomandibular ligaments(called the bouchet's white flowers ) , as well as the muscles styloglossus , stylopharyngeus , stylohyod (the so called Riolano's bouchet). (Fini et al.,2000).

Furthermore literature does not contain information regarding how these changes may affect the processes of swallowing, phonation, breathing and chewing. We present an osteometric study on the length of the styloid process of collection of over 250 dried skulls belonging to the museum of Anatomy "Leonetto Comparini " at the University of Siena, collected over a period from 1900 to 1960. All skulls came from the local psychiatric hospital. Univariate analysis provides mean values and standard deviations of the main variable length. A Mann Whitney test was applied for the analysis of significant differences between the averages of the groups of skulls .

#### Materials and methods

We carried out measuring the length of the styloid process on a sample of 153 skulls of the entire collection of 250 of the local anatomical museum, having chosen the skulls in which at least one of the two processes was intact . The skulls of children, damaged skulls and skulls with pathological conditions were excluded from the study.Furthemore the skull with the styloid process which had the completely ossified stiloyd ligament of 7 cms in length was not considered for statistical purposes. The lengths of the right or left styloid processes were measured by choosing the side that appeared the best preserved for measurement purposes. The measuring starts from the sheath at the point where this became accessible below the external acoustic meatus .We employed a digital caliper with the following technical characteristics. Measuring range: 0-150 mm; resolution: 0.01 mm; accuracy: + / - 0.02mm; measurement repeatability: + / - 0.01 mm; .The data were analyzed trough a free statistical software (Salstat 2) .Average and standard deviation (sd) were calculated. A Mann-Whitney test for significance level of 0,5 was used for detecting differences between the distributions of both genders. The test was carried out on two groups of 19 male skulls and 26 female skulls, having chosen only those skulls of which the sex of belonging was certain.

#### Results

Univariate analysis of the length of the styloid process gave the following results: average: 26,57 mms ; sd : 7,40 mms ; median 25 mms , normal range : average +/- 2 sd : 41-7 mms. The histogram of the elements (fig 1) shows a large class of 48% (73) elements, with a length of the styloid process of 18-26 mms . A second class smaller as elements 37% has a styled process with a length of 27-35 mms.(fig. 1). The longest styloid process observed was 52 mms (fig. 2) and the shortest had a length of 5 mms .

In one case we discovered a skull with stylohyoid calcified ligaments on one side. The styloid process was long 50 mms on the right and 70 mms on the left where is the tip calcified (not shown). There were no significative differences between lengths



**Figure 1.** histogram representing the distribution frequency of the length of the styloid processes.



**Figure 2.** Elongated styloid process (52 mm) in a skull from the Leonetto Comparini Museum collection.

in male e female subjects. Considering that the Range of Normal Lengths was taken as  $\pm 2$  standard deviations from the mean, the percentage of elongated styloid processes was 3.9%

#### Discussion

The length of the styloid process has an average value of 26,57 mms in our biometric study .This value is in accordance with those ones established by other authors (Basekim et al. ,2005; Joung et al.,2004 ).Patil et al.(2014) in 114 dried skulls reported a mean of 2,58 cms for SP length. An abnormal long styloid process>41 mm is present in 3,9% of items. Vadgaonkar et al. (2015) reported that the prevalence of elongated styloid processes in 110 dried skulls was 4.5%. Other authors provide higher percentages of elongated styloid processes but place the value of 30 mm as the limit of normality. Gracco et al.(2017) in a north Italian population evaluated elongated styled process by digital panoramic radiographs and found that 33% of population has an elongated styled process with a length of the process more than 30 mms. The prevalence of the elongated styloid process is between 4% and 28% of the population (Silva et al., 2002). The pathogenesis of the elongation of styloid process is still debated .It was believed that there is an association with previous surgical trauma or local irritation of stylohyodeus ligament which could cause hyperplasia and reactive ossification of the process. Later the cause was believed to be a variation in development of second arch derivatives (Fini et al. ,2000). It could be a mutation in the genes implicated in the correct development of the components of branchial arches. Future genetic studies will be needed to prove this issue. Only in a case we found a calcified tip of the abnormal lengthy styloid process,70 mms long on the left.Unfortunately we have no information about the subject to search the presence in the past of surgical trauma or local irritation .Several studies have focused on the effects of changes in length and course of the styloid process, (Thot et al., 2000; Yavuz et al., 2008) highlighting the relations of the carotid artery and the glossopharyngeal nerve(Shin et al .,2009) to explain cerebrovascular symptoms or Eagle's syndrome characterized by neck pain and dysphagia (Eagle,1937). However the association for the abnormal length of styloid process and Eagle's syndrome is not always present and many cases are asymptomatic (Fini et al. ,2000). Anatomical variation in length of styloid process could have effects on ligaments and muscles which origin from it and insert on mandible and os hyoid . The stylohyoid ligament originates from the tip of the styloid process and is attached inferiorly to the lesser horn of the hyoid bone. Likewise the stylomandibular ligament originates near the apex of the styloid and is inserted medially into the mandibular angle. The shortening of the stylomandibular ligament could cause difficult in the protrusion of mandible and in mastication (Costantinides et al., 2013). The shortening of stylohyoid ligament which originates from the apex of the styloid process and is attached inferiorly to the lesser horn of the hyoid bone or its calcification-ossification could cause difficult in movement of os hyoid and in raising or lowering of larynx during swallowing(Desrhaj et al., 2011). This could be an explanation for the disturbance reported in these processes by several patients. Likewise the excessive shortening of styloglossus muscles which originates close to the apex of the styloid process and from the upper part of the stylomandibular ligament and is inserted in the septum of the tongue (Fini et al., 2000) could disturb the tongue movement . The stylohyoid muscle which originates from the base of the styloid process and is inserting on the hyoid bone near the greater horn will be longer in case of abnormal length of styloid process and so the muscle stylopharyngeus which originates from the medial aspect of the styloid process close to its base and is inserted into the lateral wall of the pharynx and elevates the pharynx during the first phase of swallowing(Tubbs et al., 2010)

To avoid the shortening of ligament and muscles the elongation of styloid process could be accompanied by a dislocation in a lower position of os hyoid with laryngeal cartilage and trachea .Lowering of hyoid happens to children with atypical deglutition. Atypical deglutition is the persistence of deciduous dentition deglutition, and in this case os hyoid is lowered about 5mm from mandible plane ( Machado and Crespo, 2012). Similarly in obstructive sleep apnea and hypopnoea syndrome( OSAHS), the hyoid bone is more distant from the mandible plane as reported in cephalometric studies ;(Riha et al., 2005; Tsai et al., 2007).However, in all these cases there is no information on the presence of an elongated styloid process by which we can infer a modification of the muscular and ligamentous structures that are placed on it. Moreover possibly hypertonia of the infrahyoid and hypotonia of the suprahyoid muscles as a consequence of the different position of hyoid bone could cause change in the voice and a greater difficulty during intubation.(Kaway et al., 1990) Our study on the length of styloid process in a population Italian confirmed the low frequency of an abnormally long process, highlights a case of ligamentum styloideus ossified and discuss the possible consequences on the muscles and ligaments which arise from the styloideus process in case of abnormally length of the same .

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## Determination of type of asymmetry in hand dimensions and its relationship with body mass index

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#### Abstract

Several documented evidences have indicated high influence of diseases on developmental instability measurable by asymmetry, there is however scarcity of reports on the type of asymmetry in hand and limited attempts to determine its relationship with body mass index (BMI) especially among Hausa population. The study was aimed to determine type(s) of asymmetry in hand dimensions and its relationship with BMI from selected tertiary institution in Kano metropolis, Nigeria. The study was cross sectional type which involved a total of 398 students (204 males and 194 females). The hand dimensions (digits length, palmer length and hand breadth) were measured using standard protocol. Body mass index was calculated from the measured height and weight. Asymmetry was determined as right-hand dimension minus left hand dimension (A = R-L). One sample t test was used to determine the type of asymmetry try in hand dimensions. Pearson's correlation was used to determine the correlation between the asymmetry and BMI. The results of the study showed that digit length, hand breadth and palmar length exhibited directional asymmetry (DA). The degree of the DA was more expressed in index digit followed by middle digit and the least was the ring digit. The nature of DA in the hand dimensions was left warded except for the hand breadth which was observed to be right warded. There was no significant correlation observed between asymmetry in hand dimensions with BMI. In conclusion, there were no significant correlations between hand asymmetry and BMI among the studied Hausa population and hand dimensions exhibited directional type of asymmetry, as such may not be used as a surrogate indicator of developmental instability.

#### Keywords

Asymmetry, Body Mass Index, Hand dimension, Hausa ethnic group.

#### Introduction

Bilateral asymmetry refers to dissimilarity of parts on either side of a straight line or plane, or about a center or axis. Asymmetry of an individual is measured as the right minus the left value of the bilaterally paired trait. Departures from symmetry in bilateral structures have the potential to elucidate the effects of genetic and physiological, but also mechanical factors, during growth and development (Van Valen, 1962; Palmer, 1994; Gawlikowska-Sroka *et al.*, 2013). Humans show bilateral symme-

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try in paired morphological traits such as ear size, digit length and breast volume. Three types of biological asymmetry exist in nature; fluctuating asymmetry, directional asymmetry, and antisymmetry. Fluctuating asymmetry (FA) is characterized by small random deviations from perfect bilateral symmetry. Directional asymmetry (DA) is characterized by a symmetry distribution that is not centered around zero but is biased significantly, towards larger traits either on the left or the right side (e.g the consistent asymmetry of the thoracic organs in humans and upper limb dimensions. Antisymmetry is characterized by being centered around a mean of zero (Van Valen, 1962; Palmer, 1994; Battles, 2009).

The existence of high influence of diseases on developmental instability measurable by asymmetry is a well-established phenomenon (Palmer, 1994; Palmer and Strobeck, 1986; Palmer and Strobeck, 2003), and as such, asymmetry is been used by proxy as a pointer to different types of stresses including environmental and occupational stress as well as developmental instability (Van Valen, 1962; Palmer, 1994; Gutnik et al., 2015). However, there is paucity of data on the type(s) of asymmetry in hand dimensions among Hausa population which might provide an insight on nature and extent of developmental instability prevalent among the Hausa. BMI, an established indicator of body adiposity and predictor of a cluster of genetic diseases has also been linked to developmental and evolutionary stress. We hypothesized in the present study that BMI and hand asymmetry are similar, being morphometric traits with developmental stress as common determinant. We therefore conducted this study to determine the type(s) of asymmetry in hand dimension and its correlation with BMI. This study which is first of its kind among the Hausa ethnic group of Kano may provide an insight on the asymmetry pattern in hand dimension and its relationship with BMI.

#### Materials and methods

The study was carried out at Bayero University Kano (BUK), College of Health Science (CHS) which comprises of four Faculties; Allied Health Sciences, Basic Medical Sciences, Clinical Sciences and Dentistry and Yusuf Maitama Sule University Kano (YMSUK), Faculty of Basic Medical Sciences (FBMS).

The study design was cross-sectional type. Using simple random sampling, a total of 398 (204 males and 194 females) of students of the CHS BUK and YMSUK Faculty of Basic Medical Sciences aged between 18 to 30 years participated in the study. Included participants are the registered students of BUK, CHS and YMSUK, FBMS which are physically fit and have no any physical malformation especially in their hands. Only student that belong to Hausa ethnic group was included. Informed consent was also gained from the participants before participation. The study was conducted in accordance with Helsinki declaration.

The sample size for study was determined using a standard formula (Cochrane, 1977):

$$n = \frac{Z^2 P q}{d^2}$$

Where; n= minimum sample size, z= standard normal deviation with confidence interval of 95% ( $\pm$ 1.96), p= proportion in the target population (50%) 0.5, q= 1-p, 1-0.5= 0.5, d = sampling error which is 5% (0.05).

 $n = \frac{(1.96)^2 \times 0.5 \times 0.5}{0.05^2} = 384$  (this was the minimum number of participants needed for the study).

The bio-data (age, sex, and location of the birth of the participants and ethnicity) were collected using structured proforma. The anthropometric parameters were measured from the participants and also recorded in the proforma.

Height was measured as a vertical distance from the standing surface to the vertex of the head using a stadiometer (RGZ, 160). Body weight of the subjects was measured using digital weighing scale (Omron, China). BMI was calculated as a ratio of weight in kg to the height per m<sup>2</sup>. The lengths of the digits, hand breadth and palmer length were measured using vanier caliper (Neiko 01407A) (Figure 1) as described as adopted from Manning et al. (1998); Umar et al. (2016) and Asuku et al. (2017).

An asymmetry index, differences between left (L) and right (R) was used. This was determined by the formulae: A = R - L. The existence of DA was detected by subjecting the mean value of asymmetry against mean of zero using one sample *t*-test. An asymmetry is directional if mean value of signed symmetry differs significantly from zero; otherwise, it is considered as FA. The nature of the asymmetry was based on the sign of asymmetry. If R - L resulted in positive value then asymmetry is considered as right warded asymmetry (that is the right dimension is greater than the left) and if R - L resulted in negative value then asymmetry is considered as left warded asymmetry (that is the left dimension is greater than the right).

The data were expressed as mean  $\pm$  standard deviation and range (minimum and maximum). One sampled t test was used to determine the type of asymmetry for each dimension. Pearson's correlation was used to determine the correlation between the hand dimension asymmetry and BMI. The data were analyzed using the Statistical Package for Social Sciences (SPSS) software version 20. *P* < 0.05 was considered as level of significance.

#### Results

Table 1 shows the descriptive statistics of the age, BMI and hand parameters of the students of BUK, CHS and YMSUK, FBMS. The mean age and BMI of the study population were 21.67 $\pm$ 2.54 years and 20.23 $\pm$ 3.55 kg/m<sup>2</sup> respectively. It was observed that in the all hand parameters, left hand dimensions showed higher values compared to the right-hand dimensions except in left thumb and left-hand breadth where the right-hand dimension was higher.

Table 2 shows type and nature of asymmetry in hand parameters among Hausa student of BUK, CHS and YMSUK, FBMS. The asymmetries were found to be left warded (negative asymmetry), where most of left-hand dimensions measurements were larger compared to the right, except for hand breadth. The mean hand asymmetries were significantly greater than zero. It was observed that all the hand asymmetry was directional asymmetry (DA) types. The degree of the directional asymmetry was more expressed in index digit followed by middle and the least was the ring digit.

| Variables                         | Mean± SD    | Min   | Max    |
|-----------------------------------|-------------|-------|--------|
| Age (years)                       | 21.67±2.54  | 18.00 | 30.00  |
| Body mass index (kg/meter square) | 20.23±3.55  | 13.76 | 40.58  |
| Right thumb (mm)                  | 63.96±5.29  | 41.92 | 78.72  |
| Right index (mm)                  | 70.53±5.15  | 45.73 | 85.45  |
| Right middle (mm)                 | 79.56±5.67  | 53.21 | 96.17  |
| Right ring (mm)                   | 73.66±5.79  | 46.62 | 89.45  |
| Right little (mm)                 | 58.59±5.59  | 30.29 | 91.60  |
| Right hand breadth (mm)           | 79.71±6.27  | 61.81 | 113.40 |
| Right palmar length (mm)          | 107.83±6.99 | 70.03 | 125.33 |
| Left thumb (mm)                   | 64.63±5.34  | 40.60 | 82.56  |
| Left index (mm)                   | 71.52±5.47  | 47.11 | 90.50  |
| Left middle (mm)                  | 80.36±5.99  | 55.01 | 97.66  |
| Left ring (mm)                    | 74.17±5.98  | 46.16 | 90.93  |
| Left little (mm)                  | 58.94±5.29  | 33.01 | 71.95  |
| Left hand breadth (mm)            | 79.06±6.26  | 55.35 | 95.35  |
| Left palmer length (mm)           | 108.55±6.89 | 85.32 | 127.04 |

Table 1. Descriptive Statistics of Age, Body Mass Index and Hand Parameters

Table 2. Type of asymmetry in hand parameters among Hausa student of BUK, CHS and YMSUK, FBMS.

| Variables              | Mean  | SEM  | t Value | P Value | Type of<br>Asymmetry | Nature       |
|------------------------|-------|------|---------|---------|----------------------|--------------|
| Thumb asymmetry        | -0.67 | 2.65 | -5.03   | < 0.001 | DA                   | Left warded  |
| Index asymmetry        | -1.01 | 2.15 | -9.34   | < 0.001 | DA                   | Left warded  |
| Middle asymmetry       | -0.79 | 2.16 | -7.35   | < 0.001 | DA                   | Left warded  |
| Ring asymmetry         | -0.49 | 2.24 | -4.42   | < 0.001 | DA                   | Left warded  |
| Little asymmetry       | -0.35 | 2.31 | -3.04   | < 0.001 | DA                   | Left warded  |
| Hand breadth asymmetry | 0.64  | 2.63 | 4.87    | < 0.001 | DA                   | Right warded |
| Palma length asymmetry | -0.70 | 3.45 | -4.07   | < 0.001 | DA                   | Left warded  |

DA; directional asymmetry, the mean asymmetry was tested against mean of zero

Table 3 shows the correlation of hand asymmetry with BMI among Hausa students of BUK, CHS and YMSUK, FBMS. It was observed that there was no significant .correlation between hand asymmetry with BMI in males. Similarly, there was no significance correlation between hand parameters asymmetry with BMI in females.

|                        | Correlation coefficient |                       |  |  |  |
|------------------------|-------------------------|-----------------------|--|--|--|
| Variables (mm)         | Male<br>BMI (kg/m²)     | Female<br>BMI (kg/m²) |  |  |  |
| Thumb asymmetry        | -0.0114                 | 0.0871                |  |  |  |
| Index asymmetry        | 0.0021                  | -0.0371               |  |  |  |
| Middle asymmetry       | -0.0185                 | -0.0181               |  |  |  |
| Ring asymmetry         | -0.0775                 | -0.0775               |  |  |  |
| Little asymmetry       | 0.0696                  | -0.0519               |  |  |  |
| Hand breadth asymmetry | 0.1359                  | -0.0700               |  |  |  |
| Palmar asymmetry       | -0.0111                 | -0.0047               |  |  |  |

Table 3. Relationship of Hand Asymmetry with BMI among Hausa student of BUK, CHS and YMSUK, FBMS.

#### Discussion

Bilateral asymmetry is one of the least understood aspects of the hand parameters. The measure of asymmetry which has mainly been used in population and genetic studies is the right and left differences (Gawlikowska-Sroka *et al.*, 2013). The present study evaluated the existence and type of asymmetry with respect to the hand parameters and its relation to BMI among the Hausa population of Kano state. All the parameters showed directional type of asymmetry. This finding was similar with the previous study (Livshits *et al.*, 1998). In humans a typical example of DA is found in upper limb dimensions (Livshits *et al.*, 1998). This DA among *Homo sapiens* is mainly interpreted as effects of handedness (Steele, 2007). It was also reported that hand parameters especially the digits exhibited directional type of asymmetry; therefore, the hand parameters may not be a good indicator of developmental instability and stress, which is measured by proxy with any structure that exhibited fluctuating type of asymmetry (Palmer and Strobeck, 1986; Palmer and Strobeck, 2003). It can be suggested that asymmetry in hand dimensions especially among Hausa population should not be consider as good indicator of developmental stress and instability.

Additionally, other conditions explained by FA such as failure of affected organism to maintain developmental homeostasis (Palmer and Strobeck, 1986; Thornhill and Moller, 1998) may also not be explained by asymmetry in hand parameters. However, since perfect symmetry of bilateral traits is said to represent ideal development, while asymmetry including DA represents an inexact presentation of developmental design (Tomkinson, 2000). Therefore, it can be appreciated that DA is developmentally controlled and likely to have adaptive significance and generally thought to have adaptive basis and the asymmetry is just a norm not just as a result of imprecise development (Van and Valen, 1962).

There is no significant correlation between the hand asymmetry and BMI among the study population. This may be explained by the fact the hand exhibited directional asymmetry rather than fluctuating type. Most of the previous studies correlated the asymmetry with health indices (Fink *et al.*, 2014) were based on FA not DA. Even among FA contrasting results were reported. For example, it was reported that FA was associated with health, using seven health measures: BMI, waist/hip ratio, systolic blood pressure, total blood cholesterol, cardiorespiratory fitness, periodontal disease, and a number of medical conditions (Barry *et al.*, 2003). These associations were demonstrated to be independent of each other, and were robust to the confounding influences of exercise (Barry *et al.*, 2003). However, it was also documented that FA was not associated with waist/ hip ratio, BP, BMI, cholesterol, fitness, or periodontal disease when investigated in both univariate and multivariate models (Milne *et al.*, 2003).

In another context, it should be noted that a well-developed, symmetrical phenotype indicates the ability of an individual to oppose the challenges of developmental stress in particular the environments (Møller and Swaddle, 1997). Therefore, asymmetry in general is imprecision of the developmental process both *in utero* and postnatally. It is also important to emphasize that different populations are exposed to different environmental conditions which may result in different expression of effect of environmental stress. Genetic variation is also another factor that may lead to difference in the manner an organism response to environmental stressor. Therefore, the expression of DA in hand dimensions among the Hausa population as well as absence of correlation of hand asymmetry with BMI may not be absolute and similar in comparison with other populations with different genetic and environmental conditions.

In conclusion, the measured hand dimensions exhibited directional type of asymmetry as such may not be used as good indicator of developmental instability by proxy among Hausa ethnic group. There is also no significant correlation between hand asymmetry with BMI among the Hausa population studied. Hence, may not be a good marker of generalized adiposity and its abnormal health related consequences.

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#### **Conflict of interest**

No existing or potential conflict of interest.

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# Variations in the arteries of the upper limb - a clinical apropos

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#### Abstract

Introduction: Blood supply to the arm and forearm is conveyed by the brachial artery and its branches, the radial and the ulnar artery. Variations in these arteries are being increasingly reported in many studies. Most of these variations observed are mostly in the course and branching pattern of these arteries and have been accounted to be due to developmental defect, from varied causes.

Materials and Methods: In the present study dissection was done on fifty seven upper limb specimens obtained from the college of Medicine and Health Sciences, Oman. Variations observed in the origin and course of the arteries in the arm and forearm, were noted and later photographed.

Results: Out of the fifty seven upper limb specimens studied variations were noted in four limbs. In one limb there was a superficial branch originating from the brachial artery, that later divided into superficial radial and ulnar arteries. In two limbs the brachial artery was superficial to the median nerve, in one of them the brachial artery coursing superficial to the median nerve had a tortuous course. Superficial course of ulnar artery in the forearm was noted in another limb.

Conclusion: Surgical procedures are being increasingly done on the upper limb arteries, these procedures are either diagnostic or therapeutic interventions, such as arteriography, flap harvesting, creating arteriovenous fistulas etc. Knowledge of the variations in the upper limb arteries is essential before any surgical intervention is planned in the upper limb arteries in order to prevent unnecessary complications.

#### Keywords

Brachial artery, ulnar artery, variations, anomalies, upper limb.

#### Introduction

The arterial supply to the upper limb is described to start with the axillary artery, which is a continuation of the subclavian artery at the outer border of first rib. At the lower border of teres major muscle the axillary artery extends in the arm as brachial artery, which later enters the cubital fossa and usually terminates at the level of the radial neck into radial and ulnar artery. The median nerve crosses the brachial artery from the lateral to medial side near the distal attachment of coracobrachialis and thereafter lies medial to the brachial artery in the cubital fossa. The ulnar artery immediately gives off the common interosseous artery in the cubital fossa that divides into the anterior and posterior interosseous arteries. (Standring, 2008)

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Variations are generally described as morphologies, which are encountered less frequently and are not abnormal and though subtle can affect the human body. (Willan & Humpherson, 1999)

Alteration in the normal course of the upper limb arteries are usually encountered in routine dissection done during undergraduate teaching.

Variations observed in the arterial pattern of the upper limb, is usually noted as absence of either the brachial artery, the radial artery, the ulnar artery, or variations in their branching pattern. Also reported are differences in the origin of these arteries and their courses which may interfere with therapeutic, diagnostic and surgical procedures. (Bidarkotimath et al., 2011)

In the upper limb arteries the variations are known to be more common in radial artery followed by the ulnar artery and less common in the brachial artery. (Chakravarthi et al., 2014)

In Compendium of Human Anatomic variation, Bergman et al (2015) noted that major variations were present in 25% of the subjects in whom brachial artery was studied. They quoted Latarjet as having described five types of branching patterns in case of 'premature' division of the brachial artery, it can terminate as: radial and ulnar-common interosseous trunk; ulnar and a radial-common interosseous trunk; common interosseous or persistent median artery and a radioulnar trunk; radial, ulnar, and a common interosseous artery and a vas aberrans and normal brachial artery.

Rodríguez-Niedenführ et al. (2001) in their study on 384 upper limb specimens reported 7 different arterial variations of the major arteries of the upper limb.

Knowledge of the variations in the arterial anatomy of the upper limb is of significant practical importance for both the vascular radiologist and surgeon and aids them in diagnostic interpretation as well as in the conduct of interventional and surgical procedures on the upper limb. (Uglietta & Kadir, 1989)

Reporting of the variations is not only of academic and clinical relevance, it can also add to the statistics and literature already available. (Patil et al., 2014)

#### **Materials and methods**

The present study was carried on 57 embalmed cadaveric upper limb specimens, which were obtained from the collection of formalin preserved 27 upper limb specimens and 15 upper limbs of cadavers, being utilised for routine dissection by the undergraduate students in the Department of Anatomy and Neurobiology, College of Medicine and Health science (COMHS), National University of Science and Technology (NUST), Sultanate of Oman, during a study period of 3 years.

These specimens and cadavers were obtained from the anatomy lab of medical school of West Virginia University (WVU) United States of America (USA). The cadavers and upper limb specimens were dissected in anatomy lab of WVU, and later sent to College of Medicine and Health sciences, NUST.

None of the cadavers and prosected limbs studied, had any pathological lesions, traumatic lesions or surgical procedures in the upper limb.

The arteries in the upper limb were cleaned appropriately and their course, relations and branches were studied in detail and the variations were noted. Photographs were taken under good lighting, using digital camera and were labelled later.

Approval for conducting the research was taken from the Research and Ethics Committee, of the college.

#### Results

In the 57 upper limbs studied, variations were observed in 4 upper limb specimens.

In an upper limb specimen of the right side, a branch (superficial brachial artery) arose from the brachial artery that coursed laterally in the arm crossing the median nerve. After entering the cubital fossa, this branch divided into radial and ulnar artery. Both the radial and ulnar artery had a superficial course in the forearm. The ulnar artery passed superficial to the superficial flexors, and continued as the superficial palmar arch in the hand. The radial artery coursed superficial to the brachioradialis muscle and had a normal course in the hand.

The brachial artery after entering the cubital fossa continued as the common interosseous artery, which later divided into anterior and posterior interosseous arteries. All the recurrent branches arose from the common interosseous artery. The median nerve crossed the brachial artery in the lower third of the arm. (Figure 1)

In another specimen of the left side, the brachial artery was tortuous and superficial to the median nerve, termed as superficial brachial artery (SBA). It crossed the median nerve and descended on the lateral side of the arm, it later entered the cubital fossa where it divided into the ulnar and radial artery, their courses were normal later in the forearm.

The common interosseous artery which usually arises from the ulnar artery arose from the radial artery, it later divided into the anterior and posterior interosseous artery. (Figure 2)



**Figure 1.** BA=Brachial artery; SBBA= Superficial branch of brachial artery; MN= Median nerve; SRA= Superficial radial artery; SUA= Superficial ulnar artery. SBBA arising from the BA and coursing laterally in the arm crossing the MN. After entering the cubital fossa, the SBBA divided into SRA and SUA. BA after entering the cubital fossa continued as the common interosseous artery.



Figure 2. Brachial artery is tortuous and superficial to the median nerve. Common interosseous artery was seen as a branch of radial artery in this specimen.



**Figure 3.** BA=Brachial artery; RA= Radial artery; SUA= Superficial ulnar artery; CIO=Common interosseous artery. The ulnar artery after originating from the BA in the cubital fossa had a superficial course in the forearm i.e. SUA. The CIO arose as a branch of the RA in the cubital fossa, the radial artery had a normal course in the forearm.

Similar variation was seen in another limb of the left side, where the brachial artery was superficial to the median nerve, in the arm. But in this limb the brachial artery was not tortuous and the common interosseous artery arose from the ulnar artery as is seen generally.

In another right sided limb specimen, the ulnar artery after originating from the brachial artery in the cubital fossa had a superficial course in the forearm, termed as superficial ulnar artery. It descended superficial to the pronator teres, flexor digitorum superficialis and flexor carpi radialis muscles in the upper two thirds of the forearm and in the distal one third, followed a typical course for the ulnar artery. At the wrist it was accompanied medially by the ulnar nerve and the tendon of the flexor carpi ulnaris, it then traversed the flexor retinaculum, and continued across the palm as the superficial palmar arterial arch. The common interosseous artery arose as a

branch of the radial artery in the cubital fossa, the radial artery had a normal course in the forearm. (Figure 3)

#### Discussion

Anomalies of the upper limb arteries are seen to be more common. This has been assumed due to their multiple and plexiform sources, the temporal succession or emergence of principal arteries, anastomoses and periarticular networks and functional dominance followed by regression of some paths. (Gujar, et al., 2014)

Rodrrguez-Niedenfuhr et al (2001) defined superficial brachial artery as the brachial artery coursing in front of the median nerve. If the superficial brachial artery branched at elbow level into radial and ulnar arteries, coexisting with a normal brachial artery that continues as the common interosseous trunk it was termed as superficial brachioulnoradial artery. The prevalence of superficial brachial artery noted in their study was varying from 0.2-25%, whereas the total incidence of superficial brachial artery was 2 out of the 384 upper limbs (0.52%).

In present study the superficial brachial artery was seen in 2 limbs, one of them followed the course of the superficial brachioulnoradial artery.

Neelamjit Kaur et al. (2011) in their case report also reported similar course of the brachial artery as seen in our case.

Wadhwa et al. (2008) reported a constellation of multiple upper limb anomalies with unilateral muscular, neural and arterial variations. The arterial variation noted in their case was, the division of the superficial brachial artery into radial and ulnar arteries in the cubital fossa. The deep branch of brachial artery continued as the common interosseous artery, but only the ulnar artery had a superficial course in the forearm.

In another study done on 130 upper limbs, superficial radial and superficial ulnar artery arose from the bifurcation of a superficial brachial artery in the cubital fossa but in their case the normal or deep brachial artery was not seen. (Kachlik, et al., 2011).

Variations in the origin of common interosseous artery have also been reported. In one of the limbs in the present study, the superficial brachial artery was seen tortuous in its course and the common interosseous artery arose from the radial artery and not from the ulnar artery as is seen normally.

The incidence of origin of common interosseous from radial artery is uncommon.

Jayakumari et al (2006) noted that in addition to the presence of double brachial artery seen in their case, they also noticed the common interosseous arising from the radial artery.

In a study done on 102 upper limb specimens, the common interosseous artery arising from radial artery was seen in 12.7% cases and the superficial ulnar artery in 1.8% cases. (Baral & Koirala, 2012).

Similar origin of the common interosseous artery from radial artery was also reported by Udayavar (2004).

Arole et al (2016) reported in their case, the presence of a superficial brachial artery arising from the axillary artery, which later continued as the superficial ulnar artery, the main brachial artery continued as the radial artery, and the common interosseous artery arose as a branch from the radial artery. In one of the limbs we noticed that the superficial brachial artery was tortuous in the arm. Presence of tortuous brachial artery has been reported earlier, and is noted to be less common than a tortuous radial artery.Due to its larger diameter the tortuous brachial artery does not seem to cause problems clinically. (Sirisha, et al., 2015)

The term superficial ulnar artery is applied to an artery which arises from the axillary, brachial or superficial brachial arteries and courses over the origins of the superficial forearm muscles to join at the midlevel of the forearm with the ulnar artery, sometimes replacing it. (Reddy & Vollala, 2007)

In the present study we found two limbs where the ulnar artery after originating from the brachial artery had a superficial course in the forearm, and since only the ulnar artery had a superficial course it cannot be termed as a brachioulnoradial artery as described by Rodriguez-Niedenfuhr et al (2001).

The prevalence of the superficial ulnar artery is reported to be 0.7–9.4% and the incidence of a combined superficial radial artery and superficial ulnar artery is far less seen. (Kumka and Purkiss, 2015).

The superficial ulnar artery can course over the forearm flexors either after passing over the antebrachial fascia or under the antebrachial fascia a subcutaneous position, crossed by the median cubital vein. It reaches the lateral border of the flexor carpi ulnaris either at the midforearm level or after passing deep to the palmaris longus.(Reddy and Vollala, 2007).

In the present study the superficial ulnar artery was seen superficial throughout the forearm, passing above to the tendon of flexor carpi ulnaris muscle.

In an analysis of intraoperative findings during harvest of ulnar free forearm flap for head and neck reconstruction, aberrant superficial ulnar artery was observed in 5 of 322 (1.5%) cases.(Hakim, et al., 2014).

Casal D et al, (2012) reported superficial brachioulnar artery, found bilaterally where the superficial brachioulnar artery originated at midarm level from the brachial artery, pierced the brachial fascia immediately proximal to the elbow, ran over the pronator teres muscle subsequently it ran deep to the palmaris longus muscle and later was lateral to the flexor carpi ulnaris muscle.

Of all vascular surgical procedures, approximately 4% of upper extremity ischemia is seen to require surgical intervention. Apart from occlusive disease in the upper extremity, presence of emboli, trauma (iatrogenic or noniatrogenic), collagen vascular disease, and thromboangiitis obliterans is found to be more common distal to the axillary artery.(Roddy et al., 2001)

The clinical use of brachial artery for diagnostic and curative treatment of many diseases, such as chronic renal failure, coronary artery disease, aortic and peripheral vascular disease, and also for use in interventional radiology is well documented. In individuals undergoing arteriography of the brachial artery unexpected complications can be noted in asymptomatic patients with arterial anomalies. (Jo, et al., 2005)

Uneven tortuous course of superficial brachial artery in relation to the median nerve as noted in one of the limb in the present study, may lead to compression of the median nerve in the arm. The clinician may confuse it with compression that usually is caused by more common causes, such as radiculopathy and carpal tunnel syndrome or pronator teres syndrome. The superficial tortuous brachial artery may be mistaken subcutaneous veins during cannulation and can lead to limb ischemia. (Wadhwa et al., 2008) During harvesting ulnar free forearm flap presence of superficial ulnar artery can lead to failed flap raising. Even during harvesting the radial forearm flap, if circumferential incision of a large skin paddle encounters the superficial ulnar artery running underneath it might be injured thus the perfusion of the whole hand can be at risk. (Hakim et al., 2014)

Careful palpation or by using vascular doppler, the presence of superficial ulnar artery and its course can be diagnosed preoperatively in order to prevent accidental division of these vessels.(Ghosh et al., 2016)

Cases of superficial arteries being mistaken for veins are also reported, such an error can lead to intra-arterial injections, (Senanayake, et al., 2007) misinterpretation of angiographic images or severe disturbances of hand irrigation during surgical procedures on the arm or forearm. (Ghosh et al., 2016)

#### Conclusion

Variations in the course of the arteries of upper limb are known to exist, and may be treated unimportant in asymptomatic patients. But knowledge of these variations are definitely indispensable for clinicians and surgeons who undertake procedures involving the vessels in the upper limb.

The lookout for such variations preoperatively by palpation for arteries passing superficially or through imaging in cases where the artery is absent or having variation in its course or branching pattern, can prevent unnecessary complications that can risk the procedure.

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# Face-to-face vs distance learning in human anatomy education: a longitudinal study of students' perspective and learning outcomes during COVID-19 pandemic

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## Abstract

On March 9, 2020 attendance classes in Italian universities were suspended due to Covid-19 Pandemic. Thanks to the fast actions put in place by Sapienza University Governance and to the efforts made by all components of the university, the face-to-face courses were turned into on-line courses in only one week. This sudden change has been an even more exciting challenge for the Faculties of Medicine, whose members were also involved in the frontline battle against the virus. Anatomy academics, recognizing the challenges as opportunities to innovate anatomy teaching, set up at the same time: a specific survey to investigate students' perspective on educational preferences and their mood; a longitudinal quantitative study to compare, for the first time in the same student's population, exam grades after face-to-face classes and after online classes. The students, although with different motivations, considered valid both modes of attendance. Exam grades statistical analysis showed that anatomy exam marks after the online course had a higher average value (statistically significant) and with an excellent correlation factor, compared to the marks obtained at the end of the face-to-face course. Considering our data as a whole, we can suggest that face-to-face classes and online classes, rather than being interchangeable education modes, should be considered as modes with different characteristics that offer different educational benefits. These advantages may have different relevance for individual students, depending on their specific needs and individual preferences. This suggests the opportunity to propose customizable courses, centered on the student's needs.

## Keywords

Anatomy education, COVID-19, computer-assisted learning, e-learning, web-based learning.

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# 1. Introduction

In the Italian universities the academic year 2019/20 began, as usual on October 1, 2019, nothing foreshadowed the advent of a pandemic, due to the SARS-CoV-2 virus.

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Following government indications (DPCM 8-3-2020 GU n. 59 8-3-2020), on March 9, 2020 schools and educational institutions were closed to students, attendance classes were suspended, so universities had to organize and implement distance learning activities in a few days.

Sapienza University of Rome governance has made available resources to support the transition from face-to-face teaching to online teaching, after just one week, on March 16, 2020. During the second semester, a very rapid infrastructural adjustment of classrooms to support the education digitalization was carried on. Professors, with a great sense of responsibility and attachment to the university institution, have responded quickly to the need for continuity of educational activities with great commitment, allowing to switch instantly the education provided face-to-face to online education. The students immediately adhered to the new educational delivery method with order and discipline. All technical, administrative, and library staff gave their full support, which was essential for the continuation of educational activities in online mode. Anatomy teaching consist mainly of "hands-on" practical experience, in which cadaveric dissection is considered the "gold standard" in anatomy education (McLachlan, 2004; Darras et al., 2018; Singal et al., 2020) has had to adapt to the emergency, trying to ensure the continuity of teaching to the students. The transformation of teaching activities in presence into online lessons made it possible to ensure the continued delivery of anatomy teaching, respecting social distancing and providing a safe learning environment, during the COVID-19 pandemic.

However, several challenges as well as opportunities were recognized by anatomy academics in this period including time, resources, technical capability, and teaching innovation that will continue to support the learning experience of our students during the COVID-19 emergency period, and will improve our existing face-to-face teaching when the situation begins to return to normality (Brasset et al., 2020; Byrnes et al., 2020; Evans et al., 2020; Iwanaga et al., 2020; Longhurst et al., 2020; Moszkowicz et al., 2020; Pather et al., 2020). According to several authors, using new e-learning platforms allows the research of increasingly rich and complex materials and stimulates the creation of knowledge-building communities (Scardamaglia and Breiter, 2004). Moreover, they call for a collaborative, reflective, and metacognitive approach to study through the comparison of the objectives and content of educational activity (Bereiter; 2004; Varisco, 2009; Alby and Zucchermaglio, 2008, 2009; Trentin, 2011; Zucchermaglio and Alby, 2016). Studies in literature state that social media as well have shown to have potential as learning environments, if developed within educational projects (Siemens and Weller 2011; Manca and Ranieri 2013). Blended learning, defined as the combination of conventional face-to-face learning and asynchronous or synchronous e-learning, is effective, complementary to traditional education for teaching human anatomy, as well (Khalil et al., 2018; Relucenti et al. 2019). Anatomical education has been greatly enriched by recent on-line accessible technological improvements in simulation and material sciences, namely 3D printing (Clifton et al., 2020), virtual interactive anatomy dissection table, and 3D reconstruction models (Moszkowicz et al., 2011, 2020).

For what concern the human and clinical anatomy integrated course of the medicine and surgery undergraduate course at Faculty of Medicine and Psychology at the Sapienza University of Rome, COVID-19 pandemic gives the possibility to compare the face-to-face teaching (used in the first semester) with on-line teaching (utilized in the second semester), in the same first-year medicine and surgery undergraduate students.

Therefore, the present study aims to examine students' perceptions, comparing both face-to-face and on-line learning, considering that anatomy successful learning may be obtained, in the post-COVID-19, through a complimentary use and integration of both educational methodologies.

#### 2. Materials and methods

2.1 The Human and Clinical Anatomy integrated course of Sapienza Medical School at S. Andrea Hospital

The human and clinical anatomy integrated course (HCA) of Sapienza Medical School at S. Andrea Hospital is divided into 3 modules (HCA1-2-3) that are spread over the first two years, lectures in the three modules are delivered by four professors. Each one of them gives lectures in all three modules, and exams are admistred by all four professors. The HCA 1 program includes the musculoskeletal and cardiovascular systems, it is delivered during the first year first-semester; the HCA 2 program includes the lymphatic, respiratory, urinary, gastrointestinal, and genital systems, it is delivered during the first year second-semester; the HCA 3 program includes tegumentary, endocrine, and nervous systems with sensory organs, the second year first semester. Lessons were delivered by both anatomy and clinical teachers, specifically in HCA 1 clinical lectures were delivered by neurosurgeons and internal medicine physicians; in HCA 3 clinical lectures were delivered by neurosurgeons and neurologists. Practicals include: attending to light microscopy laboratory to manage with glass slide recognition, attending to gross anatomy sectory room, to attend prosections ad practice dissection.

At the end of each semester, students have an oral formative examination, representing a part of the student's overall assessment for the summative examination for the *HCA* course. The assessment was conducted by the same four teachers for the entire three semesters period. The maximum mark/grade achievable in each HCA 1 or HCA 2 examination was 30/30 cum laude, which was the result of the sum of the marks attributed independently by three teachers (10/10 for each teacher, Laude corresponds to an extra point) in each examination at the end of the semester. Marks were established by the Academic Rules and Regulations for Italian Universities (the minimum pass mark is 18/30, while the top mark is 30/30 cum laude).

At Sapienza Medical School of S. Andrea Hospital, in the academic year 2019-2020, 110 students attended face-to-face (over 67% of classroom attendance) the *HCA* 1, in the October/December 2019 period. Traditionally, medical undergraduate degree students attend integrated theoretical lectures, workshops, and practical laboratory classes. During practical classes, students work in small groups under teaching tutor supervision, using cadaveric prosection, plastinated specimens, plastic models, plastic bones, radiographs, MRI and CTI scans using multimedia systems (Familiari et al., 2013) and a digital touch screen anatomy table (SECTRA®).

To facilitate human anatomy learning and teacher-student relation, one of the human anatomy teachers created a professional Facebook profile (named ProfMi-

chelaRelucenti) and a YouTube channel (named Michela Relucenti) dedicated to human anatomy topics. The Facebook profile was used by the teacher to share useful information, such as the lesson calendar (with related topics, specifying time and location), dates and location of examinations, the presence of scientific seminars of possible interest to the students, the deepening of some anatomy issues, as well as the reference to the YouTube channel (Relucenti et al., 2019).

2.2 The Human and Clinical Anatomy integrated course of Sapienza Medical School at S. Andrea Hospital: educational transition during COVID-19 pandemic

Starting with the second semester, remote/distance learning was implemented, for the same students, following Sapienza University instructions for remote/online learning published on the Sapienza website (https://www.uniroma1.it). Considering the lockdown, HCA 2 asynchronous theoretical as well as practical lessons were prepared as PowerPoint files with integrated recorded videos, explained by teachers (both voice and face were shown in each slide by one teacher, whereas the other three teachers showed only voice in each slide). Teachers uploaded lessons on a Google Drive folder shared with students at the beginning of each week from March 16 to May 25, 2020. Files were available to all the students following the course. At the beginning and the end of the semester, some lessons were delivered in synchronous mode, using the hangouts/meet conferencing tool (Google, CA, US). Practical lessons were developed basing on the contents offered by the digital anatomical table (SECTRA®). One of the four anatomy teachers (R.H.) was able to connect remotely thanks to the special permission of Sectra. Besides, the company offered 100 student licenses for free to use with the Uniview platform, so that students could access the anatomical table remotely. With these "remote-practical" lessons shown on the video, the teacher tried to guide the students on which cases to search and what to observe (they cannot edit but only browse the cases). A Professional Facebook profile and a YouTube channel dedicated to human anatomy topics remained active, being utilized by students. Almost the same students who attended the face-to-face HCA1 attended the online *HCA* 2 in the March/May 2020 period.

#### 2.3 S. Andrea hospital medical degree course students survey

Two surveys were designed and then distributed to S. Andrea students (SAS) who attended the face-to-face *HCA1* as well as the online *HCA* 2 courses. The first was a pilot survey, that provided the ground to design the second one, a multiple-choice survey. The pilot study consisted of 16 questions, some with dichotomous answers and others with open answers, involving 10 voluntarily recruited students, belonging to the Degree Courses in Medicine and Surgery. A qualitative content analysis (Graneheim and Lundman, 2004; Hsieh and Shannon, 2005; Alby and Fatigante, 2014) was performed on the open-ended questions to identify emic categories for the closed-ended questions of the final multiple-choice survey. Specifically, the final survey consisted of 17 questions (16 multiple-choice questions and 1 open-ended question), arranged in three set, each one concerning specific topics. The final survey had an initial incipit (that constitutes the informed consent, in which the questionnaire is presented, the average filling in time is communicated, the privacy regulations were

 Table 1. Students' opinion on classes usefulness.

|    | Questions  | Answers   |  |  |  |
|----|--|---|--|--|--|
| 1. | What did you find most useful on attending first-semester face-to-face anatomy classes?          | Possibility to clarify doubts, ask questions;<br>possibility of interactive discussion with<br>teacher; possibility to deepen topics covered in<br>the text; Other  |  |  |  |
| 2. | What did you find most useful on attending second-semester's online classes?                     | Possibility to clarify doubts, ask questions;<br>possibility of interactive discussion with<br>teacher; possibility to listen and re-listen to the<br>lessons; to be able to have more concentration<br>and attention; possibility to organize<br>autonomously the times of attendance of<br>classes and study; do not waste time moving<br>around; other |  |  |  |
| 3. | What did you find most useful on your independent activities on the anatomical digital table?    | Allows to visualize the topographic relations<br>between the various organs; allows to combine<br>the study of the macroscopic anatomy with<br>the microscopic one; it is possible to study on<br>images similar to those used in diagnostics;<br>other   |  |  |  |
| 4. | Have you been given clear information on how to attend the online classes?                       | Yes/no  |  |  |  |
| 5. | Was the procedure to connect and follow the online classes simple?                               | Yes/no  |  |  |  |
| 6. | Some online anatomy classes showed the teacher, others had audio only, which one did you prefer? | The one in which the teacher was also seen;<br>the one with audio only  |  |  |  |
| 7. | Justify your choice: I preferred to see teacher because  | Gestures and expressions support<br>understanding; gestures and expressions<br>support attention and interest; both of the<br>above; other  |  |  |  |
| 8. | Justify your choice: I preferred the one with audio only because                                 | Seeing the teacher is not relevant; the teacher's box covers the slides; both of the above; other   |  |  |  |

explained, a reference e-mail was provided for further questions). The first set contained questions on students' classes usefulness perception (Tab 1).

Students' opinion on overall *HCA2* course organization, their preferences and aspects to improve, were asked in the second question set (Tab 2).

Information about Covid-19 impact on students' skill an mood were asked in third question set (Tab 3).

The questionnaire was distributed as a Google Modules file to the SAS who attended the face-to-face *HCA1* as well as the online *HCA2* courses. Answers collection extended from May to June 2020. Survey participation was anonymous, voluntary, and free of charge. The final survey was completely filled by 104 students, their answers were collected as a data set and then analyzed by descriptive statistical

|    | Questions  | Answers  |  |  |  |
|----|--|--|--|--|--|
| 1. | Indicate, among those listed, which aspects<br>of the organization of the anatomy course<br>in on-line mode are particularly effective<br>for anatomy study. | Access to teaching materials (video, digital<br>anatomical table, images, slides, texts);<br>bi-weekly organization of the supply; rapidity<br>in making the course available online; listen<br>to and re-listen to the lectures; organize<br>independently the times of attendance and<br>study; do not waste time moving around; other |  |  |  |
| 2. | Would you improve something of the organization of the online anatomy course?  | Yes/no   |  |  |  |
| 3. | What would you improve?  | Open-ended   |  |  |  |
| 4. | Do you miss face-to-face classes?  | Yes/no   |  |  |  |
| 5. | Indicate, among the following, the aspect that you miss the most   | Physical use of the table and infrastructure;<br>direct interactions with the professor;<br>interactions with fellow students; other   |  |  |  |
| 6. | Do you prefer face-to-face or online classes?  | Face to face; online; I do not have a preference   |  |  |  |

Table 2. Students perspective on HCA 2 course management.

Table 3. Covid-19 impact on students' skill and mood.

|    | Questions  | Answers  |  |  |  |
|----|--|--|--|--|--|
| 1. | Have you experienced any study-related difficulties due to the Covid-19 emergency?                                   | Yes/no   |  |  |  |
| 2. | What were the biggest difficulties for you?  | Time management problems; concentration difficulty; increased anxiety; low efficiency and productivity in the study; other |  |  |  |
| 3. | From the adjectives listed below, choose<br>those that describe your prevailing mood<br>during this emergency period | Sad; industrious; Cheerful; Calm; Worried;<br>Lazy   |  |  |  |

analyses. We did not proceed with a psychometric validation of the questionnaire for the following two reasons: 1) the exploratory purpose of the study; 2) the needing to build a questionnaire to obtain information from the students of a specific course, that is the need for a situated instrument linked to their educational activities, without therefore having the purpose of generalizing its use. The research was carried out on students according to the Helsinki Declaration.

# 2.4 Marks evaluation: HCA 1 vs HCA 2

The *HCA* 1 oral formative examinations were exclusively conducted in a face-to face mode between January/February 2020 (winter session, WS), whereas the *HCA* 2 oral formative examinations were conducted using the hangouts/meet conferencing tool (Google, CA, US), according to Rules and Regulations of Sapienza University of

Rome, between June/July 2020 (summer session, SS). To assess the effect of different education methods on SAS students' examination outcome, we compared *HCA1* marks with *HCA2* marks (*HCA1* N=104, *HCA2* N=103). Statistical analysis was conducted by using the paired two-sample, two-tailed t-test. Statistical significance was established at P<0.05. The median, upper, and lower limits of the 95% confidence interval for both the difference and the mean were also considered. The mark evaluation 30 cum laude was accounted for as 31 in the informed system of the Sapienza University, and used for the statistical analysis. Correlation coefficient r was also conducted; statistical significance was established at P<0.05. The 95% confidence interval for r was also considered. The data were analyzed using MedCalc<sup>®</sup> statistical software version 19.5.3 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org; 2020).

# 3. Results

## 3.1 SAS Survey data

# 3.1.1 SAS opinion on HCA1 face-to-face and HCA2 on line classes and practicals: pros and cons

SAS student said that HCA1 face-to-face classes have several benefits: the possibility to interactively discuss with the teacher and to deepen the links with the clinical practice (43.9%), to deepen what is discussed in the books (25.8%) and to ask questions to the teacher to clarify doubts (22.7%). Some students (7.6%) also stated that attending facilitates study and provides an overview of the topic (Fig. 1).



Figure 1. Evaluation of first-semester face-to-face anatomy classes.



Figure 2. Evaluation of second-semester online classes.

In HCA2 online lessons, several positive aspects were found, as the possibility to listen to the lessons several times (21.5%) and to organize autonomously both lessons attendance times and study times (21.5%). Many students also found it advantageous not to waste time in traveling to reach university (19.3%) and to have lessons enriched with multimedia content absent books, useful to integrate study (17.2%). Some students also state that during online lessons they can be more attentive and focused (12.9%) and that these online lessons are useful to increase textbook comprehension (7.6%). (Fig. 2).

As concerns the online practicals organization, the anatomical digital table with remote access was used by 61.8% of the students who answered the questionnaire. Most found it useful and formative experience (89.7%), as it allows to combine the study of macroscopic anatomy with microscopic (50%), allows to visualize the topographic relationships between the various organs (23.1%), and allows to study on images similar to those used in clinical diagnostics (19.2%). Finally, some students believe that using the table remotely allows more time than using it in the presence (7.7%). (Fig. 3).

Regarding the technical aspect of online classes and practicals, students report ease of connection (96.1%) and clarity about online lessons organization (96.1%). The lessons in which it was possible to see the teacher on the video were preferred over those in which only audio was available to most students (84%), this preference was justified by the possibility of observing gestures and expressions of the teacher, considered elements of support of attention, and understanding. A minority of students (16%) expressed a preference for lessons only with audio, considering the video image of the teacher as an element of distraction from reading the slides, the element on which it is useful to focus.



Figure 3. The anatomical digital table usefulness.

#### 3.1.2 SAS Overall evaluation of online HCA2 organization: Preferences and aspects to improve

Students were asked for an overall evaluation of online HCA 2 organization, they expressed appreciation mainly (23.3%) for the possibility to access to a wide amount of learning materials (video, digital anatomical table, images, slides, texts), then they liked the possibility of independently organize the time devoted to class attendance and that devoted to study (21.4%). Other factors considered important were the possibility to listen to the lessons whenever you want (19.5%), but also not to waste time travelling to university (17.6%). Finally, the rapidity in making the online course available in the face of sudden emergency (11%) and the bi-weekly organization of the delivery of lessons, the same as the course in attendance (7.2%), was appreciated. In addition, students emphasize the importance of learning material availability, provided to a greater extent during the online course with respect to the face-to-face classes. (Fig. 4).

Most of the students (71.1%) state that they miss face-to-face classes. They suffer from the lack of interaction with each other (70.4%), but also the interaction with the teacher (22.2%), and the physical use of the anatomical table and infrastructure (7.4%). Only 23.7% of students express an opinion on something to improve in the online course. Among the most mentioned aspects, there is the introduction of moments of synchronous interaction with the teacher to ask questions to create more participatory lessons. Finally, when an even more direct question is asked, students respond that overall they prefer to attend face-to-face classes (51.3%), although a not low percentage prefer online classes (40.8%), and a percentage of 7.9% state that they have no particular preference.



Figure 4. Students' opinion on HCA 2 course organization, preferences and aspects to improve.

#### 3.1.3 COVID-19 impact on SAS learning skills and mood

The spread of COVID-19 that has upset the normality of Italian daily life, forcing population to social distancing and self-isolation, had an impact also on the student population.

Most of SAS (61.8%) say they have had difficulties in learning because of the COVID-19 emergency, especially difficulty concentrating (33.6%), low productivity and efficiency in learning (28.3%), time management difficulties (19.5%), increased anxiety and stress (17.7%), difficulty in finding study material (0.9%) (Fig. 5).

When SAS are asked to express their state of mind prevailing during this period, they state that they feel worried (29.6%), confirming a certain state of anxiety that also emerged in previous responses, but also that they can work at full speed (busy: 22.5%), showing the presence of initiative although, as a general mood, they are rather sad (15.4%). (Fig. 6).

# 3.2 Online vs face-to-face classes: examination marks as outcome evaluators

#### 3.2.1 SAS exam grades

In order to evaluate the effect of the education transition (from face-to-face lessons and praticals, to online lessons and practicals) due to Covid-19, SAS examination marks obtained in the WS at the *HCA1* examination, were compared with examination marks obtained in SS *HCA2* examination. SAS who attended face-to-face *HCA1* 



Figure 5. Covid-19 pandemic related study difficulties.



Figure 6. Students' mood during Covid-19 pandemic.

|                                  |                             | Exam session                    |                                 |  |
|----------------------------------|-----------------------------|---------------------------------|---------------------------------|--|
|                                  |                             | WS: HCA1 1                      | SS: HCA1 2                      |  |
| SAS number                       |                             | 104                             | 103                             |  |
| Marks/grades                     | Mean<br>95% CI mean         | 26,98 (±2,41)<br>26,51 to 27,45 | 27,84 (±2,20)<br>27,41 to 28,27 |  |
| (1011. pass=10/30)<br>max 31/30) | Median<br>Lowest to highest | 27<br>19/30-31/30               | 28<br>19/30-31/30               |  |

Table 4. Statistical analysis of SAS marks obtained in the WS (HCA1) and SS (HCA1).

WS: Winter session anatomy 1 marks, SS: summer session anatomy 2 marks.

Paired t-test WS vs SS: P=0,0014 (95%CI of the difference 0,4677 to 1,8818).

Correlation coefficient r between WS and SS, r =0.80 (P<0,0001; 95%Cl of r 0,72 to 0,86).

course and underwent face-to-face *HCA1* examination obtained a mark mean value of 26,98±2,41 (Tab 4).

SAS who attended online the *HCA2* course and underwent online *HCA2* examination, a mark means value of 27,84±2,20, which is a statistically significant higher value (P=0,0014). Median grades values (27 for *HCA 1* examination, 28 for *HCA 2* examination) were also considered and their correlation coefficient r was calculated. Its high value (r=0.8039) resulted statistically significant (P≤0,0001).

# 4. Discussion

In the context of the COVID-19 pandemic and the related changes necessary in the maintaining active the medical education field, some articles argued on the "forced disruption" of anatomy education (Pather et al., 2020); some pointed on the strengths (development of new online resources, upskilling in new technologies and resources) and opportunities (academic collaboration, working remotely, incorporation of blended learning in future curriculum development) as well as on the weaknesses (time constraints, lack of practical sessions and cadaveric exposure, issues with assessment) and threats (reduced student engagement, diminished teacher/ student relationship) of the adaptations to anatomical education (Longhurst et al., 2020); some pointed specifically on the problem of the no access to cadavers (Iwanaga et al., 2020; Singal et al., 2020); other articles underlined the need for technologies enabling communication such as video conferencing technology, collaborative tools, social media and networking platforms (Byrnes et al., 2021; Moszkowicz et al., 2020); other underlined the challenge related to teaching human anatomy to students with intellectual disabilities (Pacheco et al., 2020). On the other hand, in all the articles the undisputed usefulness of these online learning methodologies is also underlined, together with Evans et al. (2020), in medical education innovation (Byrnes et al., 2021; Iwanaga et al., 2021; Longhurst et al., 2020; Moszkowicz et al., 2020; Pacheco et al., 2020; Pather et al., 2020; Singal et al., 2020). These new learning resources will be very useful in the future post COVID-19 period when there is a return to a primarily face-to-face learning. Such integrated approach will allow personalization of learning, its increasing flexibility, supporting the learning experience of students, "developing a learning environment that caters for students with a different pace of learning and those that require multiple learning channels, as well as facilitating opportunities for deeper learning" (Ozer et al., 2017; Guy et al., 2018; Evans et al., 2020). COVID-19 pandemic related learning organization at Sapienza University of Rome, contains the basic elements described in the literature (Familiari et al., 2013; Relucenti et al., 2019; Byrnes et al., 2020; Brasset et al., 2020; Hennessy et al, 2020; Iwanaga et al., 2020; Moszkowicz et al., 2020), as well as the use of virtual reality in anatomy (Birbara et al., 2020). Few studies, in addition, analyzed so far, the students' perspectives in this pandemic context, regarding current anatomy education (Franchi, 2020; Srinivasan, 2020). Franchi (2020) is a medical student and in its article describes how the lack of practical teaching with cadavers, through dissection, can adversely impact training and lead to losses for students; this author further outlined that the learning environment was less than ideal, not allowing the development of personal and professional competencies. On the contrary, Srinivasan (2020) reported that students were satisfied with the understanding and learning of anatomy using zoom video conferencing platform (zoom Video Communications, inc., San Jose, CA). In this study, two statements were proposed to the students attending an anatomical course using zoom video conferencing platform. The first question was about the students' satisfaction with the understanding and learning of anatomy using zoom. At the response rate of 88.88% of students (n. 16), about 87.5% of students (n.14) of Singapore University were satisfied.

At the second question, concerning one way in which zoom might be enhanced further to maximize learning in anatomy, several students reflected on the need for more interaction in the e-tutorial by zoom platform using Poll Everywhere; some students also proposed the use of regular individual quizzes or mini quizzes (not counted to overall grade) to assess their own progress in learning anatomy, or the addition of transcripts to slides for further explanations.

To compare the results of the study with those present in the literature, two peculiar features of the present course must be considered: a) the number of students attending, higher than the class analyzed in the study in Srinivastan (2020); b) the current reality of the use of dissection of corpses in Italy. In our country is currently available for study and teaching a small number of corpses and the use of dissection is accompanied by the use of prosection. Dissection courses are offered mainly as an activity of the student's choice, in the form of monographic courses. These courses are attended by a limited number of students (also enrolled in the years following the first two, those in which anatomy is studied), who are often involved in the preparation of prosections, used by students of the first and second year of the course (Macchi et al., 2003; 2007; 2014). The analysis of the results of our survey shows that both modes of attendance (face-to-face and online) are considered valid by students although with different motivations. In the face-to-face lessons the immediacy of the interaction with the teacher is appreciated, especially to deepen the clinical and surgical aspects of anatomy (e.g. discussing clinical cases with the teacher) and the possibility to ask questions that are immediately answered. In the online lessons, the possibility of organizing the study time independently according to one's own rhythm and needs is greatly appreciated. Finally, a significant percentage of students find it advantageous not to waste time moving around, listening to the lessons several times, claiming to be more attentive and concentrated during the online lessons, compared to face-to-face lessons.

If in the first case, therefore, the possibility of direct interaction with the teacher is appreciated, in the second case the possibility of a more finely integrated study is appreciated, in which the frequency of lessons, the consultation of books and the use of digital teaching material are used together according to individual times and needs. This preference of the students could be an indication to be followed after the end of the pandemic emergency period, maintaining the accessibility of online teaching materials for deferred use, so as to allow learning according to everyone's personal rhythms and times. The characteristic feature of the lessons in presence, which during the online lessons has been missed the most, is the direct and immediate interaction with the teacher and with the classmates. An effort needs to be made to create and develop meaningful social interactions also in the online mode. In summary, SAS students have not shown any preferences for one of the two teaching modes. Face-to-face and online lessons are simply each one having different advantages, which can be suited to the needs of the individual student. The practical lessons carried out online have been greatly appreciated, in fact, the normal activities in presence have been effectively replaced by the activities carried out by the students using the digital anatomical table.

The analysis of the results regarding the state of mind and emotions of the students showed that the psychophysical stress suffered during the lockdown period has affected the study causing difficulties in concentration, time management, and reducing productivity. However, there was no significant conditioning in student performance. In fact, feelings of concern and anxiety, while present, have been harnessed and channeled into a hardworking attitude of commitment and work. Maintaining both a virtual attendance to the classroom and a focus on the study, from this point of view, has helped to maintain a proactive attitude, of those who have an influence on the events that affect them, having a balancing effect compared to an anxious attitude of withdrawal, more aimed at grasping the risks of a pandemic.

The analysis of the marks obtained by students in the anatomy examination shows that the marks of the examinations taken at the end of the distance learning course had a higher average value (statistically significant) and with an excellent correlation factor, compared to the marks obtained with examination in presence at the end of the course in presence.

These positive results show that in the lockdown period of the second semester, SAS have however had good learning opportunities, through the didactics delivered in online mode. It is possible that students who normally would not have attended face-to-face classes have benefited from a better access to educational activities and materials. In the analysis of the results should obviously also be considered factors independent of the didactic actions implemented by the degree courses. The inability to go out and devote time to social activities has increased the time dedicated to the study, this may have affected the best preparation and therefore hesitate in a better grade on the examination. However, the amount of time has not always been qualitatively good, in fact the students stated in the survey that they suffered from psychophysical stress that, in some cases, would have created difficulties in the concentration necessary for the study.

You may wonder if the higher grades in the summer session are due to a more permissive behavior of teachers in the conduct of the examinations at a distance than those taken in attendance. This is a possibility, however it should be noticed that the teachers who delivered the course and carry out the examinations in the second semester were the same who delivered the course and carried out the examinations in attendance in the first semester, so they probably rely on the same method of judgment. All our data analyses suggest that face-to-face classes and online classes, rather than being interchangeable modes, should be considered as modes with different characteristics that offer different educational benefits. It is possible that these benefits may have a different relevance for individual students, depending on their specific needs and individual preferences. This suggests the opportunity to propose customizable courses, centered on the student's needs. Due to the pandemic continuation, Italian universities, according to ministerial directives, are adopting from September 21 the dual mode of teaching (blended mode). In our Sapienza University we are delivering in person the anatomy course scheduled for the first semester. Students access both in person, compatibly with the necessary safety measures, and remotely with online live lessons, except for activities with mandatory in-person attendance, such as small groups laboratories. Students have access to pre-recorded lectures and off-line educational material. Students who cannot attend in person - including international and off-campus students - can attend remotely. University facilities supply the largest number of physical classrooms at their disposal with multimedia equipment; access to classrooms is scheduled to guarantee all students, regardless of the year of enrolment, the possibility to attend in person as much as possible, with particular attention to first-year students. The program calendar is optimized so that students can have continuous and non-split periods of presence in the classroom. At the same time, it is also limited the movement of students to distant classrooms/locations; to access the classroom students are asked to register through a reservation system set up for this purpose (Sapienza Prodigit software). This blended modality is meant to provide the best integration between activities that can be carried out both at a distance and in presence (classroom activities), safeguarding the practical activities that are carried out in small groups within the laboratories. Activities are carried out in compliance with the conditions of social distancing and sanitization, imposed by the COVID-19 pandemic. Further studies might explore the students' perceptions of this educational modality.

# 5. Conclusions

Once the COVID pandemic has been brought under control, it will be necessary to continue to rethink medical teaching, by implementing different teaching techniques complementary to conventional face-to-face education. Our data, referring to the Italian context, document that distance learning mode in the teaching of Human Anatomy has been perceived by most students as useful and positive. Although within the limits of an exploratory study, we have highlighted how distance learning modality, in which the lessons are more integrated with the study moments, respecting the times and the individual needs of the students.

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# The teaching of anatomy during the Covid-19 pandemic

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#### Abstract

A COVID 19 pandemic led to the closure of Mozambican schools. The government urged educational institutions to start the distance learning process during this period of sudden and unprecedented interruption. In Mozambique, 7.993.520 students at all levels were affected, with 213.9309 from higher education. This work aims to describe a teaching experience in anatomy at a private university in Mozambique.

#### Keywords

COVID-19, E-learning, Mozambique, Anatomy, Teaching.

# Introduction

In December 2019, a disease developed in a city in China quickly turned into a global pandemic leading to several countries to proclaim a partial or total state of emergency, with the closure of borders, schools and economic activities leading to a consequent increase in poverty for the most disadvantaged population.

As a way of controlling the COVID-19 pandemic, most countries have closed all schools and universities, impacting 87% of the world's student population, equivalent to 1.5 billion students in 165 countries [1].

From the 23rd of March, the President of the Republic of Mozambique announced the closure of all educational institutions.

In Mozambique, 7.993.520 students from all levels were affected, with 213.930 specifically from the tertiary level [1].

The government urged educational institutions to start the distance learning process during this period of sudden and unprecedented interruption. Distance learning is characterized by interaction at a distance between teacher and student. The teacher must be involved in receiving feedback from student [2]. *Distance learning* can be defined as a method of studying in which lectures are broadcast or classes are conducted by correspondence or over the Internet, without the student's needing to attend a school or college. Refers to a variety of programs that are away from the main campus. *E-learning* is frequently associated with activities involving computers and interactive networks simultaneously, generally involves completing a course of instruction on the internet [3]. E-learning is not only internet class, but it involves a more active learning by the student where all the teaching is given by electronic means, whether synchronous or asynchronous.

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# **Case report**

In this context, the university introduced the Teams platform to teach. Tutorials about platform were sent to lectures and students to familiarize themselves with the system, maybe it would have been helpful if the significance of the Microsoft Teams platform had been explained to students.

WhatsApp working groups were created between the Information technology department and the lectures and students to communicate the difficulties and to help with the installation of the system.

Theoretical classes and seminars have started. Practical classes are suspended. The assistants of the practical classes were placed to give theoretical classes, so as not to be without work.

The online classes are made up of the same number of students from the presential classroom. Class I of 100 students and II and III of 50 students in anatomy I and Class I and II in anatomy III, respectively, with about 50 students respectively. Each group is allowed to communicate (or work) with each other offline.

Each lecturer is required to should deposit on the platform the analytical plans and objectives of each class, the slides, the electronic books, the sheets, the questionnaires and other materials. The course coordinators monitor these classes and check whether the lecturers complied with the requirement to conduct timely updates. Each class has up to 8 supervisors to monitor the classes.

The institution opened spaces in the campus with WiFi for lectures to give classes online.

Theoretical classes of gross anatomy were taught using the TEAMS platform. The practical anatomy classes this way is a challenge. At this stage there were no practical classes. No anatomy laboratory component adapted. Lecture/theory sessions are often given online.

Lectures and students, very quickly, without a transition process, were forced to change and adapt to teaching and learning strategies.

As determined by the country's educational authorities, there was no continuous assessment, because there were many difficulties for students to access the internet, and at the end of the semester an online theoretical exam was taken.

## Comments

The practical classes modules will be made after the President of the Republic authorizes the commencement of classes. The themes were grouped in large devices, such as digestive, respiratory, nervous system, etc, for anatomy III and osteology of the upper and lower limbs for anatomy I.

During the classes and in the Whatsapp groups of the classes, it was possible to listen to the students' opinions. The online classes brought several difficulties for students and lectures: 1) Lack of knowledge of online platforms by lectures and students. 2) Need to have a computer with microphone and camera to teach classes by lectures or for students to present on topics in seminars. These tools only exist on laptops. On desktop computer, it is necessary to associate a mobile device to talk to students, spending double on the internet. 3) Impossibility to teach with a cell phone. 4) Need to have good quality Internet and the ability to teach classes with high consumption of megabytes.

Difficulties of lecturers: 1) Lack of knowledge of online platforms by lecturers and students. 2) Teach without seeing students. 3) Students do not answer questions immediately, taking up to 2 to 3 minutes. 4) Less and less students in class. 5) Not being able to schedule absences or make assessments as directed by the institution. 6) Lecturer's demotivation. 7) Difficulties to access the platform. 8) Teams platform consumes a lot of internet. 9) Some lecturers did not teach classes due to financial difficulties to support the internet.

Difficulties of students: 1) Students' lack of knowledge of online platforms. 2) Absence of an android application phone to receive classes. 3) Financial inability to buy a good quality phone. 4) Not all neighborhoods in the city of Maputo have a good mobile telephone network and access via optical cable from TV Cable. 5) Many students traveled to their home provinces, where the connection is poor. 6) The internet network fluctuates, making it difficult to attend the class. 9) Lack of motivation for studying alone and without seeing the lecturer.

#### Perspective

Tests were cancelled for this semester. But not seeing the end of COVID, we have to think of ways to give practical classes to students, probably using anatomy software. But this software is very expensive and institutions in poor countries usually do not have the capacity to acquire these platforms. In the same way that several online courses on various topics have been released all over the world, we propose that this software also be released at this time of pandemic, but only inconvenience in online courses would be the English language, because students do not speak English. A study in the UK and Ireland showed that 43% of universities started using digitized cadaveric resources and 3D virtual anatomical platforms were in an attempt to emulate canceled practical sessions [3].

#### **Competing interests**

The authors declare no competing interest.

# **Authors' contributions**

Mahomed Sidique Abdul Cadar Dadá: Teacher of anatomy. Writing the article Abdul Habib Dadá (anatomy monitor). Help with draft, correction and looking for students' problems

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# Study of hippocampal size and age

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#### Abstract

Objective (or background): The hippocampus is a thoroughly studied structure of the temporal lobe. In contrast to our current knowledge of hippocampal anatomy, neurophysiology and pathophysiology, scientific literature on the relationship between the hippocampal size and age is limited. Our study aims to further the understanding of this relationship. Methods: 16 hippocampi were anatomized, photographed, measured and analyzed in comparison to age and gender using Pearson and bootstrap analyses with IBM SPSS®. Results: The results for all three independent variables of size, age and gender were not statistically significant. Conclusions: We were unable to show a statistically significant result on the correlation between the size of the hippocampus and age due to small sample size.

Keywords

Hippocampus, hippocampal atrophy, aging.

Abbreviations list: cornu ammonis (CA), dentate gyrus (DG)

# Introduction

The hippocampus is a thoroughly studied structure of the temporal cerebral lobe<sup>1</sup> due to its key role in the systems involved in learning, memory, emotional behavior, motor and homeostatic control.<sup>2</sup> It inferiorly borders the temporal horn of the lateral ventricle,<sup>3</sup> resembling a seahorse, and is composed of a head with hippocampal digitations, a body and a tail, both with fimbriae. It is anatomically connected medially, ventrally and posteriorly to the fornix, and inferolaterally connected to the parahippocampal gyrus through the subiculum. It contains a bilaminar internal structure comprised of the cornu ammonis (CA) and dentate gyrus (DG). Cornu ammonis can be subdivided into four structures according to its display of pyramidal neurons, namely from CA1, which is continuous with the subiculum, to CA4, which is the nearest region to the DG.<sup>2</sup>

The hippocampus is susceptible to different means of damage and, among them, epilepsy, hypoxia, ischemia, and encephalitis are associated with amnesic effects.<sup>1</sup>

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Cardiovascular disease, vascular risk factors, diabetes, obesity, obstructive sleep apnea, and psychiatric disorders, among others, are factors associated with hippocampal atrophy with increasing age.<sup>4,7</sup> Moreover, pathologies of the brain related to a loss of hippocampal function, such as Alzheimer's disease and dementia, are also associated with atrophy of the hippocampus and its internal structures.<sup>4,5,6</sup> The rate of hippocampal atrophy in cognitively normal individuals increases with age, which substantially increases onwards from midlife.<sup>9</sup>

In contrast to our current knowledge of hippocampal anatomy, neurophysiology and pathophysiology, scientific literature on the relationship between the hippocampal size and age is limited.<sup>5</sup> Our study aims to further the understanding of this relationship.

## Methods

The available and relevant clinical information about the cadaveric specimens, sex and age, were collected from the national network of services of death verification in Brazil.

Anatomization: We dissected 8 brains that were set in formaldehyde for 2 days from individuals of variable age and sex. We isolated each hippocampus from the adjacent cortical structures by starting with an incision with a scalpel on the ipsilateral superior temporal sulcus, between the superior and middle temporal gyri, reaching the temporal horn of the lateral ventricle. Then, we extended the cut anteriorly and posteriorly, following the ventricle and avoiding damage to the hippocampus. We resected the middle and inferior temporal gyri up to a few centimeters anteriorly to the preoccipital notch, followed by the partial resection of the fusiform gyrus. After removing the choroid plexus in the temporal horn (Figure 1), we identified the borders of the hippocampus for its resection: the hippocampal fimbriae as a medial boundary for both the body and the tail, and the collateral eminence as the lateral boundary. Then, we separated the hippocampus and parahippocampal gyrus from the superior temporal gyrus anteriorly, the fornix posteriorly, and from the remaining fusiform gyrus at the collateral sulcus. The parahippocampal gyrus was digitally removed from the image of the hippocampi during the image processing step, which is explained as follow.

Photography: A camera with 12 megapixels was set up to take a picture of the resected hippocampi individually from a distance of 20cm perpendicular from its superior surface. Each image was processed with a computer in order to trace each hippocampus and substitute the background of the image with a millimeter grid (Figure 2).

Measurement: The millimeter grid was used in order to measure the hippocampal size (mm<sup>2</sup>), subtracting the area not covered by the hippocampus from the total area of the background grid.

Analysis: We analyzed the hippocampal size of 8 individuals, both right and left independently, thus 16 samples in total. We then analyzed these 16 samples in comparison to age and sex, using Pearson analysis and Bootstrap analysis utilizing IBM SPSS®. A constant code was given to each side (1 - right, 2 – left) and sex (1 – male, 2 – female), while the variables were assigned as age (from 26 years to 81 years) and size (from 36mm<sup>2</sup> to 453mm<sup>2</sup>).



Figure 1. Picture of exposed hippocampus, after dissection of temporal lobe gyri and removal of choroid plexus.

## Results

The collected hippocampi and their respective sizes, side (right or left), age and sex are shown on Figure 3.

Two types of analysis were conducted to evaluate the results of the relationship between the hippocampal size, age and sex - Pearson correlation and Bootstrap analysis.

Pearson analysis (Table I) shows the correlation between size and each side of the hippocampus, sex and age (-0.25, 0.064, -0.163 respectively). All three correlations were not significant (p>0.05).



Figure 2. Picture of hippocampi over the millimeter grid background.



# Hippocampal size

Figure 3. Graph of hippocampal size (mm2) in regard to age, sex and side.

To confront the sample size limitation, we used Bootstrap analysis (Table I). Up to 1,000 data sets were created for comparing two independent samples based on the 16 hippocampal samples. The 16 hippocampal samples comprise of both right and left

|      |                        |                            |       | Side   | Sex                 | Age    |
|------|------------------------|----------------------------|-------|--------|---------------------|--------|
|      | Pearson correlation    | 1                          |       | -0.025 | 0.064               | -0.163 |
|      | Sig. (2-tailed)        |                            |       | 0.927  | 0.814               | 0.547  |
| 0.   | Bootstrap <sup>d</sup> | Bias                       |       | -0.038 | -0.015 <sup>e</sup> | -0.003 |
| Size |                        | Standard Error             |       | 0.274  | 0.177 <sup>e</sup>  | 0.342  |
|      |                        | 95% Confidence Interval Up | Lower | -0.653 | -0.332 <sup>e</sup> | -0.806 |
|      |                        |                            | Upper | 0.437  | 0.389 <sup>e</sup>  | 0.548  |

**Table 1.** Pearson Correlation and Bootstrap analysis.

d. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples.

e. Based on 991 samples.

sides of the hippocampus of each individual. The results for all three independent variables were similar to the Pearson correlation and no statistically significant result was found.

## Discussion

The standardized approaches to dissection and image collection were essential to avoid artifacts in our dataset.

As the hippocampal formation is very complex, it was crucial to have careful attention to its gross anatomy and boundaries during resection. The lateral approach that was used in this study is a useful route to reach the lateral and superior aspects of the hippocampus, which is presented as a C-shaped bulging structure in the temporal horn of the lateral ventricle.<sup>2,3</sup> To reach the hippocampus without damaging it during anatomization, the initial, careful approach to the temporal horn from the superior temporal sulcus was an essential step. After successfully reaching the temporal horn, the excision of the middle and inferior temporal gyri could be done with proper attention to the ventricle space in order to prevent damaging the structures of interest. The expanded access to the hippocampus with the resection of the gyri permitted the clear view of the borders of interest for the separation of the hippocampus and parahippocampal gyrus from the adjacent structures.

The hippocampus is located superiorly to the parahippocampal gyrus, which is composed of the subiculum posteriorly and the uncus and entorhinal cortex anteriorly.<sup>2</sup> The subiculum is continuous with the CA1 field,<sup>10</sup> therefore it would be difficult to separate them physically using only the hippocampal sulcus as a landmark for separation.<sup>2</sup> From a superior view, the subiculum can be seen medial to the hippocampus, hence it was the most evident part of the parahippocampal gyrus to be digitally removed during the background replacement step. Such digital removal of the parahippocampal gyrus, rather than the physical removal, was permitted by the technique of photography, which was taken exactly 20cm from the superior surface of the hippocampus. Hence, structures inferior and lateral to the hippocampus would not interfere with the measurements.

Hippocampal atrophy is associated with aging, multiple risk factors and pathologies, making it difficult to distinguish between the causes of atrophy in a given sample. It is also possible that a preclinical stage of pathology leading to the atrophy of the hippocampus are not detected in seemingly healthy patients.<sup>9</sup> A limitation of the present study is the unavailability of the clinical history of the cadavers. Such information would help distinguish the factors that would cause atrophy in the samples.

The results of the analysis demonstrated no statistical significance between hippocampal size, age and sex, so it is difficult to make any assessment based on these results without further samples. The results do show a trend towards a smaller hippocampal size with increased age, but one out the 8 patients dissected had an unusually large hippocampal size at an older age. Larger hippocampus and decreased atrophy could be explained by other variables, such as education level and physical activity.<sup>8</sup> Perhaps more samples would improve the significance of the association between age and the decreasing size of the hippocampus.

# Conclusion

In our research we were unable to show a correlation between the size of the hippocampus and age due to a small sample size. The insignificance of the results was due to one sample that was unusually large for its older age, and, without it, there appears to be a trend towards a decreased size in hippocampus as age increases. More samples might need to be obtained in order to reach significant results.

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# Da Vinci's Vitruvian Man, Golden Ratio and Anthropometrics

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# Abstract

Objectives: Divine golden ratio (Phi = 1.618) observed in several human body parts such as, heart, dentition and upper limb tends to present homeostasis in anatomy and physiology. This cross sectional survey study investigates the relationship of golden ratio with BMI, GPA, gender and blood serotypes in Jordan.

Methods: Demographic features including GPA, body weight, lengths of some body parts and ABO/Rh blood groups were measured for 380 undergraduate students of matched age (19.14±0.76) and matched sex ratio. Navel-Foot/Height, Forearm-Hand/Armpit-hand and BMI were calculated. Golden ratio fitness was calculated based on phi and standard deviation of collected data.

Results showed significant gender differences in the means for all body lengths measured, GPA, BMI, and calculated ratios. Golden ratio fitted bodies students were 40.5% (Females > males). The overall prevalence of overweight and obesity was 15% (females < males), while 80% (females > males) have normal weight. Among the overweight and obese students 77.2% (44/57, females less than males) lies outside the golden ratio. The overall prevalence of GPA grades above good were 51.3% (females > males) and among them 47.7% (93/195, females > males) fit golden ratio while 67.2% of the good or below GPA grades (121/180, females < males) did not fit golden ratio. No significant difference between ABO-Rh system related to gender or golden ratio fitness. Statistically, a significant association was found between golden ratio and the three parameters BMI, GPA and gender.

Conclusions: Students who are golden ratio fitted bodies were more likely to have better BMI and GPA especially females.

## Keywords

Vitruvian man, Golden ratio, ABO serotypes, Rh factor, BMI, Anthropometrics.

# Introduction

Vitruvian man the iconic drawing by Leonardo da Vinci which represent the art and science of human body relationships and proportions as it translates Marcus Vitruvius Pollio work and ideas of golden ratio in human body (Figure 1). The embedded text in the iconic drawing of Leonardo Da Vinci's describes the relationship between different proportions of different human body lengths (Maloney and Fried, 2011; Gielo-Perczak, 2001). Gyorgi Doczi, an anatomist who suggests the golden ratio as the ideal proportion in human body lengths (Doczi, 1981).

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In mathematics, two quantities are in golden ratio if their ratio is the same as the ratio of their sum to the larger of the two quantities (Livio, 2008). Golden ratio or Phi ( $\varphi$ ) constant (1.618) observed in the universe suggests a role in balance and equilibrium as it were associated with esthetically and healthy bodies' (Jefferson, 2004). In human body, golden ratio or divine anthropometric was observed in DNA, heart, dentition and musculoskeletal mechanics as well as in upper limb measures which tends to present physical and biological homeostasis (Persaud and O'Leary, 2015; Yalta et al., 2016; Livio, 2008; Wang et al., 2017).

Golden ratio fitness represent human body parts in harmony and aesthetics as such, anatomy of human body represented by anthropometric measures has been investigated for potential link with golden ratio at different levels such as coronary blood vessels, genome, and hand skeleton (Persaud and O'Leary, 2015). Several factors influence human body anthropometrics (Anatomy) such as, age, gender, health status, and environment and consequently these factors have impact on the golden ratio (Katzmarzyk and Leonard, 1998).

The Phi ( $\varphi$ ) constant (1.618) has been investigated for its association with many anthropometric measurements such as BMI and Height, in order to increase our understanding of development, growth, forensic medicine, physiotherapy, pathology, dentistry, medical imaging and many other applications in human life (WHO, 1995; Utkualp and Ercan, 2015; Livio, 2008; Saraswathi, 2019). A study suggest a golden ratio role in potential prediction of health problems based on anthropometric measures of the face (Saraswathi, 2019). However, a review of the evidence presented by investigations in different populations for the role of the Phi in anatomy and physiology were mixed (Iosa et al., 2018).

BMI is a worldwide adopted measure used to represent body fat composition which can be a predictor of health risk based on the distribution of body mass over a square of body height. BMI was classified into four grades; underweight (<18.5), normal (18.5 – 24.9), over weight (25 – 29.9) and obese (30) (WHO, 2004), however, its cut off points is different among different populations (Wildman et al., 2004; Misra, 2015). BMI value was observed to be influenced by sex, age and region (Perissinotto et al., 2002; Meeuwsen et al., 2010; Madanat et al., 2011; Madanat et al., 2007).

ABO and Rhesus (Rh) blood serotypes are key features in blood transfusion and are considered as a significant polymorphic and immunogenic system. ABO genotypes are polymorphs of three major alleles (A, B, O) which results in several phenotypes, while Rh system consisted of two structural genes; D and CcEe genes. Rhpositive individuals inherit one or two RHD genes, which result in expression of RhD antigen and are typed Rh-positive (Yamamoto et al., 1990; Avent and Reid, 2000).

ABO and Rh blood serotypes distributions exhibit variations based on population, time, and region (Liu et al., 2017; AlSuhaibani et al., 2015). In Jordan, A phenotype has the highest percentage while the AB phenotype has the lowest percentage (Hanania et al., 2007). Moreover ABO/Rh groups have been linked to several factors in humans such as, intelligence quotient (IQ) (Sarvottam et al., 2018), diseases (Franchini and Lippi, 2015) and behavior (Tsuchimine et al., 2015). For example, A-antigen containing groups were associated with higher levels of BMI, LDL, VLDL, triglycerides, cholesterol and blood pressure compared with other blood groups of patients with hypertension (Arora et al., 2018). Males with O blood group has a higher bone density than other blood groups (Davidson et al., 1990). Additionally, variations between



Figure 1. Leonardo da Vinci's drawing of Vitruvian man and body dimensions (Gielo-Perczak, 2001).

ABO groups was reported regarding bone density using lumbar and femoral bone densitometries (Aghighi et al., 2017).

The main aim of this study is investigating the association between human body parameters; ABO blood group, Rh factor, body mass index (BMI), gender, grade point average (GPA) and the golden ratio described by the Vitruvian man proportions among Jordanian undergraduate students.

# Methodology

This cross sectional study had been approved by the Institutional Review Board (IRB) at The Hashemite University in March 2019. A sample consisted of 380 undergraduate students aged 18-20 years of both sexes were recruited from the Hashemite University in April-June 2019. Aims, objectives, techniques, risks and benefits were explained. Security and confidentiality of collected data were warranted. Participants were asked kindly to read and sign consent form. Demographic features as, age, sex, GPA and study year were collected using a short survey. Body dimensions of different body parts including upper and lower limbs were measured and used to calculate two ratios (Figure 1) described by Leonardo da Vinci in his Vitruvian man drawing; one is based on human height and the other was based on human width for comparisons (Table 1 and 3). Calculated ratios were further classified into golden or not golden ratios fitness using a range of golden phi ( $\varphi$ ) (0.618) within one standard deviation (Gielo-Perczak, 2001) (Table 3). Body height and weight were measured using measuring tape and electronic scale to the nearest of 0.1cm and 0.1kg, respectively. Height was measured without shoes and students were standing in the standard anatomical position. BMI (called BMI-H) was calculated using the traditional Adolphe Quetelet equation: BMI = Weight (Kg) / Height<sup>2</sup> (m<sup>2</sup>). In this study a new formula for BMI index (called BMI-W) was proposed using a ratio of weight distributed over the Leonardo da Vinci square of Vitruvian man (i.e. height multiplied by open hand width) calculated using the formula: BMI= weight (Kg) / (Height\*Width) (m<sup>2</sup>). BMI for both calculated ratios (H and W) were classified into four grades; underweight (<18.5), normal (18.5 – 24.9), over weight (25 – 29.9) and obese (30 or more) (WHO, 2004). Blood serotypes (ABO and Rh) were determined using red blood cells agglutination test, finger-puncture blood samples were collected on slides and Anti sera-A, Anti sera-B, and anti-D antibodies were added.

Collected data for body-parts lengths and demographic features were summarized as means and standard deviations. Two ratios of the human body proportions described by Leonardo Da Vinci in his famous drawing the Vitruvian were calculated for all participants and summarized as means and standard deviations (Gielo-Perczak, 2001) (Table 1). The two calculated ratios, one is based on human height and the other based on human width, were classified either golden or not golden ratios; a ratio that falls within the range of phi ( $\varphi$ ) constant (0.618)  $\pm$  1 standard deviation of the corresponding calculated ratio were considered fitting the golden ratio (Gielo-Perczak, 2001). Ratios calculated:

- 1- Navel-Foot length / Head-Foot length (based on height)
- 2- Forearm-Hand length / Armpit-Hand length (based on width).

In addition, some of the ratios stated by Da Vinci in his Vitruvian man drawing were also calculated (Table 3):

"The greatest width of the shoulders contains in itself the fourth part of the man. Elbow to the tip of the hand will be the fifth part of a man. Elbow to the angle of the armpit will be the eighth part of the man. The whole hand will be the tenth part of the man."

# Statistical Analysis

Statistical analysis of collected data were done using Microsoft Excel and SPSS statistical package version 21. Data collected were expressed as means and standard deviations (SD). Descriptive and inferential statistics were used to answer the research questions. Groups were compared using parametric and nonparametric tests accordingly. Significant difference was set at a p-value less than 0.05.

## Results

#### General features (Table 1)

A total of 380 students with a mean age of  $19.14 \pm 0.76$  were participated, 51.6% (196/380) were females and 48.4% (184/380) were males. Anthropometric measures showed that males were taller, wider, and heavier than females. On the other hand, females have higher GPA, better golden ratio and lower BMI than males. Statistical analysis showed a significant difference between females and males students in the means of weight, height, open hand width, head-navel length, navel-foot length,
| Anthropometric and Demographic Feature | Female (n=196)<br>(Mean ± S.D) | Males (n=184)<br>(Mean ± S.D) |
|--|--------------------------------|-------------------------------|
| Weight*                                | $58.30\pm12.45$                | $74.90 \pm 14.75$             |
| Height*                                | $160.48\pm5.92$                | $175.69 \pm \ 6.54$           |
| Open Hand Width*                       | $163.34\pm6.78$                | $180.88\pm7.48$               |
| Head Navel*                            | $63.79\pm3.31$                 | $70.72\pm3.46$                |
| Navel Foot*                            | $96.69 \pm 4.19$               | $104.97 \pm 4.80$             |
| Hand*                                  | $17.75\pm0.92$                 | $19.59\pm1.07$                |
| Forearm*                               | $42.91 \pm 2.31$               | $45.23\pm2.99$                |
| Armpit*                                | $72.36\pm3.46$                 | $79.10\pm4.07$                |
| Age§                                   | $19.08\pm.74$                  | $19.20\pm.78$                 |
| BMI-H*                                 | $22.64 \pm 4.62$               | $24.24\pm4.42$                |
| BMI-W*                                 | $22.22\pm4.42$                 | $23.54 \pm 4.24$              |
| Navel-Foot/Height (Vertical)*          | $0.603\pm0.014$                | $0.596\pm0.014$               |
| Forearm-Hand/Armpit-Hand (Horizontal)* | $0.593\pm0.024$                | $0.572\pm0.029$               |
| GPA <sup>&amp;</sup> (Median)          | 3                              | 2                             |

 Table 1. Descriptive and Inferential Statistical Summary of Anthropometrics Measures, Demographic Feature and Golden Ratio Fitness Data (n=380).

| Variable                              |              | Female (n=196)<br>(Frequency) | Males (n=184)<br>(Frequency) |  |
|---------------------------------------|--------------|-------------------------------|------------------------------|--|
| Golden Ratio Fitness <sup>&amp;</sup> | Yes          | 99                            | 55                           |  |
|                                       | No           | 97                            | 129                          |  |
| Rh Factor                             | Rh+ve        | 175                           | 163                          |  |
|                                       | Rh-ve        | 21                            | 21                           |  |
| Blood Group#                          | А            | 78                            | 60                           |  |
|                                       | В            | 39                            | 42                           |  |
|                                       | AB           | 13                            | 6                            |  |
|                                       | 0            | 66                            | 76                           |  |
| GPA <sup>#</sup> &                    | Satisfactory | 10                            | 37                           |  |
|                                       | Good         | 63                            | 70                           |  |
|                                       | Very Good    | 73                            | 58                           |  |
|                                       | Excellent    | 47                            | 17                           |  |
| BMI-H <sup>#&amp;</sup>               | Underweight  | 16                            | 3                            |  |
|                                       | Normal       | 161                           | 143                          |  |
|                                       | Overweight   | 11                            | 27                           |  |
|                                       | Obese        | 8                             | 11                           |  |

\*: T-test (2-tailed), p < 0.05.

<sup>§</sup>: Mann-Whitney test (2-tailed), p = 0.103.

<sup>&</sup>: Pearson Chi-Square test, p value < 0.05.

\*: Dichotomies grouping.

hand length, forearm length, armpit length, BMI-H (Means or Category), BMI-W (Means or Category), and GPA (Median or Category), while there were no significant differences between males and females mean age (Table 1). Blood group O and A (37.3% and 36.3%) were the most prevalent and 88.9% of students were Rh+ve. ABO and Rh frequency distributions showed no significant difference between females and males statistically (Table 1). Interestingly, results showed that only 23 students out of the 380 students have their height equal to their open hand width while the majority of students have higher width than height (295/380).

The overall prevalence of golden ratio among all students was 40.5 % (154/380) (99/196 females and 55/184 males). Data showed that males who did not fit the golden ratio were 70.1% of all males and 49.5% of all females did not fit golden ratio (Table 1). Comparing frequency distribution of female and male students based on golden ratio fitness revealed a significant differences (Pearson Chi-Square (F/M); p = 0.001, OR = 2.39, 95% CI: 1.57– 3.65). Females were more likely to fit golden ratio than males (OR 2.39).

#### BMI distribution relative to golden ratio

Results showed that the mean value of BMI-W was significantly lower than the mean value of BMI-H (T-test, p = 0.001). This difference between both BMI means is caused by differences in the open hand width and height of a person as data showed that the mean height is significantly lower than the mean width (Table 1). Data showed that the mean value of students BMI-H or BMI-W who fit golden ratio were lower than students who did not fit golden ratio and statistically this difference was significant (T-test, p = 0.001). This means that golden ratio fitted bodies have better BMI (i.e. lower values) (Table 1).

Results revealed that females have higher percentage in underweight and normal weight category than males, while males have higher percentage in overweight and obese category than females. Statistically this gender differences in BMI was significant (Pearson Chi-Square test, P = 0.001)) (Table 1). This means that females have better BMI than males. In addition, frequency distribution of BMI-H, categorized as four levels, showed that among the Hashemite University students aged 19 years, the overall prevalence of overweight and obesity was 15% (10% and 5%, 57/380, 19 females and 38 males) and 85% (80% and 5%) for normal weight and underweight levels.

BMI-H levels were compared based on golden ratio and results support a significant difference (Pearson Chi-Square test (All BMI levels), P = 0.019 ( $X^2$  (3, N = 380)) (Table 2). Moreover, BMI-H levels dichotomized as ( $\leq$ Normal) and (>Normal) then compared according to golden ratio fitness and data showed that among the overweight and obese students (>Normal), 77.2% (44/57, 13 females and 31 males) of them lie outside the golden ratio while in the normal weight and underweight students ( $\leq$ Normal), 56.3% (182/323, 84 females and 98 males) of them lie outside the golden ratio cance. Statistically this difference was significant (Pearson Chi-Square ( $\leq$ Normal/>Normal); p = 0.003, OR = 2.62, 95% CI: 1.36– 5.06). Students who have  $\leq$ Normal BMI were more likely to fit golden ratio than students who have BMI >Normal (OR 2.62) (Table 2).

In summary, percentage of golden ratio fitted bodies were higher in normal weight females but not males. Whereas, the percentage of non-golden ratio fitted

|                          | ** * 11      | Golden ratio fitness (Frequency) |            |
|--------------------------|--------------|----------------------------------|------------|
|                          | Variable     | Yes (n=154)                      | No (n=226) |
| Gender*                  | Female       | 99                               | 97         |
|                          | Male         | 55                               | 129        |
| Rh Factor                | Rh+ve        | 142                              | 196        |
|                          | Rh-ve        | 12                               | 30         |
| Blood Group <sup>#</sup> | А            | 57                               | 81         |
|                          | В            | 28                               | 53         |
|                          | AB           | 11                               | 8          |
|                          | 0            | 58                               | 84         |
| GPA#*                    | Satisfactory | 13                               | 34         |
|                          | Good         | 46                               | 87         |
|                          | Very Good    | 60                               | 71         |
|                          | Excellent    | 33                               | 31         |
| BMI-H§                   | Mean         | 22.41                            | 24.09      |
| BMI-W§                   | Mean         | 21.78                            | 23.59      |
| BMI-H <sup>#</sup> *     | Underweight  | 10                               | 9          |
|                          | Normal       | 131                              | 173        |
|                          | Overweight   | 10                               | 28         |
|                          | Obese        | 3                                | 16         |

| Table 2. Summary and association between some body parameters and golden ratio (n: |
|--|
|--|

<sup>§</sup>: T-test, p value < 0.05

\*: Pearson Chi-Square test, p value < 0.05

\*: Dichotomies grouping

bodies were higher in obese and overweight females and males (Table 1). Females have a better BMI grades and higher golden ratio percentage than males.

# ABO and Rh blood groups distribution relative to golden ratio fitness

In blood types A, B, O, Rh+ve and Rh-ve, the percentage of non-golden ratio fitted bodies was higher than golden ratio fitted bodies, while in AB blood group, the golden ratio fitness percentage was higher. Comparing frequency of blood groups; ABO and Rh for golden ratio fitness showed no significant differences (Pearson Chi-Square test, P = 0.064 ( $X^2$  (3, N = 380)) (Table 2). Moreover, ABO blood groups were categorized as a dichotomous variable (i.e. A and Not A) and were tested for association with golden ratio fitness and results showed no significant association for all dichotomized blood groups (ABO and Rh) (Pearson Chi-Square test for all, P > 0.05, ( $X^2$  (3, N = 380)).

| #  | Ratio                           | Leonardo da Vinci′s Gold-<br>en Ratio<br>Mean ± S.D | Leonardo da Vinci's<br>Recorded Ratio<br>Mean ± S.D           |
|----|---------------------------------|---|---|
| 1. | Hand/Head – Foot                | 0.100   | $0.111 \pm 0.01$  |
| 2. | Elbow – Hand/Head – Foot        | 0.200   | $0.262 \hspace{0.2cm} \pm \hspace{0.2cm} 0.01 \hspace{0.2cm}$ |
| 3. | Shoulder – shoulder/Head – Foot | 0.250   | $0.123\pm0.03$  |
| 4. | Navel-Foot / Head-Foot          | 0.618   | $0.600\pm0.014$   |
| 5. | Forearm-Hand / Armpit-Hand      | 0.618   | $0.583\pm0.029$   |

Table 3. Comparisons of Leonardo da Vinci's Theoretical Ratios and recorded ratios.

#### GPA distribution relative to golden ratio fitness

Results showed that females (120/375) have higher GPA grades than males (75/375) and statistical analysis demonstrates significant gender difference in favor of females (Pearson Chi-Square; p = 0.001, ( $X^2$  (3, N = 380)) (Table 1). GPA grades were categorized as a dichotomous variable into above good (i.e. very good and excellent) and good (i.e. good and satisfactory) and were tested for association with golden ratio fitness and results showed that 24.8% (93/375) of all students have above good GPA grades and fit golden ratio (Table 2). Statistically a significant association was present (Pearson Chi-Square (good/above good); p = 0.004, OR = 1.87, 95% CI: 1.23– 2.84) (Table 2). High GPA grades were more likely to fit golden ratio than low grades (OR 1.87). Moreover, comparing Leonardo da Vinci's Theoretical Ratios for human body in his drawings of Vitruvian man and recorded ratios for students showed similar values except for the Shoulder – Shoulder/Head – Foot ratio (Table 3).

# Discussions

#### Main Findings

Results of this study gives insight about human body anthropometric data, weight, BMI GPA and ABO blood groups of similar age students and their association with the golden ratio or gender. Results confirmed gender differences in human body anthropometric data, BMI, GPA, and consequently calculated ratios. In addition, findings revealed that students who are golden ratio fitted bodies were more likely to have better BMI and GPA especially females. Also, findings showed that gender or golden ratio fitness has no association with ABO or Rh blood groups.

#### BMI, Gender and Golden Ratio

Golden ratio fitness has been investigated for potential link with different human body levels such as coronary blood vessels, genome, and hand skeleton (Persaud and O'Leary, 2015). In this study females have golden ratio more than males. Several factors such as, age, gender, health status, and environment influence human body anthropometrics and consequently these factors have impact on the golden ratio (Katzmarzyk and Leonard, 1998). Gender differences in bone length development were reported which could impact the calculations of golden ratios and BMI as both were dependent on body lengths (Clark et al., 2007).

BMI has been shown to differ according to age, gender, ethnic group, lifestyle, health, and genetic variations (Mokdad et al., 2003; Williams et al., 2015; Nuttall, 2015), for example, obesity was associated with females in ELSA-Brazil study (Pinto et al., 2018) and a study in Jordan reported that women were more obese than men at the age of 25 or above (Khader et al., 2008) while another study in Jordan reported that obesity were more in males at age 14-16 (Abu Baker and Daradkeh, (2010)). This study revealed that males were more obese than females at the age of 20. These gender variations in results may be related to differences in the age of the sample selected.

BMI cut off points is different among different populations (Wildman et al., 2004, Misra, 2015). BMI grades used in this study were based on the world health organization classification (WHO) which belong to Asians (WHO, 2004; Perissinotto et al., 2002). Males and females of different ages showed differences in BMI – body fat composition (Meeuwsen et al., 2010) and this was supported by our results. BMI standard values were reported to be influenced by age, sex, and geography (Perissinotto et al., 2002) and similar conclusions were reported in Jordan (Madanat et al., 2011, Madanat et al., 2007). In this study, geography, age were controlled as Jordanian students of similar age and equal gender ratio were participated, and results confirm gender differences in BMI in favour of females (lower BMI) (Table 1). Additionally, gender variations in body lengths is reflected on the calculations of BMI as supported by the results of this study which might be explained by reported sex differences in bone length development in several populations (Clark et al., 2007).

#### Proposed new BMI Formula

BMI is one of the indices that can be used to diagnose obesity, however, this ability is not fully precise and researchers recommend using new indices to predict BMI risk levels such as body fat percentage BF%, skinfold thickness and waist to hip ratio (WHR) (Romero-Corral et al., 2008; Ortega et al., 2016; Adab et al., 2018; Hall and Barwell, 2015). Based on our results that showed significant differences between body width and height we recommend the use of a BMI formula based on weight/ height\*width instead of the traditional BMI that based on weight/height\*height which could be more accurately estimate of body weight distribution in the body. This new formula result in a lower new BMI-H\*W than old BMI-H\*H (Table 2).

#### Vitruvian Man

The Da Vinci drawing was for a man and called "Vitruvian man" which raise a question of what about woman is there a "Vitruvian woman". Our results showed that the majority of students of both gender have higher body width than height (295/380) and this contrary to the Vitruvian man drawing which hypothesized equal height and width and only 23 of the 380 students (10F and 13M) have their height

and width equal. Of those 23 Vitruvians 16 students have normal BMI and 5 students fit the golden ratio. Researchers demonstrates gender difference in body lengths which is supported by the results of this study (Oranges et al., 2016). A Turkish study found that nearly one third of the sample have matched Da Vinci proportions in his Vitruvian man circle and square (Yılmaz et al., 2005).

#### GPA, Gender and Golden Ratio

Academic performance has been reported to differ according to gender in turkey (Dayioğlu and Türüt-Aşik, 2007) which is comparable with our results and another study in Pakistan were females have better academic performance than males (Arshad et al., 2015). Gender and ethnicity differences in GPA was also reported in students in favour of females (Holmes and Slate, 2017). Moreover, academic performance was linked with anthropometrics such as BMI (Alswat et al., 2017; Taras and Potts-Datema, 2005; Asirvatham et al., 2019). For example, in Sri Lanka, normal weight BMI were associated with better GPA, however, the study sample was from different university colleges students at different year levels (Wehigaldeniya et al., 2017). Our study showed a gender difference in favour of females and support an association with the golden ratio as an anthropometric measure. Higher grades were also associated with golden ratio fitness, GPA scores in university students were linked to body anthropometrics (Deliens et al., 2013). Another study link academic performance with physically active persons (Santana et al., 2017) and the general health status (Shaw et al., 2015). Moreover, using a large sample size and applying mendelian randomization was able to support a link with academic performance (Tyrrell et al., 2016).

#### ABO, Rh, Gender and Golden Ratio

This study revealed no significant association between (ABO and Rh serotypes) and golden ratio or gender. A previous study confirmed no associations between gender and the distribution of blood groups among medical students in Pakistan (Abbasi et al., 2018; Butt et al., 2018). Several studies suggested a relation between health and blood groups (Smith et al., 2018). For example, BMI and risk of hypertension were investigated in students and results showed that B blood group may has a role (Bhattacharyya et al., 2010; Chandra and Gupta, 2012). To the best of our knowledge there are no studies on the association between blood groups and golden ratios, however, association was performed with other body anthropometrics measures such as BMI which depends on weight and height. The distribution of ABO blood groups or Rhesus according to BMI showed no significant differences with BMI scores above normal (Smith et al., 2018). This is also confirmed by our results as no association was seen between blood groups and BMI-H (Pearson Chi-Square test, P = 0.964 and 0.064  $(X^2 (3, N = 380))$ , weight (ANOVA test, P > 0.05), height (ANOVA test, P > 0.05) and open hand width (ANOVA test, P > 0.05). A recent study that relies on obesity indices such as, lean body mass (LBM) other than BMI reported significant difference in the distribution of ABO or Rh blood groups (Siddiqui et al., 2019) and this association was also reported by other studies (Suadicani et al., 2005; Eren and Çeçen, 2019). In a previous study no statistical difference were reported between blood groups and obesity although sample was small and 85% were females (Asafa et al., 2019).

#### Conclusion

This cross-sectional research provides informative data regarding body dimensions and different body lengths proportions of Jordanian undergraduate students at their first and second year of study. It revealed that students who are golden ratio fitted bodies were more likely to have better BMI and GPA especially females. No association between golden ratio fitness and ABO or Rh blood groups.

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The authors declare no conflict of interest.

#### **Author Contributions**

All authors have made substantial contributions to all of the following: (1) Conception and Design of the study (All authors), (2) Acquisition of data (Khaldun M. Jacoub and Samya A. Omoush), (3) Analysis and Interpretation of data (Jihad A. M. Alzyoud and Abd Al-Rahman S. Al-Shudiefat), (4) Drafting the article and Revising it critically (All authors) (5) Final approval of the version to be submitted (All authors).

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# Lower limb posture and joint mobility in young Soccer players

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#### Abstract

Soccer practice can induce marked changes in the lower limbs with dreaded short and long term consequences. We evaluated the possible effects of sport practice on lower limb posture and their relationships with ankle joint mobility (AJM). In 61 male Soccer players mean age 11.6±1.8 years, and in 50 Volleyball and Basketball players matched for age, sex and body mass index, lower limb posture and AIM in both plantar flexion and dorsiflexion were evaluated on the sagittal plane in supine position using, respectively, images analysis and an inclinometer. A multivariate analysis of variance was carried out to assess if the practice of different sports affects the leg posture (FP: angle between foot and leg) and foot posture (LP: angle between the foot and the line perpendicular to the ground). The sport practiced showed a significant multivariate effect on the lower limb posture. Soccer players showed a greater LP angle (169.2±4.3° vs  $164.9\pm4.5^{\circ}$ ; p<0.001) and a lesser FP angle in both Basketball and Volleyball players  $(298.0\pm12.6^{\circ} \text{ vs } 305.6\pm10.9^{\circ}; p<0.002)$ . These differences were not present between the Basketball and Volleyball players. Soccer players showed a reduced AJM (127.6±15.7° vs. 138.8±21.6°; p<0.002) compared to the other subjects evaluated. The AJM was found directly correlated to the FP angle (p<0.005). The results of this study indicate that young Soccer players could show an altered posture of the leg and foot and a reduced AJM. The alterations of these parameters seem to be a consequence of the sport practice.

Keywords

Ankle joint mobility, Lower limb posture, Flexibility, Sport, Injury prevention.

# Introduction

The study of the effect of sport on young players is of noteworthy importance considering the large number of subjects involved and how sport can affect the development [Strong et al., 2005; Merkel, 2013; Bergeron et al., 2015].

Soccer is the most practiced sport, especially by males, in many countries around the world. The practice of Soccer in sports settings as well as in recreational and school ones can begin from the first years of life; therefore, even young subjects can have a history of a multiple years of sports practice [FIFA Communications Division,

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2007; Study Center FIGC, 2017; Statistics Canada, 2014; Department for Digital, Culture, Media & Sport - England, 2018; National Physical Activity Plan Alliance, 2018].

Basketball and Volleyball are also two of the most practiced sports by young subjects, but they differ from Soccer where the ball is managed with the feet, a condition that can induce muscle-connective adaptations at the level of the lower limb and expose the ankle to a greater number of traumas [Faude et al., 2013; Golanò et al., 2014; Read et al., 2016].

The study of the effects of sport on ankle mobility is considered important because the ankle is a load-bearing joint of the body with fundamental biomechanical and postural functions [Basnett et al., 2013; Golanò et al., 2014; Brockett & Chapman, 2016]. The ankle is formed by a ginglymus of hinge-type synovial joint formed by the medial and lateral malleolus, which forms a mortise to receive the trochlear surface of the talus [Kaueyer & Malone, 1980; Golanò et al., 2014; Brockett & Chapman, 2016]. The anatomy of the articular surfaces of the talocrural joint together with other passive factors (e.g. capsuloligamentous structures surrounding the joint) and dynamic factors (e.g. muscle-action) determine joint mobility, by allowing and limiting it, in dorsiflexion and plantar flexion on the sagittal plane [Lin et al., 2004; Golanò et al., 2014; Brockett & Chapman, 2016].

Previous studies underlined that the practice of certain sports can significantly modify ankle mobility. In this sense, it has been reported that the practice of Soccer can induce a reduction in ankle joint mobility, while, this effect does not seem to occur in young Basketball and Volleyball players [Hattori & Ohta, 1986; Francia et al., 2021; Moreno-Perez et al., 2020]. The levels of AJM reduction detected in Soccer players were such to be able to increase the risk of ankle sprain, and affect the quality of gait as well as the balance, also due to a partial deafferentiation from the articular and periarticular structures caused by repeated injuries [Kaueyer and Malone, 1980; Kaufman et al., 1999; Carlson et al., 2000; Aronow et al., 2006; de Noronha et al., 2006; Basnett et al., 2013; Evans et al., 2018; You et al., 2009].

To date, there is no clear evidence regarding the effects that a reduced AJM can have on lower limb posture of young Soccer players [Ribeiro et al., 2003; Hoch et al., 2012]. The complexity of this condition can lead to a significant variation in ankle mobility, detectable in young Soccer players, suggesting that another parameter of great importance such as posture could be altered in these subjects [Conradsson et al., 10; Fong et al., 11; Basnett et al., 2013]. The possible detection of postural anomalies is important because they could be studied and treated in order to prevent the same joint and postural abnormalities and injuries as well as to improve sports performance [Kaueyer and Malone, 1980; Faude et al., 13; Young et al., 2013].

The main aim of this study was to evaluate the possible effects of sport practice on lower limb posture and their relationships with the AJM.

#### Materials and methods

A total of 111 young male athletes, 61 Soccer, 20 Basketball, and 30 Volleyball players participated in this study. Data were collected on age, height, weight, years of sports practice, other sports practiced, number of weekly training sessions, lower limb-dominance, and history of injuries. Body mass index (BMI) was calculated

|                          | Soccer<br>group  | Control<br>group | p-value     | Volleyball       | Basketball       | p-value     |
|--------------------------|------------------|------------------|-------------|------------------|------------------|-------------|
| Age (years)              | 11.6±1.9         | 11.9±1.6         | 0.60*       | 12.7±1.3         | 10.7±1.2         | <0.001*     |
| BMI (Kg/m <sup>2</sup> ) | 18.5±2.3         | 19.7±3.6         | $0.115^{*}$ | 19.0±2.9         | 20.8±4.4         | $0.178^{*}$ |
| Years of activity        | 5.5±1.9          | 3.2±2.4          | < 0.001*    | 3.5±2.7          | 2.7±1.8          | 0.358*      |
| Total AJM (°)            | 127.6±15.7       | 138.8±21.6       | 0.002       | 137.3±19.4       | 140.7±24.9       | 0.552       |
| Plantar Flexion AJM (°)  | 24.7±7.4         | 31.4±7.4         | < 0.001*    | 31.2±5.7         | 31.7±9.6         | 0.843       |
| Dorsal Flexion AJM (°)   | $102.9 \pm 13.0$ | $107.5 \pm 17.0$ | 0.113       | $106.4{\pm}16.1$ | $109.0{\pm}18.6$ | 0.231       |
| Right AJM (°)            | $65.6 \pm 8.4$   | 69.5±10.5        | 0.029       | 69.3±10.1        | $70.0{\pm}11.4$  | 0.816       |
| Left AJM (°)             | 62.3±8.9         | 69.3±12.1        | < 0.001     | $68.4{\pm}10.8$  | 70.7±14.0        | 0.512       |
| Dominant AJM (°)         | 65.0±8.9         | 69.8±10.7        | 0.011       | 69.4±10.3        | $70.4{\pm}11.5$  | 0.539*      |
| Non Dominant AJM (°)     | 62.3±8.6         | 69.1±11.9        | 0.002       | 68.3±10.6        | 70.2±13.9        | 0.586       |
| $\Delta$ R/L AJM (°)     | 3.2±7.1          | 0.2±7.0          | 0.025*      | 0.9±7.8          | 0.7±5.6          | 0.434       |
| S/ R test (cm)           | -5.2±7.5         | -8.0±8.3         | 0.091       | -8.4±9.1         | -7.5±7.0         | 0.627*      |

**Table 1.** Main characteristic, ankle joint mobility and hamstring flexibility and comparison between Soccer vs controls composed of Volleyball and Basketball players.

Values are means  $\pm$  standard deviation. Comparisons were performed using T-test or Mann-Whitney test (\*). Abbreviations: N.: number; AJM: ankle joint mobility; BMI: body mass index; R/L: right/left; S/R: Sit and Reach  $\Delta$ : difference; °: degree; cm: centimeters.

as body weight in kilograms divided by height in meters squared ( $kg/m^2$ ). Detailed characteristics of study participants are shown in Table 1,2.

Before enrolment, subjects underwent a physical examination including inspection of lower limb to detect the presence of deformity, injuries, and trauma, that could affect ankle joint mobility, hamstring flexibility, or posture. Individuals with the presence of current foot and ankle problems at baseline, such as orthopaedic or surgical complications, congenital foot or leg deformity or who did not practice the same sport for at least six months continuously were not enrolled.

All young players and their parents or guardians were informed on the purpose of the study and its experimental procedures before obtaining their written informed consent and the enrolment in the study. The protocol and the consent forms were approved by the Paediatrics Ethics Committee of Meyer Children's Hospital in Florence. The study was performed according to the principles expressed in the Declaration of Helsinki.

#### Ankle joint mobility

Ankle joint mobility (AJM) was evaluated using a standardized protocol (Clarkson, 2013; Francia et al., 2017; Francia et al., 2018). The patient was lying supine with the feet over the edge of the outpatient examination table, and the ipsilateral knee was extended and put over a rigid support 5-cm high. The maximum range of dor-

|                            | 6               |                 |         |
|----------------------------|-----------------|-----------------|---------|
|                            | Soccer          | Control         | p-value |
| FP angle                   |                 |                 |         |
| Standing - left angle (°)  | 122.6±4.9       | 123.9±4.2       | 0.306*  |
| Standing - right angle (°) | 122.0±6.0       | 121.0±4.9       | 0.396   |
| Standing Tot. angle (°)    | 244.6±8.9       | 244.7±6.9       | 0.93    |
| Lying - left angle (°)     | 149.2±6.7       | 152.7±5.9       | 0.004   |
| Lying - right angle (°)    | $148.8 \pm 6.7$ | 152.9±6.0       | < 0.001 |
| Lying Tot. angle (°)       | 298.0±12.6      | 305.6±10.9      | 0.002*  |
| LP1 angle                  |                 |                 |         |
| Standing - left angle (°)  | 87.6±3.1        | 86.5±3.2        | 0.095   |
| Standing - right angle (°) | 87.1±3.6        | 85.8±3.6        | 0.072   |
| Standing Tot. angle (°)    | 174.7±5.8       | 172.2±6.3       | 0.049   |
| LP2 angle                  |                 |                 |         |
| Lying - left angle (°)     | 85.0±2.5        | 82.5±2.6        | < 0.001 |
| Lying - right angle (°)    | 84.3±2.3        | 82.4±2.4        | < 0.001 |
| Lying Tot angle (°)        | 169.2±4.3       | $164.9 \pm 4.5$ | < 0.001 |

**Table 2.** Soccer and Control players leg-foot inclination angles with vertex at the center of the lateral malleolus, one half-lines passing through head of the fibula and one through: head of fifth metatarsal bone (FP angle) or parallel to the ground (LP1 angle) or perpendicular to the ground (LP2 angle).

Values are means ± standard deviation. Comparisons were performed using T-test or Mann-Whitney U Test (\*). Abbreviations: (°): degree; (Tot): right+left.

sal and plantar flexion was determined after marking the fifth metatarsal bone with the dermographic pen and positioning the inclinometer (Fabrication EnterprisesInc, White Plains, NY) along the diaphysis of the bone, with one extremity placed on the distal condyle. The subtalar joint was in a neutral position while the ankle joint was in the resting position that it naturally takes on the sagittal plane.

In a recent paper, it has been reported that the mean standard deviation of three consecutive readings of the ankle range of motion (ROM) in young subjects, as carried out in this study, was very limited: 1.1±0.9 degrees of plantar flexion and 1.4±1.1 degrees of dorsiflexion (Francia et al. 2019).

#### Flexibility

Flexibility was evaluated using the Sit and Reach test. Participants barefooted were asked to sit on the ground with their feet approximately hip-wide against the testing box. While keeping their knees extended, putting one hand on the other, and slowly reaching forward as far as they could. Once fully extended forward, the participant could touch a metric tape and this distance was recorded (López-Miñarro et al., 2009). All measurements were performed by the same observer with more than 10 years of experience, recording the mean of 3 consecutive readings.

#### Angle of inclination of the foot and leg

Lower limb posture on the sagittal plane was assessed by photographic images of: a) Foot posture (FP), the angle with vertex at the center of the lateral malleolus and straight lines passing through the head of the fifth metatarsal bone and the second through the center of the head of the fibula; b) Leg posture (LP), the angle with vertex at the center of the lateral malleolus and straight lines passing through the head of the fibula and the second parallel to the ground (perpendicular in the case of subjects lying; Fig. 1).

The analysis of the images was acquired in two different postures: in the upright position and lying supine on the examination table with the patient's feet over the limit, same posture maintained during the evaluation of the ankle ROM without knee rigid support. The angles were calculated from the photographic images using Auto-CAD software.

When the results relating to the lower right and left limb were considered together, the description "tot", which stands for total, was used (i.e., right + left = tot.)



**Figure 1.** Lower limb posture on the sagittal plane. **Angles considered: FP angle:** vertex at the center of the lateral malleolus (O) and straight lines passing through the head of the fifth metatarsal bone (A) and the second through the center of the head of the fibula (B); **LP angle:** the angle with vertex at the center of the lateral malleolus (O) and straight lines passing through the head of the fibula (B) and the second perpendicular to the ground (C).

**Table 3.** Post-hoc pairwise comparison between groups of players considering leg-foot inclination angles in the lying position with vertex at the center of the lateral malleolus and half-lines passing through head of the fibula and through the FP angle (head of fifth metatarsal bone) or the LP2 angle (perpendicular to the ground).

| Den en dent Verieble | Comparison               | Mean       | <b>9</b> 5%       | 95% CI            |         |
|----------------------|--------------------------|------------|-------------------|-------------------|---------|
| Dependent variable   |                          | Difference | CI <sub>INF</sub> | CI <sub>SUP</sub> | p-outue |
| Lying Tot. FP angle  | Soccer vs Basketball     | -9.68      | -16.94            | -2.43             | 0.006   |
| Lying Tot. FP angle  | Soccer vs Volleyball     | -6.30      | -12.58            | 02                | 0.049   |
| Lying Tot. FP angle  | Volleyball vs Basketball | -3.38      | -11.51            | 4.75              | 0.585   |
| Lying Tot. LP2 angle | Soccer vs Basketball     | 5.63       | 2.97              | 8.29              | 0.000   |
| Lying Tot. LP2 angle | Soccer vs Volleyball     | 3.43       | 1.12              | 5.74              | 0.002   |
| Lying Tot. LP2 angle | Volleyball vs Basketball | 2.20       | 78                | 5.18              | 0.191   |

Pairwise comparisons were performed using post-hoc Tukey's HSD. CI inferior (<sub>INF</sub>) and superior (<sub>SUP</sub>) 95% confidence intervals of the mean difference. (Tot): right+left.

#### Statistical analysis

Data were reported as mean±standard deviation (SD). ROM values were expressed in degrees (°). Statistical normality test was performed using Shapiro-Wilk tests. A multivariate analysis of variance (MANOVA) was conducted to assess if the practice of different sports affects the lower limb posture (i.e., Lying tot. FP angle; and Lying tot. LP angle; dependent variables). The assumption of multivariate normality (Doornik-Hansen test: p = 0.723) and homogeneity of the variance (Levene's F test: Lying tot. FP angle p = 0.332; Lying LP tot. angle p = 0.900) and covariance (Box's M test: p = 0.172) matrices were assessed and met.

When the different sports showed a significant multivariate effect on the lower limb posture, a univariate analysis of variance (ANOVA), followed by post-hoc Tukey's HSD pairwise comparisons, was performed for each dependent variable (Tab. 3). Basketball and Volleyball players were grouped because, as demonstrated previously<sup>16</sup> and in the present study, they showed a similar lower limb posture and AJM, whereas they differed from Soccer players (Tab. 1,2).

The comparisons between the two groups (Soccer vs. non-Soccer players) were made using the independent T-test or the nonparametric test: Mann-Whitney.

The association between the joint mobility and posture parameter has also been evaluated separately for soccer and non-soccer players as well as considering all subjects assessed using Pearsons or Spearman's correlation coefficients. The analyses were performed using Stata (StataCorp, v.13) and SPSS Statistics (IBM, v.20) software. The  $\alpha$  level of statistical significance was set at 0.05.

# Results

According to the inclusion criteria, age and BMI were fully comparable between groups (Tab. 1). The sport practiced showed a significant multivariate effect on the

posture of the leg (Wilk's  $\Lambda = 0.761$ ,  $F_{(4, 214)} = 7.814$ , p < 0.001). Follow up ANOVAs indicated that the sport practiced significantly affected both Lying tot. FP angle ( $F_{(2, 108)} = 6.220$ , p = 0.003, eta-squared ( $\eta^2$ ) = 0.103) and Lying tot. LP angle ( $F_{(2, 108)} = 15.013$ , p < 0.001,  $\eta^2 = 0.218$ ). Post-hoc pairwise comparisons showed that Lying tot. FP angle and Lying tot. LP angle were not different between the Basketball and Volleyball players. However, Soccer players showed greater Lying LP tot. angle and lesser Lying tot. FP angle than Basketball and Volleyball players (see Tab. 2).

Compared to the control group, the young Soccer players showed reduced plantar flexion AJM (p<0.001) and total AJM (p=0.002; Tab. 1). A significant difference in mobility was found in the group of young Soccer players by comparing left and right limbs (p<0.001) and non-dominant dominant limb (p=0.035). This difference was not found in the control group. Basketball and Volleyball players showed no differences in the joint mobility of the ankle and about the angle between the leg and the foot calculated in non-weight-bearing condition (Tab. 1,2).

Considering all subjects assessed the total AJM and ankle dorsiflexion was found to be directly related to the Lying tot. FP supine position angle (respectively: p < 0.005 and p < 0.001) and inversely correlated with the Lying LP tot. angle (respectively p = 0.015 and p = 0.009).

The sit and reach test did not show any significant differences between Soccer players and controls.

# Discussion

In this study, we aim to verify whether the practice of Soccer could affect the posture of the leg and foot of young players as well as verifying the negative effect on ankle joint mobility (FIFA Communications Division, 2007; Statistics Canada, 2014; Department for Digital, Culture, Media & Sport - England, 2018; National Physical Activity Plan Alliance, 2018).

Regarding the analysis of the lower limb posture carried out, while, on one hand the evaluation of the young subjects in upright position did not show particular differences between the Soccer and non-Soccer groups, on the other hand the analysis of the images of the players in the lying position showed significant differences between the groups (Tab. 2).

The multivariate analysis showed a significant effect of the type of sport practiced on the lower limb posture. Instead Basketball and Volleyball players showed overlapping results.

In particular, the angle with the vertex at the center of the lateral malleolus and with half-lines passing through the distal extremity of the fifth metatarsal and through the head of the fibula was minor in Soccer players compared to controls (Tab. 2, FP angle). This result is evident, despite the analysis that considered the inclination of the leg in relation with the perpendicular line to the ground, which showed a lower inclination of the leg in Soccer players (Tab. 2, LP2 angle).

The results achieved suggest that, if on one hand, the modifications sport-related considered cannot prevail on postural needs in orthostatic condition, on the other hand, the foot, in young Soccer players, takes a posture in dorsal flexion if evaluated in non-weight-bearing condition (Tab. 2, LP2 angle). Moreover, this result was only obtained in non-weight-bearing condition which suggests that the tests performed in this study may allow recognizing the early effects of Soccer practice on the posture of the lower limb in addition to those on AJM. Therefore, the posture's modifications detected could have negative consequences for young Soccer players.

One of the study results confirmed a significant reduction in AJM in young Soccer players (Tab. 1). Even if, the real causes of the limited AJM and the altered posture of the lower limb that can be shown by young Soccer players are not known, it is well known that the peculiarity of this sport is to directly manage the ball with the feet. In addition to a high risk of incurring in traumas, this activity, could involve, differently from other sports such as Basketball or Volleyball, the toning not only of the flexor muscles of the foot but more generally of the leg muscles.

In this sense, the results of this study could indicate that the main role in determining the variations of AJM could be played by the effects induced by repetitive hitting the ball with greater or lesser strength. Such activities may require high strength in both concentric (hitting) and isometric (stabilizing the joint) activity executed by the dorsal flexor muscles of the ankle involved (Kellis and Katis, 2007; Lees et al., 2010).

Moreover, the strengthening of these muscles in the anterior and lateral part of the leg would justify, at least in part, the difference between Soccer and non-Soccer groups investigated regarding the posture assumed by the foot if evaluated in a nonweight-bearing position.

This condition could also justify the apparent paradox detected in the group of young Soccer players and related to the presence of a condition of leg extension associated with a reduced total angle between leg and foot in addition to a reduced AJM in plantar flexion. The latter is often associated with rigidity of the triceps of the sura; this stiffness would hinder an extension of the leg and the dorsiflexion of the foot.

It can therefore be hypothesized that the reduced AJM and modified posture of the lower limb found in this study share the same causal factors. In fact, the results achieved on AJM and the posture evaluated in lying supine position were correlated.

According to the data reported in literature, the AJM assessed in the Volleyball and Basketball players is similar and resulted to be in line with the reference values reported for subjects matched for age (Boone and Azen 1979; Grimston et al., 1993; Lin et al., 2004; Kellis and Katis, 2007; Soucie et al., 2011). Similarly, the posture evaluated resulted similar in Volleyball players and Basketball players in both the positions examined.

Numerous studies showed that the reduced AJM is a risk factor for several dreadful adverse events and this relationship could also concern the postural anomalies detected. For this reason, it would be important not only monitoring these parameters but also verifying the effect on the performance and history of injuries in addition to study the effectiveness of exercise protocols aimed at recovering AJM (de Noronha et al., 2006; Fong et al. 2011; Hoch et al., 2012; Read et al., 2016).

# Conclusions

The results of this study confirm that young Soccer players can show a reduced ankle joint mobility and an altered posture of the leg and the foot that can be seen in non-weight-bearing position. The alterations of these parameters seem to be a consequence of the Soccer practice. While the possible negative effects induced by a limited AJM are known, the possible short and long term effects that an altered posture of the lower limb can have on young subjects are unknown. Considering the importance of the parameters investigated, further studies aimed at clarifying this relationship seem necessary.

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# Medical Embryology and its Importance in the 21<sup>st</sup> Century Curriculum: A mini review

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#### Abstract

Embryology is an essential tool in clinical practice especially for managing various medical disorders. As a course in medical schools, it is neither easy nor straightforward to understand and teach in the 21<sup>st</sup> century curriculum, as such, it is easy to overlook. As a discipline, embryology has entered a phase of unparalleled transition in its understanding base. This translates to a phase of abundant modification in the medical curriculum. A main critical issue of learning embryology is how extensively newer molecular medical embryology can be paired with the traditional approach to developmental of anatomy. Another issue would be which venue to be considered most effective in embryology instruction. The medical curriculum in during 21<sup>st</sup> century have different objectives, as well as different educational approach. As a consequence, this paper outlines a variety of aspects in which embryology could be presented as well as how it may be incorporated within the medical curriculum.

### Keywords

Medical embryology, Clinical embryology, Medical education, Approaches to learning, Medical curriculum.

# Introduction

Embryology as a discipline in the West originated from researches conducted by Ancient Greek Philosophers. The philosophers had struggled to answer the foremost metaphysical query "What is man? And "What is the Universe?" Anaximander and Empedocles (611-547 BC and 504-433 BC respectively) had tried to compare the different stages of embryonic development as well as cosmos development (Needham & Hughes, 1959). Studies on medical embryology as an philosophical discipline was initially perceived in the Hippocratic Corpus. However, *On the Generation of Animals* by Aristotle (383 – 322BC) became the maiden scientific edition that gave a comprehensive theory of embryology (reproduction and development in various organisms) – a theory that remained unchallenged until the Renaissance, and which greatly influenced later theories in philosophy and embryology (Bury, 1911). The vital role played by human embryology in human's understanding of himself, and in medicine cannot be overlooked. It is worth noting that the knowledge of embryology has greatly contributed to the advancement in medical science. Several investigations have also shown that learners who consider embryology as a beneficial aspect of their universi-

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ty education are doing it because they believe it helps learners to enhance their clinical skills (Scott, Charles, and Holland, 2013). Other than studying the evolutionary nature of different anatomy of the body, the analysis of a variety of scientific topics, including the foundation of gametogenesis as well as fertilization, congenital abnormalities, and the consequence of intrauterine incidents like congenital disease and the growth of the foetus, that are clearly important to a range of specialities like obstetrics and paediatrics (Alfalah et al., 2019).

Like other disciplines, embryology must justify its position in the present-day medical curriculum, one that is overcrowded. Curriculum committees in schools are most likely to ask questions such as "why this subject should be taught?" and "what should be taught?". In this paper, the viewpoint on the role or importance of embryology in medical education is presented. Educational programs, as well as curricular objectives, differ and there is no correct answer to explain how embryology classes should be performed. What is emphasized in a particular program may be marginalized in another program? This paper does not prescribe a one-fit-all model. Rather, it presents options that will facilitate the effective contribution of embryology within the medical education.

# Modern embryology curriculum

In most cases, justice is done to content in a particular course, however it is not much constraint to change the subject to "what the student should be taught during their medical education?" If this is resolved, then more focus is channeled to coordination, timing, and packaging. Recently, content regarding embryology has been described in vague phrases, old versus new. The phrase old here refers to developmental phase, while the phrase new is thought to be referred to as molecular aspect. Frankly, there are not many instances where the entire information in a particular area has been transformed or improved as rapidly like medical embryology, all within a decade. But then, it has been shown a seemingly a large decrease in time allocation to embryology in the curriculum of medical schools. Thus, one has to select what material to present to the students.

Neither "old" nor "new" knowledge is adequate for a comprehensive learning of embryology. Restricting oneself to growth-related anatomy limits adequate comprehension of causation. In the same vein, an understanding of molecular events without a comprehending the knowledge of the morphologic contextual in how they're occurring could cause misconceptions. The problem, subsequently, is which vast information in this area is the learner supposed to master as well as what must be the level of exposure?

Presently, most medical institutions within USA as well as Australia, are gradually shifting from the classroom-based study of embryology, preferring to impart the knowledge during the student's clinical years (Muller et al., 2008; Drake et al., 2009). One, therefore, wonders if the subject is accorded the same value to the same level. Al-Neklawy (2017) showed that teaching embryology via online classes might constitute a successful additional tool while performing embryology classes. The results of this study showed that the majority of Egyptian medical students (90%) were satisfied with embryology classes performed online (Al Neklawy, 2017). Moreover, the wide availability of embryology resources online might facility online learning (Hill, 2018). The bone of contention is the problem-based approach used in the modern medical curriculum, which encourages the teaching of embryology as a basic medical science (Drake, Lowrie and Prewitt, 2002). It is important to note that over 64% of medical schools in the United States combine embryology with gross anatomy. It is also worth noting that 22% of medical schools teach embryology alongside human structure, human function, and histology (Drake, Lowrie and Prewitt, 2002). American Medical Schools are mostly graduate schools that offer fast-track programs with many subjects condensed together. And so, students can retain information by comprehending the physiological and anatomical relationships between both subjects.

Allowing medical students to learn multiple subjects simultaneously helps them to acquire a comprehensive understanding of each subject. For instance, students in their first years must have a working knowledge of the early development of the fetus, development of the organ system as they occur in stages, genetics, and how embryology is applied in the clinic. The goal is to make embryology clinically relevant while in the classroom (Hamilton and Carachi, 2014). It is important to note that a problem-based approach will hasten the student's understanding of complex diseases, as well as its treatment. It will also ease the learning process (Scott, Charles, and Holland, 2013). As such, even though there may be a reduced focus on embryology as an independent subject, its integration with physiology and anatomy is of great benefit, and implicitly recognizes the value of embryology.

#### Learning approaches to embryology

The pedagogical strategy to embryology do not vary significantly with those which are employed in academic education for other topics, but the source material does raise certain particular difficulties. Of course, embryology is introduced at a majority of medical schools as a lecture-based subject, usually without a laboratory, albeit with differing numbers of material hand-outs. The amount of allocated hours assigned to a formal embryology teaching ranges greatly. Thus, the forms and quantities of content officially discussed differ similarly. Regarding institutions adopting the problem based learning (PBL) method, there are no standardized classes, and much of the learning is conducted through the study of specific clinical cases. Several institutions often dedicate conventions to address the divide between basic science teaching and clinical practice of the basic science portion of the medical curriculum. Such workshops, which can require attendance from both fundamental science and physicians, may be very helpful to introduce students to content that is not extensively discussed in classes or books on embryology. The science of conception, birth abnormalities, the foetus as a patient and childbirth are subjects that have proved to be well suited for such venues. Textbooks prove to be a mainstay with several classes in academic embryology. Two of the field's longstanding texts Moore & Persaud, 1998, and Sadler (2000) were originally largely anatomically focused as well as used commonly among conventional courses. More over, two other textbooks also combined biological and molecular approaches (Carlson 1999; Larson 2001). Web-based instruction is rapidly being used in nearly every medical school in the USA. Placing the outlines of whole classes, including still images, on safe websites for the benefit of the students is becoming more popular. If the ability to hold the Internet grows, so do the volume and sophistication of the text-related statistics. Animations developed throughout the years for chosen embryological developments like embryo transfer, fertilization, gamete, embryo folding, intestine rotation, and heart septation, but due to the complexities and cost, there is currently no broad scope for standard animation. Nonetheless, these animations can be extremely efficient for individual use.

# **Team based learning**

Team-based learning (TBL) is an educational business training technique (Michaelsen et al., 1997), and this approach has been implemented increasingly by medical schools (Searle et al., 2003). There is increasing proof that team-based learning (TBL) is a productive way to integrate collaborative peer instruction in small groups and learning engagement. Progressively, classes in both undergraduate and graduate medical school are conducted using TBL. During the preclinical years, when medical schools develop comprehensive and interdisciplinary classes, TBL is especially valuable because of its focus on collaboration, material mastery, and problem solving for clinical use. For medical gross anatomy and medical embryology, TBL is an appealing approach to follow since it allows the student to study anatomical details on which they improve anatomical principles important for skills in clinical practice (Nieder et al., 2005; Vasan et al., 2009). Multiple medical schools have implemented TBL in fundamental science implementation in recent years (Nieder et al., 2005), clerkships (Levin et al., 2004), and internship opportunities (Haidet et al., 2002). As several medical schools in the preclinical years are developing comprehensive and multidisciplinary classes, TBL is incredibly beneficial because of its focus on collaboration, topic competence and clinical practice problem-solving skills. TBL is an appealing technique for anatomical education, as it allows learners to study anatomical details upon which to acquire anatomical principles regarding the solution of medical symptoms (Nieder et al., 2005). A reasonable planning and attendance is also needed. TBL offers more constructive learning for students as opposed to passive education often linked with conventional lectures (Nieder et al., 2005).

# The importance of embryology in anatomy

Embryology is very important in the present-day medical curriculum. It sounds trite, but pregnancy conditions for the birthing of each individual remain true. Consequently, it is of utmost significance that while an infant develops from a zygote (which is in turn formed from the union of a sperm and an oocyte), a lot of things can go wrong. Whether it is traced to environmental or genetic factors, it results in premature births, and several birth defects such as maldevelopment, spina bifida, congenital heart problems, abnormal positioning of the kidneys, etc. Research has shown that at least 7.9 million infants globally are born with some form of congenital defects each year (Christianson, Howson, and Modell, 2006). A study conducted at the Centres for Disease Control and Prevention (2006) concluded that "Birth defects affect 1 in every 33 babies (about 3% of all babies) born in the United States each year."

This accounts for over 20% of neonates. Doctors must be able to trace the root causes before diagnosis, treatment, and possibly prevention can be made. This explains why it is more than important to study the abnormalities of physiological development, a discipline known as teratology while doing anatomy courses. For instance, it is important that obstetricians and gynaecologists have a working understanding of anatomy as well as physiology to provide optimal prenatal care as well as postnatal care, and for paediatricians treating students who have been affected by these congenital defects.

There are various explanations why embryology is a fitting part of the traditional medical syllabus. Since multiple classes or people might not possess the same academic habits, nor do they have the same technical requirements, such factors cannot really be favored or described in rank order. The basic philosophical rationale for researching embryology is to consider how our bodies originated. Like in many other basic science fields, for its own sake this can be seen as gaining information, but beyond that there are many explanations why this information is essential. Birth deformities appear to rate higher in the disorders that their physicians offer to patients. While the recorded occurrence of birth defects ranges based on the screening severity, any identifiable form of birth defect may eventually be assigned to approximately 5 percent of live-born individuals. Recognizing the origin of birth defects has proved to be amongst the hardest to break diagnostic notions. Traditional embryology offered morphological clues as to where trends of pathological morphological development vary from ordinary, but the origin of most birth defects has scarcely been derived from that information solely. Modern molecular technology has highlighted the roots of numerous genetically engineered defects occur at birth, and has presented some hints about the genesis of other. A course in embryology must deliver a theoretical foundation for understanding processes that underlie both normal as well as abnormal developments. Thus, embryology is a very important field in medicine and its associated disciplines.

#### The advantage of combining embryology with anatomy

Having a working knowledge of developmental embryology allows medical students to understand better, the anatomy. Students, for example, will be familiar with the nervous system and the venous system, because embryology explains in detail the development of the veins in the vertebra, arrangement of plexus, and of course, embryology of the cranium and the caudal regions. Knowing the embryology regarding the venous structure greatly aids with understanding the arrangement of the transverse foramen's venous system (Magro et al., 2013). When the student understands the development of organs in embryology, such as the urogenital and cardiovascular systems, their understanding of anatomy will be reinforced. For example, knowing how the lungs are segmented, how the heart is formed, and where the dermatomes are located makes it easier to visualize their development. More so, Carlson (2002) identifies the "formation of heart chambers, the development of gross vascular patterns, lung segmentation, gut rotation, and development of major parts of the urogenital system" as aspects of anatomy for which learning is approached through developmental anatomy. Frankly, developmental anatomy is a foremost aspect of embryology, considering that it focuses on the changes undergone by the body, cells, organs, and germ cells during development (Leonard et al., 2000).

Embryology plays a very vital role in understanding how birth defects relate to genetics, for instance, the sox and hedgehog genes for development of the limbs, situs inversus where there is a transposition of the heart and other vital organs to the opposite side of the body during embryological development. The development of the pancreas is another example. Here, the head, the body, and the tail undergo separate developments, and then unite, leading to a higher risk of defects (Sadler et al., 2007). If there is an abnormality in pancreatic development, food substances, especially lipids, will remain undigested. It is important that healthcare professionals understand the causes of these defects to be able to handle the rising conditions, and also prevent them. This explains why it is important to study and understand the normal and abnormal formation of organs.

#### **Clinical application: treatment**

Understanding embryology creates a strong foundation for other aspects of medical knowledge. Both - descriptive and experimental embryology - provides an opportunity to investigate the etiology of particular congenital malformations. Studying embryology is crucial to understand the etiology of congenital anomalies and possibly evaluate the possibilities for their prevention. As stated by Carlson (2002), "understanding the developmental stages that a structure or region undergoes during its embryogenesis gives important clues not only to its normal structure but also to reasons why anatomical variation can occur (e.g., the pattern of the major veins of the trunk and abdomen)". The fact is, one cannot fully lay claim to understanding anatomy when one doesn't understand embryology. Congenital defects of the heart like aortic coarctation can be used as a case study. By having a working knowledge of embryology, the medical student will know that this disorder can be treated at birth by interventional cardiac catheterization or prevented via alteration or eradication of the medications or environmental factors responsible for its development (Stark, De Leval, and Tsang, 2006). Imperforate anus is another common congenital defect. Understanding how the cloaca develops in the human embryo, as well as the occurrence of imperfect fusion, helps with the treatment of this condition. Treatment may be done at birth if it has not reached the advanced stage. A surgical procedure may be done later on (Hosokawa et al., 2017). While these treatments are common, their existence is attributed to embryology. Thus, they continually serve as a testimony of the importance of embryology in the modern clinical curriculum.

#### Conclusion

Embryology is considered an increasingly vital aspect of the medical curriculum. An understanding of embryology translates to an understanding of the human anatomy. Medical students will be able to make sense of the variation in organ structure in humans after they have had a perfect understanding of the organ embryology. Understanding the embryology helps with treatment and even prevention of abnormal development of organs. But then, it is important to note that there is no "allround" correct way of presenting this subject in the modern medical curriculum. The watchword in the modern curriculum is basing the content and presentation of the course in relation to desired results. As such, medical schools must tailor the teaching of embryology to match with their curricular objectives. Instructors also have the responsibility of creating a platform that is effective for presenting the subject.

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# The location of the mental foramen in relation to the biometrics of the lower dentition and mandibular arch: A cross-sectional study

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#### Abstract

Purpose: To evaluate the position of the mental foramen among Malaysian Malays and Chinese based on the biometrics of the lower dentition and mandibular arch dimensions.

Materials and methods: The horizontal and vertical position of mental foramen in relation to the midline, roots of teeth, mesiodistal dimension of the anterior and posterior teeth, and the mandibular arch length and width were measured and evaluated in 65 Cone-Beam Computed Tomography scans.

Results: The location of mental foramen was predominantly located either between 1st and 2nd premolar or below 2nd premolar. There were no significant differences for all measurements of the mandible in relation to gender and ethnicities. There was a positive correlation of the mesiodistal diameter of the teeth with the mandibular arch length and width. However, mandibular arch length and width did not affect the location of the mental foramen.

Conclusions: As the size of the teeth increases, the mandibular arch length and the width increase concurrently. However, regardless of the changes in the biometrics of the mandibular dentition and the arch dimensions, the anatomical position of the mental foramen remains the same. The mental foramen was located at the ratio of 0.5 vertically and 0.27 horizontally in the mandible. The inter-foramina distance of 51.36 mm suggests that Malaysian Mongoloids had sufficient space to receive five implants rigidly joined with a bar.

Keywords -

Mental foramen, anthropometry, CBCT, Malaysian Mongoloids.

# Introduction

In clinical dentistry, arch width and shape of the jaws are of particular interest to the orthodontists and prosthodontists. For the orthodontists, dental arch expansion is one of the methods used to solve dental crowding as extraction of teeth may not be required. However, stability has always been controversial in the dental arch expansion.<sup>1</sup> As a result, different formulae, indices and methods using tooth size has been suggested to predict the ideal inter-premolar and inter-molar width for the purpose of arch expansion. Because of this, several publications have investigated the maxillary and mandibular arch length and interdental width to develop baseline data.<sup>2, 3</sup>

In the mandible, identification of actual position of mental foramen (MF) is of

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substantial clinical significance in dentistry especially during surgical interventions and anaesthetic procedures. Pre-operative imaging examinations are necessary to aid in developing a comprehensive treatment plan for patients who requires dental implant, flap surgery or anaesthetic procedures in the mental region.<sup>4</sup> Traditionally, panoramic imaging is used as a preoperative diagnostic tool in treatment planning of implant placement in inter-foramina region. However, the drawback of panoramic imaging includes the distortion and magnification of anatomic structures which often results in either over- or underestimation of the real size. On the other hand, CBCT has clearly proven its reliability of negligible distortion, superimposition, and magnification of the images. Hence, high accuracy can be achieved by using CBCT scans.

Population-specific 3D database is needed to determine the location of mental foramen in relation to the biometrics of lower dentition and mandibular arch dimension. These findings are essential to maximise the best mechanic and function of implants where its shape and size must meet both technical and clinical requirements.<sup>5</sup> The two most common location of the MF is between the first and second premolar or apical the second premolar, but variations have been reported. Green (1987) reported that the most common location of the mental foramen in Caucasians and Middle Eastern was between the first and second premolars, but was more distally placed in the Mongoloids, Melanesian and Negroids.<sup>6</sup>

Knowing that the position of mental foramen can vary according to different populations, the purpose of this research is to determine the biometrics of lower dentition and mandibular arch dimensions in the ethnic Malay and Chinese Malaysians of Mongoloid ancestry. The results obtained can be used as baseline to plan the number and position of implants for edentulous case.

# Materials and methods

# Ethical approval

The study was approved by the Faculty of Dentistry, University of Malaya and MAHSA University Ethics Committee (RMC/EC13/2019).

#### Sample collection

Sample size was estimated based on two means formula. A standard deviation (SD) of 2.3 mm was estimated. A minimum of 58 adult subjects was needed to be recruited, considering level of significance at 0.05 and 80% of the power of the study. In our study, 65 CBCT scans of patients who underwent treatment at Faculties of Dentistry at University of Malaya and MAHSA University for diagnostic purposes were carefully selected. This was a retrospective cohort study using convenient sampling method.

The following are the inclusion criteria for CBCT:

- High quality CBCT scans with respect to sharpness and contrast of images
- Images of mandible without any radiolucent/radiopaque lesions.
- Images with identifiable mental foramen.
- Healthy periodontal structures.

- No missing teeth at the lower arch and must be caries-free from teeth 37-47.
- No history of orthodontic treatment. The exclusion criteria for CBCT include:
- Presence of any supernumerary teeth.
- Severe malocclusion or obvious diastema.
- Presence of tooth/teeth with abnormal anatomy.
- Has any history of dental treatment (tooth restoration, prothesis etc)

CBCT imaging measurement in panoramic, sagittal and axial views were performed using i-CAT Vision software (Imaging Sciences International, Inc. Hatfield, USA).

The panoramic image was obtained using the Implant Screen windowpane of the software. Fig 1 illustrates the various points of measurement on the panoramic view of a CBCT scan. Two reference points were drawn prior to the measurements. First reference line was straight line drawn from the nasal spine down to the symphysis menti, which divided the mandible into left and right side. The second reference line was a line drawn parallel to and in contact with the lowest margin of the body of the mandible. The distance between symphysis menti and mental foramen (MF-Mid-line) was recorded with a straight horizontal line drawn from the mental foramen to the first reference line. The distance between mental foramen and posterior border



**Figure 1.** Parameters for measurement. MF-Midline: Mental foramen to the symphysis; MF-Post: Mental foramen to the posterior border of the mandible. Yellow line denotes the inter-foramina distance. MF-Sup denotes the distance from the middle of mental foramen to the alveolar crest, and MF-Inf denotes the distance from the middle of the mental foramen to the lower border of the mandible.



**Figure 2.** The position of mental foramen in relation to the longitudinal axis of the lower dentition. Position 1 MF is situated anterior to the first premolar; Position 2 MF is in line with the first premolar; Position 3 MF is situated between the first and second premolar; Position 4 MF is in line with second premolar; Position 5 MF is situated between second premolar and first molar; Position 6 MF is in line with first molar.

of ramus (MF-Post) was recorded with a horizontal line paralleling with the second reference line. The distances from the alveolar crest of bone to the mental foramen (MF-Sup) and the mental foramen to the lower body of mandible (MF-Inf) were measured with a vertical line drawn perpendicular to the second horizontal line. Distance between the left and right mental foramen was measured from the point of intersection (which marks the location of mental foramen)

on the left to the right side.

Based the description by Ngeow & Yuzawati,<sup>7</sup> the location of mental foramen was classified in relation to the longitudinal axis of mandibular teeth, as shown in Fig 2.

Fig 3(a) demonstrates the tooth size measurement carried out on the axial planes of the Multiplanar

Reconstruction (MPR) screen of the i-CAT Vision software. The mesiodistal width of molars, premolars and anterior were measured from interproximal anatomical contact



**Figure 3.** (a) The measurement of teeth size: A=Anterior tooth size, B = Premolar tooth size, C = Molar tooth size (b) the measurements of arch widths, namely the intercanine, inter-first-molar and inter-second molar.
of each tooth as seen on the occlusal view (Fig 3a). The arch length, i.e., the horizontal distance from the incisal point perpendicular to the midpoint of a line joining the distobuccal cusp tip of permanent second molars was also obtained.<sup>8</sup>

The arch widths were measured in the mandible as the followings (Fig 3b):

- a) Inter-canine width Measured as the distance between cusp tips of the permanent canines. If attrition is noticed, the middle of the facet on the tooth will become the measuring point.
- b) Inter-first molar width Measured as the distance between the mesiobuccal cusp tips of permanent first molars.
- c) Inter-second molar width Measured as the distance between the distobuccal cusp tips of permanent second molars.

#### Results

From 65 CBCT scans studied, 32 scans belonged to Malay (18 male & 14 female) and 33 scans were from Chinese patients (15 male & 18 female). The initial investigation revealed that the baseline characteristics of all parameters between the genders and ethnicities were found to have no significant differences (Independent *t-test*; p > 0.05).

Fig 4 shows the distribution of various positions of the mental foramen. The findings indicate that most of patients had their left mental foramen located in between the first and second premolar (Position 3) & in line with second premolar (Position 4), which accounted for 50.8% and 26.4% respectively. However, it was also noticed that there are 2 patients (3.1%) who had the left mental foramen located below first premolar (Position 2) and 4 (6.2%) who had their left mental foramen located between second premolar and first molar (Position 5). Similarly, most patients had their right mental foramen located at Position 3 and Position 4, accounting for 63.1% and 30.8% of patients respectively. However, it was also noticed that there was 1 (1.5%) patient who had his right mental foramen located at Position 2 and 3 (4.6%) patients showing the presence at Position 5.





**Figure 4.** The distribution of various positions of the mental foramen.

der of the mandible (MF-Sup) on the right side was 15.75mm (SD  $\pm$  2.20) and on the left side, it was 15.22mm (SD  $\pm$  2.46). The inter-foramina distance was 51.36 (SD ± 6.20) [95%CI = 42.61 to 61.63 mm]. The overall mean distance from the middle of mental foramen to the inferior border of the mandible (MF-Inf) on the right side was 15.23mm (SD  $\pm$  1.91) and on the left side was 15.27mm (SD  $\pm$  1.80). These results give a ratio of 0.51 on the right side, and 0.50 on the left side between the superior (MF-Sup) and the mandibular height (MF-Sup + MF-Inf). There was no statistically significant difference between side, with the overall mean ratio being 0.50 (max-minimum = 0.37-0.62). Besides, the overall mean distance from the middle of mental foramen to the midline of the mandible (MF-Midline) on the right side is 25.21mm (SD  $\pm$  3.84) and on the left side is 26.17mm (SD  $\pm$  3.90). The overall mean distance from the middle of mental foramen to the posterior border of the mandible (MF-Post) on the right side was 69.95mm (SD  $\pm$  4.56) and on the left side was 68.43mm (SD  $\pm$  4.54). The ratio of the MF-Midline distance to the mandibular length (MF-Midline + MF-Post) was 0.26 on the right side, and 0.28 on the left side. There was no statistically significant difference between side, with the overall mean ratio being 0.27 (max-minimum = 0.19-0.38).

The breakdown of measurements from various specific anatomical structures to the mental foramen in Position 3 and Position 4 is shown in Table 1. Overall, it can be observed that the MF-Sup and the MF-Midline measurements were larger in cases where the mental foramen was located in Position 4.

MF- sup is the distance from mental foramen to the superior border of the man-

 Table 1 Measurements from specific anatomical structures to the mental foramen for 2 MF different positions.

|       | Location of    |       | Mean distance (SD)    |                       |                       |                       |  |
|-------|----------------|-------|-----------------------|-----------------------|-----------------------|-----------------------|--|
| Side  | mental foramen | No. – | (MF-Sup)              | (MF-Inf)              | (MF-midline)          | (MF-post)             |  |
| Right | Position 3     | 41    | 15.54mm<br>(SD ±2.06) | 15.77mm<br>(SD ±1.66) | 24.35mm<br>(SD ±3.25) | 70.92mm<br>(SD ±4.48) |  |
|       | Position 4     | 20    | 16.14mm<br>(SD ±2.45) | 14.09mm<br>(SD ±1.73) | 26.32mm<br>(SD ±4.65) | 67.64mm<br>(SD ±4.38) |  |
| Left  | Position 3     | 33    | 14.61mm<br>(SD ±2.54) | 16.07mm<br>(SD ±1.69) | 25.65mm<br>(SD ±3.89) | 69.69mm<br>(SD ±4.33) |  |
|       | Position 4     | 26    | 15.81mm<br>(SD ±2.38) | 14.30mm<br>(SD ±1.57) | 26.99mm<br>(SD ±3.64) | 66.69mm<br>(SD ±4.38) |  |

| Table 2. Correlation between mandibular arch dimensions and position of mental foramen on both si | ides |
|---|------|
|---|------|

| Verichles                   | *R_L                  | OMF                   | Р     | P *L_LOMF             |                       |       |
|-----------------------------|-----------------------|-----------------------|-------|-----------------------|-----------------------|-------|
| variables                   | Position 3            | Position 4            | value | Position 3            | Position 4            | value |
| Arch length                 | 41.93mm (SD<br>±2.65) | 40.53mm (SD<br>±2.40) | 0.051 | 41.74mm<br>(SD ±2.70) | 41.25mm (SD<br>±2.68) | 0.491 |
| Inter-canine width          | 30.46mm (SD<br>±2.20) | 30.23mm (SD<br>±1.81) | 0.682 | 30.34mm<br>(SD ±2.31) | 30.40mm (SD<br>±1.80) | 0.921 |
| Inter-first molar width     | 51.55mm (SD<br>±3.36) | 50.82mm (SD<br>±2.85) | 0.402 | 51.79mm<br>(SD ±3.25) | 50.55mm (SD<br>±2.94) | 0.134 |
| Inter-second molar<br>width | 61.85mm (SD<br>±3.23) | 60.71mm (SD<br>±2.31) | 0.162 | 62.12mm<br>(SD ±3.17) | 60.67mm (SD<br>±2.70) | 0.068 |

\*R\_LOMF is the location of the mental foramen on the right side of mandible whereas L\_LOMF is the location of mental foramen on the left side of the mandible.



Figure 5. The correlation between total teeth size and inter-ach length. Right side (R), Pearson correlation = 0.650 (P<0.001); Left side (L), Pearson correlation = 0.704 (P<0.001).

dible; MF – Inf is from the mental foramen to inferior border of the mandible, MF – Midline is from the mental foramen to midline of the mandible and lastly, MF – Post denotes the distance between mental foramen and posterior border of the mandible.

The anterior teeth sizes were 17.94 (SD  $\pm$  .13) [95%CI = 16.06 to 19.62 mm]. In comparison, the premolar teeth sizes were 14.69 (SD  $\pm$  0.83) [95%CI = 13.30 to 20.39 mm] and the molar teeth sizes was 22.55 (SD  $\pm$  1.18) [95%CI = 16.03 to 24.50 mm].

The mean arch length was 41.56 (SD  $\pm$  2.60) [95%CI = 37.00 to 45.51 mm]. The mean intercanine distance was 30.53 (SD  $\pm$  2.09) [95%CI = 26.71 to 34.10 mm]. The mean inter- molar (first molar) distance was 51.67 (SD  $\pm$  3.43) [95%CI = 44.55 to 54.29 mm]. The mean inter- molar (second molar) distance was 61.78 (SD  $\pm$  3.23) [95%CI = 55.38 to 66.78 mm]. The detailed arch length and inter-arch widths are shown in Table 3. The correlation study between mandibular arch dimensions and the position of mental foramen on both sides shows no significant correlation (P > 0.001).

The results from correlations test showed that there was statistically significant correspondence between the total size of teeth and total arch length (P < 0.001 on both sides, and arch width (P < 0.001). The total tooth size is directly proportional to the increment of mandibular arch length and width (Figs 5 and 6).

Moreover, further analysis was undertaken to determine the correlation between mandibular arch dimensions and positions of mental foramen on both sides. Interestingly, statistic result showed no significant differences between the mandibular arch dimension and the position of the mental foramen as shown in Table 2.



**Figure 6.** Correlation between total teeth size in relation to inter-canine width, inter-first molar width and inter-second molar width. On the right side, their respective Pearson correlation are 0.576, 0.500 and 0.452 (P<0.001); on the left side, their respective Pearson correlation are 0.519, 0.493 and 0.496 (P<0.001).

# Discussion

Clinical and radiographic evaluation of inferior alveolar canal, incisive canal and mental foramina of the mandible is an essential preoperative assessment for the success of many of the surgical interventions. This includes injection of local anaesthesia during routine dental procedures, implant placement, periodontal surgeries and orthognathic surgeries.<sup>9</sup> CBCT scanned images has been deemed as a gold standard in these and other clinical scenarios. It is widely acknowledged that the radiological information provided surpasses the risks associated with absorbed radiation doses. CBCT scans allows for the establishment of quantitative correlation between different anatomical structures, which may then minimize complications and help predict favourable treatment outcomes.<sup>10</sup>

The mental foramen is an important landmark to identify prior to local anaesthesia injection and for the planning dental implant placement in the anterior mandible. In addition, the mental nerve that emerges from the mental foramen is the important anatomical structures that needs to be identified, preserved and avoided during surgery. The location of the mental foramen is usually determined by relating its relationship the lower dentition. Previous study involving the Malays reported that the most common location of the mental foramen is Position 4.<sup>7, 11, 12</sup> The current findings suggest that there is ethnic influence on the position of the mental foramen in Mongoloids, as the addition of the Southern Chinese into the sample pool possibly skewed the most common position to be between the first and second premolar (Position 3). In the current study, the location of mental foramen was predominantly located either between 1<sup>st</sup> and 2<sup>nd</sup> premolar or below 2<sup>nd</sup> premolar regardless of gender and ethnicities on both left and right sides. Another possible explanation for the difference with regards to the Malays is the fact that previous studies were done on dentopantomographs, while the current one is undertaken using CBCT.

The current results shown here were in accordance with previous studies in other populations such as the Australoids and Caucasian (American, Anglo-Saxon, central European and South African Caucasian) showing that the commonest location of the mental foramen was positioned between the first and second premolar tooth, followed by a position below the second premolar.<sup>6, 13, 14</sup> However, in others like the Mongoloids (Chinese, Japanese, Thai, Korean, Malay), Melanesian and Negroids, the mental foramen was most commonly located below the second premolar.<sup>7, 14-17</sup>

The MF is usually located in the body of the mandible at an equal distance from the superior and inferior border below.<sup>18, 19</sup> In this current study, the distance from upper border of mandible or alveolar crest to MF was at a range of 11.03 - 20.12mm (Mean:  $15.75\pm2.20$ mm) on the right side, and at a range of 9.83-20.58mm (Mean:  $15.22\pm2.46$ mm) on the left side. The mean distance between the MF to the inferior border of mandible was  $15.23\pm1.91$ mm on the right side, and  $15.27\pm1.80$ mm on the left side. An average ratio of 0.5 is obtained by dividing the MF-Sup against the height of mandible at the mental foramen (Table 3). This finding concurs with the same ratio reported by Apinhasmit et al. (2006), Wang et al. (1986) and Tebo and Telford (1950).<sup>18, 20, 21</sup>

The MF-Sup range is within the means reported by various studies (Table 3). The reason for this wide variation maybe due to resorption and deposition (remodelling) causing the changes in the size of the mandible. In addition, the location of the mental foramen appears to be affecting this measurement. The fact that these figures were between 10-20 mm of height suggests that short or standard implants can be placed above the mental nerve, while avoiding the mental foramen/nerve if done early before gross resorption sets in. The MF-Sup measurements were larger in cases where the mental foramen in located in Position 4 than those in Position 3. Such finding has not been reported before and shall be kept in mind during treatment planning.

Besides that, the mean distance between the MF to the inferior border of mandible was  $15.23\pm1.91$ mm on the right side, and  $15.27\pm1.80$  mm on the left side. This finding concurs with those reported for 2 other group of Mongoloid ethnicities. Chung et al. (1995) found that the average distance from the inferior border of the MM to the centre of the MF was 15.5 mm for Korean men and 14.0 mm for Korean women.<sup>22</sup> Guo et al. (2009) found that MFs were located 15.56 mm above the inferior border of the mandible.<sup>15</sup> These findings are important as there are clinical applications associated with locating the MF by relating it to the symphysis and lower border of mandible, as suggested by Delgadillo-Ávila et al. 2015.<sup>23</sup>

However, Currie et al.'s study showed only fair agreement between examiners when scoring the MF position in relation to the premolar crowns, and moderate agreement when scoring the position in relation to the premolar apices in dentopantomographs.<sup>24</sup> Their results casted doubt on the reliance to conventional radiography alone, although the MF is more commonly visible on dentopantomographs than periapical radiographs.<sup>25</sup> In addition, although the MF is reported to be visible in the majority of dentopantomographs, it is only clearly identifiable in 49% of cases.<sup>26</sup> Ngeow et al. (2010) attributed this to ageing, where they reported that the visibility reduced tremendously in patients aged >50 years.<sup>12</sup>

Because of these inconsistencies, the authors resorted to turn back to the most fundamental approach to determine the location of the mental foramen, namely by

R:0.45

L:0.53

R:0.51

L:0.52

R:0.48

L:0.44

R:0.49

L:0.49

R:0.53

L:0.48

R:0.51

L:0.50

R = 26.7 + 4.1

 $L = 26.5 \pm 4.1$ 

| manaloic.                            |   |  |  |           |  |  |  |
|--------------------------------------|---|--|--|-----------|--|--|--|
| A (1                                 | Apicobasal morphometric measurements  |  |  |           |  |  |  |
| Authors                              | Upper (MF-Sup)  | Lower (MF-Inf)   | Mandible Height  | Ratio     |  |  |  |
| Matsuda Y (1927) /<br>Multiple races | $\begin{array}{l} R = 13.5 - 14.0 \mbox{ mm} \\ L = 14.5 - 15.0 \mbox{ mm} \end{array}$ | $\begin{array}{l} R = 13.5 - 14.0 \mbox{ mm} \\ L = 14.5 - 15.0 \mbox{ mm} \end{array}$                                  |  | ~ 0.5     |  |  |  |
| Tebo & Telford<br>(1950) / Unknown   |   | $\begin{array}{l} R = 15.2 \pm 1.54 \ mm \\ L = 15.0 \pm 1.57 \ mm \end{array}$  |  |           |  |  |  |
| Wang TM et al.<br>(1986) / Chinese   |   | $\begin{array}{l} R = 14.68 \pm 1.78 \mbox{ mm} \\ L = 14.71 \pm 1.77 \mbox{ mm} \\ Mean = 14.70 \mbox{ mm} \end{array}$ | $\begin{array}{l} R = 30.30 \pm 3.03 \mbox{ mm} \\ L = 30.26 \pm 2.83 \mbox{ mm} \\ Mean = 30.29 \mbox{ mm} \end{array}$ | 0.426     |  |  |  |
| Neiva et al. (2004) /<br>Caucasian   | Mean to CEJ = 15.52 ± 2.37 mm   | $\begin{aligned} Mean = 12.0 \pm 1.67 \\ mm \end{aligned}$   |  | 0.56      |  |  |  |
| Apinhasmit et al.<br>(2006) / Thai   |   | $\begin{array}{c} M = 15.40 \pm 1.73 \\ mm \\ F = 13.89 \pm 1.40 \ mm \\ Mean = 14.88 \pm 1.77 \\ mm \end{array}$        | $\begin{split} M &= 31.13 \pm 2.45 \\ mm \\ F &= 27.80 \pm 2.3 \ mm \\ Mean &= 29.97 \pm 2.9 \\ mm \end{split}$          | 0.496     |  |  |  |
| Yeşilyurt et al. (2008)<br>/ Turkish |   | $\begin{array}{l} R = 9.44 \pm 5.84 \\ L = 9.46 \pm 5.88 \end{array}$  | $\begin{array}{l} R = 19.44 \pm 11.94 \\ L = 20.10 \pm 12.09 \end{array}$  | 0.45-0.47 |  |  |  |
| Guo et al. (2009) /<br>Chinese       | $Mean = 16.56 \pm 2.53$ mm  | $Mean = 15.56 \pm 1.74$ mm   |  | 0.52      |  |  |  |

 $R=16.5\pm2.1~mm$ 

 $L = 14.3 \pm 2.1 \text{ mm}$ 

R = 14.53 mm

 $L=14.01\ mm$ 

R = 14.0 mm

L = 15.0 mm

 $R = 12.9 \pm 2.16$ 

 $L=12.5\pm1.9$ 

 $R = 13.9 \pm 1.7 \text{ mm}$ 

 $L = 13.7 \pm 1.5 \text{ mm}$ 

**Table 3.** The vertical measurements related to the apicobasal morphometric of the mental foramen in the mandible.

adopting morphometric study. This approach has been used as early as 1950 by Tebo & Telford (1950) and have been adopted by several authors in determining the location of the mental foramen in some major populations of the world.<sup>20</sup> Table 4 shows a summary of the distance for the mental foramen from the symphysis and posterior border of the mandible in selected studies. As can be observed the mental foramen is located between ~ 19.0 mm and ~ 29.0 mm. This distance is the lowest in the Turkish<sup>27</sup> and highest in the Pakistani.<sup>28</sup> In all ethnic groups listed, the mental fora-

 $R = 15.75 \pm 2.20 \ mm \ R = 15.23 \pm 1.91 \ mm$ 

 $L = 15.22 \pm 2.46 \ mm \quad L = 15.27 \pm 1.80 \ mm$ 

Sankar et al. (2011) /

Rehman et al. (2015)

Chandramohan et

al. (2016) / South

Subramanian et al.

(2019) / Zambian

Present study /

Malay & Chinese

Mishra & Mittal

(2015) / North

/ Pakistani

Indian

Indian

Indian

 $R=13.7\pm2.8\ mm$ 

 $L=16.4\pm2.9\ mm$ 

R = 14.86 mm

 $L=15.36\ mm$ 

R = 13.0 mm

L = 12.0 mm

 $R = 12.54 \pm 3.1 \text{ mm}$ 

 $L=12.1\pm2.8\ mm$ 

 $R=15.9\pm2.9\ mm$ 

 $L = 12.5 \pm 2.9 \text{ mm}$ 

| A (1  | orphometric measuremer  | nts  |                      |
|---|---|--|----------------------|
| Authors   | MF-Midline  | MF-Post  | Ratio                |
| Tebo & Telford (1950)<br>Unknown  | $\begin{array}{l} R = 26.8 \pm 2.33 \mbox{ mm} \\ L = 26.6 \pm 2.32 \mbox{ mm} \end{array}$   | $\begin{array}{l} R = 74.6 \pm 6.09 \ mm \\ L = 74.9 \pm 5.90 \ mm \end{array}$  | 0.22                 |
| Wang et al. (1986)<br>Chinese   | $\begin{array}{l} R = 28.12 \pm 1.86 \mbox{ mm} \\ L = 27.99 \pm 1.86 \mbox{ mm} \\ Mean = 28.06 \mbox{ mm} \end{array}$  | $\label{eq:R} \begin{array}{l} R = 74.11 \pm 3.79 \mbox{ mm} \\ L = 74.17 \pm 3.75 \mbox{ mm} \\ Mean = 74.14 \mbox{ mm}. \end{array}$ | 0.28                 |
| Santini A, Land M. (1990)<br>Chinese<br>British   | Mean = $27.6 \pm 1.6 \text{ mm}$<br>Mean = $25.9 \pm 2.0 \text{ mm}$  |  | 0.27                 |
| Chung et al. (1995)<br>Korean   | $\label{eq:mr} \begin{array}{l} MR = 24.4 \pm 1.5 \mbox{ mm} \\ ML = 24.9 \pm 1.8 \mbox{ mm} \\ FR = 23.9 \pm 1.9 \mbox{ mm} \\ FL = 24.0 \pm 2.3 \mbox{ mm} \\ Mean = 24.4 \mbox{ mm} \end{array}$ |  | NA                   |
| Curtright et al. (2003)<br>American White<br>American African   | WM: $22.5 \pm 0.4$ mm<br>BM: $22.9 \pm 0.6$ mm<br>WF: $20.5 \pm 0.4$ mm<br>BF: $21.9 \pm 0.4$ mm<br>Mean = $22.0$ mm  |  | NA                   |
| Apinhasmit et al. (2006)<br>Thai  | $\begin{array}{l} M = 29.30 \pm 3.42 \mbox{ mm} \\ F = 27.94 \pm 1.87 \mbox{ mm} \\ Mean = 28.83 \pm 3.05 \mbox{ mm} \end{array}$   | $\begin{array}{l} M = 70.92 \pm 4.39 \mbox{ mm} \\ F = 64.95 \pm 3.49 \\ Mean = 68.85 \pm 4.98 \mbox{ mm} \end{array}$                 | 0.30                 |
| Yeşilyurt et al. (2008)<br>Turkish  | $\label{eq:R} \begin{split} R &= 19.18 \pm 11.19 \ mm \\ L &= 19.37 \pm 11.33 \ mm \end{split}$   | $\label{eq:R} \begin{array}{l} R = 48.58 \pm 29.03 \mbox{ mm} \\ L = 48.27 \pm 28.86 \mbox{ mm} \end{array}$                           | ~ 0.28               |
| Ilayperuma et al. (2009) <sup>33</sup><br>Sri Lankan  | $\begin{array}{l} R = 24.87 \pm \ 6.07 \ mm \\ L = 24.77 \pm \ 6.07 \ mm \\ M = 26.02 \pm \ 5.12 \ mm \\ F = 25.62 \pm \ 4.20 \ mm \end{array}$   |  | NA                   |
| Sankar et al. (2011)<br>South Indian  | $\begin{array}{l} R = 27.2 \pm 2.4 \ mm \\ L = 27.7 \pm 2.4 \ mm \end{array}$   | $\begin{array}{l} R = 70.7 \pm 4.2 \ mm \\ L = 70.7 \pm 4.2 \ mm \end{array}$  | 0.28                 |
| Santini & Alayan (2012)<br>Hokien-Hylam Chinese<br>European - Anglo- Saxon (English)<br>or Dark Ages (Scottish)<br>Indian | $\begin{array}{c} 27.97 \pm 1.66 \text{ mm} \\ 25.89 \pm 2.04 \text{ mm} \\ 26.35 \pm 1.81 \text{ mm} \end{array}$  | $\begin{array}{c} 70.68 \pm 3.95 \text{ mm} \\ 71.80 \pm 4.35 \text{ mm} \\ 67.97 \pm 4.85 \text{ mm} \end{array}$                     | 0.28<br>0.26<br>0.28 |
| Moogala et al. (2014)<br>Indian   | $\label{eq:R} \begin{split} R &= 28.24 \pm 5.09 \ mm \\ L &= 27.45 \pm 3.7 \ mm \end{split}$  | $\label{eq:R} \begin{split} R &= 69.61 \pm 6.03 \mbox{ mm} \\ L &= 69.17 \pm 6.0 \mbox{ mm} \end{split}$                               | ~ 0.29<br>~ 0.28     |
| Mishra & Mittal (2015)<br>Indian  | R = 25.28 mm<br>L = 23.04 mm  | R = 65.47 mm<br>L = 76.11 mm   | ~ 0.28<br>~ 0.24     |
| Rehman et al. (2015)<br>Pakistani   | R = 29.0<br>L = 30.0  | $\begin{array}{l} R=72.0\\ L=73.0 \end{array}$   | ~ 0.29               |

**Table 4.** The horizontal measurements related to the anteroposterior morphometric of the mental foramen in the mandible.

| A  | Anteroposterior morphometric measurements   |   |                                      |  |  |  |
|--|---|---|--------------------------------------|--|--|--|
| Authors                                    | MF-Midline  | MF-Post   | Ratio                                |  |  |  |
| Chandramohan et al. (2016) South<br>Indian | $\begin{array}{l} R=25\pm3.1\\ L=25.7\pm2.4 \end{array}$  | $\begin{array}{l} R = 64.5 \pm 5.9 \\ L = 65.6 \pm 5.3 \end{array}$                                       | ~ 0.28                               |  |  |  |
| Subramanian et al. (2019) Zambian          | $R = 28.5 \pm 2.7 \ mm \\ L = 28.6 \pm 2.2 \ mm$  | $\label{eq:R} \begin{split} R &= 73.4 \pm 5.5 \ mm \\ L &= 73.5 \pm 5.1 \ mm \end{split}$                 | ~ 0.28                               |  |  |  |
| Present study<br>Malay<br>Chinese          | $\label{eq:R} \begin{split} R &= 26.17 \pm 3.90 \mbox{ mm} \\ L &= 25.21 \pm 3.84 \mbox{ mm} \end{split}$ | $\label{eq:R} \begin{split} R &= 68.43 \pm 4.54 \mbox{ mm} \\ L &= 69.95 \pm 4.56 \mbox{ mm} \end{split}$ | L = 0.28<br>R=0.26<br>Mean =<br>0.27 |  |  |  |

men was commonly located apical to the second premolar, except for the British,<sup>14, 29</sup> American White and African American.<sup>4</sup> For these groups of ethnic, the mental foramen, the distance between the mental foramen ranged between 22.0 and 25.9 mm.

The results of the present study were close to that observed on the British and Indian skulls and in skulls of unknown ethnicity of a number of studies.<sup>14, 20, 29, 32</sup> It is smaller than that reported in Thai subjects, Chinese, Pakistani, Zambian, Caucasian and some Indian subpopulations.<sup>13, 14, 18, 19, 21, 28-30, 34</sup> The current finding is bigger than that reported for the Turkish, Korean, Sri Lankan, American White and African American, and at least one Indian subpopulation.<sup>4, 22, 27, 31</sup> In addition, the current MF-Midline distance is within the length reported among Mongoloids examined.<sup>14, 18, 21, 22</sup>

According to Apihamsmit et al. (2006), these measurements were also not usually clinically relevant due to the wide range of values in dimensions. However, they suggested that a ratio could be clinically useful in locating the MF. The overall horizontal position of the MF, i.e., the ratio of MF-Midline (MF-Midline + MF-Post) was 0.27 in the current study. Table 4 provides a comparison of the mean distances of the MF to the posterior mandibles in different populations, summarised as the proportion ratio. In summary, it can be observed that the anterior-posterior relative position of MF on mandibles were significantly different among different ethnic groups from different geographic locations. The relative position of the MF ranged from 0.26 to 0.29 on the right side of mandibles and from 0.23 to 0.29 on the left side of mandibles, which is within the range reported for different populations. The current finding is close to the finding in other Mongoloids,<sup>14, 21</sup> except for the Thais.<sup>18</sup> Such a proportion is useful to predict the location of the mental foramen in our populations.

In this study, the Malays and Chinese (who have Mongoloid ancestry) presented with mean intercanine and inter-first molar distances which are higher than that reported in the Chinese<sup>2</sup> and the Malay from another study.<sup>35</sup> The difference with the Malays may be attributed to the low sample size in Rozali et al.'s study, while the Chinese study had younger subjects.<sup>35</sup> One interesting finding that has not been reported elsewhere is the positive correlation between right and left total tooth size and mandibular arch dimensions. As shown in the graphs, as the total teeth size increases, the mandibular arch width and length increases concurrently. However, the anatomical

| Study                              | Ethnicity  | Inter-foramina<br>distance  |
|------------------------------------|--|-----------------------------|
|                                    | Predynastic Egyptians: Badari  | 44.3 mm                     |
|                                    | Dynastic Egyptians: Sedment  | 44.0 mm                     |
|                                    | Dynastic Egyptians: Kerma  | 44.3 mm                     |
|                                    | Dynastic Egyptians: Qau  | 43.9 mm                     |
|                                    | Tamils   | 45.1 mm                     |
| Marant & Advanthava (1026)         | Nepalese   | 46.2 mm                     |
| Molalit & Auyalitilaya, (1950)     | Tibetans A   | 45.7 mm                     |
|                                    | Hylam Chinese  | 46.7 mm                     |
|                                    | Fukien Chinese   | 46.8 mm                     |
|                                    | Tibetans B   | 47.0 mm                     |
|                                    | English: Dunstable   | 45.4 mm                     |
|                                    | Anglo-Saxons   | 45.3 mm                     |
|                                    | Anglo-Saxons – Spitafields   |                             |
|                                    | Male   | $45.1\pm0.21~\text{mm}$     |
|                                    | Female   | $43.4\pm0.27\ mm$           |
|                                    | Anglo-Saxons – Farringdon Street   |                             |
| Classer 1027 39                    | Male   | $43.9\pm0.24\ mm$           |
| Cleavel, 1937                      | Female   | $42.9\pm0.25\ mm$           |
|                                    | Punjabi male   | $43.9\pm0.21~mm$            |
|                                    | Australian   |                             |
|                                    | Male   | $47.4\pm0.21~\text{mm}$     |
|                                    | Female   | $45.8\pm0.32~mm$            |
| Neiva et al. (2004)                | Caucasian  | $55.23\pm0.32\ mm$          |
| Guo et al. (2009)                  | Chinese  | $49.25\pm5.34~mm$           |
|                                    | Indian   |                             |
|                                    | Class I  | $41.81\pm4.87~mm$           |
| Bhargava et al. (2016)             | Class II   | 34.99 ±2.34 mm              |
|                                    | Class III  | 40.06 ±3.24 mm              |
|                                    | Overall  | 38.95 ±4.58 mm              |
|                                    | Brazilian  |                             |
|                                    | Male   | $41.93 \pm 3.98 \text{ mm}$ |
| Marieiro et al. (2017)             | Female   | 39.99 ± 3.80mm              |
|                                    | Overall  | $40.52\pm3.92mm$            |
|                                    | South Indian   |                             |
| Vane Swetah & Jayanth Kumar (2019) | Male   | 50.62 ± 3.63 mm             |
| (_01)                              | Female   | $50.02 \pm 3.72 \text{ mm}$ |
| Present study                      | Malaysian Mongoloids   | $51.36 \pm 6.20$ mm         |
| - reserve study                    | internation of the second of t | 21.00 ± 0.20 mm             |

Table 5. The inter-foramina distances of various selected ethnic groups.

position of the mental foramen remains the same despite changes in the mandibular arch dimension. This finding can be adopted when determining the teeth size and inter-arch alignment when planning to restore the dentition of edentulous cases.

The inter-foramina distance was studied in morphometric studies of the mandible in the 1930s.<sup>36</sup> However, there have been a pause in such study until the revisits by several authors in the millennium.<sup>5, 13, 15, 37, 38</sup> Table 5 illustrates the current finding against all available information relating to these studies. In general, it can be seen that the inter-foramina distance of the current study samples is larger than most difference races, including the Chinese who are of Mongoloid origin. However, Neiva et al. (2004) reported the largest inter-foramina distance ( $55.23 \pm 534$ ) in 22 Caucasian skulls examined.<sup>13</sup> The current finding is closer to the measurement reported by Guo et al. (2009) and is in agreement with the measurement reported by Vane Swetah & Jayanth Kumar (2019).<sup>15, 37</sup> Difference in measurements in the studies performed after the millennium against those reported in the 1930s may be related to the technique used, or difference in the population whereby the current samples may have benefitted from better living condition and nutrition. In the case of Bhargava et al. (2016) and Bastião Marieiro et al. (2017)'s findings, their subjects were fully edentulous.<sup>5, 38</sup> In addition, the method of obtaining linear measurement used by appear to cause shorter inter-foramina distance.<sup>38</sup>

In edentulous mandible, the mandibular arch form, arch size, and the inter-foramina distance are factors that influence the selection of size and position of implants and the prosthetic design (fixed or removable).<sup>5</sup> Bhargava et al. (2016) proposed a classification system that incorporated the inter-foramina distance in addition to the generally accepted classification system based on the vertical restorative space.<sup>5</sup> They reported that the space requirement for five implants needs to be approximately 44.5–48.5 mm to accommodate the placement of five implants with a minimum diameter of 3.3 mm in the mandibular inter-foramina region. They found that a universal treatment plan cannot be followed in their Indian subjects due to the short interforamina distance of 38.9 mm in their subjects. In agreement with them, Marieiro et al. (2017) reported most of their subjects could be rehabilitated using a protocol with 4 implants of 3.3mm diameters. In contrast, the finding of current study suggests that Malaysian Mongoloids had sufficient space to receive the so-called OD-5 prosthesis, i.e., five implants rigidly joined with a bar.<sup>38</sup>

Lastly, while this study did provide useful metrical and morphological parameters of the mandible of Mongoloid Malaysians, there were some limitations due to insufficient CBCT scans to undertake any age-related changes in different age groups. This study does present some limitations, including the insufficient CBCT scans recruited for the different age groups of adult Malaysians to recognize any age-related changes, and the facts that all subjects were dentate. However, this limitation shall be mitigated in the future when we embark on a larger sample size for a similar study.

#### Conclusion

Regardless of the changes in the biometrics of the mandibular dentition and the arch dimensions, the anatomical position of the mental foramen remains the same, i.e., located either between 1<sup>st</sup> and 2<sup>nd</sup> premolar or below 2<sup>nd</sup> premolar. The anteroposterior and apicobasal morphometric measurements of the mental foramen yielded a 0.5 and 0.27 ratio respectively. The inter-foramina distance of 51.36 mm suggests that Malaysian Mongoloids had sufficient space to receive five implants rigidly joined with a bar.

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# Availability of data and material

The data that support the findings of this study are available from the authors upon reasonable request.

#### **Disclosure statement**

No potential conflict of interest was reported by the authors.

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No funding was received for conducting this study.

# List of abbreviations

| CBCT | Cone beam computed tomography |
|------|-------------------------------|
| MF   | Mental foramen                |
| MPR  | Multiplanar Reconstruction    |

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# Saphenous Vein Graft between Brachial Artery and Cephalic Vein in Antebrachial Arteriovenous Anastomosis – Simulation of the Procedure

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#### Abstract

Patients with chronic renal failure and vascular diseases require the vascular access for hemodialysis procedure to be performed with the most possible comfort for the patient. Native vein graft has a longer-lasting term in the patients' limb, lower risk of graft infection and lower price. Native vein graft is constructed from the great saphenous vein, if the diameter of the vessel is smaller than the diameters of vessels (brachial artery, cephalic vein) it is connected to the cubital fossa region due to the risk of graft folding, that might occur, if the graft diameter is bigger than the diameter of one or both vessels, on which anastomosis is made. The most important sizes, that were taken before graft placement, are the length of the forearm compartment, the distance between the brachial artery and cephalic vein in the cubital fossa region, distance from an expected incision in the brachial artery to the middle of the forearm compartment, the distance between incision in cephalic vein to the middle of the forearm compartment, length of the great saphenous vein (the graft) and diameters of blood vessels, used in the procedure. Finally, the right position of the graft should be determined for the successful outcome of anastomosis creating procedure.

#### Keywords

Arteriovenous graft, hemodialysis, vascular access, anastomosis.

# 1. Introduction

The arteriovenous graft is a type of vascular access. For the graft insertion most, preferred places are arm or forearm with the most often chosen blood vessels – the brachial artery and cephalic or basilic vein, or lower limb, where the femoral artery and femoral vein are chosen for arteriovenous connection [24]. Left or right extremity is chosen, depending on patient comfort (right-handed, left-handed), anamnesis (vascular diseases, emboly, previous surgeries). The graft is recommended for nephrology department patients with chronic renal failure, which is one of the most prevalent worldwide public health problems in the field of nephrology, that affects functions of the kidney. Some of the curing methods for chronic renal failure are kidney

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transplantation, hemodialysis or peritoneal dialysis [27]. While the patient is waiting for the kidney donor, he/she needs to be undergoing a hemodialysis procedure. The arteriovenous graft is a frequent procedure in the hemodialysis unit due to easier access to blood vessels, which lets the medical staff perform hemodialysis faster and improve patient comfort. The procedure for inserting the graft into the patient's extremity, as well as the graft maintenance, requires the integrity of vascular surgery, nephrology, infectiology, radiology professionals and dialysis department specialists for successful treatment and care of the patient.

In Pauls Stradiņš Clinical University Hospital, the Latvian Transplant Centre in Riga, Latvia, as well as in other hospitals in the world, prosthetic graft is a method of vascular access for dialysis, however, many problems, associated with prosthetic graft may occur [27]. The patient's native vein is more preferred than prosthesis due to the lower risk of infection and sepsis. Arteriovenous graft lasts 5-10 years longer than synthetic graft, which is important for patients, who are waiting in queue for the kidney donor [14, 19]. In some patients' cases, native vein graft is a better option, due to the lower expenditure. According to information from the transplantology and hemodialysis center specialists in Pauls Stradiņš Clinical University Hospital, prices of synthetic vascular grafts in Latvia vary from 600 Eur and more per vessel (the price is provided as an information from "Baltijas Dialīzes Serviss" ("The Baltic Centre of Dialysis"), 7 March 2018), but native vein does not have any material costs for the patients, except the surgery procedure, which is usually state paid, if the patient has health insurance, however the diagnosis and surgery affect patient's mental and physical health.

The arteriovenous graft is used to provide the patient with initial vascular access for hemodialysis. Saphenous vein graft is an approach for the arteriovenous anastomosis when one end of a saphenous vein is inserted into the artery and another end into the vein to make the connection between blood vessels. Saphenous vein graft is often used in coronary bypass surgery [3]. To minimize the risk of complications, the great saphenous vein was chosen for the procedure over the synthetic graft.

This study aims were to establish the length of the great saphenous vein for creating antebrachial anastomosis and to insert the saphenous vein into the anterior compartment of the forearm to make the graft between the brachial artery and cephalic vein.

# 2. Materials and Methods

#### 2.1. Preparation

Materials and corpse, that were used in the procedure, were provided by the Laboratory of Anatomy of Department of Morphology at the Institute of Anatomy and Anthropology, Rīga Stradiņš University. In the procedure, the right arm and left leg of the corpse were used. The saphenous vein graft model for the hemodialysis procedure was made in the Complete Anatomy 2020 app for the better visualization and imagination of an actual arteriovenous graft.

The cubital fossa and forearm region of the right arm of the corpse were chosen for the anastomosis creating procedure due to the easier vascular access of the brachial artery and cephalic vein (Figure 1.). Regular forearm graft placement was chosen with arteriovenous graft location from the cubital fossa to the middle of the forearm compartment. The middle of the forearm compartment was established by measuring the distance from the middle of the cubital fossa region to the flexor retinaculum of the hand and dividing the distance by two. The great saphenous vein from the anterior compartment of the left leg was chosen for the procedure (Figure 1. a). During the study, several anatomy books, atlases (*Sobotta, Complete Anatomy 2020 3D* anatomy platform), medical studies from *PubMed.gov* (*National Center for Biotechnology Information, U.S. National Library of Medicine*), *Medicina (1996-2020, MDPI (Basel, Switzerland), ResearchGate (2008-2020 ResearchGate GmbH)* databases, "*Vascular Access: Principles and Practice*" book, "*Journal of Vascular Surgery*" were used. Publications, used in our study, were observed and read from November, 2019 to August, 2020.

Key inclusion and exclusion criteria for publications included the following: studies describing the use of the great saphenous vein in arteriovenous anastomosis creation in the forearm compartment, as well as the risk of infection of the graft and native vein use in other surgical manipulations, such as coronary bypass creation for the purposes of surgical education and training were analyzed in this article. There were included studies from 2000 to present day. Only publications in the English language were used. In the present study publication data from 17 countries (Australia, Belgium, Canada, France, Germany, Greece, India, Israel, Italy, Korea, Latvia, Lebanon, Muscat, Singapore, Taiwan, United Kingdom, United States of America) was included. There were excluded articles about people less than age 18, animal studies, reviews, editorials and studies describing dissection techniques for anatomy teaching without performing any procedure and clinical/functional outcome. Stenosis, vascular abnormalities and emboly on the body side, where procedure was planned to be performed, were excluded.

# 2.2. Diameters and Thrombosis Risk Reduction

The brachial artery, cephalic vein and great saphenous vein diameters were measured by using Vernier calliper to make sure, that the diameter of the great saphenous vein is not bigger than the diameters of blood vessels the graft would be connected to.

## 2.3. Tissue Dissection and Working Surface

After the blood vessel diameters were measured and the possibility of the saphenous vein use in vascular anastomosis creation between the brachial artery and the cephalic vein was established, skin and adipose tissue were dissected from the brachial artery and cephalic vein in the cubital fossa region by using a surgical scalpel, surgical and anatomical tweezers. Median and cutaneous nerves with branches were moved aside to free the brachial artery and cephalic vein. Connective tissue was removed from blood vessels. Skin and fat tissue were removed from the great saphenous vein in the anterior compartment of the leg. Connective tissue was removed from the great saphenous vein. Moving nerves, as well as closely lying to the working surface blood vessels allows lowering the risk of damaging closely located anatomical structures of the body.



**Figure 1.** Modeling of the saphenous vein graft in Complete Anatomy 2020 app. (a) Cubital fossa and forearm compartment of the right arm: 1 – brachial artery, 2 – cephalic vein, 3 – great saphenous vein; (b) lower part of the left leg: 4 – great saphenous vein. Adapted from Complete Anatomy 2020 app (Copyright © 2020 3D4Medical. All rights reserved.), modified by Banceviča et al., 2020.

#### 2.4. Measurements

Locations for incisions in the brachial artery and cephalic vein were chosen and marked. Incisions were marked as close to the middle of the cubital fossa region, as possible for the ability to flex and extend the arm after the graft placement, so movements of the forearm could be performed, as before graft insertion. The following measurements were done for establishing sufficient length of the great saphenous vein for the graft creation.

# 2.5. Distances

1 - the distance between incision location in the brachial artery to incision location in the cephalic vein was measured; 2 - the distance between incision location in the brachial artery to the middle of the forearm compartment was measured; 3 - the distance between incision location in cephalic vein to the middle of the forearm compartment was measured and summed to establish the length of the saphenous vein graft. The standard error of the Vernier calliper of  $\pm$  0.05 mm was used for reinsurance of the measurement and size sufficiency in the anastomosis creating procedure.

#### 2.6. Anastomosis Creation

The great saphenous vein was measured in the anterior compartment of the leg, ligation and dissection of great saphenous vein branches were done. Ligation and dissection of the great saphenous vein from the leg were done by using surgical forceps and *Surgipro 7-0* sutures [7]. Diameters of the great saphenous vein graft ends were measured to establish incision sizes in the brachial artery and cephalic vein. Saphenous vein graft ends sized incisions were made in the brachial artery and cephalic vein. One saphenous vein graft end was connected to the brachial artery and the second end was connected to the cephalic vein by using *Surgipro 7-0* sutures and straight stainless steel hemostat. The vascular anastomosis was done. The angle of the graft folding was established.

#### 3. Results

#### 3.1. Diameters of Blood Vessels

The great saphenous vein diameter was 2.12 mm, diameter of the brachial artery was 4.48 mm, diameter of the cephalic vein was 2.46 mm, which allowed the procedure to be performed with insurance of graft not being folded and not causing any pathologies and circulatory disorders with consecutive thrombosis and graft abruption.

#### 3.2. Sizes and Distances

The size of the anterior compartment of the forearm was 254.7 mm, the size of the great saphenous vein in the anterior compartment of the leg was 322.75 mm. The length of the great saphenous vein for antebrachial arteriovenous anastomosis creation between the brachial artery and cephalic vein should be 304.81 mm, if the distance between brachial artery and cephalic vein in the cubital fossa and forearm region is 18.01 mm. The distance from the saphenous graft connection to the brachial artery and cephalic vein region to the middle of the forearm compartment was 128.40 mm. The distance from an incision in the cephalic vein to the graft folding place was 107.3 mm and the distance from an incision in the brachial artery to the graft folding place was 129.9 mm (Table 1.).

The angle of the graft folding was 80°.

# 4. Discussion

In this study, we observed the possibility of great saphenous vein use in arteriovenous anastomosis in the forearm compartment between the brachial artery and cephalic vein. Sizes of the anterior compartment of the forearm, distance from bra-

| Nr. | Measured part  | Length / diameter<br>/ distance,<br>mm |
|-----|--|--|
| 1.  | Diameter of great saphenous vein                                     | $2.12\pm0.05$                          |
| 2.  | Diameter of brachial artery  | $4.48\pm0.05$                          |
| 3.  | Diameter of cephalic vein  | $2.46\pm0.05$                          |
| 4.  | Length of anterior compartment of the forearm                        | $254.70\pm0.05$                        |
| 5.  | Length of great saphenous vein                                       | $322.75\pm0.05$                        |
| 6.  | Distance from brachial artery to cephalic vein                       | $18.01\pm0.05$                         |
| 7.  | Distance from incision in cephalic vein to the graft folding place   | $107.30\pm0.05$                        |
| 8.  | Distance from incision in brachial artery to the graft folding place | $129.90\pm0.05$                        |

#### Table 1. Sizes of the measured parts.

chial artery to a cephalic vein in cubital fossa region, distance from an incision in the brachial artery to the graft folding place, distance from an incision in cephalic vein to the graft folding place, length of the great saphenous vein in the anterior compartment of the leg below the knee, diameter of the brachial artery, diameter of the cephalic vein and diameter of the great saphenous vein are crucial for the successful outcome of the procedure. We have detected sufficient sizes for the possible use of the patient's vein to make the anastomosis between the brachial artery and cephalic vein in the cubital fossa region of the forearm and used the native patients' graft as a connective material, instead of prosthetic graft material, which is commonly used in similar procedures in nephrology unit.

According to our study, it is possible to use the native vein of the patient, as a graft in the hemodialysis unit in cases of chronic renal failure [16]. The research provides new insight into the use of native vein of the patient in nephrology, hemodialysis and vascular surgery fields [15].

According to many epidemiological types of research [11, 25], in graft placement methods synthetic material is used in vascular anastomosis creation in patients with chronic kidney failure. The method of prosthetic vascular graft insertion has major extracavitary infection outcomes due to claudication and tissue loss [2, 13]. The most used material in prosthetic grafts is polytetrafluoroethylene (PTFE) vascular grafts, known for the elasticity and greater compliance, than grafts, used before, and Dacron grafts. The risk of infection in polyurethane graft use in graft placement surgery is 33-37.5%. The risk of Dacron graft infection is 29% [9, 23]. The infection in polyurethane grafts is more common than Dacron graft infection [4]. The great saphenous vein is used as a graft in coronary bypass surgery [5], the use of prosthetic grafts is less common in coronary bypass procedure [20]. The risk of saphenous vein graft failure in a coronary bypass surgery is 20-25%, which shows, that native vein graft material allows the procedure to be more successful, than in the case of prosthesis use [21]. Arteriovenous fistulas have a lower incidence of infection of 0.2 to 0.4 per 1000 fistula days, compared to the grafts, which proves the lesser risk of infection of the native body's material use in vascular access [18].

In the forearm compartment, graft diameter should be small due to the diameters of the brachial artery and cephalic vein, which are relatively small to other blood vessels in the human body and the blood flow rate in these vessels is relatively lower [11]. The importance of diameter measurement of the graft is connected with the possibility of graft folding, when it is being connected to other blood vessels, to the brachial artery and cephalic vein. If the diameter of prosthetic or biological graft is bigger than the blood vessel diameter, used in the vascular anastomosis, there is a possibility of graft ends folding, which could lead to the abnormal blood flow with a result of emboly [26]. To reduce the risk of thrombosis caused by neointimal hyperplasia and decreased blood flow, measurement of diameters is crucial before starting the actual procedure [6]. It can be done, by using ultrasound and angiography as a non-invasive method of diagnostics. If the diameter of the great saphenous vein is bigger than the diameter of blood vessels, that are planned to be connected to form arteriovenous anastomosis, it is recommended to use either a prosthetic graft or the biological graft, that is taken from the own patient's extremities, for example, the radial artery.

Several studies have evaluated the risk of such complication occurrence as bacterial and viral infection and graft abruption after and during prosthetic graft insertion and connection procedure [8, 17]. A disadvantage of the prosthetic graft connection method is the lack of autologous material (graft) for the bypass creation, which leads to overstimulated blood flow or opposite the insufficient blood flow due to thrombus formation on the prosthesis wall and increased risk of emboly. The main reason of bypass failure, that occurs due to the non-autologous graft use in connection procedure, is horizontal or vertical graft folding. Graft folding elevates the risk of graft infection and emboly [11, 22, 28]. Our results demonstrate that native vein can be used in arteriovenous anastomosis creating procedure and lower risks of occurrence of infectious disease in future rehabilitation of the patient, who outwent the graft placement surgery and is outgoing the hemodialysis.

The risk of blood clot formation in the lower extremity after great saphenous vein extraction is minimal due to the leg blood circulation system. Veins, that could continue to function are short saphenous vein, which is anastomosing with great saphenous vein, posterior tibial veins, fibular veins, anterior tibial veins. The oxygen-deficient blood would flow up the large circle of blood to the right atrium of the heart starting from medial and lateral plantar veins, anastomosing with posterior tibial veins, from a lateral marginal vein, anastomosing with small saphenous vein and dorsal and plantar metatarsal and digital veins, anastomosing with marginal veins of the foot.

The great saphenous vein graft allows to elongate of the graft lasting in the patient's limb, which is important for patients, who require kidney transplantation and are waiting for the donor. Our study provides information for future researches and clinical use of native patients vein in transplantology and vascular surgery departments of hospitals.

#### 4.1. Limitations of the Study

This study has several limitations. First, we cannot exclude, that in some patients' body the native vein graft will be rejected or vasodilation will occur faster, than using

the prosthetic graft, which will depend on individual reaction to the new structure in the forearm compartment, hypoxia, nutrition, different growth factor (plateletderived growth factor, vascular endothelial growth factor) activity, the presence or lack of everyday physical activity, changes in body temperature, hypotension et cetera [9, 10, 12]. A second limitation, we had no possibility to perform the procedure clinically on the patient due to the limited access to the hospital and lack of the researches, in which the great saphenous vein was used as arteriovenous anastomosis connective structure, except cardiovascular surgery researches with the saphenous vein in coronary bypass surgery [1].

#### 4.2. Strengths of the Study

To our knowledge, this is the first study, that investigates the use of great saphenous vein as a graft in forearm arteriovenous anastomosis. The novelty of the study allows transplantology and vascular surgery units to start using native patients' vein instead of synthetic material, prosthesis and provide patient the longer-lasting effect of the graft with a much lesser risk of graft failure due to the vascular muscle cell proliferation and inflammation, which depends on such factors, as a platelet-derived growth factor, vasoconstrictive substances, angiotensin, proinflammatory molecules and thromboxane, in vein-to-artery and vein-to-vein anastomosis, taking into account both vein connection ends [10]. The study extends the possibility of native vessel use in transplantology, vascular surgery departments and lowers the risk of different complications, that usually occur in procedures of prosthetic graft integration as a foreign structure in patients' bodies.

#### 4.3 Future Research

Further research is needed to establish the saphenous vein graft function in alive patients' bodies. Future studies will include the simulation of blood flow in the forearm compartment of the corpse by using sodium chloride physiological solution and saphenous graft placement in patients' forearm compartment for establishing the graft function with the real blood flow.

# 5. Conclusions

To sum up, our results provide evidence, that the great saphenous vein can be used as a vascular graft in the anastomosis creation in the anterior compartment of the forearm. There is a risk of graft folding with following graft rejection by native patients' tissues, if the graft is placed too high due to the flexion and extension of the forearm and due to the larger diameter, than diameters of blood vessels, which are being connected in the graft placement procedure. The right position and size of the graft have to be chosen for the successful outcome of the procedure.

# **Supplementary Materials**

Supplementary Materials can be found at AppStore, Microsoft, GooglePlay "Complete Anatomy 2020", the information about graft costs can be found in the protocol of procurement commission Nr. PSKUS 2018/1.

# **Author Contributions**

Conceptualization, L.B., D.K. and A.M.; Methodology, L.B., D.K. and A.M.; Validation, L.B., D.K. and A.M.; Formal Analysis, L.B., D.K. and A.M.; Investigation, L.B., D.K. and A.M.; Resources, L.B., D.K., A.M. and M.P.; Data Curation, L.B., D.K., A.M. and M.P.; Writing – Original Draft Preparation, L.B. D.K. and A. M.; Writing – Review & Editing, L.B., D.K., A.M. and M.P.; Visualization, L.B.; Supervision, D.K., A.M. and M.P.; Project Administration, D.K., A.M. and M.P. All authors have read and agreed to the published version of the manuscript.

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# **Conflicts of Interest**

The authors declare no conflict of interest.

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# Root canal anatomy and morphology of permanent maxillary Lateral Incisors in an Iranian population

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#### Abstract

A meticulous knowledge of the root canal anatomy and morphology of the teeth is imperative to achieve successful root canal and also many other dental and surgical treatments on them. The purpose of this study was to study the root canal anatomy and morphology of permanent maxillary Lateral incisors in Kerman. 100 extracted intact permanent maxillary Lateral incisor teeth were collected from different dental clinics in Kerman. The anatomic and morphologic characteristics of the selected samples such as the number of roots, the apical root curve direction and the length of the teeth were determined by macroscopic evaluation and length assessment of each sample. After staining, decalcification and clearing of each tooth the existence and location of additional and lateral canals was also carefully explored by using magnifying tools. All maxillary Lateral incisors had just one root and one root canal and the average length for this tooth was 22.5 mms in this study. The curvature of the roots in 67% of the samples was distally, in 5%; bucally, in 2%; palatally and in 3%; mesially. 23% of the teeth had straight roots and root canals. Also, 29% of the teeth had lateral canals that in 89% of the cases were located in the apical thirds, in 11%; in the middle and in none of the cases in the coronal thirds. As a conclusion lateral canals and curved apex which are thorough challenges in dentistry are prevalent in maxillary lateral incisors among this south eastern Iranian population.

#### Keywords

Anatomy, lateral incisors, Maxillary, Morphology, Root canal.

# Introduction

Maxillary Lateral Incisors are a pair of upper teeth that are located laterally from both maxillary central incisors and medially toward the midline of the face from the maxillary canines. Like all incisor teeth, their function is tearing and cutting food during mastication. These teeth generally have no cusp, but the rare condition known as the Talon cusp has been most, observed and reported in them. The surface of these teeth, which is used in mastication is called, incisal ridge or incisal edge.

The maxillary lateral incisor teeth resemble the maxillary central incisors from many aspects, but are smaller in all dimensions and the mesio-distal width of them is evidently smaller than that of the maxillary Centrals. These teeth have the most variability in crown shape within the dental arch after the maxillary third molars and it is possible to be congenitally missing bilaterally .(Wikipedia, accessed 01/03/2020).

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Disto-incisal angles of the maxillary lateral teeth are more rounded in comparison with those of the Central incisors and the root canal space of these teeth is wider in the mesio-distal than in the bucco-lingual, in contrast with the Canine teeth. Both maxillary Central and Lateral incisors have pulp horns and since the width of the incisal ridge, in the Lateral teeth is less than that of the Centrals the outline form of the access cavity to their pulp space is oval shape in comparison with the Central incisors which is triangular (Hargreaves *et al.* 2016, Nelson 2015, Ingle *et al.* 2011, Seltzer *et al.* 1988).

Previously it has been proven that the anatomic characteristics of the teeth and their related root canal morphology could follow many various patterns within different racial populations in different parts of the world (Hargreaves *et al.* 2016, Ingle *et al.* 2011).

Maxillary lateral incisors generally have a single root with a single canal but this generalization is not always true since a number of research and case report articles have described them to have two roots with two, three and even four root canals. Many anatomical anomalies and variations such as gemination, concrescence, fusion or dens invagination, have also been reported to happen in maxillary lateral incisors, since these teeth are located at a high prone to risk embryological position in the oral cavity (Lim *et al.* 2012, Mohan *et al.* 2012, Nosrat *et al.* 2015, Peix-Sánchez *et al.* 1999, Pereira *et al.* 2000, Shokouhinejad *et al.* 2009).

All maxillary incisors (Centrals, Laterals and Canines) may have various configurations of root canals in different racial groups and populations. (Vertucci *etal.* 1984, Caliskan *et al.* 1995, Sert *et al.* 2004) Because of these reported racial variations, current cross sectional study was carried out to study the external and internal root canal anatomy and morphology of the extracted permanent maxillary lateral incisor teeth in Kerman a city in the south east of Iran, using macroscopic observation along with staining, decalcification and clearing the selected samples.

#### Materials and methods

After, approval of the University research and the ethics in research committees;

Approval Code: K/90/18, 100 permanent maxillary Lateral incisors with intact and completely formed apices which had been extracted because of progressive caries, periodontal diseases, complete or partial denture treatment planning were randomly collected from 5 dental centers within 5 different municipal districts of the city of Kerman, without considering the side, age and gender as selective criterions

The attached periodontal ligament tissues were separated from the root surfaces by scaling instruments, then the samples were washed and brushed under distill water and were immersed and kept within 5.25% Sodium Hypochlorite solution (Shimin-Tehran). The length of the teeth was measured from apex to the incisal ridge considering that in cases with root curvature this measurement could underestimate root length up to 1 mm, also the apical root curvature directions of the samples were visually assessed and recorded in a table along with their measured lengths. After determining macroscopic anatomical characteristics, the root canals of the teeth were stained, decalcified and finally cleared in order to study the internal morphology of these teeth, for this purpose, access cavities to the pulp chambers were prepared with a high-speed Turbine (Bien-Air, Swiss) and diamond burs, (Diatech-Germany). Then their organic pulp tissues were dissolved and removed by immersing the teeth in 5.25% Sodium Hypochlorite (Shimin-Tehran) for nearly 12 hours and finally were washed and dried in the room temperature. The locations of the apical foramina for all samples were determined by putting a no 10 K file (Maillefer-Swiss) inside the canal, until it reached to the root apex. India ink (Shimin-Tehran) was injected into the pulp chambers of the teeth by an irrigating syringe and a 27 Guage needle. Ink was moved into the canal systems by negative pressure to the apical end of the teeth from a central suction system. Afterwards the stained samples were dried and demineralized by immersion in 14% Nitric acid solution (Shimin-Tehran) for almost 10 days. The Acid solution was changed daily and also was checked for enough demineralization of the teeth by taking frequent X-rays.

After enough demineralization the samples were dehydrated in Ethanol (Taghtir-Iran) for 12 hours and finally were made transparent by immersion inside 5% Methyl Salicylate (Merck-Germany). The teeth were maintained inside this solution until they completely became transparent. The stained, decalcified and cleared samples were finally carefully observed under the Stereomicroscope (Olympus-Japan) at ×2 to ×3 magnifications (Vertucci *et al.* 1984, Caliskan *et al.* 1995, Sert *et al.* 2004, Kuzekanani *et al.* 2019).

#### Results

All maxillary Lateral incisors had just 1 root and one root canal in this study. The assessed average length for this anterior tooth was 22.5 mms. The curvature of the roots in 67% of the samples was distally, in 5% bucally, in 2% palatally and in 3%; mesially, 23% of the teeth had straight roots and root canals. Also, 29% of the teeth had lateral canals that in 89% of the cases were located in the apical thirds, in 11% in middle thirds of the roots and in none in the coronal thirds.

#### Discussion

Many investigators and clinicians have reported more than one root and one root canal for the maxillary Lateral teeth in the literature and through the case reports. (Christie *et al.* 1981, Fabra 1985, Pecora *et al.* 1991, Caliscan *et al.* 1995, Walvekar *et al.* 1997, Peix- Sanchez *et al.* 1999, Collins 2001, Shokouhinejad *et al.* 2009, Sert *et al.* 2004, Kottoor *et al.* Mohan *et al.* 2009, 2012, Nosrat *et al.* 2015) in contrast, more than one root and one root canal was not observed among randomly selected extracted maxillary Lateral teeth in this study. In agreement with the results of current study, many other investigators have not found more than one root and one root canal for maxillary Lateral teeth in different parts of the world (Green et *al.* 1956, Chapman *et al.* 1969, Pindea *et al.* 1972, Dedeus *et al.* 1975, Vertucci *et al.* 1984).

As, it has been mentioned before, results obtained from different studies on root canal anatomy and morphology of the teeth, are so much dependent on the methodology of the research (Kuzekanani *et al.* 2018, Kuzekanani *et al.* 2020). According to some recent valuable studies, no significant statistical difference has been found between clearing of stained and decalcified teeth and the Cone beam Computed Tomography method in detecting additional canals in the extracted human teeth and these two methodologies currently are more approved than other methodologies for studying the root canal anatomy and morphology of the teeth (Neelakantan *et al.* 2010, Dalili Kajan *et al.* 2018).

Results of different studies on root canal morphology of the maxillary Lateral teeth along with the years and places of the research, also the used methodologies are summarized in the Table 1.

| Author                  | Methodology/<br>year            | Country   | 1<br>canal(%) | 2<br>canals(%) | 3<br>canals(%) | 4<br>canals(%) |
|-------------------------|---------------------------------|-----------|---------------|----------------|----------------|----------------|
| Caliskan et al          | Clearing<br>1995                | Turkey    | 95.1          | 4.9            |                |                |
| Christie et al          | Clinical<br>Raiography<br>1981  | Canada    |               | Case<br>report |                |                |
| Peix-Sánchez            | Clinical<br>Radiography<br>1999 | Spain     |               |                | Case<br>report |                |
| Collins                 | Clinical<br>Radiography<br>2001 | Australia |               | Case<br>report |                |                |
| Fabra-Campus            | Clinical<br>Radiography<br>1985 | Spain     |               | Case<br>report |                |                |
| Pecora et al            | Clinical<br>Radiography<br>1991 | Brazil    |               | Case<br>report |                |                |
| Pineda&Kuttler          | Radiographic<br>1972            | Mexico    | 100           |                |                |                |
| Sert&Bayirl             | Clearing<br>2004                | Turkey    | 97            | 3              |                |                |
| Thompson et al          | Clinical<br>1985                | USA       |               | Case<br>report |                |                |
| Vertucci                | Clearing<br>1984                | USA       | 100           |                |                |                |
| Walvekar &<br>Behbehani | Clinical<br>Radiography<br>1997 | Kuwait    |               |                | Case<br>report |                |
| Pereira                 | Clinical<br>Radiography<br>2000 | Brazil    |               | Case<br>report |                |                |

 
 Table 1. Variations in root canal anatomy and morphology of Maxillary lateral incisors in different populations.

| Author        | Methodology/<br>year            | Country | 1<br>canal(%) | 2<br>canals(%) | 3<br>canals(%) | 4<br>canals(%) |
|---------------|---------------------------------|---------|---------------|----------------|----------------|----------------|
| Kottoor       | Clinical<br>(CBCT)<br>2012      | India   |               |                |                | Case<br>report |
| Nosrat        | Clinical CBCT))<br>2015         | USA     |               |                |                | Case<br>report |
| Mohan         | Radiographic<br>2012            | India   |               | Case<br>report |                |                |
| Shokouhinejad | Clinical<br>Radiography<br>2009 | Iran    |               | Case<br>report |                |                |

Considering that the variations in the external and internal anatomical and morphological features of the teeth influence the outcome of many dental treatments, all clinicians practicing in different dental disciplines must be attentive to possible complexities present. In root canal treatments most of iatrogenic procedural errors such as missed canals, canal transportations and also perforations arise from an insufficient knowledge of root canal anatomy and morphology of the teeth. (Pan *et al.* 2019) To avoid these procedural errors using the recently introduced facilities such as the CBCT, dental microscopes and loups are highly recommended in complicated recognized cases on first common peri-Apical radiographs (Schwarze *et al.* 2002, Patel *et al.* 2007, Pan *et al.* 2019).

#### Conclusion

All maxillary Lateral incisors were single root and single canal in this Iranian population; in 67% of cases had distal curve and also in 29%, showed lateral canals that in 89% of the cases were located in the apical thirds, although many case report

articles from different parts of the world and even in Iran have described maxillary lateral incisors to have one or two roots with, one, two, three and four root canals. By review of the literature it is concluded that, Turk, Asian and south(Latin) American populations are more probable to show additional roots and root canals than other racial groups in maxillary lateral incisors.

# **Conflicts of interests**

Nothing to be declared.

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