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The Influence of Physical Activity on Functional Performance and Urinary Incontinence in Elderly Women

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Introduction

Urinary incontinence (UI) is the involuntary urine loss, with the prevalence of 17% to 24% in women over 65 years-old. It has a multifactorial cause, being a condition that affects many people in all ages mainly postmenopausal women, it is also found in athlete women who have a high level of training associated mainly to high impact activities.

Objective

The aim of this study is to conduct a literature review that relates the influence of physical activity on functional performance and on UI among women on the stage of aging.

Method

The bibliographical review was done in Medline, Scielo, Pubmed and Sports-Disco databases using the keywords physical activity or exercise, postmenopause or aging and urinary incontinence and stress urinary incontinence.

Conclusion

Elderly women engaged in a regular exercise program have a lower incidence of UI.

Keywords

► physical activity
► exercises
► urinary incontinence
► aging

Abstract

Introduction

Urinary incontinence (UI) is the involuntary urine loss, with the prevalence of 17% to 24% in women over 65 years-old. It has a multifactorial cause, being a condition that affects many people in all ages mainly postmenopausal women, it is also found in athlete women who have a high level of training associated mainly to high impact activities.

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Conclusion

Elderly women engaged in a regular exercise program have a lower incidence of UI.
the occurrence of chronic diseases. Moreover, the regular physical activity practice improves the quality of life and increases life expectancy.

The urinary incontinence (UI) is a frequent complaint among women, it can range from 32% and 64%. The most common type is the stress urinary incontinence (SUI) characterized by involuntary urine loss on physical exertion or exercising, reflecting on her daily routine, professional and sports activities Abrams et al.4 Several factors might influence this condition, as age, medication (diuretics and α-blockers), endocrine dysfunctions (diabetes), central or peripheral neuropathy (multiple sclerosis), prolapse, pelvic floor denervation, obesity and smoking.5 The urinary symptoms are also present in the transition from perimenopause to postmenopause. The urinary tract trophic changes caused by hypoestrogenism increases the susceptibility to infections, bladder filling disorders, dysuria, vaginal dryness, and dyspareunia. The atrophy of urinary tract tissues can also increase the incidence of UI by the presence of estrogen receptors and the common embryology of the bladder, urethra, and vagina influencing the onset of UI symptoms after menopause.5

The UI leads to occupational, domestic and sexual activities restrictions, causing discomfort that includes needing constantly panty liners to prevent urine leakage, frequent changing of clothing, the odor of urine and skin rashes, affecting negatively the quality of life.6

**Functional and morphological aspects of the urinary bladder**

There are functional differences among the several parts of the bladder. The ureterovesical junction (UVJ) is the name that is assigned to the transition zone between the bladder and the urethra, and it has an important role in the urine storage and voiding. Such function is maintained by a synergistic process that engages central and autonomic nervous systems, detrusor muscle and urethral sphincter.7,8

The urine storage occurs under low pressure, so bladder relaxes during the filling stage. Disorders in the storage functions can lead to lower urinary tract symptoms (LUTS), such as urgency, frequency and urge incontinence, caused by contraction of the smooth muscle of bladder on this stage. The emptying requires coordination between bladder contraction and urethra relaxation. Disorders in the emptying stage can lead to voiding dysfunctions such as incomplete emptying of the bladder sensation on post voiding. The LUTS rises significantly with age, irrespectively of whether they are men or women, is a major problem for the elderly.9

The urinary bladder stores adequate volume of urine, allowing the accumulation of volumes without increasing the intravesical pressure. Its muscle fibers spread in every direction and once the intravesical pressure increases, the emptying happens through the contraction of smooth muscle (detrusor muscle) and the relaxation of striated muscle from external urethral sphincter, to allow the urination to occur.

The bladder wall is composed of three layers: the innermost layer, the mucosa, which contains transitional epithelium tissue and is impermeable to urine; middle layer, muscular, the most developed one and which made bladder to be known as a dense muscular organ; and the outermost layer, adventitia, composed of connective tissue since serosa is just found in the upper region of the bladder.10 The muscular layer (detrusor muscle) consists in a central region of fibers arranged in a circular configuration containing 3 irregularly arranged layers of smooth muscle, intermixed with collagen fibers which makes difficult to distinguish the internal, circular and external layers of the detrusor muscle. The contraction of this muscular layer is related to urination reflex, as the muscle stretches out when the bladder is filled with urine, and this condition affects the autonomic innervation, that is under voluntary control. Thus, parasympathetic nervous system fibers are responsible for urination reflex, they are located amongst the muscular fibers layers and are responsible for bladder contraction. The sympathetic nervous system fibers are responsible for bladder relaxation and compose a plexus on the adventitia, innervating the blood vessels.9

The cells of external and internal layers tend to be arranged longitudinally and the ones from middle layer circularly. The configuration of these fibers helps the tissue architecture, enables a passive action on the tissue when under mechanical stretch, facilitating to return to the original position; along with collagen cells, the elastin cells are important for the maintenance of a regular tissue resistance.9

There is a high intercellular cohesion level that protects the bladder against the excessive volume. The collagen concentration on the vesical wall is closely related to the protection level available. The smooth muscle cells of the bladder help the collagen production accordingly to the bladder wall distension triggers.11

The smooth muscles exhibit an extreme variability, not only in ultrastructural details, but also in their contractile, regulatory, and electrophysiological properties and in their sensitivities to drugs and neurotransmitters.9

**The bladder extracellular matrix**

The cells of a tissue are generally attached to a complex extracellular macromolecules network known as extracellular matrix (ECM) that sets cells and tissues together, providing an organized structure where cells can migrate and interact with each other and also support most of the mechanical stress the tissue is exposed to.

The extracellular matrix (ECM) molecules are composed of two main classes of macromolecules: glycosaminoglycans (GAGs) and proteins. The ECM protein components are also classified in structural such as collagen and elastin and adhesive as fibronectin and laminin.12,13

The bladder ECM is composed of proteins, proteoglycans and GAGs that provides support to bladder cells and components, playing an important role in protecting the urothelium and in urine storage. The GAGs protective membrane (main chondroitin) that covers the urothelial cells form a barrier against several toxic substances.14

Most of the bladder collagen is found in the connective tissue outside the muscle bundles, in the perimysium. The main types of collagen found in the bladder are: collagen type I that represents ~75% of the bladder collagen, they are
disposed in wavy shape and under volumetric load they stretch and can become 3% to 5% longer; and collagen type III that represents ~25% of the bladder collagen, usually found around type I collagen, widely distributed throughout the vesical wall. Collagen type II seems to undergo conformational changes to adjust to the intravesical volume.15

During regular aging occurs elastic fibers fragmentation, a decrease in smooth muscle cells, disarrangement and broadening of collagen and increase of amorphous ground substance, causing the reduction of elastic properties in the urinary bladder.16

Aging and urinary incontinence

The UI is a usual issue on women aging, with prevalence of 17% to 24% in women over 65 years-old, and increase ~75% in women above 75 years-old.17

Based on epidemiological studies, the occurrence of UI in elderly women is related to diabetes, hypertension, and obesity.18

The correlation between the estrogen serum levels and the urogenital system atrophy is directly related to the incidence of UI. However, as there is a clear relationship between the higher prevalence of UI and age, so it is a huge challenge to establish whether the prevalence is due to the estrogen decrease in menopause or part of the regular aging process.19

Morphological, structural and histological studies over the urinary bladder found weight increase, thickening of the bladder wall, thinning of the urothelial layer, reduction of muscle mass and increase in collagen quantity and, therefore, disorders in the bladder storage and filling processes.20

Kitta et al.21 analyzed how the ovariectomy in middle age and young adult female rats affects the urinary continence mechanism during sneeze reflex and noticed apoptotic changes in the urethral tissue. Hence, aging and estrogen deficiency affect baseline urethral function and can induce SUI in postmenopause women.

Several factors can be the reason behind the filling disorders observed during aging, including detrusor muscle fibrosis, and therefore, vesical contraction limitation, collagen deposition, and accumulation. During aging process, the smooth detrusor muscle progressively develops fibrosis by collagen deposition and may change the vesical contractility, resulting in involuntary vesical contractions.22

The aging is also related to the reduction of urethral sphincter function in a woman, a reduction on striated muscle cells in rhabdosphincter of humans and an increase on urethral muscle fibers apoptosis.23

The dysfunctions on the pelvic floor muscle (PFM) are frequent and bring uncomfortable problems including several conditions that affect the performance of daily activities, sports, sexual and social activities, having consequences as urinary and fecal incontinence, lower urinary tract abnormalities, sexual dysfunctions, pelvic pain and pelvic organs prolapses.

The aging is a well-known factor that affects the PFM and lower urinary tract function due to hormonal deficiency, is likely one of the causes of pelvic floor muscle dysfunctions on elderly women, that can lead to pelvic organs prolapse and UI.24,25

The levator ani and the coccygeus muscle are connected to the pelvis inner surface and along with the urogenital diaphragm muscles compose the pelvic floor muscles (PFM). The levator ani consists of three main muscles: the puboccocygeus, the puborectalis and the iliococcygeus.26

The pubococcygeus and the puborectalis muscles have a U shape and arise from pubic bone through both sides of midline and stretches behind the anus. They are formed mainly by striated muscular fibers type I, which are important to maintain continuous tone during resting state and keep the urogenital hiatus closed. The iliococcygeus arises laterally from tendinous arch reaching the gap on the posterior part of the pelvis, providing thereby support to the pelvic organs.27

The PFM’s are functionally essential to maintain continence and pelvic support. Ultrasonography studies show that under the sudden rise of intra abdominal pressure (around 150 cmH2O) the proximal urethra undergoes a displacement of the midsagittal plane close to 10mm, therefore the lower abdominal content is forced caudodorsally, due to a simultaneous contraction of the diaphragm and the abdominal wall muscles. The bladder neck downward move that is visible on the ultrasonography makes the surrounding tissues to move downwards. The abdominal pressure is transversally transferred to the urethra, so that its anterior wall is deformed toward the posterior, thereby helping to close the urethral lumen and prevent urine loss caused by increased intravesical pressure. The PFM’s injury reduces the support layer and provides less resistance against deformity while increased abdominal pressure, thus the urethral lumen closing is ineffective, increasing the incidence of SUI.27

The muscles in elderly have around 35% less strength, exhibit mass loss and atrophy mainly in type II fibers, when compared with a young adult.28 These changes are not due to neural recruitment but to aging on muscle contractility. Furthermore, if PFM’s are damaged or the innervation is impaired, muscle contraction will take even longer to make the same strength.27,28

The presence of estrogen receptors in the pelvic floor muscles means that the reduction of this hormone concentration in the body can also promote changes in this musculature.25,29

Bocardi et al.20 after observing the aging effect over PFM function and electromyographic activity in healthy and nulliparous women from 18 to 69 years old, noticed no difference among the several groups of different ages when comparing the pelvic floor function and muscular strength. However, a low negative correlation was found between age and the electromyographic activity of the pelvic floor muscles revealing the trend that higher the age, lower the electromyographic activity of these muscles.

The pelvic floor is directly related to the urinary continence mechanism, as the muscular function is better on physically active women.31 Women that have UI usually exhibit pelvic floor dysfunctions. Strengthening these muscles results in an efficient improvement in urinary losses.32
Method

A systematic bibliographical review that examined the influence of physical activity on functional performance and on urinary incontinence among elderly women. The research was performed by 2 (two) editors on articles from January 2000 to January 2017. This study follows platform PRISMA8 protocols over articles selection and eligibility criteria.

The research was done on the following databases: Medline, Sports Disco, Pubmed, and Scielo. In each database, these keywords were checked: urinary incontinence, post-menopause, aging, physical activity, exercise, sport and fitness in Portuguese and its corresponding term in English, with the Boolean operator AND. A manual search was also conducted in all references mentioned in the articles selected for this study. The complete PubMed database research strategy can be found in Table 1: The eligibility criteria were: aging women population, who performed a regular physical activity for more than three months, there was no initial restriction regarding publications quality. The short-listed articles were fully analyzed (Fig. 1).

From the review of titles and abstracts, it was selected for fully reading the cross-sectional and longitudinal randomized clinical trials, which includes a method for assessing incontinence and/or functional performance, type of physical activity performed and incidence of UI.

It was excluded repeated studies, protocols of articles recorded in databases, studies involving pharmacological agents, noninterventional studies or those which didn’t specify the type of intervention performed.

Results

The electronic search identified 218 articles on Medline, Sports Disco, Pubmed and Scielo databases, after removing the duplicates.

After reading the titles, 206 articles were excluded for they were not related to the subject or for being unavailable.

Table 1 Complete database research strategy

<table>
<thead>
<tr>
<th>Keywords in English</th>
<th>Keywords in Portuguese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity OR exercises AND postmenopausal OR elderly OR aged OR older AND urinary incontinence</td>
<td>Atividade física OR exercícios AND pós-menopausa OR mulheres idosas OR envelhecimento AND incontinência urinária</td>
</tr>
<tr>
<td>Physical activity OR exercises AND postmenopausal OR elderly OR aged OR older AND stress urinary incontinence</td>
<td>Atividade física OR exercícios AND pós-menopausa OR mulheres idosas OR envelhecimento AND incontinência urinária de esforço</td>
</tr>
</tbody>
</table>

Fig. 1 Studies selection process flowchart, as per PRISMA check-list.
UI. It is presumed that high impact activities (like jogging and running) between high levels of physical activity and reduction of UI in women having kidney failure. No correlation was found compared to the relation for UI incidence in 471 women of 346 women over 65 years old. The UI was evaluated through the ICIQ-SF. The physical activity level was evaluated using a set of 08 tests and the fitness index was calculated. Active and sedentary behaviors were then recorded from standardized questionnaires. The UI was found in 28% of the participants with higher prevalence in obese and lower physical ability ones. There was a trend toward a higher level of physical activity and walking once a day in women with no UI when compared with those with UI.

Danforth et al.34 and Morrisroe et al.35 evaluated the incidence of UI using the questionnaire which has the following question: “In the last 12 months, how often have you had leakage or loss of urine?” The answer possibilities were: less than once a month, once a month, 2 or 3 times per month, once a week, every day. Women that have the loss more than once a month were defined as incontinent.

Morrisroe et al.35 evaluated the fitness performance of Latin women in the United States, applying the Guaralnik Short Physical Performance Battery for more than 60 years. To measure the number of steps they take per day, it was given a pedometer to be used throughout the day. It was shown that physical activity practice is related to lower incidence of UI. The interventions made to improve physical performance may help to prevent UI, as a better functional performance helps the toilet visits, thus keeping a vesical control.

Virtuoso et al.31 analyzed the UI incidence and perineal muscle function in 39 elderly women (over 60 years old) active and inactive in regular physical activity. For such they applied the section “Atividade Física de Recreação, Esporte, Exercício e Lazer” from questionnaire “Questionário Internacional de Atividade Física adaptado para idosos”, where

Table 2 Summary of included studies (n = 8)

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Type</th>
<th>Age</th>
<th>N</th>
<th>Population characteristics / intervention</th>
<th>Assessment method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moreno-Vecino et al. (2015)</td>
<td>Transversal</td>
<td>&gt; 65 years-old</td>
<td>471</td>
<td>Walking</td>
<td>ICIQ-SF” Fitness assessment</td>
<td>[UI sedentary group (p = 0.08), ↓ UI walking group (p = 0.01), ↓ UI smaller the flexibility (p &lt; 0.01)]</td>
</tr>
<tr>
<td>Morrisroe et al. (2014)</td>
<td>Transversal</td>
<td>60–93 years-old</td>
<td>248</td>
<td>20 minutes exercising 3X per week</td>
<td>ICIQ’ Pedometer and physical performance</td>
<td>[functional performance ↓ UI symptoms (p = 0.05)]</td>
</tr>
<tr>
<td>Tak et al. (2012)</td>
<td>Randomized Controlled Multicentric</td>
<td>Average of 84 years-old</td>
<td>155</td>
<td>Group exercising on functional ability to use the toilet Pelvic floor training</td>
<td>Physical performance questionnaire Three-day voiding diary</td>
<td>[functional performance has not been associated to the ↓ of UI (p = 0.01)]</td>
</tr>
<tr>
<td>Vinsnes et al. (2012)</td>
<td>Randomized Controlled Multicentric</td>
<td>&gt; 65 years-old, average of 84,3 years-old</td>
<td>68 (TG n = 35 / CG n = 33)</td>
<td>TG: transfer, walk ability, balance, muscle strength, endurance and ADL training CG = habitual care</td>
<td>24 hours Pad-Test</td>
<td>↓ UI on TG (p = 0.03)</td>
</tr>
<tr>
<td>Virtuoso et al. (2011)</td>
<td>Transversal</td>
<td>&gt; 60 years-old</td>
<td>28 active on physical activities 11 inactive</td>
<td>Exercising the last 6 months</td>
<td>ICIQ’</td>
<td>↓ UI in women who performs physical exercises (p = 0.288)</td>
</tr>
<tr>
<td>Townsend et al. (2012)</td>
<td>Prospective cohort</td>
<td>37–54 years-old (average of 45,9)</td>
<td>–</td>
<td>Walking (35%), vigorous activities (18%) e calisthenic exercises (15%)</td>
<td>ICIQ’</td>
<td>↑ physical exercises ↓ UI</td>
</tr>
<tr>
<td>Danforth et al. (2007)</td>
<td>Prospective cohort</td>
<td>54–79 years-old (average of 65,9)</td>
<td>2335</td>
<td>Walking (≥ 50%)</td>
<td>ICIQ’</td>
<td>↑ walking ↓ UI 26% ↑ physical exercises ↓ UI (p &lt; 0.01)</td>
</tr>
<tr>
<td>Kikuchi et al. (2012)</td>
<td>Transversal</td>
<td>&gt;70 years-old</td>
<td>346</td>
<td>Exercising the last 12 months</td>
<td>ICIQ’</td>
<td>↓ exercises ↓ UI</td>
</tr>
</tbody>
</table>
elderly women were considered as active in case they perform 150 minutes or more of moderate or vigorous physical activity during a regular week. It was included among the activities: aerobics, swimming, dancing, and bodybuilding. They also applied a validated questionnaire about the urinary tract dysfunctions symptoms and the definition of UI type (stress or urge incontinence) as determined by the International Continence Society (ICS). The participants were inquired making use of a 4 points rating scale (never, sometimes, once a week, all the time or during daytime and nighttime). The pelvic floor function was evaluated by digital palpation using the PERFECT scheme and perineometry. The results show that physically active elderly women seem to have better pelvic floor function than the inactive ones.

Danforth et al. evaluated through a questionnaire, the relation between physical activity and the risk to establish UI, in women between 54 and 79 years old. The women were inquired about how much time they spend per week on physical activity, choosing from 0 minutes to 11 hours or more and also indicating which activity was performed among: running, swimming, dancing, aerobics, calisthenic exercises, squash, cycling or low-intensity exercises like yoga (in or outdoor). From the results, they noticed that women that performed walking presented 26% lower chance of UI compared with the those who performed other activities (aerobics, running, cycling, dancing, water aerobics, among others). There was no evidence of a relation between physical activity and emergency or mixed UI. Among: running, swimming, dancing, aerobics, calisthenic exercises included: sitting and getting up from chair and bed, upper-limb mobility and walking. The intervention was performed by a physiotherapist specialized in pelvic floor musculature training, with experience in group training and affinity with the elderly. The Physical Performance Test (PPT) was applied. The involuntary urine loss was measured through the three-day voiding diary. The reduction of UI was noticed in 40% of the intervention group and in only 28% of the control group. The frequency of urine loss episodes decreased in both groups in six months (51% in the intervention group and 42% in the control group). The physical performance improved in the intervention group and worsened in the control group during the study. The physical performance improvement was related to joining in an exercise program.

**Discussion**

The physical activity is an integral part of promoting healthy, active and independent aging, and urinary complaints cannot be neglected, as it is established that women can quit exercising if they experience urinary losses during the exercises, directly affecting their physical fitness and functional performance.

Individual strategies and training for UI control should be embraced and not allow this symptom to be part of the aging process since UI is one of the major public health problems in aging, affecting negatively the life quality of this population. The UI during physical activity is an obstacle that can hinder people from exercising, mainly in women with more severe losses, increasing the possibility of inactivity and obesity.

The urine loss issue in young female athletes has been underestimated. Several studies show increased risk of UI in this population and tends to be even worse over the years. Approximately 30% of female athletes experience some urinary loss during exercise. Young women who practice low-intensity exercise for at least 1 hour or more per week, have less UI compared with the group of sedentary women. However, studies that show correlation of UI and physical activity in elderly women are limited, some studies include in its research only physical activity practice, and some include an association between daily activities orientations and physical activity and some include an association between physical activity and UI. Elderly women who practice low/moderate physical activity have lower UI complaints when compared with sedentary women. Danforth et al. found a decrease in UI risk of 20 - 25% in elderly women who walk, as well as active young women also have a lower incidence of UI. Sedentary women should be advised to participate in a low or moderate physical activity program. The exercise program should be unconditionally embraced to prevent or reduce urinary losses and enhance physical.

The pelvic floor muscle exercising, when performed with the help of a qualified physiotherapist, is an important factor both for the prevention and for the UI treatment, and it is the first line therapy in women with UI. The exercises for the pelvic floor muscles when associated with the regular physical activity contribute effectively to UI reduction and should be embraced into physical activity practice.
Physically active women have a stronger pelvic floor. The women who participated in a low-intensity exercise program for one hour or more per week had fewer UI complaints compared with the nonparticipating group.\textsuperscript{45} On the other hand, some high impact activities may increase SUI symptoms during exercise. Therefore, it is recommended for this population to practice low or moderate intensity exercises and it is advised to contract the pelvic floor muscles in every situation of intra abdominal pressure increasing.\textsuperscript{31} Nygaard and Shaw\textsuperscript{46} suggest that women with some loss during exercise, carry out prevention strategies such as emptying the bladder before activity. No association between physical activity and UI is established, there is still the hypothesis that women with some loss should avoid physical activity.\textsuperscript{35}

Nygaard et al.\textsuperscript{32} assessed the prevalence of UI in middle-aged women (from 39 to 65 years old) and concluded that intense physical activity performed for more than 10 years may be associated with a moderate increase in SUI. Thus, walking is an effective modality to be practiced by elderly women to promote functional performance improvement, it has no negative effects on UI and helps the overweight control and obesity\textsuperscript{33,41} since weight reduction is associated with UI reduction.\textsuperscript{49}

Hannestad et al.\textsuperscript{45} found that the risk of severe incontinence among obese women was three times higher than in normal weight women. Low or moderate physical activity helps maintain the body weight, reduces body fat, decreases intra abdominal pressure, and strengthens the pelvic floor.\textsuperscript{33} There is, therefore, the hypothesis that women that practices physical exercises have a lower body mass index, which may also contribute to the lower incidence of UI complaints.

The multimorbidity contributes to a sedentary lifestyle and inactivity, so health programs should include changes in women’s lifestyles. These changes should include weight loss, control of chronic diseases, maintenance of mobility and functional capacity, and restoration of life quality.\textsuperscript{50} Morrisroe et al.\textsuperscript{35} found that the decline in health, obesity and low physical performance is associated with a higher incidence of UI, and they associated the best physical performance to the ability to easily move to the bathroom, reducing urinary losses.

This study has faced some limitations. The first was the lack of clinical trials proving that elderly women that practice physical activity has a lower incidence of UI. Therefore, in most of the studies, questionnaires were sent to assess the extent of the UI and which physical activity they perform. Future studies should be conducted on elderly women population to conclude which activity is most effective in preventing and controlling UI.

**Conclusion**

The data indicate that elderly older women who participate in regular exercise programs have a lower incidence of UI. Therefore, it is necessary to endorse physical activity for the studied population.

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Physical Activity and Urinary Incontinence in Elderly Women

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Bilateral Anatomical Variation in the Formation of Trunks of the Brachial Plexus - A Case Report

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¹Laboratório de Anatomia Humana, Departamento de Biologia e Farmácia, Universidade Santa Cruz do Sul – UNISC, Santa Cruz do Sul, Rio Grande do Sul, Brazil


Abstract

This study presents a bilateral variation in the formation of trunks of brachial plexus in a male cadaver. The right brachial plexus was composed of six roots (C4-T1) and the left brachial plexus of five roots (C5-T1). Both formed four trunks thus changing the contributions of the anterior divisions of the cervical nerves involved in the formation of the cords and the five main somatic motor nerves for the upper limb. There are very few case reports in the scientific literature on this topic; thus making the present study very relevant.

Keywords

► brachial plexus
► trunks

Introduction

Most of the upper limb nerves arise from by the brachial plexus (BP), which has a complex anatomical structure that begins in the root of the neck and extends to the axilla (armpit region). The BP is formed by the anterior rami of the lower four cervical nerves (C5-C8) and first thoracic nerve (T1), which form the roots of the brachial plexus.¹⁻³ Generally, the roots of BP nerves pass through the space between the anterior and middle scalene muscles along with the subclavian artery and join with each other by anastomosis leading to the formation of the upper trunk (C5 and C6), medium trunk (C7), and lower trunk (C8 and T1), accompanying the axillary artery under cover of the pectoralis major muscle. These three trunks pass through the cervicoaxillary canal, posterior or anterior the clavicle or behind; each trunk splits into anterior and posterior divisions: the anterior divisions from the upper and middle trunks form the lateral cord, the anterior divisions from the lower trunk form the medial cord, and the three posterior divisions from the posterior cord.²⁻⁴ The supraclavicular branches (descending branches) of BP arise from the trunk or from cervical nerves, parallel to the trunk, and are as follows: dorsal scapular nerve, suprascapular nerve, long thoracic nerve, and subclavian nerve. The short infraclavicular branches, namely axillary nerve, subscapular nerves, thoracodorsal nerve, and medial and lateral pectoral nerve, arise from the cords and innervate the shoulder muscles. The long infraclavicular branches, which extend longitudinally to the upper limb, are: the radial nerve, the ulnar nerve, and the median nerve.⁴⁻⁵

Variations in the formation of the BP are common and are usually related to the contribution of anterior rami of C4 and T2 nerve roots. When the superior root of the plexus is C4 and the inferior root is C8, it is a prefixed Brachial Plexus. When the superior root is C6 and the inferior root is T2, it is a postfixed BP. Variations may also occur in the formation of trunks, divisions and cords, in the origin and/or combination of branches, and in the relationship with the axillary artery and scalene muscles.²⁻³⁻⁵

The BP is of great importance in medical and surgical procedures and in making a diagnosis of orthopedic injuries, such as neurovascular compression of the subclavian artery and BP injuries.⁵ Brachial plexus blocks, such as the interscalene and inter-sternocleidomastoid blocks, are commonly employed anesthesia techniques for proximal upper extremity surgery.⁷ The knowledge of the anatomical variations of the BP is very important to anatomists, radiologists, anesthesiologists, and surgeons, mainly for making accurate diagnosis of nerve injuries and providing the most effective and appropriate treatment for injuries in the axillary region and upper limb. Moreover, from a clinical point of view, in terms
of anesthesia and surgical interventions, it is necessary to understand BP variations, morphometry, and possible anastomosis.1,9

Due to their importance in surgical procedures involving the upper limb, variations of the brachial plexus have been addressed in the scientific literature in terms of their origin, formation, and distribution. Therefore, studies aimed at contributing to the morphological knowledge of BP and its variations are of paramount importance as a source of information and guidance for safe surgical procedures in brachial plexus injuries. Accordingly, the present study addresses a bilateral anatomical variation in the formation of trunks of the brachial plexus.

Case Report

During a routine dissection of a 60 year-old Caucasian male cadaver fixed in 10% formalin, in the Human Anatomy Laboratory of the University of Santa Cruz do Sul, RS, Brazil, we found a bilateral variation in the formation of the trunks of the brachial plexus; there were four trunks instead of the usual three.

The right brachial plexus/RBP (Fig. 1) was composed of six roots (C4-T1), which formed four trunks: first trunk (C4-C5); second trunk (C6); third trunk (C7) and fourth trunk (C8-T1). The four trunks formed anterior divisions, and only the three superior trunks formed posterior divisions.

The posterior cord was formed by posterior divisions of the three superior trunks (C4-C7); the medial cord was formed by anterior divisions of the two lower trunks (C7-T1); and the lateral cord was formed by anterior divisions of the first three trunks (C4-C7).

The median and ulnar nerves were formed by anterior divisions of the four trunks, where one branch originated from the lateral cord, called lateral root (C4-C7), and the other branch originated from the medial cord, called medial root (C8-T1). The axillary nerve was formed by the union of the two posterior divisions of the first (C4-C5) and second trunk (C6). The radial nerve originated from the posterior cord (C4-C7) and had no communication with the C8 and T1 roots; the musculocutaneous nerve was formed by the three anterior divisions of the three superior trunks (C4-C7).

The left brachial plexus/LBP (Fig. 2) was composed of five roots (C5-T1), which formed four trunks: first trunk (C5);
second trunk (C6); third trunk (C7), and fourth trunk (C8 and T1). All four trunks formed anterior and posterior divisions.

The posterior cord was formed by posterior divisions of the four trunks (C5-T1); the medial cord was formed by anterior divisions of the third (C7) and fourth trunk (C8 and T1); the lateral cord was formed by anterior divisions of the first three trunks (C4-C7).

The median nerve was formed by anterior divisions of the four trunks (C5-T1), where one branch originated from the lateral cord, called lateral root (C5-C7), and the other branch was originated from the medial cord, called medial root (C7-T1). The ulnar nerve was formed by anterior divisions of the third (C7) and fourth trunk (C8 and T1). The axillary nerve was formed by posterior divisions of the first (C5) and second trunk (C6). The radial nerve was formed by the posterior divisions of the four trunks (C5-T1); the musculocutaneous nerve was formed by anterior divisions of the two superior trunks (C5 and C6).

The Tables below show a summary of the contributions of the roots to the formation of the trunks of the brachial plexus: ►Table 1, typical pattern described in the literature according to Standring; ►Table 2, right brachial plexus; and ►Table 3, left brachial plexus.

**Discussion**

The present study demonstrates a bilateral variation in the formation of the BP trunks, in which the RBP was composed of six roots (C4-T1) and the LBP of five roots (C5-T1). The nerve fibers that contribute to the formation of the upper limb nerves showed variations due to the presence of four trunks. There are very few reports in the scientific literature addressing cases of BP with four trunks. Investigating 60 BPs which belonged to 30 cadavers, Chaudhary et al. found four trunked brachial plexuses on the left side in three cases. Of the three cases, one BP (prefixed and postfixed) was composed of roots C4-T2 which formed four trunks: first trunk (C4-C6), second trunk (C7), third trunk (C8), and fourth trunk (T1 and T2). In the other two cases, the BPs (postfixed) were composed of C5-T2 roots, which formed four trunks: first trunk (C5 and C6), second trunk (C7), third trunk (C8), and fourth trunk (T1 and T2). Although the same variation of four trunked plexus was found in the present study, these authors found different variations in terms of the fibers from which they originate and trunk endings.

During routine dissection of a female cadaver, Singla et al. found two trunks instead of the usual three in the

Yildiz et al. found the same variation in a male cadaver in the LBP. RBP: upper trunk (C5 and C6) and the lower trunk (C7, C8, and T1) with an absence of the middle trunk. Yildiz et al. found the same variation in a male cadaver in the LBP.

In another study, Shetty et al. found unilateral variations in the formation of the trunks in five (11.3%) of 44 cadavers investigated. In one cadaver (2.27%), the middle trunk was formed by the union of C7 and C8 roots, and the lower trunk was formed by the T1 root. In another cadaver (2.27%), the upper and middle trunks were fused with each other. In the other three cadavers (6.81%), the C5 root pierced the anterior scalene muscle before joining C6 to form the upper trunk.

The prevalence of anatomical variants of the brachial plexus is addressed in different specimens in the literature. One of the first common variants described is the prefixed or postfixed types of BP. In the present study, a prefixed RBP was found, in which the C4 roots contribute to its formation, but the T1 root is not smaller or absent and is similar in caliber to the other roots. Ogoiba et al. and Bowden argue that the prevalence rates for the prefixed type range from 12 to 30%, and that the postfixed type occurs in 0% to 11% of the cases. In a study conducted in Brazil, it was found a prevalence of the prefixed type in 20% to 24% of cases, and the postfixed type in 0% to 6% of the cases.

Fazan et al., evaluated 54 BPs in 27 cadavers and found normally formed BPs in 39 cadavers (72%) and prefixed BPs in 13 cadavers (24%); two males and eleven females (54% RBPs and 46% LBPs). Only three BPs were postfixed and they were found in male cadavers, two in the right antimer and one in the left antimer. According to the authors, intercordal neural communications between the lateral and medial cords are relatively common, especially between the lateral cord and the ulnar nerve or between the median nerve and the musculocutaneous nerve. They found communications between the lateral cord and the ulnar nerve in 30% of cases. The same variation was found by Harris in 26 (86.66%) of the 30 upper limbs studied by this author.

Investigating 32 upper limbs in 16 formalin-fixed Brazilian cadavers of both sexes, Olave et al. found a communicating branch between the musculocutaneous nerve and the median nerve in ten cases (31.3%), nine males, four in the right antimer and six in the left antimer, and two with bilateral variation. Chiarapatistanakom et al. found the same communication branch in 21.8% of 57.3% cases investigated. In 75 (90.5%) of the cases studied by Tountas and Bergman, the musculocutaneous nerve originated from the BP, 2% originated from the median nerve, and 1.4% from the posterior cord. In the present study, it was found a communicating branch between the musculocutaneous and median nerves in the BP in both antimeres. In the RBP, the radial nerve was formed by the posterior cord (C4-C7) showing no communication with the C8 and T1 roots, and the axillary nerve was formed from the fusion of the two posterior divisions of the first (C4-C5) and second trunk (C6).

According to Albertoni et al., the prevalence rates of anatomical variants of the BP varies depending on the population studied and can be up to 48%. In some individuals, the trunk divisions or the cord formations may be absent in different parts of the BP; However, the makeup of the terminal branches is invariable. Moreover, the lateral or medial cords may receive fibers from anterior divisions directly inferior or superior to the usual levels respectively, resulting in the fusion of different calibers the terminal branches. According to Albertoni et al., the prevalence rates of anatomical variants of the BP varies depending on the population studied and can be up to 48%. In some individuals, the trunk divisions or the cord formations may be absent in different parts of the BP; However, the makeup of the terminal branches is invariable. Moreover, the lateral or medial cords may receive fibers from anterior divisions directly inferior or superior to the usual levels respectively, resulting in the fusion of different calibers the terminal branches.

### Table 1

<table>
<thead>
<tr>
<th>Cords</th>
<th>Nerve</th>
<th>Roots</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral</td>
<td>Musculocutaneous</td>
<td>C5, C6, C7</td>
<td>+ C4</td>
</tr>
<tr>
<td></td>
<td>Roots lateral of</td>
<td>(C5), C6, C7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>the nerve median</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial</td>
<td>Ulnar</td>
<td>C7, C8, T1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roots medial of</td>
<td>C8, T1</td>
<td></td>
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<tr>
<td></td>
<td>the nerve median</td>
<td></td>
<td></td>
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<tr>
<td>Posterior</td>
<td>Axillary</td>
<td>C5, C6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Radial</td>
<td>C5, C6, C7, C8, (T1)</td>
<td></td>
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</tbody>
</table>

(+) inclusion in the formation; (-) absence in the formation.

### Table 2

<table>
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</tr>
<tr>
<td></td>
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<td>C4, C5, C6, C7</td>
<td>+ C4</td>
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<tr>
<td></td>
<td>the nerve median</td>
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<td></td>
</tr>
<tr>
<td>Medial</td>
<td>Roots medial of</td>
<td>C8, T1</td>
<td>- C7</td>
</tr>
<tr>
<td></td>
<td>the nerve ulnar</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roots medial of</td>
<td>C8, T1</td>
<td></td>
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<tr>
<td></td>
<td>the nerve median</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior</td>
<td>Axillary</td>
<td>C4, C5, C6, C7</td>
<td>+ C4</td>
</tr>
<tr>
<td></td>
<td>Radial</td>
<td>C4, C5, C6, C7, C8, (T1)</td>
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</tbody>
</table>

(+) inclusion in the formation; (-) absence in the formation.

### Table 3

<table>
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<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral</td>
<td>Musculocutaneous</td>
<td>C5, C6</td>
<td>- C7</td>
</tr>
<tr>
<td></td>
<td>Roots lateral of</td>
<td>C5, C6, C7</td>
<td></td>
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<tr>
<td></td>
<td>the nerve median</td>
<td></td>
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</tr>
<tr>
<td>Medial</td>
<td>Ulnar</td>
<td>C7, C8, T1</td>
<td>- C7</td>
</tr>
<tr>
<td></td>
<td>Roots medial of</td>
<td>C7, C8, T1</td>
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(+) inclusion in the formation; (-) absence in the formation.
variations in the BP are not influenced by gender or race and are more common in a certain antimer.

Gonzáles et al.\(^{21}\) stated that severe BP injuries can cause shoulder paralysis due to disrupted innervation of the muscles. An epidemiological study on traumatic BP injuries in adults performed by Flores\(^{22}\) showed that the BP is very susceptible to injuries due to its anatomical position and relationship with other anatomical structures; therefore knowledge of anatomy is essential for treatment and injury prevention.

According to Olave et al.,\(^{18}\) there may be nerve fibers that run for some distance in another nerve, to reach their innervation territory and return to the nerve they belong to be distributed, which are sometimes denominated anastomotic communications and allow the understanding of the variations in the innervation territories of nearby nerves. Moreover, the presence of anatomical variations of the peripheral nervous system is often used to explain unexpected clinical signs and symptoms. Therefore, the knowledge of the distribution, route, and patterns of the rami of nerves, cords, trunks, and roots that make up the BP are fundamental for clinical investigations involving peripheral nerve injuries.

Considering that every individual may have some type of anatomical variation, the present study aims to deepen the knowledge of anatomical variations of the BP, which can be an important source of information to help healthcare professionals to acknowledge and interpret BP variations make accurate diagnosis, improve surgical safety, and provide effective treatment facilitating recovery.

References

Biceps Brachii Muscle with Third Head a Case Study

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Abstract

Introduction Biceps brachii muscle belongs to the flexor group of muscles in arm. As Biceps brachii is double headed muscle. It is one of the known variable muscles of human body in terms of number and morphology.

Material and Method During routine human cadaveric dissection for the purpose of teaching medical undergraduates in our department. The upper limb of embalmed adult male cadaver was dissected as per the standard method to note the origin, insertion, and nerve supply of biceps brachii.

Results The three headed biceps brachii was found unilaterally on right side in adult male cadaver. The long and short head had normal origin, but third head originated from humerus below insertion of coracobrachialis and inserted with main muscle belly and innervated by musculocutaneous nerve.

Conclusion Variant biceps brachii may confuse a surgeon who performs procedures on the arm and may lead to iatrogenic injuries. The surgeons and traumatologists have to keep such muscular variations in mind. So the knowledge of existence of its variation is important for anaesthetists and surgeons.

Keywords ► biceps brachii ► number ► third head ► variation

Introduction

The biceps brachii as the name suggests, biceps mean any skeletal muscle having two heads and brachii means arm. There is a biceps muscle in both forelimbs and hindlimbs i.e., biceps femoris. Biceps brachii muscle belongs to the flexor group of muscles in arm. It is also called biceps flexor cubiti.

It is the only flexor muscle of the arm which crosses the shoulder as well as the elbow joint. As Biceps brachii muscle has two heads, short head and long head. Long head originates from the supraglenoid tubercle of scapula and it is both intraarticular and extrasynovial and short head originates from the tip of coracoid process of scapula. Distally, these two heads join to form a common tendon which inserts on the posterior rough part of radial tuberosity, and some aponeurotic fibers form the bicpital aponeurosis which merges with deep fascia of forearm, this arrangement serves to protect the brachial artery and median nerve during phlebotomy of median cubital vein.

The Biceps brachii muscle is innervated by the musculocutaneous nerve (root values: anterior division of ventral primary rami of C5, C6) and supplied by brachial and anterior circumflex humeral arteries. This biceps brachii is a biarticular muscle which means that it helps to control the motion at two different joints shoulder and elbow. The biceps brachii acts as true prime supination of forearm assisted by supinator muscle and act as a synergist to the true prime flexor the brachialis muscle at elbow joint. The long head acts as humeral head depressor and glenohumeral joint stability. The short head aids in adduction of humerus, also act as fixator to stabilize shoulder joint.

Case History

During the routine undergraduate cadaveric dissection performed in our department of upper limb a three headed biceps brachii was found unilaterally in the right arm of 45–55-year-old adult male cadaver. The human
cadaver used in the dissection was obtained through our departmental body donation program following all ethical guidelines.

The arm was dissected carefully to expose the full length of Biceps muscle from proximal to distal attachment. All other related structures were also exposed. The additional head was examined for the origin and course up to its insertion and then photographs were taken (Fig. 1).

The third head was a flat muscular belly which originated from the shaft of humerus near the insertion of coracobrachialis. Third head was present deep to other two heads. It lies lateral to the brachial artery and median nerve. Third head was supplied by a twig from musculocutaneous nerve. Other two heads of Biceps were supplied by musculocutaneous nerve. Third head descended and merged with other two heads to form common tendon and was inserted on posterior part of radial tuberosity. No other variations were observed.

**Discussion**

Variations are inherent in all the species and is necessary to ensure diversity within the species. Biceps brachii is one of the most variable muscles of the human body Occurrence of this variation is population specific with a rate ranging from 2% (Nayak et al., 2006) to 37% of different ethnic groups, out of which Indian population had 2% variation and highest in Columbians i.e., 37%. It is one of the most variable muscle in human being, third head being a common variation, though supernumerary heads up to 8 have been recorded in previous literature. Most of the articles mention supernumerary head of biceps occurs unilaterally, and bilateral occurrence is rare. Supernumerary heads are found more commonly in males.

After searching the literature, we came to know extra heads may present as a belly similar to usual heads or may be present as a group of accessory fascicles arising from either of these: - Head or shaft of humerus, coracoid process, pectoralis major or minor tendon, capsule of the shoulder joint or V- Shaped insertion of deltoid muscle. The most common origin is from the proximal part of humerus as in our case, hence also called as humeral head. Below are some of the researches on third head with different origins.

Kumar et al. stated that they found bilateral third head of biceps brachii in one male cadaver out of 48 cadavers. Its origin was from the anterior limb of V shaped deltoid insertion on the humerus and it fused with the common bulk of the muscle before insertion.

Donmez et al. found supernumerary heads in two male cadavers. In one case the origin was from the medial lip of the intertubercular groove and in another case it was from the lateral lip of intertubercular groove. In both the cases, the three heads united with one another at the middle third of the arm and formed a common tendon before the insertion. Balasubramanian found supernumerary head in one old adult male cadaver originated from upper and medial part of brachialis and fused with the common muscle belly for insertion.

Singh et al. observed additional head in both the arms arose from humeral shaft between the insertion of coracobrachialis muscle above and origin of brachialis below in adult male cadaver bilaterally.

Govindarajan and Vellaichamy found third head arising from capsule of shoulder joint and fused with main muscle in middle of right arm in adult male cadaver.

Shashikala and Ashwini conducted a study on ten cadavers from which third head was found only in one case originated from V shape insertion of deltoid muscle unilaterally and inserted with main muscle.

![Fig. 1](image_url)  
**Fig. 1** Third head of biceps brachii arising from humerus near insertion of coracobrachialis (THB-third head of biceps, SHB-short head of biceps, LHB-long head of biceps, CBM-coracobrachialis muscle, MCN - musculocutaneous nerve, MN-median nerve).
Lokanadhan and Devi\textsuperscript{11} found supernumerary head bilaterally in two cadavers out of 40 cadavers. In one cadaver the third head originated from lower anterior part of humerus on its medial side and fused with common bulk of muscle before its termination into bicipital aponeurosis. In another male cadaver it had its origin from upper anterior part of humerus on its medial side. The third head fused with common bulk of muscle before the origin of bicipital tendon.

Cheema and Singla\textsuperscript{12} found three unilateral supernumerary heads in biceps brachii muscle out of 63 adult cadavers in North Indian Population. Two of the supernumery heads had their origin from the shaft of the humerus near the insertion of coracobrachialis and one had its origin near the origin of brachialis. Their insertion was into the muscle belly in two cases and into the bicipital aponeurosis in one. The supernumery heads were innervated by a branch from musculocutaneous nerve.

As in our case third head is arising from humeral shaft also called humeral head which is most common location of supernumery head and is supplied by musculocutaneous nerve and inserted into main muscle belly before insertion which is in agreement with the previous reports.\textsuperscript{8,12} The presence of third head of biceps brachii has its functional and clinical implications. From functional point of view third head increases the strength of biceps tendon in elbow flexion and pronation of forearm irrespective of shoulder position. From clinical point of view third head can cause unusual bone displacement subsequent to fracture humerus. If supernumery head is present unilaterally it can confuse the surgeon for any soft tissue tumor during operation. Variant origin of third head may lead to intramuscular course of musculocutaneous nerve and can cause nerve compression during contraction of biceps especially important in cases of professional body builders and weight lifters where the biceps become hypertrophied due to vigorous exercise. This additional head of biceps can be used as a flap in plastic surgeries. the nerve or the vascular structure is piercing the accessory head, then it would probably cause difficulty during elevating or transferring the flaps.

Explanation for the third head from different views: 

Evolutionary view– Phylogenetically third head of biceps brachii represent long head of coracobrachialis, particularly in those cases where the third head arose from the insertion area of coracobrachialis, as in our study also.\textsuperscript{13} Embryological view–Probably the third head of biceps brachii is a portion of brachialis muscle whose insertion is shifted from ulna to radius and innervated by musculocutaneous nerve.\textsuperscript{2} Circula-
tory view–During the time of formation of brachial plexus because of presence of many circulatory factors the third head of biceps brachii is present.\textsuperscript{14}

Conclusion

Presence of additional head and its nerve supply is important for clinicians and anaesthetist for selective nerve blocks and to treat the nerve impairments.

References

Cadaveric Study of Angiosomes of Anterior Tibial and Dorsalis Pedis Artery

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Abstract

Introduction Angiosome formed by Anterior tibial artery and dorsalis pedis artery supply the ankle and the dorsum of foot. Both the vessels shows variation in termination and branching pattern.

Materials and Methods 50 free formalin preserved limbs were dissected for the study.

Results and Conclusion Anterior tibial artery showed variation in termination in 16%, most common of them was formation of loop with perforating branch of peroneal artery in 10%, bifurcation in to medial and lateral tarsal artery in 4% and trifurcation in 2%. Branching pattern of dorsalis pedis artery was variable in 18%, medial and lateral tarsal arteries were not branches of dorsalis pedis artery in 14% and 16% respectively. Arcuate artery was absent in 10%, when present it was branch of dorsalis pedis artery in 96%, perforating branch of peroneal artery in 2% and loop of medial and lateral tarsal artery in 2%. First dorsal metatarsal artery was not branch of dorsalis pedis artery in 4%. Lateral tarsal artery was constantly found in all the dissected limbs. Thorough knowledge of variation about branching pattern of arterial tree is clinically important for accuracy in interpretation of angiographs, Doppler study of lower limb vessels and optimum use of vascular pedicle for myocutaneous flaps. Importance of palpation of peripheral pulse in lateral tarsal artery is discussed in the study.

Keywords ► dorsalis pedis artery ► angiosome ► vascular surgery ► lateral tarsal artery ► myocutaneous graft

Introduction

Anterior tibial artery (ATA), Posterior tibial (PTA) artery and Dorsalis pedis (DPA) artery are routinely used to access peripheral pulsation of lower extremity. Anterior Tibial (ATA) artery is a terminal branch of Popliteal artery. It approaches ankle joint midway between the two malleoli deep to superior extensor retinaculum, then lies between the tendons of Extensor hallucis longus and Tibialis anterior accompanied by Deep peroneal nerve on its lateral aspect. It continues distal to ankle joint as Dorsalis pedis artery (DPA).

Medial and Lateral malleolar anastomotic network is formed by branches of Posterior tibial artery (PTA), Peroneal artery (PA), Anterior tibial artery (ATA), and Dorsalis pedis artery (DPA).

Anterior Medial malleolar artery (AMMA) and Anterior Lateral Malleolar Artery (ALMA) are the branches of ATA.
Peroneal artery is branch of Posterior tibial artery. Perforating branch of Peroneal artery (PBPA) anastomoses with Anterior lateral malleolar artery (ALMA) and Lateral tarsal artery (LTA). Dorsalis pedis (DPA) artery passes deep to inferior extensor retinaculum and gives Medial Tarsal Arteries (MTA) and Lateral Tarsal Artery (LTA). Dorsalis Pedis gives Arcuate artery (AA) and First Dorsal Metatarsal Artery (FDMA) and Dorsalis pedis itself enters the sole to complete Plantar arch by anastomosing with the branches of Posterior Tibial artery. Branches of DPA artery contribute to the formation of malleolar anastomosis and also contribute to formation of plantar arch. This makes DP artery an important contributor to limb salvage surgery.

Second (SDMA), third and fourth dorsal metatarsal arteries are the branches of Arcuate artery (AA).

Here we present the branching pattern of Angiosomes on dorsum of foot formed by Anterior tibial artery and Dorsalis pedis artery on dorsum of foot.

Materials and Methods

50 Free Formalin preserved limbs of unknown sex and age were taken from Department of Anatomy Dr D Y Patil Mediact College Hospital and Research center Pimpri, Pune; and dissected. Vertical incision was taken on anterior aspect of leg five centimeters above the anterior aspect of ankle joint midway between the lateral and medial malleoli. The incision was extended on dorsum of foot till base of the second toe. Distal limit of the incision was extended on both the sides till the lateral and medial border of the foot. Skin flaps were reflected. Anterior Tibial artery was identified between tendons of Tibialis anterior and Extensor hallucis longus and studied with respect to its termination along with the branching pattern of dorsalis pedis artery pattern on the dorsum of foot. Arterial tree distal to termination of Anterior Tibial artery was carefully dissected and observations were tabulated. The limbs showing gross anomalies were excluded from the study. The research was conducted according to the principles of the Declaration of Helsinki.

Results

The results are tabulated in Table 1.

Anterior tibial artery (ATA) continued as dorsalis pedis artery in 42/50 limbs (84%).

Anterior tibial artery showed variation at its termination in 8/50 (16%) limbs as follows:

Anterior tibial artery bifurcated in medial tarsal (MT) and lateral tarsal (LT) in 2/8 limbs.

Anterior tibial artery trifurcated in anterior lateral malleolar (ALMA), LT, dorsalis pedis (DPA) in 7/8 limbs.

Anterior Tibial artery anastomosed with the perforating branch of peroneal artery (PBPA) in ¾ limbs.

AMMA (Anterior medial malleolar artery) was branch of Anterior tibial artery in 47/50 limbs (94%).

AMMA was not a branch of ATA in 3/50 (6%) limbs in such cases; AMMA was branch of DPA in ½ limbs.

AMMA was branch of loop formed by ATA and PBPA in ¾ limbs.

Anterior lateral malleolar artery (ALMA) was branch of Anterior tibial artery in 45/50 (90%) limbs.

When, ALMA was not a branch of ATA in 5/50 (10%) limbs, in such cases:

ALMA was branch of PP in 5/50 (10%) limbs.

MTA (Medial tarsal artery) was branch of DP (dorsalis pedis) 43/50 (86%) limbs.

When, MTA was not a branch of DPA in 7/50 (14%) limbs, in such cases:

MTA was branch of ATA in 2/7 limbs.

MTA was branch of loop formed by ATA and PBPA in 5/7 limbs.

LTA (lateral tarsal artery) was a branch of DPA in 42/50 (84%) limbs.

When, LTA was not a branch of DPA in 8/50 (16%) limbs, in such cases:

LTA was branch of ATA in ¾ limbs.

LTA was branch of Loop formed by ATA and PBPA in 3/7 limbs.

LTA was branch of PBPA in ¾ limbs.

DPA (dorsalis pedis artery) was continuation of ATA in 43/50 (86%) limbs.

When, DPA was not continuation of ATA in 7/50 (14%) limbs, in such cases:

DPA was branch of MTA in 2/7 limbs. DPA was branch of LTA in 1/7 limbs.

DPA was branch of loop formed by ATA and (PBPA) in 3/7 limbs.

DPA was a branch of PP in 1/7 limbs.

AA (arcuate artery) was present in 45/50 (90%) limbs. AA was absent in 5/50 limbs (10%).

AA was branch of DPA in 43/45 (86%) limbs.

AA was branch of loop formed by MT and LT in 1/45 limbs.

AA was branch of PBPA in 1/45 limb.

FDMA (First dorsal metatarsal artery) was branch of DPA in 48/50 (96%) limbs.

FDMA was not a branch of DPA in 2/50 (4%) limbs.

FDMA was a branch of loop formed by ATA and PBPA ½ limbs.

FDMA was branch of LTA in ½ limbs.

SDMA (second dorsal metatarsal artery) was branch of AA in 44/50 (88%) limbs.

SDMA was not a branch of AA in 6/50 (12%) limbs. SDMA was a branch of LTA in 2/7 limbs.

SDMA was a branch of DPA in 2/7 limbs.

SDMA was branch of loop formed by ATA and PBPA in 2/6 limbs.

Variation in termination of ATA was seen in 8/50 limbs 16% of the limbs.

Variation in branching pattern of DPA was seen in 18% of the limbs.

Dorsalis pedis artery showed variation with respect to its origin and branching pattern as follows:

DPA was not continuation of ATA in 14%.

DPA did not give MTA and LTA in 14% and 16% respectively.

AA was absent in 10%.

Table 1

<table>
<thead>
<tr>
<th>Angiosomes on Dorsum of Foot</th>
<th>Paranjape et al.</th>
</tr>
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<tbody>
<tr>
<td>Anterior Tibial artery continues as Dorsalis pedis artery in 42/50 limbs (84%).</td>
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<tr>
<td>Anterior tibial artery showed variation at its termination in 8/50 (16%) limbs as follows:</td>
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<tr>
<td>Anterior tibial artery bifurcated in medial tarsal (MT) and lateral tarsal (LT) in 2/8 limbs.</td>
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<tr>
<td>Anterior tibial artery trifurcated in anterior lateral malleolar (ALMA), LT, dorsalis pedis (DPA) in 7/8 limbs.</td>
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<tr>
<td>Anterior Tibial artery anastomosed with the perforating branch of peroneal artery (PBPA) in ¾ limbs.</td>
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<tr>
<td>AMMA (Anterior medial malleolar artery) was branch of Anterior tibial artery in 47/50 limbs (94%).</td>
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<tr>
<td>AMMA was not a branch of ATA in 3/50 (6%) limbs in such cases; AMMA was branch of DPA in ½ limbs.</td>
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<td>Sp no</td>
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<td>47</td>
<td>L</td>
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</tbody>
</table>

(Continued)
When present AA it was not a branch of DPA in 4% limbs. DP did not give FDMA in 4% limbs. Extra branch in form of AMMA was branch of DPA in 2% limbs. Extra branch in form of SDMT was branch of DPA in 4% limbs.

Thus variations found in the above study can be grouped as: Type A:

Most common variation (⅝, 62.5% of the variations seen) seen in our study was formation of a loop by perforating branch of peroneal artery and anterior tibial artery. In 4/5 (80%) limbs with this type of variation, anterior tibial artery was rudimentary and thin where as the perforating branch of peroneal artery was the prominent and larger and formed main source of supply to dorsum of foot. In 1/5 (20%) ATA and branches or PBPA itself were equally dominant and formed a loop. Branches from loop further supplied dorsum of foot.

Type B:

ATA did not continue as DP but terminated. (ATA bifurcated or trifurcated in our study). ATA bifurcated in MT and LT in two right limbs (2/8, 25% of the variations). Pattern of bifurcation was different in both the cases.

ATA trifurcated in one right limb (⅛, 12.5% of the variations). Examples of these variations are as follows: Type A:

In all cases, Anastamotic loop was formed in the vicinity of ankle joint. Loop once formed showed different type of branching pattern when traced further as seen in specimen no 10 –Fig. 1 and specimen 43 –Fig. 2.

Table 1 (Continued)

<table>
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<th>Sp no</th>
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<th>ATA continues as</th>
<th>AMMA P/A branch of</th>
<th>ALMA P/A branch of</th>
<th>MTA P/A branch of</th>
<th>LTA P/A branch of</th>
<th>DPA P/A continuation of</th>
<th>AA P/A branch of</th>
<th>FDMA branch of</th>
<th>SDMA branch of</th>
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<tr>
<td>48</td>
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<td>DPA</td>
<td>ATA p</td>
<td>ATA p</td>
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<td>ATA p</td>
<td>P DPA</td>
<td>DPA</td>
<td>AA</td>
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<td>49</td>
<td>R</td>
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<td>DPA p</td>
<td>DPA p</td>
<td>ATA p</td>
<td>P DPA</td>
<td>AA</td>
<td>AA</td>
</tr>
</tbody>
</table>

Abbreviations: A, absent; AA, arcuate artery; ALMA P/A, anterior lateral malleolar artery; AMMA P/A, anterior medial malleolar artery; anast PBPA, anastomosis with perforating branch of peroneal artery; ATA –UP Branch; P, anterior tarsal artery; Upper Branch, present; ATA, anterior tibial artery; DPA, dorsalis pedis artery; FDMA, first dorsal metatarsal artery; LTA, lateral tarsal artery; MTA, medial tarsal artery; p, present; PBPA, LO branch, P-Perforating Branch of Peroneal Artery, Lower Branch, Present; SDMA, second dorsal metatarsal artery; Sp no, specimen number.
Fig. 1: specimen no 10; Table 1: ATA was rudimentary and PBPA was dominant vessel. DP artery was continuation of PBPA and entered first intermetatarsal space. LTA was branch PBPA. MTA was branch of loop. Further branching pattern of DP was normal.

Fig. 2: specimen no 43; Table 1: ATA and PBPA, both vessels were dominant. Anastomosis between PBPA and ATA was seen at two levels. PBPA gave two branches (upper branch UB and lower branch LB) 6 cm above the ankle joint. Upper branch (UB) of PBPL anastomosed with a small branch from ATA at the same level. ATA continued distally toward ankle joint and gave another branch which anastomosed with lower branch (LB) of PBPL at the level of Ankle joint. LB of PBPA continued as DP artery. LTA was seen arising at the level of lower anastomosis. ATA deviated medially and continued as MTA. Further branching pattern of DPA was normal.

Fig. 3: specimen no 35; Table 1: In this case ATA did not continue as DPA. ATA bifurcated and also anastomosed with PBPA. PBPA and ATA were prominent. ATA bifurcated at the level of ankle joint. Both branches of ATA anastomosed with PBPA. Anastomosis was noted at two different levels. One branch anastomosed with PBPA at the same level as bifurcation. Second branch anastomosed with PBPA which further continued as DPA. DPA continued as FDMA. PBPA before anastomosing with second branch of ATA gave another branch which gave SDMA and other metatarsal arteries. MTA and LTA were branches of ATA.

Type B:

Fig. 4: specimen no 1; Table 1: ATA terminated instead of continuing as DPA.

Fig. 5: specimen no 22; Table 1: ATA trifurcated in LMA, LTA and DPA. MMA and MTA were branches of DPA. FDMA was branch of DPA. SDMA was branch of LTA. Arcuate artery was absent.

Discussion

Knowledge of vascular anatomy of foot is of outmost importance in clinical medicine. Absence or abnormality of peripheral pulsation suggests some kind vascular disease. Vascular disease can be in the form of thromboangiitis obliterans as in Burgers disease or microvascular angiopathy associated with diabetes or atherosclerotic changes. Variation in normal anatomy and branching pattern of ATA as evident from various studies is also one of the reasons for abnormal or absent peripheral pulsation. Successful treatment of pathology related to foot depends on prevention of tissue ischemia by establishment of sufficient vascular supply to distal fore foot. This is possible only with competent and viable anastomosis of distal and proximal foot vessels via bypass graft of occluded segment or endovascular interventions like percutaneous transluminal angioplasty and stents. Not only for treatment of vascular diseases, ATA and DPA are also used as chief vessel
for pedicle or free myocutaneous graft of extensor digitorum brevis muscle. Whichever is the modality of treatment, thorough knowledge vascular anatomy is essential. Blood vessels supplying foot and ankle are divided into 6 angiosomes supplied by three branches of posterior tibial artery (supplying plantar aspect of foot), two branches of peroneal artery (supplying foot and ankle from anterolateral aspect) and ATA and its continuation DPA (supplying ankle and dorsum of foot)\textsuperscript{3}.

Anterior tibial artery (ATA) continued as Dorsalis pedis (DPA) in 74%,\textsuperscript{2} 86%,\textsuperscript{3} 92%,\textsuperscript{4} 54.76%,\textsuperscript{5} 24.1\%\textsuperscript{6} and 86% as seen in our study. In 16% of the limbs studied by us, DPA was not continuation of ATA (\textsuperscript{7}Table 2).

DPA was formed by equal contribution from ATA and PBPA in 4% (Swathi, 2010),\textsuperscript{2} 6% in our study, in such cases we found formation of a loop contributed by PBPA and ATA at the level of ankle joint, all distal branches were given from the loop including DPA, MTA and LTA; hence branching pattern of entire arterial tree on dorsum of foot was different. This pattern is similar to Type E pattern described by Vasudha and Ramesh (2012)\textsuperscript{8} in 12.1\% cases. In such cases Anterior tibial and Peroneal arteries have equal contribution to blood supply on dorsum of foot. Other studies do not mention about this type of branching pattern (\textsuperscript{8}Table 2).

DPA was branch of PBPA as follows: In 16%,\textsuperscript{2} 8%,\textsuperscript{4} 6.7\% in our study (We have excluded those limbs where DPA was branch of loop formed by ATA and PBPA 6%). Isolated cases of PBPA continuing as DPA has been reported by Tuncel et al.,\textsuperscript{11} Ali and Mohajir\textsuperscript{12} and Shetty et al.\textsuperscript{13} DPA deviated laterally in such cases and is very likely to be missed during manual examination pedal pulse. Vasudha and Ramesh\textsuperscript{8} mention similar lateral deviation in 6% of the cases (\textsuperscript{8}Table 2).

**Fig. 5** (a) Anterior Tibial Artery (ATA); (b) Anterior Lateral Malleolar Artery (ALMA); (c) Lateral Tarsal Artery (LTA); (d) First Dorsal Metatarsal Artery; (e) Medial Tarsal Artery (MTA); (f) Anterior Medial Malleolar Artery (AMMA).

<table>
<thead>
<tr>
<th>Author</th>
<th>ATA continues as DPA</th>
<th>DPA = PBPA and ATA</th>
<th>DPA branch of PBPA</th>
<th>ATA Terminated</th>
<th>AA - P/A, %</th>
<th>AA branch of</th>
<th>FDMA branch of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swathi (2010)\textsuperscript{2}</td>
<td>74%</td>
<td>4%</td>
<td>16%</td>
<td>8%</td>
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<td>Yamada et al. (1993)\textsuperscript{3}</td>
<td>86%</td>
<td>–</td>
<td>6.70%</td>
<td>9.10%</td>
<td>A-33%</td>
<td>–</td>
<td>DPA-93%</td>
</tr>
<tr>
<td>Vijayalakshmi et al. (2011)\textsuperscript{4}</td>
<td>92%</td>
<td>–</td>
<td>8%</td>
<td>–</td>
<td>P-76%</td>
<td>–</td>
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<tr>
<td>Rajeshwari et al. (2013)\textsuperscript{5}</td>
<td>54.76%</td>
<td>–</td>
<td>–</td>
<td>9.52%</td>
<td>A-16.6%</td>
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<tr>
<td>Vasudha and Ramesh (2012)\textsuperscript{6}</td>
<td>24.10%</td>
<td>12.10%</td>
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<tr>
<td>Present study</td>
<td>86%</td>
<td>6%</td>
<td>2%</td>
<td>6%</td>
<td>P-90%, A-10%</td>
<td>DPA-86%, Loop-2%, PBPA-2%</td>
<td>DPA-96%, LTA-2%, Loop-2%</td>
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<tr>
<td>Dilandro et al. (2001)\textsuperscript{7}</td>
<td>–</td>
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<td>P-16.7%</td>
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<td>El Saeed et al. (2008)\textsuperscript{8}</td>
<td>–</td>
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<td>DPA-90%, LTA -10%</td>
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<td>Lee and Dauber (1997)\textsuperscript{9}</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
<td>DPA- 90.6%, LTA-9.4%</td>
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<td>Gabrielli and Olave (2008)\textsuperscript{10}</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
<td>DPA-86%</td>
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</table>

Abbreviations: AA, P/A, Arcuate Artery, Present /Absent; ATA, Anterior Tibial artery; DPA, dorsalis pedis artery; FDMA, First Dorsal Metatarsal Artery; Loop, Loop formed by ATA and PBPA; LTA, Lateral Tarsal Artery; PBPA, Perforating Branch of Peroneal Artery.
ATA terminated as a medial and a lateral branch in 8% and DPA was the branch of lateral branch in 6% and medial branch in 2%. Vasudha and Ramesh mention termination of ATA by bifurcation in larger LTA and smaller DPA in 9.1%. Termination of ATA by bifurcation in MTA and LTA in 9.52% cases was noted by Rajeshwari et al. (2013). In our study ATA terminated in MTA and LTA in 6% of the limbs, DPA was a branch of LTA 2% limbs and MTA in 4% limbs. Vijayalakshmi and Varsha reports a case of double DP artery where ATA trifurcated in LTA and two DP arteries, which dipped in to first metatarsal space. Lateral of the two continued as plantar arch and medial one continued as FDMA. We have also encountered trifurcation of ATA in 2% of limbs in our study. However the branching pattern was different. The three branches at termination of ATA were lateral malleolar artery, lateral tarsal artery and dorsalis pedis artery. FDMA was a branch of DP. AA was absent and all other dorsal metatarsal arteries were branches of LTA (Table 2).

Our study shows variation in branching pattern of DPA in 18% of limbs in form of absent AA in 10%. FDMA was not a branch of DPA in 4% and in 4% DPA gave SDMA which normally is a branch of Arcuate artery (AA) (Table 2).

AA was present in 76%, AA was absent in 33% where as AA was found only in 16.7% cadavers studied by Dilandro et al. AA was absent in 16.6% cases studied by Rajeshwari et al. and 2.38% of these cases AA was replaced by loop formed by two LTA that gave branches to second and third and fourth dorsal metatarsal arteries. AA was branch of DPA in 90% and LTA in 10%. In our study, AA was present in 90% and absent in 10%. When present, AA was branch of DPA in 86%, branch of loop formed by LTA and MTA in 2% and was a branch of PBPA in 2% of the limbs. Absence of AA ranges from 10% in our study, 16.6% by Rajeshwari et al., 33% to 83.5% by Dilandro et al. (Table 2).

FDMA was branch of DP in 93%, FDMA from DP in 90.6% and LTA in 9.4% as mentioned by Lee and Dauber, FDMA is branch of DP in 86% by Gabrieli and Olave. FDMA was branch of DPA in 96% in our study, when FDMA was not a branch of DPA, it was branch of LTA in 2% and loop formed by ATA and PBPA in 2%. 2nd to 4th DMA were not branches of AA in 12% but they were branches of LTA and from loop formed by ATA and PBPA (Table 2).

Vasudha and Ramesh mention that only 15.2% cases showed normal branching pattern of DPA on dorsum of foot. From all the studies mentioned above it is evident that DPA has lot of variation in its branching pattern and size. DPA was absent in 4.2%, 10% and 10–12% as found by Vijayalakshmi et al. We have found that DPA was hypoplastic and thin and difficult to dissect when it was a branch either of MTA and LTA, it deviated laterally when it was continuation of PBPA and had variable in position when it was a branch of loop formed from ATA and PBPA. All these limbs also showed variable branching pattern of DPA. Variation in origin of DPA when associated with absence of AA, most common finding in such cases is FDMA is a branch of DPA and remaining dorsal metatarsal arteries rise from loop formed by ATA and PBPA or from LTA.

LTA was constantly found in all the 50 dissected limbs in present study. It travelled laterally and supplied extensor digitorum brevis muscle and anastomosed with branches of lateral malleolar and lateral plantar arteries. Absence of LTA has not been mentioned in any of the studies mentioned above. On the contrary occurrence of three LTA are mentioned by Rajeshwari et al. (2013). They found three LTA in 2.38% and two LTA in 14.29%. Presence of two LTA is also reported by Hamada et al. (1993) and also EL SAEED E M et al (2008). From all the studies available in literature it is evident that there exists a strong association related to variation in termination of ATA and branching pattern and origin of DPA. In such cases blood supply to arterial tree on dorsum of foot is supplemented by LTA. Taking this in to consideration authors feel that, in addition to palpation of DPA, should the pulsation of LTA be routinely performed at the proximomedial edge of first muscle belly of Extensor digitorum brevis either manually or by doppler probe on the lateral aspect of navicular needs to be discussed and evaluated. Authors are of the opinion that recording the pulsations of Lateral tarsal artery should be a part of routine clinical examination.

DP is a versatile vessel used for assessing pedal pulse as a part of assessment of PVD, for calculation of ABPI which is an important marker of cardiovascular disease. Change in value of ABPI is crucial for assessing the progress of PVD and in treatment of foot ulcers. DPA is used as a vascular pedicle for myocutaneous grafts of EDB muscle. In absence of proximal vessels, DP is used for arterial reconstruction to vascularize distal foot as an effort to salvage the ischemic lower extremity in diabetic patients thereby avoiding limb amputation. DPA, LTA and plantar arteries are used for endovascular interventions like percutaneous transluminal angioplasty and stent placement in case of morbid patients where surgical bypass is contraindicated. Martin et al. quotes that DPA can be used for long term cannulation in ICU patients because long term cannulation of DP holds similar risks to that of radial artery and has advantage when immobilization of patients hand is undesirable or radial artery is inaccessible due to extensive trauma, burns, or damage from previous catheterizations. Direct vascularization of the artery feeding the ischemic angiosome is considered more beneficial because of better wound healing thus reducing the chances of amputation. Presence of Lateral calcaneal artery has been demonstrated by Chang et al. and Zeng-Yuan et al. They have also used this vessel as a recipient pedicle in foot reconstruction without sacrificing main circulation.

ATA and DP is easily accessible for palpation of pedal pulse however their variation and or absence as documented by various studies in literature warrants sole dependency on DP for its use in various clinical procedures with special reference to limb salvage surgery or Myocutaneous grafts. To have more clarity about the anatomy of arterial tree on dorsum of foot which would definitely help clinicians for direct revascularization of ischemic artery specific angiosome or during assessment of PVD, we plan to study the anatomy of lateral arterial chain and planter network of foot as an extension and addition to our study of angiosome formed by Anterior tibial artery and Dorsalis pedis artery.
Conclusion

Anterior tibial artery is used to calculate ABPI (Ankle brachial pressure index). ABPI is a marker of cardiovascular disease and crucial parameter in treatment of peripheral vascular diseases and management of vascular ulcers of leg and foot. The branching of arterial tree on the dorsum of foot is helpful while planning Myocutaneous flaps for reconstruction surgeries of the foot during treatment of thromboangiitis obliterans of diabetes mellitus, vehicular accidents and other soft tissue and bone defects. Knowledge of variation in Dorsalis pedis artery is necessary for revascularization of reconstitution distal foot vessels is main motive of diabetic foot surgery. Authors are of opinion that, pedal pulsation should be routinely assessed in Lateral tarsal artery in addition to Dorsalis pedis artery. Variations in DP artery must be taken into account while assessing peripheral pulsations as variable branching pattern of dorsalis pedis artery leading to its variable position is responsible for diminished or weak Dorsalis pedis pulsation which is an important clinical indicator of peripheral vascular disease.

References

Degree of Myelination (g-ratio) of the Human Hypoglossal Nerve

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Abstract

Introduction The degree of myelination (g-ratio) can be useful to the evaluation of the fiber morphology during peripheral nerve regeneration and in studies in the area of microsurgery. Therefore, the aim of this analyze was to investigate the g-ratio of the human hypoglossal nerve.

Materials and Methods The hypoglossal nerve was bilaterally analyzed in human specimens obtained from necropsies (6 subjects). The nerves were analyzed using histology, and the morphometric parameters (axon diameter and myelinated fiber diameter) were measured using Image Pro-Plus Software 6.0.

Results Quantitative comparison of the g-ratio demonstrated no statistically significant difference between the left and right human hypoglossal nerves. In addition, the values of the g-ratio of both hypoglossal nerves were considerably higher than the normal values for the peripheral nerve fibers. This demonstrates a low degree of myelination of the hypoglossal nerve, bilaterally.

Conclusion Knowledge of the variations of the human hypoglossal nerve, are clinically important especially in the area of microsurgery (techniques used for reinnervation).

Introduction

A variety of ways of transferring and suturing (microsurgery/techniques used for reinnervation) the hypoglossal nerve (XII) to the distal segment of the facial nerve have been reported. However, while the macroscopic anatomy of the XII nerve1 and its cervical loop, have been well documented2,4 a few studies have been done on the microscopic anatomy of this nerve, especially on the degree of myelination (g-ratio) bilaterally. Recently, some authors5 have reported that the g-ratio describes the relationship between axon size and myelin thickness, and deviations in the g-ratio are thought to be involved in abnormal development and...
disease. Moreover, the g-ratio can be useful to the evaluation of the relationship between nerve conduction velocity, fiber morphology during peripheral nerve regeneration and in studies in the area of microsurgery. Therefore, we decide to present here the data about the g-ratio of the human XII nerve (bilaterally) extracted of the histological collection of our research group.

Methodology

The histological material of nerves were obtained from the collection of the Laboratory of Histology and Pathology of the Universidade de Santa Cruz do Sul, RS, Brazil, these tissues were collected from necropsies (dissected from the point where it crosses the internal carotid artery to its ending at the tongue - bilaterally). Six subjects (males [age = 65.6 ± 11.5] were used in our study. None of the subjects used in this study had any illness or injury that could alter the fibers of the XII nerve.

Morphometric Measurements

The g-ratio (degree of myelination) was calculated by measuring the diameter of axons and dividing by the total diameter of that axon plus the surrounding myelin sheath (myelinated fiber diameter)6 (Fig. 1). To estimate the axonal diameter (μm), the axonal area of each individual fiber was measured and the value obtained was converted to the diameter of a circle with an equivalent area. The sum of the axonal diameter and myelin sheath represent the myelinated fiber diameter (μm). Myelin sheath thickness was estimated using the measurement tools of the Image Pro-Plus Software [Image Pro-Plus 6.0; Media Cybernetics, Silver Spring, MD, USA] (four vertical and horizontal lines in each fiber counted in areas of interest) (Fig. 1). All statistical analyses were performed using GraphPad Prism 5.0 software. The comparisons between the right and left XII nerve from each subject were performed using a paired t test.

Results

The main result found in our analyze is the significant difference in axonal diameter and myelinated fiber diameter between the left and right human XII nerves, the left XII nerve being bigger when compared with the right XII nerve. The axon diameter in the left nerve (9.2 ± 0.3 μm) was found to be 8.7% larger than that on the right nerve (8.4 ± 0.4 μm) (p = 0.0001) (Fig. 1).

There was also a significant difference between the myelinated fiber diameter of the left (10.2 ± 0.7 μm) and right (9.6 ± 0.7 μm) nerves, the former being found to be 5.9% larger (p = 0.0001). On the other hand, quantitative comparison of the g-ratio demonstrated no statistically significant difference between the left (0.90 ± 0.1) and right (0.88 ± 0.1) nerves (p = 0.0798) (Fig. 1).

Discussion

For this study, were used histological slides of the XII nerve, made previously by our research group for the comparison of numerous morphometric parameters between the recurrent laryngeal nerve and the XII nerve of humans. However, in this study,7 no comparison between the g-ratio of right and left XII nerve was performed. Thus, to search for more scientific articles that could be confronted with our results, we perform a basic search in the following scientific databases:

- PubMed (NCBI, 2017),
- Bireme (BVS, 2017),

The search terms were as follows: human hypoglossal nerve, degree of myelination and g-ratio. Our research shows that there are no human studies in the literature showing comparative data between the g-ratio of the left and right XII nerves.

Fig. 1 Images demonstrating the differences between the left (A) and right (B) nerves in terms of myelinated fiber diameter. Representation of one of the fields (areas of interest) used in the study. The fibers located inside this square or intersected by the upper and/or left edge of the areas of interest were counted (*); the fibers intersected by the lower and/or right edge of the areas of interest were not counted (x). Images demonstrating the myelin sheath thickness of fibers were counted within the areas of interest, calculated by means of a specific software tool (four vertical and horizontal lines in each fiber) of the Image Pro-Plus 6.0; Media Cybernetics, Silver Spring, MD, USA. Semithin sections (1 μm) were stained with toluidine blue.
However, although we have found no studies in the literature that showed some data that could be compared with our results, these limitations do not change the main goals and results of our study, to provide new information and basic knowledge about the human XII nerve bilaterally, which may be essential for understanding and improving the techniques used for reinnervation.

In addition, in our study, the values of the g-ratio of both hypoglossal nerves were considerably higher than the normal values (between 0.6 and 0.7) for the peripheral nerve fibers. This demonstrates a low degree of myelination of the hypoglossal nerve, bilaterally.

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Atlanto-Occipital Synostosis in a Colombian Population Sample

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Abstract

Introduction The Cervico-capital rotation process involves important joints with respect to the neurological field. Among these joints, the atlanto-occipital joint accounts for 40% of the total rotation process and its anatomical changes may affect the joint, i.e. when the synostosis of both bones occurs. This anomaly has a reported incidence in the world population of 0.14% to 0.75%. To determine whether this incidence range also corresponds to the Colombian population, atlanto-occipital fusion in Colombian patients was studied.

Materials and Methods The presence of atlanto-occipital fusion was assessed in a random sample of 105 skulls of a Colombian population. Morphometric features were evaluated by using a Vernier caliper.

Results Out of the total sample two cases of atlanto-occipital fusion were identified. One of them exhibited a bilateral fusion between the anterior arch of the foramen magnum and the lateral masses of the atlas; the other one exhibited a left hemisynostosis that compromises the anterior arch foramen magnum and left lateral mass of atlas. The overall frequency of occurrence was 1.9%.

Conclusion A greater incidence of atlanto-occipital fusion was found in contrast to the range of world incidence reported in the literature.

Introduction

Knowledge of cranio-vertebral abnormalities and their morphology is important for clinicoradiological studies. This leads to clinical assessments with more accurate diagnoses, as in those cases where synostosis is present, i.e. the atlanto-occipital fusion that affects the mentioned joint and that is formed by the junction between the lateral mass superior articular facet of the atlas and the corresponding occipital condyle. The atlanto-occipital membrane joins these two bones.1 The atlanto-occipital joint is a synovial joint with a loose and thin articular capsule. Atlanto-occipital synostosis is defined as a congenital fusion of the atlas to an occipital base.2,3 This anomaly was first described by Rokitansky in 1884, and since then, several terminologies such as synostosis, occipitalization of Atlas, fusion of atlanto-occipital or ankylosis of atlanto-occipital fusion have been used.2 Cervico-capital rotation process involves important joints with respect to the neurological field. Among these joints, the atlanto-occipital joint accounts for 40% of the total rotation process,4 and anatomical changes may affect the joint i.e. when synostosis of both bones occurs. This anomaly has a 0.14% to 0.75% of reported incidence in world population.2
varieties of this anomaly have been described. These include complete or incomplete types, complete synostosis being the most common.\textsuperscript{5} Synostosis may be associated with other skeletal malformations, such as spina bifida of atlas, occipital vertebra, basilar invagination, Klippel-Feil syndrome, Arnold-Chiari malformation and cervical stenosis.\textsuperscript{5}

Knowledge of atlanto-occipital synostosis is important from the clinical point of view due to the vertebral artery position and the first spinal nerve (C1). Vertebral artery compression may cause a cerebral blood flow (CBF) decrease and C1 compression can cause neurological symptoms as well.\textsuperscript{7} Different clinical manifestations of this synostosis have been described, namely cephalgia, cervical pain, abnormal neck position anomalies, decreased range of movement, dizziness, dysphagia and dysarthria.\textsuperscript{8}

**Materials and Methods**

This was an observational and cross-sectional study with a descriptive coverage. Quantitative and qualitative information was collected. 105 adult skulls without reported injury of the occipital region were randomly selected. These skulls belonged to the Departments of Anatomy of Caldas, Manizales and Autonoma universities. The presence of synostosis between the occipital bone and C1 was assessed. There was no distinction between ethnicity or sex. Among exclusion criteria were those skulls with presence of trauma or gross malformations in the cervico-occipital region. Synostosis was assessed between the occipital bone and the C1. A Vernier caliper and a camera were used for morphometric descriptions.

**Results**

Out of 105 skulls studied, 1.9\% showed synostosis between the lower portion of the occipital bone and the C1. One of skulls had complete synostosis and another one hemisinos- tosis, as follows.

Skull 1 exhibited a hemisinosostosis between the occipital bone and the first cervical vertebra (C1), characterized by complete fusion on the right side and incomplete fusion on the left side (\textsuperscript{Fig. 1} and \textsuperscript{2}). This region did not exhibit posterior condyloid foramina (\textsuperscript{Fig. 2}). This suggests the presence of synostosis in corresponding ducts of emissary veins within sigmoid sinus. On the right side, a developed channel between the lateral mass and the lateral occipital bone was observed, which makes way for the course of the right vertebral artery. Its diameter was 4.5 mm at its greatest end and 2.5 mm at its lowest end. On the left side, a lateral mass fusion of the vertebrae C1 with occipital bone was observed. The transverse foramen and the extra space makes way for the course of the left vertebral artery. Its diameter was 4.5 mm at its greatest end and 2 mm at its lowest end. The anterior tubercle of C1 vertebra showed no synostosis with the occipital bone. However, it was deviated from the median line of 4 mm, with reference to the pharyngeal tubercle (\textsuperscript{Fig. 1}).

Skull 2 exhibited a bilateral synostosis between the occipital bone and the first cervical vertebra (C1) (\textsuperscript{Fig. 3} and \textsuperscript{4}). This region presented no posterior condyloid foramina, which suggests the presence of synostosis in corresponding ducts of emissary veins within sigmoid sinus, similarly to case 1. On the left side, a developed channel

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\textsuperscript{Fig. 1} (Standard basal skull with C1 and occipital synostosis. Synostosis on the right side of the lateral masses and hemisinosostosis on the left side is highlighted.)

\textsuperscript{Fig. 2} (Incomplete synostosis between C1 and occipital bone.)

\textsuperscript{Fig. 3} (Complete synostosis: in a standard basal skull, a deviation of the above process C1 to the right side is noted. Intrajugular process fused with occipital bone is also noted. This divides jugular foramen into two.)
between lateral mass and occipital bone is identified which makes way for the course of the left vertebral artery. Its diameter was 6 mm at its greatest end and 5 mm at its lowest end. On the right side, there was a complete fusion of the lateral mass that prevented the development of the C1 foramen transversarium. This suggests that the artery had an intracranial course from the C2 foramen transversarium. The anterior tubercle of C1 suffered no synostosis with the occipital, although it was deviated from the midline, 1.5 mm, with reference to the pharyngeal tubercle.

Discussion
The frequency of atlanto-occipital synostosis in this study was 1.9%. This is significantly higher compared with the information reported in the literature, which references values in range from 0.14 to 0.75%.

In the skull with hemisinostosis, the reduced diameters of the small ends of the ducts formed by the passage of the vertebral artery in its exocraneal course lead to a suspected blood flow decrease due to stenosis of both arteries. This is due to the contrasting values of the diameter of the vertebral artery reported in the literature from 3.5 to 4 mm.

This would affect the movement of the anterior, posterior, inferior posterior, cerebellar and basilar arteries. On the other hand, the skull with atlanto-occipital bilateral synostosis shows a transverse foramen diameter within normal parameters in its left side, in contrast to the right side transverse agenesis foramen which suggests that the vertebral artery undergoes no narrowing in its course toward the inside of the skull after piercing the atlantoaxial membrane.

Atlas deviations of the anterior tubercle suggest an asymmetrical cruciate ligament, since the fovea for the odontoid process moves unilaterally. This could have generated instability on the atlantoaxial joint. The case of bilateral synostosis in one of the skulls is related to a deep invagination of the basilar region, consistent with reports in the literature.

Conclusion
In a direct morphological study in skulls of a Colombian sample, it was determined that the frequency of the atlanto-occipital synostosis is higher by 253% compared with that reported in the literature. This type of synostosis alters the occipital and spinal region morphologically and can generate variations in the course of the vertebral artery compatible with some clinical manifestations.

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References
Effects of Aqueous Extract of *Persea americana* (Mill.) Leaf on the Myocardium of Left Ventricle of High Salt Fed Adult Wistar Rat

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Abstract

**Introduction** This study was designed to show that *Persea americana* extract possess the ability to protect the myocardium of left ventricle against injury caused by high salt diet in adult Wistar rats.

**Method** Forty healthy Wistar rats of both sexes weighing 120–150 g were randomly assigned into 8 groups of 5 rats each (Groups A, B, C, D, E, F, G and H). Rats in groups A, F, G and H were fed with standard laboratory pellets, while groups B, C, D and E were fed on the high-salt diet for four weeks. Concomitantly, daily administration of 50 mg kg⁻¹, 100 mg kg⁻¹ and 150 mg kg⁻¹ of the *Persea americana* extract were given orally to groups C&F, D&G and E&H respectively while rats in groups A and B were administered distilled water. The rats were sacrificed under ketamine anesthesia (30mg/kg i.m). The left ventricle of the heart was excised, processed in paraffin wax and stained with haematoxylin and eosin and Verhoeff-Van Gieson stains. One-way ANOVA was used to analyze data, followed by Student Newman-keuls (SNK) test for multiple comparison.

**Result** Results revealed that there was statistically significant (*p* < 0.05) difference in body weight change across all experimental groups; which was significantly lower in high salt fed groups. It was revealed that there were morphological alterations in the myocardium of left ventricle in group B while *Persea americana* protected myocardium in other experimental groups.

**Conclusion** In conclusion, high salt diet induced myocardium alterations which were significantly protected by oral administration of *Persea americana* extract.

Introduction

Cardiovascular disease is a class of disease that involves the heart, the blood vessels (arteries, capillaries, and veins) or both.¹ Cardiovascular disease refers to any disease that affects the cardiovascular system, principally cardiac disease, and peripheral arterial disease.² Though the causes of cardiovascular disease are many but hypertension and atherosclerosis are the most common. Also there are numbers of physiological and morphological changes that alter cardiovascular function with aging and this may lead to increased risk of cardiovascular disease even in healthy asymptomatic individuals.³

High salt intake has deleterious effect on the body system, one of the main organ systems vulnerable to the adverse effects of excessive sodium in the diet is the cardiovascular system; excess sodium predisposes to high blood pressure (BP);⁴ Raised BP is a major risk factor for Left ventricular hypertrophy (LVH)⁵
and LVH is an important predictor of CVD. Reduction in salt intake may have effect a direct effect on LVH independent of BP.

Various phytochemicals have been found in Persea americana leaf these include: saponins that has hemolytic activity and cholesterol binding properties, Tannins noted for astringency and bitter taste, hastening the healing of wounds and inflamed mucus membrane. Flavonoids that prevent oxidative cell damage and have strong anticancer activity and protect against all stages of carcinogenesis, Alkaloids that are used as basic medicinal agents for their analgesic and bactericidal effects, and Phenols which have been extensively research as disease preventive agents.

It has been established that high salt diet causes damage to the cardiovascular system leading to cardiovascular disease especially hypertension; components like such as tannin, flavonin and alkaloids with suggested antihypertensive effects have been discovered in avocado leaf aqueous extract and its use for treatment of hypertension has also been documented, but the effect of avocado aqueous leaf extract on the microanatomy of the myocardium of left ventricle when exposed to high salt has not been fully documented. This study aimed at providing complementary information on the cardio-protective effect of avocado (Persea americana) aqueous leaf extract on the myocardium of left ventricle of high salt-fed Wistar rats.

**Materials and Methods**

**Animal Care and Management**

Forty adult Wistar rats of both sexes between 120–150 g obtained from Animal Holdings of Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife were used for this research. The animals were housed in Animal Holdings of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife. They were maintained on standard laboratory pellet before commencement of the experiment and clean water was provided ad libitum. They were maintained in natural day and night cycle and temperature. The rats were assigned into eight groups of five animals each.

**Plant Material and Preparation of Extract**

Fresh avocado (Persae americana) leaves were obtained from the avocado fruit trees in the town of Ilesa, Osun State. The leaves specie was authenticated by a taxonomist in the Department of Botany Obafemi Awolowo University, Ile-Ife; the collected plant samples were washed thoroughly with running tap water. The leaves were air-dried at room temperature, the dried leaves were pulverized using an electric blender, soaked in distilled water and kept at 4°C for 48 hours with occasional shaking. The mixture was then filtered and the filtrate was concentrated to gel at 40 ± 1°C in a rotary evaporator, and then freeze dried. This crude extract was used without further purification. Required dosage was prepared from the freeze dried extract.

**Preparation of High Salt Diet**

High salt diet containing 8% sodium chloride was prepared specially by replacing 0.3% sodium chloride-containing standard diet with 8% sodium chloride.

**Animal treatment**

Group A was the control, group B was negative control, while groups C, D, E, F, G and H were the test groups. Rats in groups A, F, G and H were fed with standard laboratory pellets, while groups B, C, D and E were fed on the high-salt diet for four weeks; concomitantly, daily administration of 50 mg kg-1, 100 mg kg-1 and 150 mg kg-1 of persea americana extract were giving orally to groups C&F, D&G and E&H respectively. The extract solution was administered orally, using oral cannula and duration of the experiment was 4 weeks.

**Measurement of Body Weight**

The body weights of the animals were taken using a top loader weighing balance.

**Sacrifice of Animals**

At the end of the experiment, the rats were sacrificed under ketamine anesthesia. The left ventricles of the heart were excised and weighed.

**Histological and Histochemical Analyses**

The excised left ventricle was fixed in neutral buffered formalin for 24 hours, and processed using paraffin wax embedding method. Sections of 5 μm thicknesses were produced from the paraffin embedded tissues. Haematoxylin and Eosin method was used to demonstrate the general histarchitecture of the left ventricle and Verhoef Van-Giesons Stain was used for elastic-collagen fiber differential staining.

**Determination of Relative Heart Weight (%)**

At sacrifice, the heart weight was determined using a top loader sensitive balance (Mettler-Toledo Garvens GmbH, Giesen, Germany). The relative weight of the heart (%) to the body weight at sacrifice was evaluated.

**Photomicrography**

Stained sections were viewed under a LEICA research microscope (LEICA DM750, Switzerland) with digital camera attached (LEICA ICC50) and digital photomicrographs were taken at various magnifications.

**Quantification of Staining Intensity**

Image analysis and processing for Java (ImageJ), was used to analyze and quantify Verhoeff-Van Gieson staining intensity. Imported RGB images were converted to grayscale images on ImageJ. The software quantifies staining intensity by measuring the pixel value of each pixel in grayscale images following threshold of areas of staining activity and converting the pixel value to brightness value or gray value, in a scale of 0 to 255 from less brighter (that is more intensity) to more brighter (that is less intensity).

**Statistical Analysis**

One-way ANOVA was used to analyze data, followed by Student Newman-Keuls (SNK) test for multiple comparisons. GraphPad Prism 5, Version 5.03 (GraphPad Software, Inc., La Jolla, CA) was used as the statistical package. Statistically significant difference was set at $p < 0.05$. 
Results

Body Weight Change

One way ANOVA revealed that there was statistically significant difference in body weight change across all experimental groups ($p < 0.05$). Post hoc analysis showed that percentage body weight was significantly lower in high salt fed groups; this reduction was reversed in a dose dependent fashion across all treatment groups (Fig. 1).

Histological Findings

Histology showed well arranged clearly branched myocardial fiber with well placed nucleus and no alterations in A Control, E (Fig. 2e), F (Fig. 2f), G (Fig. 2g), and H (Fig. 2h); Mild distortion of myocardial fiber branching arrangement in C (Fig. 2c) and D (Fig. 2d). The crossbanding pattern of cardiac cells was distorted and there was degeneration of myocardial fiber, and nuclear displacement in high salt-fed group (Fig. 2b). The increase in collagen fiber deposit was evident on histological sections of left ventricle of the rats in the high salt-fed group (Fig. 3b). Collagen content measured by digital densitometry is shown as result of collagen content in each specimen (Fig. 4). The amount of elastic fibers in Figs. 3b–c were reduced when compared with the control group (Fig. 3a). Elastic fiber in Figs. 3d–h, were same as in 3a as evident in the intense staining intensity and also elastic fiber content measured by digital densitometry is shown in each specimen (Fig. 5).

Discussion

The effects of concomitant treatment with Persea americana (Mill) aqueous extract (PAE) and sodium (NaCl) load via high salt feed (HSF) on the left ventricle of Wistar rats were investigated in this study.

One of the main organ systems vulnerable to the adverse effects of excessive sodium load in the diet is the cardiovascular system; this has been demonstrated in this study.

![Fig. 1](image)

Fig. 1 Effect of *Persea americana* on Body Weight Change of Rats fed with High Salt Diet. $n = 8$, values are expressed as mean ± SEM. 'statistical difference relative to control at $p < 0.0001$.

![Fig. 2](image)

Fig. 2 Shows photomicrographs of left ventricles of experimental group of Wistar rats: A (Control), B (Negative Control), C (50 mg/kg PAE + HSF), D (100 mg/kg PAE + HSF), E (150 mg/kg PAE + HSF), F (50 mg/kg PAE + SF), G (100 mg/kg PAE + SF), H (150 mg/kg PAE + SF). I - Intercalated disc; M - Muscle fiber; CM - cardiomyocytes, N - Nucleus; DM - Degenerated muscle fiber; DN - Displaced Nucleus (H&E x400).
A high-sodium diet increases potassium excretion by increasing distal sodium delivery. It has been suggested that potassium depletion inhibits insulin secretion and is associated with glucose intolerance.\textsuperscript{13,14}

Another study stated that salt load results in insulin resistance that results in abnormal glucose uptake and energy release.\textsuperscript{15} The reduction in weight as documented by this present study may probably be as a result high salt
induced ulceration of the stomach; this is in support of a previously done study. The weight gain in groups treated with HSF+ graded doses of PAE in a dose dependent fashion when compared with negative control group could be as a result of increased glucose uptake.

It has been reported that aqueous extract of Persea americana causes reduction of body lipid; it was observed in this study that there was weight loss in groups treated with SF + PAE when compared with control group; this may be as a result of breakdown of body lipid probably by increased catabolism of lipid in the adipose tissue.

This study showed that high salt diet induced left ventricular hypertrophy; in control group, the micrograph showed normal histology of the myocardium with regular striations without histological alterations; these striations were distorted in the negative control group following the administration of high salt feed (4 weeks). High salt induced hypertrophy in the negative control group probably because excess sodium intake induces lactic acid formation by switching on the sodium-induced cellular anaerobic thermogenesis (SICAT). Also, reported that switching on SICAT may result in acid-induced cell death.

High salt may have also induced hypertrophy in the negative control group by enhancement of rennin–angiotensin system probably by increased rennin and aldosterone production that result in vasoconstriction. Increased Angiotensin Converting Enzyme (ACE) activity results in increased plasma level of angiotensin II; angiotensin II has been reported to cause cardiac remodeling.

It was also observed that collagen fiber deposit in negative control group was higher than that of the control and extract alone groups while there was a marked decrease in elastic fiber deposit in negative control group when compared with all groups, elastic fiber deposition is similar in the control and extract alone groups. Ushiki reported that collagen fiber functions to provide structural support while elastic fibers provide resilience and contractility; high collagen fiber deposition in negative control group as observed in this study is thus suggestive of reduced contractility and resilience, and cellular degeneration.

The protective effect of PAE against myocardial damage in this study can be explained by its Angiotensin Converting Enzyme (ACE) inhibition. PAE probably induced inhibition of ACE that lead to reduction in production of aldosterone from the adrenal glands, natriuresis, and decreased plasma volume. The inhibition of ACE activity resulted ultimately in decrease in reabsorption of water and sodium from the distal convoluted tubule resulting in natriuresis and decrease in plasma volume. This may be the mechanism underlying prevention of left ventricular hypertrophy in this study.

Conclusion

The results of this study indicate that high salt diet caused significant histomorphological changes on myocardium of the left ventricle of rats as evidenced by myocardioctye distortion and ventricular hypertrophy. Persea americana extract has protective properties against these perturbations probably via inhibition of renin-angiotensin-aldosterone system. Further studies on the effect of persea americana extract on markers of activity of renin-angiotensin-aldosterone system will be required to support this suggestion.

Conflict of Interest

There was no conflict of interest among the authors and every necessary detail was agreed upon during the preparation of the work.

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Skin Development in H. huso Larvae

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Abstract

Introduction  Fish skin is important for life and protection mechanisms. Sturgeon skin also has an economical potential for its leather.

Materials and Methods  This article is a light microscopy study for knowledge the development of epidermis and dermis in one species of sturgeon (Huso huso). The fishes from different days of week-1 to week-6 were caught from Shahid Marjani propagation center and were processed.

Results  From 1(dph) the epidermis as two layers and dermis as a very thin collagen layer had formed. Sensory buds in epidermis and chromatophore cells in dermis of head and also arranged the dermal endothelial cells were features of 3 (dph). At 10 (dph) there was an increase in epidermal cell line, existence the circular cells as club cells in epidermis and increase in dermal thickness observed. From the character of 20 (dph) was organized epidermis to three layers and collagen fibrils as parallel to body. From 25-40 (dph) an increase in cell lines of epidermis up to 10 rows in some areas was observed.

Conclusion  The most important changes were the events occurred in dermis. The self-organized collagen fibrils to several lamellae, invading the fibroblasts to derm and forms of stratum campactum and stratum spongiosum.

Keywords
► huso huso
► skin development
► histolog

Introduction

Skin is a complex tissue and the first extensive interface between the animal and its external environment. In fishes, skin has a major role from the view of adaptation to the environment. In addition to physical protection, it helps to maintain the osmotic balance, accommodates hydrodynamics, and has sensory receptors and can takes part in respiration by the exchange of oxygen and carbon dioxide through the numerous blood vessels near the skin surface. Because it aids to skeletal support, in swimming mechanics has basic role and muscle force transmit down the body through the skin.¹ It differs from the skin of other vertebrates for the living surface cells which are in direct contact with the aqueous environment and presence a mucous layer instead of keratin.² The mucus coat maintains the water balance, and enzymes and antibodies which protects fish from infectious of bacteria. In several species, the skin also protect through control of coloration by expansion and contraction of the chromatophores.

The structure of fish skin has interested researchers for a long time. It is composed of three compartments: epidermis, dermis and hypodermis. Fish epidermis can be subdivided into surface, intermediate and basal layers. In contrast to terrestrial vertebrates which epidermis is covered by a layer of dead and keratinized cells, in fish it comprises from living cells exceptified sites that are subjected to abrasion such as lips and pads and the epidermal surface of some species capable of emerging from the water.³ The surface epidermal cells are as a single cell layer through its microfilament can protects the animal against different shocks. It is not renewed periodically, but when dead it can replace.
The intermediate layer is composed of various types of cells, the unicellular glands such as mucous goblet cells and club cells that produce alarm substances, epidermal ionocytes in some species are ion transporters and maintain the homeostasis of body fluids, sensory buds and the most dominant undifferentiated cells that when necessary can divide rapidly and serve as a reservoir for dead cells. The basal layer cells are as a single cell layer which keeps the epidermis to the basement membrane by means of hemidesmosomes. However other functions such as production of the early collagen stroma, interaction with the underlying mesenchymal cells for development of the dermal skeleton also imputation to basal layer in developmental stages. Variation in cell composition and in thickness of epidermis that usually concern to intermediate layer is related to the ecological adaptations of the species.2,3

The dermis divided into superficial region (stratum laxum) and the deep region (stratum compactum). Stratum laxum is composed of a loose connective tissue and the stratum compactum is a dense collagen plywood-like tissue with fibrocytes that in some places the vertical bundles of collagen fibers from this penetrated between muscles. So collagen organization is to strengthen the skin against tensile forces. The lamellar form of stratum compactum can causes the transparency of tissue.4 Under the collagenous fibers, the chromatophores are located and therefore the transparency can be important for the function of these cells. The thick dermis of naked fishes is interest to the leather industry.

The hypoderm separates the derm from the muscle fibers and is composed of a space containing of loose connective tissue and chromatophores, the fibrocyte like cells bordered the hypoderm on both sides and named dermal endothelium.5

The sturgeon, Huso huso is one of the most valuable species of Acipenseridea that widely distributed in the southern basin of the Caspian Sea and as other sturgeon, this species also has diminished and is in danger of extinction.6 This species is used for commercial food products such as caviar and smoked sturgeon meat. Sturgeon skin has special morphological, histological and physiological characteristics such as a beautiful appearance and a well balanced thickness and flexibility, makes it suitable for production of high quality leather and related products as clothing, shoes and handbags.7,8 Sturgeon production is considered as a business with great economic potential. This production can often accompany by the risk of infection and knowledge about skin development as a protective barrier against aquatic environment, can reduce the risk of diseases in aquaculture. The present study will help to understand the process of skin development and also to diagnose and treat fish skin diseases.

Materials and Methods

Five Huso huso larvae from each of 1 (dph), 3 (dph), 7 (dph), 10 (dph), 15 (dph), 20 (dph), 25 (dph), 30 (dph) and 40 (dph) were obtained from Shahid Marjani propagation center in Gorgan. The larvae were kept in fiberglass tanks to 21 (dph) and then, they were transferred to pond until the age of 45 days. The specimens were fixed in 10% neutral buffered formaldehyde and transferred to laboratory, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin, cut into 6µm thick sections. Then mounted on glass slides and stained with Haematoxylin-eosin (H&E) for general studies, Masson’s Trichrome (M.T) for collagen, PAS and Alcian blue (AB) for mucous substances. Then they were studied using light microscopy.

Results

In the first day after hatching (1dph), the epidermis of Huso huso consisted of two cell layers, the surface layer and the basal layer. They were cuboidal and columnar cells with not reaction to PAS and AB, although a mucous layer was observed on the surface of epithelium. There was a very thin collagenous layer observed by M.T staining, but the main tissue constitute the dermis was mesenchyme (►Figs. 1 and 2). It must be noticed that from the first day, the thickness of epiderm varied depending on the body region.

At 3 (dph), the skin had no change compared with 1 dph (►Fig. 3). At (7dph), in some parts of head a third to fourth row was formed and the epidermis had thickened. Sensory buds began to form on the epidermis of the head. An obvious collagen layer named as primary dermal stroma with a few chromatophore was observed and in some regions, it was lined by squamous cells, called dermal endothelial cells (►Figs. 4 and 5).

At 10 (dph) and 15 (dph) the epidermis composed of 2–4 row of epithelial cells with large circular or elongated cells and few reaction to PAS and AB staining. At 15dph an increase in large and light circular or elongated cells that some of them had positive reaction to PAS and AB staining. The sensory buds were obvious and formed only on the head epidermis. The primary dermal stroma was thickened and it was shown chromatophores at the deep of derm. The skin was separated from the muscle fibers by a distance and the deep and surface of the primary stroma was lined by dermal endothelial cells (►Figs. 6–9).

Fig. 1 Histological section of the head skin in 1 day old larvae (AB). Abbreviations: ML, mucous layer; Ep, epiderm; Me, mesenchym; bar = 6. 6µm.
At (20 dph), there were remarkable changes in the feature and organization of skin. The epidermis was composed of three cell layer. The superficial squamous to cuboidal cell layer, intermediate flat and large circular to elongated cell layer and the basal cuboidal cell layer. The collagen fibers of derm had changed their orientation from an irregularly to parallel fibrils covered by endothelial cells. Adjacent dermal endothelial, the deep surface of the dermis was lined by chromatophores. In some areas that muscle fibers had been formed, the dermal stroma penetrated by vertical bundles of collagen fibers into muscles. These bundles were also lined by dermal endothelial cells (→Fig. 10).

**Fig. 2**  Histological section of the head skin in 1 day larvae (M.T). Abbreviations: Ep epiderm; De, derm; bar = 3.3 μm.

**Fig. 3**  Histological section of the head skin in 3 day larvae (AB). Abbreviations: Ep, epiderm; De, derm; ML, mucous layer; bar = 3.3 μm.

**Fig. 4**  Histological section of the head skin in 7 day larvae (M.T). Abbreviations: Ep, epiderm; De, derm; DEC, dermal endothelial cells; Ch, chromatophore; bar = 6.6 μm.

**Fig. 5**  Histological section of the head skin in 7 day larvae (H&E). Abbreviations: TB, taste bud; bar = 3.3 μm.
At 25–40 (dph), remarkable changes in skin thickness were observed. The epidermis of certain regions of the head were thickest, composed up to 10 row and in different parts of the trunk it was up to 4 row that as in previous stage they were organized in three layers. The superficial cells were squamous to cuboidal, located on the surface of the epithelium and its outer surface seemed to have microridge. The epithelium of intermediate layer composed from two morphologically distinct cell types, the flat cells with condensed chromatin spread throughout the epithelium and the large elongated or circular cells were arranged in two rows in some areas with thick epidermis. This was a common feature for club cells, ionocytes and mucous cells, by light microscopy they were easily identified with their reaction to PAS and AB staining. Mucous cells were characterized by their containing filled with PAS positive secretion, distributed in the middle and superficial layers and open onto the surface, the ionocytes also have free surface and no reaction to PAS but most club cells were covered by the superficial layer. The sensory buds in Huso huso were observed in the head, lip, bronchial and fascial skin as fusiform pale staining structures containing tall columnar cells with oval nuclei. The basal part of the

**Fig. 6** Histological section of the head skin in 10 day larvae (H&E). Abbreviations: Tb, taste bud; DEC, dermal endothelial cells; CC, circular cell; bar = 6.6 μm.

**Fig. 7** Histological section of the head skin in 10 day larvae (M.T). Abbreviations: De, derm; DEC, dermal endothelial cells; CC, circular cell; bar = 6.6 μm.

**Fig. 8** Histological section of the trunk skin in 15 day larvae (AB). Abbreviations: Cl, club cell; GC, goblet cell; bar = 6.6 μm.

**Fig. 9** Histological section of the head skin in 15 day larvae (PAS). Abbreviations: Cl, club cell; GC, goblet cell; Tb, taste bud; bar = 3.3 μm.

**Fig. 10** Histological section of the trunk skin in 20 day larvae (M.T). Abbreviations: SL, Superficial layer; IL, intermediate layer; BL, basal layer; DEC, dermal endothelial cells; Ch, chromatophore. Bar = 6.6μm.
epidermis was a single cell layer of cuboidal cells. From 25 (dph) Most changes were obvious at derm of trunk. The collagen fibers were organized to layers called lamellae, so at 40 (dph) in certain areas of trunk the lamellae had increased to 10–12 layers. Other feature of derm from 25 (dph) was the change from a cellular to cellular dermis and primary derm changed into a secondary derm called stratum compactum. From 40 (dph) up of derm in trunk regional so showed a thin cellular layer with a few pigment cells considered as stratum spongiosum. It must be mentioned, at the deep surface of stratum compactum also pigment cells were visible and this region considered as hypodermis (Fig. 11). This layer was situated between stratum compactum and muscle. It shows some empty spaces and the collagen fibers from derm pass through this layer and penetrate deep between muscle bundles as thick connective tissue septa or myosepta (Fig. 12).

Discussion

The integument is the first barrier and the outer protective that separates the fish from aquatic environment. There are many descriptions on fish skin and also a few data about the embryonic and larval stages. The aim of this study was to get information about differentiation of epidermis and derm of skin development in Huso huso that is a valuable species of Acipenseridae.

Epidermis Development

At 1 (dph) the epidermis of Huso huso larvae consisted of two cell layers. Some studies showed these layers were formed at different stages, the surface epithelium during the blastula and the inner layer or epidermal basal layer at gastrula period. The surface layer functions like periderm in mammalian embryo protect the embryo and their cells that arise at mid-blastula, is the first specified cell type, all of the genes affected this development are related to epiboly process. The basal layer contains both differentiated and undifferentiated cells. Regulation of epidermal basal layer development is regulated by bone morphogenetic protein during gastrulation.

In Huso huso the third row of epidermis and sensory buds were formed from 7 (dph) in head epidermis, this is consistent with previous research on Acipenser percicus that the sensory buds were formed from the end of week 1. The large circular and elongated cells without positive reaction to carbohydrate staining were present from 10 (dph), these cells could be club cells and ionocyte that in the later stages were increased. Those cells that were covered by surface cells named club cells and those with free surface area and no reaction to PAS named to ionocytes. The first mucous cells with positive reaction to PAS and AB only seemed to appear from 15 (dph), and they could be diagnosed from club cells and ionocytes. In Acipenser percicus also from week-2 the mucous cells could be distinguished from club cells. It can be supposed that from the first stages of development, the mucous layer on epidermis was secreted by the undifferentiated epithelial cells to protect the larvae from microorganism infection. The epidermis with three layers was obvious from 20 (dph).

The 25 (dph) – 40 (dph) stages were associated with increasing in cell rows and thickness of epidermis especially in head. At 40 (dph) the skin was evolved and had the adult feature. The epidermis of Huso huso as many other species composed of non keratinized stratified epithelium. Keratinization of fish skin is uncommon, except some species such as the head of Solea senegalensis. The function of keratin is in part compensated by mucous secretion. In this study, the apparent changes in epithelial thickness were observed from 2 to 10 rows. According to previous findings, epidermal thickness differ based on species, age and body region. The thickness of head epidermis was greater than trunk, because it can serve as a protection against physical injury and mechanical stress. The stratified epithelium consisted of three layers, it is similar to the findings in other species such as loach (Iksookimia Koreensis), Senegal sole, Torrent catfish (Libagus media diposalis) and eel goby (Odon tamblyopus lacepedii). The intermediate layer was composed of various cells with similar appearance between some of them,
each of these have specific function. Club cells release alarm signal factors and by olfactory stimulation exhibit a fright reaction, ionocytes are one of the most important cells maintain the homeostasis of body fluids in developmental stages and in Huso huso it can be one of the subtypes of this cell.,

Skin Development in H. huso

Dermis Development

In Huso huso, the presence of the first collagenous fibrils were from 1 (dph). During the first week of development, the primary dermal stroma fills the subepidermal space and the fibrils distributed throughout the thickness irregularly. Between 10–20 days of development, an organization of derm to regular parallel fibrils happened and these events were occurred in a space without cells (fibroblast). Starting from 25 (dph) the primary derm organized to layers called lamellae and it was invaded by fibroblasts. These modifications lead us to consider the collagenous derm as secondary dermal stroma or stratum compactum.

From the first days of dermis development, some like mesenchymal cells look like fibroblast were found to differentiate to dermal endothelial cells delimits the deep dermal region. In zebrafish (Danio rerio), also at hatching, a primary dermal with different thickness according to body region is perceived, in 5 (dph) the primary derm forms a regular layer and to 10 (dph) the collagen fibrils organized up to three layers or lamellae. During the following days the number of lamellae are added (Dominique et al., 2004).

According to previous studies on zebrafish, during the early stages of evolution the dermis, cytoplasm of basal layer cells show features revealing active protein synthesis and they can involve in collagen production but with presence the fibroblast in dermal stroma, interaction between epidermis and derm is effective in collagen synthesis and although from one stage to later the epidermal basal cells with contribution the dermal endothelial cells and fibroblasts ceases collagen deposition (Dominique et al., 2004). From the 3 (dph) the chromatophores appeared in the deep of derm, then gradually with more development more cells expanded throughout the hypoderm of head and trunk and also in stratum spongiosum of trunk. The stratum spongiosum only is obvious in trunk but not a conspicuous layer observe in head region, the part of derm lying immediately below the epidermis composed of loose connective tissue and assumed as Stratum spongiosum. A few chromatophore cells are discernible in this region of derm in trunk. The thick dermis of Huso huso causes utilization of skin in leather industry.

Conclusion

This study aimed to describe the morphogenesis of the skin by light microscopy in one species of sturgeon. It allows us to find the organization and composition of skin. Some findings are agreement with other species, although there are differences from the view of timing. Further analysis are necessary to differences between morphology and the immunohisto-chemical and also electron microscopy for more precise characterization of skin in Huso huso.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Description of Morphological Abnormalities in *Rhinella arenarum* (ANURA: BUFONIDAE)

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Abstract

The rise of anatomical abnormalities in anurans over recent years has increased the interest in producing more and better information about the abnormalities observed in specimens within natural environments that are currently being altered. The aim of this work was to identify and describe the osseous abnormalities found in *Rhinella arenarum*. Four samplings were conducted between March 2009 and February 2010 in the Claro river, located in San Francisco in the Ayacucho Department in the province of San Luis. Specimens of *R. arenarum* with visible abnormalities were observed and collected manually. They were euthanized in the laboratory, preserved in 10% formaldehyde, and stored in the Herpetological Collection Unit of the National University of San Luis (CH-UNSL). The Alizarin-Alzian technique was also applied to the specimens. Photographs of the abnormalities were taken, and observations were made with a stereoscopic microscope. The following abnormalities were identified and described: femur ectromelia, tibial and fibular ectromelia, ectrodactyly and brachydactyly. Further work could focus on determining the causes of the abnormalities observed in this species, conducting an integrated study, and attempting to link this with the worldwide phenomenon of amphibious declination.

Keywords

► abnormalities
► *Rhinella arenarum*
► brachydactyly
► ectrodactyly
► ectromelia

Introduction

Amphibians are considered good indicators of healthy environments.¹⁻³ Reports on amphibian deformations have increased dramatically in recent years.⁴ The causes and implications of these abnormalities remain poorly explained, but several researchers have suggested they may indicate an emerging threat for populations of amphibians and other forms of life.⁵⁻⁷

Several causes or groups of causes are proposed as originators of abnormalities in amphibians, including UV-B radiation,⁸⁻¹⁰ trematodes,¹¹⁻¹⁴ retinoids,⁶ pesticides,¹⁵⁻¹⁷ other chemical pollutants¹⁸⁻²¹ and predation.²²,²³ However, knowledge about the abnormalities resulting from heredity, development, traumatic factors, and particularly, knowledge regarding the variation in abnormality rates across species, life stages, types of habitat, and geographical areas remain unexplained, thus making the identification of differences between natural and artificial abnormalities problematic.¹¹

There is evidence of anuran specimens with supernumerary extremities in Argentina.²⁴ The interest in dealing with this issue has grown in recent years in Argentina due to the increasing number of anurans with abnormalities.²⁰,²⁵⁻³⁰ These findings have also occurred in the province of San Luis.³¹⁻³³ Consequently, the objective of this work was to identify and describe the abnormalities found in *Rhinella arenarum* from a natural environment in the province of San Luis.

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Materials and Methods

Description of the Site and Natural History
The work was conducted in the Claro river, one of the main tributaries of the San Francisco river, in the town of San Francisco (S 32° 36.7771 - WO 66° 08.0357), Ayacucho Department, province of San Luis. San Francisco del Monte de Oro is located to the North of the province, in a valley framed by the Sierras Centrales and the Socoscora hills. One of the characteristics of this area is the presence of Caranday palms (*Trithrinax campestris*), surviving in the Southernmost habitat for this species.

The climate is dynamic, with strong daily and seasonal temperature fluctuations. During summer, the temperature ranges between 17°C and 38°C. Nights are often cold in winter, with minimal temperatures below 0°C. However, daytime temperatures are temperate, ranging between 15°C and 20°C.

Sampling Method
Four nocturnal samplings were made between March 2009 and February 2010, and six abnormal specimens of *Rhinella arenarum* (2 juveniles and 4 adult individuals) were observed and collected. Specimens were collected using the Collection by Visual Encounters method (CVE), described by Crump and Scott Junior.34

Laboratory
Specimens were taken to the laboratory, euthanized with an MS-222 solution, fixed with 10% formaldehyde, and stored in the Herpetological Collection Unit of the National University of San Luis (CH-UNSL: 0431; 0432; 0433; 0434; 0435 and 0436). Differential staining of cartilage, bone and subsequent diafanization these animals was apply according to the technique developed by Wassersug.35 All observations were made using a stereoscopic microscope (Olympus SZ51). Photographs of the abnormalities found were taken with a digital camera (Canon EOS Rebel T5).

Morphological Analysis and Terminology
The abnormalities observed were described according to the terminology proposed by Meteyer.36 We adhered to Fabrezi and Goldberg37 for the description of skeletal structures.

Results and Discussion
CH-UNSL0431 Juvenile specimen: Ectromelia of the right hindlimb (Fig. 1).
CH-UNSL0432: Juvenile specimen: Ectrodactylyia of the left hindlimb. Absent metatarsal bone in digit V, curved digit III and digit II, and merged cartilages. It is not possible to differentiate digit I from digit II, although associated with these there are two phalanges and an ossified distal phalanx with a more robust appearance than that observed in the right limb with normal development. Absence of prehallux (Fig. 2).
CH-UNSL0433 Adult specimen: Ectrodactylia of the right back limb. Complete absence of metatarsal bones and digits, except for the prehallux. Undetermined ossified traces were also observed. The fibula presents a slight curvature in relation to the left limb (Fig. 3).
CH-UNSL0434 Adult specimen: Brachydactyly and ectrodactylia of the right hindlimb. Digit I presents one phalanx, and digit IV presents 2 phalanxes. Complete absence of digit V. The prehallux is cartilaginous in both limbs (Fig. 4).
CH-UNSL0435 Adult specimen: Brachydactyly of the right hindlimb. Digit III has 2 phalanxes, the last of which is...
forked; digit IV has 2 phalanxes, and the last one is forked, swollen and cartilaginous (Fig. 5).

CH-UNSL0436 Adult specimen: Ectromelia in the tibiofibula of the left hindlimb, where only the proximal portion of the ossified and strong tibial and fibular traces are observable. The basal portion of the tibiofibula in the lateral view is swollen and shorter than the normal tibiofibula (Fig. 6).

All the abnormalities observed were located in the hindlimbs. The most common abnormality was ectrodactyly, followed by brachydactyly and ectromelia. Multiple abnormalities in a single limb were observed in one specimen. The cases recorded present different forms in a single abnormality.

The abnormalities in the hindlimbs of the captured specimens are the most common abnormalities observed in natural areas. However, the causes of these morphological abnormalities have not yet been identified. This makes it difficult to determine the difference between natural abnormalities and anthropogenic abnormalities.

We have examined unilateral abnormalities in the hind extremities in this study, which coincides with findings of studies conducted by Ouellet et al. and Johnson et al. However, while the most frequent abnormalities reported in the literature are additional extremities (polymelia) or reduced limbs (hemimelia), we did not observe these abnormalities. The variation of abnormalities in the limbs recorded in this study suggest etiological heterogeneity.

Conclusion

These cases may represent the first documented report of morphological abnormalities in an anuran population of San Luis North area, in Argentina. We believe it is important to establish precedents for abnormalities in different anuran populations to document the increase of occurrence rates and abnormality recurrence.

Collection Permission

The collection of the Rhinella arenarum specimens that were used to conduct this study was authorized by Resolution N° 2525 of the Biodiversity Program of San Luis Province.

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Morphology of the Left Atrial Appendage: Prevalence and Gender Difference in a Kenyan Population

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Introduction

The left atrial appendage (LAA) or left auricle is a small muscular pouch attached to the left atrium. It is constricted at its atrial junction hence demarcating it from the left atrium.¹ Its morphology varies in size, shape and the number of lobes. Based on these features, the LAA has been classified into Windsock, Chickenwing, cactus or cauliflower types.²,³ These morphologies may influence an individual’s risk of cardioembolic stroke.⁴,⁵ The risk being highest in cauliflower morphology and least in Chickenwing morphology.²,⁴ This variation in risk arises from a difference in contractility in the morphologies since low contractility predisposes to thrombi formation.⁶–⁸

Cardioembolic stroke (CES) risk shows gender and racial variations. It is higher in the females and blacks compared...
with males and other races. The highest risk of CES occurs in individuals with atrial fibrillation (AF) patients who have a 5-fold increase in risk. Despite low prevalence of AF in females and blacks, these groups are known to have a higher risk of CES in the event of AF. It is unclear whether the higher predisposition in blacks and females is due to presence of high risk morphology in these groups. This study therefore aimed to determine frequency of LAA morphological types in a Kenyan population and assess for existence of gender differences. This knowledge may further elucidate the basis of variation in CES risk.

Materials and Methods

A total of ninety one (91) human hearts, 50 male and 41 females, were obtained after exclusion of 4 hearts (3-dilated cardiomyopathy, 1-aortic valve replacement) from autopsies conducted at Chiromo, Nairobi city and Kenyatta National Hospital mortuaries following ethical approval from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH-UoN/ERC). This study was done in conformation to the Declaration of Helsinki. Informed written consents were obtained from the next of kin of the deceased prior to subject inclusion. Subject confidentiality was ensured by use of case numbers instead of names throughout the study for the purposes of identification. Hearts with any obvious gross cardiomegaly, observable gross pathology for example endocarditis, observable congenital defects such as LAA agenesis and heart septal defects, history of surgery and deformed LAA were excluded with the help of expert advice from the attending pathologists. Normal heart specimens of males and females of age ranging from 18–80 years who died of non-cardiac pathology and had grossly normal anatomy of the LAA were included in this study.

During the autopsies, routine standard Y incision were made and the thoracic cage opened. The pericardium was excised longitudinally and the hearts were harvested by excising the great vessels 2 cm from the base and the inferior vena cava was excised close to the thoracic diaphragm. The left atrium and the left atrial appendage were identified. On the external surface, the length of the LAA was measured using a vernier caliper, Pittsburg™, as the longest dimension from the left atrial-LAA junction (which is constricted) to the apex. Moreover, angulation of bend was measured, in presence of a bending morphology (Windsock and Chickenwing), using a goniometer. An incision was made on the posterior wall of the left atrium extending from the roof of the left atrium to the atrio-ventricular junction (Fig. 1). The two flaps were then reflected laterally so as to expose the endocardial surface of the left atrium. This allowed for internal assessment of the LAA (Fig. 1). The LAA morphology type was determined by external observations of shape, measurement of length and confirmation of number of lobes which was done using a probe into the LAA. According to classification by Wang et al and Di Biase et al. (2012), a lobe is regarded as a visible outpouching marked by an external crease and can internally accommodate a 2 mm probe. The different morphological types were determined using the shape, overall length, degree of bend from the principal axis, and number of lobes. Chickenwing morphology > 4 cm, with a dominant lobe which bend some distance from the ostium < 100° (Fig. 2a). Cactus morphology < 4 cm with secondary lobes (Fig. 2b). Windsock morphology was regarded as having length > 4 cm with a dominant lobe and bend > 100° (Fig. 2c). It could have secondary lobes. Cauliflower morphology < 4 cm with no forked lobes (Fig. 2d). The shape, number of lobes and length were recorded in the data sheet.

Data were analyzed using Statistical Package for Social Sciences (SPSS) (Version 21.0, Chicago, Illinois). The frequencies of the morphological types was determined. Percentage prevalence of the morphological types based on gender was performed. Chi square was applied to check for gender differences. A p-value of < 0.05 was considered statistically significant at 95% confidence interval.

Results

All the four varieties of LAA were observed in the current study (Fig. 3) with the highest prevalence of cauliflower (29.7%) and least cactus (14.3%). Cauliflower, Chickenwing and Windsock morphologies were of almost similar prevalence (Fig. 4).

Cauliflower was the most prevalent morphology in males compared with females who had Chickenwing as the most prevalent morphology. Cactus morphology was the least prevalent in both the male and female group. Gender
difference in the morphologies was however not statistically significant ($p = 0.537$) (Table 1).

**Discussion**

Cauliflower, Chickenwing and Windsock morphologies were more frequent and present in almost similar prevalence while cactus morphology was the least frequent. The similar proportions of the LAA morphologies in this study is contrary to other studies which report a wider variability in the proportions (Table 2). On account of the similar proportions observed, it is conceivable that each individual from this study population had a relatively equal probability of having any of the four morphological types as opposed to other populations which had various preponderance of specific morphologies. It is however unclear whether such a pattern of similar proportion is characteristic of black populations since there was no literature among black populations for comparison.

Cauliflower morphology was the most prevalent morphology in this study and is higher than existing data from other populations (Table 2). Although almost similar proportions of cauliflower have been recorded recorded among American, Turkish and Finish populations, cauliflower was not the most prevalent morphological type among these populations. In contrast, Cauliflower morphology was least frequent among Italians. This morphology is associated with the highest risk of cardioembolic stroke in the event of atrial fibrillation [AF]. Infact, it is thought to have, as high as, an 8-fold risk compared with Chickenwing morphology which is noted as the least risky morphology. Despite the high frequency of cauliflower (29.7%), existing similar proportions of non-cauliflower morphologies suggest that the frequency of LAA morphology might only partially explain high CES risk in this black population. Additional studies assessing multiple risk factors among black CES patients are required to completely understand the basis of CES predisposition in blacks.

Chickenwing morphology was common in this study population (27.4%) and this was only comparable to findings by a study by Di Biase et al. who currently report the highest prevalence in literature. Other studies report Chickenwing as the least prevalent morphology. Chickenwing morphology is suggested to be the least risky morphological type with regards to CES in the event of AF. Although higher risk of CES is seen among Blacks when compared with other races, the high prevalence of the least risky morphology in this black population and low prevalence reported among non-black populations is paradoxical. It is therefore plausible that other factors might be implicated in influencing cardioembolic stroke risk variation between these races. Moreover, despite the advantage of lower risk of CES in Chickenwing morphology, the bending morphology confers a procedural challenge during insertion of LAA occlusion devises and has higher periprocedural risk of complication during AF catheter ablation. The high prevalence of Chickenwing morphology in our population should be taken into consideration during surgical management of CES risks.

The prevalence of Windsock morphology was high (28.6%), only second to cauliflower morphology in our study population. Windsock morphology was also the most common morphology type in other studies. Cactus morphology on the other hand shows a large interpopulational variability in literature (Table 2). It was the least common in the present study in accord with findings in an American population but differing greatly with the Japanese who found it as the most prevalent morphology.

The current study also showed that males and females did not differ significantly in the prevalence of LAA morphological types. Females are known to have higher risk of CES compared with males in the event of AF. This absence of gender difference in in the prevalence of high risk LAA morphology types therefore suggests a possibility of other factors underlying the difference in CES risk. For example, various hematological differences between males and females with atrial fibrillation have been suggested to play a role in the variation in CES risk. These include a higher concentration of prothrombotic factors such as tissue plasminogen activator, Von willebrand factor in females compared with males. Further studies are therefore warranted to elucidate the basis of gender variation in risk of CES.

![Fig. 2 Image showing LAA morphology types. (a) Chickenwing; (b) cactus; (c) Windsock; (d) cauliflower. Classification by Di Biase et al. (2012).](image-url)
Fig. 3  (a) chickening; (b) Windsock; (c) Cactus; (d) Cauliflower (arrow pointing to LAA, LAA-left atrial appendage). Comparison CT images based on classification of Di Biase et al. (2012).
Conclusion

There are no statistically significant gender differences in the LAA morphology types in this sample black Kenyan population. The Cauliflower, Chickenwing and Windsock morphologies were more frequent and present in almost similar prevalence. Cactus morphology was the least frequent. High frequency of both the risky cauliflower morphology and protective Chickenwing morphology warrants assessment of LAA morphological types in management of high risk CES patients in this setting.

Acknowledgments

The contribution of my colleagues Mr. Brian Bundi, Mr. Brian Wambua and Ms. Swafiya Salim who assisted in the conceptualization of this work is highly appreciated. We also acknowledge Mr. Washington Ochieng (senior technician) and Dr. Edwin Walong (attending pathologist) for making data collection at KNH mortuary possible.

References


Morphological and Morphometric Study of the Supratrochlear Foramen

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Abstract

Introduction The supratrochlear foramen (SF) is located in the distal portion of the humerus, formed in between the coronoind fossa and the olecranon fossa. It is associated with a narrowing of the medullary canal in the distal humerus, in addition to being a phylogenetic characteristic, more common in ancient populations. The objective was to evaluate the incidence of the supratrochlear foramen, its shape, dimensions and translucency of the septum.

Materials and Methods A total of 141 humerus adults, 72 right and 69 left-handed individuals from the collection of the Human Bone of the Academic Center of Vitória-Brazil. The format was classified as oval, round, irregular and cribiform. The vertical (VD) and transversal (TD) diameter were measured and the translucency of the septum was observed.

Results Of the 141 humerus, 19.8% (28) had SF 39.3% (11) on the right side and 60.7% (17) on the left side. The oval was the most recurrent, found in 39% of the cases and the translucency was identified in 78% (110) of the bones. The mean VD was 3.653 mm and 3.492 mm on the right and left sides, respectively. The mean of the TD was 4.853 mm on the right side and 5.427 mm on the left. The area averages were 19.633 ± 20.57 mm² on the right side and 19.919 ± 19.24 mm² on the left side.

Conclusion The knowledge of SF favors the preoperative procedures of patients with fractures in the distal humerus, necessary for the surgeries in the region.

Keywords hysterus ► supratroclelar foramen ► anatomical variations ► radiology ► phylogeny

Introduction

Bones are irregular structures that have elevations, protrusions and open areas for passages of vessels and nerves, these impressions are called bone accidents. These accidents are generated by several factors, such as fixation of the skeletal muscles, vessels and nerves transiting the bone surface, ligaments attaching to the ends of the bones and some viscera using them as a point of support.¹ Some accidents may not be present in the most individuals, being considered anatomical variations, such as the supratrochlear foramen. The supratrochlear foramen is located in the distal portion of the humerus and is formed by the absence of the septum that separates the coronoind fossa and the fossa of the olecranon (Merckel, 1825).² Some authors suggest that the supratrochlear septum is not perforated in the embryonic development, and that it is intact up to 7 years of age.³

Its development occurs more frequently during adolescence or in adulthood, due to the incomplete ossification of that region.⁴ Anthropologists argue that SF is more common in individuals of ancient civilizations when compared with contemporary populations, as well as present in primates, being considered a phylogenetic characteristic.⁵ There have been reports that the presence of SF is associated with a narrowing of the medullary canal in the distal humerus.⁶,⁷

Knowledge of this association plays an important role in the intramedullary fixation procedure, since this region is commonly affected by fractures, especially in early childhood. The width of the canal is statistically smaller in humerus with SF, which can directly affect the entry point of an intramedullary fixation rod. In this context, the objective was to evaluate the incidence of the supratrochlear foramen, its shape, dimensions and translucency of the septum of this region.

**Methods**

Eighty-six adult human skeletons were used, where 55 had right and left side humerus, 17 only right side humerus and 14 left side humerus, totaling 141 bones. Being 72 rights and 69 left, coming from the collection of the Human Bone Collection of the Academic Center of Vitória-Brazil, authorization number CAAE 43228015.0.0000.5208. The presence of SF was observed, and when present they were classified according to their shape (oval, round, irregular and cribiform) (Fig. 1). Measurements of vertical diameter and transverse diameter were also performed using a Lee Tools® 150-mm stainless steel digital caliper. The SF area was calculated through the ImageJ 150i program, from photographs of the bones. The translucency of the septum was also observed, placing the lower end of the humerus against the light of a negatoscope (Fig. 2). For the data analysis, a normality test was used, and then the parametric (Student-t-test) and non-parametric (Mann-Whitney) tests were applied.

**Results and Discussion**

Of the 141 bones studied, 19.8% (28) presented SF. The highest prevalence occurred in left side 60.7% (17), corroborating other authors. However, Nayak et al described a prevalence of 44.5% on the right side and 26.8% on the left side, differing from our study. Already Singhal and Rao identified a prevalence of 27.9% on the right side and 27.8% on the left side. The intermittent pressure of the olecranon and the coronoid process in the septum of the humerus can potentially lead to bone resorption and the formation of SF. If mechanical stress is the causative agent, we can infer that SF is more frequent in the side that the individual has a higher prevalence of use, such as the right side in the case of rightists.

SF is a phylogenetic feature, also found in primates, and which can be inhibited by a more robust member. Explaining the higher frequency of SF on the left side observed in our study. This fact is due the intermittent pressure in the region resulting in hyperemia and strengthening of the bone septum, which occurs on the right side in right-handed individuals, generating a more robust member compared with the left one.
According to Paraskevas et al. (2012), there is an association between SF and presence of the supracondylar process, however, in this study, only one humerus was identified with the presence of the supracondylar process, not associated with SF (Fig. 3).

Regarding the SF format, the oval type was the most recurrent, found in 39% of the cases, corroborating the study by Kumar et al. (2015) (Fig. 4). The translucency was identified in 78% (110) of the analyzed bones; if there is a poor radiological interpretation, SF can be confused with osteolytic or cystic lesion at the distal humerus, an important finding during radiological examinations. In relation to the SF measurements, the mean vertical diameter of the SF was 3.653 mm (0.5700–7.860 mm) and 3.492 mm (1.230–8.330 mm) on the right and left sides, respectively. The mean cross-sectional diameter was 4.853 mm (0.3900–9.870 mm) on the right side and 5.427 mm (1.770–11.25 mm) on the left side. The difference between the two sides was not significant (Table 1).

The area averages were almost identical, 19.633 ± 20.57 mm² on the right side and 19.919 ± 19.24 mm² on the left side (Table 2). The knowledge of the VD and TD of the SF

<table>
<thead>
<tr>
<th>Table 2 Area values of the supratroclear foramen</th>
</tr>
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<tbody>
<tr>
<td>Area values</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
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</tr>
<tr>
<td>medium</td>
</tr>
<tr>
<td>Minimum</td>
</tr>
<tr>
<td>Average</td>
</tr>
<tr>
<td>Standard deviation</td>
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</table>

*Measurements were in mm2. Mean ± standard deviation.

Table 1 Measurements of the Supratroclear foramen

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Right side</th>
<th>Value p &lt; 0.05</th>
<th>Test t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical Diameter</td>
<td>3.653 ± 1.983</td>
<td>3.492 ± 1.746</td>
<td>0.824</td>
</tr>
<tr>
<td>Horizontal Diameter</td>
<td>4.853 ± 2.934</td>
<td>5.427 ± 2.540</td>
<td>0.587</td>
</tr>
</tbody>
</table>

*All measurements were in millimeters (mm). Mean ± standard deviation.
helps in preoperative procedures of orthopedic surgeries, as well as the awareness of the various forms and dimensions of this foramen may help to avoid errors in the interpretation of radiographs.\textsuperscript{16,18}

**Conclusion**

The knowledge of SF improves the understanding of the distal humerus region, favoring the preoperative procedures of patients with fractures in this region, necessary in the practice of orthopedic surgeons and radiologists.

**Acknowledgments**

To the Laboratory of Anatomy of the Academic Center of Vitoria, Federal University of Pernambuco.

**References**

A Contribution to the Anatomical Study of the Mandibular Premolars

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Abstract

Introduction  During the practical classes of Dental Anatomy, some students have difficulties in identifying the mandibular first premolars, especially in determining the proximal surfaces.

Objective  The objective of this study was to evaluate the anatomical structures of those teeth, to determine the criteria for identifying the proximal surfaces in individual teeth.

Materials and Methods  Two hundred mandibular first premolars belonging to 100 individuals, at the age of 18 to 25 years old and from both sexes were evaluated according to some features of the dental crown. The associations of interest were estimated using the chi-square test or Fisher’s Exact Test and kappa statistic. The level of significance was 5%.

Results  The distal occlusal fossa (DOF) was the widest fossa (94.5%), and the distal surface constituted the proximal surface with the largest dimension (94.5%). It was found that in 60% of the teeth, the DOF was located closer to the lingual surface. The grooves from the mesial and distal occlusal fossa, toward the lingual surface, were absent in most teeth (64.5% and 91.5%, respectively).

Conclusions  The criteria for determining the proximal surfaces when evaluating individual first mandibular premolars should be: DOF will be the widest occlusal fossa; the proximal surface with the largest buccolingual dimension will be the distal surface. The occlusal fossa which is closer to the lingual surface may also be evaluated, being considered as the DOF. However, this should be considered the last of the three highlighted criteria, since it generates a greater margin of error in relation to the others.

Keywords
► anatomy
► premolar
► dental crown
► dental education

Introduction

Normal shapes of the teeth, suitable arrangement on dental arch and natural occlusion are closely related to a functional performance and a physiological action. The shape of the tooth has an important role in masticating and protecting periodontal tissue.¹ Knowledge of tooth morphology and function is fundamental to all aspects of dental practice,² and it is essential to establish a correct diagnosis, to indicate the appropriate treatment and to avoid complications during

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certain procedures like dental extractions, endodontic treatments, and surgical procedures. The discipline of Dental Anatomy is a component of the Basic Sciences in the program of Dentistry Schools that comprises the study and organization of the tooth as an isolated entity and as an integrant of both dental and masticatory systems. The professional (dental surgeon) who is committed to the preservation of human teeth should have a clear understanding of the characteristics and fundamentals of dental morphology. The learning process is mainly based on the identification of natural teeth, the study of stone or resin macro models, the computer-assisted learning program or even internet resources.

Most dental anatomy textbooks describe the human dentition appropriately, but sometimes they fail in providing details, such as the variation in external root anatomy and internal root canal systems, as in the case of the mandibular first and second premolars. According to Kraus and Furr (1953), the mandibular first premolar shows an extremely wide range of morphologic variability. From all the teeth of the human denture, the first mandibular premolar is the most interesting morphologically.

The first mandibular premolar is usually described as unirradicular, with an oval cross-section. However, there may be variability in root morphology, such as the presence of three roots and variation in the number of canals.

As noticed relating to the roots of the mandibular first premolars, morphological variations in the dental crown of these teeth can also be observed. Kraus and Furr (1953) published a relevant study about the morphology of the first mandibular premolars, describing seventeen different structures of the dental crown and the variations related to some of them, such as the number of lingual cusps, the position of the enamel ridge, and the grooves on the lingual surface.

Dental anomalies involving the mandibular first premolars are rare, but they may occur, as in the case report of a 33-year-old man who presented bilateral Dens invaginatus (dens in dente) that is a rare developmental anomaly resulting from invagination of a portion of the dental crown. It is an important dental anomaly, due to the possible pulpal involvement.

There are few studies about premolar morphology and the ones that exist do not study all the morphological characteristics, or use different methodologies.

During the practical classes of Dental Anatomy, we can see some students with difficulties in identifying the first premolars, especially in determining the proximal surfaces. We can see the literature describing some features that may facilitate the identification of proximal surfaces of those teeth, although there are few studies in which researchers performed standardized measures, and provided more detailed information. According to Madeira and Rizzolo (2016), the distal occlusal fossa is the largest, and from both occlusal fossae (mesial and distal), the distal one is the closest to the lingual surface. These features may be facilitating the criteria for identifying the tooth’s side. However, these characteristics are not routinely described in dental anatomy textbooks or in research papers.

The aim of this study was to evaluate the morphology of mandibular first premolars in young individuals, in which anatomical criteria were determined to identify the proximal surfaces, improving the anatomical study when those teeth were analyzed individually.

Therefore, it is expected that this study upgrades the published data about the mandibular first premolars, improving the identification of the hemi-arch of the individual mandibular first premolar, during morphological study in Dental Anatomy Classes.

**Materials and Methods**

This project was approved by the Ethics Committee of São Paulo State University (UNESP), School of Dentistry, Araraquara (CAAE 17513313.6.0000.5416).

A clinical evaluation was performed and it was based on 200 mandibular first premolars belonging to 100 students of São Paulo State University (UNESP), School of Dentistry, Araraquara, at the age of 18 to 25 years old, from both sexes.

As an inclusion criterion, individuals with healthy mandibular first premolars were evaluated. The analyses of individuals with those teeth with caries lesions, fractured and/or restored teeth, or with the mandibular first premolars absent were not included.

The following structures were evaluated: size of mesial occlusal fossa (MOF) and distal occlusal fossa (DOF) (1 = discreet; 2 = small; 3 = medium; 4 = large), occlusal fossa located closer to the lingual surface, presence/absence of grooves in the lingual surface that emerged from MOF and DOF, and from which proximal surface showed larger buccolingual dimension.

It was used a visual criteria for the classification of the occlusal fossae. Photographs of occlusal surface of mandibular premolars were made, and discreet, small, medium and large fossae were exemplified, in order that the examiner could follow those patterns during the analysis.

The buccolingual dimension of the proximal surfaces was also evaluated by visual criteria, without any metric instrument. The apexes of the buccal and lingual cusps were taken as reference points, and the distance from the apex of the buccal cusp to the apex of the lingual cusp was analyzed in the mesial and distal surfaces.

The analyses were made by a qualified examiner. For calibration of the examiner, the teeth were analyzed in duplicate, with an interval of at least 7 days between the analyses. From the 100 students who constituted the sample, 15 (30 teeth) were randomly selected. The minimum sample size was estimated considering a minimum agreement of 0.50, power of 80% and α = 5%. The reproducibility was estimated using Kappa (k) Statistics when the data were dichotomous and Kappa statistic with linear weighting (kp) when the data were ordinal. It should be clarified that this step was necessary to ensure that evaluations were performed with an adequate accuracy.

To get the reproducibility of the two analyzes (initial and after interval), the measurements obtained from the initial evaluation were considered to compose the data of the study.
since the other 85 students, who constituted the sample, were evaluated only once.

The associations of interest were estimated using Chi-square test or Fisher’s exact test. The agreement between the categories of the anatomical structures of the teeth (44 × 34) was assessed using kappa statistic. The significance level was 5%. To determine the number of teeth the rule of thumb was used, which recommends from 5 to 10 sample units per category of row and column variables. Therefore, considering that the test of association used in this study with the highest number of categories was 4 × 4 (= 16), the minimum sample size should comprise from 80 to 160 teeth.

The criteria for identifying proximal surfaces of the mandibular first premolars were determined after the statistical analysis.

Results

From the 100 students, 80 were female. The intra-examiner reproducibility was performed in two different moments. Seven parameters were evaluated; four of them presented “Optimal” intra-examiner agreement (widest occlusal fossa, occlusal fossa closer to the lingual surface, proximal surface with the largest buccolingual dimension and groove from MFO; \( \kappa = 1.0 \)), and three presented “good” agreement (MOF, DOF, groove from DOF, ranging from 0.609 to 0.762) (Kappa - \( \kappa \)).

The frequencies of the features observed in teeth 34 and 44 are presented in Table 1. It can be observed that the size of most MFO for both teeth was “discreet” and “small,” with no statistically significant difference between the teeth (\( p = 0.244 \)). However, for DOF, the most frequent sizes were “small” and “medium,” and there were no significant differences between the teeth (\( p = 0.966 \)). The widest occlusal fossa for both teeth was the distal one (Fig. 1).

For most of the evaluated teeth, the proximal surface with the largest buccolingual dimension was the distal surface, with no difference between the teeth (\( p = 1.0 \)) (Table 1). We could observe that the distal surface presented a rounded configuration, whereas the mesial surface had a pointed contour, as if it presented two segments (Fig. 1).

The occlusal fossa located closest to the lingual surface was also the distal fossa, however a high frequency of symmetrical occlusal fossae (44% and 29% for 44 and 34, respectively) was observed, with no difference between the teeth (\( p = 0.77 \)) (Table 1).

Grooves from MFO and DOF were not present in most individuals for 34 and 44 (Table 1). Table 2 presents the coincidences of each anatomical structure evaluated in 34 and 44, in the same individual.

According to Kappa statistics, the agreement between 34 and 44 ranged from “weak” to “regular,” except for the distal occlusal fossa that presented the “greatest” coincidence. Therefore, teeth 34 and 44 are not symmetrical, i.e, the size and morphology of the anatomical features assessed in the dental crown are not coincident on the right and left sides of the first mandibular premolars, in the same individual (Table 2). The frequencies of the anatomical features observed in 44 and 34, according to sex, are presented in Table 3. It can be observed that there was no statistically significant association between the observed features and sex in 44 and 34 (\( p > 0.05 \)).

From the 200 analyzed teeth, most presented DOF as the widest fossa, (94.5%), as well as the distal surface constituted the proximal surface with the largest buccolingual dimension (94.5%) (Fig. 1).

When the occlusal fossa located closest to the lingual surface was evaluated, it was observed that in 60% of the examined teeth, the DOF occupied this position (Fig. 1). In 36.5%, MOF and DOF occupied a symmetrical position, and in 3.5% MOF was closer to the lingual surface.

Regarding the grooves from the occlusal fossae, the groove from MOF (MOF groove) was more frequent (35.5%) compared with the groove from DOF (DOF groove) (8.5%), with no

Table 1 Frequency of the anatomical features observed in teeth 44 and 34

<table>
<thead>
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<th>Anatomical Features</th>
<th>44</th>
<th>34</th>
<th>( p )</th>
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<td>MFO</td>
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<tr>
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<tr>
<td>Small</td>
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<td>Medium</td>
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</tr>
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<tr>
<td>DOF</td>
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<td>Large</td>
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<td>Widest Occlusal Fossa</td>
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<td>44</td>
<td>29</td>
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</tr>
<tr>
<td>Proximal surface with the largest buccolingual dimension</td>
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<tr>
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<tr>
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<tr>
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<td>2</td>
<td>2</td>
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<tr>
<td>Groove from MFO</td>
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</tr>
<tr>
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<td>68</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
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<td>Present</td>
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There was no significant association between the groove from MOF and the size of MOF \((p = 0.323)\), and no association between the groove from DOF and the size of DOF \((p = 0.840)\).

When the occlusal fossa located closest to the lingual surface was the DOF, the groove from MOF was generally absent \((p = 0.004)\). However, there was no significant association between the presence and absence of the groove from DOF, and the occlusal fossa located closest to the lingual surface \((p = 0.504)\).

There was a statistical significant association between the sizes of the mesial and distal occlusal fossae \((p < 0.001)\). The distal occlusal fossae were generally larger than the mesial fossae.

### Discussion

In the discipline of Anatomy at São Paulo State University (UNESP), School of Dentistry, Araraquara, the methodology used to teach Dental Anatomy is based on direct observation of natural teeth, visualizing the general and specific features of each tooth. Other complementary methodologies such as macro models, dental sculpture and computer-assisted learning programs can be used. However, all these methodologies require knowledge of the specific anatomical features of each tooth. The purpose is that the student understands that knowing these features is important to
Table 3 Frequency of anatomical features observed in 44 and 34 according to sex

<table>
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<tr>
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<td>DOF</td>
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<td>Medium</td>
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<td>12</td>
<td>0.322</td>
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<td>11</td>
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<td>Proximal surface with the largest buccolingual dimension</td>
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<tr>
<td>Distal</td>
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<tr>
<td>Groove from MOF</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>55</td>
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<td>0.957</td>
<td>48</td>
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<td>0.682</td>
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<tr>
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<td>7</td>
<td>32</td>
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</tr>
<tr>
<td>Groove from DOF</td>
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</tr>
</tbody>
</table>

establish the function, and they must be accurately reproduced in the patient. Therefore, a poor contour, the absence of a groove, and a ridge out of position would compromise the function.12

In this study, we evaluated the occlusal features of the first mandibular premolar, because it is considered to be one of the most complex teeth, and students present difficulties in identifying it, particularly in relation to the determination of the hemi-arch.

It was verified that teeth 34 and 44 were not symmetrical in the same individual. Morphodifferentiation of teeth is clearly under genetic control, but the extent to which environmental factors can influence the process is unknown. Different teeth have characteristic occlusal topographies, and the variance in these topographies can be measured and used to determine the relative similarity between individuals. Heredity estimations revealed that genetic factors strongly influence occlusal morphology of mandibular primary molars.16 According to Consolaro, epigenetics may explain why teeth of the same patient are not exactly the same on both sides. The genetic information that DNA carries is to develop teeth bilaterally equal. However, it does not happen due to the action of environmental features.17

The occlusal surface of the first mandibular premolar presents an ovoid form,11,12,18 frequently with the presence of two cusps, buccal and lingual, which are almost always united by an enamel ridge, which limits a fossa on each side (mesial and distal occlusal fossae).12,19,20

Our results showed that the widest occlusal fossa was the distal. According to Figun and Garino, even though the occlusal fossae are irregularly arranged, the distal fossa is the widest one.17 Some other authors reported that the DOF is the widest one.12,18 Other authors10,19,20 did not mention whether there were differences between the occlusal fossae.

According to our results, the distal surface presented the largest buccolingual dimension, and it presented a rounded configuration, whereas the mesial surface had a pointed contour. Figun and Garino (2003) also verified that the distal segment is the largest portion of the occlusal surface, and the mesial segment presents a large slope and a small dimension.11 Pagano et al. (1965)18 reported that the occlusal surface presents a large/moderate reduction of the mesiolingual segment; the convex lingual surface is continuous with the contact surface and a large buccolingual dimension of the distal surface.18

The mesial surface presenting the largest size compared with the distal surface is considered to be one of the anatomical features, which is common to all teeth. Therefore, it was verified that the first mandibular premolar presents an exception to this rule, i.e., it presents the distal surface larger than the mesial one. The anatomy professor should point out this issue during the Dental Anatomy classes to improve the identification of the hemi-arch, and also the reconstruction of this tooth during oral rehabilitation procedures.

Besides the DOF being the widest, we also verified that it is the one which is located closest to the lingual surface. We observed only two authors who reported that the distal fossa is located closer to the lingual surface.11,12

The groove from MOF frequently continues to the lingual surface of the tooth.12,20 In our study, the groove from MOF was also the most frequent compared with the groove from DOF, though with no statistically significant difference between them. According to Pagano et al.,18 there are some different situations related to the occlusal grooves: grooves from DOF, short grooves from MOF and DOF, or mesiolingual groove crossing the mesial marginal ridge.18

We observed that the grooves toward the lingual surface from MOF and DOF were not present in most individuals. Nevertheless, Madeira and Rizzolo reported that it is rarely absent.12

Considering the results confirmed by statistical tests, we determined the most reliable criteria for the identification of the proximal surfaces of the first mandibular premolars, which will probably improve the individual study of these teeth during Dental Anatomy classes.
Conclusion

According to the results, the following criteria were determined to identify the proximal surfaces of the mandibular first premolar:

- The distal occlusal fossa will be considered the largest occlusal fossa;
- The distal surface will be considered the proximal surface presenting larger buccolingual dimension and a rounded shape.

The occlusal fossa located closer to the lingual surface can also be evaluated, being considered the distal fossa. However, this feature should be considered the last of the three indicated criteria, because it generates a greater degree of error regarding the others.

References

Resistance of the Collateral Ligaments of the Femorotibial Joint in Domestic Dogs (*Canis lupus familiaris* LINNAEUS, 1758)

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(e-mail: marcelo.faria@univasf.edu.br).

This work aimed to demonstrate the resistance of the femoral-tibial joint collaterals of 60 dogs of undefined breed, euthanized at the Zoonoses Control Centre of the city of Petrolina (PE), North-Eastern Brazil.

**Materials and Methods** The femur-tibial articulation was dissected to expose the collateral ligaments. Bones were cut on the distal third of the femur and the proximal third of tibia and fibula. The knee joint was kept bound only by the collateral ligaments and then submitted to destructive mechanical tests, evaluating the deformation of the ligaments (mm) per unit of time, (seconds), and the joint resistance.

**Results** We could observe no side effects of contralateral joints. In a destructive mechanical assay, we detected that the medial collateral ligaments ruptured earlier than the lateral ones. The resistance of the lateral and medial ligament of the right limb of females was respectively 537.1N ± 413.6 and 360.1N ± 228.6; while in males, were of 632.4N ± 333.4 and 440.4N ± 227.1. About the left antimere, the females presented 442.4N ± 288 resistance on the lateral ligament and 417.4N ± 190.6. In males, the resistance was 605.8N ± 316.1 and 548.6N ± 2,333.7, respectively.

**Conclusion** The results of our research will allow the development of synthetic tissues to replace the natural ligament of dogs in cases of rupture or degeneration.

**Keywords**
- arthrology
- material resistance
- knee

**Introduction**

There is a low agreement on the origin of dogs. Paleontological studies suggest that canids have been existing for over 30 million years, together with the other mammalians and primates. Researchers, through archaeological records and genetic sequencing of wolves and dogs, try to discover the real origin of dogs.

Archaeological records of the fossilized specimen, allowed to estimate how long ago the first canine teeth appeared. However, until now it has not been possible to recognize the differentiation between dogs and wolves, due to the similarity between the two species. DNA allows the determination of the location and species that have domesticated them.

During prolonged periods of drought or famine, some wild animals, in search of food, approached humans. These events
marked the beginning of domestication of dogs. The man began to interfere in the evolution of canids using artificial selection between different species (consider the option of between/among, and species, subspecies or varieties, as considering the complex taxonomy of canids) of wolves. From these experiences originated the different breeds of domestic dogs that are existing today. A well-accepted theory states that the domestic dog descends from the gray wolf (Canis lupus lupus), which has been wiped out by civilization. Modern studies have shown that dogs retain much of the wolf’s wild instincts: territorialism, hunting, and attitude for shepherding.

The daily habits in the cities contribute to the solitude of human beings, increasing the presence of domestic animals, such as dogs and cats in homes, which become families members. The enhancement of concern about pets welfare influences the frequency of visits to the veterinarian.

Articulation is the contact between bone surfaces, allowing sliding between them, bone growth and movement in response to muscle contraction. The femorotibial joint² is a synovial joint (diarthrosis), that is, it has the synovial fluid, which has the functions of lubrication, nutrition, shock absorption, increased congruence between the bone parts, besides allowing the locomotion. The articular capsule, composed of the synovial and fibrous membrane, envelops the femorotibial joint. The former is responsible for producing the synovial fluid, the latter, for maintaining the stability of the joint and movement. According to its movements, it is classified as hinge and uniaxial, allowing almost exclusively flexion and extension.

The femorotibial joint includes the condyles of the femur, the structures of the proximal epiphysis of the tibia and the interposed articular meniscus or semilunar cartilage. The surface of the condyles of the tibia does not conform to the femoral condyles and are in contact with only a small part of them.⁵ According to König and Liebich (2002),⁴ the femorotibial joint has two ligaments on both sides, which are extra-articular ligaments, called lateral collateral and medial collateral ligaments, that lie between the epicondyles of the femur and the tibia. The lateral collateral ligament inserts, without union with the lateral meniscus, in the lateral condyle of the tibia and the head of the fibula. The medial collateral ligament maintains contact with the medial meniscus and enters the medial condyle of the tibia. The primary function of these ligaments is to prevent lateral and medial movement of the knee.

According to Dyce et al.,⁵ palpation of the femorotibial joint reveals the following features of the skeleton: the patella with its patellar ligament; the troclear ridges and the outer surfaces of the femoral condyles; the sesamoid bones (at the origin of the gastrocnemius muscle); the head of the fibula; the margin of the lateral condyle adjacent to the fibula; the tuberosity; the extensor sulcus; the medial surface of the tibia and the medial and lateral collateral ligaments. The sharpest internal feature of the joint is free communication between various synovial compartments.

The femorotibial joint is very complex, and the largest in the body, consolidated by muscles and ligaments deficiently, which consequently suffer severe trauma.⁶ It is a condylar joint, composed of medial and lateral femoral condyles, making contact through the meniscus interposed to the proximal articular face of the tibia. It consists of the distal end of the femur, the proximal end of the tibia and the patella. This articulation is cable of performing flexions, extensions, and slight rotations. The femorotibial joint is the main joint to bear the weight of the body. The collateral ligaments attach to the joint capsule, allowing support to this structure, being, however, totally extra-articular and responsible for the varus and valgus movements of the tibia.⁷ According to Leopizzi,⁸ a dog’s knee is a bi-articulated “transmission joint,” stabilized by secondary restraints that are required from the biomechanical demand and the ligament system. The movement of the knee is determined by ligaments and not by its bone structure. According to Slocom and Slocom,⁹ it is a joint with two degrees of freedom. The former allows flexion and extension movements on the transverse axis at the level of the femoral condyles, the latter, the rotation around the long axis of the tibia. Dejardin et al.¹⁰ reported that the femorotibial joint is a diarthrodial joint of the gingivitis and uniaxial type, mainly consisting of flexion and extension movements. The joint is composed of a cavity, articular capsule, articular cartilage, synovial fluid, Subchondral bone, articular ligaments and two meniscus – one medial and one lateral.¹¹ According to Oda,¹² the lateral ligaments (lateral and medial) are responsible for the sustentation of the femorotibial joint. They demarcate movements of the vagus type, where the head of the femur establishes with its body an angle greater than 135° (medial collateral ligament) and virus presenting angulation less than 120° (lateral collateral ligament).

According to Vasseur (apud Oda, 2008),¹² the juncture among the femur, tibia, and patella is formed by two functionally differentiated joints. The femorotibial joint gives weight support and the femoropatellar joint, increases mechanical efficiency of the quadriceps muscle, facilitates the movement of extension and keeps the patella in the trochlear groove.

Biometry (from the Greek bios = life, metron = measurement) is the study of the physical characteristics of an individual or a population, taking into account their dimensions, mass, and volume or simply their conformation and visual, physical aspects.¹³ There are two methods for determining biometrics: somatoscopy, also known as zooscopy, and somatometry or zoometry. The first, however descriptive analyze the characteristics, such as coat color, eye color, skull conformation. Is not very reliable, since even within the same species, there are several breeds. The somatometry is considered more reliable because it bases on measurements of dimensions, mass, and volume.

Given the importance of the ligaments in the stabilization of the joints and the several pathologies that affect them, the present study aimed to determine the resistance and deformation of the collateral ligaments of the femorotibial joint in dogs, correlating them with body biometric parameters and sex.

Materials and Methods

This study was approved by the Ethical Committee on Human and Animal studies of the Federal University of the São Francisco Valley under protocol number 22041021.
Collection of Material
Sixty previously euthanized medium sized dogs of undefined breeds, both males and females, were collected from the Zoonoses Control Center (CCZ) of the city of Petrolina (Pernambuco State, Brazil). After the collection, the animals were transported to the laboratory of the anatomy of domestic and wild animals (Laboratório de Anatome dos Animais Domésticos e Silvestres) of the Univasf, located in the agrarian sciences Campus.

Body Biometry
To measure the body Biometry, we used a metric tape and a millimetric caliper, with the aim of obtaining:
- Height: determined by measuring the distal extremities of the thoracic limbs to the shoulder region, between the spinal processes of the thoracic vertebrae, located between the scapulae, referred to as the withers;
- Length: conferred through the interval along the spine, between the nuchal crest and the tail insertion;
- Scapular girdle (width): determined through the measurement between contralateral scapular spines;
- Pelvic girdle (width): determined by measuring the coxal tuberosities of the iliac wings;
- Height of the thorax: checked by taking the measurements between the base of the sternum and the spinous processes of the seventh or eighth thoracic vertebrae;
- Height of the abdomen: checked by measuring between the umbilical scar and the spinous processes of the third or fourth lumbar vertebrae;
- Cranial chest length: determined by the interval between the frontonasal suture and the nuchal ridge;
- Nasal length: obtained through the range between the apex of the nose and the frontonasal suture;
- Head width: checked by measuring the space between the lateral sides of the contralateral zygomatic arches;
- Head height: determined through the interval between the nuchal crest and the angle of the mandible.

Body mass was determined by a digital analytical balance (Techline®, São Paulo – SP, Brazil) which expresses the value in kilograms.

Dissection of Material
In the Laboratory of Anatomy of Domestic and Wild Animals of Univasf, the material was processed, and the dissections were made for the femoral-tibial joint to be revealed with its respective collateral ligaments (►Fig. 1). All the muscles surrounding the knee joint were separated and cut.

Other supporting structures, such as capsule, lateral and medial menisci, cranial patellar ligaments, medial patellar caudal patellar ligaments, lateral femoropatellar ligament, meniscotemoral ligament, caudal tibial ligament of the lateral meniscus, cranial cruciate ligament, caudal crossover, interosseous ligament, vessels and nerves, were cut using surgical material (anatomical tweezers, surgical scissors).

Then, with the aid of a band saw, the distal third of the femur was fractured, as well as the proximal third of the tibia and fibula, suspended only through the collateral ligaments.

Biometry of Collateral Ligaments
After dissection, the dimensions of the collateral ligaments of the femorotibial joint, such as the thickness (medial-lateral) and the width (cranio-caudal) of both antimeres, were determined.

Destructive Mechanical Testing
After dissection, the joint parts were transported to the Laboratory for the study of the Materials (Laboratório de Ensaios de Materiais) of the mechanical engineering course, in the Campus of the city of Juazeiro of the same University, located in Município de Juazeiro, Bahia State (Latitude - 09° 24’ 42,” Longitude 40° 29’ 55,” Altitude 368 m). A mechanical device was developed with claws that allowed the bone structures to fit together with their respective collateral ligaments.

The apparatus used for the experiment was the Universal Machine for electromechanical and micro processed tests Digital Line (EMIC® - DL10000, São José dos Pinhais–PR, Brazil). The superior claw anchored to the distal epiphysis of the femur. The inferior, the proximal epiphysis of the tibia (►Figs. 2 and 3). Force (N) was then applied, analyzing the displacement in millimeters (mm) of the upper jaw, evaluating the deformation of the ligaments (mm) per unit of time (s). It is important to emphasize that the angulation of this joint is of fundamental importance in the destructive mechanical test, mimicking the orthostatic position of the part in the living animal.

After the fixation of the bone pieces in the fixation devices, the traction was promoted to evaluate the proposed hypotheses (►Fig. 1).

Statistical Analysis
For statistical analysis, the SAS 9.2 help program and the Satterthwaite test were used, with significance (r) less than 0.05, as well as to obtain the correlations used a significance higher than 0.7 (SAS, 2010). Thus, the data were tabulated and linear scatter plots were prepared, to establish existing correlations.

Results and Discussion
►Tables 1 and 2 describe the results of the experiments, making numerical details (mean and standard deviation) of
both body biometric data and data on the collateral ligaments of the femorotibial joint, respectively. It is important to highlight that the statistical treatment was very detailed, to establish correlations between the body biometry and the biometry of the collateral ligaments of the femorotibial joint, as well as to elucidate differences inherent to the contralateral ligaments. The graph above displays the correlations between the collateral ligaments of the femorotibial joint. Data were obtained from the SAS 9.2 help program and the Satterthwaite test, considering a significance level (r) greater than 0.7:

- The medial-lateral thickness of the left medial collateral ligament showed a significant correlation with the medial-lateral thickness of the lateral ligament of the same antimere;
- The resistance of the right medial collateral ligament obtained a correlation with the resistance force related to the lateral ligament of the same antimere;
- The strength of the lateral collateral ligament of the right antimere showed a correlation with the force of the medial collateral ligament of the same antimere and the displacement of the right lateral collateral ligament;
- The displacement of the left lateral collateral ligament was correlated with its resistance force.

Table 1: Biometric data of dogs collected by the CCZ of Petrolina (PE) - Petrolina, 2016

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<td>Males</td>
</tr>
<tr>
<td></td>
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<td>Males</td>
</tr>
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</tr>
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</tr>
<tr>
<td>Length (cm)</td>
<td>61.0741</td>
<td>65.4394</td>
</tr>
<tr>
<td>Shoulder girdle (cm)</td>
<td>47.0741</td>
<td>52.3333</td>
</tr>
<tr>
<td>Pelvic girdle (cm)</td>
<td>38.7778</td>
<td>44.6970</td>
</tr>
<tr>
<td>Chest height (cm)</td>
<td>27.2593</td>
<td>29.0909</td>
</tr>
<tr>
<td>Abdomen height (cm)</td>
<td>24.9630</td>
<td>28.2424</td>
</tr>
<tr>
<td>Nasal length (cm)</td>
<td>7.1519</td>
<td>7.5879</td>
</tr>
<tr>
<td>Cranial carton length (cm)</td>
<td>9.9296</td>
<td>10.7364</td>
</tr>
<tr>
<td>Head width (cm)</td>
<td>8.4963</td>
<td>9.1758</td>
</tr>
<tr>
<td>Head height (cm)</td>
<td>7.8556</td>
<td>7.8909</td>
</tr>
</tbody>
</table>

Abreviations: cm, centimeter; kg, kilogram.

Table 2: Biometry of the lateral and medial collateral ligaments of the femorotibial joint of medium-sized dogs from the Centro de Controle de Zoonoses de Petrolina (PE). Width (W) and thickness (T) are expressed in centimeters. The strength of resistance (FR) is expressed in Newtons. The displacement of the claws of the destructive mechanical test (D) is expressed in millimeters - Petrolina, 2016.

<table>
<thead>
<tr>
<th>Collateral Ligaments</th>
<th>Aspect</th>
<th>Average</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Right medial collateral ligament</td>
<td>L</td>
<td>2.4741</td>
<td>2.8970</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.8915</td>
<td>0.9273</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>360.1</td>
<td>440.4</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>16.1111</td>
<td>17.3333</td>
</tr>
<tr>
<td>Left medial collateral ligament</td>
<td>L</td>
<td>2.5852</td>
<td>2.9576</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.7600</td>
<td>0.6776</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>417.4</td>
<td>548.6</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>17.4815</td>
<td>19.0303</td>
</tr>
<tr>
<td>Right lateral collateral ligament</td>
<td>L</td>
<td>2.3815</td>
<td>2.4788</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.6996</td>
<td>0.5927</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>537.1</td>
<td>632.4</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>25.9259</td>
<td>38.4545</td>
</tr>
<tr>
<td>Left lateral collateral ligament</td>
<td>L</td>
<td>2.4630</td>
<td>2.1879</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.7041</td>
<td>0.6233</td>
</tr>
<tr>
<td></td>
<td>FR</td>
<td>442.4</td>
<td>605.8</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>20.0741</td>
<td>24.3939</td>
</tr>
</tbody>
</table>
The data treated through statistical studies showed that there was no significant difference between the collateral ligaments of the femorotibial joint, as considering the correlation between the thicknesses and the displacements of the claws, without significant difference, that is, they have the same length and thickness. However, during the destructive mechanical tests, the medial collateral ligaments ruptured anteriorly to the lateral ones, evidencing more resistance of the latter.

In 10% of cases, before the rupture of the collateral ligaments, fibular fractures occurred, pointing out that, in certain animals, the ligament is as resistant as the bone itself.

In 19.2% of the animals, we could observe disarticulation between the tibia and fibula, with rupture of the interosseous ligament before the rupture of the collateral ligaments.

The statistical analysis highlighted that the resistance and the dimensions of the ligaments have no correlation with the mass and the body dimensions of the animals, that is, they do not depend on the size of the dogs.

Rodrigues,\textsuperscript{15} reported that traumatic injuries represent 80% of the pathologies of the human knee because the osteo-ligament system is very complex and often subjected to trauma. We could verify the complexity of the articulation through the number of tissue excisions, promoted during dissections of the collateral ligaments when ligaments, muscles and other adjacent tissues were rebounded.

In the limited existing literature concerning the collateral ligaments of the femorotibial joint, it was possible to verify that the rupture of the medial is more constant, but does not manifest, characteristically, a significant joint effusion, because the collateral ligaments are positioned as extra-articular structures in humans.\textsuperscript{16} According to Reider,\textsuperscript{17} the medial collateral ligament of the human knee humans is more frequently injured due to the combined forces or a valgus, external rotation and valgus force. The rupture of the medial collateral ligament occurs as a result of a blow on the lateral side of the knee, with the leg fixed (\textit{\textsuperscript{-}Fig. 4}).

The magnitude of the valgus lesion causes a rupture, first, of the medial collateral ligament and, probably afterward, of the cranial cruciate ligament (\textit{\textsuperscript{-}Fig. 5}).

In the present study, using the femorotibial joints of dogs, we could observe that the collateral ligaments were extra-capsular and that in 34.5% of the right antimere and 29.2% of the left, there was also the same relation, that is, the medial collateral ligament fractured more easily than the lateral one (\textit{\textsuperscript{-}Fig. 6}). The fracture occurs because of the support of the mass of the dog, which fosters a higher deposition of fibroblasts to support the acting forces.\textsuperscript{4} In the other animals, there was no significant difference between the resistances. We could not comment on the cranial cruciate ligament since we did not study it and we removed it before our tests.

\textbf{Fig. 4} Diagram demonstrating how rupture of the medial collateral ligaments occurs (Reider, 1996).\textsuperscript{17}

\textbf{Fig. 5} Diagram showing the medial collateral ligament and ruptured cranial cruciate ligament (Reider, 1996).\textsuperscript{17}
The size of most of the ligaments of the body is associated with the body size. We could not see such relation between the development of the collateral ligaments of the femorotibial joint and the mass or the size of the body.

**Conclusion**

Given the obtained results, we could observe no correlations between the somatometric data and the resistance of the femorotibial collateral ligaments, that is, they do not depend on the size of the animals. The destructive mechanical essay pointed out that the medial collateral ligaments are more easily prone to ruptures than the lateral ones. This research will help the development of synthetic tissues to substitute the natural ligament in cases of rupture or degenerative injury since their resistance has already been assessed in dogs.

**Acknowledgments**

The authors would like to thank the Center for the zoonoses control of the city of Petrolina (PE) for the supply of corpses and the National Council for Scientific and Technologic development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq) for the scientific initiation grant.

**References**

Are Corpses the Best Method for Practical Anatomy Assessment, According to Health Course Students?

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Abstract

The aim of this study was to evaluate the student’s conception in reference to the teaching materials used for the practical assessment of the discipline of Human Anatomy. The study was quantitative and qualitative, exploratory and descriptive. The materials were industrialized synthetic anatomical models, models made of inexpensive material by students, human cadaveric material, photocopied images and software. The study included 97 students, who after analysis indicated cadaveric materials (35%) as the best educational tool to be applied for the practical assessments of Anatomy. The photocopied images (3%) were the material with which the students presented greater difficulty. The content analysis of the discursive questions showed 5 factors in learning Human Anatomy: little time per table; difficulty in identifying, locating and memorization of anatomical structures; the quality of material used; comparing these materials; and cadaveric material. The study proved to be relevant for the evolution of teaching quality, in addition to the cadaveric material as the best tool to be used in practical assessments of Human Anatomy. We suggest the use of this material the more frequent, in laboratories that have the same materials as analyzed here, because the corpse can never be substituted in teaching and learning of Anatomy.

Keywords
► gross anatomy
► education
► software
► photocopied images
► anatomical structures
► manufacture
► corpses prossected.

Introduction

Anatomy analyzes biological structures and the morphology of different organisms (both in macro and microscopic terms) as well as the function and organization of the structures present in the body as a whole or in its particularities. Learning is a multimodal process, which obtains satisfactory results confronted with diverse types of materials and methods, making the classes more dynamic and interesting to the students. However, the scarcity of didactic
resources and the bureaucracy in acquiring them affects the quality of student learning.\textsuperscript{3}

In addition to theoretical knowledge, practical study complements the contents studied at the classroom, allowing more dynamic anatomical study and correlated with the profession. One of the main factors that hinders the learning of practical content in laboratories is the discrepancy between the number of students and pieces of quality.\textsuperscript{4}

Institutions and professors invest in developing innovative teaching methods to adapt discomfort regarding to the study of Human Anatomy. At the same time the aim is to create innovative alternative and skills of those that invest training creative and critical professionals in the health and science sector.\textsuperscript{5,6}

In the current situation, the technological dominance in anatomical study simplifies the learning of contents that need visual acuity.\textsuperscript{2} Thus, it is necessary to train the professor,\textsuperscript{7} to elaborate pedagogical techniques that facilitate and support the transition between the virtual application to the reality of anatomical knowledge,\textsuperscript{8} resulting in successful learning.\textsuperscript{2}

Bureaucratic difficulties to obtain quality material, especially the cadaveric material,\textsuperscript{9} and the reality of worn materials, the photocopy of atlas, demonstrates practicality by providing visual and nominal information on the various structures of the human body in a simplified way.\textsuperscript{10} Another material that can be employed in those circumstances is the industrialized synthetic material, available for anatomy laboratories, but with inadequate size or morphology, thus preventing a more detailed view of the necessary structures of the learning process.\textsuperscript{11}

Careful observation is important to teaching Human Anatomy,\textsuperscript{6} thus the modeling technique proposes to transcribe the cadaveric pieces as a way of topographic memorizing, the structures if they improve the student’s interaction with the discipline.\textsuperscript{12–14}

However, the use of cadaveric resources is essential for training health sciences professionals, making it the effector more humanized actions, although some students state this kind of material brings visual repulsion, inducing nightmares and unpleasant odor of the solution with formaldehyde, blocks the learning process.\textsuperscript{15} Nevertheless, most of the students are aware that studying Anatomy with the help of the corpse and enhance their degree knowledge of morphology in all its particularities.\textsuperscript{16}

Certain setbacks faced by the students against the didactic resources in the practical classes of the human morphology is perceptible. The evaluation of materials, and its conditions, by the students will contribute to the focus of the professors much more effective teaching strategies that contribute to the learning process. In this context, the aim of this study was to analyze the perceptions of students regarding the efficiency of teaching resources, alternative and classic, in the applicability of practical assessment of the discipline of Human Anatomy.

**Methodology**

Both exploratory-descriptive, quantitative and qualitative. It has been developed at the Laboratory of Human Anatomy at the Petrolina campus at the Human Anatomy – University of Pernambuco (UPE). The participants were students of Physiotherapy, Nursing, Nutrition and Undergraduate in Biological Sciences, enrolled during of the semester of 2016. All participants have signed an Informed Term of Consent.

Eligibility criteria included volunteers of both sexes who had already been approved at the the discipline of Human Anatomy or who were enrolled in that discipline at the time of the data collection. And those who did not meet the criteria for participation in the research, were excluded.

The study was developed through a knowledge gymkhana. The material used were industrialized synthetic anatomical models, manufacture models of low cost materials by students, prossected corpses, photocopied images and software. All of the above resources belong the Petrolina laboratory of Humana Anatomy, UPE.

The recruitment of volunteers was made through invitation, resulting in the participation of 97 volunteers. The study was divided into two stages: the first one, corresponding to the practical part of the study, the practical assessment; and the second, related to critical-reflexive perception which it was made to answer the guiding questions.

The collection of data was performed between April and May 2016, at times and days agreed between the researchers and the participants, at the Human Anatomy Laboratory of the institution. Before the collect period, the didactic material used in the gymkhana was randomly chosen, although respecting general patterns for identification.

Ten tables were arranged in two rows with 5 tables, listed in ascending order from 1 to 5. The materials used in this study were arranged as follows: \textit{Table 1} - computers with software (Anonica | Goodwill Enterprise Development, LTD.), with three-dimensional images of anatomical structures; \textit{Table 2} - photocopied images of atlas of Human Anatomy; \textit{Table 3} - anatomical structures manufacture by the students themselves during practical classes of the referent curricular component; \textit{Table 4} - industrialized synthetic parts of the laboratory collection; and finally table 5 - corpses prossected.

Participants received an answer sheet (\textit{Fig. 1}) before entering the laboratory in pairs. The stipulated time for the resolution of each table by the researchers was two minutes, allowing the participants to identify the nomenclature of the four anatomical structures present in each table.

The second stage of the study began after the contest. Participants were directed to an anteroom to the laboratory so that they could answer the discursive questions of the answering sheet. After collecting the end data, the researchers began to analyze the answers. The quantitative findings

**Table 1** Categories associated with the score obtained, after data collection

<table>
<thead>
<tr>
<th>Punctuation</th>
<th>Concept</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>6–</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>11–</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>16–</td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>
Table 2 Results of the feedback from participants showing the best method

<table>
<thead>
<tr>
<th>Workbench</th>
<th>Used material</th>
<th>N</th>
<th>%</th>
<th>Standard-deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Software</td>
<td>27</td>
<td>28</td>
<td>9.3 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>Photocopy</td>
<td>3</td>
<td>3</td>
<td>9.0 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>Manufactured model</td>
<td>15</td>
<td>15</td>
<td>9.0 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>Industrialized model</td>
<td>18</td>
<td>19</td>
<td>8.81 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>Prossected corpses</td>
<td>34</td>
<td>35</td>
<td>9.0 ± 0.1</td>
</tr>
</tbody>
</table>

Table 3 Feedback from participants for the worst material

<table>
<thead>
<tr>
<th>Workbench</th>
<th>Used material</th>
<th>N</th>
<th>%</th>
<th>Standard-deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Software</td>
<td>12</td>
<td>12</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>Photocopy</td>
<td>43</td>
<td>44</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>Manufactured model</td>
<td>19</td>
<td>20</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>Industrialized model</td>
<td>6</td>
<td>6</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>Prossected corpses</td>
<td>17</td>
<td>18</td>
<td>6.2 ± 0.3</td>
</tr>
</tbody>
</table>

Table 4 Individual result participants after the gymkhana

<table>
<thead>
<tr>
<th>Correct answers</th>
<th>N</th>
<th>%</th>
<th>Result</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 5</td>
<td>1</td>
<td>1</td>
<td>Mark 1</td>
<td>Unsatisfactory</td>
</tr>
<tr>
<td>6 to 10</td>
<td>9</td>
<td>10</td>
<td>Mark 2</td>
<td>Median</td>
</tr>
<tr>
<td>11 to 15</td>
<td>44</td>
<td>45</td>
<td>Mark 3</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>16 to 20</td>
<td>43</td>
<td>44</td>
<td>Mark 4</td>
<td>Excellent</td>
</tr>
</tbody>
</table>

were categorized by the researchers, so that each item corresponded to 1 point, which added up, to obtain a maximum of 20 points, associated to a specific categorization, as shown in Table 1.

The qualitative analysis was performed through the method proposed by Minayo, whose analysis starts with the search for the meanings, manifest and latent of the material collected by all the speeches of those involved in the research. Three steps have to be followed. In the first, the data were organized with the transcription of the answers obtained through the guiding questions, followed by double-cross reading the material, organizing it into categories; then the data were ordered, performing strenuous and repeated reading of the texts, with the detection of sense nuclei. The final analysis was made by means of triangulation with the theoretical reference and the material collected.

Quantitative data were processed and analyzed using the SPSS program (SPSS Inc., Chicago, IL, United States of America, Release 15, 2008). Initially, the data was entered through double typing, consolidated and validated. The categorical data was presented in absolute and relative frequencies, as well as the mean and the standard deviation.

All participants signed the Informed Consent and Informed Term, in line with the Resolution 466/2012 of the National Health Council, which values the confidentiality of the participants. The present study was approved by the Ethics and Research Committee of Human Research from University of Pernambuco after registration CAAE: 51066115.1.0000.5207.

Results

The study included 97 volunteer students, 30 from the Biological Sciences course, 51 from the Physiotherapy course, 02 from the Nursing course and 14 from the Nutrition course. The sociodemographic characteristics of the volunteers showed that the mean age of the participants was 21.9 (±3.5) with female prevalence in the health courses.

Graph 1 shows the heterogeneity of the punctuation given to the materials used in each workbench, for which in the individual opinion of each participant, the cadaveric material was the best method applied in the gymkhana. The axis of ordinates presents the score attributed to the best method applied in the gymkhana, the latter being represented in the axis of abscissae.

In a first aspect it was possible to observe the absence of an outlier, and deeper perceives a equidistant mean score for 3 of the 5 methods (photocopy, manufacture models and cadaveric pieces), and a mean with similar value maximum score attributed to the two remaining methods (software and industrialized synthetic models).

Graph 2 presents the variations of the punctuation attributed to each table referring to the difficulties with identification and interpretation of anatomical structures. Thus, there is association between question 5 (“What punctuation would you give to the workbench where you had the most difficulty?”) and question 4 (“In your opinion, what was the worst method in terms of difficulty? Identify the workbench number.”) on the answer sheet.

Like the previous graph, it can be seen the absence of outliers and mean score variation compared between each method of the gymkhana. The software and the photocopy

►
| Age: _______ | Sex: _______ | Course: ____________________ |

1) Identify the structures marked on the pieces on the workbench.

**Workbench 1)**
- a) __________________________
- b) __________________________

**Workbench 2)**
- a) __________________________
- b) __________________________

**Workbench 3)**
- a) __________________________
- b) __________________________

**Workbench 4)**
- a) __________________________
- b) __________________________

**Workbench 5)**
- a) __________________________
- b) __________________________

2) In your opinion, what was the best method applied? Identify the workbench number.

3) What a method would you rate the best one applied? (Punctuation: 1 - 10)

4) In your opinion, what was method you found most difficult? Identify the workbench number.

5) What punctuation would you give to the workbench where you had the most difficulty? (Punctuation: 1 -10)

6) List workbench in ascending order by facility to 5 methods applied.
   - 1 - __________________________
   - 2 - __________________________
   - 3 - __________________________
   - 4 - __________________________
   - 5 - __________________________

7) What difficulty do you find in the practical assessment of Human Anatomy, when applied by the professor of the discipline, in relation to the materials used in its elaboration? Why?

8) In your opinion, do you agree with the use of corpses and prossected pieces? Why?

---

**Fig. 1** Answer sheet received by participants for answering the questions of gymkhana into the human anatomy laboratory. The duration in each table was two minute.
Method showed the same mean, however the score attributed to photocopy coincides with the highest one, 5. The manufactured model method presented a mean value consistent with the minimum score, 5. The others methods had a mean score with apparent non-normal deviation of the opinions.

- Table 4 shows the categorization of the correct answers offered by the researchers, with scores interpretation at the first stage of the study.

In the analysis of the results expressed above, most of the volunteers reached high classificatory concepts. 89% of the participants reached 11 to 20 correct answers, being assigned to scores 3 and 4, with the classifications of satisfactory and excellent. A clear interpretation is allowed on how much didactic information the volunteers learn from the discipline of Human Anatomy.

The efficiency of the methods for practical assessment are consistence with the acquisition of knowledge on behalf of the student about the disciplines of Human Anatomy in the health courses.

The discursive questions allowed the researchers to identify important categories for data analysis. Thus, guiding questions were used: “What difficulty do you find in the practical assessment of Human Anatomy, when applied by the professor of the discipline, in relation to the materials used in its elaboration? Why?” and “In your opinion, do you agree with the use of corpses and prossected pieces? Why.”

After the qualitative analysis, the researchers identified the categories: short time; difficulty, localization and memorization anatomical structures; cadaveric material quality; and comparison between materials. When the participants were asked about the importance of the use of cadaveric pieces and their continuation, their acceptance for the practical teaching of Human Anatomy was notorious, their continuous use in classes and assessments and practical tutorials, as shown in - Graph 3.

Discussion

The pedagogical strategy in higher education is associated with the teaching-learning process, and as essential what is taught as what is learned. Based on this perception, there are gaps in knowledge left throughout the student’s academic development, for multifactorial reasons, which limits the performance of this student, the adherence to new knowledge.

Adopting alternative techniques and materials for Human Anatomy education process is a measure taken to the evident discrepancy between the demand of students and the supply of quality didactic-practical materials available in the institutions. Thus, professors should look for pedagogical tendencies in a concise and convincing way that reflect learning, such as manufactured anatomical models, cadaveric and prosthetic pieces, photocopies, industrialized synthetic models and conscious of the evolutionary framework of technology, to insert virtual reality and high-tech, software, video-lessons, social networks, as feasible possibilities to the pedagogical practice in education and teaching of Human Anatomy.

In respect to the analysis of the comparison between the methods studied here, the participants reported that the photocopied images showed less efficiency in the teaching and learning process of Human Anatomy. It is known to the scientific areas that photography cadaveric material is not
allowed for bioethical reasons.\textsuperscript{15} Therefore, images provided by atlases and textbooks are used as didactic support. However, the acquisition of this type of bibliography becomes inaccessible to many undergraduate students, either because of its high cost or because of the low number of copies available in libraries, which often do not meet the high demand of students.\textsuperscript{20}

The use of photocopied images, evaluated in the present study, is the most common practice among higher education students. Copying images of atlases and textbooks, present in the collection of the university’s library or laboratory, allows them access in a more accessible, non-profit manner.\textsuperscript{21}

The modeling technique is equivalent to assimilating one piece to another, using artistic materials such as modeling mass and biscuit.\textsuperscript{14} The three-dimensional anatomical models manufactured by students themselves, with low cost materials,\textsuperscript{12,13} transcribe the cadaveric material.

The models used in the present study were produced by students. However they did not present as a promising option for the practical assessments of Anatomy, obtaining considerable rejection by the participating students. This result, corroborated by Collipal Larre and Silva Mella’s study,\textsuperscript{22} the use of these models, for the practical anatomical study, results in an easy, unreal, artificial and invariable teaching. However Falcão et al. study,\textsuperscript{23} unlike the above study and the results obtained, they observed that construction of similar structures is possible for cadaveric. In addition, the material obtained a good acceptance by students in practical classes and in schools and colleges visits.

The use of cadaveric material in teaching Human Anatomy obtained a significant score, in terms of acceptance by the participating students. The data of the authors of the present study corroborate Costa, Costa and Lins’ study\textsuperscript{15} who stated that the use of corpses, even just for demonstration of anatomical structures is considered indispensable for to the teaching-learning process of the human anatomical studies. Satisfaction, from the use of the corpse in teaching Anatomy, is due to the contribution of better technical formation of health professionals. It permits knowledge of the anatomical variations. It contributes for humanistic training of future health professionals; and it promotes advances and innovation in surgical techniques.\textsuperscript{15,24}

As pointed out by Souza et al. study,\textsuperscript{16} the use of corpses in the process of building basic knowledge for undergraduate, postgraduate, and other levels of knowledge is essential. However, there is a positive consensus with regard to the use of new technologies, as a means of complementing the knowledge acquired with the use of corpses.

The use of software, the more functionality the material offers, more attractive and stimulating it becomes for the student.\textsuperscript{9} In the present study, it was possible to perceive the great potential of the software that was considered as the second best method in the opinion of the participants of the study (28%).

With technological advances in education, innovative alternatives have emerged in teaching and learning Human Anatomy, facilitating offering playful visualization of anatomical structures.\textsuperscript{25} According to Souza et al. study\textsuperscript{16} and Collipal Larre and Silva Mella,\textsuperscript{22} the students’ perceptions for this updated study are efficient and do not put aside traditional methods of teaching practice, continuing to report that they consider the use of corpses as relevant.

Anatomists have commercially developed software programs in education that include computerized 3D interactive models of human body structures.\textsuperscript{26} Three-dimensional modeling is the process of obtaining anatomical models from measurements of a real object in three dimensions (length, width and height), allowing to produce a computerized graph of the desired structure.\textsuperscript{27} 3D interactive virtual model exhibits that can be handled efficiently, showing the studied structures, layer by layer.\textsuperscript{28,29} Thus, students have already begun to realize that the use of this technology in virtual dissection may aid in anatomical study, in the absence of cadaveric material.\textsuperscript{5,29,30}

In accordance with aforementioned authors, the software in the present study was presented as a method accepted by the students, and another alternative in the teaching-learning process of Human Anatomy.

Analyzing the means values of data, the industrialized synthetic material was well accepted by the participants (19%). They become they become dynamic, close to reality, providing specific and detailed characteristics as observed by Portugal et al.,\textsuperscript{31} when they compared synthetic models with cadaveric female pelvis, proving to be an effective tool for the study.

Thus, it is necessary to validate how didactic alternative models support the visual construction of the structure, but does not replace the morphology of the corpse. On the other hand, attention is paid to the richness of details and interest for their similarity in the organization, as in the human body.\textsuperscript{32}

The qualitative analysis of the present study obtained five categories that allowed the identification of items that provide better learning of Human Anatomy, as can be seen in – Graph 4.

**Shortage of Time**

The practical assessment of Human Anatomy is considered by the students as an unpleasant evaluative method.\textsuperscript{33} The inclusion of the discipline in the first year of higher education, associated with the great amount of information and complexity treated of the discipline, gives visibility to the students’ inexperience in academic activities and specific anatomical nomenclatures. According Santos et al.,\textsuperscript{33} the shortage of time compromises the final result of the students.

The participants were asked to describe the difficulties they faced during the practical classes of Human Anatomy, the report on reduced time presents as the greatest obstacle to learning:

- **The time for each table is very reduced, this implies the nervousness. (A3)**
- **Too short time for hard and meticulous assessment. (A30)**
- **The time, some structures that we seek to identify and are not readily apparent. (B3)**

The evaluation as assessment need to be performed in a quiet atmosphere, allowing students’ knowledge to flow
naturally, without neuroses or anxiety states, that block their performance.\textsuperscript{12,33}

The Santos et al. study\textsuperscript{,33} demonstrates the meaning of the evaluation, according to the student, in assessment with microscopy slides showing the significance that the evaluation represents and its necessity. However, the way it is done - several possibilities of answers, processed in a short period of time - leads the students to a high level of stress.\textsuperscript{34}

In respect to the psychological state of the student, De Azambuja Montes and Vieira de Souza\textsuperscript{35} presented some difficulties encountered by the students of Human Anatomy, amongst which: the programmatic content of the discipline, that is usually quite extensive and is not always appropriate to the course objectives; The lack of have didactic material (cadaveric and/or synthetic pieces) and, most of the time, insufficient for the number of students; the emotional reaction of the students to the contact with the study material (corpses); and the current evaluation pattern makes the student’s resourcefulness difficult. Like a way of solving this disorder to the students, these same authors cite the more active action of the student, who it is the actor of his own learning, creating a pedagogical interaction with the professor and the contents of the discipline.

The use of alternative methods in teaching Human Anatomy facilitates learning, increases the amount of information and develops the artistic abilities of the students, as in the case of manufactured anatomical models.\textsuperscript{12} Therefore, it is highly important to implement innovative models that lead to a higher level of anatomical knowledge apprehension.\textsuperscript{5,12}

**Difficulty, Localization and Memorization of Anatomical Structures**

In the present study, the students’ difficulties were verified in the regarding to questioning. According De Azambuja Montes and Vieira de Souza\textsuperscript{36} and Falcão et al.\textsuperscript{23} teaching Human Anatomy presents challenges due to the complexity of the terminology and the difficulty in visualizing structures, which hinders their understanding for most of the students, as mentioned in the students’ report, quoted below:

Visualization of structures. (B23, B24, B30)
Memorization of anatomical terms and some structures visualization. (B27)
Difficulty in identifying structures because they are not easy to visualize. (B28)
Difficulty in remembering the names of some anatomical structures and locating accidents. (A11)

Memorizing information leaves the students submissive in this performance of the assessment. They don’t considered knowledge a something important for the construction of their future in the desired profession. Thus, the evaluation of learning is not really meaningful for the student, who is not attentive in learning what was passed in class, but rather in memorizing names and functions, just to answer questions of proof.\textsuperscript{37} In this sense, the students erroneously report that Anatomy is a discipline that they only read. They don’t read to understand the contents, principally when involving a detailed knowledge with a descriptive approach. This fact hinders the good anatomical learning process.\textsuperscript{4}

The location of anatomical structures and their comprehension are the second major problem encountered in the performance of practical assessments. The teaching-learning process related to the morphological aspects provides an intricate way, since the memorization of such structures becomes an inflexible and discouraging task for a good part of the students,\textsuperscript{3,38} as corroborated with the reports of the participants of this study.

In this category, it is important to emphasize that it is incumbent upon teachers to seek innovative didactics to arise the interest and attention of academics, presenting positive results in learning the discipline.\textsuperscript{12} However, the creation of new alternatives for teaching Human Anatomy are extremely necessary, especially methodologies that place the student as an active subject.\textsuperscript{38}

**Quality of the Material**

In anatomical studies, the essential condition in which the anatomical pieces are found is indispensable: adequate dimensions and good conditions with respect to quality for all to have
access, without difficulty of understanding or interpretation, this is patent from the participants’ report below.

The greatest difficulty is related to identifying what the professor really wants [...] color confusion. (C36)
The cadaveric material is generally deteriorated. (C4)

Pre-existing bodies are not conditioned and adequately handled by universities, resulting in the degradation of the material with consequent difficult to visualize the anatomical structures. The cadaveric material is generally deteriorated. (C4)

Pre-existing bodies are not conditioned and adequately handled by universities, resulting in the degradation of the material with consequent difficult to visualize the anatomical structures.

In some worn-out corpse parts, it is often difficult to identify the structure. The same happens with the synthetic parts. (C8)
The indication of the structures in the cadaveric piece, because often the state in which the corpse is found difficult to view and identify. (C26)
The corpses parts in bad condition, which makes it difficult to visualize the structures. (C7)

A viable and low maintenance cost alternative is the creation of anatomical models by students, which can be sustained for long periods. Although these have not been approached as one of the best methods, several studies point to it as a facilitator in the process of learning Human Anatomy, evaluation and good performance in future professional activities.

Comparison between Materials
The use of alternative resources to learning of Human Anatomy is showing efficiency. Numerous pedagogical techniques have been indicated, integrating teaching to the current reality.

The resulting information, according to the opinion of the participants, was not directly related to the methods, but the confrontation between the materials used in the practical assessment proves are the best method to learn:

The difference between a model piece and the reality can bring doubts to students. (C11)
The study in PVC pieces or materials manufactured by students makes it difficult to learn because it does not have much resemblance to cadaveric parts (in many cases). The use of very old cadaveric material parts also makes learning difficult. (C35)
The use of synthetic parts alone makes it difficult to identify structures and memorize anatomical terms. (B58)
Some pieces are not reliable. (A76)

As a consequence of the scarcity and inadequate preparation of cadaveric material, the inclusion of new methods is seen as a new way for teaching Human Anatomy, including the insertion of technology. However, the receptivity of the methods is not unanimous to all participants. Many of them emphasize that alternative materials are important, and good methods, but they do not substitute prospected cadaveric material.

Disagreeing with the authors above, Falcão et al. and Przybysz and Scolin report that the manufactured material, for the most students, does not contribute effectively to the anatomical understanding, as expected. As the synthetic models results, they do not match the Portugal study in which the method fits with didactic potential, to allow the best anatomical teaching and learning.

Corroborating the study of images, manufactured and synthetic models try to reproduce the corpses’ parts, but it is the point of view of the modeler or photographer who will be represented in the piece or image (photo), which may be a factor that subsequently complicate adherence to knowledge.

Cadaveric Material
The use of dissected human bodies represents the oldest form and one of the most used today to teach Human Anatomy through dissection procedures. The study of certain anatomical contents is difficult to understand due to the complexity of the structures submerged to the overlapping of anatomical elements.

The difference between the atlases, the books and the pieces used, especially cadaveric. Because they are reliable generally.

The material used, cadaveric parts, are often difficult to visualize. It depends on the state and form presented to us. (C15)

Identify the structures in the practical classes. (B14)
I believe that the corpses parts still make it very difficult to visualize, it is difficult to perceive some structures, because they suffer alterations in coloration due to the products that use for their preservation.

The study of cadaveric material in the teaching-learning process of Human Anatomy is a unique and indispensable activity because it allows understanding the shape, location and relations of the different between organs and structures that make up the human body.

Cadaveric material continues to be considered indispensable to study Human Anatomy, providing skills and competences the students from health courses, as well as the humanistic side of the professional future.

According to Oliveira et al. to allow the study of cadaveric parts, for a longer period autolysis, it is necessary to use fixation and preservation methods, such as formaldehyde. Karam study, formalization, a low cost technique based on the application of formaldehyde, is the main form of preservation and fixation of corpses, and anatomical pieces, used up to present days. However, the same authors point out several harmful factors associated with the application of this solution to the cadaver body, such as darkening, gaining weight and stiffness of the soft parts of material, besides presenting strong and unpleasant odor.

This chemical solution causes adverse reactions to those that manipulate it, such as mucosal irritation, burning, irritation in the eyes and nostrils, dermatitis among others. The use of chemical fixatives should be increasingly employed in the ideal concentrations, reducing the destruction of structures and the inconvenience caused to those involved in their study.

However, the use of corpses remains as the most efficient and indispensable method to acquire knowledge of human morphology, as reported by De Menezes, Collipal Larre and
We students assimilate better what was proposed by the professor and thus learning is better. (B1)

Their utilization makes it more evident what part of the body, showing the reality to students. (B10)

Because we study by a real method. (B6)

We know, in fact, how the human body presents itself and how we, the future health professionals, will use of corpses and prosected pieces.

Conclusions

Analyzing the perceptions of students of the Petrolina campus of the University of Pernambuco, regarding the materials used in the practical assessment of Human Anatomy, was relevant in times of evolutionary changes attributed to the quality of teaching.

It is essential to report that among five materials used in the practical assessment the participants reported that the best method of the use cadaveric material. Nevertheless, the use of photocopies did not obtain acceptance (photocopied material of printed anatomical atlases), which the participants presented greater difficulty during the gymkhana.

The use of software obtained a great acceptance by the participants, too. Important information lays on the technological era in modern times and this method can be used as a complement to the traditional study of Human Anatomy. The deficient topics in Human Anatomy learning lead us to believe that there is still a need to reformulate the techniques of practical assessment and adherence to new models that arise the interest aimed at the construction of knowledge in the students’ mind. While the students’ positioning regarding the application of cadaveric material used in the Anatomy practice assessment has been widely accepted, it is necessary that this material be preserved and that seek ways to acquire this type of material to be used, daily, in practical classes of the referring curricular component. No method will ever replace the use of the corpse which can be presented as complement that contributes to the process of knowledge acquisition.

Thus the cadaver was presented as the best method to be used in practical assessment of Human Anatomy at the University of Pernambuco. We need to emphasize the importance of its use in practical classes and practical assessments, according to the representative acceptance by the students.

The alternative methods, approached here to the traditional teaching of Human Anatomy, serve as a complement for the teaching-learning process, and never as a substitutes.

References


Acknowledgments

All authors thank all the students of the health courses of the Petrolina campus of the University of Pernambuco, for participating in this study and contributing to the dissemination of our results and all the staff LABPEAH (Study and Research Laboratory in Human Anatomy).
Toxoplastic Infection-induced Injury in the Ileal Myenteric Plexus in Rats Depends on the Dose of Toxoplasma gondii Oocysts

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Abstract

Introduction The present study evaluated the effects of different inocula of Toxoplasma gondii oocysts on the ileal myenteric plexus in rats.

Materials and Methods Male Wistar rats, 60 days old, were distributed into four groups: control group (CG; which received saline solution) and groups that were infected with 100 (TG100), 1000 (TG1000), and 5000 (TG5000) T. gondii oocysts. Thirty days after infection, the rats were sacrificed, and the ileum was collected to make whole-mount preparations that were subjected to immunofluorescence staining to observe the general neuronal population (HuC/D), nitrergic neurons (nNOS), and enteric glial cells (S100). Morphometric and quantitative analyses of myenteric neurons were performed.

Results The infections with different T. gondii inocula did not cause neuronal or glial loss, but cause neuronal hypertrophy in general population and nitrergic subpopulation in infected groups. Conclusion: Changes in neuronal morphology were observed in the TG5000 group, including the presence of vacuoles, translocation of Hu protein to the nucleus, and dendritic distortions, suggesting functional alterations in these cells.

Introduction

Toxoplasma gondii is an intracellular parasite that causes toxoplasmosis and is considered one of the most evolutionarily successful protozoa on Earth.¹ One-third of the world’s population has had contact with this parasite.² In Brazil, the prevalence of anti-T. gondii immunoglobulin G (IgG) antibodies in the human population varies from 59% to 91% in different regions.³,⁴ Based on SAG2 gene analysis, T. gondii has three main distinct clonal lineages: genotypes I, II, and III.⁵,⁶ The majority of human infections is associated with type II genotype strains;⁵ and its transmission can occur when the host ingests tissue cysts that are present in under-cooked meat or oocysts in contaminated water and food and via the vertical transmission of tachyzoites.¹,⁷ When sporulated oocysts are ingested by any warm-blooded animal, sporozoites are released in the small intestine, and the parasite penetrates enterocytes within 30 minute.⁸ Tachyzoites can be found in the blood 4 hour after the ingestion of oocysts, and most tachyzoites remain in the lamina propria where they multiply inside several cell types, with the exception of erythrocytes.⁹ The presence of these parasites in the intestine can cause sloughing of the intestinal mucosa and enteritis, with edema and necrosis in the lamina propria.⁹ Unknown is how often T. gondii infection causes gastrointestinal symptoms in humans.
Materials and Methods

All of the procedures that involved the use of animals were approved by the Ethics Committee on the Use of Animals in Experimentation of the State University of Maringá (CEAE, Universidade Estadual de Maringá, no. 081/2012) and were in accordance with the ethical principles adopted by the Brazilian Society of Science in Laboratory Animals (SBCAL).

Experimental Design

Twenty-five male Wistar rats (Rattus norvegicus), 60 days old, were randomly distributed into four groups: control group (CG; \( n = 5 \); which received saline solution) and groups infected with 100 (TG100, \( n = 6 \)), 1000 (TG1000, \( n = 7 \)), and 5000 (TG5000, \( n = 7 \)) Toxoplasma gondii oocysts of the ME49 strain (genotype II). The oocysts were obtained from the Veterinary Parasitology Laboratory, Universidade Estadual de Londrina (Londrina, PR, Brazil) and previously sporulated and resuspended in 1 mL of sterile saline solution. Control rats received only the sterile saline solution. The rats were then maintained for 30 days in a animal house with controlled temperature (22 ± 2°C) and a 12/12 hour light/dark cycle and fed standard rodent chow (Nuvilab, Quintia SA, Colombo, PR, Brazil) and water ad libitum.

Sample Collection and Tissue Preparation

T. gondii infection was confirmed by the presence of anti-T. gondii IgG in serum using the direct agglutination method.\(^{10}\) Thirty days after infection, the rats were deeply anesthetized with halothane vapor.\(^{11}\) Laparotomy was performed to collect the distal ileum, which was tied at both ends and distended with 4% buffered paraformaldehyde (pH 7.4) for 3 hour. The samples were opened at the mesenteric border and stored in phosphate-buffered saline (PBS; 0.1 mol/L, pH 7.4) with 0.08% sodium azide. The whole-mount preparations were then washed in PBS-T (3 × 10 minute) and incubated with secondary antibodies (\( \rightarrow \text{Table 1} \)) for 2 hour at room temperature under shaking. The whole-mount preparations were then washed in PBS-T (3 × 10 minute) and mounted on glass slides using Prolong Gold Antifade Reagent (Molecular Probes, Life Technologies, Eugene, OR, USA).

Morphoquantitative Analysis

The neuronal density and morphometry analyses were performed on images that were captured under a fluorescence microscope with a high-resolution camera coupled to a computer. The analyses were performed using ImagePro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA). The images were captured by randomly sampling across all of the whole-mount preparations, with no specific visual fields chosen, and the same field was not captured more than once. Immunoreactive neurons (HuC/D\( ^+ \)) and nNOS\( ^+ \)) and glial cells (S100\( ^+ \)) were counted in 30 images per animal. The area of each image was \( \sim 0.36 \text{mm}^2 \), and the total quantified area was 10.82 mm\(^2 \) per animal. The results are expressed as the number of enteric neurons or glial cells per cm\(^2 \). For morphometry, the area of 100 HuC/D\( ^+ \) cell bodies and 100 nNOS\(^+ \) cell bodies per animal were measured, and 100 HuC/D\( ^+/\text{nNOS}^- \) neurons per animal were also measured. Only neurons for which we could clearly delimit the cell body were chosen, and the area is expressed in \( \mu\text{m}^2 \).

Statistical Analysis

The data were analyzed using the Statistica 7.1 and GraphPad Prism 3.1 software. The quantitative data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s test. Morphometric data were set in delineation blocks (ANOVA) followed by Tukey’s test. All of the statistical tests considered a 5% level of significance. The results are expressed as mean ± standard error.

Table 1 Primary and secondary antibodies used for immunofluorescence

<table>
<thead>
<tr>
<th>Primary Antibodies</th>
<th>Secondary Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td>Dilution</td>
</tr>
<tr>
<td>HuC/D Mouse</td>
<td>1:500</td>
</tr>
<tr>
<td>nNOS Rabbit</td>
<td>1:500</td>
</tr>
<tr>
<td>S100 Rabbit</td>
<td>1:500</td>
</tr>
</tbody>
</table>

Abbreviations: HuC/D, general neuronal population; nNOS, nitricergic subpopulation; S100, enteric glial cells.

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Results

Clinical Aspects
None of the characteristic clinical signs of toxoplasmosis were observed in the infected rats. The TG100, TG500, and TG5000 groups presented serum anti-T. gondii IgG 30 days after inoculation, whereas the CG remained negative.

Neuronal and Glial Density
None of the myenteric cellular populations (neurons and glial cells) presented significant population density changes in the infected groups (TG100, TG1000, and TG5000) compared with the CG (►Table 2).

Morphometric Analysis
The general HuC/D⁺ neuronal population and HuC/D⁺ /nNOS⁻ subpopulation presented hypertrophy in all of the infected groups compared with the CG (p < 0.01), most prominently in the TG100 group (►Table 3). The nNOS⁺ subpopulation showed hypertrophy of the cell bodies in the TG100 and TG5000 groups (p < 0.01). Some of the rats in the TG1000 group exhibited translocation of Hu protein to the nucleus, causing a reduction of cytoplasmic immunoreactivity and compromising visualization of the cell body limits. Special attention was necessary to ensure that the entire area of the neuronal body, including the nucleus and cytoplasm, was being measured.

Qualitative Analysis
HuC/D⁺ /nNOS⁺ neuronal immunoreactivity was generally weak in the infected rats (►Fig. 1). Higher HuC/D⁺ nuclear immunofluorescence was observed in neurons in the TG1000 group, indicating the translocation of Hu protein from the cytoplasm to the nucleus (►Fig. 2A). Some neurons in the TG5000 group presented an irregular shape and no clear definition of the cell body limits (►Fig. 2B). A substantial presence of intra-cytoplasmic vacuoles was observed in neurons in the infected groups (►Fig. 2C). Some nNOS⁻ neurons in the infected groups were swollen and presented distortions in the dendritic projections (►Fig. 2D). Although the Hu/D antibody that was used is considered a pan-neuronal marker, we were able to observe some nNOS⁺ neurons that did not show immunoreactivity to Hu protein (►Fig. 3).

Discussion
Infection with different inocula of T. gondii (ME49 strain, genotype II) did not cause neuronal or glial cell loss but caused morphologic changes in myenteric neurons of the ileum in rats, such as hypertrophy, the translocation and degradation of Hu protein, the presence of vacuoles, and the distortion of dendrites in nitrergic neurons. The neuronal alterations that were observed in infected animals in this experimental model were not sufficient to provoke clinical signs of toxoplasmosis, such as diarrhea. Toxoplasmosis is often a subclinical infection in both rats and humans,12 which has also been observed in previous rat studies.13–16 Our data showed that the infection did not cause neuronal loss in the general population (HuC/D⁺) or nitrergic subpopulation (nNOS ⁺). T. gondii infection in rats after 30 days does not compromise the survival of myenteric neurons, regardless of the infective stage of the parasite that is used for the inoculation, which was also demonstrated in previous studies that evaluated the ileum (acute and chronic phase) and colon (chronic phase) in rats that were infected with tachyzoites.16–18 The density of myenteric glial cells also did

Table 2 Quantification of the general population (HuC/D⁺) and subpopulations (nNOS⁺ and nNOS⁻) of myenteric neurons and glial cells (S100⁺) in the distal ileum in rats infected with different inocula of T. gondii for 30 days. The results are expressed as neurons per cm² (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Group</th>
<th>HuC/D⁺ general population</th>
<th>HuC/D⁺ /nNOS⁻ subpopulation</th>
<th>HuC/D⁺ /nNOS⁺ subpopulation</th>
<th>S100⁺ Glial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16829 ± 3966</td>
<td>12839 ± 3322</td>
<td>3988 ± 689</td>
<td>25752 ± 3063</td>
</tr>
<tr>
<td>TG100</td>
<td>15749 ± 2096</td>
<td>11333 ± 1981</td>
<td>4295 ± 742</td>
<td>25475 ± 2350</td>
</tr>
<tr>
<td>TG1000</td>
<td>19443 ± 2309</td>
<td>14760 ± 1533</td>
<td>4691 ± 790</td>
<td>26454 ± 2508</td>
</tr>
<tr>
<td>TG5000</td>
<td>17116 ± 1864</td>
<td>12848 ± 1251</td>
<td>4299 ± 685</td>
<td>24773 ± 1812</td>
</tr>
</tbody>
</table>

No significant difference was found between groups (p < 0.05; ANOVA).

Table 3 Cell body area (μm²) of the HuC/D⁺ general population, nNOS⁻ subpopulation, and nNOS⁺ subpopulation of myenteric neurons in the distal ileum in rats infected with different inocula of T. gondii for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>HuC/D⁺ general population</th>
<th>HuC/D⁺ /nNOS⁻ subpopulation</th>
<th>HuC/D⁺ /nNOS⁺ subpopulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>290.7 ± 117.3</td>
<td>289.9 ± 121.5</td>
<td>300.0 ± 87.82</td>
</tr>
<tr>
<td>TG100</td>
<td>351.1 ± 112.9a</td>
<td>364.6 ± 131.4a</td>
<td>321.1 ± 93.99a</td>
</tr>
<tr>
<td>TG1000</td>
<td>318.5 ± 111.0ab</td>
<td>333.0 ± 122.3ab</td>
<td>296.7 ± 87.27b</td>
</tr>
<tr>
<td>TG5000</td>
<td>322.9 ± 133.2ab</td>
<td>317.1 ± 138.4ab</td>
<td>325.6 ± 101.6c</td>
</tr>
</tbody>
</table>
not change as a result of toxoplasmic infection. The survival of myenteric glial cells may have contributed to the maintenance of the number of neurons in the general population and nitrergic subpopulation because glial cell loss usually precedes neuronal loss.¹⁹

The dose of parasites affected the myenteric neuronal population in the duodenum. Rats that were infected with T. gondii presented progressive, dose-dependent general neuronal loss in the duodenum in ascending order (10, 100, 500, and 5000 oocysts). The nitrergic myenteric neuronal subpopulation

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**Fig. 1** Micrographs illustrating immunoreactivity to HuC/D (green) and nNOS (red) in the myenteric plexus of the ileum in rats. (A–C) Control group; (D–F) TG5000 group. The immunofluorescence of nNOS is weaker than HuC/D protein (arrowhead). Scale bar = 50 µm.

**Fig. 2** Micrographs showing immunoreactivity to HuC/D (A–C) and nNOS (D) in the ileal myenteric plexus in infected rats. Hu protein translocated (arrow), and no labeling was observed in the cytoplasm (arrowhead in A). HuC/D + neurons (green) in rats in the TG5000 group presented an irregular shape with no clear cellular limit (arrowhead in B) and cytoplasmic vacuoles (arrowhead in C). Nitrergic neurons (red) also showed distortions in rats in the TG5000 group (D). Scale bar = 50 µm.
was shown to increase at doses of 100, 500, and 5000 T. gondii oocysts.\textsuperscript{20} Although infection with different doses of T. gondii oocysts in mice is more harmful to the ileum than the duodenum,\textsuperscript{21} we did not know whether this holds true for rats. Studies by our group suggest that duodenal myenteric neurons in rats are more sensitive than ileal myenteric neurons to toxoplasmic infection.\textsuperscript{20} Within the general neuronal population (HuC/D +), we found that \textasciitilde{}25\% of the neurons were nitrergic (nNOS +) in all of the groups. The different doses of T. gondii oocysts did not cause any changes in the proportion. Although HuC/D is considered a pan-neuronal marker, we observed the absence of Hu protein immunoreactivity in some neurons that were immunoreactive to nNOS protein (\textasciitilde{} Fig. 3). Similar results have also been reported in humans.\textsuperscript{22} Although we did not observe myenteric neuronal loss, toxoplasmic infection caused hypertrophy in HuC/D +, nNOS +, and HuC/D +/nNOS- neurons. This hypertrophy might have occurred in response to the inflammation that was caused by T. gondii in the intestine, a remarkable feature of this parasitic infection.\textsuperscript{21} In fact, the cell body area increased in all of the groups but in descending order of the dose of the parasites. This indicates that higher parasite doses might impair neuronal plasticity. The mechanisms that are involved in these effects should be explored in further studies. Other studies by our group found morphometric alterations in myenteric neurons during toxoplasmic infection. For example, we observed hypertrophy in myenteric neurons in the distal ileum in rats that were infected with tissue cysts of the ME-49 strain of T. gondii for 24 hour.\textsuperscript{18} We also observed hypertrophy in nitrergic subpopulation and total population of myenteric neurons of proximal jejunum in rats that were infected for 36 days with tissue cysts of the ME-49 strain of T. gondii.\textsuperscript{23}

Despite the fewer morphometric alterations in myenteric neurons in rats in the TG1000 and TG5000 groups, we suggest that toxoplasmic infection compromises neuronal plasticity in the ileum when it is exposed to higher doses of the parasites. Considering that neuronal plasticity is important for the survival and physiologic balance of neurons,\textsuperscript{24} myenteric neurons in rats that received higher doses of the parasites may be more impaired because of the infection. The qualitative analysis appears to support this hypothesis.

We observed the nuclear translocation of Hu protein especially in the TG1000 group. This could indicate an imbalance in the trafficking of molecules between the cytoplasm and nucleus, thus compromising cellular metabolism. Hu protein is an RNA-binding protein that is present in both the cytoplasm and nucleus, with many functions in neuronal biology, such as cellular development and plastic adaptations.\textsuperscript{25} Changes in the localization of Hu protein may occur in response to stimuli that change the mRNA regulation by Hu\textsuperscript{26} or modify or selectively degrade Hu protein, thus modifying its immunoreactivity.\textsuperscript{25}

The consequences of translocation of this protein are not yet completely understood. This event may be indicative of neuronal injury (Rivera et al., 2011),\textsuperscript{27} and Hu protein loss in the cytoplasm may indicate changes in general protein synthesis. Some HuC/D + neurons presented a reduction of cytoplasmic immunoreactivity, including almost no labeling in some cases in the TG1000 group. Some HuC/D +, nNOS-, and nNOS+ neurons in the infected groups also showed the presence of vacuoles in their cytoplasm. Although cytoplasmic vacuolation occurs in neurons that are exposed to injury, the presence of these vacuoles is not necessarily related to neuronal death.\textsuperscript{28} Additionally, HuC/D +, nNOS-, and nNOS+ neurons with an abnormal aspect were frequently observed in the TG5000 group. These neurons presented distortion and no distinct demarcation of the cytoplasm.

**Conclusion**

The different doses of T. gondii oocysts did not cause glial or neuronal death in the ileal myenteric plexus in rats, but they provoked hypertrophy of their cell bodies. The higher doses (1000 and 5000 oocysts) caused less hypertrophy. The appearance of cellular injury signals, such as cytoplasmic vacuoles, Hu nuclear translocation, and dendritic distortion, may indicate less neuronal plasticity that is caused by this infection.

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