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Morphological and morphometric study of calcification of the petrosphenoidal ligament in a sample of dry human skulls from two States in the Brazilian Northeast

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ABSTRACT

Recent studies have suggested that calcifications in the petrosphenoidal ligament (PSL) may increase the likelihood of abducens nerve injury, resulting in idiopathic paralysis of the lateral rectus muscle of the eyeball. However, the literature is still scarce in determining the occurrence of these calcifications and associated factors. Therefore, the objective of this study was to evaluate the occurrence of calcifications in the PSL in dry human skulls from Northeastern Brazil. The presence of areas indicative of calcifications in skulls belonging to the Federal University of Alagoas and the Federal University of Pernambuco were evaluated. Each ligament was classified into four patterns based on morphometric parameters, with the aid of a digital caliper: (1) Absence of calcifications; (2) < 50%; (3) 50% to < 100% and, (4) complete calcification. Statistical analyzes were tabulated in the jamovi statistical software, version 2.2.5, with a significance level of 5%. 65.5% of the skulls showed some degree of calcification. 56.4% had calcification on the right side and 40% on the left side. The most common classification was type 2. The frequency of calcification was statistically higher on the right side. The frequency of calcification in the PSL was high in the sample of skulls evaluated and more frequent on the direct side. More studies are necessary to better elucidate its occurrence, relationship with the sides of the skull, gender, age and possible clinical complications.

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RESUMO

Estudos recentes têm sugerido que calcificações no Ligamento petroesfenoidal (LPE) podem aumentar a probabilidade de lesão do nervo abducente, resultando em paralisias idiopáticas do músculo reto lateral do globo ocular. No entanto, a literatura ainda é escassa na determinação da ocorrência destas calcificações e fatores associados. Assim, o objetivo deste estudo foi avaliar a ocorrência de calcificações no LPE em crânios humanos secos do Nordeste brasileiro. Foram avaliadas a presença de áreas indicativas de calcificações em crânios pertencentes a Universidade Federal de Alagoas e a Universidade Federal de Pernambuco. Cada ligamento foi classificado em quatro padrões a partir de parâmetros morfométricos, com o auxílio de um paquímetro digital: (1) Ausência de calcificações; (2) < 50%; (3) 50% a < 100% e, (4) calcificação completa. As análises estatísticas foram tabuladas no software estatístico jamovi, na versão 2.2.5, com nível de significância de 5%. 65.5% crânios apresentaram algum grau de calcificação. 56.4% apresentaram calcificação no antímero direito e 40% no antímero esquerdo. A classificação mais comum foi o tipo 2. A frequência de calcificação foi estatisticamente maior no antímero direito. A frequência de calcificação no LPE foi elevada na amostra de crânios avaliados e mais frequentes ao antímero direto. Mais estudos são necessários para elucidar melhor sua ocorrência, relação com os antímeros do crânio, sexo, idade e possíveis complicações clínicas.

Palavras-Chave:
Crânio, Base do Crânio, Ligamentos, Calcificação Fisiológica

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Introduction

The abducens nerve is occasionally involved in skull base fractures and its injuries can result in paralysis of the lateral rectus muscle of the eye, convergent strabismus and diplopia (Tubbs et al., 2012; Warwick & Williams, 1979). The long course of the nerve, through the pontine cistern and its abrupt curvature over the petrous part of the temporal bone, makes it vulnerable to injuries resulting from caudal displacement of the brain stem and consequent stretching of the nerve (Warwick & Williams, 1979).

The petroclival region has been identified as one of the body parts of greatest vulnerability of the abducens nerve, due to its intimate relationship with an osteofibrous canal, known by the eponymous Dorello Canal (Kshettry et al., 2013; Tubbs et al., 2012; Umansky et al., 1991). This canal was described by Primo Dorello in 1905 when studying possible factors associated with transient paralysis of the abducens nerve in inflammation of the middle ear (Gradenigo syndrome), and was later known as Dorello's canal (Ambekar et al., 2012; Ferraro et al., 2018). However, the first anatomical description of this canal is due to Wenzel Leopold Gruber in 1859, who also described the petrosphenoidal ligament (PSL), a fibrous band that forms the roof of a canal that allows the passage of the abducens nerve, called it of petrosphenoidal canal (Ambekar et al., 2012; Umansky et al., 1991). Therefore, the PSL is known by the eponymous Gruber's ligament (Ambekar et al., 2012).

The Dorello canal or petrosphenoidal canal is limited superiorly by the PSL, while the apex of the petrous part of the temporal bone and the superolateral part of the clivus form its other limits (Icke et al., 2010). It has been described that the abducens nerve is found in the middle part of this canal, surrounded by a kind of second fibrous tube, strongly attached to adjacent structures, including the PSL (Tubbs et al., 2012, 2014; Umansky et al., 1991). This adhesion makes the nerve more vulnerable to stretch injuries in cases of caudal displacement of the brain stem, resulting, for example, from superior expansive lions, lumbar puncture in patients with cranial hypertension and tumors in the petroclival region (Kshettry et al., 2013; Tubbs et al., 2012).

The PSL is a fibrous band, shaped like a butterfly or triangle, surrounded by venous blood, fixed posteriorly to the apex of the petrous part of the temporal bone and anteriorly to the lateral margin of the back of the sella turcica of the sphenoid bone, inferior to the process posterior clinoid (Skrzat et al., 2017; Touska et al., 2019; Umansky et al., 1991; Warwick & Williams, 1979). It has an average length of 13.4 ± 3.3 mm and an average width of 6.1 ± 3.2 mm near the posterior clinoid process and 4.2 ± 1.6 mm at the petrous apex (Icke et al., 2010).

Due to its relationship with the abducens nerve, some studies have suggested that calcifications in this ligament can trap and compress the abducens nerve, resulting in idiopathic paralysis of the lateral rectus muscle of the eye (Ekanem et al., 2023; Icke et al., 2010 ; Özgür

& Esen, 2015; Skrzat et al., 2017; These calcifications can also increase adhesions of the abducens nerve, increasing its vulnerability (Ekanem et al., 2023).

Despite being described by many researchers, the occurrence of calcifications in the PSL shows discrepant values between studies. Furthermore, doubts still persist about its relationship with age factors, sex, antimeres and geographical differences. Therefore, the objective of this study is to determine the occurrence of PSL calcifications in a sample of dry human skulls from two States in the Brazilian Northeast, through a classification based on morphometric parameters.

Materials and Method

Study design

This is a cross-sectional study, with analyzes of morphological and morphometric aspects of skull base calcifications.

Ethical Aspects

Studies carried out with human corpses or parts thereof are regulated by Law No. 8,051, of November 30, 1992, which provides for the use of unclaimed corpses for the purposes of studies or scientific research; and Law No. 10,406, of January 10, 2002, Art. 14, which rectifies the free disposal of one's own body, in whole or in part, after death (Law No. 8,051, 1992; Law No. 10,406, 2002; Lemos et al., 2020). Thus, the present study followed Brazilian legislation, and was approved by the Human Anatomy Sector at UFAL and the Forensic Anthropology and Osteology Laboratory of the Anatomy Department at UFPE.

Context and sample

The context was composed of all dry human skulls belonging to the Descriptive and Topographic Anatomy laboratory, of the Institute of Biological and Health Sciences (ICBS/UFAL) and the Laboratory of Forensic Anthropology and Osteology (Department of Anatomy/UFPE). The sample was constituted according to the following eligibility criteria:

Inclusion

We included intact skull bases (with the skullcap removed), from adult individuals, without restrictions on sex and ethnic group.

Exclusion

Skulls sectioned in a sagittal direction and with evidence of trauma to the skull base or craniofacial deformities were excluded. A non-probabilistic convenience sampling was carried

out, seeking to select all eligible dry skulls, according to the criteria previously described.

Data collect and research tools

The skulls were evaluated for the presence of partial or complete calcifications, with the aid of a digital caliper with a precision of 0.01 mm (MTX®, Tools World, Guarulhos, SP, Brazil - MTX-316119).

The presence of areas indicative of calcifications was assessed bilaterally at the PSL fixation points, as per the following description (Skrzat et al., 2017; Touska et al., 2019; Umansky et al., 1991; Warwick & Williams, 1979):

- Posterior attachment: Apex of the petrous part of the temporalis (medial to the trigeminal impression).
- Anterior attachment: Lateral margin of the dorsum of the sella turcica of the sphenoid bone, inferior to the posterior clinoid process.

The presence of calcifications and the pattern of presentation, whether bilateral or unilateral, were recorded on an assessment form developed for this study. Each ligament was classified into four types of morphological patterns (Adapted from Touska et al., 2019):

- Type 1 – Absence of calcifications;
- Type 2 – Presence of calcifications < 50% of the ligament;
- Type 3 – Presence of calcifications involving 50% to < 100%;
- Type 4 – Completely calcified ligaments, forming foramina.

In all skulls, distances between the PSL anatomical fixation points were measured, as previously described. In the presence of partial calcifications, the lengths of the calcified ligament areas were also measured. These calcification measurements were added together, allowing the determination of the total amount of calcification for each antimere of the skull. Then, the percentage of calcification was determined, based on data regarding the distance between the PSL fixation points.

Measurements were carried out using a digital caliper with an accuracy of 0.01 mm. Three measurements were carried out, and the average of the three measurements was considered.

Cases that presented a line of union between the two calcified ends of the ligaments were classified as type 4 (Touska et al., 2019).

In skulls classified as type 4, the following additional morphometric analyzes were performed: (1) length of the calcified bar, (2) largest diameter and (3) smallest diameter of the formed foramen. Furthermore, the formula for calculating the area of ellipses was used to calculate the area of the respective foramen:

Area = a x b x π, where (a) represents the largest radius, (b) the smallest radius and π is equal to 3.14. The diameter was calculated by multiplying the radius by 2.

All analyzes were performed by a single examiner through previous calibration. To this end, 20% of the sample was analyzed and classified by the examining researcher and an anatomist with experience in the area (expert). Next, the inter-examiner Kappa agreement coefficient was calculated. The inter-examiner Kappa coefficient was 0.84, indicating an almost perfect agreement with the "Expert".

Statistical Analysis

Data were tabulated in the Jamovi program, version 2.2.5, and analyzed using descriptive and inferential statistics. Statistical differences related to the frequencies of calcifications between the antimeres of the skull were determined using the McNemar test. For morphometric data, the Shapiro-Wilk test was initially performed to evaluate data distribution, which demonstrated a non-parametric distribution ($p<0.05$). In this way, statistical differences related to the distance between the PSL fixation points and the antimeres of the skull were determined by the Wilcoxon W test for paired groups. Statistical differences related to the length of calcifications were determined using the Mann-Whitney U test (independent groups). In all analyses, a significance level of 5% ($p<0.05$) was adopted.

Results

From a sample of 55 skulls, it was observed that 36 skulls (65.5%) presented some calcification in the PSL, without distinction of antimere. 31 skulls (56.4%) showed calcification in the right antimere and 22 skulls (40%) in the left antimere (Table 1). In skulls that present some calcification, both in the direct and left antimere, the most prevalent classification was type 2 (calcifications smaller than 50% of the ligament extension) (Figure 1, Table 2). In the right antimere, 2 skulls (3.6%) showed complete calcification of the PSL and only 1 (1.8%) in the left antimere. The McNemar test demonstrated that the frequencies of calcifications were statistically different between the skull antimeres, with the right antimere showing a higher frequency of calcification ($p=0.039$) (Table 3).

Table 1.

Frequency of ligament calcifications

General Frequency		
	N	**%**
Without calcification	19	34.5%
With calcification	36	65.5%
Frequency in right antimere		
	N	**%**
Without calcification	24	43.6%
With calcification	31	56.4%
Frequency in left antimere		
	N	**%**
Without calcification	33	60%
With calcification	22	40%

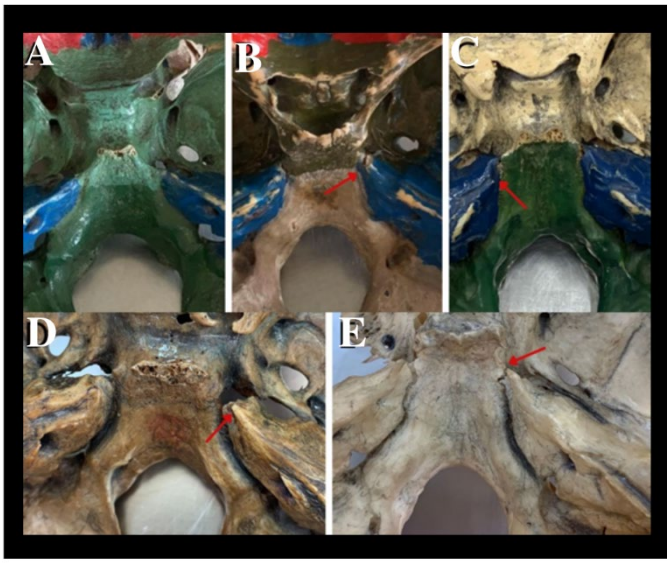

Figure 1.

Morphological patterns of calcification of the petrosphenoid ligament. A) type 1; B) type 2; C) type 3; D) type 4; E) type 5

Table 2.

Classification of the degree of ligament calcification

Right antimere		
	N	**%**
Type 1	24	43.6%
Type 2	25	45.5%
Type 3	4	7.3%
Type 4	2	3.6%
Left antimere		
	N	**%**
Type 1	33	60 %
Type 2	20	36.4 %
Type 3	1	1.8%
Type 4	1	1.8 %

Table 3.

Association between the presence of calcification in skulls' antimeres

	Left antimere			
	Without calcification (n)	**With calcification (n)**	**Total**	**p**
Right antimere				
Without calcification (n)	19	5	24	0.039*
With calcification (n)	14	17	31	
Total	33	22		

** McNemar test*

n= Skull Number

Shapiro-Wilk test demonstrated that the quantitative data did not present a normal distribution ($p<0.05$). The median distance between the PSL anatomical fixation points in the right antimere was 5.44 (Interquartile Range - AIQ 3.16) and 5.65 (AIQ 2.48) in the left antimere. In skulls with some degree of calcification, the median length of calcification in the right antimere was 1.5 AIQ (2.15) and 1.27 (AIQ 2.1) in the left antimere. In the right antimere, 2 skulls showed complete calcification, forming a foramen. The mean area of the foramen was 8.51 mm2 (Standard deviation - SD 2.54, median 8.61, AIQ 1.8). In the left antimere, only one skull showed complete calcification, with a foramen area of 4.95 mm2. No statistically significant differences were observed between antimeres for the distance between the ligament attachment points and the length of the calcification (Table 4). Due to the small sample, statistical comparisons were not made regarding the area of the foramen formed in complete ligament calcifications.

Table 4.

Statistical differences for ligament distances and lenght of calcifications between right and left antimeres

	Distance among ligament fixation points[a]		
	Median	Interquartile extent (AIQ)	p*
Right antimere	5.44	3.16	0.121
Left antimere	5.65	2.48	
	Calcification lenght[b]		
	Median	Interquartile extent (AIQ)	p**
Right antimere	1.5	2.15	1.000
Left antimere	1.27	2.1	

** W de Wilcoxon test (paired groups)*

*** Mann-Whitney test (independent groups)*

[a] N= 55 in right antimere and 55 in left (paired comparation)

[b] N=31 in right antimere and 22 in left

Discussion

Anatomical knowledge of the petroclival region is important, due to the wealth of anatomical structures and their clinical implications (Kshettry et al., 2013). In this study, we addressed the occurrence of calcifications in the PSL, a fibrous band that forms the roof of the Dorello or petrosphenoidal canal, through which the abducens nerve passes (Ambekar et al., 2012; Umansky et al., 1991).

Actual studies have suggested that calcifications in the PSL may increase adhesions of the abducens nerve to the petrosphenoidal canal, increasing the possibility of injuries in cases of caudal displacement of the brainstem (Ekanem et al., 2023; Icke et al., 2010; Özgür & Esen , 2015; Skrzat et al., 2017; Touska et al., 2019; Tubbs et al., 2014). Furthermore, calcification of this ligament has been associated with paralysis of the lateral rectus muscle of the eye due to probable compressive injury to the abducens nerve (Skrzat et al., 2017; Touska et al., 2019; Tubbs et al., 2014). Paralysis of the lateral rectus muscle can be clinically identified as a deviation in the orthophoric position of the eyes, deficiency in the abduction movement of the gaze, esotropia (deviation towards the nose) and horizontal binocular diplopia (Curi et al., 2013; Pinheiro et al., 2023). Therefore, given its clinical importance, it is necessary to investigate the occurrence of calcifications in the PSL.

In this study, using a sample of 55 dried human skulls from two States in the Northeast Region of Brazil, a high occurrence of calcifications in the PSL was observed (65.5%). However, the majority were small calcifications, involving an extension of less than 50% of the ligament. Corroborating our results, a study carried out with a sample of 175 dry skulls from Southeastern Brazil also identified a high occurrence of PSL calcifications. Researchers observed that 83.42% of the skulls exhibited some degree of ligament calcification (De Oliveira et al., 2022).

However, other studies have shown a lower occurrence of PSL calcifications, with percentages such as 8% (sample of 100 dry human skulls in the North American region) (Ekanem et al., 2023), 8.8% (CT scans of 46 patients in Turkey) (Özgür & Esen, 2015), 10.8% (CT scans of 240 patients in the United Kingdom) (Touska et al., 2019) and 11.5% (CT scans of 200 patients in Kuwait) (Kumar et al., 2023). These differences may be related to calcifications ways are avaluated. In the studies by Özgür and Esen (Özgür & Esen, 2015); Kumar et al. (Kumar et al., 2023) and Ekanem et al. (Ekanem et al., 2023) the skulls or tomography images were classified as absence of calcifications, partial calcifications and total calcifications, without explaining the methodology for this classification. In this study, we used morphometric parameters to classify the skulls into 4 patterns: absence of calcifications (Type 1), presence of calcifications < 50% of the ligament (Type 2), presence of calcifications involving 50% to < 100% (Type 3)

and completely calcified ligaments (Type 4). We believe that, by using morphometric measurements, we make the evaluation more sensitive for calcifications smaller than 50% (Type 2), which was the pattern most observed in this study.

Touska et al. (Touska et al., 2019) argued that calcifications involving a ligament extension of less than 50% do not present great clinical significance. Thus, in their study, researchers classified tomography images into three patterns: absence of calcifications, partial calcification (involving an extension of 50% to less than 100% of the ligament) and total calcifications (100% of the calcified PSL). If we count the percentages of only types 3 and 4, results of this study are similar to those observed by Touska et al. (Touska et al., 2019). In the right antimere, it was observed that 10.9% of the skulls present calcifications between 50% and 100% (Type 3 and 4). In the left antimere, a percentage of 3.6% was observed.

In this study, it was observed that the frequency of calcifications was statistically higher in the right antimere compared to the left antimere. It was not possible to assess differences related to sex or age, as this information was absent for the majority of skulls. Corroborating our results, Ekanem et al. (Ekanem et al., 2023) also observed a higher frequency of PSL calcification in the right antimere. Regarding sex and age, Kumar et al. (Kumar et al., 2023) did not observe statistical associations between the frequency of partial or total calcifications and these variables.

In this study, there were not observed statistical differences, related to the length of the calcifications or the distance between the ligament attachment points and the skull antimeres. We did not find others studies that conducted morphometric analyses, allowing comparisons with this study. Therefore, further research must be conducted to confirm these results.

As limitations of the study, we highlight the small number of skulls, due to difficulties in obtaining skull bases with the petroclival region intact. Another limitation was the lack of data regarding the age and sex of the skull sample, making it impossible to assess whether these variables statistically interfere with the occurrence of different patterns of PSL calcifications. Therefore, we suggest carrying out more studies, especially conducted with tomography images, to better evaluate the occurrence of the 4 patterns of calcifications, their relationships with the antimeres of the skull, sex and age, as well as the presence of symptoms related to nerve damage abducent.

Conclusion

The occurrence of calcifications in the PSL was high in a sample of skulls from two states in the Brazilian Northeast, the majority of which were type 2, involving an extension of less than 50% of the ligament. The right antimere showed a statistically higher frequency of calcification and no statistical directions were observed related to the data on the length of the

calcifications and the distance between the fixation points. More studies should be conducted to better elucidate the occurrence of these calcifications, their relationship with the antimeres of the skull, sex and age and possible clinical complications.

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