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MORPHOLOGICAL AND MORPHOMETRIC STUDY OF THE DIGASTRIC MUSCLE IN HUMAN FETUSES

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ARTIGO ORIGINAL

RESUMO

Introdução: O músculo digástrico, essencial para funções como fala e deglutição, possui uma dupla origem embriológica que contribui para suas variações morfológicas. Uma compreensão aprofundada de suas características durante o desenvolvimento fetal é crucial para a prática anatômica e cirúrgica. **Objetivo:** Investigar as características morfológicas e morfométricas do músculo digástrico em fetos humanos, avaliando a influência da idade gestacional, sexo e lateralidade. **Materiais e Métodos:** Vinte e seis fetos humanos (13 masculinos e 13 femininos), fixados em formalina e sem anomalias macroscópicas, foram analisados. Eles foram obtidos em conformidade com a Lei 8.501 de 1992 na Universidade Federal de Sergipe. A idade gestacional foi determinada por uma equação baseada na medição do comprimento calcanhar-hálux. As análises morfométricas incluíram medições de comprimento e largura do ventre anterior, ventre posterior e tendão intermediário usando um paquímetro digital. A análise morfológica classificou os componentes digástricos de acordo com a tipologia de De-Ary-Pires et al., **Resultados:** A idade gestacional média foi de 29,4 (±3,58) semanas. Foi observada uma correlação positiva significativa entre a idade gestacional e o comprimento e largura de todos os componentes do músculo digástrico. Morfologicamente, o ventre anterior foi predominantemente classificado como Tipo I, enquanto o tendão intermediário exibiu variações entre os Tipos I, II e III. Todos os ventres posteriores foram classificados como Tipo I. Nenhuma diferença significativa de sexo foi encontrada. A simetria bilateral foi predominante na maioria das estruturas, exceto pela largura dos pontos anterior e médio do ventre anterior. **Conclusão:** A idade gestacional é o principal determinante do desenvolvimento morfológico e morfométrico do músculo digástrico durante o período fetal. Sexo e lateralidade não tiveram influência significativa. Estudos adicionais são sugeridos para comparar o desenvolvimento fetal e de adulto a fim de elucidar as implicações clínicas das variações anatômicas.

Palavras-chave: Músculo digástrico, ventre anterior, ventre posterior, variação, anatomia, classificação, assimetria, idade gestacional.

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ABSTRACT

Introduction: The digastric muscle, essential for functions such as speech and swallowing, has a dual embryological origin that contributes to its morphological variations. A thorough understanding of its characteristics during fetal development is crucial for anatomical and surgical practice. **Objective:** To investigate the morphological and morphometric characteristics of the digastric muscle in human fetuses, assessing the influence of gestational age, sex, and laterality. **Materials and Methods:** Twenty-six human fetuses (13 male and 13 female), fixed in formalin and without macroscopic abnormalities, were analyzed. They were obtained in compliance with Law 8,501 of 1992 at the Federal University of Sergipe. Gestational age was determined by an equation based on the heel-toe length measurement. Morphometric analyses included length and width measurements of the anterior belly, posterior belly, and intermediate tendon using a digital caliper. Morphological analysis classified digastric components according to De-Ary-Pires et al.'s typology. **Results:** Mean gestational age was 29.4 (±3.58) weeks. A significant positive correlation was observed between gestational age and the length and width of all digastric muscle components. Morphologically, the anterior belly was predominantly classified as Type I, while the intermediate tendon exhibited variations among Types I, II, and III. All posterior bellies were classified as Type I. No significant sex differences were found. Bilateral symmetry was predominant in most structures, except for the width of the anterior and middle points of the anterior belly. **Conclusion:** Gestational age is the main determinant of morphological and morphometric development of the digastric muscle during the fetal period. Sex and laterality had no significant influence. Further studies are suggested to compare fetal and adult development to elucidate the clinical implications of anatomical variations.

Keywords: Digastric muscle, anterior belly, posterior belly, variation, anatomy, classification, asymmetry, Gestational age.

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Introduction

The digastric muscle is located in the suprahyoid region of the neck, comprising two bellies, anterior and posterior, connected by the intermediate tendon. The anterior belly attaches to the digastric fossa of the mandible, while the posterior belly attaches to the mastoid notch of the temporal bone[1 2]. The intermediate tendon passes through the stylohyoid muscle and attaches to the body and greater horn of the hyoid bone via its fibrous loop[1].

Together with other neck muscles, the digastric muscle contributes to speech, mastication, and swallowing. It elevates and stabilizes the hyoid bone during swallowing and speech, and, along with the infrahyoid muscles, helps depress the mandible against resistance[3].

The embryological origin of the anterior belly of the digastric muscle derives from the first pharyngeal arch, and its variations result from the complex development of this arch, while the posterior belly originates from the second pharyngeal arch[2]. The digastric muscle is innervated by the mylohyoid nerve, a branch of the inferior alveolar nerve, and by the digastric branch of the facial nerve[3], while its blood supply is provided by the submental artery, muscular branches of the posterior auricular artery, and occipital branches of the external carotid artery[4].

Additionally, the bellies of the digastric muscle delineate triangles of the neck. The inferior border of the mandible base, together with the posterior and anterior bellies, defines the submandibular triangle. This triangle generally contains the marginal mandibular branch of the facial nerve, the facial and lingual arteries and veins, the submandibular gland, and other structures. The anterior bellies and the body of the hyoid bone delimit the submental triangle, which contains submental lymph nodes that receive lymphatic drainage from the mental region, tongue apex, lower lip, and incisor teeth[5]. The present study aimed to investigate the morphological characteristics and morphometry of the digastric muscle.

Materials and Methods

A total of 26 human fetuses (13 male and 13 female), fixed in formalin and without evidence of macroscopic abnormalities in the neck region, were used. All specimens belonged to the Human Anatomy Laboratory of the Federal University of Sergipe, Sergipe, Brazil, and were obtained in accordance with Law 8,501 of 1992, which governs the use of unclaimed cadavers for studies and research. Gestational age of the fetuses was determined using the equation: $GA = 8.2982 + (0.38764 \times F)$, where GA is gestational age and F is the heel-toe length measurement[6], recorded with a digital caliper accurate to 0.01 mm.

Morphometric Analysis

Using a digital caliper accurate to 0.01 mm and under natural light, the lengths of the anterior belly, intermediate tendon, and posterior belly of the digastric muscle were measured. The widths at the anterior, middle, and posterior thirds of each belly and its tendon were also recorded.

Morphological Analysis

In the morphological analysis of the digastric muscle, its components were examined and classified according to the typology of De-Ary-Pires et al.,[7]:

Anterior Belly (AB) Type I – One belly originating from the inferior border of the mandible, near the symphysis.

Anterior Belly (AB) Type II – Two bellies, with additional bundles connected to the mandible or mylohyoid muscle, either ipsilaterally and/or contralaterally.

Anterior Belly (AB) Type III – Three bellies, with additional bundles connected to the mandible or mylohyoid muscle, either ipsilaterally and/or contralaterally.

Anterior Belly (AB) Type IV – Four bellies, with additional bundles connected to the mandible or mylohyoid muscle, either ipsilaterally and/or contralaterally.

Anterior Belly (AB) Type V – Often described as an independent muscle, a rare variation known as the mentohyoid muscle (Macalister's muscle).

Intermediate Tendon (IT) Type I – The intermediate tendon appeared to pierce the stylohyoid muscle.

Intermediate Tendon (IT) Type II – The intermediate tendon was located laterally (superficial to the stylohyoid muscle).

Intermediate Tendon (IT) Type III – The intermediate tendon was located medially (deep to the stylohyoid muscle).

Posterior Belly (PB) Type I – The posterior belly of the digastric muscle originated from the mastoid notch of the temporal bone.

Posterior Belly (PB) Type II – The posterior belly originated totally or partially from the styloid process and attached, with or without a loop, to the middle or inferior pharyngeal constrictor muscle.

Statistical Analysis

For the analysis of study variables, descriptive statistics and hypothesis tests were applied. Descriptive measures, such as mean, standard deviation, median, and quartiles, were used to characterize the sample.

Normality of the data was assessed using the Shapiro-Wilk test to verify the normal distribution of the data[8]. Based on this analysis, the appropriateness of parametric or non-parametric tests was determined.

To investigate the association between quantitative variables, Spearman's correlation was applied for non-normally distributed variables, and Pearson's correlation for normally distributed variables[9]. Correlation coefficients ranged from 1 to 1, with values closer to the extremes indicating strong correlation — negative or positive — while values near zero suggested weak correlation. The adopted classification for correlation strength was: weak (0 to 0.4), moderate (0.4 to 0.7), and strong (0.7 to 1).

In the analysis of the relationship between side and the length or width of the variables, the paired t-test was used for normally distributed data[10]. For data not meeting normality assumptions, the Wilcoxon test was applied which is appropriate for comparing paired measures in non-normal distributions[11].

For comparisons between sexes regarding the variables of interest, the independent t-test was used when normality assumptions were met[12]. For variables that did not meet normality assumptions, the Mann-Whitney test was applied[13] wich is suitable for comparisons between two independent groups in non-normal distributions.

All statistical analyses were performed in JAMOVI software version 2.6.26, adopting a significance level of 5% ($p < 0.05$).

Results

The mean age of the fetuses was 29.4 (3.58) weeks with a median of 29.9 [26.7; 31.4] weeks. The statistical values of the length of the digastric muscle components can be seen in Table 1.

Table 1. Fetal age (semanas) and lengths of digastric muscle structures.

	Age	RAB	RPB	RIT	LAB	LPB	LIT
N	26	26	26	26	26	26	26
Mean	29.4	17.2	20.6	10.9	16.5	19.9	11.7
Median	29.9	17.7	20.0	10.8	17.0	19.7	11.6
Standard deviation	3.58	3.81	3.36	3.67	3.63	3.78	2.69
Minimum	22.3	9.97	16.5	3.67	10.2	12.0	4.75
Maximum	39.2	22.7	28.2	19.2	22.2	28.3	15.5
25th percentile	26.7	14.2	17.6	9.29	13.7	17.2	10.8
50th percentile	29.9	17.7	20.0	10.8	17.0	19.7	11.6
75th percentile	31.4	20.6	22.9	13.5	19.3	21.9	13.8

LAB = Left Anterior Tendon

LIT = Left Intermediate Tendon

LPB = Left Posterior Belly

RAB = Right Anterior Belly

RIT = Right Intermediate Tendon

RPB = Right Posterior Belly

Analyzing the widths on the right and left sides, the midpoint of the anterior belly stood out with a larger mean compared to the other points. On the posterior belly, the posterior point has the greatest width. The intermediate tendon presents uniformity and low variation between the points on both sides (**Tables 2 and 3**).

Table 2. Description of structure widths on the right side.

	AT RAB	MT RAB	PT RAB	AT RPB	MT RPB	PT RPB	AT RIT	MT RIT	PT RIT
N	26	26	26	26	26	26	26	26	26
Mean	5.30	6.42	4.59	2.14	3.45	4.43	1.33	1.21	1.35
Median	5.02	6.29	3.91	2.03	3.36	4.52	1.19	1.13	1.29
Standard deviation	1.30	1.74	2.51	0.556	0.824	1,000	0.686	0.536	0.462
Minimum	3.12	3.26	1.84	1.27	2.02	2.16	0.570	0.620	0.600
Maximum	8.38	10.7	12.6	3.43	5.98	5.97	3.70	2.70	2.49
W for Shapiro-Wilk	0.949	0.984	0.853	0.963	0.923	0.961	0.845	0.882	0.974
p Shapiro-Wilk	0.221	0.950	0.002	0.443	0.051	0.417	0.001	0.006	0.734
25th percentile	4.47	5.32	2.76	1.70	2.87	3.89	0.850	0.853	1.02
50th percentile	5.02	6.29	3.91	2.03	3.36	4.52	1.19	1.13	1.29
75th percentile	5.87	7.76	5.73	2.47	3.85	5.17	1.51	1.44	1.62

AT RAB = Anterior Third of Right Anterior Belly

AT RIT = Anterior Third of Right Intermediate Tendon

AT RPB = Anterior Third of Right Posterior Belly

MT RIT = Middle Third of Right Intermediate Tendon

MT RPB = Middle Third of Right Posterior Belly

MT RPB = Middle Third of Right Posterior Belly

PT RAB = Posterior Third of Right Anterior Belly

PT RIT = Posterior Third of Right Intermediate Tendon

PT RPB = Posterior Third of Right Posterior Belly

Table 3. Description of structure widths on the left side.

	AT LAB	MT LAB	PT LAB	AT LPB	MT LPB	PT LPB	AT LIT	MT LIT	PT LIT
N	26	26	26	26	26	26	26	26	26
Mean	4.89	6.08	4.43	2.47	3.46	4.58	1.39	1.32	1.32
Median	4.61	5.75	3.78	2.27	3.38	4.81	1.29	1.10	1.23
Standard deviation	1.34	1.50	2.34	0.970	0.698	1.01	0.759	0.782	0.447
Minimum	2.81	3.31	1.96	1.05	1.81	2.13	0.520	0.500	0.700
Maximum	8.50	9.81	10.4	6.25	5.26	5.80	4.40	4.39	2.60
W for Shapiro-Wilk	0.946	0.969	0.816	0.765	0.975	0.915	0.748	0.758	0.941
p Shapiro-Wilk	0.184	0.600	<.001	<.001	0.743	0.034	<.001	<.001	0.141
25th percentile	4.12	5.02	2.93	2.09	3.06	3.78	1.03	0.838	1.00
50th percentile	4.61	5.75	3.78	2.27	3.38	4.81	1.29	1.10	1.23
75th percentile	5.71	7.17	4.79	2.58	3.73	5.44	1.55	1.46	1.60

AT LAB = Anterior Third of Left Anterior Belly

AT LIT = Anterior Third of Left Intermediate Tendon

AT LPB = Anterior Third of Left Posterior Belly

MT LAB = Middle Third of Left Anterior Belly

MT LIT = Middle Third of Left Intermediate Tendon

MT LPB = Middle Third of Left Posterior Belly

PT LAB = Posterior Third of Left Anterior Belly

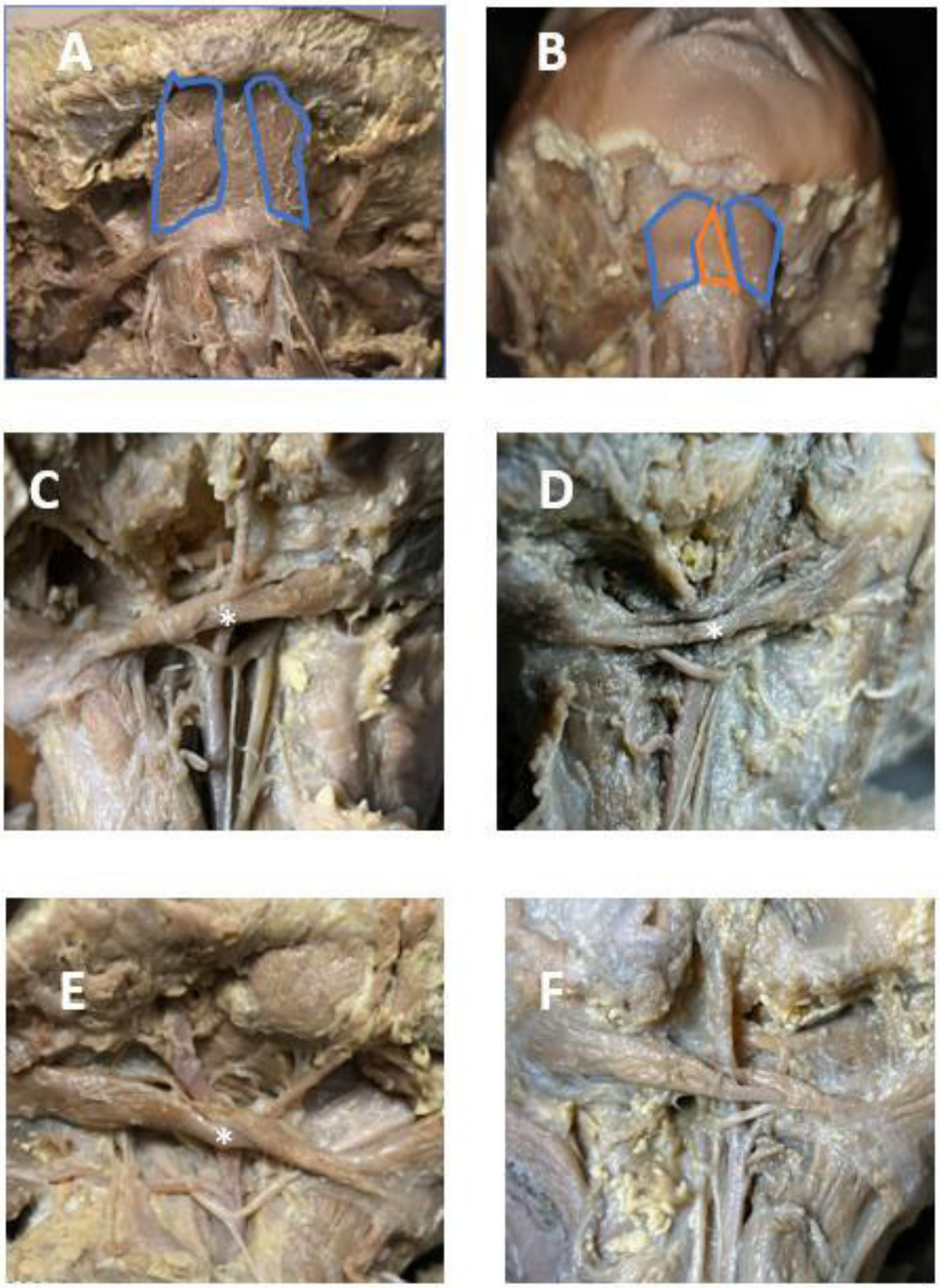
PT LIT = Posterior Third of Left Intermediate Tendon

PT LPB = Posterior Third of Left Posterior Belly

On the right side, the morphological classification of the anterior bellies of the digastric muscle included 22 type I AB (**Figure 1A**) and four classified as type II (**Figure 1B**), while the left side had 23 type I AB and three type II. For the intermediate tendon, on the right side, nine were classified as type I, five as type II and six as type III (**Figure**

1E). On the left side, 11 type I TI (**Figure 1C**), seven type II (**Figure 1D**) and three type III were found. Some IT could not be classified due to disruption of the stylohyoid muscle. All posterior bellies were classified as type I (**Figure 1F**).

Figure 1. Morphological classifications of the digastric muscle



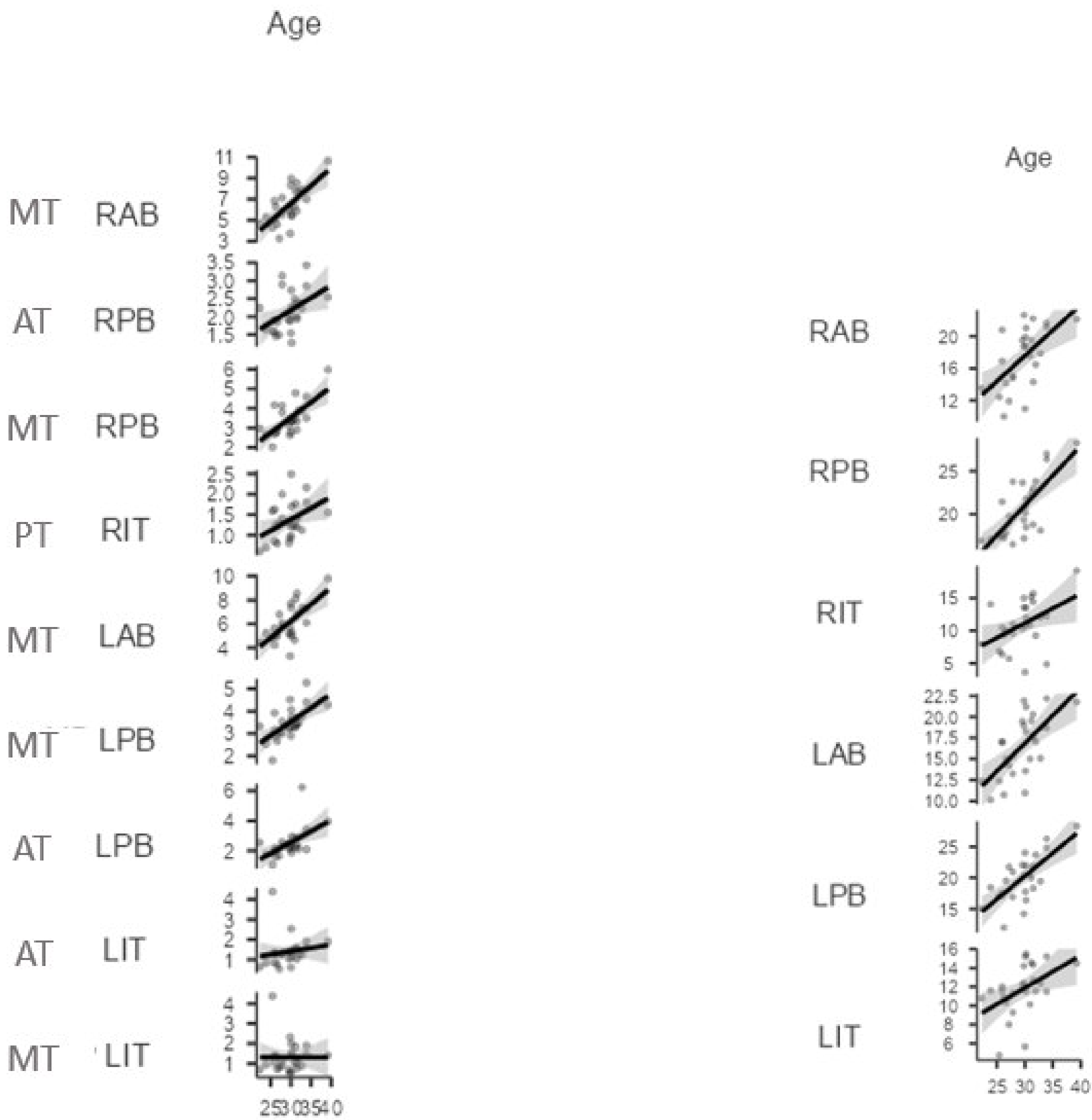
Legend

A) Bilateral AB type I; **B)** Right AB type II and left type I; **C)** Left IT type I; **D)** Left IT type II; **E)** Right IT type III; **F)** PB type I; **Blue:** main bellies; **Orange:** accessory belly; ***:** intermediate tendon in relation to the digastric muscle.

Data analysis revealed a significant positive correlation between gestational age and the length of the main components of the digastric muscle, especially the anterior and posterior bellies, as well as the intermediate tendons on both sides (**Figure 2.**). Similarly, there was a positive correlation between age and several width measurements, notably at the middle thirds of the bellies and the ends of the intermediate tendons. These findings reinforce the role of gestational age as a possible determining factor in the growth of these structures.

No significant differences were found between the sexes, and analysis of the relationship between the sides revealed symmetry in most structures, except at the anterior and middle thirds of the anterior belly, which showed significant asymmetry in width.

Figure 2. Correlation between age and digastric structures in scatter plots.



Discussion

The present study highlighted the developmental characteristics of the digastric muscle in human fetuses. Gestational age emerged as the most influential variable for the structures analyzed. This finding is consistent with the embryological knowledge of the digastric muscle. The anterior belly arises from the first pharyngeal arch, whereas the posterior belly originates from the second arch. Furthermore, muscle development occurs in different stages, with fiber differentiation taking place mainly from the sixth gestational week onward[14]. This implies that variations of the digastric muscle stem from processes that occur early in life.

Fetal sex or the side of the muscle did not appear to have a significant influence on digastric muscle differentiation. In the study by Zdilla et al.,[15] involving a sample of 23 cadavers, no significant difference was found between sides in a general analysis of length, anterior belly area, or intermediate tendon. However, in paired analysis, the left anterior bellies were longer than the right ones. Their sexual dimorphism analysis showed that men had significantly larger left anterior bellies than women, suggesting that variable influences on the digastric muscle may differ between the fetal period and adulthood.

Our study identified cases of accessory anterior bellies. It is known that variations of the anterior belly of the digastric muscle can occur unilaterally or bilaterally, as well as cases of duplication, incomplete belly fusion, or even absence[4 16]. Since it develops from two pharyngeal arches, variations of the digastric muscle are not limited to its anterior bellies. De-Ary-Pires et al.,[7] describe five anterior belly types, three intermediate tendon variations, and two posterior belly types.

Although digastric muscle variations can be asymptomatic, it is important to consider them in clinical and surgical contexts. They can cause confusion during procedures in the neck region, such as lipectomies or facial reanimation surgeries[17]. Odontogenic infections, in some cases, can spread to the submental space and form abscesses[18]. Occasionally, extraoral incisions are necessary to treat such abscesses, and the risk of the incision is low because the structures within the triangle are sparse and do not include arteries[5].

Khan et al.,[19] reported cases of patients undergoing wide resection of oral carcinoma, including segmental mandibulectomy and fibula flap reconstruction. In such cases, the digastric tendon was used to anchor and reinforce the lower lip flap, improving oral competence and aesthetics. Additionally, Seok et al.,[20] reported correction of post-traumatic anterior open bite after bilateral mandibular fractures by injecting botulinum toxin into the anterior belly of the digastric muscle, resulting in immediate correction.

Therefore, recognizing the anatomical structure of the digastric muscle and its variations is crucial to guiding clinical and surgical approaches. There is also a need for further studies comparing fetal and adult development to investigate which variables influence structural differences, thus informing decision-making in various healthcare procedures.

Conclusion

The present study revealed that, during the fetal period, gestational age is the main variable influencing the development of the digastric muscle. Moreover, factors such as muscle side and sex showed no significant relationship with its structural characteristics. Additional studies are necessary to achieve a better understanding of the development and anatomy of the digastric muscle in both fetuses and adults.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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