THE MORPHOLOGY AND MORPHOMETRY OF THE DIGASTRIC MUSCLE IN A PREDOMANTLY FETAL SOUTH AFRICAN POPULATION

by

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Submitted in the fulfilment of the requirements for the degree of

MASTER OF MEDICAL SCIENCE (ANATOMY)

In the School of Laboratory Medicine and Medical Sciences, Collage of Health Sciences, University of KwaZulu-Natal

2019

DECLARATION

I, Mr Khayelihle Guambe, declare as follows:

1. The work described in this thesis has not been submitted to UKZN or any other tertiary institution for the purposes of obtaining an academic qualification, whether by myself or any other party.

2. That my contribution to the project was as follows:

    Development and design of the research protocol
    Collection, analysis, and interpretation of the data
    Formulation of the manuscripts
    Compilation of the dissertation

3. That the contributions of others to the project was as follows:

    Prof. K.S. Satyapal (Supervisor), Dr. B.Z. De Gama (Co-Supervisor), Dr. P. Pillay (Co-Supervisor)

    Assistance with the editing, planning, and structuring of the dissertation
    Assistance with the editing, planning, and structuring of the manuscripts
    Provided constructive criticism
    Review of the dissertation and manuscripts before examination submission

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Signed (Co-supervisor): ____________________ Date: ____________________

Signed (Co-supervisor): ____________________ Date: ____________________

DEDICATION

To my family, especially my parents for their support and sacrifices.

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to the following individuals for their assistance in the preparation of this dissertation:

- To my family without whom I would not be here today. Thank you to Mum, Mfihlakalo, Nkanyiso, Sandile and Gcwalisiwe for being my pillars of strength.
- To my supervisors Professor KS Satyapal, Dr. BZ De Gama and Dr. P Pillay for always motivating and inspiring me to do my best. Thank you for your endless support and for believing in my capabilities.
- To Dr. BZ De Gama for your guidance, patience and constant motivation.
- The staff of Clinical Anatomy, University of KwaZulu-Natal, Westville campus, for the support and guidance.
- College of Health Sciences, University of KwaZulu-Natal for their Postgraduate funding in the first year of my degree.
- National Research Foundation (NRF) for funding in the final year of my degree.
- Ms. S Govender for her support and guidance during data analysis.
- Ms. N Ngubane for her friendship, support, guidance, and advice.

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ACRONYMS

ABDM: Anterior Belly of the Digastric Muscle

PBDM: Posterior Belly of the Digastric Muscle

IT: Intermediate Tendon

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ABSTRACT

Introduction: The digastric muscle is a suprahyoid muscle made up of two muscle bellies namely, the anterior and posterior bellies. These bellies originate from the digastric fossa of the mandible and the mastoid notch of the temporal bone respectively and insert via a common intermediate tendon (IT) onto the hyoid bone. This study aimed to investigate and document the morphology and morphometry of the digastric muscle in cadaveric specimens in the South African population.

Materials and methods: Macro-and micro-dissection was conducted on 40 fetuses (26 males, 14 females) between a gestational age range of 22-31 weeks old and 10 adults (8 males, 2 females) between the age range of 33-84 years old.

Results: In fetuses, the anterior belly inserted via a narrow and broad belly onto the body, lesser – and greater horns of the hyoid bone. The posterior belly originated from the mastoid notch of the temporal bone as well as the lateral surface and tip of the mastoid process. Trifurcation of the posterior belly was reported in 2.5% of the cases. The IT location varied as it travelled on the superior and inferior border of the stylohyoid muscle in 5% and 2.5% of the specimens, respectively. In adults, the anterior accessory bellies were documented as they occurred unilaterally (20%) and bilaterally (30%).

Conclusion: The fetal morphology reflected anatomical variations in the digastric muscle that have not been previously documented compared that of the adults. Comprehensive understanding of these anatomical variations may be of surgical relevance during corrective surgery and radiological imaging of the head and neck.

Keywords: Digastric muscle, anterior belly, posterior belly, morphology, morphometry, variations.

CHAPTER 1

INTRODUCTION

1. Background

The digastric muscle is part of the suprahyoid muscle group with three other muscles namely, the mylohyoid, sternohyoid and geniohyoid. These muscles insert on the hyoid bone along with the digastric muscle (Harrison *et al*., 2009; Chaurasia, 2010). The digastric muscle is formed by two bellies, the anterior and posterior belly. The anterior belly of the digastric muscle (ABDM) originates from the digastric fossa, in the lower internal surface of the mandible, while the posterior belly of the digastric muscle (PBDM) originates from the mastoid notch of the temporal bone (Chaurasia, 2010; Standring *et al*., 2016). These muscle bellies are connected by an intermediate tendon (IT) which pierces the stylohyoid muscle and attaches by a facial loop into the body of the hyoid bone (Drake *et al*., 2015; Standring *et al*., 2016). Conjointly with the other muscles, they anchor the hyoid bone by elevating this bone against traction of the infrahyoid muscles (Drake *et al*., 2015).

The anterior and posterior muscle bellies of the digastric muscle develop from two different embryonic precursors namely, the 1st and 2nd pharyngeal arch, and therefore are supplied by different nerves (Drake *et al*., 2015; Standring *et al*., 2016). The ABDM is innervated by the mylohyoid nerve, a branch of the inferior alveolar nerve of the mandibular branch of the trigeminal nerve, while the PBDM is innervated by the digastric branch of the facial nerve (Drake *et al*., 2015; Standring *et al*., 2016). In the suprahyoid region, the digastric muscle compartmentalizes different anatomical triangles found in the anterior region of the neck such as the submandibular triangle known as the ‘digastric triangle’, the submental and carotid triangles (Harrison *et al*., 2009; Drake *et al*., 2015; Standring *et al*., 2016). These triangles contain neurovascular structures and lymph nodes of the neck (Standring *et al*., 2016).

Anatomical variations exist in the digastric muscle and are common, particularly in the ABDM and have been reported in the literature. Various authors have described duplication, trifurcation and quadrification of the accessory belly, which may exist in different formations (Aktekin *et al*., 2003; Yamakazi *et al*., 2011; Kyung *et al*., 2011; Nayak *et al*., 2017). These muscle bellies may have well defined muscular or tendinous origins either from the digastric fossa, mandible, or the hyoid bone (Loukas *et al*., 2005; Liquidato *et al*., 2007; Mascaro *et al*., 2011; Yamakazi *et al*., 2011). Furthermore, these muscles may travel either unilaterally, bilaterally or traverse the midline of the mylohyoid muscle (Rani *et al*., 2013; Quadros *et al*., 2013; Azedero *et al*., 2015). Similarly, variations in the course of the IT have been documented in relation to the stylohyoid muscle where the IT travelled lateral or medial to the stylohyoid muscle (Zhao *et al*., 2015). However, variation in the PBDM are not common and few authors have reported the presence of an accessory posterior muscle belly (Ozgursory and Kucuk, 2009; Mehta *et al*., 2011).

REVIEW OF LITERATURE

1.1. Embryological Development of the Digastric Muscle

The digastric muscle is derived from pharyngeal arches which have five paired primordia made from the trilaminar embryonic discs through which facial features develop during the 4^{th} week of embryonic life (Sadler, 2011) (Figure 1). These arches surround the stomodeum and mesenchymal tissue and further differentiate into muscle mass, cartilage and bone which each acquire a different vascular supply (Standring *et al*., 2016). Since the digastric muscle is formed from two of the five pharyngeal arches it receives different innervation and vascular supply (Standring *et al*., 2016).

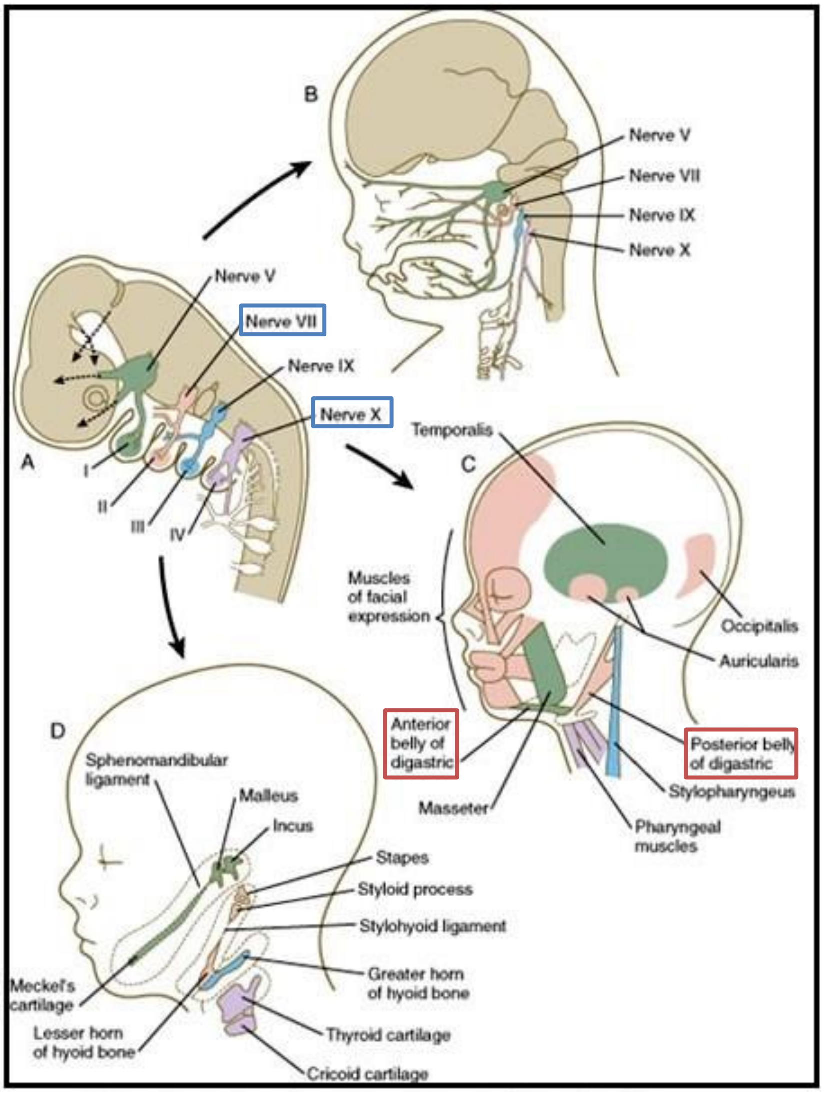

Figure 1: Illustration of the formation of the digastric muscle from the pharyngeal arches, (*Adapted from Sadler, 2011*).

The ABDM is derived from the myoblast of the 1st pharyngeal arch along with the muscles of mastication, tensor veli palatini, tensor tympani and mylohyoid muscles. The mandibular division of the trigeminal nerve accompanies this arch and gives off the nerve to mylohyoid muscle which innervates both the mylohyoid muscle and the ABDM (Standring *et al*., 2016). The PBDM is derived from the muscle mass of the 2nd pharyngeal arch or the mandibular arch along with the muscles of facial expression, platysma, stapedius, stylohyoid and auricular muscles. The facial nerve innervates the musculature of the 2nd pharyngeal arch and its branch, the posterior auricular nerve, supplies the PBDM (Standring *et al*., 2016).

1.2. Morphology of the Digastric Muscle

1.2.1. Anterior Belly of the Digastric Muscle (ABDM)

According to various reports in the literature, the morphological description of the digastric muscle reflected a standard anatomical origin, insertion and vascularization of the digastric muscle (Harrison *et al*., 2009; Chaurasia, 2010; Moore *et al*., 2013; Drake *et al*., 2015). The ABDM is described as shorter in length, and descending downwards and backwards from the digastric fossa of the inferior base of the mandible (Figure 2) (Drake *et al*., 2015; Standring *et al*., 2016).

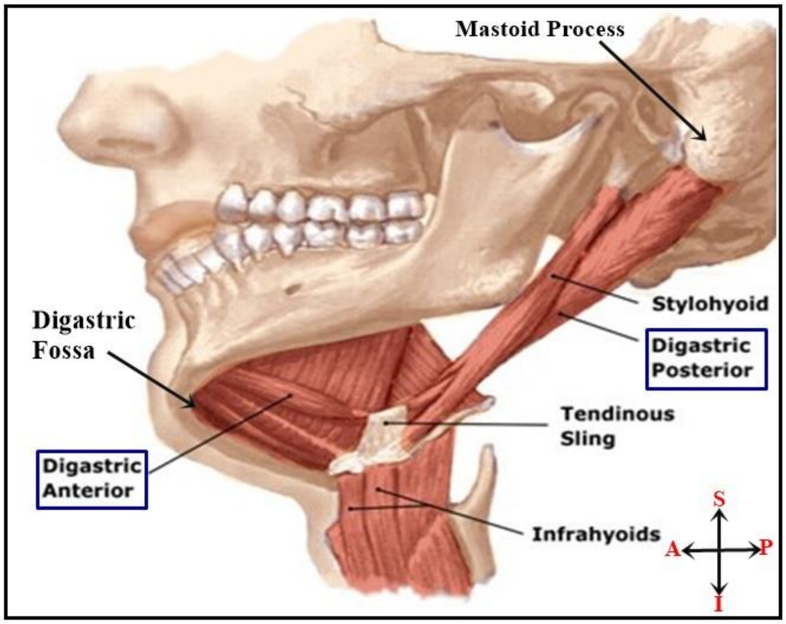

Figure 2: Lateral view of the standard anatomy of the digastric muscle, (*Adapted from Netter, 2010*).

In addition, anatomical variations have been extensively described in anatomical literature where an additional accessory belly in the submental region presenting with different morphological patterns such as an “X”, “Y” and weave pattern, has been reported (Mangalagiri and Razvi, 2009; Mascaro *et al*., 2011; Harvey *et al*., 2015).

It is evident from the literature reviewed that the occurrence of more than one accessory belly supersedes the existence of the main ABDM and are frequently reported as either unilateral or bilateral (Azeredo *et al*., 2016; Chaithra Rao *et al*., 2016; Nayak *et al*., 2017). In addition, accessory bellies varying in lengths compared to the main ABDM have been reported (Kyung *et al*., 2011; Zdilla *et al*., 2014; Azeredo *et al*., 2016). Furthermore, the accessory bellies reported had various points of origin which included the medial side of the ABDM, digastric fossa of the mandible, the intermediate tendon, the hyoid bone and the junction between the IT and ABDM (Rani *et al*., 2013; Buffoli *et al*., 2016). These accessory muscles have been reported to insert either into the hyoid bone, mylohyoid muscle, midline raphe of the mylohyoid muscle, intermediate tendon or digastric fossa (Mangalagiri and Razvi, 2009; Rani *et al*., 2013).

1.2.2. Variations in the Anterior Belly of the Digastric Muscle (ABDM)

The occurrence of the anomalous digastric muscle has been commonly reported with the ABDM, and anatomical descriptions and classifications of these variations exist in literature reports. Ozgur *et al*. (2010), classified two types of anatomical variations based on the arrangement of their muscle fibers. The atypical type (Crossover type) had muscle fibers that crossed the midline of the mylohyoid raphe, while those that did not cross the midline were classified as the Digastric type. Recently, Zhao *et al*. (2015) proposed four types (Types I – IV) of the ABDM based on the numerous bellies of origin. Despite the above-mentioned classification methods of the ABDM, the unilateral variations are more frequently reported in the ABDM than the bilateral variations.

1.2.2.1. Unilateral Asymmetric Anterior Accessory Belly

A unilateral accessory belly varying in origin and length from the main ABDM has been previously reported by some authors (Mangalagiri and Razvi, 2009; Kyung *et al*., 2011; Azeredo *et al*., 2016). In a study from 2009, Mangalagiri and Razvi reported accessory bellies originating from a common tendon centrally and dividing into upper and lower muscle fibers (Mangalagiri and Razvi (2009) (Figure 3).

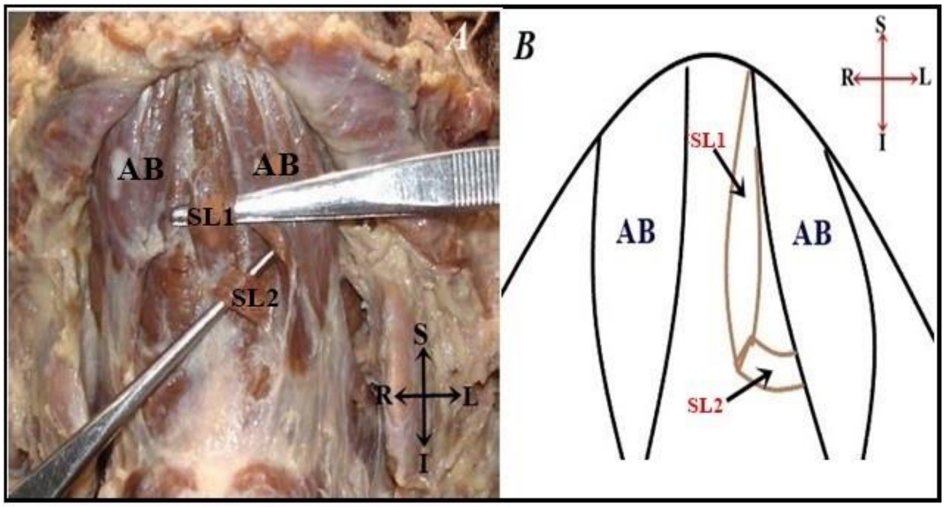

Figure 3: Upper and lower muscular bellies of the ABDM with different insertion points depicted by photograph (A) and associated schematic (B). *(Adapted from Mangalagiri and Razvi, 2009).*

Key:

Anterior belly of digastric muscle (**AB**)

Upper muscle slip (**SL1**)

Lower muscle slip (**SL2**)

In 2013, Quadros *et al*. documented a left-sided accessory belly medial to the ABDM, which coursed posteriorly and gave off fibers to the hyoid bone and intermediate tendon for attachment (Figure 4).

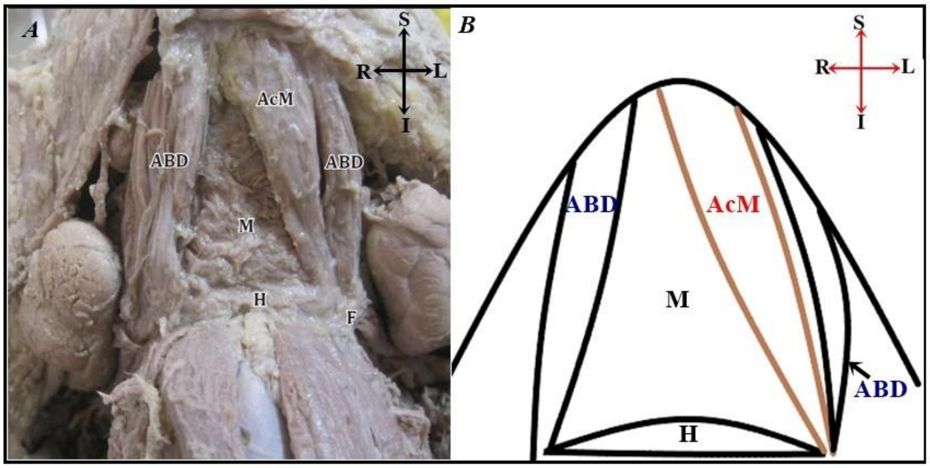

Figure 4: Left-sided anterior accessory belly of the digastric muscle depicted by photograph (A) and associated schematic (B), *(Adapted from Quadros et al., 2013).*

Key:

Anterior belly of the digastric muscle (**ABD**)

Accessory muscle (**AcM**)

Fascial sling of digastric muscle (**F**)

Hyoid bone (**H**)

Mylohyoid muscle (**M**)

While, Zdilla *et al*. (2014) described a right-sided oblique accessory belly originating from the opposite right digastric fossa of the mandible, which crossed the midline and inserted on the right ABDM and into the IT of the digastric muscle (Figure 5).

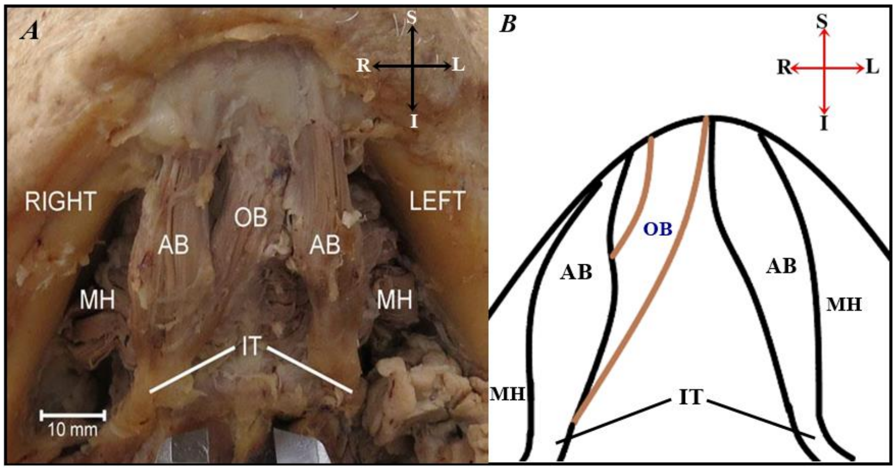

Figure 5: Oblique accessory belly of the ABDM depicted by photograph (A) and associated schematic (B), *(Adapted from Zdilla et al., 2014).*

Key:

Anterior belly of the digastric muscle (**AB**)

Mylohyoid muscle (**MH**)

Oblique belly (**OB**)

Similarly, Accioly Lins *et al*. (2017) documented an accessory belly originating from the left digastric fossa of the mandible with its elongated muscle fibers reaching into the contralateral left-sided ABDM while some fibers inserted onto the intermediate tendon (Figure 6).

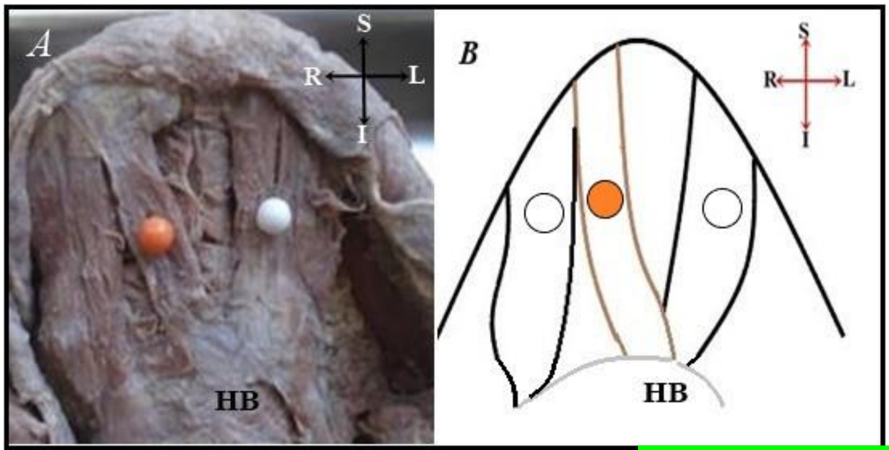

Figure 6: Unilateral variant ABDM in the suprahyoid region depicted by photograph (A) and associated schematic (B), (*Adapted from Accioly Lins et al., 2017*).

Key:

Anterior belly of digastric muscle (**White**)

Right accessory belly (**Orange**)

1.2.2.2. Bilateral Asymmetric Anterior Accessory Belly

Trifurcated accessory bellies comprising of an anterior, posterior and left-sided belly were reported on both sides of the mandible by Turan-Ozdemir *et al*. (2004). Anteriorly, the accessory belly arose from the digastric fossa of the mandible, while the posterior and left-sided bellies arose from the right and left intermediate tendon, respectively (Turan-Ozdemir *et al*., 2004). In 2007, Reyes *et al*. reported a bilateral triangulated accessory belly which originated from a base located along the mylohyoid raphe and an apex pointed towards and inserting onto the intermediate tendon.

Similarly, Kyung *et al*. (2011) presented a trifurcation of the accessory bellies with an inferior muscle bundle; these originated in the digastric fossa of the mandible deep, and medial to the anterior bellies while, the inferior muscle bundle arose from the distal end of the right anterior belly (Raju *et al*., 2014). The afore-mentioned accessory muscular bellies had a similar mode of insertion as they all converged at the midline and inserted onto the mylohyoid raphe of the mylohyoid muscle (Figure 7).

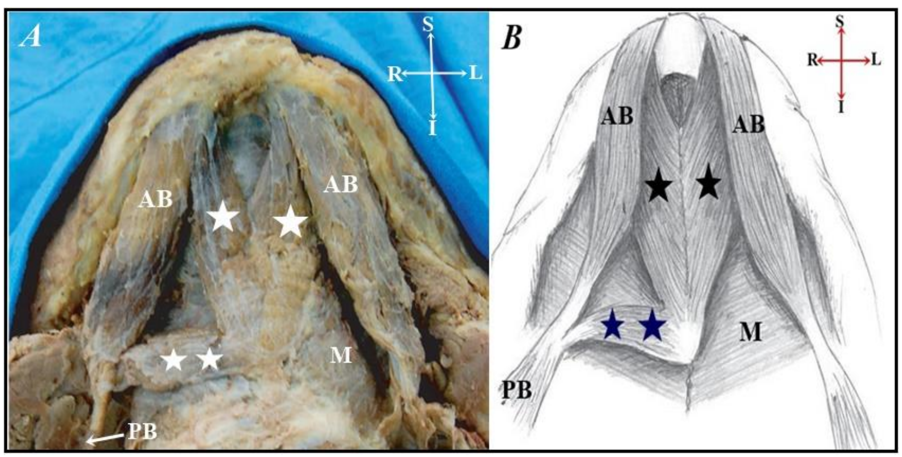

Figure 7: Bilateral anterior accessory belly of the digastric muscle with triple bellies (namely, the right, left and posterior belly) depicted by photograph (A) and associated schematic (B), (*Adapted from Kyung et al., 2011*).

Key:

Anterior belly of the digastric muscle (**AB**)

Posterior belly of the digastric muscle (**PB**)

Mylohyoid muscle (**M**)

Accessory bellies (★)

A case report by Loukas *et al*. (2005), described a bilateral belly. The right accessory belly split and exhibited a medial and lateral head which arose postero-inferiorly from the body of the mandible and inserted with its respective heads to the midline raphe of the mylohyoid muscle and into the main right ABDM (Loukas *et al*., 2005). Furthermore, the muscle fibers of the right accessory belly decussated distally and merged with the mylohyoid muscle at the midline (Loukas *et al*., 2005). The left accessory belly arose from a tendon on the digastric fossa medial to the left ABDM, and then inserted on the mylohyoid raphe (Loukas *et al*., 2005) (Figure 8).

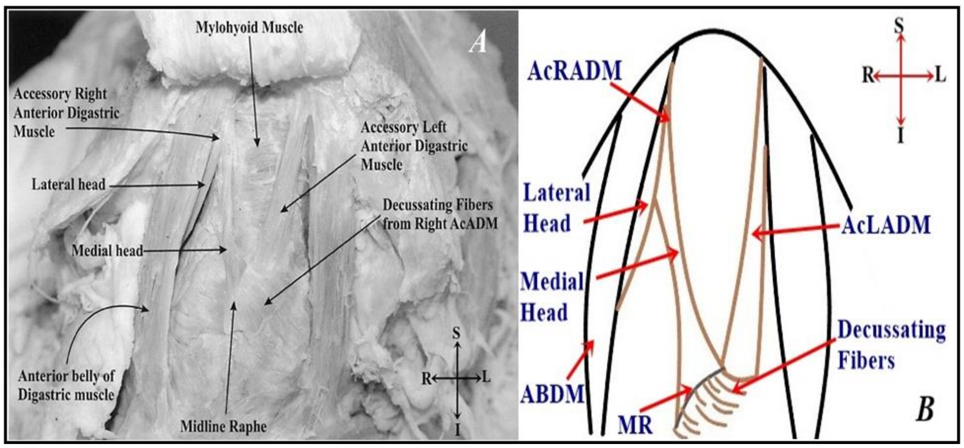

Figure 8: Asymmetric accessory bellies of the ABDM inserted into the midline of the mylohyoid raphe depicted by photograph (A) and associated schematic (B), *(Adapted from Loukas et al., 2005).*

Key:

Anterior belly of the digastric muscle (**ABDM**)

Accessory right anterior digastric muscle (**AcRADM**)

Accessory left anterior digastric muscle (**AcLADM**)

Midline raphe of mylohyoid muscle (**MR**)

A rare, variant anterior accessory muscle belly consisting of long and short segments decussated at the midline deep to the anterior bellies of the digastric muscle (Mascaro *et al*., 2011). The accessory belly then overlapped over the midline to insert on the digastric fossa of the mandible (Mascaro *et al*., 2011) (Figure 9).

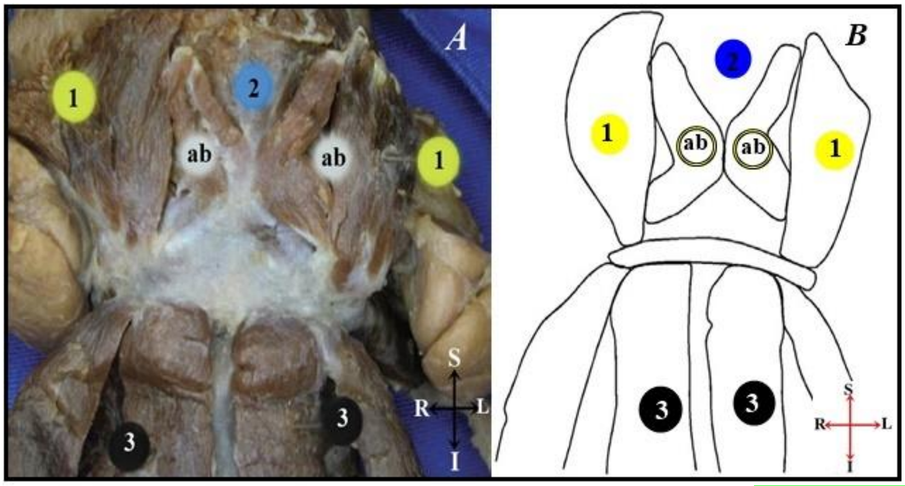

Figure 9: Accessory bellies of the ABDM with an "X" configuration depicted by photograph (A) and associated schematic (B), *(Adapted from Mascaro et al., 2011*).

Key:

Anterior belly of digastric muscle (**1**)

Mylohyoid muscle (**2**)

Sternohyoid muscle **(3**)

Accessory anterior bellies of the digastric muscle (**ab**)

Furthermore, multiple accessory anterior muscular bundles, *viz*. right, left, transverse and crossover bundle, originating from the ABDM bilaterally have been reported by Yamakazi *et al*. (2011). These muscles inserted into the midline of the mylohyoid raphe and the digastric fossa of the mandible (Yamakazi *et al*., 2011). An additional transverse bundle traversed along the right and left intermediate tendon (Yamakazi *et al*., 2011) (Figure 10).

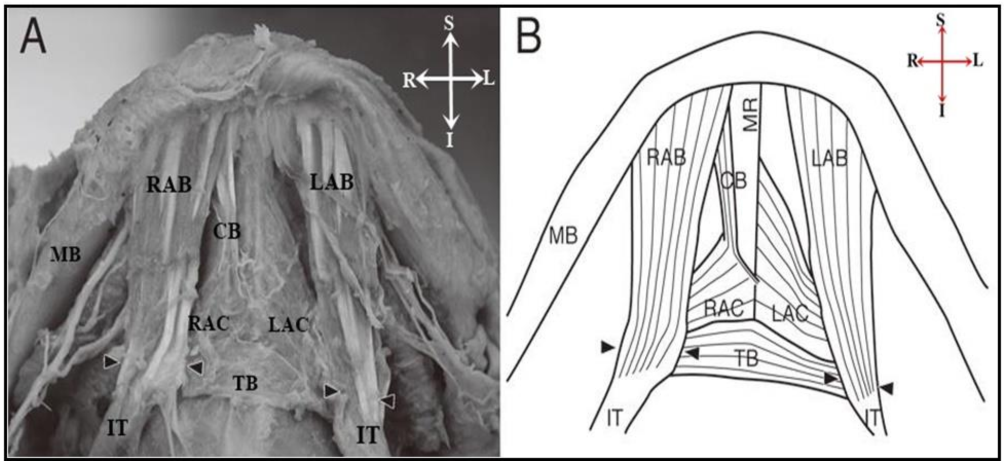

Figure 10: Bilateral anterior accessory bellies of the digastric muscle in the submental region depicted by photograph (A) and associated schematic (B), *(Adapted from Yamazaki et al., 2011).*

Key:

Right anterior belly of the digastric muscle (**RAB**)

Left anterior belly of the digastric muscle (**LAB**)

Right accessory belly (**RAC**)

Left accessory (**LAC**)

Transverse bundle (**TB**)

Crossover bundle (**CB**)

Mandibular bone (**MB**)

Intermediate tendon (**IT**)

Rani *et al*. (2013), discovered asymmetric accessory bellies on either side of the digastric fossa, which then extended downwards and backwards to insert into the anterior surface of the body of the hyoid bone (Figure 11).

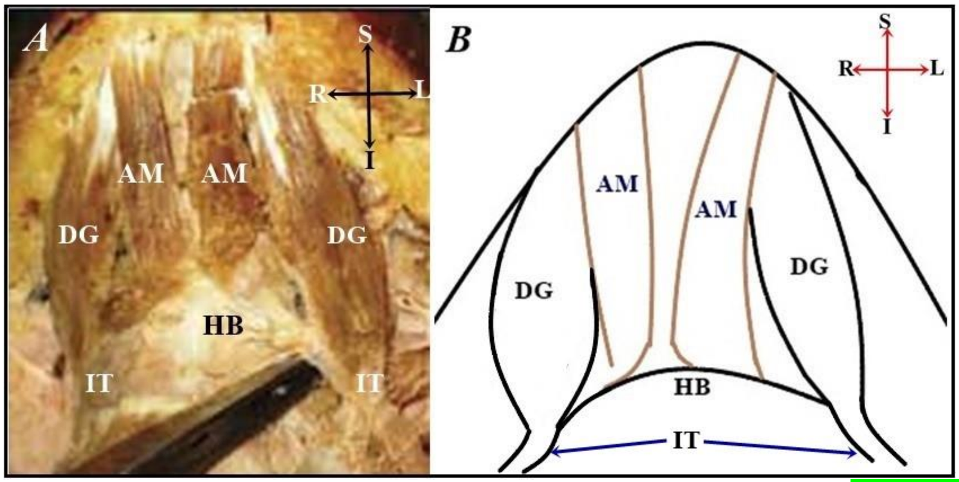

Figure 11: Bilateral asymmetric variant accessory bellies of the ABDM depicted by photograph (A) and associated schematic (B), *(Adapted from Rani et al., 2013).*

Key:

Anterior belly of digastric muscle (**DM**)

Accessory muscle (**AM**)

Hyoid bone (**HB**)

Intermediate tendon (**IT**)

Mylohyoid nerve (**MN**)

Bilateral, long and short accessory segments of the ABDM originating from the internal surface of the mandible, anteriorly and posteriorly (Chaithra Rao *et al*., 2016). The longer anterior segment descended along the medial margin of the main ABDM and inserted on the hyoid bone, while the short segment arched over and interconnected to each other by a thick connective tissue to the hyoid bone (Chaithra Rao *et al*., 2016) (Figure 12).

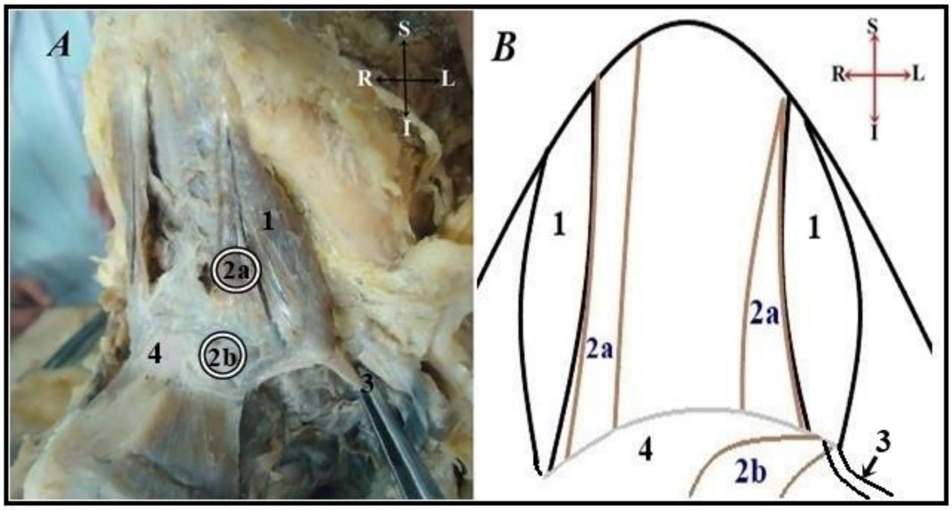

Figure 12: Bilateral variant anterior accessory bellies of the digastric muscle with long and short accessory segments depicted by photograph (A) and associated schematic (B), (*Adapted from Chaithra Rao et al., 2016*).

Key:

Anterior belly of the digastric muscle (**1**)

Long accessory segment (**2a**)

Short accessory segments (**2b**)

Intermediate tendon (**3**)

Hyoid bone (**4**)

Azeredo *et al*. (2016), discovered a unique and rare variant muscle, where the accessory bellies originated from a transverse tendinous arch which extended from either side of the lower border of the ABDM just above the hyoid bone (Azeredo *et al*., 2016) (Figure 13).

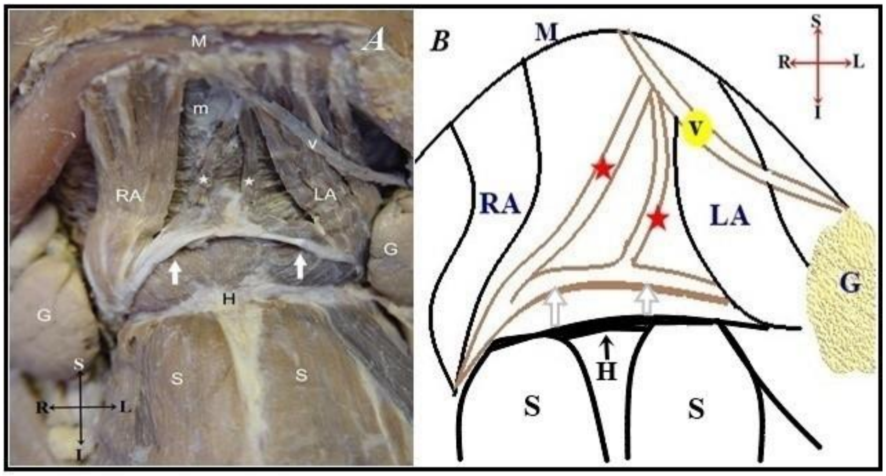

Figure 13: Two thin accessory bellies of the ABDM arising from the tendinous arch depicted by photograph (A) and associated schematic (B), (*Adapted from Azeredo et al., 2016*).

Key:

Right anterior belly of digastric muscle (**RA**)

Left anterior belly of digastric muscle (**LA**)

Mylohyoid muscle (**M**)

Submental vein (**V**)

Submandibular gland (**G**)

Hyoid bone (**H**)

Sternohyoid muscle (**S**)

Tendinous arch (**White arrows**)

Right and left accessory bellies (★)

Nayak *et al*. (2017), documented a triple asymmetrical accessory belly with two right-sided upper and lower muscle segments which originated from the digastric fossa of the mandible and distal part of the ABDM, respectively. While, the left accessory belly coursed obliquely and extended anteriorly from the mylohyoid muscle and attached to the terminal end of the contralateral left main ABDM posteriorly (Nayak *et al*., 2017) (Figure 14).

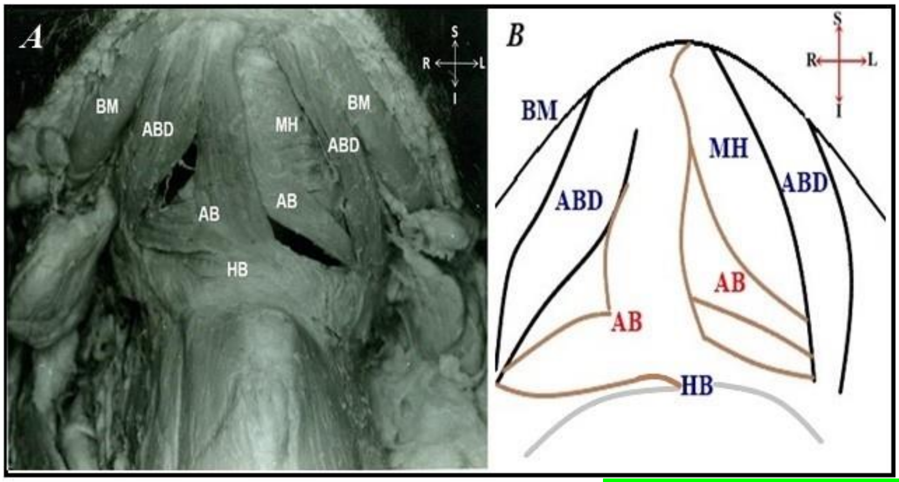

Figure 14: Anomalous ABDM in the suprahyoid region depicted by photograph (A) and associated schematic (B), (*Adapted from Nayak et al., 2017*).

Key:

Anterior belly of digastric muscle (**ABD**)

Accessory bellies of digastric muscle (**AB**)

Base of the mandible (**BM**)

Hyoid bone (**HB**)

Mylohyoid muscle (**MH**)

The occurrence of the accessory muscle belly of the ABDM encountered in the literature reviewed as previously described in the text has been grouped according their anatomical organization i.e. unilaterally or bilaterally or either symmetrical or asymmetrical in Table 1.

Table 1: Incidence and occurrence of the accessory belly of the ABDM

Author/s (year)	Sample size	Sex	Age	Country	Category	Main findings
Unilateral asymmetric anterior accessory belly						
Bakirci *et al*. (2007)	-	-	60	Turkey	Case Report	A transverse unilateral accessory belly
Liquidato *et al*. (2007)	10	-	-	Brazil	-	Unilateral right-sided triangular belly.
						Unilateral right-sided belly
Mangalagiri and Razvi (2009)	-	Male	60	India	Case Report	Upper and lower muscle slips with a tendinous origin
	-	Male	74	India		
Quadros *et al*. (2013)	-	Male	50	India	Case Report	Unilateral accessory belly
Zdilla *et al*. (2014)	-	Female	64	USA	Case Report	Oblique unilateral accessory belly
Dakhane and Fating (2014)	-	Male	60	India	Case Report	Unilateral left-sided accessory anterior belly originated from the mandibular digastric fossa.
Accioly Lins *et al*. (2017)	-	-	-	Brazil	Case Report	Left unilateral accessory belly
Bilateral symmetric and asymmetric accessory bellies						
Aktekin *et al*. (2003)	-	Female	35	Turkey	Case Report	Superficial and symmetrical accessory bellies
Turan-Ozdemir *et al*. (2004)	-	-	-	Turkey	Case Report	Three accessory bellies, the anterior, posterior and single
Loukas *et al*. (2005)	-	Male	73	West Indies	Case Report	Bilateral and asymmetrical accessory bellies
Bakirci *et al*. (2007)	-	Male	80	Turkey	Case Report	Bilateral symmetrical accessory anterior bellies
Liquidato *et al*. (2007)	10	-	-	Brazil	-	Bilateral accessory bellies
Reyes *et al*. (2007)	-	Male	80	India	Case Report	Bilateral triangulated anterior accessory bellies
Ozgur *et al*. (2010)	-	-	-	Turkey	Anatomical variations	Digastric muscle classified into the Digastric fossa type and Atypical type or crossover type
Yamazaki *et al*. (2011)	-	-	-	Japan	Short Communication	Bilateral asymmetric accessory bellies with crossover bundle
Kyung *et al*. (2011)	-	Male	72	Korea	Case Report	Bilateral accessory muscle bellies

Author/s (year)	Sample size	Sex	Age	Country	Category	Main findings
Mascaro *et al*. (2011)	-	Male	40	Brazil	Case Report	Bilateral muscular bellies arranged in a cross 'X' arrangement along the midline
Rani *et al*. (2013)	-	Female	65	Singapore	Case Report	Bilateral symmetric muscle bellies
Kalniev *et al*. (2013)	-	-	-	Bulgaria	-	Thin bilateral anterior bellies
						Intermediate tendon coursed deep to the stylohyoid muscle
						Absence of the ABDM
Chaithra Rao *et al*. (2016)	-	Male	55	India	Case Report	Bilateral accessory bellies
Nayak *et al*. (2017)	-	Male	60	India	Case Report	Right upper and lower muscular segments, and a left accessory belly

1.2.3. Posterior Belly of the Digastric Muscle (PBDM)

The PBDM is a large muscle belly passing forwards and downwards inferior to the sternocleidomastoid muscle. It originates from the mastoid notch of the temporal bone and then descends antero-inferiorly to attach to the hyoid bone (Drake *et al.*, 2015; Standring *et al.*, 2016) (Figure 15). The PBDM has been regarded as a critical landmark of the neck due to its relationship with important craniofacial nerves *viz.* facial nerve and hypoglossal nerve (Zhao *et al.*, 2015; Standring *et al.*, 2016).

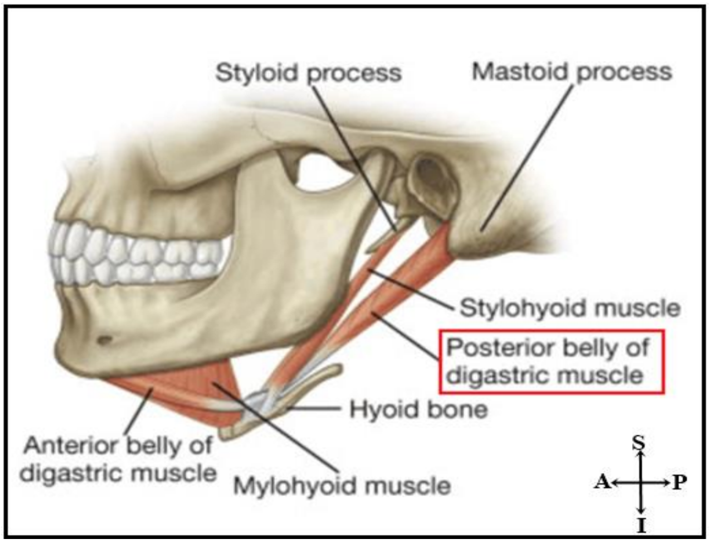

Figure 15: Illustration of the typical PBDM, *(Adapted from Drake et al., 2015).*

Variability in the PBDM exists. Ozgursoy and Kucuk (2006), reported a bifid PBDM originating with two portions, a superior main PBDM and an inferior accessory posterior muscle strap. These muscles originated from the mastoid notch of the temporal bone and coursed antero-inferiorly below the stylohyoid muscle. The two muscle bellies then inserted to the sternohyoid muscle (Figure 16).

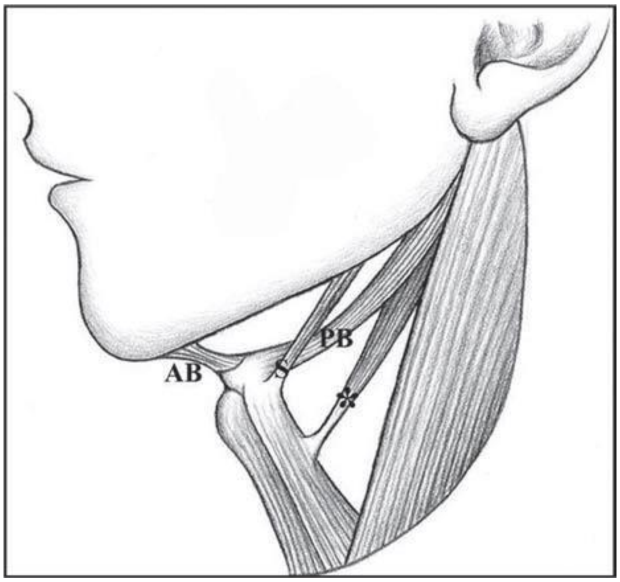

Figure 16: Diagrammatic presentation of a unilateral bipartite PBDM, (*Adapted from Ozgursoy and Kucuk, 2009*).

Key:

Anterior belly of the digastric muscle (**AB**)

Posterior belly of the digastric muscle (**PB**)

Stylohyoid muscle (**S)**

Posterior accessory belly (*****)

In a study conducted by Mehta *et al*. (2011), a PBDM with a bilateral bipartite origin was observed on a 45-year-old male cadaver. It originated from the medial aspect of the mastoid notch as the medial (upper) and lateral (lower) posterior belly. Inferiorly, the muscular slips united forming a short common continuous intermediate tendon (Mehta *et al*., 2011) (Figure 17).

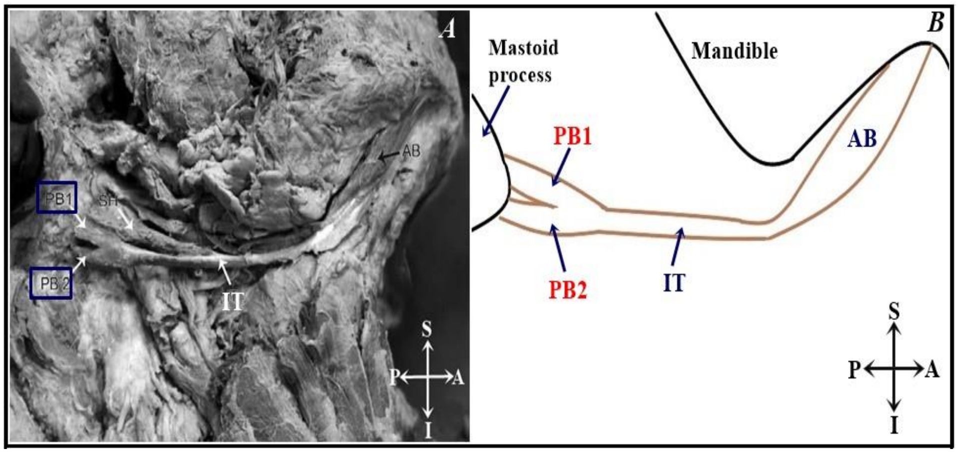

Figure 17: Posterior belly of the digastric muscle with a bifid origin consisting of an upper and lower belly depicted by photograph (A) and associated schematic (B), (*Adapted from Mehta et al., 2011*).

Key:

Anterior belly of the digastric muscle (**AB**)

Upper posterior belly (**PB1**)

Lower posterior belly (**PB2**)

Intermediate tendon (**IT**)

Stylohyoid muscle (**SH**)

Furthermore, a study conducted by Zhao *et al*. (2015), observed an additional anomalous muscle strip at the superior border of the sternocleidomastoid muscle. The muscular strip was regarded as the variant accessory PBDM in the absence of the omohyoid muscle (Zhao *et al*., 2015). The variant muscle arose from the mastoid notch of the temporal bone inferior to the PBDM and descended antero-inferiorly to insert into the muscles of the infrahyoid region. Furthermore, Zhao *et al*. (2015), divided the origin of PBDM as Type I PBDM originating from the mastoid notch of the temporal bone and Type II PBDM as partly originating from the stylohyoid process with either of the types attached by a fibrous sling to the inferior and middle constrictor muscles (Zhao *et al*., 2015) (Figure 18).

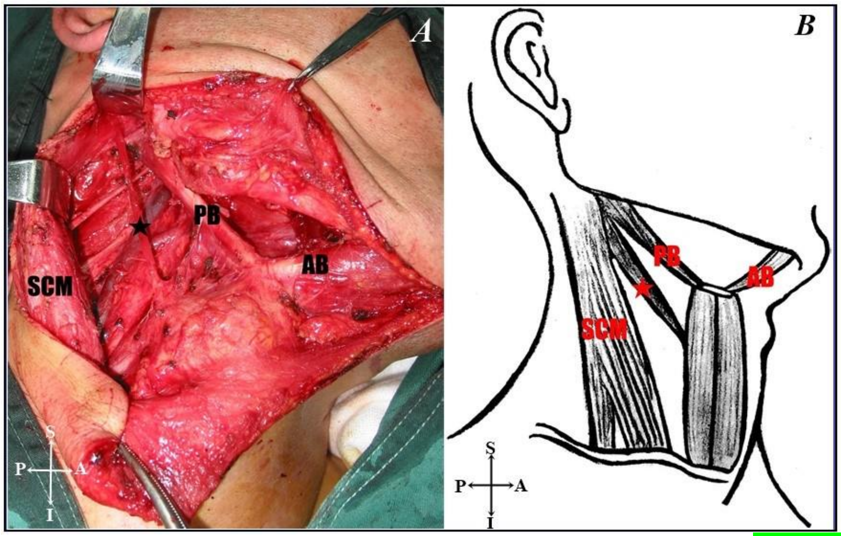

Figure 18: Duplicated PBDM showing the additional posterior belly depicted by photograph (A) and associated schematic (B), (*Adapted from Zhao et al., 2015*).

Key:

Anterior belly of the digastric muscle (**AB**)

Posterior belly of the digastric muscle (**PB**)

Sternocleidomastoid muscle (**SCM**)

Duplicated posterior belly of the digastric muscle (★)

1.2.4. Intermediate Tendon (IT)

The two muscular components of the digastric muscle *i.e.* the ABDM and PBDM interconnect via the IT. This tendon extends obliquely above the lesser horn of the hyoid bone and attaches via a fibrous loop into the body and greater horn of hyoid bone (Moore *et al*., 2013; Drake *et al*., 2015; Standring *et al*., 2016). Anterior to the PBDM, the tendon traverses through the distal portion of the stylohyoid muscle prior to inserting into the hyoid bone (Drake *et al*., 2015; Standring *et al*., 2016; Chaithra Rao *et al*., 2016; Nayak *et al*., 2017). However, variations in the trajectory of the IT in relation to the stylohyoid muscle have been reported and described as travelling either superficial (lateral) or deep (medial) to the stylohyoid muscle (Zdilla *et al*., 2014).

1.3. Morphometry of the Digastric Muscle

Morphometric analysis of the ABDM and IT of the digastric muscle in relation to its relevance for clinical implications and sexual dimorphism were documented (Zdilla *et al*., 2016). The measured parameters in their study were the muscle belly length, muscle belly area, IT length and width. The results obtained revealed the left-sided ABDM to be significantly longer than the right (Zdilla *et al*., 2016). Table 2 reflects the different morphometric measurements of the digastric muscle encountered in the literature reviewed (Table 2).

Table 2: Length and width parameters of the ABDM and its accessory belly

Author/s (year)	Sample Size	Sex	Age	Country	Main ABDM				Accessory Belly			
					Right side (mm)		Left side (mm)		Right-sided (mm)		Left-sided (mm)	
					Length	Width	Length	Width	Length	Width	Length	Width
Turan-Ozdemir *et al*. (2004)	-	-	-	Turkey	-	-	-	-	27 & 32	9 & 11	29	9
Liquidato *et al*. (2007)	10	Male	-	Brazil	-	-	-	-	35 & 24; 9; 15; 45 & 25 (cases 1- 4)	29; 39; 5; 6 (cases 1- 4)	12; 15 (cases 3 - 4)	38; 7 (cases 3 - 4)
Bakirci *et al*. (2007)	-	Male	80	Turkey	49.9	9.9	47	8.8	46.3	10. 5	52.9	7
Kyung *et al*. (2011)	-	Male	72	Korea	-	-	-	-	30	7	33	7
Zdilla *et al*. (2014)	-	Female	64	USA	31	13	33	10	41	10	-	-
Azeredo *et al*. (2016)	-	-	-	Brazil	55	14	50	14	-	-	-	-
Chaithra Rao *et al*. (2016)	-	Male	60	India	35	12	53	14	22 & 12	9 & 8	24 & 12	7 & 9
Zdilla *et al*. (2016)	23	Male and Female	-	Caucasian	38.7				-	-	-	-

The PBDM length and extended length were measured in a study by Ankolekar *et al*. (2015) on the PBDM. The anatomical landmarks used for the length was from the tip of the mastoid process to the loop of the hypoglossal nerve, while the extended length was measured from the tip of the mastoid process to the hyoid bone (Figure 19).

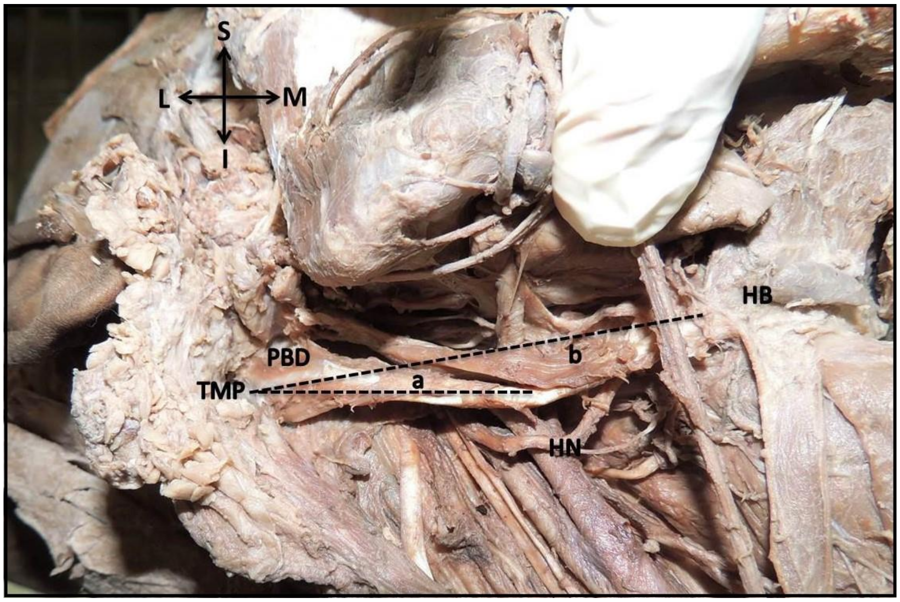

Figure 19: Measurements of the parameters in the PBDM, (*Adapted from Ankolekar et al., 2015*).

Key:

Posterior belly of the digastric muscle (**PBD**)

Hyoid bone (**HB**)

Hypoglossal nerve (**HN**)

Tip of mastoid process (**TMP**)

Length of the PBD (**a**)

Extended length of the PBD (**b**)

The bipartite PBDM described by Mehta *et al*. (2011) had asymmetric muscle bellies. On the right side, the lengths of the medial and lateral muscle bellies were measured as 3.4cm and 3.1cm, respectively. The short muscle belly formed by these muscles was measured to be 1.6cm long while on the left side, the superior and inferior muscle bellies were 4.1cm and 4.3cm in length respectively and formed a short muscle belly which was 1cm in length (Mehta *et al*., 2011). The average length of the left-sided PBDM was longer compared to the right-sided PBDM, 3.77 ± 1.08cm and 3.15 ± 0.05cm (mean ± SD) respectively. Whereas, the extended lengths were symmetrical on both sides, with an average right length of 6.7 ± 1.23cm and 6.7 ±0.75cm (mean ± SD) on the left (Ankolekar *et al.*, 2015).

1.4. Clinical Relevance

The digastric muscle demarcates boundaries of the digastric and submental triangles in the neck, where they are regarded as a landmark for localizing important neurovascular structures during head and neck surgery (Ankolekar *et al*., 2015). In the submental region, the facial artery and vein, lymph nodes and submental vessels travel superficial and medial to the ABDM. Thus, the ABDM is used to locate the submandibular duct and lingual nerve by the surgeon (Chaithra Rao *et al*., 2016). The structures deep to the inferior border of the PBDM include the cervical branches of the external carotid artery (Ankolekar *et al*., 2015). In facial nerve dissection, the PBDM is palpated to locate and assess the depth of the nerve during dissection of the parapharyngeal space or in parotidectomy (Ozgursoy and Kucuk, 2006; Zdilla *et al*., 2016). This crucial dissection in the suprahyoid region is often performed during the removal of metastatic lymph nodes in cases of carcinoma of the floor of the mouth (Mehta *et al*., 2011). However, an anomalous digastric muscle might interfere with the therapeutic imaging and surgical examination of the neck region as the accessory bellies that are prevalent in the ABDM could be rendered as a tissue mass in this area (Chaithra Rao *et al*., 2016). Thus, knowledge of the digastric muscle is relevant when performing radiography or interventional surgery in the submandibular region (Ankolekar *et al*., 2015).

2. Research Aims, Objectives and Questions

2.2. Research Aim/s:

i. To investigate and document the morphology and morphometry of the digastric muscle in cadaveric specimens in the South African population.

2.2. Research Objectives:

i. Document the morphology of the digastric muscle

ii. Document the morphometry of the digastric muscle

iii. Document any variations of the digastric muscle in fetuses and adults

2.3. Research Question

What is the morphology and morphometry of the digastric muscle in adult and fetal cadaveric specimens?

3. Materials and Methods

3.1. General Sample Size

Fifty bilateral micro-and macro-dissected head and neck (n = 100) cadaveric specimens comprising of 40 fetal and 10 adults were dissected at the Department of Clinical Anatomy, University of KwaZulu-Natal, Westville Campus. Of these specimens, 34 (26 fetuses; 8 adults) were males and 16 (14 fetuses; 2 adults) were females. Using a Digital Vernier caliper (150mm) (Mitutoyo Digimatic Caliper Series 500, USA), the fetal foot length was measured and the age (in weeks) was then estimated using the formula $y = 7.130 + 0.503x$, where y = gestational age in weeks and x = foot length in mm (Pandey *et al.*, 2016). Ethical clearance was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BE530/17) (Appendix 1).

3.2. Methodology

The present study involved micro-dissection of fetal and macro-dissection of adult cadaveric head and neck specimens. Microdissection was conducted using the stereo microscope (Carl Zeiss Stemi DV4 Stereo Microscope) whilst the adult cadaveric specimens followed routine dissection. The dissection of both fetuses and adults was carried out as per guidelines in the Grant's Dissector (Tank and Grant, 2012).

The morphology of the digastric muscle was observed after the removal of skin, superficial fascia and the platysma muscle anteriorly without damaging the underlying ABDM. The superficial muscles originating from the mastoid process were gently removed to expose the PBDM and the underlying neurovascular vessels. This study documented the origin and insertion of the ABDM and PBDM, and the course of the IT. The classification methods proposed by Zhao *et al*. (2015) was used to classify the digastric muscle:

i. The origin of the ABDM was classified into four types:
    a. Type I: originated as one muscle belly
    b. Type II: originated as two muscle bellies
    c. Type III: originated as three muscle bellies
    d. Type IV: originated as four muscle bellies
ii. The course of the IT was classified as:
    a. Type I: pierced the stylohyoid muscle
    b. Type II: located lateral (superficial) to the stylohyoid muscle
    c. Type III: located medial (deep) to the stylohyoid muscle
iii. The origin of the PBDM was classified into two types:
    a. Type I: originated from the mastoid notch of the temporal bone
    b. Type II: partly originated from the stylohyoid process.

The parameters measured were muscle belly length, muscle belly width at mid-muscle belly, intermediate tendon length and width at mid-tendon. Measurements were recorded with the aid of a Digital Vernier caliper (Mitutoyo 0-8 in [0-203.2mm] ABSOLUTE digimatic caliper series 500, accuracy ± 0.001 in [0.025mm]). The caliper was placed alongside the digastric muscle to acquire its length and width (Figure 20). The measurements obtained were analysed to obtain descriptive and inferential statistics using the Statistical Package for Social Sciences software (SPSS version 25.0). A p-value of < 0.05 was regarded as statistically significant.

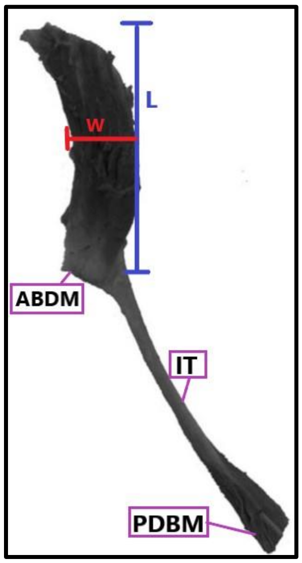

Figure 20: Parameters used to measure the ABDM, (*Adapted from Zdilla et al., 2016*).

Key:

Anterior belly of the digastric muscle (**ABDM**)

Posterior belly of the digastric muscle (**PBDM**)

Intermediate tendon (**IT**)

Length (**L**)

Width (**W**)

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CHAPTER 2

SCIENTIFIC MANUSCRIPT I

The literature reviewed provides comprehensive knowledge of the anatomy of the digastric muscle along with its variations being extensively documented. The anatomical variations in the digastric muscle were more evident in the anterior belly of the muscle. These variations included the presence of the accessory belly occurring either unilateral or bilateral. Therefore, variations in the origin, insertion and shape of the anterior accessory belly were documented. On the other hand, variations in the PBDM were reported to be very rare. From the literature reviewed, there is a paucity of reports describing the digastric muscle in fetal cadaveric specimens. This manuscript seeks to document the morphology and morphometry of the digastric muscle in a fetal sample. Therefore, the objectives of this manuscript will be to:

i. Document the morphology of the digastric muscle in fetuses, with respect to its origin and insertion
ii. Document the morphometry of the digastric muscle
iii. Document morphological variations in the digastric muscle

TITLE PAGE

Title	**:**	Morphologic and morphometric analysis of the digastric muscles: A fetal study
Subtitle	:	Anatomical study of the digastric muscle

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Number of Figures	:	16
Number of Tables	:	2
Type of article	:	Original communication

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ABSTRACT

Introduction: The digastric muscle is located in the suprahyoid region originating with two muscle bellies namely, the anterior and posterior belly. They originated from the digastric fossa of the mandible and mastoid notch of the temporal bone respectively. The intermediate tendon (IT) joined these bellies and inserted into the hyoid bone. This study aimed to investigate the morphology and morphometry of the digastric muscle in a fetal cadaveric sample.

Materials and methods: Forty fetal (n=80) head and neck specimens were micro-dissected bilaterally. The origin of the anterior belly of the digastric muscle (ABDM) was classified into four types, the course of the IT was classified into three types while the origin of the posterior belly of the digastric muscle (PBDM) was classified into two types.

Results: The ABDM originated from the digastric fossa of the mandible in all of the specimens. The ABDM inserted to the lesser of the hyoid bone (80%), and into the body and greater horn of the hyoid bone (20%). The PBDM arose from the mastoid notch of the temporal bone, and from the lateral surface and tip of the mastoid process in 57.5% and 42.5% of the specimens, respectively. The PBDM originating with three bellies was documented (2.5%). The IT coursed superiorly and inferiorly to the stylohyoid muscle in 5% and 2.5% of the specimens.

Conclusion: The fetal morphology reflected anatomical variations in the digastric muscle that have not been previously documented and may assist the surgeon during clinical interventions, such as digastric muscle transfer surgery.

Keywords: Digastric muscle, anterior belly, posterior belly, morphology, morphometry, variations.

INTRODUCTION

The digastric muscle is found in the suprahyoid region and is made up of two bellies or "heads" of origin *viz*. the anterior and posterior belly originating from two distinct bony landmarks *viz*. the mandible and temporal bone (Standring *et al*., 2016). The varying origin is due to the two bellies being embryologically derived from separate sources of branchial arches i.e. the first and second arches (Drake *et al*., 2015). The anterior belly of the digastric muscle (ABDM) originates from the digastric fossa on the internal border of the mandible and the base of the mandible inferiorly, then descended posteriorly, superficial to the mylohyoid muscle (Standring *et al*., 2016). Whereas, the posterior belly of the digastric muscle (PBDM), originates as a thin slender muscle belly from the mastoid notch of the temporal bone adjacent to the stylomastoid foramen from which it passes antero-inferiorly deep to the sternocleidomastoid muscle to become continuous with the intermediate tendon (IT).

The two muscle bellies join at the IT which passes through the stylohyoid muscle and inserts by a fibrous connective tissue to the body and greater horn of the hyoid bone (Harrison *et al*., 2009; Chaurasia, 2010; Standring *et al*., 2016). The anterior and posterior bellies of the digastric muscle receive their innervation mainly from the facial and the mandibular branch of the trigeminal nerve, respectively (Standring *et al*., 2016).

Anatomical literature describes the digastric muscle as having the most variable ABDM, with the presence of more than one accessory belly with duplication, trifurcation and quadrification of accessory bellies which are either unilateral or bilateral (Kyung *et al*., 2011; Kalniev *et al*., 2013; Rani *et al*., 2013). The origin of the ABDM is extensively variable from (i) the digastric fossa, (ii) the relevant anterior belly, (iii) the contralateral anterior belly, (iv) the intermediate tendon, (v) the mylohyoid raphe, (vi) the internal and (vii) external surfaces of the mandible, (viii) the digastric fossa and even the opposite digastric fossa (Aktekin *et al*., 2003; Fijumura *et al*., 2003; Turan-Ozdemir *et al*., 2004; Liquidato *et al*., 2007; Ozgur *et al*., 2010; Quadros *et al*., 2013; Buffoli *et al*., 2016). Several authors documented that the accessory bellies may also cross over the midline of the mylohyoid raphe to insert into the contralateral digastric fossa of the mandible and into the distal portion of the ABDM (Mangalagiri and Razvi, 2009; Nayak *et al*., 2017). Hegeman *et al*.

(2010), also described a quadrificated accessory belly arranged in an "X" shaped pattern in the submental triangle.

Unilateral occurrences of the accessory bellies have been frequently reported as either symmetrical or asymmetrical originating in the locations mentioned above (Mascaro *et al*., 2011; Yamakazi *et al*., 2011; Rani *et al*., 2013; Nayak *et al*., 2016). Bilateral accessory bellies have been documented to display different anatomical patterns, i.e. weave pattern, 'X' and 'Y' configurations (Turan-Ozdemir *et al*., 2004; Loukas *et al*., 2005; Reyes *et al*., 2007; Liquidato *et al.*, 2007; Sevinç *et al.*, 2009; Azeredo *et al*., 2016). Thus, the classification criterion suggested in these studies concerning the unilateral and bilateral occurences was based on the morphological and numeral types. Authors have classified these variations of accessory bellies as either (i) the digastric fossa type, where the accessory belly originated and inserted on the same side; or (ii) the crossover type where the accessory belly crossed the midline of the mylohyoid raphe (Ozgur *et al*., 2010). However, Zhao *et al*. (2015) classified these muscles according to number of the accessory bellies present. They classified four types of the ABDM originating with one and up to four muscle bellies (Zhao *et al*., 2015).

With regards to the PBDM, the occurrence of an accessory or duplicated posterior belly is very rare in the literature and few have been documented. For example, an accessory posterior muscle belly emanating from the mastoid notch of the temporal bone descending and attaching to the infrahyoid muscles inferiorly has been reported by Ozgursory and Kucuk (2006). Mehta *et al*. (2011) also reported a duplicated belly emanating from the mastoid notch to become continuous with the common tendon. Zhao *et al*. (2015) later described an additional origin of the PBDM through fascial slips from the styloid process, and the middle and lower pharyngeal constrictor muscles. As the number of paediatric surgical interventions in the neck may involve the digastric muscle, its anomalies may have surgical significance and implications. Therefore, the aim of this study was to investigate and document the morphology and morphometry of the digastric muscle in a South African fetal population.

MATERIALS AND METHODS

A bilateral micro-dissection of the digastric muscle on 40 fetuses (n = 80; 26 males and 14 females) by aid of a Carl Zeiss Stemi DV4 Stereo Microscope was conducted. The fetuses were obtained from the Department of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences, College of Health Sciences at the University of KwaZulu-Natal, South Africa. The fetal gestational age was calculated using the Pandey *et al*. (2016) method and ranged from 22 – 31 weeks old. Ethical clearance was obtained from the University Biomedical Research Ethics Committee (BE530/17).

After removal of the skin and superficial fascia, the platysma and sternocleidomastoid muscles were reflected to expose the underlying digastric muscle. The digastric muscle was observed and classified according to Zhao *et al*. (2015) classification method. The ABDM with one, two, three or four muscle bellies was classified into four types (Type I – IV). The IT was classified into three types: Type I which pierced the stylohyoid muscle; Type II which coursed superficial to the stylohyoid muscle; and Type III which coursed deep to the stylohyoid muscle. The PBDM was classified into two types: Type I (narrow) originated from the mastoid notch of the temporal bone, and Type II (broad) PBDM arose from the styloid process, middle and inferior pharyngeal constrictor muscles (Zhao *et al*., 2015).

The morphometric parameters measured were muscle belly length (ABDM: from the digastric fossa of the mandible to the body of the hyoid bone; PBDM: from the tip of the mastoid to the posterior end of the IT) , muscle belly width across the middle of the muscle belly (from the lateral border to the medial border of the muscle belly), IT length (from the posterior end of the ABDM to the anterior end of the PBDM) and IT width across the IT (from the lateral border to the medial border of the IT). A Digital Vernier caliper (Mitutoyo 0-8 in [0-203.2mm] Absolute digimatic caliper series 500, accuracy ± 0.001 in [0.025mm]) was used to acquire the measurement. The measurements obtained were analysed by use of descriptive and inferential statistics with the Statistical Package for Social Sciences software (SPSS version 25.0). A p-value of < 0.05 was regarded as statistically significant.

RESULTS

A) Morphological Analysis of the Digastric Muscle

I) Origin of the muscle belly

1. The ABDM originated from the digastric fossa and inferior border of the base of the mandible in 100% ($^{40}/_{40}$) of the specimens (Plate 1).

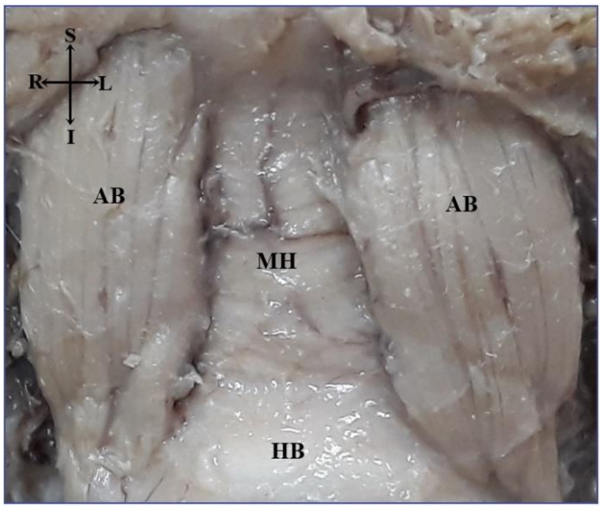

Plate 1: Dissection of the suprahyoid region showing the anterior muscle bellies of the digastric muscle.

Key:
Anterior belly of the digastric muscle (**AB**)
Hyoid bone (**HB**)
Mandible (**M**)
Mylohyoid muscle (**MH**)

2. The Type I PBDM (narrow type) was observed in 57.5% ($^{46}/_{80}$) of the specimens (Plate 2). In 5% ($^{4}/_{80}$) of the specimens, the PBDM was variable.

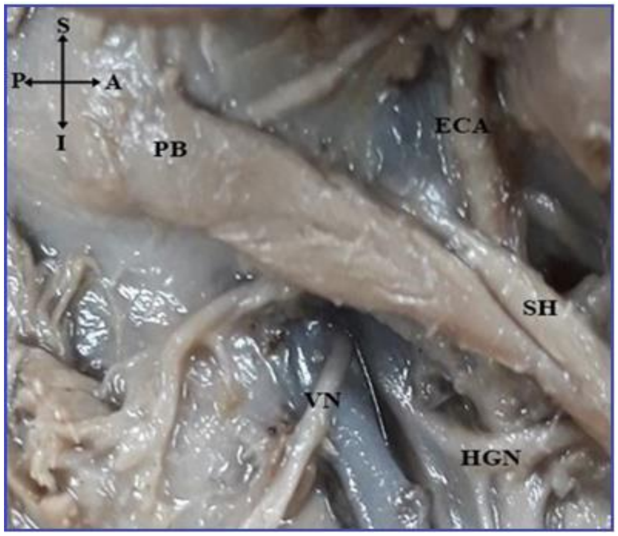

Plate 2: Photograph showing the origin of the posterior belly of the digastric muscle with a narrow muscle belly.

Key:

External carotid artery (**ECA**)

Hypoglossal nerve (**HGN**)

Posterior belly of the digastric muscle (**PB**)

Stylohyoid muscle (**SH**)

Vagus nerve (**VN**)

3. An additional Type III of the PBDM was observed in 42.5% ($^{34}/_{80}$) of the specimens, where the PBDM originated from the mastoid notch, lateral surface and tip of the mastoid process (Plate 3).

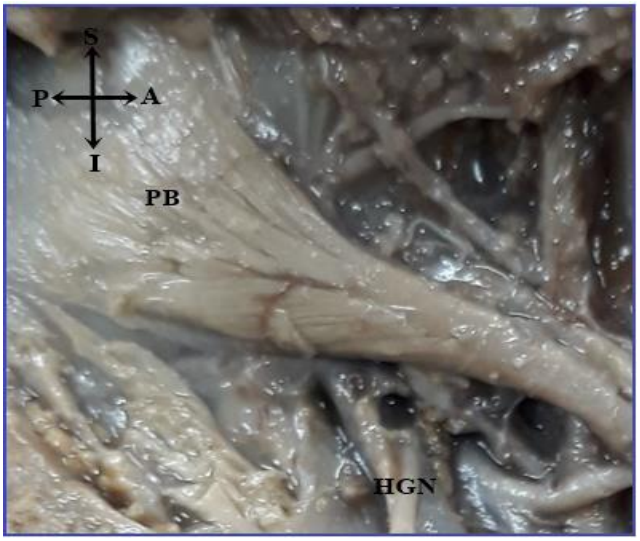

Plate 3: Photograph showing the origin of the PBDM with a broad muscle belly.

Key:

Posterior belly of the digastric muscle (**PB**)

Hypoglossal nerve (**HGN**)

a) Variations in the Anterior Digastric Muscle Belly

Variations in the ABDM were observed in 2.5% ($^{1}/_{40}$) of the specimens.

1. A bilateral 'butterfly shaped' anterior accessory belly was observed in 2.5% ($^{1}/_{40}$) of the specimens. It originated from the mylohyoid muscle deep to the main muscle bellies of the anterior digastric muscle and inserted onto the mylohyoid raphe and body of the hyoid bone (Plate 4).

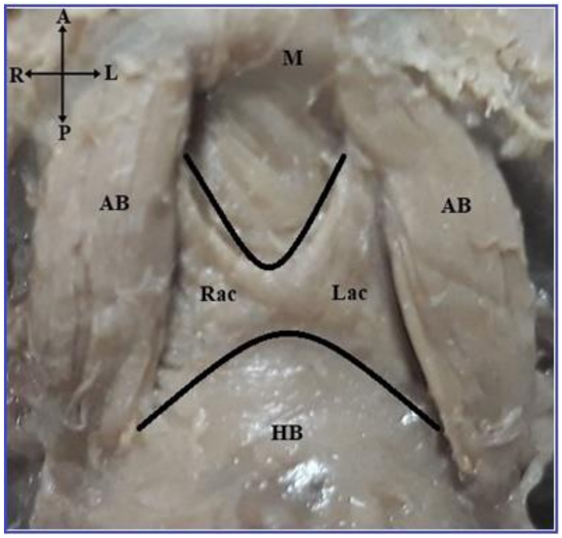

Plate 4: Photograph of a ‘butterfly’ shaped accessory belly of the ABDM.

Key:

Anterior belly of the digastric muscle (**AB**)

Hyoid bone (**HB**)

Left accessory belly (**Lac**)

Mandible (**M**)

Right accessory belly (**Rac**)

2. In another specimen, the medial borders of the right- and left-sided ABDM expanded into the submental space was observed in 2.5% ($^{1}/_{40}$) of the specimens (Plate 5).

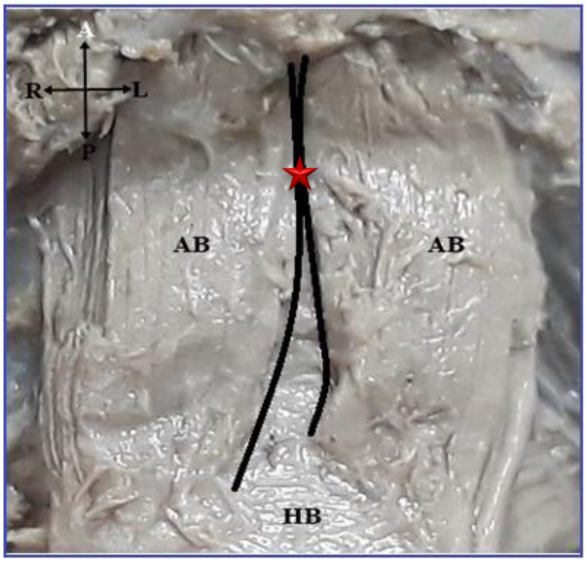

Plate 5: Photograph of a flattened ABDM.

Key:

Anterior belly of the digastric muscle (**AB**)

Hyoid bone (**HB**)

Attaching ABDM (★)

b) Variation in the Posterior Digastric Muscle Belly

A trifurcated PBDM i.e. an upper, middle and lower belly was observed in 2.5% ($^{2}/_{80}$) of the fetal specimens (Plate 6). The upper belly originated from the mastoid process, coursed antero-inferiorly and inserted through a small tendon onto the superior border of the conjoined muscle belly of the middle and lower bellies. Both the middle and lower bellies originated from the lateral border of the mastoid process and became continuous anteriorly through a common muscle belly. Thereafter, all three muscle bellies joined to form the common IT.

Plate 6: Trifurcated origin of the posterior belly at the mastoid process.

Key:

Ansa cervicalis nerve (**AC**)

Anterior belly of the digastric muscle (**AB**)

Posterior belly of the digastric muscle (**PB**)

Hypoglossal nerve (**HGN**)

Mylohyoid muscle (**MH**)

Stylohyoid muscle (**SH**)

Vagus nerve (**VN**)

Upper belly of the PB (**UB**)

Middle belly of the PB (**MB**)

Lower belly of the PB (**LB**)

II) The Intermediate Tendon (IT)

1. The course of the IT of the digastric muscle was observed piercing through the stylohyoid muscle, was observed in 77.5% ($^{62}/_{80}$) of the specimens (Plate 7).

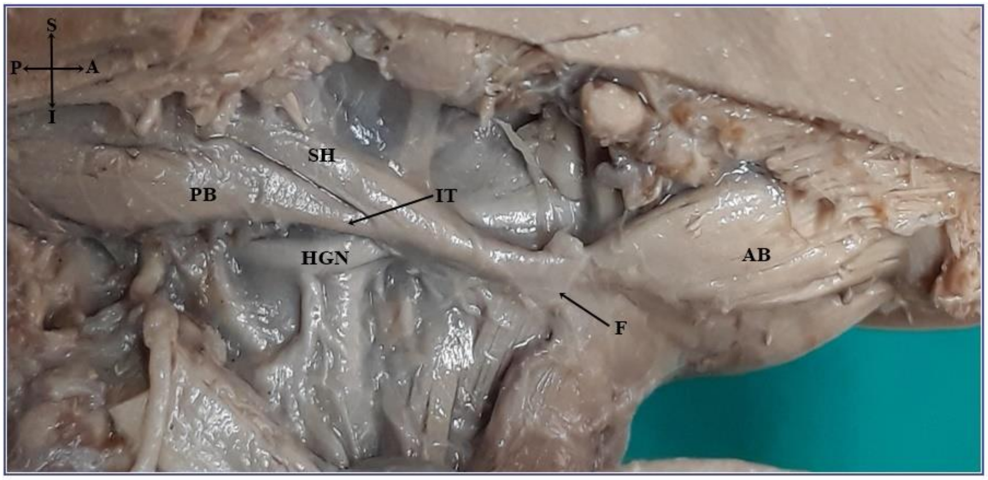

Plate 7: Photograph of the IT course through the stylohyoid muscle.

Key:

Anterior belly of the digastric muscle (**AB**)

Posterior belly of the digastric muscle (**PB**)

Intermediate tendon (**IT**)

Fascial loop (**F**)

Hypoglossal nerve (**HGN**)

Stylohyoid muscle (**SH**)

2. The IT coursed lateral or superficial to the stylohyoid muscle was observed in 10% ($^{8}/_{80}$) of the specimens before inserting into the hyoid bone (Plate 8).

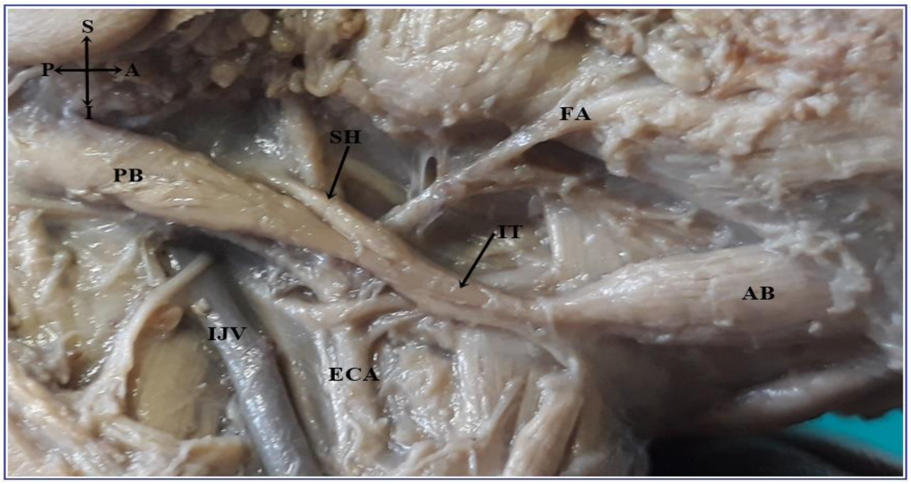

Plate 8: Intermediate tendon located lateral to the stylohyoid muscle.

Key:

Anterior belly of the digastric muscle (**AB**)

Posterior belly of the digastric muscle (**PB**)

External carotid artery (**ECA**)

Facial artery **(FA**)

Internal jugular vein (**IJV**)

Intermediate tendon **(IT**)

Stylohyoid muscle (**SH**)

3. The IT travelled medial or deep to the stylohyoid muscle was observed in 5% ($^{4}/_{80}$) of the specimens and attached into the hyoid bone (Plate 9).

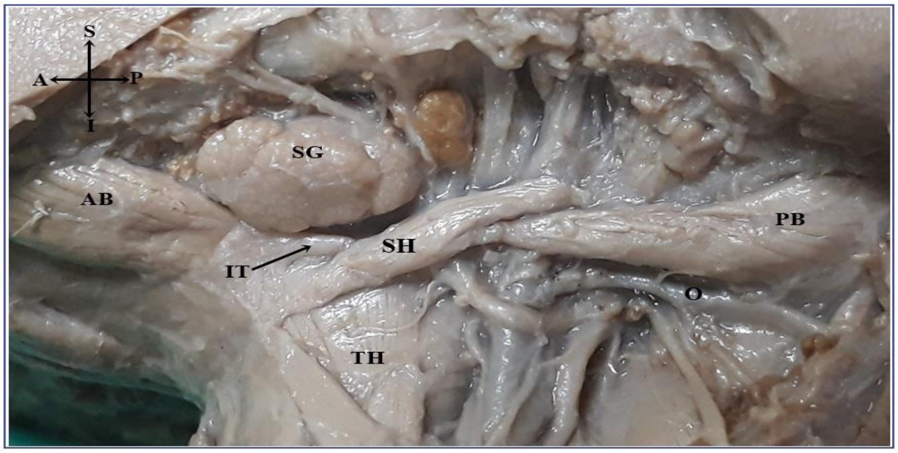

Plate 9: Intermediate tendon located medial to the stylohyoid muscle.

Key:

Anterior belly of the digastric muscle (**AB**)

Posterior belly of the digastric muscle (**PB**)

Intermediate tendon **(IT**)

Occipital artery (**O**)

Submandibular gland (**SG**)

Stylohyoid muscle (**SH**)

Thyrohyoid muscle (**TH**)

a) *Variations in the IT Course*

The IT had a variable course which was observed as superior, inferior, medial (deep) and lateral (superficial) to the stylohyoid muscle was observed in 22.5 % ($^{18}/_{80}$) of the specimens.

1. The IT was located superior to the stylohyoid muscle was observed 5% ($^{4}/_{80}$) of the specimens (Plate 10).

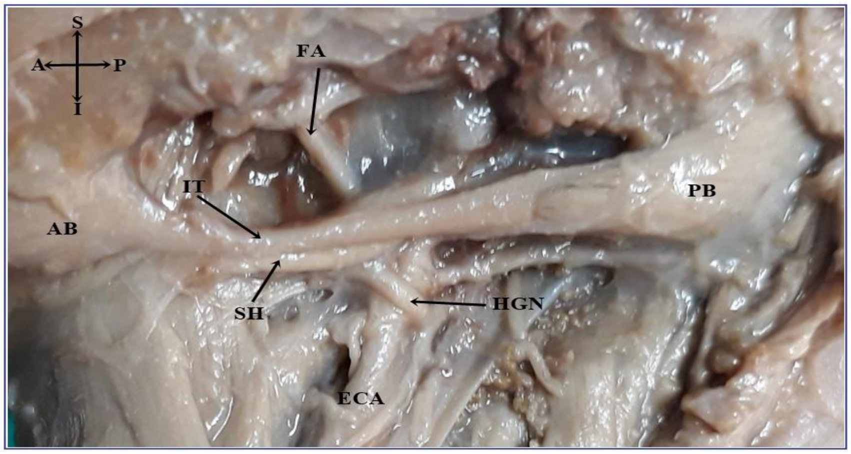

Plate 10: Intermediate tendon located superior to the stylohyoid muscle.

Key:

Anterior belly of the digastric muscle (**AB**)

Posterior belly of the digastric muscle (**PB**)

External carotid artery (**ECA**)

Facial artery (**FA**)

Hypoglossal nerve (**HGN**)

Intermediate tendon (**IT**)

Stylohyoid muscle (**SH**)

2. In 2.5% ($^{2}/_{80}$) of the specimens, the IT was observed inferior to the stylohyoid muscle prior to inserting onto the hyoid bone (Plate 11).

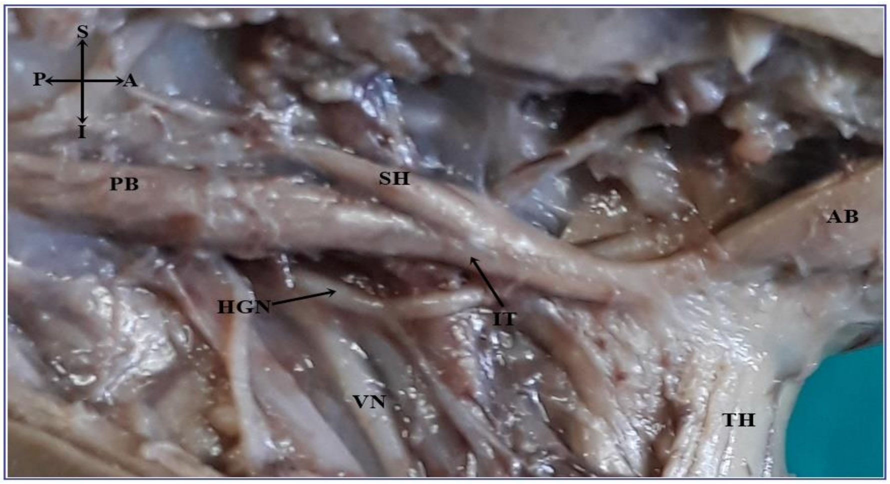

Plate 11: Intermediate tendon located inferior to the stylohyoid muscle.

Key:

Anterior belly of the digastric muscle (**AB**)

Posterior belly of the digastric muscle (**PB**)

Hypoglossal nerve (**HGN**)

Intermediate tendon **(IT**)

Stylohyoid muscle (**SH)**

Thyrohyoid muscle (**TH**)

Vagus nerve (**VN**)

III) Insertion of the Digastric muscle

The anterior and posterior muscle bellies inserted through a short common IT into the body and greater horn of the hyoid bone. However, a variable insertion was observed in the ABDM.

1. The ABDM inserted through a narrow muscle belly into the lesser horn of the hyoid bone, in 80% ($^{64}/_{80}$) of the specimens (Plate 12).

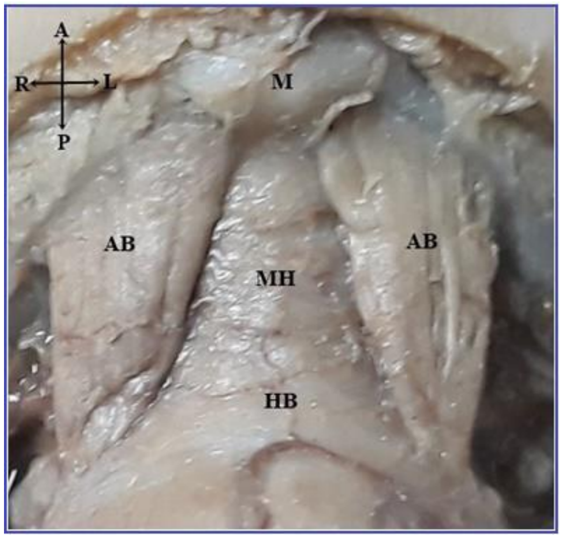

Plate 12: Photograph of the narrowed insertion of the ABDM at the hyoid bone.

Key:

Anterior bellies of digastric muscle (**AB**)

Hyoid bone (**HB**)

Mandible (**M**)

Mylohyoid muscle (**MH**)

2. In 20% ($^{16}/_{80}$) of the specimens, the ABDM had a broad muscle belly inserting onto the body, lesser and greater horn of the hyoid bone (Plate 13).

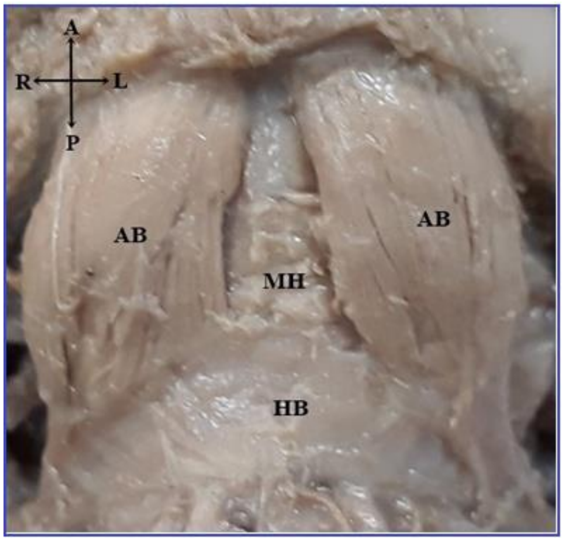

Plate 13: Photograph showing the broader insertion of the ABDM at the hyoid bone.

Key:
Anterior bellies of digastric muscle (**AB**)
Hyoid bone (**HB**)
Mylohyoid muscle (**MH**)

B) Morphometric Analysis of the Digastric Muscle

i. Anterior Belly of the Digastric Muscle

The average lengths between the right and left-sided ABDM in males, were 11.46 ± 1.75mm and 11.23 ± 1.92mm *(mean ± SD)*, with an average width of 4.14 ± 0.65mm and 4.00 ± 0.58mm (*mean ± SD*), respectively. While, females had an average length of 11.07 ± 1.41mm and 10.74 ± 1.56mm *(mean ± SD)* for each of respective side. The muscle belly width was determined to be 4.65 ± 1.10mm on the right and 4.54 ± 0.90mm *(mean ± SD)* on the left side (Plate 14).

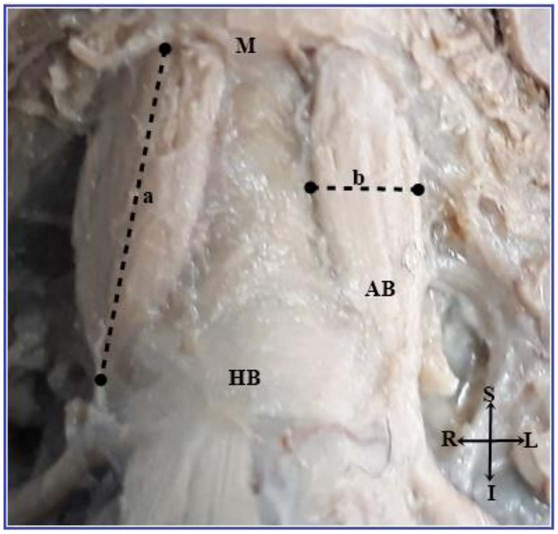

Plate 14: Parameters measured in the ABDM.

Key:

Anterior belly of the digastric muscle (**AB**)

Hyoid bone (**HB**)

Mandible (**M**)

Length of the AB (**a**)

Width of the AB (**b**)

ii. Intermediate Tendon

The minimum and maximum lengths of the IT in males, were 7.78mm and 7.88mm, respectively. While, in females the IT length ranged between 7.83mm and 8.12mm. The average IT width between right and left sides was 0.68 ± 0.18mm and 0.63 ± 0.19mm for males, and 0.61 ± 0.23mm and 0.62 ± 0.18mm *(mean ± SD)* for females (Plate 15).

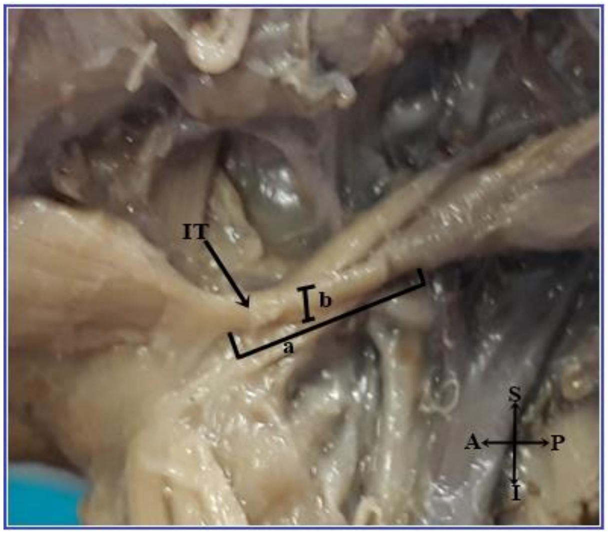

Plate 15: Parameters measured in the IT.

Key:

Intermediate tendon (**IT**)

Length of the IT (**a**)

Width of the IT (**b**)

iii. Posterior Belly of the Digastric Muscle

The average muscle belly length documented from males in right and left sides, was 11.67 ± 1.54mm and 11.5 ± 1.13mm *(mean ± SD)*, respectively. Each side had a corresponding average width of 0.68 ± 0.18mm *(mean ± SD)*. Whereas, the average PBDM width from females on the right and left-sides, was 2.82 ± 0.58mm and 2.82 ± 0.81mm *(mean ± SD)*, respectively (Plate 16).

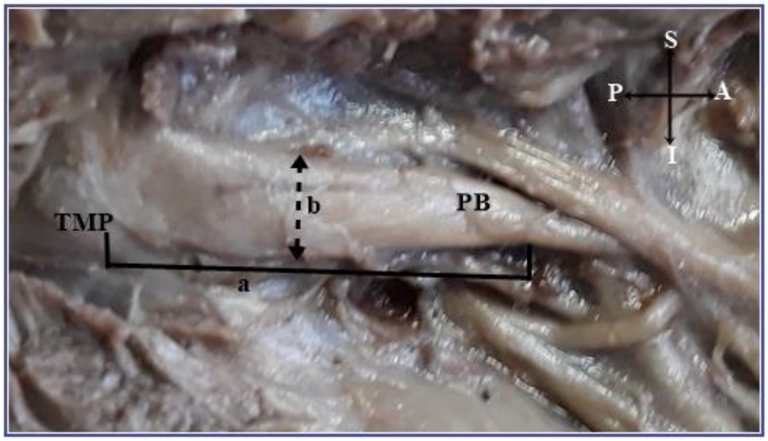

Plate 16: Parameters measured in the PBDM.

Key:

Posterior belly of the digastric muscle (**PB**)

Tip of the mastoid process (**TMP**)

Length of the PB (**a**)

Width of the PB (**b**)

The morphometry of the digastric muscle and the IT are reflected in Table 1 below.

Table 1: Comparison between the average length and width of the digastric muscle in males and females

Digastric muscle	**Length Average T-Test *(mean ± SD)***			**Width Average T-Test *(mean ± SD)***		
	Right (mm)	**Left (mm)**	***P-value***	**Right (mm)**	**Left (mm)**	***P-value***
Males						
ABDM	11.46 ± 1.75	11.23 ± 1.92	0.196	4.14 ± 0.65	4.00 ± 0.58	0.205
IT	7.78 ± 1.27	7.88 ± 1.31	0.553	0.68 ± 0.18	0.63 ± 0.19	0.187
PBDM	11.67 ± 1.54	11.5 ± 1.13	0.519	2.89 ± 0.64	2.85 ± 0.59	0.555
Females						
ABDM	11.07 ± 1.41	10.74 ± 1.56	0.150	4.65 ± 1.10	4.54 ± 0.90	0.603
IT	8.12 ± 1.24	7.93 ± 1.28	0.360	0.61 ± 0.23	0.62 ± 0.18	0.924
PBDM	11.53 ± 1.92	11.21 ± 2.85	0.640	2.82 ± 0.58	2.82 ± 0.81	0.990

There was no statistically significant difference between males and females ABDM lengths from the right side ($P = 0.406$) and left side ($P = 0.439$). However, there was a statistically significant difference between males and females left-sided anterior belly widths ($P = 0.034$) (Table 2). The IT lengths of paired right and left sides from males and females were not statistically significant, $P = 0.548$ and $P = 0.766$ were documented, respectively. However, females were found to have significantly longer IT than males on both sides.

The PBDM revealed no statistically significant difference between the right and left muscle belly lengths from both males and females, $P = 0.750$ and $P = 0.789$ were documented, respectively. No statistically significant differences were also documented between the right- and left-sided muscle belly widths ($P = 0.669$ and 0.987) (Table 2).

Table 2: Comparison of the right and left digastric muscle against males and females

Digastric muscles	Side (s)	Gender	Length Average T-Test *(mean ± SD)*		Width Average T-Test *(mean ± SD)*	
			Average (mm)	*P* - value	Average (mm)	*P* - value
ABDM	**R**	M	11.61 ± 1.79	0.406	4.17 ± 0.66	0.211
		F	11.07 ± 1.41		4.65 ± 1.10	
	L	M	11.30 ± 1.79	0.439	3.97 ± 0.50	**0.034***
		F	10.74 ± 1.56		4.54 ± 0.90	
IT	**R**	M	7.79 ± 1.34	0.548	0.69 ± 0.17	0.365
		F	8.12 ± 1.24		0.61 ± 0.23	
	L	M	7.76 ± 1.29	0.766	0.65 ± 0.16	0.667
		F	7.93 ± 1.28		0.62 ± 0.18	
PBDM	**R**	M	11.29 ± 1.38	0.750	2.92 ± 0.61	0.669
		F	11.53 ± 1.92		2.82 ± 0.58	
	L	M	11.44 ± 1.10	0.789	2.81 ± 059	0.987
		F	11.21 ± 2.85		2.82 ± 0.81	

* Statistically significant ($p < 0.05$)

DISCUSSION

Morphological variations in the digastric muscle are common and involves the ABDM which has been described as the most variable when compared to the PBDM. Furthermore, unilateral variations are more dominant compared to bilateral variations. Various authors suggested that the abnormal migration of the neural crest cells and deficient mesoderm in the pharyngeal arches may cause these variations (Loukas *et al*., 2005; Raju *et al*., 2014; Buffoli *et al*., 2016). As a result, numerous reports have studied and classified anomalies concerning this muscle since these variations may be significant during surgery. In this study, variations were observed in the origin and insertion of the digastric muscle belly, and in the course of the IT.

The origin of the ABDM from the digastric fossa and inferior base of the mandible corroborated with standard anatomical literature (Standring *et al*., 2016). Since variations are common in the ABDM, the accessory belly may arise from the anterior belly, the contralateral anterior belly, the IT, the mylohyoid muscle and the mandible (Rani *et al*., 2013; Buffoli *et al*., 2016). In a study by Mascaro *et al*. (2011), they described 'X' configured bellies that originated from the respective anterior digastric muscle and inserted in the digastric fossa while, other authors reported accessory bellies forming a 'Y' configuration, weave pattern and triangular shaped belly (Mangalagiri and Razvi, 2009; Hegeman *et al*., 2010; Harvey *et al*., 2015). In this study, a bilateral anomalous belly was observed in the main anterior digastric muscle, which had flattened rectangular-shaped bellies with intersecting medial borders. In another case, accessory anterior bellies formed a 'butterfly' configuration in the submental region. These bellies originated deep to the anterior digastric muscle and attached to the midline raphe of the mylohyoid muscle centrally. These findings appear to be unique.

The PBDM demonstrated two modes of origin in which a narrow muscle belly (Type I) arose from the mastoid notch of the temporal bone, and a broad muscle belly (Type III) arose from the lateral surface and tip of the mastoid notch. The Type I posterior belly followed the classification described by Zhao *et al*. (2015), while the Type III appeared to be a new additional classification proposed by this study. Although variations in the PBDM are very rare, authors have reported a posterior belly emanating with dual bellies (Ozgursory and Kucuk, 2009; Mehta *et al*., 2011; Zhao *et al*., 2015). In this study, a posterior muscle belly with three bellies of origin is reported. The upper, middle and lower belly originated in the mastoid process of the temporal bone and formed a common belly which was continuous with the IT. This finding was deemed rare but important, since excess musculature in this region has been suggested as a potential cause of compression of the neurovascular vessels travelling beneath the PBDM (Mehta *et al*., 2011).

Although the digastric muscle inserts via a short common IT which perforates the distal part of the stylohyoid muscle (Drake *et al*., 2015). Zhao *et al*. (2015), mentioned that the IT may either travel superficial or deep to the stylohyoid muscle prior to inserting into the hyoid bone. These anatomical descriptions of the IT were also observed in this study. Moreover, the IT was located superiorly (5%) and inferiorly (2.5%) to the stylohyoid muscle. These findings appear to be unique to this study. Furthermore, the ABDM showed dual insertion into the hyoid bone either through a narrow or broad muscle belly in all specimens.

The morphometric analysis of the digastric muscle in this study revealed a statistically significant difference between paired widths between males and females ABDM ($P = 0.034$), where females had a significantly longer left-sided muscle belly than males. There was no statistically significant difference between the lengths of the anterior and posterior muscle bellies on both sides. However, males had a longer left-side ABDM on average length than females. Zdilla *et al*. (2016) reported a longer left-sided ABDM in males and suggested that the distances between the digastric fossa of the mandible, hyoid bone and the mastoid notch could be equivalent on either side, therefore the left-sided PBDM would be shorter than that on the right side (Zdilla *et al*., 2016). In addition, the IT was significantly longer in females.

CONCLUSION

This study highlighted the variability in morphology of the digastric muscle, its muscle bellies and IT, with respect to its origin and insertion, as well as the course of the IT in a cadaveric fetal sample. This study adds to the anatomical knowledge of the digastric muscle in fetuses and provides a comprehensive overview of the variety in the digastric muscle with regards to the anatomical origin of the PBDM from different sites in the mastoid process, and the insertion mode of the ABDM into the hyoid bone. Furthermore, the findings also highlighted an interchangeable course of the IT through the stylohyoid muscle. Moreover, this study provided an overview of the morphometry of the fetal digastric muscle with respects to its length and width, which revealed a statistically insignificant difference in the digastric muscle except for paired left-sided ABDM between males and females. The involvement of the digastric muscle in head and neck paediatric surgery e.g. submental artery flaps and submental rejuvenation, requires the transfer of the ABDM and its associated IT. Therefore, a thorough understanding of the anatomy and variations of the digastric muscle is important.

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CHAPTER 3

SCIENTIFIC MANUSCRIPT II

The previous manuscript discussed the morphology and morphometry of the digastric muscle in the fetal sample. Morphologically, muscular variations were documented in the origin and insertion of the digastric muscle bellies as well as in the course of the intermediate tendon. Morphometrically, the muscle belly length and width of the digastric muscle was statistically insignificant except for the paired ABDM width which revealed a statistically significant left ABDM. The following manuscript will document the anatomical variations of the digastric muscle in the adult sample, particularly in the anterior belly of the digastric muscle. Therefore, the objectives of this manuscript will be to:

i. Document variation in the anterior belly of the digastric muscle
ii. Document the occurrence of the accessory belly of the anterior belly of the digastric muscle.

This manuscript was prepared for and submitted to the International Journal of Morphology (manuscript track number: IJM 039-19).

Title	:	Anatomical variations of the anterior belly of the digastric muscle.
Subtitle	:	Morphological analysis of the ABDM

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Number of Figures	:	6
Type of article	:	Original communication

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ABSTRACT

Introduction: A routine dissection of the digastric muscle reflected that it originated by two muscle bellies namely, the anterior and posterior belly which are connected by an intermediate tendon (IT). These bellies originated from the mastoid process of the temporal bone and the digastric fossa of the mandible respectively. The digastric muscle serves as an important landmark in surgical interventions involving the submental area however, accessory bellies may interfere with surgical intervention in this area. Therefore, this study aimed to document the occurrence of the anatomical variations in the anterior belly of the digastric muscle (ABDM) in a selected number of cadaveric samples.

Materials and methods: Ten bilateral adult cadaveric head and neck specimens (n = 20) were macro-dissected in order to document the morphology of the digastric muscle.

Results: The accessory bellies in the ABDM was observed in 60% of the specimens. Unilateral and bilateral variations were observed in 20% and 30% of the specimens, respectively. These accessory bellies originated in the digastric fossa, ABDM, IT and hyoid bone, and inserted into the mylohyoid raphe, mylohyoid muscle and hyoid bone. In addition, an anomalous main ABDM was observed in 10% of the specimens inserting through a transverse tendon into the hyoid bone.

Conclusion: Variations in the digastric muscle are common especially the accessory bellies, therefore, a comprehensive understanding of these anatomical variations could be of clinical importance to the surgeons during head and neck radiological diagnosis and surgical interventions.

Key Words: Digastric muscle, anterior belly, accessory belly, anatomical variation.

INTRODUCTION

The digastric muscle originates as dual bellies; an anterior and posterior belly, from the inferior base of the cranium and the inferior border of the mandible, hence called the 'digastric'. The intermediate tendon (IT) unites these muscle bellies and curved at an angle above the hyoid bone and inserts into this bone by fascia (Harrison *et al*., 2009; Standring *et al*., 2016). The ABDM originates from the digastric fossa of the mandible, while the posterior belly of the digastric muscle (PBDM) originated from the mastoid notch of the temporal bone (Harrison *et al*., 2009; Moore *et al*., 2013). Morphologic variations in the digastric muscle have been linked with the anomalous arches during its development.

As a result, the morphologic variation in the digastric musculature includes the presence of an accessory belly in the ABDM and has been the commonly documented in the literature reviewed, with various authors describing different forms, shapes and attachments of this variant muscle. Firstly, the accessory belly may originate either from the mandible, mylohyoid muscle, IT, digastric fossa, and the main anterior or opposite anterior belly (Rani *et al*. 2013; Quadros *et al*., 2013; Azedero *et al*., 2015). Secondly, it may insert into the midline raphe of the mylohyoid muscle, the IT, the mandible and contralateral ABDM (Aktekin *et al*., 2003; Reyes *et al*., 2007; Rani *et al*., 2013). Lastly, the accessory belly may vary in its course and travel either ipsilaterally on the same side of its origin from the mandible or contralaterally crossing the mandible from its point of origin. (Liquidato *et al*., 2007; Kyung *et al*., 2011; Azeredo *et al*., 2016).

With its pivotal role in a variety of surgical procedures involving the submental region such as submental artery flaps, rejuvenation, submental rhytidectomy and lipectomy (Zdilla *et al*., 2016). Knowledge of the anatomy of the digastric muscle and its variations is of importance. especially when the accessory belly could be easily misinterpreted during radiographic imaging of the submental region. Therefore, this study is aimed to document the anatomical variations of the ABDM in a selected number of adult cadaveric specimens.

MATERIALS AND METHODS

Macro-dissection of the digastric muscle was conducted bilaterally on 10 adult cadaveric head and neck specimens (n = 20). The sample size consisted of 8 males and 2 females between the age range of 33 – 84 years old, which were obtained from the Department of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, South Africa. Ethical clearance was obtained from the University Biomedical Research Ethics Committee (BE530/17). The skin and subcutaneous fascia overlying the anterior aspect of the neck was removed. The overlying platysma and sternocleidomastoid muscles were dissected and gently retracted superiorly exposing the underlying digastric muscle. The origin, course and insertion of the digastric muscle were documented according to Zhao *et al*. (2015) classification method and digital photographs of the digastric musculature were taken using a camera (Canon PowerShot SX50 HS, 12.1 Megapixel), for morphological analysis.

RESULTS

Morphological Analysis of the Digastric Muscle

In 40% ($^4/_{10}$) of the specimens, the typical origin and insertion of the digastric muscle was observed. While, the remainder (60%; $^6/_{10}$) showed a morphological variance in the accessory bellies. The accessory bellies documented were both unilateral and bilateral. These bellies arose from the digastric fossa, the IT, the medial border of the main ABDM, the mandible and hyoid bone.

i. The main anterior bellies of the digastric muscle had a typical origin from the mandible as described in the standard anatomical literature, however, it had an abnormal insertion onto the hyoid bone via a transverse tendon situated above the hyoid bone. The medial portions of these bellies were continuous distally (Plate 1).

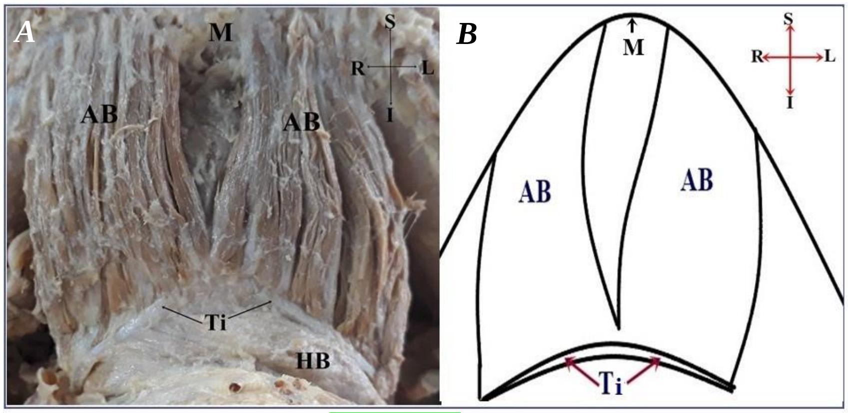

Plate 1: The photograph (A) and associated illustration (B) showing a varying main ABDM.

Key:

Anterior belly of the digastric muscle (**AB**)

Hyoid bone (**HB**)

Mandible (**M**)

Tendinous insertion (**Ti**)

ii. Unilateral left accessory belly originated via a narrow tendon from the inferior border of the mandible and coursed superficially on the left ABDM, and its fibers were inserted into the hyoid bone (Plate 2).

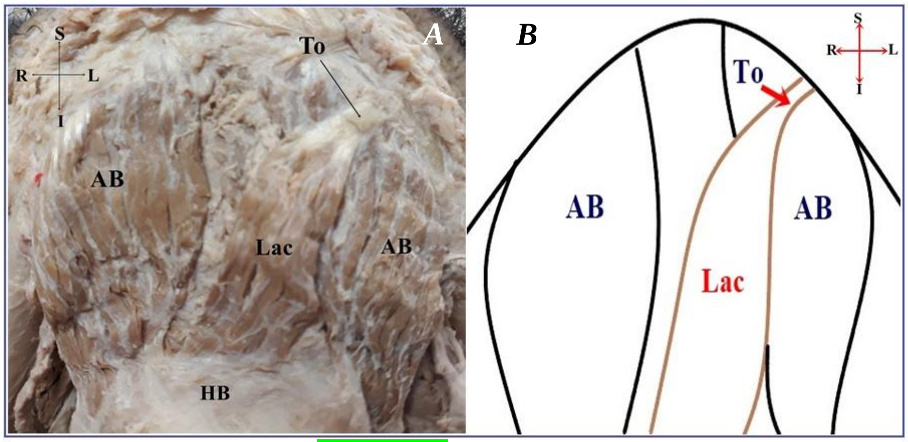

Plate 2: The photograph (A) and associated illustration (B) showing the oblique left-sided accessory belly of the ABDM.

Key:
Anterior belly of the digastric muscle (**AB**)
Hyoid bone (**HB**)
Left accessory belly (**Lac**)
Tendinous origin (**To**)

iii. A Type I (unilateral) triangulated left accessory belly originated from left ABDM posteriorly and inserted into the midline of the mylohyoid raphe (Plate 3).

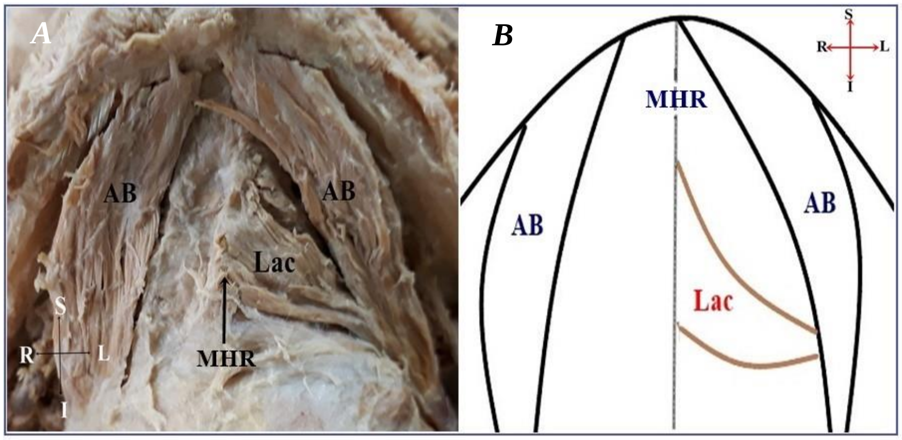

Plate 3: The photograph (A) and associated illustration (B) showing a left-sided accessory belly of the ABDM.

Key:

Anterior belly of the digastric muscle (**AB**)

Left accessory belly (**Lac**)

Mylohyoid raphe (**MHR**)

iv. A Type II bilateral accessory belly; right and left belly arranged in a triangle. This muscle originated with the vertex in the IT inferiorly and inserted via a base onto the midline of the mylohyoid raphe of the mylohyoid muscle (Plate 4).

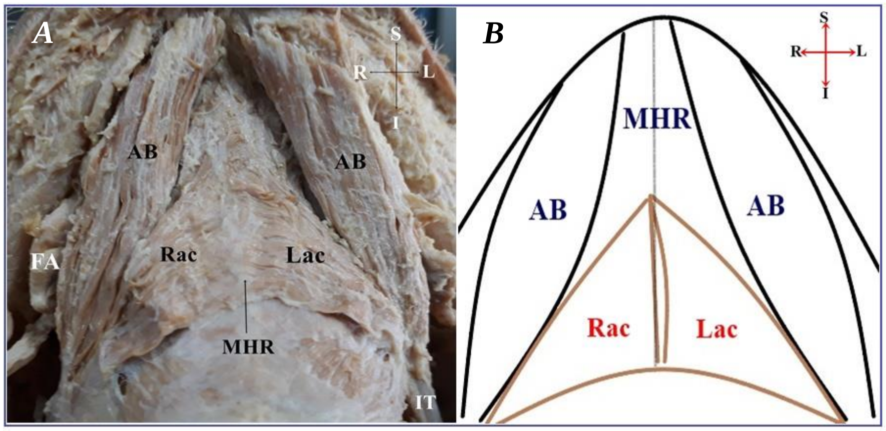

Plate 4: The photograph (A) and associated illustration (B) showing the triangulated accessory bellies of the ABDM on both sides.

Key:

Anterior belly of the digastric muscle (**AB**)

Intermediate tendon (**IT**)

Left accessory belly (**Lac**)

Mylohyoid raphe (**MHR**)

Right accessory belly (**Rac**)

v. The upper and lower muscle fibers crossed centrally into opposite directions, the upper muscle fibers originated from the digastric fossa superficial to the left main ABDM descended inferiorly and inserted onto the medial border of the right ABDM. On the right side, the lower muscle fibers originated from the body of the hyoid bone and ascended transversely to insert into the medial border of the left ABDM. (Plate 5).

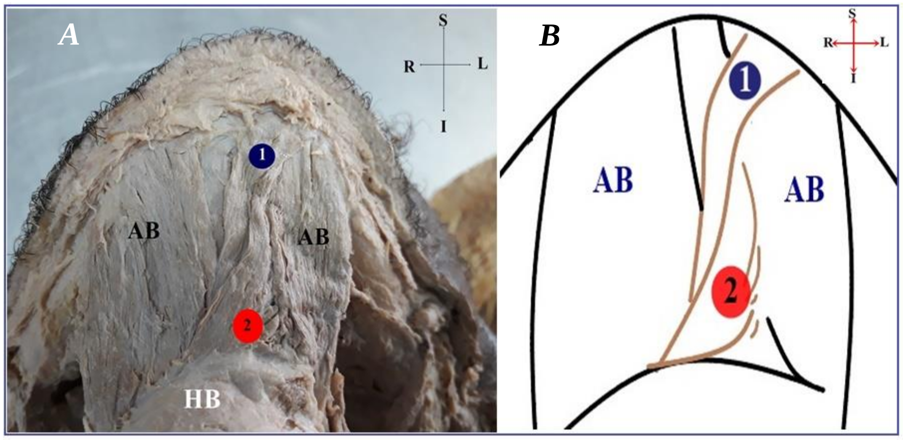

Plate 5: The photograph (A) and associated illustration (B) showing the opposite pair of accessory bellies of the ABDM.

Key:

Anterior belly of the digastric muscle (**AB**)

Hyoid bone (**HB**)

Upper muscle fiber (**1**)

Lower muscle fiber (**2**)

vi. Bilateral accessory bellies, the right and left belly originated from the respective main ABDM distally. Centrally, the anterior muscle slips originated from the internal surface of the internal surface of the mental symphysis (Plate 6).

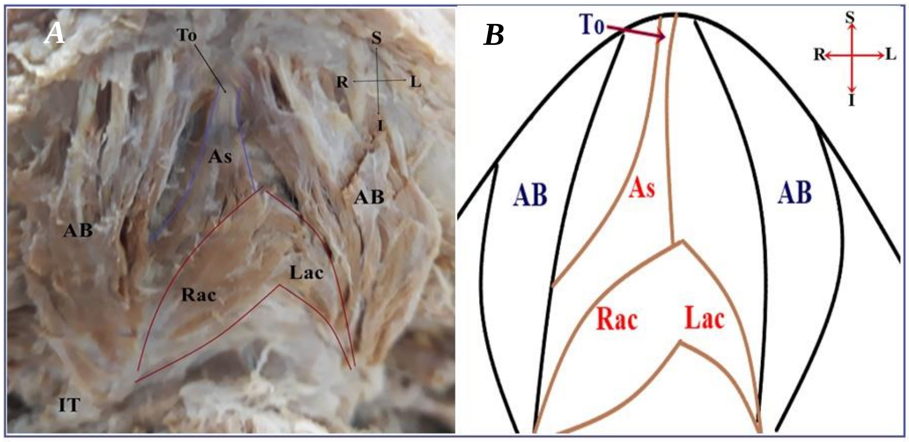

Plate 6: The photograph (A) and associated illustration (B) showing the triple accessory bellies of the ABDM (namely, the anterior muscle slip, right and left belly).

Key:

Anterior belly of the digastric muscle (**AB**)

Anterior muscle slip (**As**)

Intermediate tendon (**IT**)

Left accessory belly (**Lac**)

Right accessory belly (**Rac**)

Tendinous origin (**To**)

DISCUSSION

The morphological variations in the digastric muscle are common and are extensively reported in the anatomical literature, in particular, regarding the presence of the accessory belly of the ABDM. Abnormalities in the pharyngeal arches from which the digastric muscle is derived are claimed to contribute to the formation of these variations. For instance, anomalies in the neural crest cells of the 1st pharyngeal arch may cause the development of an accessory belly (Mascaro *et al*., 2011; Chaithra Rao *et al*., 2016). While, a deficiency during differentiation of the mesodermal cells may either result in a unilateral or bilateral accessory belly (Chaithra Rao *et al*., 2016). As a result, bilateral accessory bellies have been frequently documented in literature (Aktekin *et al*., 2003; Liquidato *et al*., 2007; Mascaro *et al*., 2011; Kyung *et al*., 2011; Chaithra Rao *et al*., 2016) and the majority of these are asymmetrical (Rani *et al*., 2013; Buffoli *et al*., 2016; Accioly Lins *et al*., 2017). This study documented both unilateral and bilateral accessory belly variations.

Different anatomical descriptions and classifications suggested by various authors have reported these anatomical variations according to their origin, composition, course and location (Ozgur *et al*., 2007; Zhao *et al*., 2015). These accessory bellies originated in the digastric fossa of the mandible, the IT, the main ABDM and the hyoid bone; and may insert into the IT, the midline of the mylohyoid raphe, the hyoid bone and the mandible (Mascaro *et al*., 2011; Quadros *et al*., 2013; Rani *et al*., 2013). Aktekin *et al*. (2003) reported a bilateral accessory belly originating from the IT crossing the midline to form an 'X shape'. A similar pattern was observed by Mascaro *et al*. (2011), but the accessory bellies did not cross the midline. While a digastric muscle with three accessory bellies has also been reported (Kyung *et al*., 2011; Nayak *et al*., 2017).

Reyes *et al*. (2007) reported a triangular shaped accessory belly that originated bilaterally from the IT and inserted into the midline of the mylohyoid raphe. A similar observation was documented in this study, unilaterally and bilaterally. A bilateral variation of the ABDM were described having three accessory bellies namely, the right, left and posterior or lower posterior accessory belly originated from either side of the digastric fossa of the mandible and IT, respectively (Kyung *et al*., 2011; Raju *et al*., 2014). Turan-Ozdemir *et al*. (2004) described similar bilateral accessory bellies, however these accessory bellies originated from either sides of the IT and the digastric fossa. Likewise, the bilateral accessory bellies documented in this study corroborated with the afore-mentioned findings documented by Turan-Ozdemir *et al*. (2004). This study further documented a unilateral oblique belly that originated through a tendon from the inferior border of the mandible and travelled superficial to the ABDM and inserted distally on both right and left halves of the hyoid bone. On other hand, an anomalous main ABDM inserted into the hyoid bone via a transverse tendon.

The digastric muscle is involved in deglutition and mastication by elevating the hyoid bone and depressing the mandible, respectively. Thus, anatomical variations in the digastric muscle may possibly alter these functions and may cause imbalance in the musculature of the anterior region of the neck, larynx and floor of the mouth especially in cases where variations occur unilaterally (Mascaro *et al*., 2011). Furthermore, excess tissue in the suprahyoid region may be misinterpreted as lymph nodes, tumour or metastases during radiological diagnosis (Mascaro *et al*., 2011; Reyes *et al*., 2007; Chaithra Rao *et al*., 2016; Accioly Lins *et al*., 2017).

CONCLUSION

This study provides a brief overview of a prevalence of the accessory belly of the ABDM in a selected adult population sample size. The results revealed varying formations of the accessory belly occurring either unilateral or bilateral. Therefore, these findings may be of significance to the anatomists and contributes into the knowledge of the morphological variations that exists in the digastric muscle.

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CHAPTER 4

SYNTHESIS AND CONCLUSION

SYNTHESIS

Anatomical variations in the digastric muscle have been well documented in the anatomical literature, particularly in the ABDM (Rani *et al*., 2013; Buffoli *et al*., 2016; Chaithra Rao *et al*., 2016). Frequently, these anatomical variations occurred bilaterally (Liquidato *et al*., 2007; Reyes *et al*., 2007; Nayak *et al*., 2017). This study therefore documented the morphology, morphometry and anatomical variations of the digastric muscle in fetal and adult head and neck cadaveric specimens.

The ABDM was found to originate from the digastric fossa of the mandible and inferior border of the mandible, while the PBDM originated from the mastoid notch, tip and lateral surface of the temporal bone. The bony origin landmarks for the ABDM documented in this study corroborated with the literature (Drake *et al*., 2015; Standring *et al*., 2016). However, the origin of the PBDM from the mastoid notch of the temporal bone corroborated with origin of the PBDM described in the anatomical literature (Standring *et al*., 2016) and with a Type I PBDM origin classified by Zhao *et al*. (2015). While the tip and lateral surface of the temporal bone were the additional origin landmarks of the PBDM made in this study.

In all of the specimens, the muscle bellies of the digastric muscle were joined to each other by an IT which then pierced through the stylohyoid muscle and inserted into the hyoid bone in 77.5% of the specimens while in 10% and 5% of the specimens, the IT travelled on the lateral and medial border of the stylohyoid muscle, respectively. These findings agreed favourably with the standard anatomical literature (Standring *et al*., 2016) and corroborated with the results of authors such as Zhao *et al*., (2015) who suggested that the IT may be placed either lateral or medial to the stylohyoid muscle. However, the IT presented variations in its course relative to the stylohyoid muscle where it travelled inferior and superior to this muscle. Furthermore, the insertion of the anterior muscle belly into the hyoid bone varied and was found to insert through a narrow and broad muscle belly into the lesser horn of the hyoid bone, and the body and greater horn of the hyoid bone respectively.

Statistical analysis revealed a statistically significant difference in the ABDM between males and females paired average widths ($P = 0.032$), where the left-sided ABDM was significantly wider in females than that of males. The IT was found to be slightly longer in females, the average length of the right-sided IT was 8.12 ± 1.24mm (*mean ± SD)* for males and 7.78 ± 1.27mm (*mean* ± *SD*) for males.

The adult specimens dissected presented with variations in the ABDM, where the presence the accessory bellies was documented unilaterally and bilaterally. Unilateral variations occurred in 20% of the specimens, these accessory bellies were oblique and triangular shaped. The bilateral accessory bellies of the ABDM were documented in 30% of the specimens and corroborated the results of authors such as Turan-Ozdemir *et al*. (2004) and Reyes *et al.* (2007) who documented the ABDM originating with three accessory bellies and the bilateral triangular shaped accessory bellies, respectively. The accessory bellies documented in this study originated from varying origins including the digastric fossa of the mandible, IT, hyoid bone and the ABDM, and inserted to the hyoid bone, mylohyoid muscle and midline of the mylohyoid raphe. These anatomical findings corroborated with the morphology of the accessory bellies described in the literature (Reyes *et al*., 2007; Rani *et al*., 2013; Chaithra Rao *et al*., 2016). However, this study further reported a unique and rare finding where an anomalous main ABDM was documented. This ABDM inserted with a broad muscle belly into a transverse tendon which then inserted into the body of the hyoid bone.

CONCLUSION

In conclusion, the findings in this study document new knowledge on the anatomy of the digastric muscle in fetuses which had not been previously documented. Firstly, the mode of insertion of the ABDM into the hyoid bone through a broad and narrow muscular belly. Secondly, an additional trajectory of the IT travelling on the superior and inferior border of the stylohyoid muscle. Thirdly, the PBDM exhibiting a narrow and broad muscle belly of origin against the mastoid process. As a result, these anatomical findings may be regarded as part of the standard anatomical description of the digastric muscle. Therefore, a thorough understanding of the digastric muscle anatomy is of outmost significance to cranio-facial surgeons since surgical interventions such as corrective or plastic surgery, in the submental region may be influenced by embryonic development and anatomical orientation of the muscle.

Recommendations for Future Studies

Future studies should examine the differences that exists in the digastric muscle across different ethnic groups and focus on the potential morphological differences in certain age groups. Secondly, the adult sample in this study was limited; possibly a larger sample may have yielded further findings that may be of interest.

Limitations of the Study

The sample size was disparate between the population groups and gender distribution among these groups. In this study, the fetal population was largely represented than the adult population comprising of a sample size of 40 and 10 respectively. This was due to the availability of cadaveric specimens in the department during the time of study, during which there was a shortage of adult cadaveric specimens. Furthermore, there was an imbalance with regards to the distribution of gender among both population groups.

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APPENDICES

APPENDIX ONE

Ethical Clearance

UNIVERSITY OF KWAZULU-NATAL™
INYUVESI YAKWAZULU-NATALI

18 October 2017

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Protocol: The morphology and morphometry of the digastric muscles in a South African cadaver population.
Degree: MMed
BREC Ref No: BE530/17

EXPEDITED APPROVAL

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 29 August 2017.

The conditions have been met and the study is given full **ethics approval** and may begin as from 18 October 2017.

This approval is valid for one year from **18 October 2017**. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **14 November 2017.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc supervisor: Prof KS Satyapal
cc co-supervisor: Dr BZ De Gama

Biomedical Research Ethics Committee
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APPENDIX TWO

Turnitin Report

Masters Thesis

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APPENDIX THREE

Data Sheets

Fetuses Morphology

Specimen number	Sex	Age (weeks)	Foot length (mm)	Origin		Insertion		Variation/accessory belly
				ABDM	*PBDM*	*ABDM at HB*	*IT*	
1	Male	24	31	Type I	Type II	Type I	Piercing	-
2	Male	29	44	Type I	Type I	Type II	Piercing	-
3	Female	29	44	Type I	Type II	Type I	Piercing	-
4	Male	23	33	Type I	Type II	Type I	-	IT- Superior
5	Female	25	35	Type I	Type I	Type I	-	IT- Medial
6	Female	27	39	Type I	Type I	Type II	Piercing	-
7	Male	30	47	Type I	Type I	Type I	Piercing	-
8	Male	26	37	Type I	Type I	Type II	Piercing	-
9	Male	23	33	Type I	Type II	Type I	-	IT- Medial
10	Male	25	35	Type I	Type I	Type I	Piercing	-
11	Male	24	24	Type I	Type I	Type II	Superior	-
12	Male	29	43	Type I	Type II	Type I	Piercing	-

13	Male	29	45	Type I	Type II	Type I	Piercing	-
14	Male	26	39	Type I	Type II	Type I	Piercing	-
15	Male	22	29	Type I	Type I	Type I	Piercing	-
16	Male	26	37	Type I	Type I	Type I	Piercing	-
17	Male	25	36	Type I	Type II	Type I	Piercing	-
18	Male	24	34	Type I	Type I	Type I		IT- Lateral
19	Female	24	34	Type I	Type II	Type I	Piercing	-
20	Female	22	30	Type I	Type I	Type II	-	IT- Medial
21	Male	24	35	Type I	Type II	Type I	-	IT- Superior
22	Female	22	30	Type I	Type I	Type I	-	IT- Inferior
23	Male	31	48	Type I	Type I	Type I	Piercing	-
24	Male	22	29	Type I	Type II	Type I	Piercing	-
25	Male	26	27	Type I	Type I	Type I	Piercing	
26	Male	25	37	Type I	Type I	Type I	Piercing	-
27	Female	29	43	Type I	Type I	Type I	Piercing	-

28	Male	26	38	Type I	Type II	Type I	Piercing	-
29	Male	24	35	Type I	-	Type I	Piercing	Bifid PBDM
30	Female	27	39	Type I	Type I	Type I	Piercing	-
31	Male	25	35	Type I	Type II	Type I	Piercing	-
32	Male	25	36	Type I	Type I	Type I	Piercing	-
33	Male	27	40	Type I	Type I	Type I	-	IT- Lateral
34	Female	24	34	Type I	Type I	Type II	Piercing	-
35	Female	25	35	Type I	Type I	Type I	Piercing	-
36	Female	33	51	Type II	Type II	Type II	-	IT- Medial
37	Male	22	29	Type I	Type II	Type I	Piercing	-
38	Female	31	47	Type I	Type I	Type II	Piercing	-
39	Male	22	29	Type I	Type II	Type I	Piercing	-
40	Female	22	30	Type I	-	Type I	Piercing	Bifid PBDM

Adults Morphology

Specimen number	Sex	Race	Age	Origin		Insertion		Variation /accessory belly
				ABDM	*PBDM*	*ABDM at HB*	*IT*	
201	Male	White	76	Digastric fossa	Mastoid notch	-	Piercing	ABDMs
202	Male	White	68	Digastric fossa	Mastoid notch	Hyoid bone	Piercing	-
203	Male	White	43	Digastric fossa	Mastoid notch	Hyoid bone	Piercing	acABDM
204	Male	White	76	Digastric fossa	Mastoid notch	Hyoid bone	Piercing	acABDM
205	Male	Black	33	Digastric fossa	Mastoid notch	Hyoid bone	Piercing	-
206	Male	White	75	Digastric fossa	Mastoid notch	Hyoid bone	Piercing	acABDM
T1	Female	White	72	Digastric fossa	Mastoid notch	Hyoid bone	Piercing	acABDM
T3	Male	White	76	Digastric fossa	Mastoid notch	Hyoid bone	Piercing	-
T5	Male	White	83	Digastric fossa	Mastoid notch	Hyoid bone	Piercing	-
T7	Female	White	84	Digastric fossa	Mastoid notch	Hyoid bone	Piercing	acABDM

Fetal Morphometry

Specimen No.	Sex	Age (weeks)	Foot Length (mm)	ABDM				IT				PBDM			
				Length		Width		Length		Width		Length		Width	
				Right	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*
1	Male	24	31	10,95	10,63	3,95	3,43	7,74	8,06	0,5	0,45	11,76	11,44	2,18	2,35
2	Male	29	44	13,63	11,76	4,06	4,36	9,56	9,62	1,03	0,62	11,76	11,57	2,54	2,54
3	Female	29	44	15,13	15,04	7,62	5,85	8,65	8,48	0,84	1,06	11,92	12,9	3,48	4,51
4	Male	23	33	10,94	10,36	4,47	3,92	6,07	7,06	0,63	0,59	13,36	11,33	3,04	3,08
5	Female	25	35	11,29	12,86	3,95	3,62	7,74	6,89	0,62	0,73	11,89	12,85	2,64	2,72
6	Female	27	39	11,26	10,94	4,01	4,97	9,77	10,52	0,73	0,68	11,05	3,27	2,86	3,27
7	Male	30	47	13,96	14,85	5,46	4,9	9,07	10,44	0,75	0,6	10,37	12,26	3,36	2,55
8	Male	26	37	10,82	9,35	5,1	5,28	8,34	7,47	0,82	0,61	9,36	11,15	2,51	2,62
9	Male	23	33	13,65	11,36	3,47	3,1	6,84	6,43	0,65	0,47	10,73	10,83	2,41	2,34
10	Male	25	35	11,23	10,62	4,08	3,64	5,52	6,43	0,69	0,67	12,86	11,66	2,75	2,68
11	Male	24	24	11,53	11,79	3,81	4,22	7,32	8,26	0,62	0,66	9,36	10,24	2,71	2,43
12	Male	29	43	7,75	7,34	3,44	3,52	7,05	6,72	0,56	0,49	9,68	9,34	2,16	2,2

13	Male	29	45	12,74	13,63	5,13	4,07	8,14	7,88	1,11	0,91	12,66	12,9	4,44	4,37
14	Male	26	39	12,54	12,11	4,97	4,68	8,06	7,31	0,67	0,96	9,31	12,75	3,53	2,96
15	Male	22	29	9,24	9,37	4,26	4,42	7,83	7,44	0,59	0,73	10,44	9,94	3,25	2,97
16	Male	26	37	11,87	11,25	4,39	3,76	10,75	9,49	0,57	0,47	12,35	12,53	3,07	3,28
17	Male	25	36	13,25	12,49	3,19	3,63	7,63	6,28	0,76	0,89	10,74	12,55	2,46	2,26
18	Male	24	34	10,27	10,76	3,75	3,94	7,53	7,28	0,64	0,65	12,81	10,9	2,99	3,43
19	Female	24	34	10,1	10,05	3,86	3,04	6,88	6,7	0,32	0,36	11,47	12,53	2,28	3,02
20	Female	22	30	9,84	9,85	3,62	4,38	10,52	9,76	0,49	0,5	8,16	7,64	2,07	2,76
21	Male	24	35	9,73	10,26	3,36	3,4	6,83	8,43	0,9	0,8	12,44	11,32	2,36	2,36
22	Female	22	30	10,07	8,84	3,76	3,52	6,46	6,34	0,54	0,4	9,56	11,05	1,74	0,75
23	Male	31	48	13,9	16,04	4,19	5,43	9,9	9,66	1,02	0,73	15,81	13,64	3,58	3,79
24	Male	22	29	11,58	11,67	5,32	4,53	8,61	9,37	0,37	0,41	11,27	10,56	4,15	3,59
25	Male	26	27	12,58	12,32	3,4	3,64	7,25	6,45	0,99	0,81	12,21	12,1	2,92	3,09
26	Male	25	37	12,15	11,44	4,72	3,87	8,35	9,71	0,5	0,95	13,45	12,51	2,35	2,82
27	Female	29	43	12,36	11,04	4,64	4,65	8,34	7,94	0,53	0,74	15,55	13,3	3,57	2,87

28	Male	26	38	11,55	11,08	3,72	3,34	5,12	6,95	0,63	0,41	11,49	11,27	2,72	2,63
29	Male	24	35	8,8	8,37	3,36	4,85	6,85	6,38	0,65	0,3	10,14	11,77	2,49	2,44
30	Female	27	39	10,28	10,3	3,54	3,52	6,6	6,62	0,46	0,42	11,54	11,52	2,86	2,92
31	Male	25	35	13,43	13,27	4,57	4,6	7,27	5,92	0,69	0,89	12,21	12,23	4,17	4,01
32	Male	25	36	9,93	8,96	3,88	3,54	7,92	7,45	0,59	0,55	10,46	10,57	2,82	2,82
33	Male	27	40	12,13	11,68	4,72	4,37	9,1	9,35	0,54	0,63	14,04	12,46	2,29	3,07
34	Female	24	34	10,47	10,64	5,24	6,18	9,32	9,36	0,31	0,72	12,41	12,84	3,57	2,82
35	Female	25	35	12,11	10,74	4,64	4,66	7,89	8,9	1,07	0,6	14,77	14,04	2,82	3,3
36	Female	33	51	10,03	9,71	4,44	4,21	8,86	7,19	0,94	0,72	11,2	13,58	3,7	3,4
37	Male	22	29	8,8	8,84	3,91	3,52	8,15	8,68	0,55	0,33	11,54	11,58	2,26	2,32
38	Female	31	47	11,25	10,97	5,96	4,98	7,85	7,47	0,4	0,53	11,19	10,17	2,74	2,16
39	Male	22	29	9,88	9,93	3,08	3,52	7,84	8,03	0,63	0,52	10,25	8,88	2,38	1,97
40	Female	22	30	10,01	10,05	4,72	4,75	6,54	7,48	0,54	0,62	11,42	10,16	2,69	2,38