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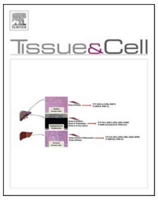

Morphogenesis and functional aspects of the muscular layer of the middle deep cervical fascia in humans

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ABSTRACT

Background: In recent years, the fasciae of the human body have received significant attention because of their crucial role in the transmission of muscle force. However, studies on the development of the fasciae, particularly the cervical fascia, remain scarce.
Purpose: This study was performed to examine the development of the fascia of the infrahyoid strap muscles, also known as the middle layer of the deep cervical fascia (MDCF), in 17 human embryos aged 6–8 weeks and 20 human foetuses aged 9–14 weeks.
Methods: Histological examination of serial sections was performed using conventional light microscopy.
Results: Three stages in the development of the MDCF were identified: the initial, formation, and maturation stages. In the initial stage (week 6 of development, Carnegie stages 18–19), the mesenchymal primordium of the epimysium of the infrahyoid muscles was observed and found to be continuous with the mesenchymal primordium of the MDCF. The infrahyoid muscles already exhibited intramuscular fibres, the primordium of the perimysium, and the endomysium. In the formation stage (weeks 7–8 of development, Carnegie stages 20–23), fibroblast-like cells and collagen fibres appeared in the primordium of the muscle epimysium and in the MDCF. Intramuscular fibres had become very evident. In the maturation stage (from week 9 of development onward), further development and organisation of the fascial structures occurred.
Conclusion: Our results suggest that the MDCF of the neck develops in parallel with the mechanical activity of this region. The relationship between the MDCF and the lymphatic and venous structures of this region suggests that the MDCF may facilitate venous and lymphatic circulation.

1. Introduction

Fascia is a connective tissue that permeates the human body, forming a continuous three-dimensional matrix that provides structural support. It is a viscoelastic matrix that envelops muscles, bones, and organs (Guidera et al., 2014). This definition has recently been expanded to include elements such as adipose tissue, adventitia and neurovascular sheaths, aponeuroses, deep and superficial fasciae, epineurium, joint capsules, ligaments, membranes, meninges, myofascial expansions, periosteum, retinaculum, septa, tendons, visceral fasciae, and all intramuscular and intermuscular connective tissues including the endomysium, perimysium, and epimysium (Zügel et al., 2018).

In the neck, the fasciae are organised into a superficial layer and various layers of deep cervical fascia (DCF). The superficial fascia is located between the dermis and the deep fascia. It consists of loose connective tissue with varying amounts of adipose tissue and contains the platysma muscle. The DCF is composed of three distinct layers: superficial, middle, and deep. The superficial layer of the DCF encircles the

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neck and envelops the trapezius and sternocleidomastoid muscles. The middle layer of the DCF is subdivided into a muscular layer [known as the muscular layer of the middle DCF (MDCF)] that envelops the infrahyoid strap muscles and a visceral layer (known as the pretracheal fascia) that surrounds the thyroid gland, larynx, trachea, pharynx, and oesophagus. The deep layer of the DCF consists of a dorsal layer (prevertebral fascia) and a ventral layer (alar or intercarotid fascia) (Stecco, 2014; López-Fernández et al., 2019; Feigl et al., 2020; Watkinson and Gleeson, 2020).

The MDCF attaches superiorly to the hyoid bone and thyroid cartilage. Inferiorly, it continues with the clavipectoral fascia, inserting into the sternal manubrium and the clavicle behind the insertion of the superficial layer of the DCF (Feigl et al., 2020; Sutcliffe and Lasrado, 2024). The carotid sheath is a condensation of the DCF and surrounds the common and internal carotid arteries, internal jugular vein, vagus nerve, and ansa cervicalis (Watkinson and Gleeson, 2020).

Studies on the development of the deep fascia in various regions (upper limbs, lower limbs, and lumbar region) in human specimens from 21 to 39 weeks of development have concluded that vessels and nerves develop in parallel with the fascia and that fibroblasts play a crucial role in muscle morphogenesis (Blasi et al., 2015). Researchers have also examined the organisation of fascial structures such as the retinaculum (wrist and ankle) and the iliotibial tract in specimens from 24 to 40 weeks of development, suggesting that movement influences the shaping of these fascial structures (Pirri et al., 2022).

However, few studies have focused on the development of cervical fasciae. Some researchers have analysed the configuration of the parapharyngeal space in the suprahyoid region in late-stage human foetuses (Katori et al., 2013). Additionally, we previously studied the development of the alar fascia in human specimens from 6 to 12 weeks of development (López-Fernández et al., 2019).

The objective of the present study was to examine serial sections of the cervical region in human specimens from 6 to 14 weeks of development to investigate the morphogenesis of the MDCF and its relationship with adjacent structures, particularly the carotid sheath and the lymphatic vessels of the neck. In addition, we aimed to provide information on the stages of MDCF development and propose its possible functions.

2. Materials and methods

The authors declare that the research was ethically conducted in accordance with the World Medical Association Declaration of Helsinki and all local ethical guidelines and laws regarding the use of human cadaveric donors in anatomical research.

The bilateral cervical region was investigated in 17 human embryos and 20 human foetuses. All specimens were obtained from the collection preserved in the Department of Anatomy and Embryology at Universidad Complutense de Madrid (UCM). All were products of ectopic pregnancies or spontaneous abortions managed in the Department of Obstetrics and Gynaecology at UCM. No clinical information or genetic analysis was available to explain the cause of the spontaneous abortions. However, all specimens were thoroughly studied morphologically and histologically, and no material suspected to contain malformation was included in this study. The study was approved by the Ethics Committee of the Hospital Clínico San Carlos de Madrid at UCM (ref. 24/740-E).

Among the embryos, the greatest length (GL) ranged from 12 to 30 mm [three specimens of Carnegie stage (CS) 18, two specimens of CS 19, four specimens of CS 20, two specimens of CS 21, three specimens of CS 22, and three specimens of CS 23]. Among the foetuses, the GL ranged from 32 to 120 mm (three specimens from week 9, four from week 10, three from week 11, four from week 12, three from week 13, and three from week 14). The parameters used to determine post-conceptional age were the GL and external and internal criteria (O'Rahilly and Müller, 2001, 2010).

All samples were fixed in 10 % formalin and embedded in paraffin for processing. Each of the studied specimens was entirely sectioned in a single plane (either transverse, frontal, or sagittal). Sections from transversely sectioned specimens were chosen to create figures and facilitate greater understanding. Sections were cut at a thickness of 7–25 μm depending on the sample size. The sections were stained with haematoxylin–eosin, Azan, Bielschowsky, or Masson's trichrome stain. Three independent observers performed an optical evaluation to discriminate anatomical structures on a screen with magnification ranging from 10 × to 400 × . Digital photomicrographs were taken with a Nikon DXM 1200 light microscope (Nikon Corp., Tokyo, Japan), transferred to a personal computer, and edited with Act One software (Act-1 Systems, Canoga Park, CA, USA) and Adobe Photoshop CS6 software (Adobe Systems, San Jose, CA, USA).

3. Results

3.1. *Embryonic period*

During week 6 of development (CS 18–19), the primordia of the infrahyoid muscles appeared, consisting of myoblasts surrounded by mesenchymal cells forming the epimysium. Mesenchymal cells and collagen fibres appeared among the myoblasts, constituting the endomysium and perimysium of the infrahyoid muscles (Fig. 1 A, B).

Laterally and medially, the primordium of the epimysium of the infrahyoid muscles extended into the aligned mesenchymal cells, forming the primordium of the MDCF (Fig. 1B, C). Along the midline, cells from the sternohyoid and sternothyroid muscles adhered to the primordium of the cricoid cartilage (Fig. 1 A, B). Laterally, the primordium of the epimysium of the omohyoid muscle continued with the primordium of the MDCF, reaching a lymphatic vessel (Fig. 1 A, C). The neurovascular bundle of the neck (common carotid artery, internal jugular vein, and vagus nerve), as well as the ansa cervicalis, were surrounded by mesenchymal cells (Fig. 1 A, C).

During weeks 7–8 of development (CS 20–23), the primordia of the infrahyoid muscles were surrounded by elongated fibroblast-like cells and collagen fibres. These were prominently visible in the lower part in relation to the sternum and clavicle, forming the muscle epimysium (Fig. 2A, B). More cranially, in proximity to the clavicle, the epimysium of the sternothyroid muscle became distinctly evident, consisting of abundant collagen fibres. Additionally, at this same level a more organised perimysium and endomysium could also be observed during this stage (Fig. 2C, D). Elements of the neurovascular bundle of the neck began to be surrounded by elongated fibroblast-like cells with an extracellular matrix of collagen fibres. These cells and matrix corresponded to the primordium of the adventitia of these structures and were prominently visible at the level of the common carotid artery. The primordium of the visceral layer of the middle DCF surrounded the thyroid gland (Fig. 2E, F). The relationship of the MDCF primordium lateral to the sternothyroid muscle with the internal jugular vein and the relationship of the epimysium of the inferior belly of the omohyoid muscle with the lymphatic vessels were very evident (Fig. 2E, F).

3.2. *Foetal period (weeks 9–11)*

During week 9 of development, secondary myotubes of the inferior belly of the omohyoid muscle were very evident, exhibiting eosinophilic cytoplasm and multiple centrally located nuclei. The epimysium of the muscle contained junctional bridges with the connective tissue surrounding the lymphatic vessels (Fig. 3A, B). Each element of the neurovascular bundle of the neck appeared to be surrounded by its adventitia. The adventitia of the common carotid artery was formed by more layers than during the embryonic period (Fig. 3A).

During week 10 of development, the carotid sheath became evident. This sheath appeared thicker between the common carotid artery and the thyroid gland as well as between the ansa cervicalis and the superior belly of the omohyoid muscle (Fig. 4A, B).

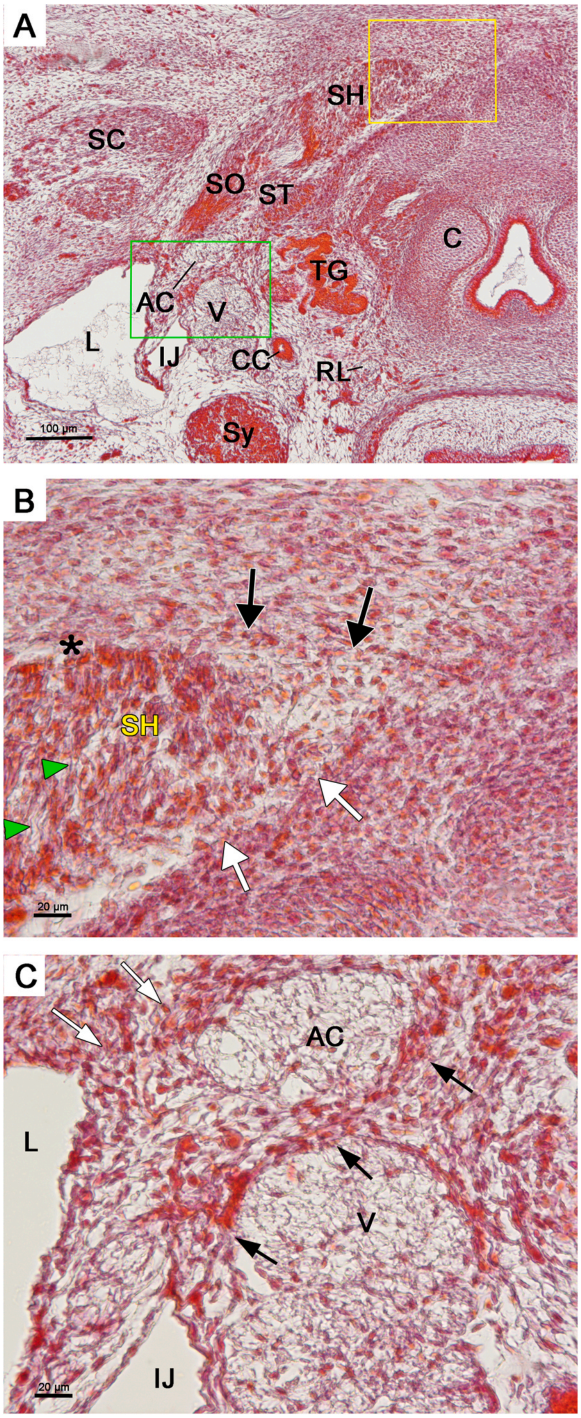

Fig. 1. . [A] Representative microphotograph of a transverse section of the human embryonic neck at Carnegie stage 18 (17 mm greatest length), stained with haematoxylin and eosin. **[B]** Higher magnification of the yellow box in [A]. Mesenchymal cells line the primordium of the sternohyoid muscle (SH), constituting the primordium of the epimysium of the muscle (asterisk). These cells align toward the midline to form the primordium of the muscular layer of the middle deep cervical fascia (black arrows). Mesenchymal cells that are aligned from the medial edge of the primordium of the sternothyroid muscle (ST), visible in [A], are also arranged toward the midline to contribute to the formation of the muscular layer of the deep medial cervical fascia (white arrows). The primordium of the perimysium and endomysium of the sternohyoid muscle (SH) are indicated by green arrowheads. **[C]** Higher magnification of the green box in [A]. The primordium of the epimysium of the superior belly of omohyoid muscle (SO), visible in [A], is continued laterally by mesenchymal cells that contribute to forming the muscular layer of the middle deep cervical fascia (white arrows), which eventually reaches the lymphatic vessel (L). The primordium of the epimysium of the sternothyroid muscle (ST) is continued by mesenchymal cells that contribute to forming the muscular layer of the middle deep cervical fascia (black arrows), which reaches the internal jugular vein (IJ). **Abbreviations:** AC = ansa cervicalis; C = cricoid cartilage; CC = common carotid artery; IJ = internal jugular vein; L = lymphatic vessel; SO = superior belly of omohyoid muscle; RL = recurrent laryngeal nerve; Sy = sympathetic trunk; SC = sternocleidomastoid muscle; SH = sternohyoid muscle; ST = sternothyroid muscle; TG = thyroid gland; V = vagus nerve. **Scale bars:** 100 µm in [A] and 20 µm in [B, C].

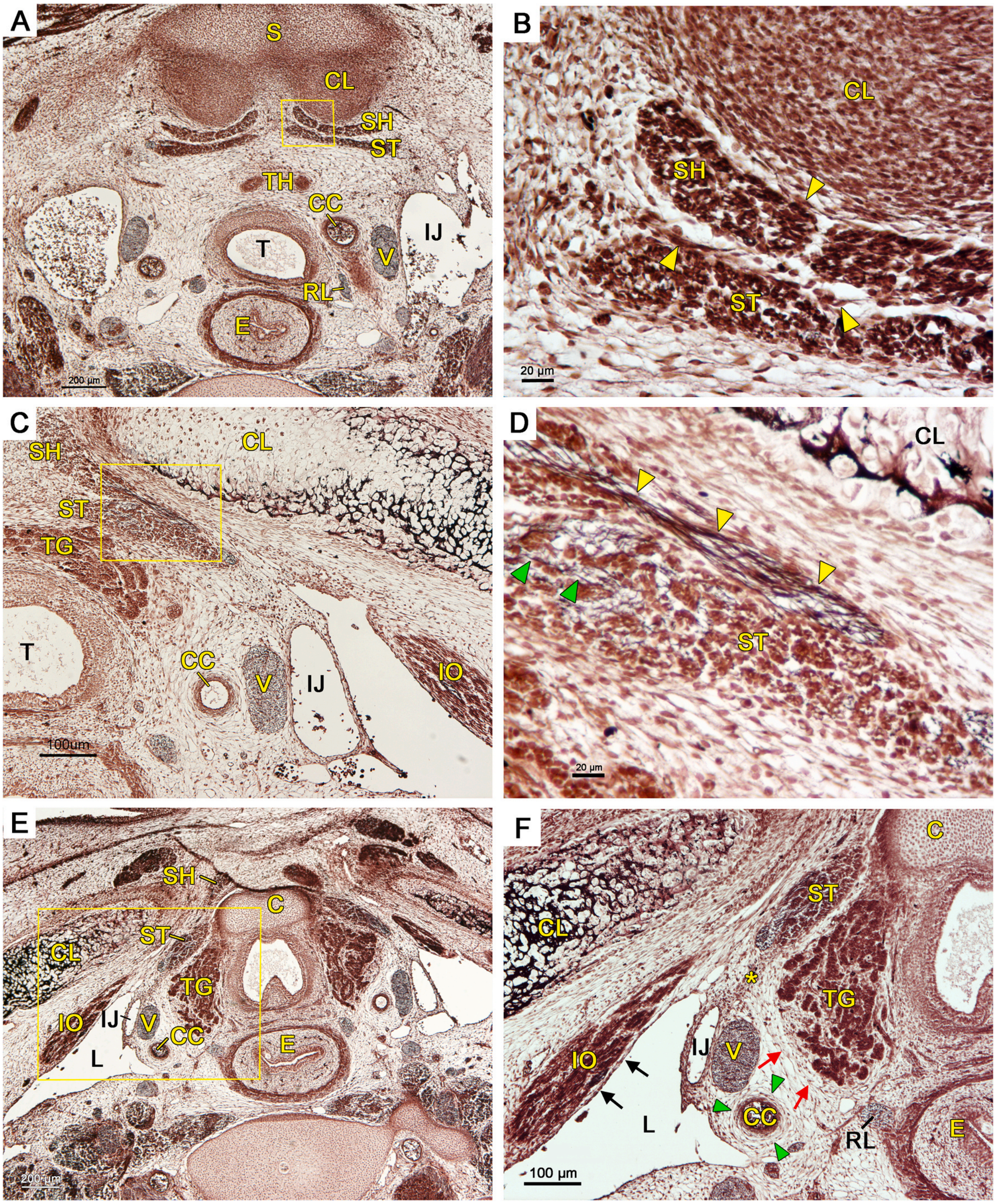

Fig. 2. Representative microphotographs of transverse sections at three different levels (from caudal to cranial, each pair from top to bottom) of a human embryonic neck at Carnegie stage 21 (22 mm greatest length), stained with the Bielschowsky technique. Left column microphotographs **[A, C, E]** correspond to panoramic views, while right column ones [B, D, F] correspond to higher magnification views of selected areas from the left images. [B] Higher magnification of the yellow box in [A]. Close to the clavicle (CL), near their origin, the sternohyoid (SH) and sternothyroid (ST) muscles are surrounded by fibroblast-like cells (yellow arrowheads). [D] Higher magnification of the yellow box in [C]. The sternothyroid muscle (ST) is covered by connective tissue fibres corresponding to its epimysium (yellow arrowheads). Furthermore, inside the sternothyroid muscle (ST), there are connective tissue fibres (green arrowheads) that form its perimysium and endomysium. [F] Higher magnification of the yellow box in [E]. The primordium of the adventitia of the common carotid artery (CC) is marked with green arrowheads. Note also the primordium of the visceral layer of the middle deep cervical fascia (red arrows). The primordium of the muscular layer of the middle deep cervical fascia (asterisk) reaches the internal jugular vein (IJ). Moreover, the inferior belly of the inferior belly of omohyoid (IO) muscle relates to the lateral wall of the lymphatic vessel (L) (black arrows). Abbreviations: C = cricoid cartilage; CC = common carotid artery; CL = clavicle; E = oesophagus; IJ = internal jugular vein; L = lymphatic vessel; IO = inferior belly of omohyoid muscle; RL = recurrent laryngeal nerve; S = sternum; SH = sternohyoid muscle; ST = sternothyroid muscle; T = trachea; TG = thyroid gland; TH = thymus; V = vagus nerve. Scale bars: 20 µm in [B, D], 100 µm in [C, F] and 200 µm in [A, E].

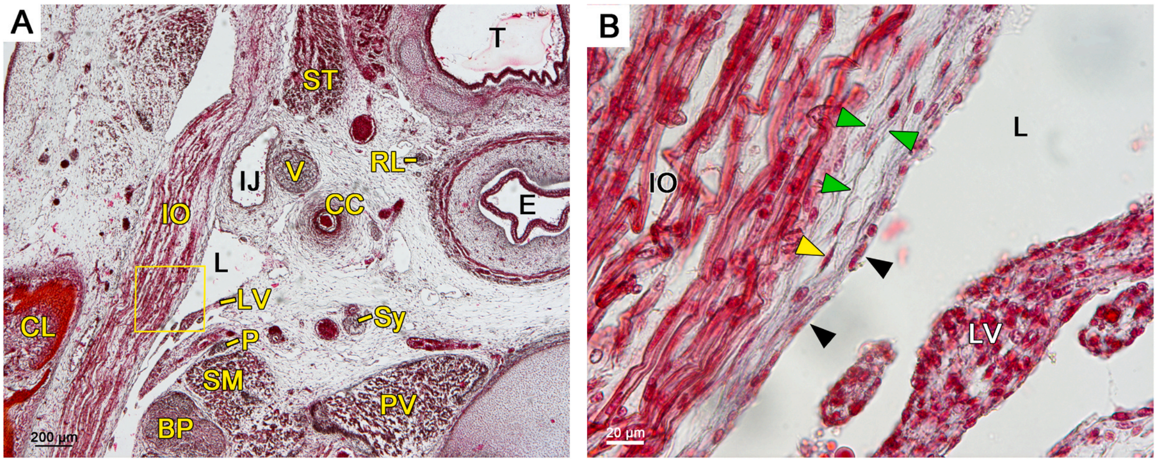

Fig. 3. Representative microphotographs of transverse sections of the lower region of a human foetal neck stained with the Azan technique. **[A]** Specimen at 9 weeks of development (40 mm greatest length). **[B]** Higher magnification of the yellow box in [A]. The epimysium of the inferior belly of the omohyoid muscle (IO) is connected to the connective tissue surrounding the lymphatic vessel (L) (green arrowheads). Within the lymphatic vessel (L), its endothelial cells (black arrowheads) and a lymphatic valve (LV) can be observed. In addition, a yellow arrowhead points to a fibroblast cell. **Abbreviations:** BP = brachial plexus; CC = common carotid artery; CL = clavicle; E = oesophagus; IJ = internal jugular vein; L = lymphatic vessel; LV = lymphatic valve; IO = inferior belly of omohyoid muscle; P = phrenic nerve; PV = prevertebral muscles; RL = recurrent laryngeal nerve; Sy = sympathetic trunk; SM = scalene muscles; ST = sternothyroid muscle; T = trachea; V = vagus nerve. **Scale bars:** 200 μm in [A] and 20 μm in [B].

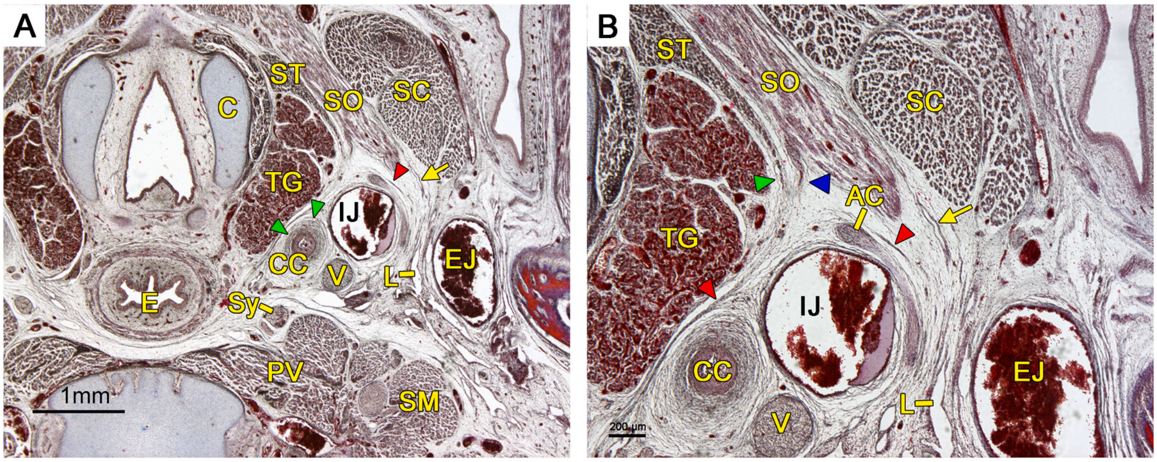

Fig. 4. Representative microphotographs of transverse sections of the upper region of a human foetal neck stained with the Azan technique. **[A]** Human specimen at 10 weeks of development (57 mm greatest length). Note the carotid sheath marked with a red arrowhead. The epimysium of the superior belly of the omohyoid muscle (SO) continues with the muscular layer of the middle deep cervical fascia, which reaches the lymphatic vessel (L) (yellow arrow). The adventitia of the common carotid artery (CC) consists of several layers of connective tissue (green arrowheads). **[B]** Higher magnification of the right part of [A]. Note the muscular layer of the middle deep cervical fascia marked with a yellow arrow and the carotid sheath marked with red arrowheads. The epimysium of the sternothyroid muscle (ST) continues with the muscular layer of the deep cervical fascia, reaching the carotid sheath (green arrowhead). The ansa cervicalis (AC) is located within the thickness of the carotid sheath. Note that the epimysium of a fascicle of the superior belly of the omohyoid muscle (SO) continues dorsally and reaches the muscular layer of the middle deep cervical fascia (blue arrowhead). **Abbreviations:** AC = ansa cervicalis; C = cricoid cartilage; CC = common carotid artery; CL = clavicle; E = oesophagus; EJ = external jugular vein; IJ = internal jugular vein; L = lymphatic vessel; LV = lymphatic valve; SO = superior belly of omohyoid muscle; P = phrenic nerve; PV = prevertebral muscles; Sy = sympathetic trunk; SC = sternocleidomastoid muscle; SM = scalene muscles; ST = sternothyroid muscle; TG = thyroid gland; V = vagus nerve. **Scale bars:** 1 mm in [A] and 200 μm in [B].

The MDCF, situated lateral to the sternothyroid muscle, was attached to the carotid sheath. In one specimen, we were able to observe how the epimysium of some muscle fascicles of the superior belly of the omohyoid muscle continued with the lateral part of the middle layer, reaching the carotid sheath (Fig. 4B). The MDCF lateral to the superior belly of the omohyoid muscle reached a lymphatic vessel (Fig. 4A, B).

3.3. *Foetal period (weeks 12–14)*

During weeks 12–14, the carotid sheath, intercarotid fascia (alar fascia), and deep layer of the DCF (prevertebral fascia) became well established. Along the midline, the fusion of the superficial layer of the DCF and the MDCF was in progress, preparing to form the cervical linea alba (Fig. 5A). The lateral part of the MDCF crossed the carotid sheath, reaching the internal jugular vein (Fig. 5B).

4. Discussion

To our knowledge, this is the first study on the development of the MDCF in humans. We identified three stages of the development of the infrahyoid strap muscle fascia or the MDCF (Fig. 6):

- Initial stage: week 6 of development (CS 18–19)
- Formation stage: weeks 7–8 of development (CS 20–23)

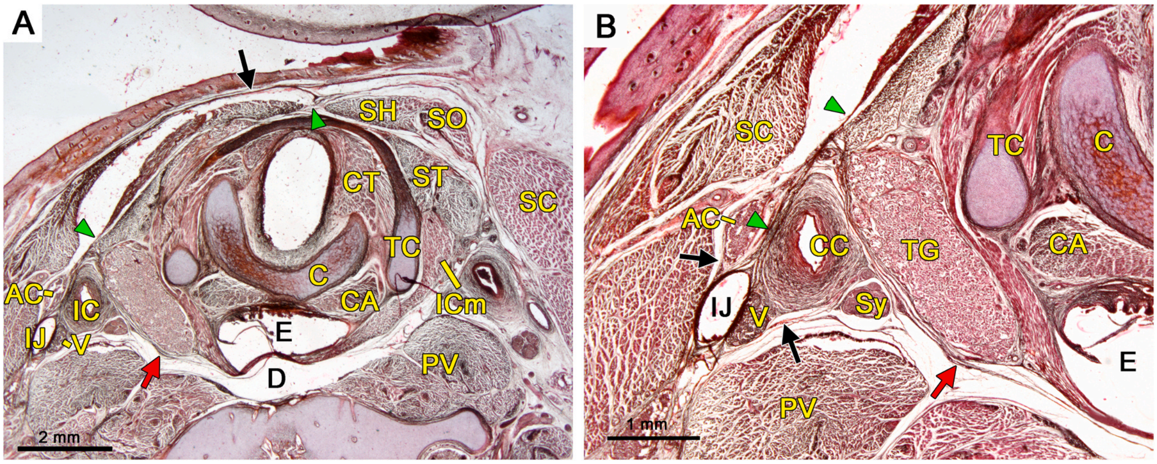

Fig. 5. Representative microphotographs of transverse sections of the human foetal neck at 14 weeks of development (120 mm greatest length) stained with the Azan technique. **[A]** The superficial layer of the deep cervical fascia can be seen (black arrow). Additionally, the muscular layer of the middle deep cervical fascia is visible (green arrowheads), as is the intercarotid fascia or alar fascia (red arrow). **[B]** Higher magnification of the left part of [A]. The muscular layer of the middle deep cervical fascia is marked with green arrowheads, the carotid sheath is marked with black arrows, and the intercarotid fascia or alar fascia is marked with a red arrow. **Abbreviations:** AC = ansa cervicalis; C = cricoid cartilage; CA = posterior cricoarytenoid muscle; CC = common carotid artery; CT = cricothyroid muscle; D = danger space; E = oesophagus; IC = inferior pharyngeal constrictor muscle; IJ = internal jugular vein; SO = superior belly of omohyoid muscle; PV = prevertebral muscles; Sy = sympathetic trunk; SC = sternocleidomastoid muscle; SH = sternohyoid muscle; ST = sternothyroid muscle; TC = thyroid cartilage; TG = thyroid gland; TH = thyrohyoid muscle; V = vagus nerve**. Scale bars:** 2 mm in [A] and 1 mm in [B].

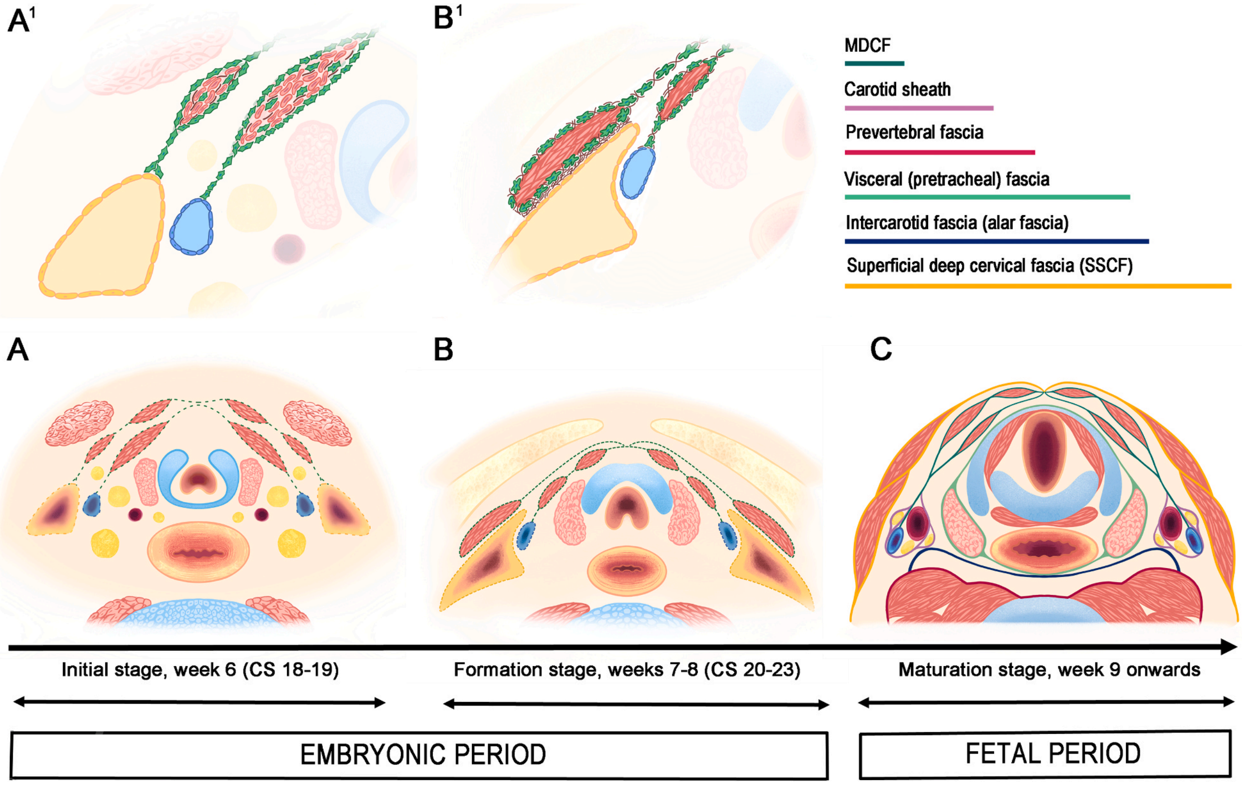

Fig. 6. Schematic representations of the different stages of infrahyoid muscles development and the muscular layer of the middle deep cervical fascia (MDCF) during embryonic and fetal periods. **[A]** Initial stage, at week 6 of development (Carnegie stage (CS) 18–19). **[A1]** The primordia of the infrahyoid muscles are formed by myoblasts, among which there are mesenchymal cells and some collagen fibres that form the primordia of their endomysium and perimysium. The primordium of the epimysium is constituted solely by mesenchymal cells and continues, medially and laterally, with the primordium of the MDCF, which is also constituted solely by mesenchymal cells. Laterally, the primordium of the MDCF reaches the lymphatic and venous vessels. **[B]** Formation stage, at weeks 7–8 of development (CS 20–23). **[B1]** At this stage, the primordia of the epimysium of the muscles and the MDCF are constituted by fibroblast-like cells and collagen fibres. Laterally, both relate to either the lymphatic vessel or the internal jugular vein. **[C]** End of Maturation stage, at week 14 of development. All cervical fascial structures are already well established: the superficial deep cervical fascia (SDCF), the MDCF and the visceral fascia (pretracheal fascia) of the neck, the carotid sheath, the intercarotid fascia (alar fascia) and the prevertebral fascia. The carotid sheath is reached and crossed by the lateral part of the MDCF, which ultimately extends to the internal jugular vein.

- Maturation stage: week 9 of development onward

Previous research has shown that in human embryos at 5–6 weeks of development, myoblasts can be identified as mononuclear, spindle-shaped cells oriented longitudinally, with scant slightly eosinophilic cytoplasm and elongated nuclei (Magro et al., 2015). In the present study, these morphological characteristics were more visible in the omohyoid muscle because of its disposition and type of section than in the sternohyoid or sternothyroid muscles, which were viewed in transverse sections. Additionally, myoblasts are known to synthesise myofilaments when contractility becomes evident (O'Rahilly and Müller, 2001).

Ultrasound studies have shown that limb and head movements in human embryos begin during week 6 of development (de Vries et al., 1982; de Vries and Fong, 2006). Mechanical stimuli alone are sufficient for fibroblastic differentiation from embryonic mesenchymal cells without the need for chemical stimuli (Subramony et al., 2013). Furthermore, it was reported that stimulation with basic fibroblast growth factor followed by traction loading enhanced collagen production as well as embryonic mesenchymal cell proliferation and subsequent differentiation into fibroblast-like cells (Subramony et al., 2014).

During this stage, we also observed that the lateral part of the primordium of the epimysium of the omohyoid muscle extended into the MDCF primordium, advancing toward a lymphatic vessel. The mechanical activity of this muscle may contribute to maintaining the patency of the lymphatic vessel lumen, thereby facilitating lymphatic circulation. By contrast, the primordium of the epimysium of the sternothyroid muscle extended into the MDCF primordium, which reached the internal jugular vein. At the contact zone, the vein exhibited a larger anteroposterior diameter. This finding suggests that the mechanical activity of the muscle, mediated through the MDCF primordium, pulls on the wall of the internal jugular vein.

During the formation stage (weeks 7–8 of development), our results confirmed the data reported by Gilroy et al (Gilroy et al., 2009). Specifically, by week 8 of development, the infrahyoid muscles were already individually separated from the surrounding muscles and positioned similarly to those in adults. These findings were more recently confirmed through three-dimensional reconstruction by Warmbrunn et al (Warmbrunn et al., 2018).

Our results also suggest that the development of the infrahyoid muscles and associated fascial structures occurs in parallel with the mechanical activity of this area. During this period, the differentiation of fibroblast-like cells and collagen fibres (epimysium) was observed in areas near the origin of the infrahyoid muscles, and the perimysium and endomysium were more organised. These findings indicate that the muscular fascial structures develop from the origin toward the terminal insertion. Cells in the fasciae, identified as fasciacytes, are dedicated to producing a hyaluronan-rich extracellular matrix that is essential for fascial gliding (Stecco et al., 2018).

Mouth opening and closing movements also begin during this period (Humphrey, 1968). Because the temporomandibular joint is not yet formed, these movements occur at the joint between the incus and the dorsal end of Meckel's cartilage (Meckelian articular complex) (Mérida-Velasco et al., 1990). In our previous study, we found that the alar fascia organised during this period to facilitate the elevation and descent movements of the pharynx and proximal oesophagus during swallowing (López-Fernández et al., 2019).

In week 9 of development (maturation stage), secondary myotubes are clearly evident in the omohyoid muscle. From week 8 of development, fusion of myoblasts reportedly results in multinucleated primary myotubes, forming longer and larger cells. These cells mature, leading to the formation of secondary (mature) myotubes (Magro et al., 2015). In specimens from week 14 of development in the present study, the MDCF and carotid sheath were well-defined, as were their relationships. Notably, the common carotid artery had an adventitia formed by numerous layers, and the MDCF inserted into the carotid sheath at the internal jugular vein area. This maintained the patency of its lumen, with the larger diameter being anteroposterior, oriented in the same direction as the insertion of the MDCF. Because this venous lumen arrangement was observed throughout all studied development stages, we consider it not to be a technical defect of the preparations but to be caused by the traction of the MDCF. Fig. 6 illustrates this relationship and, additionally, panel C specifically shows a thin fibrous tract from the MDCF crossing the carotid sheath and reaching the internal jugular vein.

Previous reports have described variations in the origin, insertion, and morphology of the two bellies of the omohyoid muscle in adults (Murugan et al., 2016; Singh et al., 2018; Nayak and Vasudeva, 2022; Kumar et al., 2023; Maślanka et al., 2023), or even the absence of one of these muscle bellies (Aithal et al., 2012; Thangarajan et al., 2014). Additionally, variable numbers of omohyoid muscle fibres can reportedly be present in the MDCF (Watkinson and Gleeson, 2020). On the right side of a 10-week-old specimen in the present study, we observed the continuity of a fascicle of fibres from the superior belly of the omohyoid with the MDCF, terminating at the carotid sheath.

4.1. Functional aspects

Fascia serves multiple functions, including force transmission, movement facilitation, stability maintenance, proprioceptive communication throughout the body, and reduction of friction associated with movement (Kumka and Bonar, 2012). There is substantial evidence of interactions between muscle fibres, intramuscular connective tissue, and fasciae (Purslow and Trotter, 1994; Purslow, 2020; Stecco et al., 2023). Studies indicate that the structure of the perimysium does not significantly contribute to passive muscle stiffness but rather acts as an organised framework for transmitting forces generated in the locomotor apparatus (Purslow and Trotter, 1994). Moreover, muscle activity plays a crucial role in promoting lymphatic circulation (Scallan et al., 2016). Fasciae are known to establish connections between adjacent structures that serve as pathways for force transmission, accounting for more than 30 % of total force transmission (Stecco et al., 2023; Maas and Sandercock, 2010).

In adults, the infrahyoid muscles are involved in the movements of the hyoid bone and thyroid cartilage during vocalisation, swallowing, and chewing, and they are mainly innervated by the ansa cervicalis (Watkinson and Gleeson, 2020). Palpation and ultrasonography studies have recently shown that the inferior belly of the omohyoid muscle contracts during neck flexion and is an agonist of the sternocleidomastoid muscle during neck rotation (Canoso et al., 2023).

The present study revealed the relationship between the primordium of the epimysium of the infrahyoid muscles and the primordium of the MDCF in the initial stage of development (week 6). This fascial tissue relationship was evident in the maturation stage (from week 9 of development onward). Two observations were relevant: first, the relationship of the MDCF with the internal jugular vein, and second, the relationship of the fascial tissue of the omohyoid muscle with the lymphatic vessels (v. Fig. 6).

In adults, the MDCF is recognised as a muscular fascia (Stecco, 2014). The primary function of the omohyoid muscle is to tense the MDCF, thereby promoting and facilitating venous circulation in the head and neck (Orts Llorca, 1986). The intermediate tendon of the omohyoid muscle typically runs adjacent to the internal jugular vein around the level of the cricoid cartilage arch. Its oblique course is maintained by a band of DCF attached below to the clavicle and the first rib, enveloping the tendon. It has been suggested that during prolonged inspiratory efforts, the muscle tension in the lower part of the DCF reduces the risk of inward-directed soft tissue collapse (Watkinson and Gleeson, 2020).

5. Conclusion

In summary, our study has established three stages in the development of the MDCF in humans. The mesenchymal primordium of the epimysium of the infrahyoid muscles is observed during week 6 of development, coinciding with the appearance of intramuscular fibres, and its development continues with the formation of the MDCF primordium. By weeks 7–8 of development, fibroblast-like cells and collagen fibres appear in both the muscle epimysium and the MDCF primordium. Maturation of these fascial structures begins in week 9. Our findings suggest that the MDCF develops in parallel with the mechanical activity of this anatomical area. The relationships of the MDCF with the lymphatic and venous structures of the region suggest that the MDCF may promote venous and lymphatic circulation.

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Author statement

The authors confirm that the manuscript has not been submitted, or is not being submitted elsewhere, and is not under consideration by another journal or other publication and that no portion of the data has been or will be published elsewhere while the manuscript is under review by Tissue & Cell.

CRediT authorship contribution statement

Jorge Murillo-González: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **José Ramón Mérida-Velasco:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Elena Martínez-Sanz:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Estela Maldonado:** Writing – review & editing, Investigation, Formal analysis. **Carmen Barrio-Asensio:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Luis A. Arráez-Aybar:** Writing – review & editing, Formal analysis. **Javier Catón:** Writing – review & editing, Formal analysis. **Pedro López-Fernández:** Writing – review & editing, Formal analysis. **Luís Otávio Carvalho de Moraes:** Writing – review & editing, Formal analysis.

Declaration of Competing Interest

No potential conflict of interest was reported by the author(s).

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Data availability

Data will be made available on request.

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