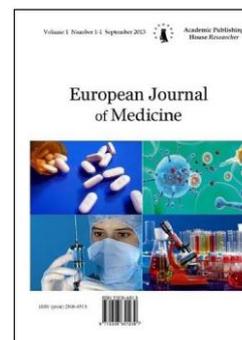




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## Spatio-Temporal Morphological Changes of Structures in Infrahyoid Triangles in Human Fetuses During Fetal Period of Human Ontogenesis

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### Abstract

Infrahyoid triangles in anterior region of neck contain crucial structures for human body like trachea, thyroid gland, nerve plexuses and magistral blood vessels. Infrahyoid muscle flaps are often used for reconstructive surgeries that is why the topography of their innervation is important for successful dissection and minimization of postsurgical complications. That is why the aim of our study was to examine topographical peculiarities of structures in infrahyoid triangles in human fetuses during late intrauterine prenatal development (PND) that would provide clinical practitioners like pediatric and oncologic surgeons with additional anatomic data.

We have examined 18 specimens of human fetuses (4-9<sup>th</sup> month of PND, 82,0-311,0 mm of parieto-coccygeal length (PCL)). All material was obtained and studied at Chernivtsy Regional Pathologists Office (Ukraine) in accordance with bilateral collaboration with the Department of Histology, Cytology and Embryology of BSMU. To reach the aim of the research we have used classic morphological methods (anthropometry, morphometry, layered dissection) as well as injection of the blood vessels (in 6 specimens by the means of dies) and three-dimensional reconstruction. The study was performed in accordance with the provisions of the Declaration of Helsinki on ethical issues of studies conducted with humans (1964-2008).

We have seen that in fetal period of human PND (80,0-310,0 mm of PCL) ansa cervicalis, that provides innervation for infrahyoid structures, is formed by anastomosis of the superior and inferior roots (C2 and C3) that are merging with internal carotid artery and internal jugular vein. The infrahyoid muscles receive nourishment predominantly from the superior thyroid artery, that is a branch of external carotid artery (91 %) or rarely bifurcation (3 %) and either as a branch of common carotid artery (6 %). Specific feature of infrahyoid triangles during fetal period of PND is the fact that it highly relies on bony boundaries such as mandible, sternum and clavicle that haven't reached their mature morphology at 3<sup>rd</sup> month of PND.

Investigations of topographical peculiarities of structures in infrahyoid triangles in fetal period of PND are important for successful reconstruction surgeries in postnatal and adult age.

**Keywords:** prenatal development, neck morphology, fetuses, embryology, triangles of the neck, human.

### 1. Introduction

Medical embryology is aimed to study normal human morphogenesis, which gives a basis for further investigations of possible pathological conditions during and after intrauterine development (O'rahilly, Müller, 2010: 74). Studies that are devoted to the problem of

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embryological sources, differentiation and topographical changes of neck structures in human fetuses during prenatal development (PND) play an outstanding role in fundamental anatomical researches (O'rahilly, Müller, 2010: 75; Naimo et al., 2015: 177; Belle et al., 2017: 162). Such investigations complement existing data on human PND and moreover, give useful data for medical practitioners such as maxillofacial surgeons, aesthetic specialists and pediatricians (Frisdal, Trainor, 2014: 416). Infrahyoid region includes crucial blood vessels, a system of muscles that is often used in reconstruction surgeries and is a place of regular development of congenital malformations (Rasool, Hassan, 2017: 330). That is why the aim of our research was to investigate topographical and developmental features of structures in the infrahyoid triangles in anterior and lateral portions of neck in human fetuses during late intrauterine development and describe the course of blood vessels, nerve plexuses and muscles' topography.

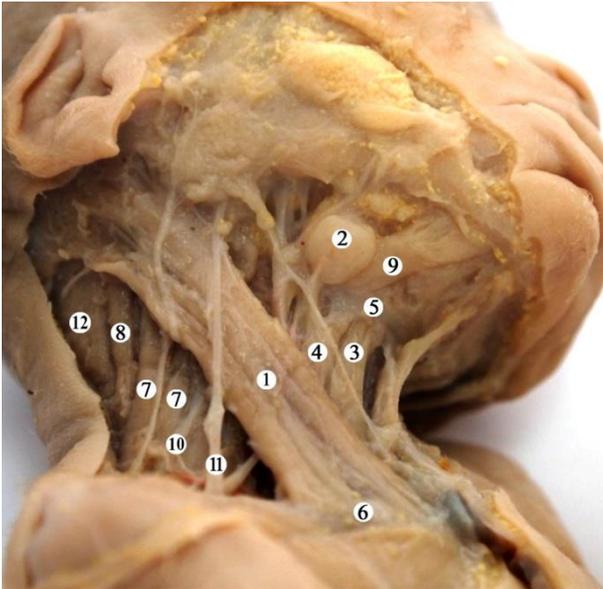
## 2. Materials and methods

We have examined 18 specimens of human fetuses (4-9<sup>th</sup> month of PND, 82,0-311,0 mm of parieto-coccigeal length (PCL)). All material was obtained and studied at Chernivtsy Regional Pathologists Office (Ukraine) in accordance with bilateral collaboration with the Department of Histology, Cytology and Embryology of Bukovinian State Medical University (BSMU). All specimens were obtained from ectopic pregnancies or spontaneous abortions, and no part of the material gave indications of possible malformation. Approval for the study was granted by the Ethics Committee of BSMU on the question of studies that involve human materials. To reach the aim of the research we have used classic morphological methods (anthropometry, morphometry, layered dissection) as well as injection of the blood vessels (in 6 specimens by the means of dies). The study was performed in accordance with the provisions of the Declaration of Helsinki on ethical issues of studies conducted with humans (1964-2008) and is a part of the scientific research of the Department of Histology, Cytology and Embryology at BSMU.

## 3. Discussion

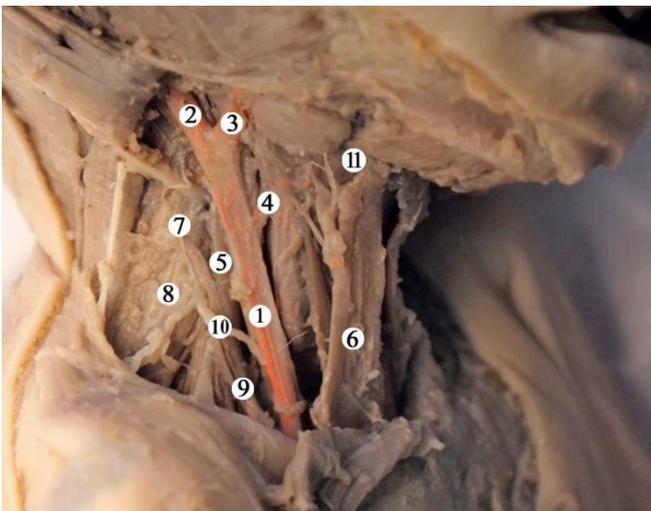
The anatomy of the neck is better understood by dividing it into regions with triangular shape. The anterior triangle of neck (AT) in human fetuses (81,0-310,0 mm PCL) is limited by the sternocleidomastoid muscle laterally (that already has developed into a mature muscular structure that spreads from the mastoid process to the clavicle), by the mandible superiorly and by the midline anteriorly (vertical line from the midpoint of the mandible that reaches the midpoint of the sternal incision). Specific feature of AT during fetal period of PND is the fact that it highly relies on bony boundaries such as mandible, sternum and clavicle that haven't reached their mature morphology at 3<sup>rd</sup> month of PND (Figure 1), which influences sized and topographical location of infrahyoid triangles (Begnoni et al., 2018).

Infrahyoid group of muscles include paired omohyoid, thyrohyoid, sternothyroid and sternohyoid that already have precise features of attachment points and fasciae coverings. It is worth to mention that at late fetal period (end of the 9<sup>th</sup> month of PND) a few neck spaces can be already distinguished. For instance, carotid space that is composed of two areas that extend from the base of the skull to the aortic arch: supra- and infrahyoid portions of neck and mediastinum. Carotid space contains internal jugular vein (IJV), common carotid artery (CCA), internal carotid artery, cranial nerves, lymph nodes and vagus nerve. In the infrahyoid region of neck, carotid space is bordered by anterior cervical space in the front, retropharyngeal space medially and by perivertebral cervical space posteriorly.



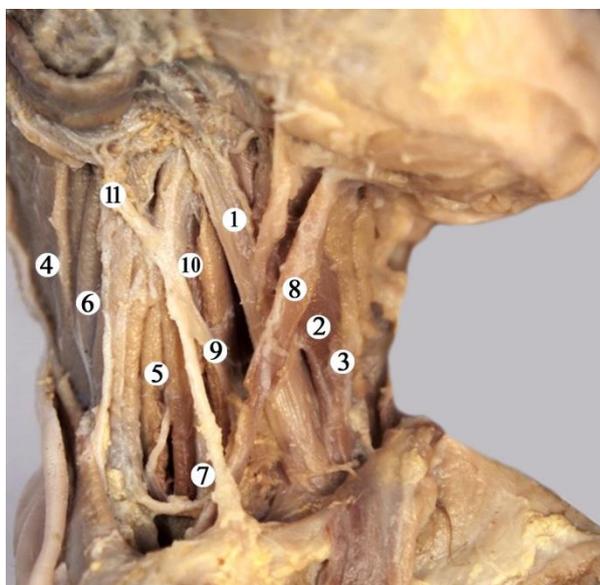
**Fig. 1.** Superficial structures in antero-lateral right projection in human fetus (195,0 mm of PCL). Macrospecimen. Magnification 4<sup>x</sup>:  
 1 – sternocleidomastoid muscle; 2 – submandibular gland; 3 – sternohyoid muscle; 4 – omohyoid; 5 – hyoid bone; 6 – clavicle; 7 – scalene muscles; 8 – levator scapulae; 9 – digastric; 10 – cervical plexus; 11 – branches of brachial plexus, 12 – deltoid muscle.

In late fetal period of human PND (271,0-310,0 mm PCL) CCA runs behind the sternocleidomastoid muscle and medially to the IJV. The IJV in fetuses has descend course, along with the lateral wall of pharynx and posteriorly to internal carotid artery. Topographical interconnection between the CCA and the vagus nerve is varying in the late fetal period (311,0-346,0 mm PCL): it changes the direction from a ventral course on intermediate one between the artery and vein (Figure 2). This is the final dorsal course that is tending to have a define adult features.



**Fig. 2.** Right lateral neck structures in human fetus (210,0 mm of PCL, male) after the dissection of platysma and sternocleidomastoid muscles. Macrospecimen. Magnification 5<sup>x</sup>:  
 1 – common carotid artery; 2 – internal carotid artery; 3 – external carotid artery; 4 – superior thyroid artery; 5 – vagus nerve; 6 – sternohyoid muscle; 7 – branches of ansa cervicalis; 8 – cervical plexus; 9 – suprascapular artery; 10 – accessory nerve; 11 – hyoid bone.

The hyoid bone (HB), that plays an important role in maintaining the commutative spatial relationship of the bones in the upper body of human fetuses, has U-shape form and is located under mandible. The HB is the bound of infrahyoid triangles, which extend from the body or horns of the hyoid downwards to the inferior border of neck and anterior borders of trapezoid muscles (starting from 80,0 mm of PCL). The HB, which is derived from the posterior portion of neural crest in human fetuses (Rodríguez-Vázquez et al., 2011: 145), divides anterior neck triangle into supra- and infrahyoid regions. Infrahyoid triangles contain such important organs like larynx, hypopharynx, cervical trachea, esophagus, thyroid and parathyroid glands. The body of the hyoid is shaped slightly curved, anterior surface is convex exhibiting a small eminence in the midline. Moreover, lateral borders of body in HB are somewhat thickened and rounded. The greater horns are joined with the body by a dense mesenchymal tissue. Connection of the lesser horn with the body and the junction between the body and the greater horn are formed by a dense stripe of closely packed cells (fetuses 85,0 mm of PCL). The prenatal morphogenesis of HB is always connected with transformation of the branchial arches (de Bakker et al., 2018: 1830). The origin of the greater horns is considered to be of the lateral cartilages of the third visceral arch, the lesser horn originates from the distal part of Reichert's cartilage (Kadir et al., 2015: 45). Superior and inferior belly of omohyoid muscle together with sternocleidomastoid subdivide infrahyoid region on smaller triangles: carotid, omotracheal, omoclavicular and omotrapezoid (Figure 3).



**Fig. 3.** Profound structures of the neck in human fetus (163,0 mm of PCL, male), anterio-lateral right view. Macrospecimen. Magnification 5<sup>x</sup>:

1 – internal jugular vein, 2 – sternothyroid, 3 – sternohyoid, 4 – trapezoid muscle, 5 – scalene muscles, 6 – levator scapulae muscle, 7 – transverse cervical artery, 8 – superior belly of omohyoid, 9 – superior laryngeal artery, 10 – vagus nerve, 11 – great auricular nerve.

The infrahyoid group of neck muscle receive nourishment predominantly from the superior thyroid artery (STA), that is a branch of external carotid artery (91 %) or rarely bifurcation (3 %) and either as a branch of CCA (6 %) (Figure 2). The sternohyoid muscle gets the blood supply from the branches of STA: superior and inferior, that are subdivided on smaller twigs (Figures 2, 3). We have observed a few cases in the middle prefetal stage (42,0-53,0 mm PCL) where the inferior branch of the STA was absent. The upper portion of the sternothyroid muscle is nourished by the STA branched twig, number of which vary from 2 to 3. It was found a variant of a common arterial trunk arising from the STA that gives branches to infrahyoid muscles like sternothyroid and sternohyoid.

Innervation of the infrahyoid structures is provided by ansa cervicalis (Kikuta et al., 2019: 222) that is formed by the means of two twigs fusion: the superior and inferior ones (C2 and C3). The superior root was observed to have ascending topography next to the lateral portion of the IJV. The terminal twigs of the nerve trunk were found next to sternothyroid and sternohyoid muscles.

We have seen two types of superior root topography: medial and lateral subtypes. The inferior root has begun from the C2 and C3. The fusion between hypoglossal nerve and ansa cervicalis was observed to have classical curved shape (fetuses 250 – 310 mm of PCL). It is important to mention that we have seen varieties in nerve course: in medial position to the IJV the topography of ansa cervicalis in total may change and to be formed between IJV and CCA. Such peculiarities during reconstructive surgeries are important for successful outcome (Yuan, Gao, 2018: 373; Som, Laitman, 2017: 242).

#### 4. Conclusion

In conclusions, it should be noted that topography of infrahyoid triangles in late fetal period of PND is characterized by mature demarcation of their borders that are represented by infrahyoid muscles and contain fascial spaces. Vascularization of infrahyoid muscles is provided by branches of external carotid artery that have a variable course. The HB horns have reached their mature morphology, but ossification has not been completed. HB plays a key role for attachment of infrahyoid muscles and margination of spaces in anterior region of neck.

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