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Observations on Muscular Attachments to Human Developing Mandible

Obserwacje przyczepów mięśniowych rozwijającej się żuchwy ludzkiej

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Abstract

Background. The development of the mandible is closely related to Meckel's cartilage, mandibular division of trigeminal nerve and muscles of mastication.

Objectives. The aim of the study was to investigate the muscular attachments to the developing mandible during prenatal life.

Material and Methods. The observations were carried out on serial sections stained according to various histological methods.

Results. At stage 16, the rod shaped Meckel's cartilage followed the general curvature of mandibular process from the otic capsule to the future symphysis menti. The stylohyoid and styloglossus muscles were visible in close relation to the ventral end of Meckel's cartilage. On the medial surface of the cartilage, the mylohyoid muscle, as well as the genioglossus and geniohyoid muscles were visible. At stage 18 the muscles established their temporary attachments to Meckel's cartilage. At the same time, near the posterior end of Meckel's cartilage the primordia of articular disc and pterygoid muscle developed. The primordium of masseter muscle was observed in close relation to the angular portion of Meckel's cartilage at stages 19 and 20. Observations on the developing coronoid process showed the attachment of the lateral pterygoid muscle to Meckel's cartilage at stage 21.

Conclusions. The muscles were connected to Meckel's cartilage by the end of 10th week of development, and after that they moved onto the developing mandible. The observations appear to confirm the hypothesis stating that the muscles attached to the Meckel's cartilage indeed influence the mandibular movements, resulting in a premature dislocation of the mandible (**Adv Clin Exp Med 2012, 21, 4, 447–454**).

Key words: mandible development, Meckel's cartilage, muscles of mastication.

Streszczenie

Wprowadzenie. Rozwój żuchwy jest ściśle powiązany z chrząstką Meckela, gałęzią żuchwową nerwu trójdzielnego oraz mięśniami żucia.

Cel pracy. Zbadanie przyczepów mięśniowych do rozwijającej się żuchwy w okresie zarodkowym i płodowym.

Materiał i metody. Obserwacje przeprowadzono na seryjnych przekrojach histologicznych barwionych uprzednio różnymi metodami.

Wyniki. W 16. stadium rozwoju zarodkowego pałeczkowata w kształcie chrząstka Meckela podążała za krzywizną wyrostka żuchwowego od torebki słuchowej do przyszłego spojenia żuchwy. W pobliżu brzusznego końca chrząstki Meckela były widoczne mięśnie: rylcowo-gnykowy i rylcowo-językowy. Na przyśrodkowej powierzchni chrząstki były widoczne mięśnie: żuchwowo-gnykowy, bródowo-językowy oraz bródowo-gnykowy. W 18. stadium rozwoju mięśnie ustaliły swoje tymczasowe przyczepy do chrząstki Meckela. W tym samym czasie, w pobliżu dystalnego końca chrząstki Meckela, rozwijały się zawiązki płytki słuchowej i mięśni skrzydłowych. Zawiązek mięśnia żwacza obserwowany był w 19. i 20. stadium rozwoju w pobliżu okolicy kąta chrząstki Meckela. Obserwacje rozwoju wyrostka dziobiastego ukazały przyczep mięśnia skrzydłowego bocznego do chrząstki Meckela w 21. stadium rozwoju zarodkowego.

Wnioski. Z chrząstką Meckela były połączone mięśnie przed końcem 10. tygodnia rozwoju, a po tym okresie przemieściły się w kierunku rozwijającej się żuchwy. Obserwacje wydają się potwierdzać tezę, że mięśnie przyczepione do chrząstki Meckela mają istotny wpływ na ruchomość żuchwy, co skutkuje przemieszczeniem się jej w tym okresie (**Adv Clin Exp Med 2012, 21, 4, 447–454**).

Słowa kluczowe: rozwój żuchwy, chrząstka Meckela, mięśnie żucia.

The interaction between bones and muscles suggests that the shape of selected parts of the skeleton depends on the degree of activity of the attached muscles. The biological effects of force on the mineralized skeleton during prenatal development have been studied extensively [1, 2]. Washburn (1947) recognized three classes of morphological features in the skull, which, to a greater or lesser extent, depend on the action of the muscles. The self-differentiating ones, which require the presence of muscles to maintain them, those that appear only in response to muscle development, and those that are largely independent of the muscles associated with them. According to this classification, muscle development and the mechanical influences within it do not determine the entire form of the cranium [3]. However, an increasing effect may occur on the type of bone, which develops on various skeletal units, including the mandible or superstructures such as the crests or the fosses [4]. Conversely, it is suggested that the degree of the masticatory muscles development may be dependent on the size of the mandibular ramus and lateral pterygoid plates [5]. The localized effect of muscle function on the remodeling of the facial bones has been attributed to mechanical loading and the resulting tension forces created within the periosteal membrane or the tendinous type of attachment [4]. The influence of masticatory muscles on craniofacial growth and development has been considered to be a very important issue due to orthodontic reasons. The connection between as well masticatory muscles thickness as function and facial morphology has been researched and discussed from an orthodontic point of view too [6].

The mandible, being the most massive bone of the human viscerocranium, develops from six independent skeletal units and is modified by different factors [7–9]. The growth of the coronoid and angular units is primarily influenced by the prenatal function of the surrounding muscles that are attached to them [4, 10, 11]. It was suggested that the masticatory muscles (temporalis, masseter, and lateral and medial pterygoids) develop from the so-called temporal-muscle primordium [12]. According to one theory, which indicates a coincidental arrangement of muscle fibers and their growth direction, whenever there is an elongation of the mesenchymal tissue by the growing skeletal primordia, muscle fibers are activated and their orientation assumes the direction of the force. As a result, there is muscle formation in the dilatation field, which parallels the development direction of the mandible [13]. A small number of studies are available describing the human prenatal muscle development in the oral region, and there is a lack of studies which comprehensively describe the entire group of perioral muscles while focusing on the correlation between both muscle groups. As a result, we conducted a study to examine the Meckel's cartilage and the mandible during their prenatal development.

The aim of this study was to trace the events in development of the masticatory muscles attached to Meckel's cartilage and ossifying the mandible and to discuss their possible influence on the formation of mandibular units.

Material and Methods

The study was performed on 30 human embryos and 5 fetuses from the Collection of the Department of Anatomy at the Poznan University of Medical Sciences. The age of investigated embryos (from 37 to 56 days) was established according to international criteria of developmental stages and was expressed in postovulatory days. The age of the fetuses (from 9 to 12 weeks) was established upon CR-L and FL and expressed in postovulatory days.

The embryos were preserved in 10% formalin and embedded *in toto* in paraffin or paraplast and the serial sections 5 μm were made in sagittal, frontal or horizontal plane. The fetuses were decalcified prior to these procedures. Some fetuses were embedded *in toto*, and some were dissected first and their mandibles were taken out for investigations. The serial sections 7 μm thick were made in the same three planes. The sections were stained with hematoxylin and eosin (H+E), with aniline blue according to Mallory's method, and silver protargol according to Bodian's methods and then observed in light microscope.

Results

The existence of muscles primordia was first observed in 39 day old (8–11 mm) embryos. During the 16th stage, the most evident is the formation of the face with the critical moment of fusion of the lateral and medial nasal prominences. The whole oral cavity is occupied by the developing tongue. The tongue's internal muscles are penetrated by the fibers of hypoglossal nerve. The lingual nerve runs laterally to the hypoglossal nerve, close to the submandibular ganglion. Meckel's cartilage extends along the whole length of the mandibular prominence. In the frontal sections between the developing external acoustic meatus and the otic vesicle, the condensation of mesenchyme is visible (Fig. 1). Beneath the condensed mesenchyme, the posterior end of Meckel's cartilage, the stylohyoid, and the styloglossus muscles are observed (Fig. 2). Along the lateral surface of Meckel's cartilage, the fibers of inferior alveolar nerve and the mylohyoid muscle are visible (Fig. 1).

According to the next stage, stage 17 (11–14 mm, 41 days) is a critical moment in the development of the primary palate, the auditory ossicles, and the beginning of the ossification process of the mandible. The primordia of genioglossus, geniohyoid, and mylohyoid muscles are well visible but without contact with surrounding supportive structures, which changes in the next observed period. In embryos at stage 18 (13–17 mm, 44 days), the oral cavity narrows. The palatine processes are vertically oriented and laterally demarcate the tongue. Meckel's cartilage broadens and flexes upwards in the symphyseal region. Frontal sections show that opposing Meckel's cartilages form parabolic shape. Genioglossus, geniohyoid, and myohyoid muscles are also well visible and fibers of the muscles attach to the inner surface of Meckel's cartilage. During this stage, the ossification of mandible intensifies. The structures of temporomandibular joint develop and the articular disc, in form of condensation of mesenchyme, is continuous with the developing lateral pterygoid muscle (Fig. 3).

In 46–49 day old embryos (16–18 mm and 18–22 mm) the rounded tongue still occupied the whole oral cavity. However, the shape of Meckel's cartilage changed in a canine region as well as on its posterior end, where it is connected with tensor palatine muscle. On the lateral surface of Meckel's cartilage we can see the mesenchymal primordia of condylar process of mandible, squamous part of temporal bone, the masseter muscle and the condensation of mesenchyme being a primordium of temporalis muscle. Between the articular surfaces of developing temporomandibular joint, the articular disc is visible and is continuous with the lateral pterygoid muscle. In the next, 21st stage (22–24 mm, 51 day) the lateral pterygoid muscle attaches itself to the internal surface of the posterior end of Meckel's cartilage. At the same time at the lateral surface of Meckel's cartilage, the masseter,

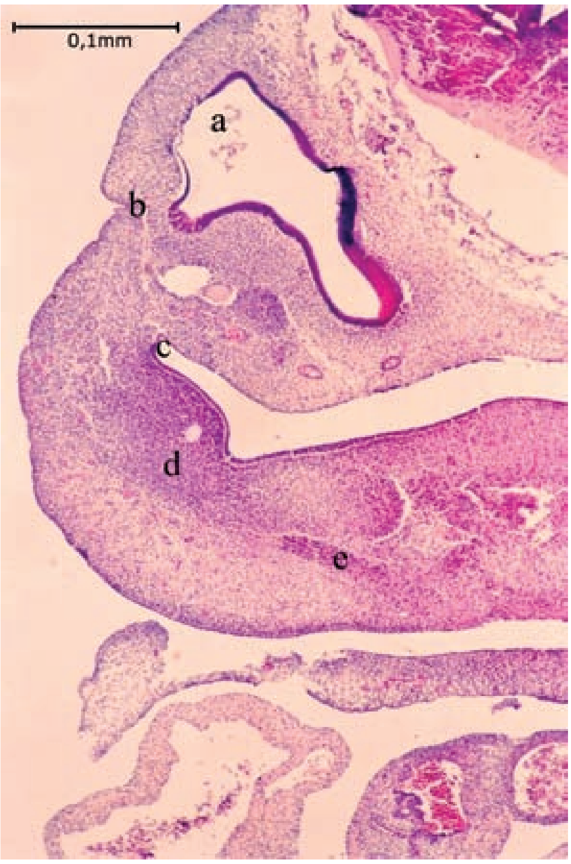

Fig. 1. Coronal section through the embryo in 16th stage (39 days). H+E. a – labyrinth, b – external auditory meatus, c – tubotympanic recess, d – Meckel's cartilage, e – mylohyoid m.

Ryc. 1. Przekrój czołowy embrionu w stadium 16. (39 dni). H+E. a – labirynt, b – przewód słuchowy zewnętrzny, c – zagłębienie trąbki słuchowej, d – chrząstka Meckela, e – mięsień żuchwowo-gnykowy

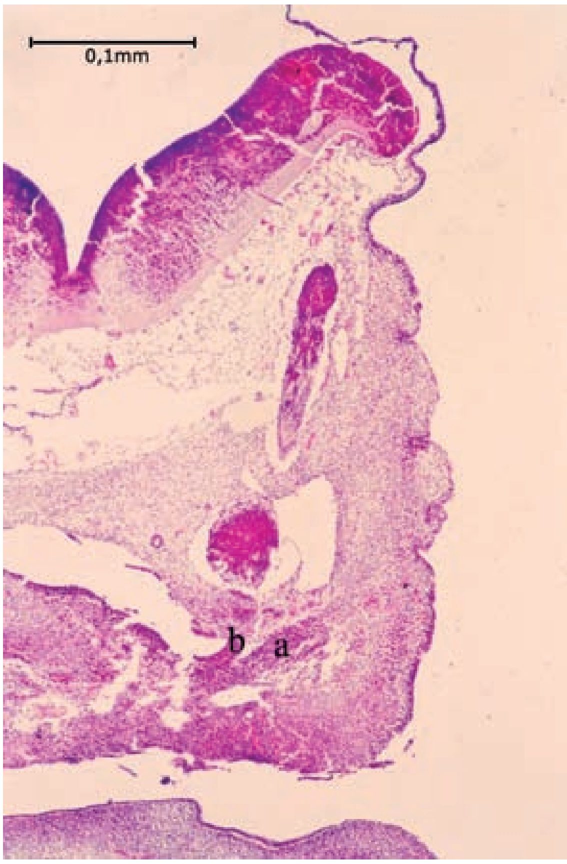

Fig. 1. Coronal section through the embryo in 16th stage (39 days). H+E. a – labyrinth, b – external auditory meatus, c – tubotympanic recess, d – Meckel's cartilage, e – mylohyoid m.

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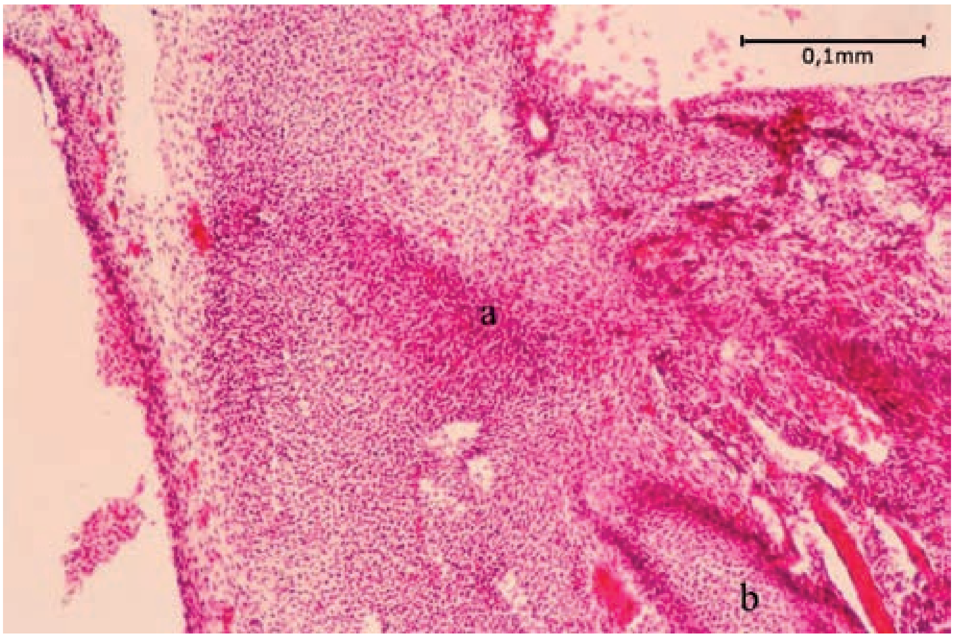

Fig. 2. Coronal section through the embryo in 16th stage (39 days). H+E. a – stylohyoid m., b – styloglossus m.

Ryc. 2. Przekrój czołowy embrionu w 16. stadium (39 dni). H+E. a – mięsień rylcowo-gnykowy, b – mięsień rylcowo-językowy

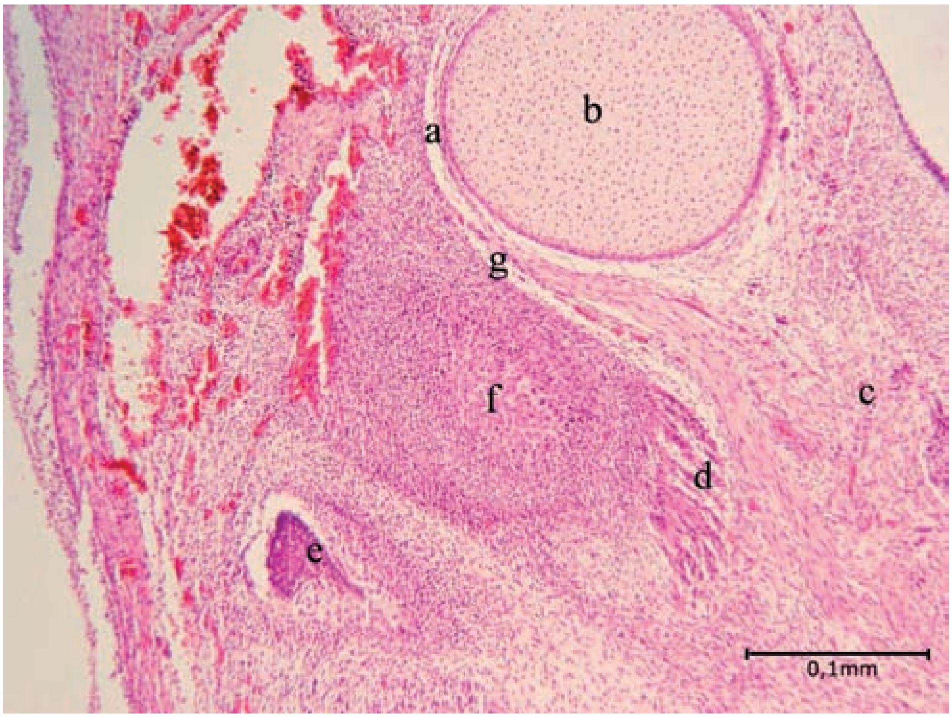

Fig. 4. Transverse section through the developing temporomandibular join in fetus (CRL 38mm). H+E. a – articular disc, b – Meckel's cartilage, c – lateral pterygoid m., d – medial pterygoid m., e – temporal process of zygomatic bone, f – condylar cartilage, g – lower join cavity

Ryc. 4. Przekrój poprzeczny przez rozwijający się u płodu staw skroniowo-żuchwowy (CRL 38 mm). H+E. a – krążek stawowy, b – chrząstka Meckela, c – mięsień skrzydłowy boczny, d – mięsień skrzydłowy przyśrodkowy, e – wyrostek skroniowy kości jarzmowej, f – chrząstka stawowa, g – dolna jama stawowa

medial pterygoid, and temporalis muscles are visible and are surrounded by the developing condylar and coronoid processes of the mandible. The muscles are perforated by adequate nerves. Both palatal processes are in vertical position but the frontal part of Meckel's cartilage retreads and stays in the closest contact between left and right side

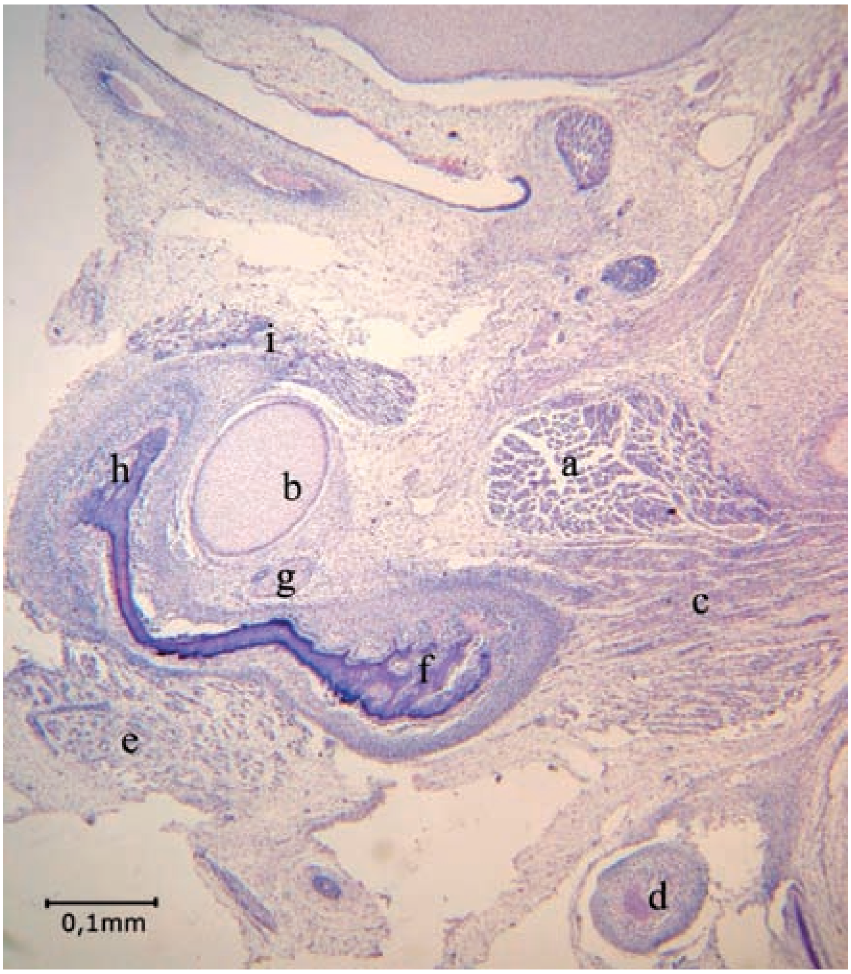

Fig. 5. Transverse section through the fetus (CRL 56 mm). H+E. a – lateral pterygoid m., b – Meckel's cartilage, c – temporalis m., d – angular cartilage of mandible, e – masseter m., f – coronoid process, g – masseteric nerve, h – condylar process and head of the mandible, i – lateral pterygoid m.

Ryc. 5. Przekrój poprzeczny płodu (CRL 56 mm). H+E. a – mięsień skrzydłowy boczny, b – chrząstka Meckela, c – mięsień skroniowy, d – kątowa chrząstka żuchwy, e – mięsień żwacz, f – wyrostek dziobiasty, g – nerw żwaczowy, h – wyrostek kłykciowy głowy żuchwy, i – mięsień skrzydłowy boczny

during 22 and 23 stages (23–28 mm, 27–31 mm, 53 and 56 days).

At the same moment, the posterior end of Meckel's cartilage becomes the attachment place for the tensor muscle of the soft palate and is near the place where the articular disc develops. The connection between the articular disc, the lateral pterygoid muscle and Meckel's cartilage was observed up to the beginning of fetal period (Fig. 4).

Starting from the 9th week of development, the beginning of the fetal period, the fibers of the lateral pterygoid muscle completely change the position from the cartilage to the developing mandible. (Fig. 5) but the articular disc of the temporomandibular joint is continuous with Meckel's cartilage. During the 9th week, Meckel's cartilage is visible along the whole length of mandible, and its distal end is still continuous with the malleus. Along the body of the mandible, the mylohyoid muscle attaches to the cartilage and first at the end of the 10th week, the major part of its fibers move from the Meckel's cartilage onto the mandible (Fig. 6). During the 11th week, the attachments of genioglossus and geniohyoideus muscles also move from the cartilage onto the mandible. Similarly, the medial pterygoid muscle attaches itself to the internal surface of the angular portion of the mandible.

Discussion

Skeletal growth and form depend on many interacting factors, one of which is mechanical loading [14]. It seems probable that the contraction of the masticatory muscles can affect bone growth in the oral region during prenatal development. Spyropoulos (1977) confirmed this theory by demonstrating that the earliest development of the muscles stays in relation to skeletal units. He proved that temporalis premyoblasts were evident at the beginning of the 6th intra-uterine week, while the first evidence of the coronoid process appeared approximately one week later. Consequently, this bone process is not self-differentiating, but it is dependent on the differentiation of the temporalis muscles. The development of the temporalis and masseter muscles and their attachment to the mandible, with the mandible growing in width, are closely related

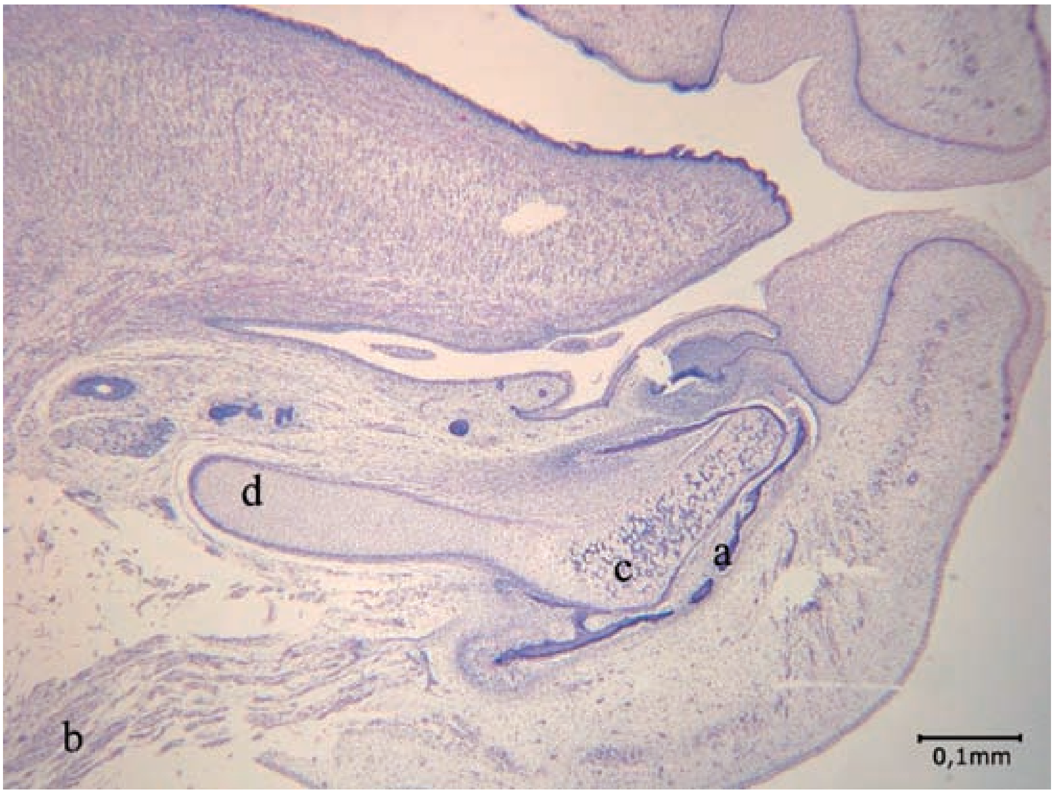

Fig. 6. Sagittal section through the fetus (CRL 56 mm). H+E. a – mandible, b – mylohyoid m., c, d – Meckel's cartilage

Ryc. 6. Przekrój strzałkowy płodu (CRL 56 mm). H+E. a – żuchwa, b – mięsień żuchwowo-gnykowy, c, d – chrząstka Meckela

when it comes to timing [10]. Our observations confirm the previous studies indicating that from the end of 6th week of the prenatal development, pointed muscles primordia are observed in relation to the developing mandible [15, 16]. The attachment of the masseter muscle is closely associated with the mandibular ramus, which undergoes remodeling during prenatal period. The consequence of this is a continual re-establishment of their fibers on the mandible. This can explain why the suckling muscles are relatively better developed than the muscles of mastication, at birth [8].

Following the appearance of the temporalis and masseter muscles, the lateral and medial pterygoids are visible and the connection between the lateral pterygoid muscle to the articular disc of temporomandibular joint was referenced [15, 16]. Furthermore, it was reported that the lateral pterygoid muscle remained attached to the disc and the condyle throughout the fetal development [17]. The earlier attachment of this muscle to the Meckel's cartilage can have an effect on the primary jaw joint movement observed during the late embryonic period [18]. In present investigation, after the first observing of articular disc and lateral pterygoid muscle primordia on the 44^{th} day, the earlier connection between Meckel's cartilage and articular disc and tensor veli palatini was indicated. In 2 days older embryos (51 day) the connection between cartilage and lateral pterygoid muscle was observed.

The Meckel's cartilage, supported the developing mandible, is also the previous structure connected with other perioral muscles [19].

In the embryonic period, the muscles forming the floor of the mouth insert into the Meckel's cartilage [20]. The cartilage is directly or indirectly affected, via the fibrous tendons by the developing bone, its growth process and its change in shape. According to authors observations, the primordia of muscles of the floor of the mouth are visible in embryos ranging from 11 to 14 mm (39 day). Radlanski et al. (2001) observed them at the lingual surface of the Meckel's cartilage in embryos ranging from 15.6 to 41 mm, and the genioglossus muscle even later [13]. Present results are consistent with the statement of Doskocil (1989), namely that in the 44th day of the development, fibers of the genioglossus, geniohyoid, and mylohyoid muscles attach themselves to the Meckel's cartilage [21]. Lee et al. (2001) observed only the mylohyoid muscle attachment and stated that at the end of the 12th week, its fibers move from the cartilage onto the mandible [15]. However, Doskocil (1989) observed such attachments changes by the end of the embryonic period [21]. According to our investigations, the muscle fiber attachments moved by the end of the 10th week. Early differentiation of the mylohyoid and digastrics muscles is important for the early mouth-opening ability and may be essential to normal development [8]. Furthermore, connections between the cartilage and the muscles present during the late period may play a role in the secondary palate formation [22–24]. Moreover, based on present study observations, the attachment of the tensor veli palatini muscle to the cartilage confirms this character of the Meckel's cartilage. In fact, the change in the shape of the Meckel's cartilage during the 8th week, along with its rapid, linear growth in the anterior direction, plays an important role in the elevation of the palatine processes [25]. At the same time, the first spontaneous muscle movement have been elicited [8]. Primarily connected with stimuli mouth opening and closure starts from 8–9 weeks of age. Mouth closure is active after the 11th week as a result of the initiation of muscle stretch reflex activity. During the 56th day of the development, the authors observed a longitudinal growth of the Meckel's cartilage and the mandible, resulting in a protrusion of the mandible. Meanwhile, the palatine processes, which are oriented vertically by this time, change the direction into horizontal. This process connected with the withdrawal of the tongue and the development of synovial cavities in the temporomandibular join may, according to Sperber (2008), depend on the early mouth movement and probably the earliest development of stylohyoid and stylogossus muscles [8].

A strong connection between masticatory muscles' and bones' development has been observed prenatally as well as postnatally. The influence of masticatory muscles on craniofacial growth and development has been researched and widely discussed by orthodontists [6, 26]. Contractions of muscles of the craniofacial area must occur to stimulate the growth of bone and cartilage. The muscles start contracting between the 6th and 8th week of embryonic development. Lack of these muscles' activity can cause, inter alia, hypertelorism, flat zygoma and midface, small and open mouth, small tongue or microretrgnathia [27]. As it was proved, the absence of muscles in mammalian embryos and fetuses significantly altered the shape and size of the mandible. The development of secondary cartilage and in turn the development of the shape and size of bones including mandible is strongly dependent on mechanical signals from muscles [28].

The observations on the serially sectioned embryos and fetuses appear to confirm the hypothesis stating that the muscles attached to the Meckel's cartilage and developing mandible indeed influence the mandibular movements, resulting in a premature dislocation of the primary mandible from the Meckel's cartilage.

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