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STEREOLOGICAL ANALYSIS OF THE HUMAN FETAL TRIGEMINAL GANGLION MICROCIRCULATORY BED

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Summary. *Blood supply is an important component for morphofunctional ability of the ganglions. The aim of this study is to quantify, by the application of stereological method, the microcirculation components of the trigeminal ganglion, added to the sensory trigeminal root as one of the most important component of the trigeminal system.The material consisted of 12 right human fetal trigeminal ganglia. Fetuses were classified into three groups according to their gestational age. Trigeminal ganglions, together with neighboring dural sheath, were dissected under the surgical microscope. After routine histological processing, trigeminal ganglions were cut into 5 μm thick slices and stained with HE, Alcian blue-PAS and Masson trichrome stain. Morphometric analysis was performed with M42 multipurpose test system under the lens magnification 63×. The number of capillaries per square millimeter increased significantly during the gestation in the third fetal age group. Average arteriolar and venular length density insignificantly increased in the mandibular, while it decreased insignificantly in the maxillary and ophthalmic division of the trigeminal ganglion. This morphometric parameter had the highest values in mandibular, lower in ophthalmic and the lowest values in maxillary trigeminal ganglion division. The presence of capillary microcirculation component increased during the gestation. Conversely, arteriolar and venular component did not show significant changes during the gestation, but its presence was the most distinct in the mandibular trigeminal ganglion division and least expressed in the maxillary portion.*

Key words: *Fetal, trigeminal ganglion, microcirculation, morphometry*

Introduction

Trigeminal (semilunar) ganglion, a very important component of the trigeminal system is a sensory ganglion associated with the sensory root of the trigeminal nerve, located in the Meckel's cave filled with cerebrospinal fluid, on the apical part of the anterior surface of the temporal pyramid.

Vieussens was the first who observed it. However, Lorenz Gasser was the first to elaborate in detail the structure of the trigeminal ganglion and his student Hirsch first called it the "gasserian ganglion" half a century later. In 1779, Proschaska called it the "triangular plexus", while Sjögvist described it in 1938 as "a well-marked triangular plexus" (1).

Blood supply is a very important prerequisite of the functional ability of the nerves and the ganglions, because any disturbances in extra- and intraneural or extra and intraganglionar blood supply, may lead to degenerative changes and have a dysfunction as a consequence.

Only a few articles have been devoted to the blood supply of the trigeminal ganglion and the nerve root (2,3). We still do not know the precise vascular pattern of the motor and the sensory root of the nerve nor of the trigeminal ganglion.

According to Frazier and Whitehead (4) and Bergman's citation (5), one of the first descriptions of the trigeminal ganglion blood supply was done by Meckel in 1748.

Previous researches adequately described the angioarchitectonics of trigeminal ganglion external blood vessels which originate from the cavernous portion of the internal carotid artery (5,6) and from the middle meningeal artery or from the accessory ramus of the middle meningeal artery (6,7,8), but the data of the intraganglionar microcirculatory bed topography are lacking (2,5). However, we did not find any study about the trigeminal ganglion microcirculatory components quantification.

The aim of this study is to quantify, by the application of stereological method, the components of the trigeminal ganglion microcirculation, as one of the most important component of the trigeminal system. Simultaneously, the distribution of microcirculatory network between different parts inside the ganglion would also be evaluated.

Subjects and Methods

Investigation was performed on 12 right human fetal trigeminal ganglia. Fetuses were of both genders and, their age ranged from the second to the eighth month of gestation. Fetuses are a part of Medical Faculty Niš Departments of Anatomy collection. All fetuses were medico legally obtained, in accordance with Internal Ethic Committee rules, from the Gynecology and Obstetrics Clinic of the Faculty of Medicine in Nis, after the spontaneous or artificial abortions. It was established that they were without anatomical deformities and systemic disease. Afterwards, fetal age was determined by measuring the crown – rump distance (CRD) according to the Patten's scale (9). Fetuses were classified into three groups according to their gestational age: I group (II month of gestation – fetuses with 10 – 19 cm CRD), II group (III and IV months of gestation – fetuses with 20 – 29 cm CRD) and III group (V to VIII months of gestation- fetuses with 30 – 39 cm crown – rump length). Trigeminal ganglions, together with neighboring dural sheath, were dissected under the surgical microscope (Olympus MTX; Olympus optical Co, Ltd, Japan). Then the ganglions were fixed in 10% buffered formalin, embedded in paraffin and, finally cut into slices 5 μm thick. Slices were further routinely histologically processed and stained with HE, Alcian blue - PAS and Masson trichrome stain. Reichart Visopan light microscope with projection screen was used for the histological and morphometric analysis. Additional analysis was performed under the Olympus BX50 imaging system.

Morphometric analysis was performed under the 63× lens magnification. Multipurpose test system M42 was used. It was calibrated with object micrometer (1:100). Test system had 42 test points. The distance between two test points (d) was 26 μm. The area per one test point (a) was 533.26 μm^2, while the whole test system area (A_t) was 22397.12 μm^2. Test system line length (L_t) was 546 μm. Morphometric analysis included measurement of the number of capillary profiles per test system area unit, as well as, arterioles and venules length density. Ten, by a selected unbiased method, fields of vision were analyzed in each case. Length density (L_V) was calculated according to the Kališnik's formula $L_V = 2 \times (QA / A_t)$, where Q_A was the number of arterioles and venules axis which hit the test system area (A_t). Its unit is μm^{-2} (10). Arterioles and venules length density was measured separately for the ophthalmic (V1), maxillary (V2) and mandibular (V3) part of trigeminal ganglion. Difference between the values of obtained morphometric parameters for different groups of fetuses and different parts of trigeminal ganglion was estimated using Student's t – test.

Results

The presence of the tripartite structure of the trigeminal ganglion (three parts marked by the connective tissue fibers) was observed on trigeminal ganglion histological slices (Fig. 1). Trigeminal ganglion is wrapped up in the connective tissue capsule and ganglion tissue is composed of the connective tissue stroma with numerous ganglion and satellite cells within. Larger blood vessels (arterioles and venules) were visible on lower magnification in trigeminal ganglion stroma (Fig. 2a, b, c). Higher magnification showed the presence of a reach capillary network among ganglion cells (Fig. 2d).

Morphometric analysis showed that trigeminal ganglion microcirculation pattern varies in relation to fetal gestational age, as well as, in the different parts of the ganglion.

Capillary component of the ganglion's microcirculation mildly increases during the gestation. The number of capillaries per square millimeter increases insignificantly ($p>0.05$) for 9.4% in the second in relation to the first group (Table 1, Chart 1A). This number then significantly ($p<0.05$) increases in the third group for

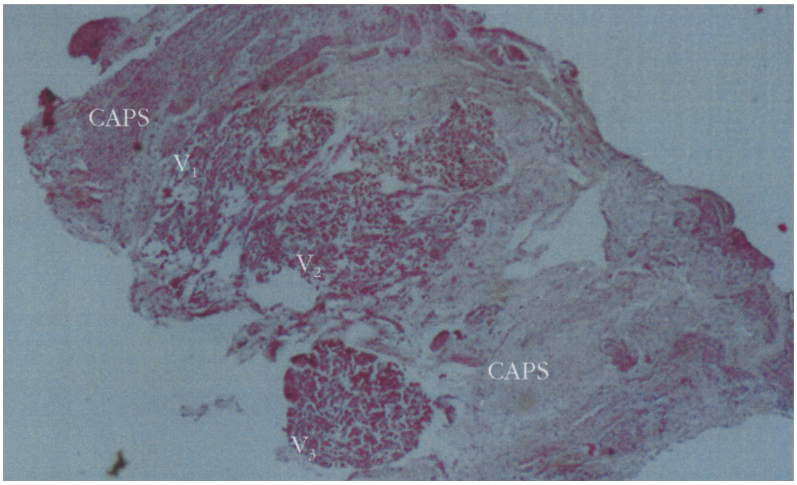


Fig. 1. Human fetal trigeminal ganglion; HE; 100×; CAPS – capsule of trigeminal ganglion; V1 – ophthalmic division; V2 – maxillary division; V3 – mandibular division.

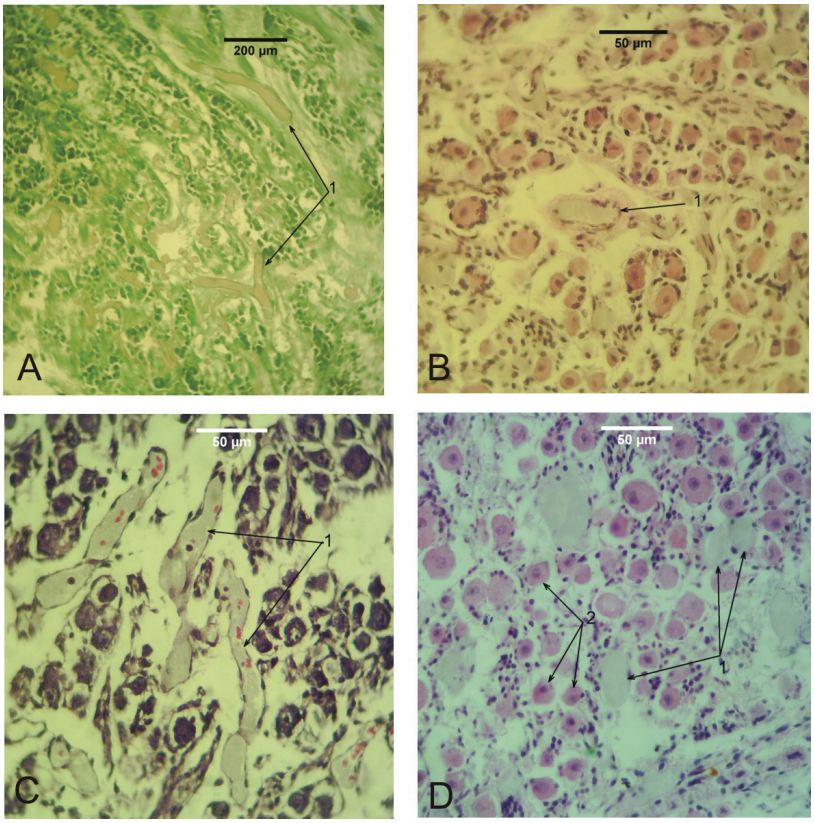


Fig. 2. A – Masson trichrome stain; 200×; 1 – larger blood vessels in trigeminal ganglion stroma; B – HE; 400×; 1 – larger blood vessel in trigeminal ganglion stroma; C – PAS; 400×; 1 – two larger blood vessels in the trigeminal ganglion stroma; D – HE; 400×; 1 – smaller blood vessels in trigeminal ganglion stroma; 2 – ganglion cells.

23.2% in relation to the second and for 65.2% in relation to the first group (Table 1, Chart 1A).

Table 1. Trigeminal ganglion number of capillaries per one test system square millimeter during the gestation

Fetal age groups	Number of capillaries per square millimeter		
	Mean	SD	SE
I (10-19 cm)	2710	138	138
II (20-29 cm)	2964	551	551
III (30-39 cm)	3652[a,b]	249	249

a – III vs. II $p<0.05$, b – III vs. I $p<0.05$

Arteriolar and venular components of the microcirculation showed significant differences between the three parts of the trigeminal ganglion.

In the ophthalmic part of the trigeminal ganglion, average length density of the arterioles and venules showed a decrease during gestation. Its values were 28.8% significantly ($p<0.05$) lower in the second group in relation to the first group (Table 2, Chart 1B). Then, it increases insignificantly, by 11.4% in the third in relation to the second, but remained lower, by 25.5% in relation to the first group (Table 2, Chart 1B). In the maxillary part of the trigeminal ganglion, arteriolar and venular average length density shows the similar trend during the gesta

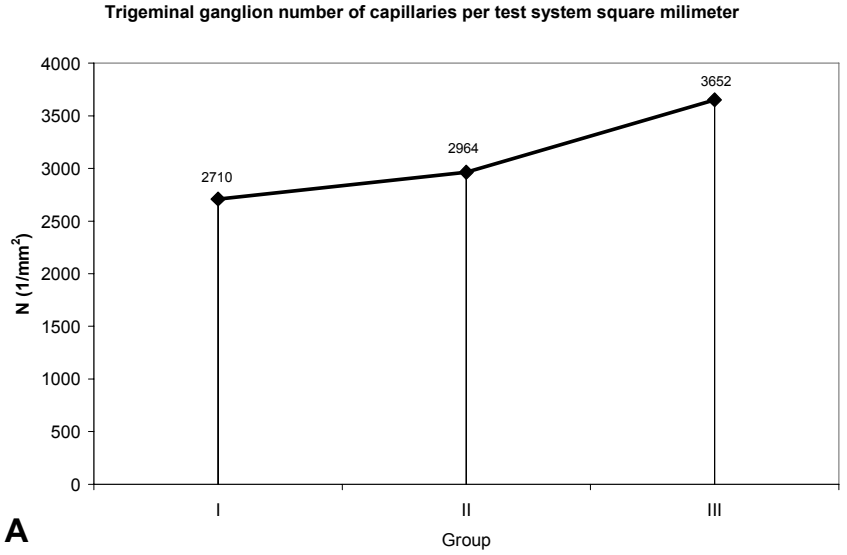
Table 2. Trigeminal ganglion parts arterioles and venules length density during the gestation

Trigeminal ganglion parts	Fetal age groups								
	I (10-19 cm)			II (20-29 cm)			III (30-39 cm)		
	Mean ($\times10^{-6}mm^{-2}$)	SD ($\times10^{-6}mm^{-2}$)	SE ($\times10^{-6}mm^{-2}$)	Mean ($\times10^{-6}mm^{-2}$)	SD ($\times10^{-6}mm^{-2}$)	SE ($\times10^{-6}mm^{-2}$)	Mean ($\times10^{-6}mm^{-2}$)	SD ($\times10^{-6}mm^{-2}$)	SE ($\times10^{-6}mm^{-2}$)
Ophthalmic (V1)	125	98	49	89**	88	44	98	25	12,5
Maxillar (V2)	107	65	32	36	92	46	45	38	19
Mandibular (V3)	125	98	57	134[a]	38	19	143[a,b]	25	12,5
n	4			4			4		

** - $p<0.05$, a – V3 vs. V2 $p<0.05$, b – V3 vs. V1 $p<0.05$

tion. Its values decreased in the second group, by 66.4% in relation to the first group (Table 2, Chart 1B). Afterwards, its values increases by 25% in the third in relation to the first, but remained lower by 57.9% in relation to the first group (Table 2, Chart 1B). These differences were not statistically significant ($p>0.05$). Finally, in the mandibular part of the trigeminal ganglion, average arteriolar and venular length density shows insignificant increase ($p>0.05$) during the gestation. It increases by 7.2% in the second and, 14.4% in the third compared to the first group (Table 2, Chart 1B). However, its increase between the second and the third group was lower and amounted to 6.7% (Table 2, Chart 1B).

Concerning trigeminal ganglion's portions average length density, mandibular part has the highest, while ophthalmic part values has lower and maxillary division has the lowest values. Mandibular and ophthalmic average length density has the same values in the first group and they were 16.8% higher than the same value of maxillary part (Table 2). This difference is not significant. Average length density of mandibular part is insignificantly ($p>0.05$) higher by 50.6% in the second group than in ophthalmic and 272.2% significantly ($p<0.05$) higher than the one of maxillary part (Table 2). Finally, in the third group, mandibular division average length density is significantly higher ($p<0.05$) by 45.9% than in ophthalmic and217.8% higher than the one of maxillary part (Table 2). In conclusion, average length density has the highest values in the mandibular portion of the trigeminal ganglion in relation to the other two parts and these differences are more distinct in the older gestational groups.



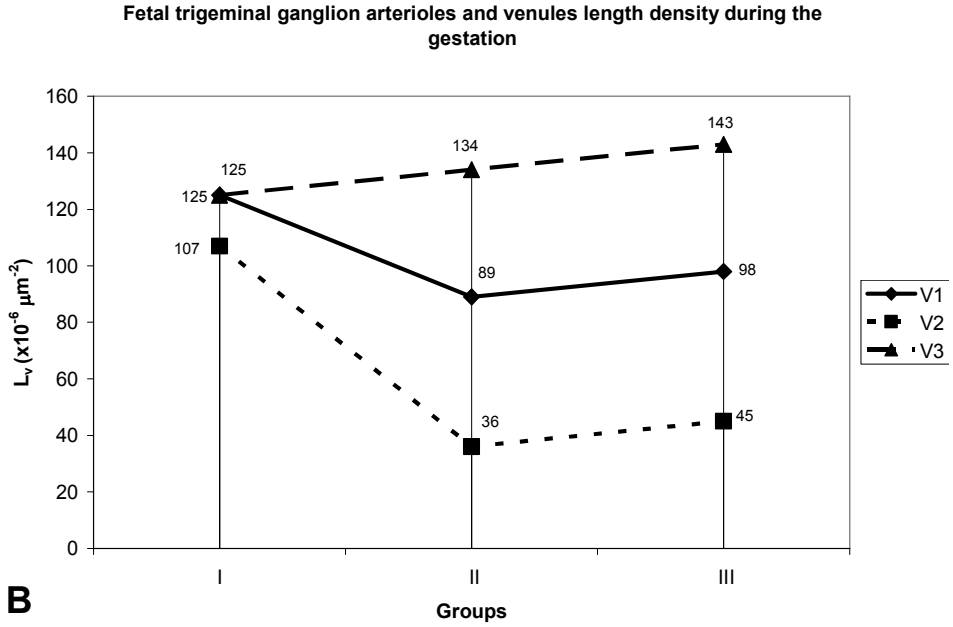


Chart 1. A – Trigeminal ganglion number of capillaries per test system square millimeter during gestation; B – Trigeminal ganglion arterioles and venules length density during the gestation.

Discussion

Structure of the trigeminal ganglion was thoroughly studied (1). It is composed of nerve cells, satellite cells, connective tissue stroma and the nerve fibers, which present its main structural components. However, in spite of the well described external sources of trigeminal ganglion blood supply (8,11,12,13), there is a lack of data about intraganglionar microcirculation or the so called microcirculatory bed (2).

Smoliar et al. (2) described the presence of capsular and stromal blood vessels in the trigeminal ganglion. These vessels were composed of long (up to 150 μm of length), as well as short (up to 50 μm of length) arterioles. These arterioles penetrate into the superficial layers of the stroma and give rise to the precapillaries, which finally branch into the capillary network. This network surrounds ganglion somata. Postcapillary chain, according to the latter authors (2) is composed of short and long segments, which drain into the surface capsular venules, or deep capsular and subcapsular stromal venules.

In our investigation, we also observed the presence of capillary network, arterioles and venules in human fetal trigeminal ganglion (Fig. 1). Morphometric analysis showed increase of the capillary component of the trigeminal ganglion during gestation. However, the second part of morphometric analysis showed a difference in the presence of arterioles and venules in different trigeminal ganglion portions. Their presence was the highest in the mandibular, lower in the ophthalmic and lowest in the maxillary part. There were no significant differences in arteriolar and venular presence during the gestation. In the present literature we did not find any data about such difference of the microcirculatory bed presence in the different parts of the trigeminal ganglion. In our opinion, these observations can be discussed from three aspects.

First, trigeminal ganglion shows a tripartite structure and according to Ziyal et al. (1) it contains dense rootlet network, making its appearance more plexiform rather than that of the classic sensory ganglion. Further, they observed a free zone, composed of loose connective tissue between its maxillary and mandibular portions. Rootlets can be easily dissected in this zone and in such way maxillary and mandibular parts of the trigeminal ganglion would be split. Moreover they divided blood supply of the trigeminal nerve into medial, middle and lateral thirds, making some kind of vascular compartmentalization of the ganglion. Such trigeminal ganglion structure is very important for transtrigeminal approach to cavernous sinus and related structures. Therefore, microcirculatory bed could be of importance during such surgical interventions in view of potential trigeminal ganglion injuries and possible bleeding (14)

The second aspect relates to the so called "idiopathic" form of the trigeminal neuralgia and the role of trigeminal ganglion in its pathogenesis. This condition is not very common (4.3 per 100 000 a year), but it is characterized by sudden, transient, intense bouts of superficial pain, strictly confined to the distribution of one or more divisions of the trigeminal nerve, usually precipitated by light mechanical activation of a trigger point or area. In 70% of the cases, only one trigeminal division is affected. Maxillary division, in which we found the lowest presence of microcirculatory bed components, is the most frequently affected (52%), while the mandibular division is affected in 39% of the cases (15). The first descriptions of this condition dated from the second century AD, but in spite of that, its etiology remained undetermined. However, recent papers (16, 17, 18, 19) more frequently cite vascular compression of the trigeminal nerve root as a possible cause of trigeminal neuralgia. Pathological substrate which was observed in the cases with trigeminal root vascular compression was a circumscribed zone of chronic demyelination beneath the indentation made by compressing artery or vein. Vacuolated neurons were also observed in the trigeminal ganglion. Recently, an ultrastructure and immunohistochemistry study of trigeminal peripheral myelinated axons in patient with trigeminal neuralgia was presented (8). It showed possible pathological changes of the trigeminal vasculature in patients with neuralgia. Vascular pathological alterations were noticed in 3 out of 6 neuralgia patients. The EM study revealed signs of apoptosis or degeneration, respectively, of some endothelial and smooth muscle cells in the wall of the trigeminal arterioles. In addition, the arteriolar basement membranes, which were thickened, showed an intense laminin, fibronectin, and collagen IV immunoreactivity. Similarly, some endothelial cells and pericytes of the intratrigeminal capillaries also showed signs of apoptosis or degeneration, respectively. Their basement membrane was very thick and showed an intense immune reaction against laminin, fibronectin, and collagen IV. The authors (8) concluded that the observed pathological changes of the trigeminal vasculature could be the primary factor, while demyelination of the trigeminal nerve fibers could be the secondary process in some patients with neuralgia.

The stimulation of trigger zones provokes abnormal discharges in demyelinated nerve fibers of trigeminal nerve root zone. These abnormal sensory impulses generations, spread through ephaptic way from fibers which sub-serve light touch to the pathways involved in the pain perception (16). Rappaport and Devor (20) cited that the site of this abnormal discharges ignition could be the trigeminal ganglion itself. Special neuron population in the ganglion can be depolarized after the stimulation of trigger zones. Such abnormal neuronal activity can enter the positive feedback mode and spread through non-synaptic and non-ephaptic "crossed afterdischarge" mechanism on the neighboring silent neurons. Nociceptors Aδ and C could be recruited during this process. According to latter cited authors, somata of afferents ending nearby in peripheral tissues reside in the corresponding trigeminal ganglion divisions, but they are not close neighbors. In contrast, axons of neighboring ganglion neurons remain in relatively close

association while they traverse trigeminal root. These bundles of axons define relatively compact groups of neurons in trigeminal ganglion, which are marked as "ignition foci". Cutaneous stimulation could initiate abnormal discharges in the demyelination zone beneath microvascular compression in trigeminal nerve root. These impulses can propagate orthodromically into the CNS, as well as antidromically and fire trigeminal ganglion focus neurons, especially in individuals which probably have points on the skin innervated by the neurons many of which are located near the ignition focus. Therefore, taking into the consideration this trigeminal ganglion structural organization, as well as the fact that reperfusion through previously compressed endoneurial vessels after the microvascular decompression, may contribute to the separation of demyelinated fibers in the trigeminal nerve root and consequent reduction of spontaneous impulse activity, we can suppose that the differences which were observed in trigeminal ganglion divisions microcirculatory bed can influence the reduction of its abnormal discharge activity, especially in its mandibular division (16). The lowest microcirculation presence in the trigeminal ganglion maxillary division is in accordance with the fact that it is the most frequently affected trigeminal ganglion division by this disorder (15).

Finally, the third aspect concerns the possible influence of trigeminal ganglion microcirculatory bed on the results of percutaneous trigeminal ganglion compression, as one of the surgical methods of treatment of the trigeminal neuralgia (21). Different microcirculation patterns in different trigeminal ganglion divisions can also influence the recovery of neurons and nerve fibers after the ganglion compression.

It may be concluded that morphometric analysis showed a specific developmental microcirculatory dynamic as a significant increase of the capillary network presence in the trigeminal ganglion during the gestation. Conversely, the presence of arterioles and venules did not significantly change during the gestation in the human fetal trigeminal ganglion. However, the latter vessels showed significantly different distribution in different portions of the trigeminal ganglion. Mandibular division had the highest and maxillary division of trigeminal ganglion showed the lowest presence of these microcirculatory bed components.

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STEREOLOŠKA ANALIZA MIKROCIRKULACIJE TRIGEMINALNOG GANGLIONA HUMANOG FETUSA

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Kratak sadržaj: *Prokrvljenost je važna komponenta morfofunkcionalne očuvanosti gangliona.Cilj ovog istraživanja bila je kvantifikacija, primenom stereoloških metoda, komponenti mikrocirkulacije trigeminalnog gangliona, pridodatog senzitivnom korenu trigeminusa, kao jedne on najvažnijih komponenti trigeminalnog sistema. Materijal za istraživanje činilo je 12 desnih trigeminalnih gangliona humanih fetusa Fetusi su bili klasifikovani u tri grupe prema gestacionoj starosti. Trigeminalni ganglioni, zajedno sa okružujućim duralnim omotačem, bili su disekovani uz pomoć operacionog mikroskopa. Nakon rutinske histološke procedure,trigeminalni ganglioni su bili sečeni na preseke debljine 5 μm i bojeni HE, Alcian blue - PAS i Masson trichromnim bojenjem. Morfometrijska analiza uradjena je uz upotrebu M42 multinamenskog testnog sistema pod uvećanjem 63×. Broj kapilara na milimetru kvadratnom raste signifikantno tokom gestacije u trećoj grupi fetusa. Prosečna dužinska gustina arteriola i venula nesignifikantno raste u mandibularnom, dok opada nesignifikantno u maksilarnom i oftalmičkom delu gangliona. Ovaj morfometrijski parametar ima najveće vrednosti u mandibularnom, manje u oftalmičkom i najmanje u maksilarnom delu gangliona. Kapilarna mikrocirklacija raste tokom gestacije. Nasuprot njoj, arteriolarne i venularne komponente ne pokazuju značajne promene u toku gestacije,ali je njihovo prisustvo najizraženije u mandibularnom delu trigeminalnog gangliona,a najmanje izraženo u maksilarnom delu.*

Ključne reči: *Fetalni, trigeminalni ganglion, microcirkulacija, morfometrija*