Age-related structural changes in the myenteric nervous plexus ganglion along the anterior wall of the proximal human duodenum – a morphometric analysis

Morfometrijska analiza ganglijskih struktura mijenteričkog nervnog spletja prednjeg zida proksimalnog dela duodenuma čoveka u toku procesa starenja

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Abstract

Background/Aim. Aging is one of the most complex biological processes which probably affect structure and function of the enteric nerve system. However, there is not much available information on this topic, particularly in humans. The aim of this study was to investigate the influence of aging on the structure of the myenteric ganglia in humans. The aim of this study was to investigate the influence of aging on the structure of the myenteric ganglia in the anterior wall of the human proximal duodenum. Methods. We examined the myenteric ganglia in the proximal duodenal anterior wall specimens obtained from 30 cadaver persons aged from 20 to 84 years. Tissue samples were classified into three age groups: 20–44, 45–64 and 65–84 years. After standard histological preparation, specimens were stained with HE, Cresyl Violet and AgNO3. Morphometric analysis of all the specimens, using a multipurpose test system M42, was performed. The data were subjected to the t-test. Results. The myenteric ganglia of very old humans contains an empty space, i.e. the respective parts of ganglia show a decreased number of neuron as compared to younger population. The average number of neuron per cm² of the duodenum in the youngest people (20–44 years) was 69,370 ± 1,750,00, in the people aged 45–64 years 69,211 ± 1,573,33, and in the oldest persons (65–84 years) 57,951 ± 1,291,52. The loss of neurons in the oldest persons was 16.46%. The applied statistic test demonstrated a significant difference between the observed groups (p < 0.0001). Conclusion. Aging does not induce changes in size and surface of neurons in the ganglia, but it decreases the number of neurons. The nerve structures in the elderly are partly emptied of bodies of nerve cells ("empty ganglia"), which indicates the existence of changed myenteric ganglia in the duodenum. These changes could be related to the duodenum motility disorder associated with aging.

Key words: myenteric plexus; duodenum; aging.

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Introduction

Aging is an universal biological phenomenon that everyone has to undergo at a certain point of life. All parts of a living organism during aging go through some changes, leading to a large number of functional disorders, generally characteristic for older people. Changes that occur during aging are deleterious; they decrease person's ability to cope with its environment. A large number of functional gastrointestinal disorders are also present in older people. It is generally known that the dysfunction of the gastrointestinal system, including dysphagia, constipation, diarrhea, irritable colin, is pronounced in the older population. A research on animal species has also shown the existence of disorders of intestinal function in elderly animals. Very rapid progress and development of neurobiology have led to a better understanding of the central nervous system, as well as aging caused changes in the enteric nervous system. It is accepted that many of age-dependent motility disorders are caused by abnormalities in nerves and muscles of the gastrointestinal tract, but a direct evidence for this is scarce. A number of studies in experimental animals showed that the number of myenteric nerve plexus neurons of the small and large intestines affect only cholinergic neurons, while nitrergic are partially spared. These findings support the hypothesis that age-related loss of myenteric plexus nerve cells of the small and large intestines is significantly lower when compared to younger animals.

Some researchers report that the number of neurons of myenteric nerve plexus ganglia submitted to the histochemical technique to stain the nerve cells through the activity of the NADH-diaphorase (NADH-d) enzyme was significantly reduced to approximately 15% in older rats. In contrast, the number of neurons stained immunohistochemically with PGP9.5 (protein gene product 9.5) was not reduced but rather increased in older animals. These findings support the idea of ganglion, cells and structures were stained with silver nitrate by the method of Mason Fontana and cresyl-violet color.

Silver nitrate staining by the method of Mason Fontana was performed as follows: the hydrated preparations were put in a previously prepared solution of silver nitrate for 2 h at 56°C, rinsed in distilled water and tones with 0.2% gold chloride solution for 2–3 min, rinsed again with distilled water and 1 min down to 5% sodium thiosulfate. Preparations were again rinsed with distilled water and 5 min immersed in nuclear-fast red and then mounted on glass slides and covered the outer husks. The result of staining was following: argentaffin granules in neural cells were black, nuclei were pink-reddish, and cytoplasm pale-pink.

Cresyl-violet staining (Cresyl violet) for nerve cells was performed as follows: hydrated preparations were left in the previously prepared solution of cresyl-violet stain during 30 min. They were then discolored in 96% alcohol which was added 1 drop of HCl and bleaching was controlled under the microscope. When they get the desired color, preparations were dehydrated and mounted on glass slides. The result of staining was: dark blue nucleus, cytoplasm, slightly lighter, nerve fibers were not colored.

Methods

Tissue samples of the human duodenum were obtained from autopsy material at the Institute of Forensic Medicine from 30 cadavers of both sexes, aged between 20 and 84 years. Ethical approval for the study protocol was obtained from the Ethics Committee. Samples were taken from the anterior wall of the proximal duodenum. After taking, tissue samples were divided into three predefined age groups: 20–44 years, 45–64 years and 65–84 years. Tissue slice preparations the size of 1 × 1 cm were fixed in 10% buffered paraformaldehyde for 48 hours. After the routine processing of conducting, series of alcohol samples were embedded in paraffin blocks, which were then cut in two ways; sections perpendicular to the longitudinal axis of the front wall of the duodenum (classical) and sections parallel to the surface of the proximal duodenum serosa until plexus myentericus and through it. Histological preparations were stained with the routine hematoxylin-eosin method, and to ensure reliable identification of ganglion, cells and structures were stained with silver nitrate by the method of Mason Fontana and cresyl-violet color.

Microscopic techniques

Tissue samples were cut with microtome on section thickness of 6 μm. Analysis was made by the M42 test system that calibrated to the proper magnification of the light microscope (Carl Zeis Jena). For measuring the average diameter of cells and their nuclei, ocular micrometer calibrated at the appropriate magnification was used. On each preparation of the anterior wall of the duodenum the number of point test-system on the area occupying ganglion structure and the number of point on nerve cell body profile of the myenteric ganglia were counted. In addition, it was numer-

ous the total numbers of neurons located in the complex nerve structure. On each preparation 10 visual fields were analyzed. The data were entered into spreadsheets. On the basis of them the number of neurons per square centimeter surface of nerve plexus, ganglion size range structure, the total area of all neurons in the ganglion structure and surface profiles of individual nerve cell bodies were mathematically calculated.

The results presented in the text and tables are expressed as mean ± standard deviation. Estimation of statistical significance between mean values was performed by the independent Student's t-test. A significance was expressed as $p < 0.05$ or $p < 0.001$.

Results

The myenteric nerve was woven between the circular and longitudinal layer of the duodenal wall smooth muscle. It was composed of ganglion cells by linking the bundles of nerve fibers to form a polygonal network between the muscle layers. On the cross sections ganglion structures of the myenteric plexus were relatively small in size and within each of them was noticed the large number of neurons (Figure 1). Neurons were interconnected with each other, and with the muscle cells through nerve fibers.

On longitudinal sections made of serosa to mucosa, i.e. through the longitudinal axis of the plexus, could be seen ganglion structures of various shapes and sizes. The shape and size of ganglion structures depend on the extent to which in particular it is affected by the cut. Around the nerve structures were irregularly scattered bundles of smooth muscle fibers (Figure 2). In the very ganglionic structures are ganglion cells that display a wide diversity of shapes and sizes and were grouped into smaller or larger groups.

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Neurons can be oval, round, spindle or polygonal with vesicular nuclei containing little chromatin. Around the ganglion cells could be seen scattered, irregularly oval nuclei and supporting glial cells whose cytoplasm was not stained by this method.

Using the method of quantification we determined the number of ganglion cells of the myenteric nervous plexus of the anterior proximal duodenum per unit area $(\text{cm}^2)$ in all age groups. The average number of neurons in the duodenal myenteric nerve plexus is shown in Table 1.

A reduction in the number of neurons per unit area of the myenteric plexus in people over 65 showed a very high statistical significance ($p < 0.0001$) compared with other age groups.

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>20–44</th>
<th>45–64</th>
<th>65–84</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of ganglion (cells/cm²), $\bar{x}$ ± SD</td>
<td>69.370 ± 1.750,00</td>
<td>69.211 ± 1.573,33</td>
<td>57.951 ± 1.291,52*</td>
</tr>
<tr>
<td>Surface area of ganglion structure (mm²), $\bar{x}$ ± SD</td>
<td>0.01169 ± 0.00174</td>
<td>0.01186 ± 0.00182</td>
<td>0.01203 ± 0.00137</td>
</tr>
<tr>
<td>Surface phase of the ganglion structure belonging to the profiles of nerve cells (mm²), $\bar{x}$ ± SD</td>
<td>0.00280 ± 0.00028</td>
<td>0.00267 ± 0.00030</td>
<td>0.00203 ± 0.00027**</td>
</tr>
<tr>
<td>($%$)</td>
<td>24.17</td>
<td>22.66</td>
<td>17.17</td>
</tr>
<tr>
<td>Surface of neurons ($\mu$m²), $\bar{x}$ ± SD</td>
<td>323.11 ± 8.71</td>
<td>338.78 ± 13.85</td>
<td>297.82 ± 48.09</td>
</tr>
</tbody>
</table>

$\bar{x}$ – Mean value; SD – standard deviation;  
* $p < 0.0001$ compared to other groups (t-test)  
** $p < 0.001$ compared to other groups (t-test)

According to this results it can be calculated that the loss of neurons of the duodenal myenteric nerve plexus in the oldest (65–84 years) compared to the youngest (20–44 years), expressed as a percentage is 16.46% and to the middle age group (45–64 years) 16.26%.

In further analysis, apart from the number of neurons per cm² surface, the area of the ganglion structure of the myenteric nerve plexus in the duodenum was also determined. The surface area of ganglion structure was expressed in mm². The results presented in Table 1 were subjected to statistical testing (t-test).

The values obtained show that the surface area of ganglion structure is equivalent regardless of age. Statistical analysis of values range of ganglion surface structure does not show a statistically significant difference with age.

In the morphometric analysis it was interesting to determine the surface occupied with the profiles of neurons inside the ganglion structure, i.e. surface phase of the ganglion structure of myenteric nerve plexus belonging to the profiles of nerve cells. The obtained values expressed in mm² are shown in Table 1.

The data statistically analyzed by the Student's t-test, showed that the total area of ganglion neurons structure of myenteric plexus of the duodenum in the oldest (65–84 years) were significantly different as compared to other age groups. The level of statistical significance was $p < 0.001$.

We came up with the idea that the difference of the total area of ganglion cells within the ganglion structure between different age groups was more apparent if the computation of the total area was expressed in percentage. The results are presented in Table 1.

It can be seen that the smallest percentage of surface in the ganglion structures occupy ganglion cells in the old people (65–84 years), i.e. 17.17%, and highest in the young adults (20–44 years), 24.17%.

In the morphometric analysis surface of individual ganglion cells of the myenteric nerve plexus of the duodenum of men at different ages was determined. The obtained data were tested by the Student’s t-test which showed no significant differences between the examined groups (Table 1).

### Discussion

Studies on the myenteric plexus of human material are very rare. In this study we used tissue samples of 30 people, age range from 20 to 84 years. Our study shows that on cross section of the duodenum the myenteric plexus is visible as a thin, folded, discontinued lamellar structure, inserted between the layers of the smooth muscle, practically, on the form of plate transverse cut off. It was noticeable that thickness of these plane was spotty; at the places of thickness groups of nerve cells were visible.

It was observed that the thickness of the plate was uneven and in thickened places were visible groups of nerve cells.

Longitudinal sections of duodenum tissue proved to be useful for the analysis of the myenteric nervous plexus. This manner of cutting tissue request strain and request more continuous native control of section under light microscope. Therefore, it is necessary to make a great number of serial sections from the serosal towards the mucosal side, make enough sections for analysis and separate only those that pass through the myenteric nerve plexus.

On longitudinal sections, myenteric plexus appears as a small or a large cluster of ganglion cells around which are irregularly scattered over bundles of smooth muscles. A fundamental objective of this study was to establish neuronal density per cm² of the surface of the myenteric plexus of the human duodenum in different age groups.

In our researches the myenteric plexus of the duodenum showed a very high neuronal density. The average number of neuron/cm² duodenum among the youngest (20–44 years) amounted to 69,370 ± 1,750, in the people aged 45–64 it was 69,211 ± 1,573.33, and in the elderly (65–84 years) 57,951 ± 1,291.52. In the oldest, it was seen a drop in the number of neurons of the 16.46% compared with the children and 16.26% compared with those in the middle-aged group. The number of neurons in the oldest age group compared to the first and second group, tested by the Student’s t-test, showed a level of statistical significance $p < 0.001$. In the available literature we found no data to compare our results obtained on human material. However, some studies show high neuronal density in the myenteric plexus of the mouse duodenum (20,212 ± 3,038 neuron/cm²) and in the rat colon (30,968 neuron/cm²). Other types of studies suggest a significantly greater percentage loss of neurons of the small intestine (over 30%) and esophagus (22–62%) of man in old age. A rational answer to the question why this is so, can be found in the fact that in all these works different techniques of tissue staining have been used.

In addition to the number of neurons/cm² interesting question is whether aging is connected to the emergence of differences in the surface area of ganglion structures of the duodenal myenteric plexus. The obtained results indicate that aging does not decrease the surface area of ganglion structure of the myenteric plexus. The obtained values are almost equal in all age groups. However, it is noticeable that ganglians of older people within their borders have often completely blank spaces in which there are no nerve cells bodies and also no nerve fibers. Ganglion spaces like this, some authors call “cavities”. They also conclude that in ganglia of myenteric plexus the frequent “cavities” occur with age.

From that reasons our research included determination of total surface inside the area of ganglion structure. The obtained results show a decreased range of nerve plexus structures of the myenteric plexus of the stomach with age, which belong to the body surface of neurons present in the ganglion. Expressed in percent 24.17% surface area of ganglion structure belongs to the body surface of neurons in young (20–44 years), and only 17.17% in the elderly (65–84 years). It can be concluded that aging process can arise ganglians that are partially emptied from the body of neurons (“empty ganglians”). Similar results have been presented in studies of human colon.

We can conclude that the process of aging lead to a decreased total surface area of ganglion cells within the range of ganglion structure of myenteric plexus. In fact, a reduction in the surface phases of ganglion structure belongs to the bodies of...
of nerve cells. This, most likely, indicates that increased surface phase belongs to connective, glial and vascular elements. In our research we did not work on the surface phase range ganglion structures belonging to the nerve fibers, connective and vascular elements for several reasons. One reason is that the presentation of glial elements and connective structures apply different coloring methods which can not clearly identify nerve cells. The second reason is contained in the fact that the structure of this phase is irregularly distributed around neurons and across the nerve cell bodies. For this reason, stained ganglion structures belonging to the nerve fibers, connective and vascular elements for several reasons. One reason is that increased fibrous components of the myenteric ganglia 13. Investigations included the determination of the value of the average body surface of individual ganglion cells in the anterior wall of the proximal part of the human duodenum of the myenteric nerve plexus in all age groups. The surface of neurons ranged from 297.82 ± 48.09 in the oldest to 323.11 ± 8.71 in the youngest. Similar values were obtained for the duodenum in studies of other authors 19. The obtained values show that there are no significant differences in body surface of ganglion cells in relation to age. It may be noted that the standard deviation of the oldest is considerably higher than in the plexus of the duodenum in younger age groups. The explanation may be in the fact that in the course of the study in the elderly we found the existence of neurons of significantly larger and much smaller surfaces.

**Conclusion**

The myenteric nerve plexus of the anterior wall of the proximal human duodenum is characterized by the presence of a large number of neurons. As all other structures of human organism, the myenteric plexus is also a subject to change during aging. During aging, a loss in the number of neurons occurs. Ganglion structures are in the elderly partly emptied of bodies of nerve cells ("empty ganglions"). The number of neurons in older people (65–84 years) decreases in relation to younger from 16.26% up to 16.46%. This finding corroborates with the fact that within the range of ganglion structure in the elderly there is some reduction in the surface phase, which belongs to the body surface of neurons located in a given structure. However, the surface area of the ganglion was not changed. We conclude that the reduction in neuronal density compensates increased fibrous components, so that the size of the myenteric ganglia is practically unchanged. With aging, there is no significant change in the size of neurons.

**REFERENCES**


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