Clinical approach of stellate ganglion blockage-literature review

Master thesis

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# TABLE OF CONTENT

1. SUMMARY .................................................................................................................................................. 3

2. ACKNOWLEDGEMENTS .......................................................................................................................... 4

3. CONFLICT OF INTERESTS ...................................................................................................................... 5

4. ETICS COMMITTEE APPROVAL ............................................................................................................ 6

5. ABBREVIATIONS ........................................................................................................................................ 7

6. INTRODUCTION ........................................................................................................................................ 8

7. AIMS AND OBJECTIVES OF THE STUDY ............................................................................................. 9

8. LITERATURE REVIEW ............................................................................................................................... 10
   8.1 Stellate ganglion neural connections, location and shape ................................................................. 10
   8.2 Stellate ganglion blockage in management of pain ............................................................................ 10
   8.3 Use of stellate ganglion blockage in the treatment of post-traumatic stress disorder ..................... 11
   8.4 Stellate ganglion blockage in management of palmar hyperhidrosis ................................................ 12
   8.5 Stellate ganglion blockage-techniques .............................................................................................. 12
   8.6 Criteria of a successful blockage ........................................................................................................ 14
   8.7 Investigation of the size of neurons in various animals ....................................................................... 15
   8.8 Investigation of the size of neurons in human ..................................................................................... 15
   8.9 Sympathetic nervous system neurons size changes upon aging ......................................................... 16

9. MATERIALS AND METHODS ................................................................................................................... 17
   9.1 Material .................................................................................................................................................. 17
   9.2. Methods ............................................................................................................................................... 17
   9.2.1 Hematoxylin and eosin, and cresyl violet staining ........................................................................... 17
   9.2.2 Immunostaining of the research ....................................................................................................... 18
   9.3 Microscopic examination ..................................................................................................................... 18
   9.4 Quantitative analysis ............................................................................................................................ 18
   9.5 Statistical analysis ............................................................................................................................... 18

10. RESULTS AND DISCUSSION ................................................................................................................ 19
    10.1 Ganglion blockage ............................................................................................................................ 20
    10.2 Study of the staining methods .......................................................................................................... 21
    10.3 Mean size of neurons in the Stellate ganglion ................................................................................... 21

11. CONCLUSIONS ....................................................................................................................................... 25

12. REFERENCES ............................................................................................................................................. 26
1. SUMMARY

AUTHOR: Dana Rachel Litnovetsky

TITLE: Clinical approach of stellate ganglion blockage

BACKGROUND: Significant role of the stellate ganglion in the person's life shaped the practice of stellate ganglion blockage treatment. Upon aging, stellate ganglion blockage should be practiced with understanding of the age-related changes in the stellate ganglion neurons. The size parameter of neurons in the stellate ganglion was chosen to be studied.

AIM AND OBJECTIVE: The goals of this study was to review stellate ganglion blockage procedure applications, to evaluate stellate ganglion blockage procedure techniques and to investigate mean neuron's square area of patients in the age range of 38-95 years old.

MATERIAL AND METHODS: Stellate ganglion blockage was revised from scientific literature between the years 1995-2017. Investigation of 17 histological slides of the stellate ganglion were examined by microscope LSM 700 (Carl Zeiss, Jena, Germany). Microphotographs were acquired and quantitative analysis was done by Axiovision software Rel. 4.8.2 (Carl Zeiss, Jena, Germany). Neurons square area was submitted into Excel software (2010). Statistical analysis performed using independent t-test. Differences were considered significant when p < 0.05.

RESULTS: From scientific literature review was found that stellate ganglion blockage caused improvement of symptoms in patients with chronic pain whom all other conservative treatments failed. In post-traumatic syndrome disorder was observed clinical improvement together with decrease in Alertness/Sedation score. In hyperhidrosis, blockage efficacy was determined by Minor’s starch test as on the treated side hyperhidrosis wasn't detected. Overall from literature were examined 2279 cases. Stellate ganglion blockage can be done by fluoroscopy or blind approach, but they don't provide imaging of the blood vessels close to the stellate ganglion and nor of the soft tissues, leading to increased risk of injury. Ultrasound is able to visualize those structures. Computed tomography and magnetic resonance imaging are efficient but are pricy and time-consuming.

When comparing increase of mean neurons size upon aging, statistically significant difference was found between age group below 46 years and age group above 60 years.

CONCLUSION: Stellate ganglion blockage can be used for chronic pain, post traumatic disorder and hyperhidrosis treatment. The superior technique to perform the stellate ganglion blockage was found to be with ultrasound guidance. A tendency of increasement in size of stellate ganglion neurons with age was detected. This finding correlates with the previous researches on a human and animal model that were conducted on the superior cervical ganglia.

Key words: Stellate ganglion; blockage; hyperhidrosis; chronic pain; post traumatic disorder
2. ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor Lecturer dr. Anita Dabužinskienė for the guidance, support and attention along the process of writing. As well to thank Anatomy institute and Lithuanian university of health sciences for establishing the best conditions for conduction of the research together with guardianship. I would also like to thank my significant other and my family for the care and inspiration for the research.
3. CONFLICT OF INTEREST

The author reports no conflicts of interest.
4. ETHICS COMMITTEE APPROVAL

Lithuanian University of Health Sciences ethics committee approval number BEC-MF-423, certified in 2018-05-23 by the State Service for Food and Veterinary.
5. ABBREVIATIONS

SGB - stellate ganglion blockage
SNS - sympathetic nervous system
NE – norepinephrine
PTSD - post-traumatic stress disorder
CNS - central nervous system
NGF - nerve growth factor
US - ultrasound
CT- computed tomography
MRI - magnetic resonance imaging
SCG - superior cervical ganglion
MI - myocardial infarction
6. INTRODUCTION

Investigation of sympathetic ganglion blockage have turned to focus of interest in many scientific researches along the years. In many fields, usage of conservative methods caused adverse effects which lowered patients’ quality of life, damaged their economic state and failed to cause improvement [1-3]. Therefore, stellate blockage may be an appropriate treatment to manage several issues people encounter during their life. Problems which effect disintegration in the society and can cause real distress.

Effect of stellate ganglion blockage on physical health, mental health and physical appearance were chosen in this research to be investigated. In particular to examine blockage treatment for pain management, post-traumatic stress disorder and palmar hyperhidrosis. Those areas contain the entire well-being of a person, therefore stellate ganglion blockage may be established as a holistic approach of therapy which will be beneficial for multiple patients.

The various techniques which are found in the area of stellate ganglion blockage were investigated in order to find the optimal technique for the physician use. Technique which is most useful for the physician will benefit the patient to achieve the best result out of Stellate ganglion blockage treatment.

In addition, it's very important to investigate properly the stellate ganglion morphology. It's location, shape and projection of axons with incorporation of age-related changes of its neurons size can contribute to a better grasp and understanding of the ganglia. Accordingly, evolving and improving the management of health concerns involving the stellate ganglion is possible and use of stellate ganglion blockage procedure is expended.
7. AIM AND OBJECTIVES

Aim: The aim of the study was to review stellate ganglion blockage procedure from scientific literature and to determine the age-related change of neurons size of the human stellate ganglia.

The objectives of the thesis:
1. To review stellate ganglion blockage procedure applications.
2. To compare stellate ganglion blockage procedure techniques.
3. To investigate mean neuron's square area of patients in the age range of 38-95 years old.
8. LITERATURE REVIEW

8.1 Stellate ganglion neural connections, location and shape

Stellate ganglion receives via rami communicantes axons from preganglionic neurons cell bodies which are located in the anterior lateral horn of T1 and T2 segments of spinal cord. Those preganglionic neurons supply the sympathetic innervation of the head and neck [4]. As for the distribution from the stellate ganglion, lateral somatic branches, rami communicantes radiate out from the stellate ganglion to the brachial plexus, medially raised rami to the cardiac plexus. In addition, two rami raise from the ganglion which are the inferior cervical cardiac ramus and the first thoracic ramus. These rami pass posterior to the subclavian artery and arch of the aorta and contributed to the plexus located posteriorly to the arch of the aorta. Rami are distributed to the subclavian and vertebral arteries and to the brachiocephalic trunk as well.

Stellate ganglion located anteriorly to C7 transverse process and prevertebral fascia. The vertebral artery is anterior to it and enters the transverse foramen of the C6 vertebra. In some, injury can develop during stellate ganglion blockage (SGB) due to entry alteration of the artery to C5. The inferior thyroid artery runs ventrally to C6 and C7, lying anteriorly to the vertebral artery. The stellate ganglion is superior to the subclavian artery, lies laterally to trachea and recurrent laryngeal nerve, longus colli muscle and esophagus. Medially to it are the scalene muscles [5].

Shapes of stellate ganglion were noticed as: spindle, and macroscopically visible union of the inferior cervical ganglion and T1 components of the cervical thoracic ganglion as dumbbell, and an inverted L shape. The mean length and width of stellate ganglion in the adult specimens are 6.65 mm to 16.51 mm [4].

8.2 Stellate ganglion blockage in management of pain

In chronic pain the sympathetic nervous system (SNS) facilitates the pain by efferent sympathetic vasoconstriction and norepinephrine (NE) release which increase the nociceptor activity. Sympathetic efferent fibers increased activity due to increased nociceptor stimulus in trauma. As well, the vasoconstriction and NE further increase the nociceptor action. Sympathetic blockage interrupts afferent and efferent fibers connection without impact on the motor or sensory function. In chronic pain in which patients tried all conservative options, SGB was found to be beneficial.
Following the treatment the patients felt improvement of symptoms upon investigation of 16 cases [1, 6, 7]. Further, neural ablation can to be applied after neural blockage was confirmed as useful in improvement of the symptoms [8].

8.3 Use of stellate ganglion blockage in the treatment of post-traumatic stress disorder

Although objective physical pain has a meaningful part in individual's life, subjective mental pain is a factor too. Post-traumatic stress disorder (PTSD) is a chronic condition of anxiety state, caused by witnessing or experiencing traumatic events. Its symptoms lead to despair in the patients as significant distress and functional impairment increase.

In 1876 the first description of PTSD was constructed. It was during the United States Civil War, Dr. Mendez DaCosta described war veterans as having irritable heart and further alarm responses. In his report was also a biological explanation of the nervous system overactivity and as such PTSD treatment could be focused to that area.

SNS is considered the factor that is essential for developing PTSD. The activation of the SNS is accomplished by the increase of catecholamines and then arousal symptoms are noted. Evidence for sympathetic contribution for PTSD was observed in an elevated urinary and cerebrospinal fluid NE and among patients with PTSD. As NE concentration was higher, worse symptoms of PTSD noted. Those findings suggest that reducing central nervous system (CNS) noradrenergic activity can be effective. Finding the best approach when dealing with mental health may be very changeling and various treatments for PTSD are often don’t reach desirable results. SGB is considered an effective in the treatment of PTSD due to the neurological connection of the stellate ganglion to the amygdala, as increased activation of the amygdala in PTSD patients was observed.

In addition, nerve growth factor (NGF) increases in the stellate ganglion when a person experience stress, elevates the NE in amygdala and then anxiety is noted. NGF increase is also promotes neurite sprouting at the end terminals which also elevates NE. So SGB, suppress NGF and as a result, NE reduces and reverse the cascade of PTSD [9]. Indeed was observed decreased Alertness/Sedation scores after SGB on 10 cases. Therefore was seen that SGB can induce a soothing effect due to reduction in brain NE [10]. In addition, after SGB among clinical participants was seen clinical improvement with significant difference in PTSD score before SGB and after on 24 cases [3].
Also, PTSD checklist for Diagnostic and Statistical Manual of Mental Disorders, was with reduced score after SGB on 166 cases [11]. By that, the use of stellate ganglion expended from treating chronic pain as was originally planned in 1925. But still there are obstacles for the use of SGB as treatment for PTSD due to the lack of randomized clinical trials and low number of physicians which are acquainted with this practice [9].

8.4 Stellate ganglion blockage in management of Palmar hyperhidrosis

If to review the stellate ganglion as evidence-based treatment which can improve multiple aspects in people lives, the debate needs to move towards the physical appearance of a person. SGB can cause a relief and increase the self-esteem of individuals dealing with palmar hyperhidrosis. Treatment initiated with topical preparations: later botulinum toxin A, tap water iontophoresis and anticholinergic drugs are used. When those measures fail, SGB is considered. As previously described NGF increase in stellate ganglion causing elevated NE, which then activates various SNS related irritably symptoms, among them hyperhidrosis. The efficacy of the treatment was seen on Minor’s starch test which was done by applying iodine which was left to dry and then brushed with cornstarch. Appearance of purplish black spots determined areas with sweating. Minor’s starch was applied before treatment and 1 hour after, and on the treated side hyperhidrosis was no longer detected [2]. However, thoracic sympathetic blockage appears be more successful in the management of hyperhidrosis, due to absence of Horner syndrome along with improvement of hyperhidrosis as was discovered in 2073 cases [12, 13].

8.5 Stellate ganglion blockage-techniques

The techniques used are fluoroscopy, ultrasound guided (US), computed tomography (CT) and magnetic resonance imaging (MRI). In fluoroscopic the patient is in a supine position, and the fluoroscopy beam is directed to anteroposterior direction until the C5-C6 intervertebral disc is visualized, to direct the needle toward the anterior tubercle of the sixth cervical vertebra. Then the fluoroscopy beam is tilted to obtain a foraminal view to visualize the junction of the vertebral body and the uncinate process. Next the needle is inserted to reach a location over the vertebral body or slightly medial to avoid injury to vessels, spinal nerves, and disc. Contrast media is injected to confirm the localization of the needle. Local anesthetic is injected.
Disadvantages with this technique over the US guided one is that there is no imaging of the blood vessels close to the stellate ganglion. Also, the fluoroscopy can't distinguish the prevertebral fascia which is the landmark for the anterior tubercle C6 as it is the marker of the location of the Stellate ganglion. In addition, soft tissue structures surrounding the stellate ganglion are poorly visualized, including the longus coli muscle, the thyroid gland, and the esophagus, leading to increased risk of injury. The specific needle placement into the subfascial plane of the longus coli is not possible [14]. For that reason US is used, moreover it allows regulation of the drug deposition under direct visual control. By that complication such as recurrent laryngeal nerve palsies, and the intrathecal, epidural, or intravascular spread may be minimized. In US guided method the patient as well is in supine position and the transducer is positioned to visualize the anterior aspect of the anterior tubercle on the C6 transverse process. The anterior tubercle of C6, the longus coli muscle, the pre-vertebral fascia, the internal carotid artery and the thyroid gland are seen. A pre-scan is needed in order to determine the path of needle insertion, as inferior thyroid artery, the esophagus, and the vertebral artery may obviate the needle insertion pathway between the carotid artery and the trachea. If this is the case, the needle is inserted laterally to the carotid artery. When advancing the needle from the lateral aspect, the best interval is between the carotid artery and the tip of the anterior tubercle. The needle is inserted para-tracheal, and it introduced to reach prevertebral fascia of the longus coli. In order to confirm the proper location which doesn't contact cerebrospinal fluid or blood, aspiration test is performed. Then a local anesthetic is injected and the fluid spread to the stellate ganglion is observed. Absence of spread indicates intravascular injection.

CT and MRI guided blockage also show the spread of local anesthetic agent. By that they are superior over fluoroscopic method and also the accuracy of needle placement allowed by the use of them contributes to the preference of it over fluoroscopy. However those methods are pricy, time-consuming so the US guided method remains the preferred method. Before those methods were developed blind approach was performed. The landmark was the anterior tubercle of C6, which lies against the cricoid cartilage. The surgeon retracted laterally the sternocleidomastoid muscle, the internal carotid artery is pulled away. The needle is then inserted perpendicular to the cricoid cartilage and the operator fingers, and passed until contact is made with the C6 tubercle. Aspiration test is preformed, then local anesthetic was injected.
However, absence of needle path visualization can cause various complications due to anatomic location of the stellate ganglion. By that various mistakes can happen because when using C6 as a landmark, the middle cervical ganglion can get involve since it is closer than the stellate ganglion. In addition, C6 width vary so can be skipped during blind approach. Due to proximity to the vertebral artery it may be harmed. Needle direction towards the posterior tubercle can cause the drug to spread around the spinal nerve roots. During the procedure aspiration test is preformed but despite it, intravascular injections and vascular injury may happen. More complication as retropharyngeal hematoma, cardiac arrest, convulsions and paralysis of the recurrent laryngeal nerve are possible [5, 15, 16].

Contraindications for the procedure are as for every other practice, including: bleeding, anticoagulant therapy, sepsis at the site of the injection. If unilateral recurrent nerve palsy or unilateral phrenic nerve palsy on the opposite side exists are contraindications as well as the method can involve the phrenic nerve or the recurrent laryngeal nerve, leading to obstruction of the airway [4].

8.6 Criteria of a successful blockage

A common indicator of effectiveness of SGB is the Horner syndrome because it is results from disruption of the sympathetic nerve supply to the eye. Horner syndrome characterized by Facial anhidrosis, ptosis and miosis, however these signs can be ambiguous. Therefore, since SB leads to vascular relaxation, decrease systemic vascular resistance and increased blood flow in the upper extremity after SGB. This can be used as a valid tool to determine whether the SGB was successful. Blood flow improvement can be measured by the time required for the arterial pulse pressure wave to travel from the aortic valve to the periphery which is the pulse transit time, and the result will show prolong time of the ipsilateral hand after SGB. Also examined blood flow in the common carotid artery using an ultrasonic blood flow meter will show increased blood flow after blocking SGB [17].

Another method for evaluating SGB is the change in temperature. Increase in temperature in the ipsilateral extremity after the blockage [18], however, skin temperature is a balance between the sympathetic vasoconstriction from NE and the gradual dilatation from the release of peptides from nociceptors during adrenergic activity. This means that changes in temperature may not always indicate necessarily the successful blockage since it depends not only on the sympathetic activity.
8.7 Investigation of the size of neurons in various animals

When investigating different species, the body size impact is examined. From comparing the neuron size of a rat, capybara and a horse in the superior cervical ganglion (SCG), was found that indeed the mean neuron size in a rat was much smaller than in the capybara or a horse. Keeping in mind that the precursor cells of neurons in those animals are originally similar in size, so the possible effect of body size is seen.

In addition, those finding were discovered in the SCG which projects it's axons to peripheral tissue, so it suggests that the neuron size is influenced from the range of the peripheral tissue innervated by it [19].

Similarly, by evaluating mice's, guinea pig's, rabbit's and sheep's neurons in the myenteric plexus of the ileum, neuronal sizes were proportionate to the body size. Peripheral tissue size spectrum impact is also evident on the mean size of neurons in the same animal like in the guinea-pig enteric ganglia. As the muscle coat of stomach is very thick and duodenum's muscle coat is twice than the ilium, found that the mean neuron size in the ileum was the smallest [20]. Likewise, when the target tissue is intentionally increased by exercise, the same result on the size of neurons is seen, as the mean size of the trained rats increased. But this can also due to response to the sympathetic activation observed by increase of the fluorescence intensity, which normally occurs after training [21, 22].

Isolating the target tissue size features obtained by reduction of urethra lumen and distention of the bladder with increase in muscle weight as a result. This caused a greater quantity of trophic factors NGF to be produced. Overall target tissues secrete certain number of neurotrophic factors that neurons compete for, which affect their size. This is likely to provide a source by which neurons and targets adjust physiologically to each other. In consequence, the responsible nerves grow and also the neurons. Upon removal of the urethral obstruction the muscle weight reduced and the average neuronal size returned to values very close to controls [23, 24].

8.8 Investigation of the size of neurons in human.

In human, health concerns are important mile stones which were investigated to affect the size of neurons. In myocardial infraction (MI) the neuron size increase and neuronal hypertrophy occurred in all conditions with no difference on the MI location. Which indicates that the injury transmits sympathetic signals to the stellate ganglion and changes the morphology of the neurons [25].
Infection wise, for example in Trypanosoma Cruzi infection which affects the myenteric plexus which causes irregularities in absorption and motility, neuronal reduction in size joined with reduction in neurons number observed.

However, the effect of infection is detected on the larger neurons destruction rather than their shrinkage. Neuronal destruction is attributed to neurotoxin-like substances which were produced, or due to an autoimmune response to the parasitic antigens that cross-react with host tissue components [26].

8.9 Sympathetic nervous system neurons size changes upon aging

SCG structure can shed light on the structure of inferior cervical ganglion which construct a part of the stellate ganglion. In the human being the findings revealed that that neuron size over the years increase since the neuron number of larger neurons increased.

Neuronal increase in older age attributed to the increased functional activity of the hypertrophied neurons when diminished activity of other neurons is noted. The amount of neuro filament immunoreactive neurons was reduced in older subjects, though the total number of neurons remained equal. This condition impairs the transport of neurofilaments polymers from neuronal body to axon and as such a reduced functional activity of the neurons can be assumed [27].

Likewise, when pelvic ganglion of one side was removed, the neurons in the pelvic ganglion which was left grew along with nerve fibers sprouting to innervate the whole bladder [23]. Postganglionic axons sympathetic sudomotor function decrease in older subjects as well [28].

Axonal alterations are also characteristic in age-related alteration in older people’s sympathetic ganglia and when adding it to the neuronal alterations, establishment of the initial site of changes is challenging. Age-related target tissue alteration may affect the axonal alteration by decrease in production of neurotrophic factors by target tissues, and subsequently the loss of their innervating nerve fibers [29]. Decrease of NGF receptor p75 in neurons was observed in the aged SCG. This phenomenon of selective vulnerability of sympathetic neurons is predetermined by the availability of neurotrophic factors produced in the target tissue [24]. Studying an animal model of SCG exposed that the size of neurons with age increase as well, its apparent that neurons which stimulated in higher amount are the ones which increase more in size and are more numerous due to pronounced stimulant effect of salivary gland-derived NGF in adult animals [30, 31].
9. MATERIALS AND METHODS

9.1 Material

17 Stellate ganglia were used, taken from the archive of the Institute of Anatomy in Lithuanian University of Health Sciences, Kaunas, Lithuania. The ganglia were without neurological pathologies taken from patients in the age range of 38-95 years old, 4 females and 13 male. The patients were divided to 3 groups by age: group I age below 46 years old, group II age between 46 and 60, group III age above 60.

9.2 Methods

9.2.1 Hematoxylin and eosin, and cresyl violet staining

During the research, detailed tissue preparation for light microscopy was studied. The tissue sample was placed in 4% 0.1 M phosphate buffer formaldehyde, after several of days sample was taken to be soaked in water. Then sample is rehydrated in Alcohol 70% for 24 hours and for the next 24 hours in 96% alcohol. Ethanol was used afterwards first alone and then with xylol in a mixture of half and half. Xylol was used next likewise alone. Liquid paraffine which was prior transformed into liquid was used in combination with xylol in half and half division in the mixture, in which the sample is placed. The blend changes its consistency to liquid as it placed into heater. The liquid blend is soaked in the paraffine once again and after that the sample is placed into a mold and the paraffine is titrated on top of it. The sample stabilized for 24 hours in which afterwards it's cut to suction of section of 5 µm.

The sample is positioned on the slide for 24 hours. Subsequently dehydration is done with xylol, Ethanol, alcohol 96% twice, alcohol 70% and distilled water in the end. The staining of the slide is done with hematoxylin and next its washed with water. Eosin is used next and washing with distilled water, alcohol 70%, alcohol 96% and ethanol is completed. The sample is dried on paper and lastly washed with xylol. Leica ultra on the top of the slide is dripped, cover with a glass cover of the slide and the slide stabilized for 24 hours. If the staining is done with cresyl violet, next the slide is washed with distilled water and subsequently the process is the same.
9.2.2 Immunostaining of the research

The sections were incubated in the citrate buffer (10 mM, pH 6.0) for 5 min, warmed 5 min in microwave and then they were left for 20 min. Afterwards they were washed in phosphate buffer for 5 min. Next 3% peroxide solution was used for 5 min and were once again washed in phosphate buffer, which was done 2 times. Incubation for 30 min in primary antibody - mouse monoclonal anti-neurofilament antibody solution (1:50, DAKO) was performed and after the sections were washed 10 min in phosphate buffer. Later, incubation for 10 min in biotinylated secondary antibody took place with washing subsequently. Additional incubation process was made for 10 min with streptavidin and washing in phosphate buffer was done later. Lastly washing with 3.3-diaminobenzidine (DAB) was done and washing afterwards with phosphate buffer. Hemalum solution was used for staining.

9.3 Microscopic examination

Histological slides were examined by a microscope LSM 700 (Carl Zeiss, Jena, Germany) and Microphotographs were acquired in the process by Axiovision software (Carl Zeiss, Jena, Germany). Microphotographs were taken to cover whole area of each individual neuron. Later they were used for quantitative analysis.

9.4 Quantitative analysis:

By Axiovision software (Carl Zeiss, Jena, Germany) the digital images were analyzed. Neurons with apparent nuclei and nucleolus were marked, their square area was submitted into Excel software (2010).

9.5 Statistical analysis:

By Excel software (2010) the mean square size of each patient was calculated. Graphs according age groups were attained. Statistical analysis performed using independent t-test which was done in the aim to find if there is difference of mean neuron size in the stellate ganglion among the age groups. Differences were considered significant when p < 0.05.
10. RESULTS AND DISCUSSION

10.1 Stellate ganglion blockage

From scientific literature review was found that SGB caused improvement of symptoms in patients with chronic pain whom all other conservative treatments failed, in 16 cases. In post-traumatic syndrome disorder was observed clinical improvement together with decrease in Alertness/Sedation score, in 166 cases. In hyperhidrosis, blockage efficacy was determined by Minor’s starch test as on the treated side hyperhidrosis wasn't detected. However, thoracic sympathetic blockage appears be more successful in the management of hyperhidrosis, due to absence of Horner syndrome along with improvement of hyperhidrosis as was discovered in 2073 cases. Overall from literature were examined 2279 cases from literature.

Therefore, the blockage is a cost effective procedure since it reduces the use of medications. The added benefit of medication termination is that the side effects of the drugs are no longer a burden. In addition, external support of a physician and nor of psychiatrist is less necessary any more. Though, Horner syndrome was observed after blockage, still the patient benefits greatly from the procedure.

The blockage can be performed by fluoroscopy, US guided, CT and MRI. US is found to be the superior method as it quick, cost effective and its imaging is most beneficial for the procedure. With US is better to visualize blood vessels and soft tissue surrounding the Stellate ganglion. Especially to detect the pre-vertebral fascia which is the landmark for the anterior tubercle to trace the stellate ganglion. The needle placed precisely with regulation of the drug deposition under direct visual control.

Fluoroscopy doesn’t provide imaging of the blood vessels close to the stellate ganglion and nor of the soft tissues leading to increased risk of injury. Also, the fluoroscopy is not able distinguish the pre-vertebral fascia. The specific needle placement into the subfascial plane of the longus coli is not possible. US on the other hand, allows regulation of the drug deposition under direct visual control. By that complication such as recurrent laryngeal nerve palsies, and the intrathecal, epidural, or intravascular spread may be minimized.

CT and MRI guided blockage also shows the spread of local anesthetic agent. By that they are superior over fluoroscopic method and also the accuracy of needle placement allowed by the use of them contributes to the preference of them over fluoroscopy. However, those methods are pricy, time-consuming so the US remains the prefabbed method.

Before those methods developed, blind approach was performed. However intravascular injections and vascular injury led to the development of interventional methods of fluoroscopy, CT, MRI, and US.
10.2 Study of the staining methods.

Fig. 1. Staining with immunoreactivity for neurofilament protein as a marker. Antibody – NFDAKO, NR M0726 (dilution 1:50). Neuron with nucleus and nucleolus is indicated by an arrow.

The staining with immunoreactivity for neurofilament protein as a marker using antibody – NFDAKO, NR M0726 (dilution 1:50) was done in order to evaluate the structures in the slide in the best way. Cytoplasm and myelin appear brown. Nucleus appears transparent and nucleolus is purple. This method was superior over cresyl violet and hematoxylin eosin. In cresyl violet staining, all the slide was painted in purple, the satellite cells were hard to distinguish and the myelin. In hematoxylin eosin all the slide was painted in red and myelin and satellite cell weren’t noticeable.

10.3 Mean size of neurons of the stellate ganglion

Each ganglion had certain quantity of microphotographs. Square area of neurons was measured by Axiovision software Rel. 4.8.2 (Carl Zeiss, Jena, Germany). The investigator circled the neuron in the software and then the software displayed the square area of the neuron in µm². Those measurements were recoded via Excel software (2010).

Fig. 2. Mean neuron square area of all examined ganglia
The ganglions were divided according age to 3 groups for comprehensive investigation. 

Group I: age below 46 years old, 4 ganglions, 131 microphotographs were acquired, were examined 702 neurons.

Group II: age between 46 to 60 years old, 6 ganglions, 578 microphotographs were acquired, were examined 4107 neurons.

Group III: age above 60 years old, 7 ganglions, 330 microphotographs were acquired, were examined 1843 neurons.

This method gave the opportunity to visualize the size changes with age. To determine significant difference between 3 groups was used t-test.

**Table 1. I and II age groups data collected in the research**

<table>
<thead>
<tr>
<th>Age groups</th>
<th>I</th>
<th>II</th>
<th>t I-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean of neuron square area (μm²)</td>
<td>611.4725</td>
<td>668.8683</td>
<td>0.73</td>
</tr>
<tr>
<td>Standard error (μm²)</td>
<td>89.49713</td>
<td>136.6448</td>
<td></td>
</tr>
<tr>
<td>Number of neurons</td>
<td>702</td>
<td>4107</td>
<td></td>
</tr>
</tbody>
</table>

\( t_{0.05} = 2.306 \)

There is no significant difference between groups I and II.

**Table 2. II and III age group neurons size**

<table>
<thead>
<tr>
<th>Age groups</th>
<th>II</th>
<th>III</th>
<th>t II-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean of neuron square area (μm²)</td>
<td>668.868</td>
<td>788.8829</td>
<td>1.873</td>
</tr>
<tr>
<td>Standard error (μm²)</td>
<td>136.6448</td>
<td>93.1002</td>
<td></td>
</tr>
<tr>
<td>Number of neurons</td>
<td>4107</td>
<td>1843</td>
<td></td>
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</tbody>
</table>

\( t_{0.05} = 2.201 \)

There is no significant difference between II and III groups.
Table 3. I and III age groups data collected in the research

<table>
<thead>
<tr>
<th>Age groups</th>
<th>I</th>
<th>III</th>
<th>t I-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data</td>
<td>Mean of neuron square area (μm²)</td>
<td>Mean of neuron square area (μm²)</td>
<td>Number of neurons</td>
</tr>
<tr>
<td></td>
<td>611.4725</td>
<td>668.8683</td>
<td>89.49713</td>
</tr>
</tbody>
</table>

$ t_{0.05} = 2.26 $  

There is a significant difference between groups I and III.

![Mean neuron's square area of 3 age groups](image)

**Fig. 3. Mean neuron’s square area of 3 age groups**

Even though there wasn’t found significant difference between the conterminal groups, the tendency of increase in the ganglion size with age is seen. Mean square area of I group was 611.4725 μm², of the II group was 668.8683 μm² and of the III was 788.8829 μm². The tendency of increase in mean neuron's square area along the years reveled. Not achieving significant statistical difference between the counter terminal groups is possibly because the changes in the neurons are slow and individual. As an evidence, significant difference was found between I and III groups which shows that in the stellate ganglion mean square area indeed increase with age. This finding correlates with the research on a human and animal model that were conducted on the superior cervical ganglia. [27,31]
Fig. 4. Percentage display of mean neuron size in the 3 age groups (mean neuron size of the first group was taken as 100%)

The difference between groups I and II was 9.39% and between II and III group was 19.64%.

Fig 5. Percentage display of standard deviation in the 3 age groups

Standard deviation showed that the scattering of the results in the II group was higher than in the III and I groups. This result can explain why a significant difference result wasn't achieved between I and II age groups and nor on the II and III groups. In addition, possibly that limited number of ganglions affected as well.
11. CONCLUSIONS

1. Stellate ganglion blockage can be used for chronic pain, post-traumatic stress disorder and hyperhidrosis treatment.
2. The best technique to perform the stellate ganglion blockage was found to be with ultrasound guidance.
3. A tendency of increasement in square area of stellate ganglion neurons with age was detected.
12. REFERENCES


