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Qualitative Histologic Assessment vs. Geomorphometric Analysis of Nerve Fiber Shape after the Intraneural Application of Liposomal Bupivacaine

Evaluación Histológica Cualitativa Versus Análisis Geomorfométrico de la Forma de la Fibra Nerviosa Después de la Aplicación Intraneural de Bupivacaína Liposomal

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SUMMARY: The preserved form of all components of the nerve fiber is a prerequisite for the proper conduction of the nerve impulse. various factors can change the shape of nerve fibers. In everyday practice, qualitative histological analysis is the gold standard for detecting changes in shape. Geometric morphometry is an innovative method that objectively enables the assessment of changes in nerve fibers' shape after local anesthetics action. A total of sixty sciatic nerves were used as material, which was intraneural injected with saline solution in the control group (n=30), and a solution of 1.33 % liposomal bupivacaine (n=30) in the test group. After the animals were sacrificed, nerve samples were taken and histological preparations were made. The preparations were first described and examined using a qualitative histological method, after which digital images were made. The images were entered into the MorphoJ program and processed using the method of geometric morphometry. Qualitative histological examination revealed no differences in nerve fibers after intraneurally applied physiological solution and liposomal bupivacaine. Using the method of geometric morphometry, a statistically significant change in the shape of axons was found after intraneurally applied saline solution and liposomal bupivacaine (p=0.0059). No significant differences in histological changes were found after the qualitative histological analysis of nerve fiber cross-section preparations. A statistically significant change in the shape of nerve fiber axons was observed after geometric morphometric analysis of digital images after intraneural application of saline and liposomal bupivacaine.

KEY WORDS: Liposomal bupivacaine; Nerve fiber; Qualitative histology; Geometric morphometry; Shape.

INTRODUCTION

The shape of the cells in each tissue is important for regulating the response to extracellular signals. Numerous factors can result in a change in cell shape, such as biological, mechanical, and molecular factors (Martin *et al*., 2010). Changes in cell shape play a vital role in the development of numerous disorders and diseases (Esfahani & Knöll, 2020).

For a long time, qualitative histological analysis was used as the gold standard in the assessment of tissue changes. Various tissue staining techniques were used to identify various structural changes in cells and tissues (Paramitha *et al*., 2017).

Histopathological diagnosis and grading of cellular changes have important implications in shaping improved therapeutic strategies. Regardless of the existence of advanced biochemical, genetic, radiological, and nuclear imaging tools, the gold standard for typing and grading is the evaluation of histological specimens using classical microscopes, which

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relies on subjective assessment of the degree of cellular change (Varejão *et al*., 2001; Navarro, 2016).

Computer-aided image analysis can improve analysis of histological entities by generating continuous variables that can be used for statistical comparisons. The application of geometric morphometry in contributing to the solution of many biological problems has been actualized in the last two decades of the 21st century, and this especially refers to the more advanced qualitative analysis of the shape of the examined structures. Geometric morphometrics has proven to be an outstanding set of tools for a wide spectrum of research in anatomy, biology, engineering, and applied mathematical fields. This popularity stems from many years of scientific and practical interest in morphology as well as the essential strength of the geometric approach to form analysis. Highly sophisticated techniques within geometric morphometrics enable the observation of the smallest changes in the shape of the examined sample (Klingenberg, 2011; Cardini & Elton, 2011; Mcnulty & Vinyard, 2015). Examining the shape and number of nerve fibers is important when assessing the neurotoxicity of individual drugs, especially local anesthetics. Local anesthetics are widely used in daily clinical practice to reduce pain in both outpatient and hospital settings (Becker & Reed, 2012).

Liposomal bupivacaine is an advanced, depo formulation of bupivacaine, where bupivacaine molecules are incorporated into carriers, liposomes, to ensure prolonged anesthetic action in just one dose. Approved indications for the clinical use of liposomal bupivacaine are postoperative wound infiltration after various surgical procedures and inter scalene brachialis plexus blockade for postoperative analgesia (Chahar & Cummings 3rd, 2012).

Counting nerve fibers can sometimes give a false negative picture of the present damage if the fact of the change in the shape of nerve fibers is ignored (Cercignani *et al*., 2017).

Therefore, the main goal of our research is to evaluate the change in the shape of nerve fibers after intraneural administration of liposomal bupivacaine using classic qualitative histological analysis and geometric morphometry.

MATERIAL AND METHOD

After obtaining the consent of the Ethics committee of the Faculty of Veterinary of the University of Sarajevo. Animal handling complied with the Principles of Care and Preservation of Laboratory animals (National Institutes of Health, 1985). A total of thirty adult Wistar rats were included in the study. Material for this study consisted of total of sixty sciatic nerves that were randomized in two groups: control group that interneural received saline (n=30), and examined group that intraneural received liposomal bupivacaine (n=30).

All animals were put under general anesthesia by intraperitoneal injection of nembutal - sodium pentobarbital, in total aseptic conditions. After they are put to sleep, the front and rear paws are fixed with rubber on a wooden board for easy access. An incision was made in the gluteal area on both sides using a dorsal approach. After it appeared under the control of the eye sciatic nerve, started with the application of tested fluids. Application of test liquids was done using 26 G needle (Terumo Europe NV, Leuven, Belgium). A saline solution was applied intraneural on the right side, and a 1.33 % solution of liposomal bupivacaine (Exparel®, Pacira Pharmaceutical Inc., USA) was applied intraneural on the left side sciatic nerve. A total of 4 ml of saline or 4ml of liposomal bupivacaine was applied per nerve. After the application, the wounds were closed and the animals were monitored for the next three days. After three days, the animals were euthanized, and nerve samples from both sides were taken for analysis.

After fixation in 10 % formalin, and dehydration in ethylene alcohol, the samples were treated with xylene as an intermediate. Then, the tissue blocks were cut with a microtome (Leica, RM 2165) into incisions 3 - 4 mm thick. The sections were then passed through a series of alcohol of decreasing concentration. The preparations were stained with the Hematoxylin Eosin method. After obtaining the appropriate sections and preparations, they were subjected to quantitative histological analysis, by light microscopy. After all nerve samples were examined and described by a qualitative histological method using a light microscope, images were taken using a digital camera attached to the microscope. All pictures are taken at a magnification of 40¥. All pictures are saved as IPG format, i.e. two-dimensional images. After obtaining two-dimensional images of the nerves, all images were subjected to the method of geometric morphometry in order to evaluate the change in the shape of the nerve fiber. One nerve fiber was selected from each image in five different parts of the image (central, top-right, top-left, bottom-right, bottom-left), a total of five nerve fibers with each image. Using the program tpsDig, four points are marked on the outer edge of each selected nerve fiber according to the principle of 12-6, 9-3 clockwise and four points are marked on the outer edge of the axons. The obtained data were entered into the MorphoJ program for the purpose of analyzing the shape of two-dimensional models of nerve fibers. The program incorporates statistical

tests (principal component analysis, generalized Procrustes analysis, discriminant analysis). The task of these tests is to determine whether there is a statistically significant difference in the shape of the nerve fiber between the control group (saline solution) and the test group (liposomal bupivacaine). After the analysis, the data were exported from the MorphoJ program in the form of graphs, tables and text.

RESULTS

Results of qualitative analysis. At the highest magnification, damage to the structure of nerve fibers with increased distance between nerve fibers and loss of tissue-vascular elements can be seen. Diffuse mononuclear infiltrate visible between nerve fibers. Nerve fibers are unevenly changed and enlarged. Schwann cells (*Schwannocytus*) are enlarged with hyperchromatic nuclei (Fig. 1).

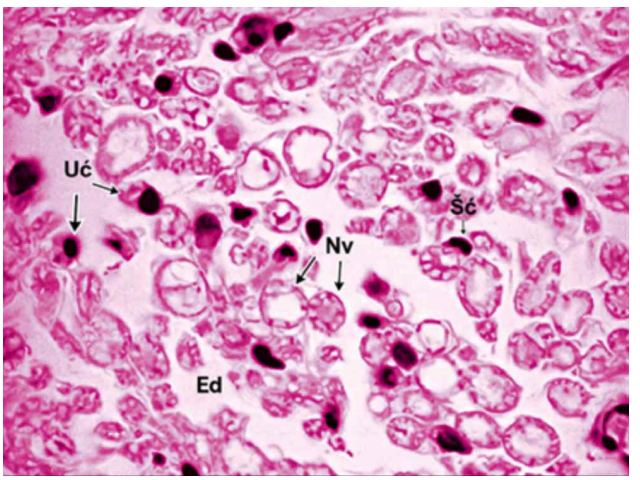

Fig. 1. Nerve fibers during intraneural saline application (HE, 40×).

The perineurium is thickened and stratified with an unclear margin to the surrounding tissue. Subperineurally located nerve fibers show different ranges of damage. Nerve fibers have uneven diameters, and in some places with a thickened myelin sheath and eccentrically laid axons. Enlarged Schwann cells with hyperchromatic nuclei. Mononuclear inflammatory infiltrate among nerve fibers. Intrafascicular edema present with mild reaction of nerve fibers. Fewer Schwann cells with hyperchromatic nuclei and mononuclear inflammatory cells among nerve fibers (Figs. 2a,b).

Results of geometric morphometric analysis. For analysis of the shape of nerve fibers and axons in groups with intraneural application liposomal bupivacaine and saline as control group we used geometric morphometric method. On two-dimesional models of the nerve fibers using TpsDig program we marked landmarks, four on nerve fibers and four on axons (Fig. 3).

Total variance was 0,01198823. The Eigenvalue variance scaled by total variance and number of variables was 0,09915. First five principal components discribed 95.407 % of total variability (Table I, Fig. 4).

For shape axon analysis first two prncipal components described 100.00 % of total variability (Table II). On Figure 5, in mopho space axons were presented after intraneural application of liuposomnal bupivacaine and saline.

After analysis of principal components and generalized Procrustes analysis of the nerve fibers shape and axons shape, we performed test of discriminant functional analysis were we analysed differences of the shape of the nerve fibers between group of nerve fibers with application

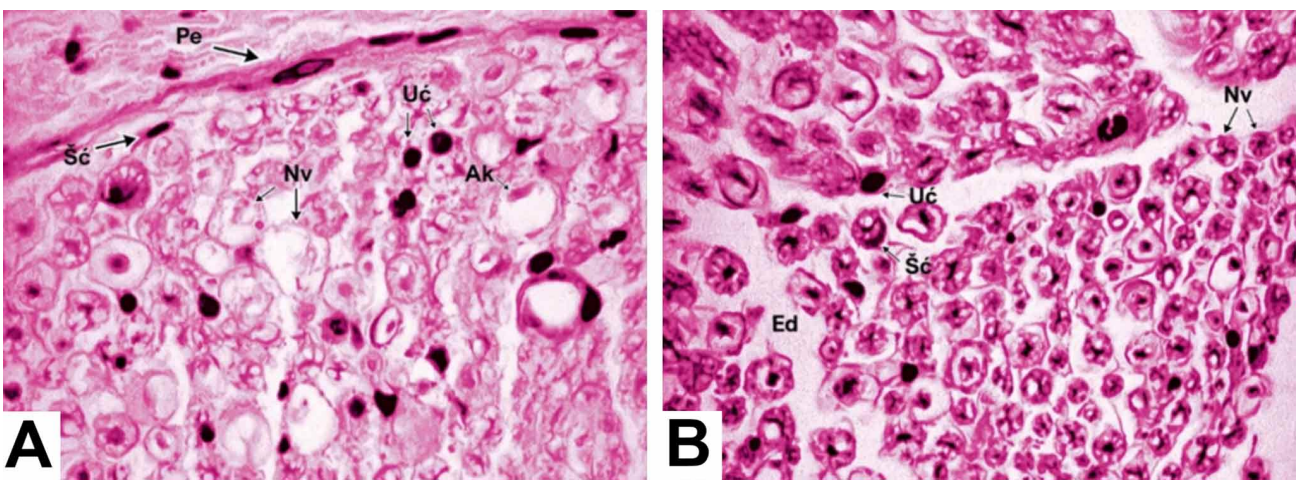

Fig. 2A, 2B. Nerve fibers during intraneural liposomal bupivacaine application(HE, 40×.

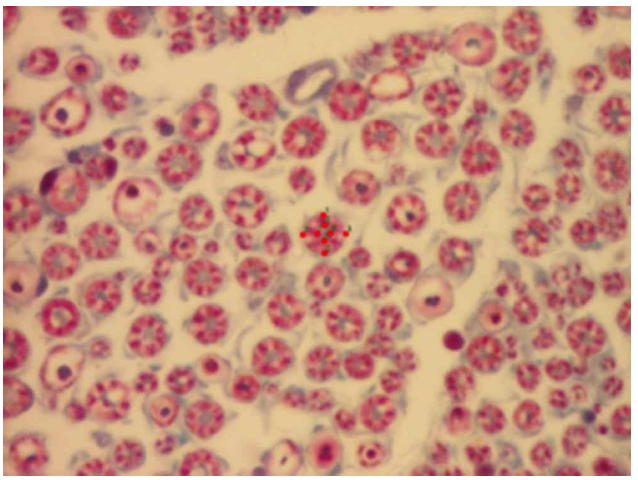

Fig. 3. Landmarks recorded on nerve fibers and axons usinf TpsDig program.

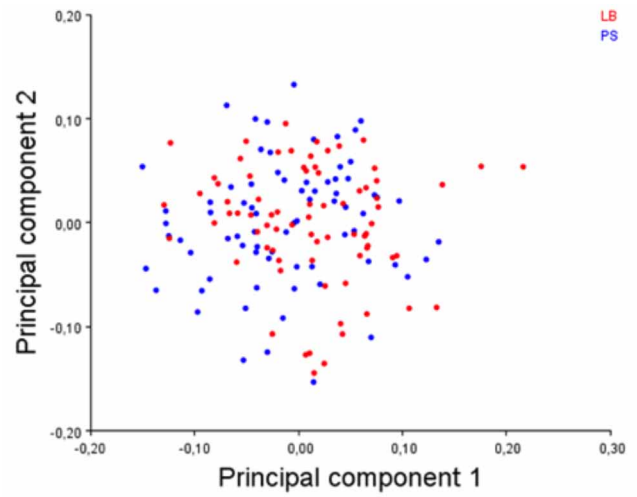

Fig. 4. Scatter plots of the first two principal components (PC1 and PC2) of the nerve fibers shape separated by application of liposomal bupivacaine (LB) or saline (PS).

Table I. Eigenvalues from a Principal Component Analysis of the shape of nerve fabers between group with aplication of liposomal bupivacaine and control group (application of saline).

Number of PCs	Eigenvalues	% Variance	Cumulative %
PC1	0,00448417	37,405	37,405
PC2	0,00327445	27,314	64,719
PC3	0,00150247	12,533	77,251
PC4	0,00127360	10,624	87,875
PC5	0,00090292	7,532	95,407

Table II. Eigenvalues from a Principal Component Analysis of the shape of axons between group with intraneural application liposomal bupivacaine or contol group.

	Eigenvalues	% Variance	Cumulative %
1	0,01233882	73,793	73,793
2	0,00438212	26,207	100,000

liposomal bupivacaine and control group, group of nerve fibers with application of saline (PS). Results of the discriminant functional analysis showed that no statistical significant differences of the shape of nerve fibers between this two groups (Table III) (P value was 0,0760).

Using discriminant functional analysis we analysed differences between shape of axons in experimental group (with intraneural application of liposomal bupivacaine, LB) and shape of the axons in control group (with intraneural application of saline, PS). Results of the discriminant functional analysis showed statistical significant differences of the axons shape between this two groups (Table IV) (P value was 0,0059).

Differences of the shape of the axons in experimental group (with intraneural application liposomal bupivacaine) and control group (with intraneural application of saline) was presented on Figure 6 with transformation grid.

Table III. Results of discriminant functional analysis of the nerve fibers shape.

Group of the nerve fibers	Group LB	Group PS	Total
Group LB	**47**	29	76
Group PS	24	**51**	75

LB-liposomal bupivacaine, PS- saline

Table IV. Results of discriminant functional analysis of the axons shape.

Group of the axons	Group LB	Group PS	Total
Group LB	**49**	27	76
Group PS	27	**48**	75

LB-liposomal bupivacaine, PS- saline

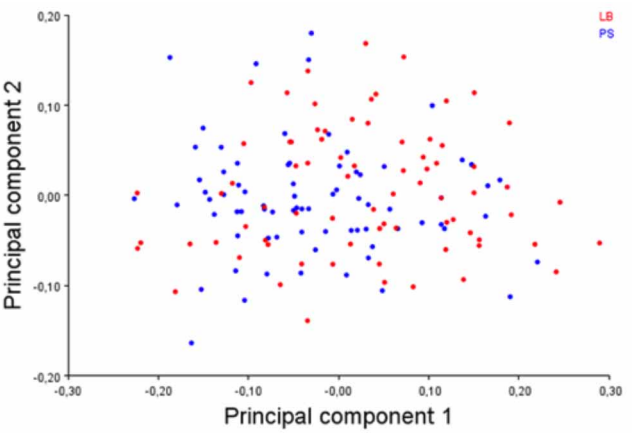

Fig. 5. Scatter plots of the first two principal components (PC1 and PC2) of the axons shape separated by application of liposomal bupivacaine (LB) or saline (PS).

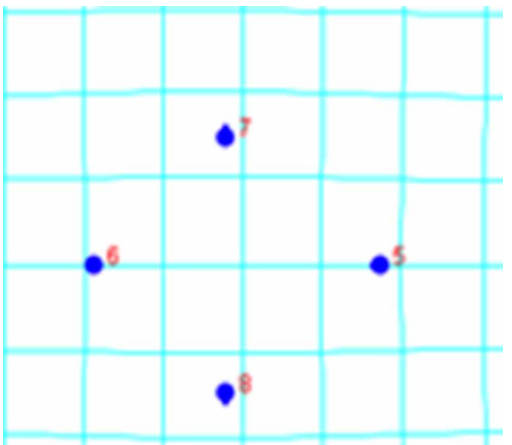

Fig. 6. Transformation grid of changes of the axons shape separated by intraneural application of liposomal bupivacaine (LB) or saline (PS).

DISCUSSION

A nerve fiber is a complex neurohistological structure that includes the axon of any neuron together with the glial sheath. Knowledge of the functional histology of nerves is essential for understanding the mode of action of local anesthetic and the mechanism of nerve damage. The anatomical specificities of the nerve and how its protective structure against external injuries is organized is perhaps the most important factor that determines the probability of injury (Ronchi *et al*., 2023).

In our study, the sciatic nerve was surgically exposed and the needle was directly applied to the nerve in all intraneural groups. In this way, there was a higher chance of nerve damage even before the application of the tested solutions, because in everyday clinical practice, the nerves are mobile in the surrounding protective connective tissue and there is less risk of the needle entering the nerve directly when performing peripheral nerve blocks (Lalkhen & Bhatia, 2012).

In our study, qualitative histological analysis did not significantly differ in the quality of changes in nerve fibers after the application of physiological solution and liposomal bupivacaine. In both groups, it was noticed that the centrally located fibers had a preserved structure without signs of degenerative changes. Peripherally located fibers were spatially disorganized with increased mutual distances and uneven diameter of nerve fibers. Schwann cells were enlarged with hyperchromatic nuclei and prominent cytoplasm. An increased number of inflammatory cells was also observed among the nerve fibers. Under a light microscope, no significantly different degree and extent of changes in nerve fibers could be observed. However, a study by Faber *et al*., found a greater degree of extensive damage after the intraneural application of bupivacaine, lidocaine, and ropivacaine, in comparison with the intraneural application of saline solution (Farber *et al*., 2013).

Previous studies have shown that there are no significantly different changes in the histology of nerve fibers after receiving liposomal bupivacaine and saline, which is consistent with our study (Damjanovska *et al*., 2015).

In contrast to our results, no significant histological changes were found in the studies of Damjanovska *et al*. (2019) during the intraneural application of liposomal bupivacaine to the sciatic nerve of pigs. This difference may be due to the anatomical specificities and differences between pig and rat nerves. Namely, it is known that the pig nerve has a polyfascicular structure with a higher proportion of connective tissue, unlike the rat nerve, which has fewer fascicles and connective tissue in its composition. Precisely, the greater amount of this binder in the pig nerve allows the needle to end up in the binder, not in the fasciculus, during intraneural applications, and automatically cause damage of lesser intensity (Damjanovska *et al*., 2019).

Qualitative histological analysis in our study proved to be somewhat difficult, due to the subjectivity of the pathologist who examines the specimens, even in a case when he/ she are blinded to the research groups. Therefore, in our research, we offered a new concept of assessing the change in the shape of nerve fibers in a more objective way using geometric morphometry.

With a more precise examination of the components of the nerve fiber, we did not find that there was a significant difference in the shape and thickness of the myelin sheath and axon after the application of saline and liposomal bupivacaine. However, when the same two-dimensional images of nerve fibers were analyzed by geometric morphometry, a statistically significant difference in axon shape after the intraneural application of saline and liposomal bupivacaine was clearly observed. Therefore, geometric morphometric analysis of the shape of nerve fibers in our study proved to be a more reliable method compared to visual analysis, which is based on poorly defined criteria and cannot be reliably reproduced, due to the subjectivity of the researcher who performs the analysis.

Previous studies have proven that geometric morphometry can be used as an effective non-invasive tool in the analysis of rat liver histological preparations (Rosioru *et al*., 2012). Geometric morphometry was mostly used to assess changes in shape for the purpose of recognizing sexual dimorphism (Kimmerle *et al*., 2008; Gonzalez *et al*., 2011; Ajanovic *et al*., 2022). In recent years, the scope of the use of geometric morphometry in various fields has increased

(Lawing & Polly, 2010). Automated, computerized grading of cell changes has proven to be highly effective in evaluating tumor changes (Tashk *et al*., 2015). Using morphometry, Ricco *et al*. (1994) assessed the role of symmetry of nuclear contours for differentiating grades of astrocytomas. The results of Buffo *et al*., showed that the symmetry of the nuclear contour together with other morphometric variables serves to distinguish between normal, dysplastic and malignant cells in colon samples (Bufo *et al*., 1990).

In our study, they offered a new concept of using geometric morphometry. With the help of specific points on the examined structures, we enabled the analysis of the smallest morphological changes. In this way, it is possible to analyze the differences in the position of the same specific points on all the structures of the examined sample. Ultimately, this allowed us to determine the difference in the shape of the smallest components of the nerve fiber, which was not possible with the classic qualitative histological method.

Due to the precision of geometric morphometry on microscopic preparations, possibilities will open for its application in diagnostic branches of medicine that are based on microscopic analysis of examined structures. Microscopic analyses, as a subjective assessment of the condition of the examined structure, would thus be objectified and the observed changes would be measurable in nature, as done in our research.

CONCLUSION

Geometric morphometry found a statistically significant change in the shape of the components of the nerve fiber, which was not possible through the classic qualitative histological findings of the image with a light microscope.

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RESUMEN: La forma conservada de todos los componentes de la fibra nerviosa es un requisito previo para la conducción correcta del impulso nervioso. Varios factores pueden cambiar la forma de las fibras nerviosas. En la práctica diaria, el análisis histológico cualitativo es el estándar de oro para detectar cambios de forma. La morfometría geométrica es un método innovador que permite evaluar objetivamente los cambios en la forma de las fibras nerviosas después de la acción de los anestésicos locales. Se utilizó como material un total de sesenta nervios ciáticos, que se inyectaron intraneuralmente con solución salina en el grupo control (n=30), y una solución de bupivacaína liposomal al 1,33 % (n=30) en el grupo de prueba. Después de sacrificados los animales, se tomaron muestras de nervios y se realizaron preparaciones histológicas. Primero se describieron y examinaron las preparaciones utilizando un método histológico cualitativo, después de lo cual se tomaron imágenes digitales. Las imágenes fueron ingresadas al programa MorphoJ y procesadas mediante el método de morfometría geométrica. El examen histológico cualitativo no reveló diferencias en las fibras nerviosas después de la aplicación intraneural de solución fisiológica y bupivacaína liposomal. Usando el método de morfometría geométrica, se encontró un cambio estadísticamente significativo en la forma de los axones después de la aplicación intraneural de solución salina y bupivacaína liposomal ($p = 0,0059$). No se encontraron diferencias significativas en los cambios histológicos después del análisis histológico cualitativo de las preparaciones de secciones transversales de fibras nerviosas. Se observó un cambio estadísticamente significativo en la forma de los axones de las fibras nerviosas después del análisis de morfometría geométrica de imágenes digitales después de la aplicación intraneural de solución salina y bupivacaína liposomal.

PALABRAS CLAVE: Bupivacaína liposomal; Fibra nerviosa; Histología cualitativa; Morfometría geométrica; Forma.

REFERENCES

Ajanovic, Z.; Dervisevic, L.; Dervisevic, A.; Sarac-Hadzihalilovic, A.; Dervisevic, E.; Biscevic Tokic, J.; Salihbegovic, A.; Jugo, A.; Aganovic, I. & Prasko, S. Sex prediction by geometric morphometric analysis of the hard palate. *Eur. Rev. Med. Pharmacol. Sci., 26(17)*:6057-64, 2022.

Becker, D. E. & Reed, K. L. Local anesthetics: review of pharmacological considerations. *Anesth. Prog., 59(2)*:90-101, 2012.

Bufo, P.; Ricco, R.; Potente, F.; Troia, M.; Serio, G. & Pesce Delfino, V. Using analytical morphometry to distinguish severe dysplasia and large bowel carcinoma. *Boll. Soc. Ital. Biol. Sper., 66(2)*:143-50, 1990.

Cardini, A. & Elton, S. GeMBiD, a Geometric Morphometric Approach to the Study of Biological Diversity: an example study of the *Red colobus* (Procolobus [Piliocolobus]) species complex. *Int. J. Primatol., 32*:377-89, 2011.

Cercignani, M.; Giulietti, G.; Dowell, N. G.; Gabel, M.; Broad, R.; Leigh, P. N.; Harrison, N. A. & Bozzali, M. Characterizing axonal myelination within the healthy population: a tract-by-tract mapping of effects of age and gender on the fiber g-ratio. *Neurobiol. Aging, 49*:109-18, 2017.

Chahar, P. & Cummings 3rd, K. C. Liposomal bupivacaine: a review of a new bupivacaine formulation. *J. Pain Res., 5*:257-64, 2012.

Damjanovska, M.; Cvetko E.; Kuroda, M. M.; Seliskar, A.; Plavec, T.; Mis, K., Podbregar, M. & Pintaric, T. S. Neurotoxicity of intraneural injection of bupivacaine liposome injectable suspension versus bupivacaine hydrochloride in a porcine model. *Vet. Anaesth. Analg., 46(2)*:236-45, 2019.

Damjanovska, M.; Cvetko, E.; Hadzic, A.; Seliskar, A.; Plavec, T.; Mis, K.; Vuckovic Hasanbegovic, I. & Stopar Pintaric, T. Neurotoxicity of perineural vs intraneural-extrafascicular injection of liposomal bupivacaine in the porcine model of sciatic nerve block. *Anaesthesia, 70(12)*:1418-26, 2015.

Esfahani, P. H. & Knöll, R. Cell shape: Effects on gene expression and signaling. *Biophys. Rev., 12(4)*:895-901, 2020.

Farber, S. J.; Saheb-Al-Zamani, M.; Zieske, L.; Laurido-Soto, O.; Bery, A.; Hunter, D., Johnson, P. & Mackinon, S. E. Peripheral nerve injury after local anesthetic injection. *Anesth Analg., 117(3)*:731-9, 2013.

Gonzalez, P. N.; Bernal, V. & Perez, S. I. Analysis of sexual dimorphism of craniofacial traits using geometric morphometric techniques. *Int. J. Osteoarchaeol., 21(1)*:82-91, 2011.

Kimmerle, E. H.; Ross, A. & Slice, D. Sexual dimorphism in America: geometric morphometric analysis of the craniofacial region. *J. Forensic Sci., 53(1)*:54-7, 2008.

Klingenberg, C. P. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour., 11(2)*:353-7, 2011.

Lalkhen, A. G. & Bhatia, K. Perioperative peripheral nerve injuries. *Contin. Educ. Anaesth. Crit. Care Pain, 12(1)*:38-42, 2012.

Lawing, A. M. & Polly, P. D. Geometric morphometrics: recent applications to the study of evolution and development. *J. Zool., 280(1)*:1-7, 2010.

Martin, A. C.; Gelbart, M.; Fernandez-Gonzalez, R.; Kaschube, M. & Wieschaus, E. F. Integration of contractile forces during tissue invagination. *J. Cell Biol., 188(5)*:735-49, 2010.

Mcnulty, K. P. & Vinyard, C. J. Morphometry, geometry, function, and the future. *Anat. Rec. (Hoboken), 298(1)*:328-33, 2015.

National Institutes of Health. *Principles of Laboratory Animal Care*. National Institutes of Health Publication No. 86-23. Bethesda (MD), National Institutes of Health, 1985.

Navarro, X. Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models: A critical overview. *Eur. J. Neurosci, 43(3)*:271-86, 2016.

Paramitha, D.; Ulum, M. F.; Purnama, A.; Wicaksono, D. H. B.; Noviana, D. & Hermawan, H. *Monitoring Degradation Products and Metal Ions In Vivo*. In: Narayan, R. J. (Eds.). Monitoring and Evaluation of Biomaterials and their Performance In Vivo. Sawston, Woodhead Publishing, 2017. pp.19-44.

Ricco, R.; Serio, G.; Caniglia, D. M.; Cimmino, A.; Lettini, T.; Lozupone, A. & Delfino, V. P. Size and shape evaluation of astrocytoma nuclei with the shape analytical morphometry software system. *Anal. Quant. Cytol. Histol., 16(5)*:345-50, 1994.

Ronchi, G.; Fregnan, F.; Muratori, L.; Gambarotta, G. & Raimondo, S. Morphological methods to evaluate peripheral nerve fiber regeneration: a comprehensive review. *Int. J. Mol. Sci., 24(3)*:1818, 2023.

Rosioru, C.; Talu, S.; Talu, M.; Giovanzana, S.; & Craciun, C. Morphometric assessments for the healthy rat hepatocytes. *Ann. Rom. Soc. Cell Biol., 17(1)*:74-9, 2012.

Tashk, A.; Helfroush, M. S.; Danyali, H. & Akbarzadeh-Jahromi, M. Automatic detection of breast cancer mitotic cells based on the combination of textural, statistical and innovative mathematical features. *Appl. Math. Model., 39(20)*:6165-6182, 2015.

Varejão, A. S.; Meek, M. F.; Ferreira, A. J.; Patrício, J. A. & Cabrita, A. M. Functional evaluation of peripheral nerve regeneration in the rat: Walking track analysis. *J. Neurosci. Methods, 108(1)*:1-9, 2001.

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