

K.V. Shevchenko, G.A. Yeroshenko, A.V. Solod, O.D. Lysachenko, O.S. Yakushko,
N.V. Boruta, D.V. Lichman, D.R. Kramarenko
Ukrainian Medical Stomatological Academy, Poltava

**CORRELATION ANALYSIS BETWEEN METRIC PARAMETERS
OF PARENCHYMATOUS COMPONENTS OF RAT SUBMANDIBULAR GLANDS UNDER
THE EFFECT OF ETHANOL**

e-mail: kvshevchenko2017@gmail.com

The effect of ethanol on the salivary glands is manifested by changes in morphometric parameters of both the final departments and excretory ducts. But taking into account that the obtained large number of morphometric indicators is rather complicated, then there is a need for a correlation analysis, where the presence of dependencies between morphometric indicators was determined using the Bravais – Pearson coefficient. It was established that diagnostic criteria for assessing the functional state of rat submandibular glands after ethanol is manifested by the dependence of the outer diameter, lumen diameter and epithelial cell height on day 12 in all parenchymal components, which corresponds to the formation of chronic alcohol dependence in rats.

Key words: submandibular glands, rats, morphometry, correlation analysis.

The work is a fragment of the research project “Experimental morphological study of the effect of cryopreserved cord blood products and embriofetoplacental complex (EFPC), diphereline, ethanol and 1% methacrylate on the morphofunctional condition of certain internal organs”, state registration No. 0119U102925.

In the 21st century, the problem of alcohol consumption is one of the most common worldwide, and every year it tends to become large-scaled due to constant stress related to national and territorial cataclysms [5]. Alcohol consumption, as one of the global problems of society, reflects the medical and ethical problems of finding ways to solve the problem, as its consequences directly negatively affect the human body. Thus, being aware of the toxic nature of alcohol, a person does not suspect that the destruction of the body occurs due to chemical reactions catalyzed by ethanol [2]. Awareness of the outcomes of alcohol consumption and its effect on the tissues of the oral cavity and its external organs, involved in maintaining its homeostasis is relevant and important both for preservation of dental health and the entire human body.

The effect of ethanol on the salivary glands is manifested by changes in morphometric parameters of both the acini [8] and excretory ducts [9]. However, given that the resulting big amount of morphometric parameters is quite complex, there is a need for correlation analysis, which can reflect relationship between changes in one parameter with the change of another one [6].

The purpose of the work was determining correlations between the morphometric parameters of the acini, excretory ducts and microvasculature of the normal rat submandibular salivary glands and in chronic ethanol intoxication.

Materials and methods. Morphometry involved study of the separate samples of semi-thin sections obtained from pieces of the submandibular glands of rats, administered which 12 mg/kg of 40° ethanol directly to stomach 4 times a day [3]. The animals were killed under 25 mg/kg thiopental anesthesia overdose on days 5, 9, 12 and 30 of the experiment. Pieces of the submandibular glands were embedded into epon-812 according to standard procedure [1]. Semi-thin sections were stained with methylene blue. The mean values of the outer diameter, the diameter of the lumen of the ducts and the height of the epithelial cells were determined using the Biorex-3 BM-500T microscope equipped with DCM 900 digital microphoto attachment with software, adapted to these studies. Statistical processing of the morphometric data and correlation analysis was performed using the Microsoft Excel software [7] to determine the relationships between morphometric parameters. The presence of correlation between morphometric parameters was determined using the Bravais-Pearson coefficient, where Pearson's r was used to obtain simultaneously information about the direction of correlation (positive +, negative -) and the strength of the relationship (from 0 to 1). If $r = 0$, it is assumed that no correlation exists, the interval of 0-0.3 indicates a weak correlation, the interval of 0.3–0.7 indicates moderate correlation, and the interval from 0.7 to 1.0 is assumed as a strong one. Pearson's method is considered the most accurate for studying the correlation [6].

Results of the study and their discussion. Correlation analysis of the metric parameters of the submandibular glands of rats of the control group has established mainly weak relationship with the mean values of the outer diameter of the acini, except for moderate relationship with the diameter of the lumen of the venules on day 9 and 30 of the experiment ($r = 0.31$ in $p < 0.05$). The diameter of the lumen of the acini of the control group showed weak relationship with all parameters of the submandibular glands, as well as the height of the epithelial cells.

Moderate correlation between the outer diameter on day 5 of the experiment and the diameter of the lumen of arterioles on day 5 ($r = 0.34$) was established, as well as negative moderate relationship with the diameter of the lumen of the venules on day 5 of the experiment ($r = -0.34$). Moderate correlation between the values of the lumen diameter and the values of the diameter of the arterioles on day 5 of the experiment has been established ($r = 0.33$). Moderate correlation between the height of the epitheliocytes of the acini of the submandibular glands on day 5 of the experiment and the diameter of the lumen of the venules on day 9 and 30 of the experiment was established ($r = 0.41$).

Strong correlations between the outer diameter of the acini on day 9 and the values of the outer diameter on day 12 ($r = 1.00$) have been found. Notably, strong positive correlations between the values of the lumen diameter and the similar ones on day 12 ($r = 1.00$) was established. Moderate negative correlations between the values of the height of the epithelial cells and the values of the diameter of the capillary lumen ($r = -0.32$) and strong positive relationship with the values of the height of the epitheliocytes on day 12 ($r = 1.00$) were established.

On day 12 of the experiment weak correlations between the values of the outer diameter and the diameter of the lumen of the rat submandibular glands were established, in contrast to the values of the height of the epithelial cells that showed moderate negative relationship with the values of the diameter of the capillary lumen on day 5 of the experiment ($r = -0.32$).

On day 30, the values showed weak relationships, the most apparent of which were moderate correlations between the outer diameter and the diameter of the acini and the diameter of the lumen of the arterioles, where positive relationship was established with the outer diameter ($r = 0.21$) and weak negative relationship was with the lumen diameter ($r = -0.21$).

Correlation analysis of the metric values of the intercalated ducts of the submandibular glands of rats of control group has shown weak relationships among which the most apparent were weak negative correlations between the diameter of the intercalated ducts and the diameter of lumen diameter ($r = -0.26$) and the diameter of the venule lumen ($r = -0.25$), whereas the diameter of the lumen of intercalated ducts showed weak negative correlations between the diameter of the lumen of arterioles ($r = -0.20$) and the diameter of the capillary lumen ($r = -0.23$) of rats of control group.

Moderate positive correlations ($r = 0.42$) between the value of the outer diameter on day 5 and the values of the diameter of the venule lumen were established in the experimental group and weak relationship with capillary lumen on day 5 ($r = -0.24$) in the same group, with the diameter of the arteriole lumen on day 9 ($r = -0.20$), with the outer diameter of the intercalated ducts ($r = -0.21$), height of the epithelial cells of the intercalated ducts ($r = 0.24$), and negative relationship with the mean values of the diameter of the arteriole lumen ($r = -0.20$) in experimental group on day 30 of the study. Moderate negative correlations ($r = -0.50$) between the values of the diameter of the lumen of intercalated ducts on day 5 of the experiment and the values of the outer diameter of the intercalated ducts on day 9 ($r = 0.54$) and day 12 ($r = 0.54$) were established, as well as values of the diameter of the lumen of the intercalated ducts on day 9 and 12. The comparison of the height of the epithelial cells of the intercalated ducts of the submandibular glands has revealed negative moderate correlations between the outer diameter of the intercalated ducts and values on day 9 and 12 of the experiment ($r = -0.31$). The rest of the morphometric parameters showed weak relationship with the values of the height of epithelial cells on day 5, except for the diameter of the arteriole lumen on day 30 where weak to moderate negative relationship was noted ($r = -0.27$, $p < 0.05$).

Strong positive correlation between the values of the outer diameter on day 9 and its values on day 12 ($r = 1.00$) was established, as well as moderate negative correlations between the diameter of the lumen of intercalated ducts in its group ($r = -0.40$) and on day 12 of the experiment ($r = -0.40$). Strong positive correlation between the values of the diameter of the lumen of intercalated ducts on day 9 and the diameter of the lumen on day 12 ($r = 1.00$) was established, as well as moderate negative relationship with the values of the outer diameter in its group ($r = -0.40$) and on day 12 of the experiment ($r = -0.40$). Moderate positive relationship with the values of the diameter of the arteriole lumen on day 30 ($r = 0.26$) was established. The comparison of values of the height of the epithelial cells on day 9 showed strong positive relationship with the values of the height of the epithelial cells of the intercalated ducts on day 12 ($r = 1.00$) and moderate positive relationship, compared to values of the arteriole lumen on day 9 and 30 ($r = 0.36$). Weak negative relationship with the values of the diameter of the arteriole lumen on day 5 ($r = -0.25$), and positive relationship ($r = 0.21$) with the values of the lumen of intercalated ducts of its group and experimental group on day 12 was established.

On day 12 of the experiment strong and moderate negative relationships were established that were similar to the above terms, as well as correlation between the outer diameter and values of the diameter of the lumen on day 12 ($r = -0.40$). The analysis of the diameter of the lumen showed weak to moderate relationship with the values of the diameter of the arteriole lumen on day 30 ($r = 0.26$). Moderate positive

correlation between the height of the epithelial cells and values of the diameter of the arteriole lumen on day 30 ($r = 0.36$) was established.

The analysis of the relationships of the intercalated ducts on day 30 revealed strong positive correlations between the outer diameter of the ducts and the lumen diameter of the ducts in its experimental group ($r = 0.75$). Moderate negative correlation between the diameter of the lumen and values of the diameter of the arteriole lumen in control group ($r = -0.32$) was established, as well as weak to moderate positive relationship ($r = 0.20$) with the diameter of the lumen of intercalated ducts in control group. Moderate negative correlations between the height of the epithelial cells and the values of the diameter of the venule lumen in the control group ($r = -0.34$) were established, as well as weak negative relationship with the outer diameter of intercalated ducts in its group ($r = -0.27$).

Correlation analysis of the metric parameters of the striated ducts of the submandibular glands has established weak correlations between the values of the outer diameter of rats of control group and the diameter of the arteriole lumen of its group ($r = 0.22$). Moderate negative correlation ($r = -0.31$) between the lumen diameter and the outer diameter of the striated ducts of control group was established, as well as weak negative relationship ($r = -0.24$) with the diameter of arteriole lumen and positive with the diameter of capillary lumen ($r = 0.27$) of its group. The comparison of the values of the height of the epithelial cells of the striated ducts of control group revealed weak correlations.

Weak correlation between the outer diameter of the striated ducts on day 5 and the values of the diameter of the lumen of the striated ducts ($r = 0.26$) and the height of epithelial cells ($r = 0.25$) of control group and the diameter of the capillary lumen ($r = 0.26$), the diameter of the arteriole lumen of its experimental group ($r = 0.24$) and on day 30 ($r = 0.29$) was established. Moderate positive relationship with the mean values of the diameter of the venule lumen of its group ($r = 0.59$) was established. Moderate negative correlations ($r = 0.25$) between the diameter of the lumen and the values of the height of epithelial cells on day 5 of the experiment were established, as well as weak negative relationship with the diameter of the capillary lumen of its group ($r = -0.24$), the diameter of the arteriole lumen on day 9 ($r = -0.25$) and day 30 ($r = -0.25$) of the experiment. Moderate positive correlations between the mean values of the diameter of the arteriole lumen ($r = 0.32$) and capillary lumen ($r = 0.36$) of its experimental group have been established.

Comparison of the mean values of the outer diameter of the striated ducts on day 9 of chronic ethanol intoxication showed strong positive relationship with its value on day 12 of the experiment ($r = 1.00$) and moderate relationship with the value of the diameter of capillary lumen on day 12 ($r = 0.38$). Strong positive correlation between the diameter of the lumen of striated ducts and their values on day 12 was determined ($r = 1.00$), as well as moderate negative relationship with the values of the diameter of capillary lumen ($r = -0.38$). Strong positive correlation between the values of the height of the epithelial cells and similar parameter on day 12 of the experiment was established ($r = 1.00$).

Weak relationship with the outer diameter on day 12 was determined, except for the mean values of the diameter of capillary lumen of its group where moderate relationship was established ($r = 0.38$). Moderate negative correlations between the diameter of the lumen of striated ducts of the submandibular glands and the value of the diameter of capillary lumen of its experimental group were revealed. Weak relationship with the height of the epithelial cells was established.

The analysis of the correlations between the metric values of the striated ducts on day 30 showed mainly weak relationship, except for positive moderate correlation between the diameter of the lumen and the height of the epithelial cells of its experimental group ($r = 0.43$).

Correlation analysis of the morphometric parameters of the granular ducts of the control group has established weak negative correlations between the outer diameter and the diameter of the capillary lumen of control group ($r = -0.24$). Moderate correlation between the diameter of the lumen of the granular ducts and the values of the capillary lumen of its group ($r = 0.30$) was established, as well as weak positive relationship with the diameter of the capillary lumen on day 5 of the study ($r = 0.24$). Weak positive correlation between the height of the epithelial cells and the values of the venule lumen of the control group ($r = 0.28$) was established.

The findings of the study on day 5 of the experiment showed moderate negative correlations between the mean values of the outer diameter of the granular ducts and the diameter of arteriole lumen ($r = -0.46$) and capillary lumen ($r = -0.54$) of its group. Moderate negative correlations between the diameter of the lumen and values of the diameter of the arteriole lumen on day 9 and 30 ($r = -0.34$) were established. Moderate positive correlations between the height of the ductal epithelial cells and the values of the diameter of the venule lumen on day 5 were determined, as well as weak negative relationship with the values of the capillary lumen on day 30 ($r = -0.26$).

Strong positive correlation between the metric values of the outer diameter on day 9 and the values of the outer diameter on day 12 ($r = 1.00$) was determined, as well as moderate positive relationship with the values of the diameter of arteriole lumen ($r = 0.35$) and capillary lumen ($r = 0.37$). Weak positive relationship ($r = 0.26$) with the diameter of lumen of granular ducts on day 9 and 12 of the experiment was established. Strong positive correlation between the diameter of the lumen of granular ducts and its values on day 12 ($r = 1.00$) was established, as well as weak relationship ($r = 0,28$) with the diameter of the arteriole lumen on day 12. Strong positive correlation between the height of the epithelial cells ($r = 1.00$) and similar parameter on day 12 was established, as well as moderate positive relationship with the values of the diameter of the arteriole lumen on day 12 of the experiment.

Moderate correlations between the values of the outer diameter of the granular ducts on day 12 and the diameter of the arteriole lumen ($r = 0.35$) and capillary lumen ($r = 0.37$) of the experimental group were established. Weak correlation between the diameter of the lumen and the diameter of the arteriole lumen on day 12 ($r = 0.28$) was determined. Moderate positive correlations between the values of the height of the epithelial cells and the values of the diameter of the arteriole lumen of the experimental group ($r = 0,39$) were established.

The findings of our study showed weak relationship with morphometric parameters of the granular ducts of the submandibular glands on day 30.

Other investigators report about significant strong correlations between the morphometric parameters of the epithelial components and elements of the microvasculature of the lobules of rat normal major salivary glands and after stimulation of the peripheral nervous system. Strong correlations were established between the metric parameters of the acini and the parameters of the exchange and capacitive sections of the microvasculature. Strong correlations were determined between the morphometric parameters of the ductal system of the intercalated and intralobular collector ducts of the sublingual gland (in control group of animals) and striated ducts of the submandibular gland, especially after administration of acetylcholine [4].

Conclusion

It has been established that the diagnostic criteria of the evaluation of the functional state of the rat submandibular glands after the effect of ethanol are the correlation between the outer diameter, the diameter of the lumen and the height of the epithelial cells on day 12 in all parenchymatous components that is associated with the development of chronic alcohol dependence in rats.

References

1. Bahriy MM, Dibrova VA, Popadynets OH, Hryshchuk MI. Metodyky morfolohichnykh doslidzhen. Bahriy MM, Dibrova VA, redactory. Vinnytsya: Nova knyha; 2016. 328s. [in Ukrainian]
2. Yefremov AYU, Belkyn VV. Biokhimiya alkoholia i yeye vliyanie na orhanizm. Nauchnye issledovaniya: ot teorii k praktike. 2015; 2(3): 455-56. [in Russian]
3. Yeroshenko GA, Shevchenko KV, Yakushko OS. Morfometrychna kharakterystyka yemnisnoyi lanky hemomikrotsyrkuliatornoho rusla slynyykh zaloz shchuriv v normi ta pry khronichnyi intoksykatsiyi etanolom. Svit medytsyny ta biolohii 2018; 3 (65): 149-152. [in Ukrainian]
4. Yeroshenko GA, Kostylenko YuP, Skrypnikov MS, Kryveha LH. Koreliatsiyni zvyazky mizh morfometrychnymy pokaznykamy velykykh slynyykh zaloz shchuriv v normi i pislia stymuliatsiyi peryferychnoyi nervovoyi systemy. Svit medytsyny ta biolohiyi. 2009; 3(1): 64-69. [in Ukrainian]
5. Konkova AV. Vlyanye alkoholia na podrostkovii orhanyzm. Integrativnye tendentsii v meditsine i obrazovanii. 2019; 2: 42-5. [in Russian]
6. Lanh TA, Sesy M. Kak opisyvat statystyku v meditsine. Annotirovannoe rukovodstvo dlia avtorov, redaktorov i retsenzentov. per. s angl. Leonov VP, redactor. Prakticheskaia medytsyna. 2011; 480 s. [in Russian]
7. Lapach SN, Chubenko AV, Babych PN. Statisticheskiye metody v mediko-biologicheskikh issledovaniyakh s ispolzovaniyem Excel. Kiev: Morion; 2000. 320 s. [in Russian]
8. Shevchenko KV, Garets VI, Fedonyuk LYa, Volkov KS, Nesteruk CO. Histophysiology of submandibular salivary glands end pieces in rats with chronic ethanol intoxication. Word of medicine and biology. 2018; 4 (66): 231-234.
9. Shevchenko KV, Yeroshenko GA, Vilkhova OV, Kramarenko DR, Yakushko OS, Yachmin AI. Remodeling of the duct system of the rat submandibular salivary glands in chronic ethanol intoxication. Wiadomości Lekarskie. 2020; 73(1): 128-133.

Реферати

КОРЕЛЯЦІЙНИЙ АНАЛІЗ МІЖ МЕТРИЧНИМИ ПОКАЗНИКАМИ ПАРЕНХІМАТОЗНИХ КОМПОНЕНТІВ ПІДНИЖНЬОЩЕЛІПНИХ ЗАЛОЗ ЩУРІВ ПІСЛЯ ДІЇ ЕТАНОЛУ

Шевченко К. В., Єрошенко Г. А., Солод А. В., Лисаченко О. Д., Якушко О. С., Борута Н. В., Личман Д. В., Крамаренко Д. Р.

Вплив етанолу на слинні залози проявляється змінами морфометричних показників як кінцевих відділів так і вивідних проток. Але з огляду на те, що отримана велика кількість морфометричних

КОРЕЛЯЦИОННЫЙ АНАЛИЗ МЕТРИЧЕСКИХ ПОКАЗАТЕЛЕЙ ПАРЕНХИМАТОЗНЫХ КОМПОНЕНТОВ ПОДНИЖНЕЧЕЛЮСТНЫХ ЖЕЛЕЗ КРЫС ПОСЛЕ ДЕЙСТВИЯ ЭТАНОЛА

Шевченко К. В., Ерошенко Г. А., Солод А. В., Лисаченко О. Д., Якушко, О. С., Борута Н. В., Личман Д. В., Крамаренко Д. Р.

Влияние этанола на слюнные железы проявляется изменениями морфометрических показателей как конечных отделов так и выводных протоков. Но учитывая то, что полученное большое количество морфометрических

показників є досить складною, то виникає необхідність проведення кореляційного аналізу, де наявність залежностей між морфометричними показниками визначали за допомогою коефіцієнту Браве- Пирсона. Встановлено що діагностичними критеріями для оцінки функціонального стану піднижньощелепних залоз щурів після дії етанолу є залежність зовнішнього діаметру, діаметру просвіту та висоти епітеліоцитів на 12 добу у всіх паренхіматозних компонентах що відповідає формуванню хронічної алкогольної залежності у щурів.

Ключові слова: піднижньощелепні залози, щури, морфометрія, кореляційний аналіз.

Стаття надійшла 10.03.2019 р.

показателей есть достаточно сложным, то возникает необходимость проведения корреляционного анализа, где наличие зависимостей между морфометрическими показателям определяли с помощью коэффициента Браве – Пирсона. Установлено что диагностическими критериями для оценки функционального состояния поднижнечелюстных желез крыс после действия этанола проявляется зависимостью внешнего диаметра, диаметра просвета и высоты эпителиоцитов на 12 сутки во всех паренхиматозных компонентах, что соответствует формированию хронической алкогольной зависимости у крыс.

Ключевые слова: поднижнечелюстные железы, крысы, морфометрия, корреляционный анализ.

Рецензент Старченко І.І.

DOI 10.26724/2079-8334-2020-2-72-229-234

UDC 572.7:612.83:57.017.642

V.S. Shkolnikov, S.O. Prykhodko, S.S. Polishchuk, O.V. Kryvoviaz, G.M. Galunko
National Pirogov Memorial Medical University, Vinnytsya

THE MORPHOLOGY OF RADIAL GLIAL SPINAL CORD OF EMBRYOS AND HUMAN FETUSES

e-mail: v.shkolnikov@gmail.com

The study of radial glia morphology and processes of targeted migration of neural stem cells in humans remains far from being resolved. The purpose of this study is to establish the morphological aspects of radial glia of spinal cord of human in prenatal period. Morphological examination of the spinal cord of human embryos and fetuses of 6-7 weeks up to 39-40 weeks was performed. Using anatomical, histological, immunohistochemical, and morphometric morphological aspects of the radial glia of the spinal cord were established. The results showed that strong expression of vimentin and CDX-2 radial glia fibers was observed up to 8-9 weeks. From 11-12 weeks, radial glia fibers retain a radial direction only in the middle part of the segments, which, in our view, is associated with the gradual involution of radial glia, which correlates with the formation of nuclear-neural complexes of gray matter. Until the moment of birth, vimentin-positive structures of radial glia gradually disappear and can be traced only in the neuroepithelium of segments.

Key words: prenatal period, spinal cord, radial glia, neuroepithelium, immunohistochemical markers.

The study is a fragment of the research project “Determination of morphological changes of the central nervous system in the prenatal ontogeny (macroscopic, histological, morphometric and immunohistochemical study)” state registration No. 0118U001043.

Neurogenic fetal brain cells generate major cell types of the nervous system during the prenatal ontogeny, which lasts from fertilization to birth. Such neurogenic cells include neural stem cells (NSC), neural progenitor cells (NPC), and linear-specific progenitors and precursors [2]. NSC are known to be multipotent cells, which are characterized by the proliferation and formation of several cell pools simultaneously: neuroblasts or glioblasts [4, 10, 15]. To date, there is no doubt that the presence of permanent neurogenesis of some areas of the brain performs due to colonies of NSC [9, 14]. Rybachuk O.A. and Pivneva T.A. (2013) emphasize that during the embryonic ontogeny period NSC cranial nerve tubes showed greater proliferative activity than neural cells of the caudal compartments [3]. Thus, it can be predicted that the above processes are inherent in the spinal cord, which precede the formation of neuronal complexes, which in turn requires further study and refinement.

It should also be noted that the identification of NSC in vivo is traditionally based on the analysis of the morphology of these cells, their mitotic activity, and the expression of certain genes and protein synthesis. The most commonly identified NSC markers are Nestin, Sox2, Msashi 1, 2, Oct 4, Nanog, etc., but none can be used as the sole criterion for NSC identification [11].

The next step is to study the mechanisms of migration of differentiated unipotent neural cells that have just formed in the paraventricular zone.

It has been established that the most extensive migration of neural cells occurs during the process of laying the cerebral cortex [5]. At the same time, Tsymbaluk V. I. and Medvedev V. V. (2010) indicate that radial glial (RG) plays a key role in ensuring nerve cell migration during the development of other neural tube compartments and cell migration. The authors have shown that immature neurons migrate along the processes of RG cells in the centripetal direction [5].

Over time, RG cells lose the ability to express nestin and vimentin [8]. However, the authors do not specify a term when such a property is lost. In contrast, Kirik O. V. and Korzhevskii D. E. (2012) indicate that the RG cell population is heterogeneous: some cells contain neural markers (they subsequently