

THE USE OF MORPHOLOGICAL STUDY TECHNIQUE FOR INVESTIGATION OF LABIAL AND PALATINE GLANDS

WYKORZYSTANIE MORFOLOGICZNEJ TECHNIKI DO BADANIA GRUCZOŁÓW WARG I PODNIEBIENIA

Volodymyr H. Hryn, Oleg O. Sherstyuk, Nataliia L. Svintsytska, Andriy V. Piliuhin, Roman L. Ustenko
HIGHER STATE EDUCATIONAL ESTABLISHMENT OF UKRAINE "UKRAINIAN MEDICAL STOMATOLOGICAL ACADEMY", POLTAVA, UKRAINE

ABSTRACT

Introduction: Due to the deterioration of environmental conditions that promotes the onset of inflammatory and autoimmune diseases, and the progress in the diagnosis, the frequency of registration of intercurrent pathology of salivary glands has markedly risen in recent years, demonstrating the increased scientific interest in the research of the common and distinctive features of their structure.

The aim: The paper was aimed at the development of the method of morphological study of human minor salivary (labial and palatine) glands by the use of plastic wax reconstruction to obtain the plastic model of the acini and ducts of human minor salivary glands.

Materials and Methods: Specimens of the glandular area of the hard palate mucosa and labial mucosa in its middle third have been studied. To gain the objective of the investigation the technique for morphological study of the human minor salivary (labial and palatine) glands is to be developed, encompassing the analysis of the spatial organization of the glandular epithelium of the labial and palatine glands together with blood microcirculatory flow by fixing the obtained specimens of the minor salivary glands in 4% glutaraldehyde solution and osmium tetroxide with subsequent embedding into the Epon-812, staining the serial semi-thin sections with phosphate buffered 0,1% toluidine blue solution, photomacrography of the distinguished boundaries of the investigated structures and obtaining of photoreconstructions.

Results and Conclusions: Thus, the use of suggested technique enables to obtain the megascopic reconstruction of the acini and ducts of the labial and palatine glands, which can be studied from different sides, getting the full visualization of the shape and size, as well as to explore the glands' inner configuration, the geometry of the lumen of the epithelial excretory ducts, to determine changes in the thickness of the wall, to get a visual representation of the microtopographic interactions between the different parts of blood microcirculatory flow and excretory ducts of the minor salivary glands.

KEY WORDS: labial gland, palatine gland, mucous membrane, plastic wax reconstruction

Wiad Lek 2017, 70, 5, 934-938

INTRODUCTION

The paper has been written within the research scientific work, carried out at the Department of Human Anatomy of the Higher State Educational Establishment of Ukraine "Ukrainian Medical Stomatological Academy", entitled "Age-related aspects of the structural organization of the organs of the human immune system, glands of gastrointestinal and urogenital system in normal condition and pathology"; State registration number 0116U004192.

The analysis of the publications shows that due to the progress in the diagnosis of diseases and the deterioration of environmental conditions that promotes the onset of the inflammatory and autoimmune diseases, the frequency of registration of intercurrent pathology of salivary glands has markedly risen [1, 2, 3, 4]. This fact explains the recent increased scientific interest in the research of the common and distinctive features of their structure [5, 6, 7].

The frequent involvement of the minor salivary glands into pathological processes leads to disturbance of their numerous and important functions, requires more detailed, up-to-date knowledge of their morphological features.

Their assessment, made on the basis of the available research techniques, is still relevant. Moreover, the potential of morphological research methods of study of the intramural glands is not fully used to date. Even the latest publications on this issue do not reveal completely the whole range of possibilities of the advanced technologies [8, 9, 10]. Therefore, for a long time, the glands of this type are of great interest not only for the clinic practitioners, but also by morphologists, where there is an understanding that the clinical interpretation of morphological factology should rely on the contemporary anatomical information [11].

For many years, both human and animal exocrine and endocrine glands, as well as their microcirculatory flow have been studied at the Department of Human Anatomy of the higher educational institution of Ukraine "Ukrainian Medical Stomatological Academy". The study of various glands, including the lacrimal gland and minor salivary ones, is a traditional domain of the Poltava school of morphologists [7, 8, 9, 12, 13].

The great variety of techniques exist for morphological study of minor salivary glands, but all current methods

have certain disadvantages, namely: they are technically sophisticated, unusable in the study of the objects with very dense arrangement of the structural units, hindering the obtaining of information about the spatial 3D organization of the structural units of minor salivary glands [14, 15, 16].

The Yu.P. Kostilenko's method of study of salivary glands, called "The methods of multilayer reconstruction of the epithelial complexes of the salivary glands based on the serial semi-thin sections" [17] is the method which is the most proximate to the proposed one, suggesting the photomicrography of the certain individual structural units of the salivary glands in each semi-thin section of the serial sampling. The next stage supposes obtaining the positive images on a glass photoplate using the magnifier in clearly adjusted magnification. After the washing, fixing, washing and drying of the profiles of tissue components of the salivary glands the photoplates (from the side of its emulsion layer) should be covered with 1% solution of nitrocellulose in amyl acetate. Then the photoplate is dipped in a solution of attenuator; as a result they keep only those areas that have been covered by the collodion film, and the other background becomes transparent. It deprives the object from the masking background of the surrounding structures. Further on as a result of the layerwise matching of the photoplates the clear contours of the investigated object appear in the thickness of the block. This method is the simplest and quite effective, though it has some limited application. It can be recommended if the investigated object is characterized by the not very dense arrangement of its structural units, for example, the salivary glands at the early stages of their development. While exploring a tightly arranged objects the method is not very effective because of the silhouettes, emerging during the layerwise matching of the microimages with excessive density in those places where a great congestion of the acini of the salivary glands occur. On their basis it is difficult to get the visual illustrations of the investigated objects. Therefore, the best method is a multi-layered plastic reconstruction. The material for the manufacturing of the plastic models based on the serial histological sections is the wax plates, which are usually made manually. It will be more appropriate to use the plates of basic dental wax base of 2 mm thick, namely, "Basic Wax basis 02", possessing the necessary elasticity and durability. They are quite transparent to carry the contours of the object on their surface.

THE AIM

The study was aimed at the developing a method of morphological study of the human minor salivary (labial and palatine) glands with the improvement of the existed one by using the method of plastic wax reconstructions and to obtain the the plastic model of the acini and ducts of the human minor salivary glands that will promote the conduct of the stereological analysis of the epithelial complexes of the minor salivary glands in conjunction with the chains of hemomicrocirculatory flow.

MATERIALS AND METHODS

Specimens of the glandular area of the hard palate mucosa and labial mucosa in its middle third have been studied.

To gain the objective of the investigation the technique for morphological study of the human minor salivary (labial and palatine) glands is to be developed, encompassing the analysis of the spatial organization of the glandular epithelium of the human minor salivary (labial and palatine) glands together with blood microcirculatory flow by fixing the obtained specimens of the minor salivary glands in 4% glutaraldehyde solution and osmium tetroxide with subsequent embedding into the Epon-812, staining the serial semi-thin sections with phosphate buffered 0,1% toluidine blue solution, photomicrography of the defined contours of the investigated structures and obtaining of the photoreconstructions by marking the additional coordinates on the obtained photoreconstructions with subsequent correct sequential arranging of the workpieces on the wax plates to create the maximum precise 3D frame of the original model of the acini and ducts of the minor salivary (labial and palatine) glands.

The suggested technique was implemented in the following way: first, the obtained specimens of the minor salivary (labial and palatine) glands were fixed in 4% glutaraldehyde solution and osmium tetroxide with subsequent embedding into the Epon-812. Serial semi-thin sections were stained with phosphate buffered 0,1% toluidine blue solution. The loss of sections greater than 3% in a set is not allowed. The next stage was the photomicrography of each section, keeping to the ultimate magnification. After that contours of investigated structures and additional coordinates have been selectively determined, using the graphical photoreconstructions. The next step was to copy the required structures and additional coordinates on the transparent plates for the preliminary evaluation, analysis and sequence for the next arrangement of the wax plates of 1-2 mm thick. The contours of investigated microobjects and additional coordinates have been obtained for correct arrangement of the workpieces on the wax plates. Thereafter the required morphological structures have been cut out from the wax plates with a sharp scalpel. Since the certain details of the section, including the additional coordinates, should keep the genuine correlation between themselves, the artificial joining bridges were temporarily kept. Then the sequential stacking of the obtained structures has been carried out, relying on the additional coordinates.

Thus, the maximum precise three-dimensional frame of the primary models has been obtained as a result of the series of wax plates-templates stacking. The final stage of the creation of the 3D wax model of the minor salivary glands has been done: thin metal needles have been placed to the points of artificial wax bridges location and the bridges itself were removed by cutting out with a warmed scalpel.

RESULTS AND DISCUSSION

Apparently, the suggested method enables to get the megascopic reconstruction of the acini and ducts of the minor

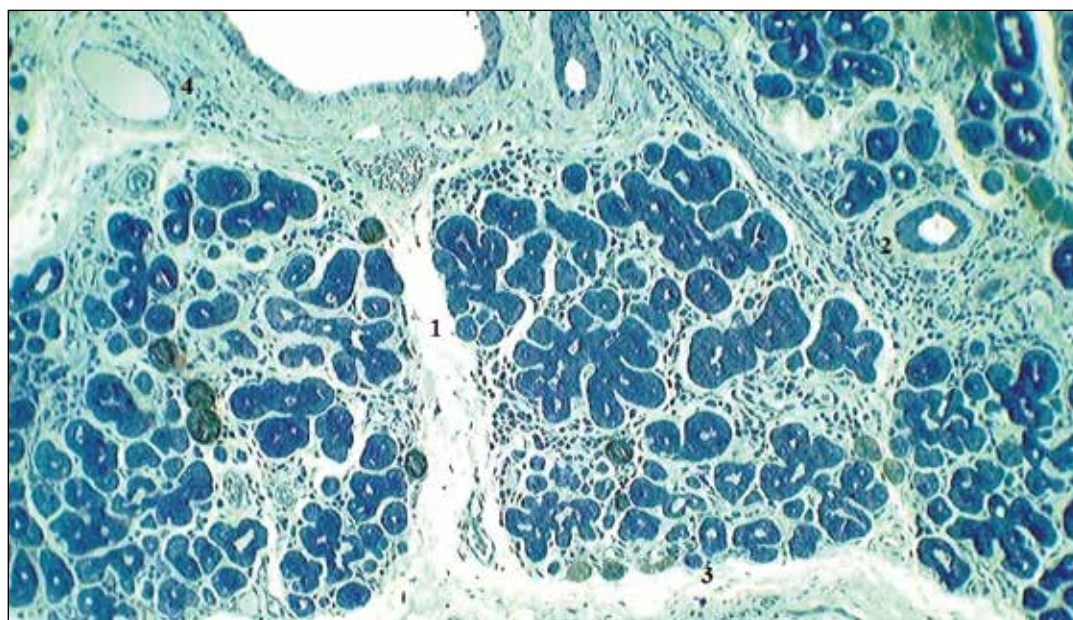


Fig. 1. Human labial gland. Semi-thin section. Toluidine blue stain. Lens, 10. 1 – interparticle intersticium, 2 – excretory duct, 3 – capsule of the gland, 4 – venule.

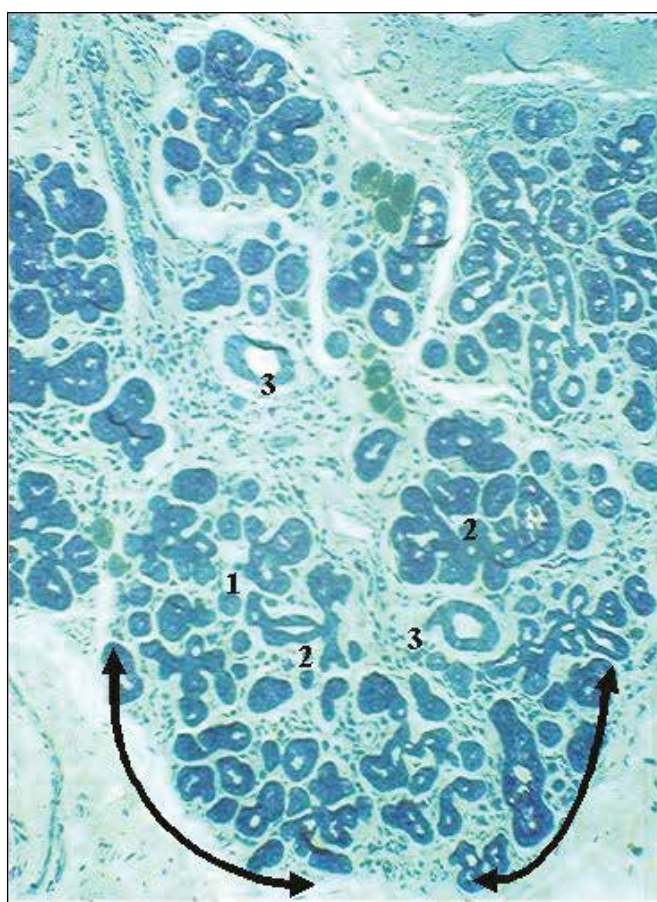


Fig. 2. Human palatine gland. Semi-thin section. Toluidine blue stain. Lens, 8. 1 – glandular particle, 2 – acinar and intercalated parts, 3 – interparticle excretory ducts.

salivary (labial and palatine) glands, which can be studied from all sides, getting a comprehensive idea about the shape and size, as well as allows to explore the inner relief of the glands, the geometry of the lumen of the epithelial excretory glandular ducts, to determine the changes in the thickness of the wall, to get a visual representation of microtopographic correlation between the different parts of blood microcirculatory flow with epithelial excretory ducts of the minor salivary glands.

The findings of the structural analysis enabled to determine the following epithelial components in the particle of the human labial gland (Fig.1): 1-acini; 2-intercalated excretory ducts, 3-intraparticle, 4-particle, 5-interparticle, 6- common excretory duct.

The overall epithelial components form a complex bifurcated system, where several structural levels of the spatial three-dimensional organization of the palatine gland can be distinguished (Fig. 2): 1 – monoacinar-intercalated, diacinar-intercalated and polyacinar- intercalated elementary units. The connecting link for them is the intercalated ducts; 2-subparticle units that represent a population group of mono-, di-, and polyacinar – intercalated elementary units. They are united by one from three or four intraparticle ducts; 3 – glandular particle that includes from three to five subparticle units; the particle duct is the common thing for them; 4 –individual gland, composed of three to five lobules, united by the interparticle and common excretory ducts.

The minor (intramural) glands of the cavitory organs of the digestive, respiratory and urinogenital systems are essential for the processes of digestion, respiration, excretion and maintenance of homeostasis. They also participate in the development of many pathological processes in organs' walls. The minor glands are involved in the acute and chron-

ic non-specific processes, as well as serve as the anatomic substrate for the development of adenocarcinomas, adenomas, retention cysts [1, 3, 8, 10]. Contemporary methods of research of pathogenic mechanisms in the development of diseases of the salivary glands changed the traditional idea about them as the secretory organs. These glands are very sensitive to changes in the internal and external environment of the body and are the "target" for immune destruction [5].

Notwithstanding the certain successes in the study of structure and functions of the minor salivary glands the available publications present contradictory and ambiguous morphological data on the structure of the excretory ducts and microcirculatory flow of the glands. Many publications do not elucidate a comprehensive approach to the study of problems of morphological rationale of the secreta movement on the bifurcated system of excretory ducts of the minor salivary glands; no stereological study and the morphometry of their ducts have been carried out. A comparative stereomorphological analysis of the structure of labyrinths of the excretory ducts has not been conducted, too, which is crucial in the interpretation of mechanisms of secreta movement on them.

CONCLUSIONS

The findings of the morphological study of the human labial and palatine glands made by the suggested method, as well as the analysis of the publication data, enable making the following general conclusions. Human minor glands have a complex, bifurcated system of excretory ducts, including intercalated, intraparticle ducts (located in the amount, taken by the individual particle), particle, interparticle (located in the interstitial spaces) and common excretory duct (located in the pre-epithelial area of the mucosa), on which secreta is discharged, mostly of the mucous nature.

The average values of the external diameter of the transversal profile of the ducts of the human labial and the palatine glands are very similar:

a) for the palatine glands they are within $35,79 \pm 1,53 \mu\text{m}$ near the intercalated portion and up to $192,03 \pm 2,35 \mu\text{m}$ near the common excretory duct;

b) for the labial glands they are within $37,63 \pm 1,38 \mu\text{m}$ near the intercalated portion and up to $181,98 \pm 2,09 \mu\text{m}$ near the common excretory duct.

The smallest lumen (the inner diameter) is detected in all intercalated ducts of the minor salivary glands: the inner diameter of the palatine and labial glands is $18,11 \pm 1,09 \mu\text{m}$ and $18,98 \pm 1,04 \mu\text{m}$, respectively.

The cells of diffuse endocrine system have been found in the walls of the excretory ducts of the human palatine and labial glands, and the cells of muscle epithelium have been visualized only within the intercalated ducts and acini.

Blood capacitive microvessels of the investigated glands (gathering and collecting venules) provide the outflow of blood from the particles and take place spaciouly along the course of interparticle and common excretory ducts. Postcapillary venules and capillaries (metabolic

microvessels) are in close connection with bifurcations of the intraparticle ducts.

In the labial and palatine glands a sharp narrowing of the external diameter and lumen in the area of the intercalated ducts has been found. Ampoule-shaped extensions (the peculiar retention points) are located within the interparticle and common excretory ducts of the investigated glands.

The presence of myoepithelium in the walls of the excretory ducts, alternation of narrowings and extensions of the ducts' lumen, tortuosity the path, the "capillarity" of their external and internal diameter are the characteristics of the normal structure of the glands that can affect the hydrodynamic features of the liquid laminar flow on the excretory ducts.

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ADDRESS FOR CORRESPONDENCE

Volodymyr Hryn

Department of Human Anatomy,
Higher State Educational Establishment of Ukraine
«Ukrainian Medical Stomatological Academy»
Shevchenko 23 str., 36011 Poltava, Ukraine
tel. +380668126497 or +380967290632
e-mail. vogrin034@gmail.com

Received: 14.06.2017

Accepted: 10.10.2017