

# Features Of Distribution And Density Of Lymphoid Cells Of The Mucosa Of The Larynx As A Manifestation Of Local Immunity In Chronic Laryngitis (Analysis Of Sectional Material)

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## **Abstract**

**Introduction:** Local immunological manifestations of chronic hyperplastic laryngitis were investigated on the basis of quantitative determination of epithelial-stromal interactions in the mucous membrane of the larynx of subjects having no respiratory problems. Autopsy examination showed that lymphoid cells were nonuniformly distributed in the laryngeal mucous membrane the pattern of distribution depending on the type of epithelium and its integrity, state of the basal membrane, stroma collagen content, and vascularization. The distribution can be viewed as a characteristic of the adaptive potential of the laryngeal mucosa.

**Methods:** The object of the study was 21 larynx (16 male, 5 female) of people aged 20-65 who died suddenly from causes not associated with the pathology of the respiratory system (acute heart failure, car accident, etc.). The larynx was fixed in 10% neutral formalin, dissected in the longitudinal direction; for histological examination, preparations were left that did not contain macroscopic signs of pathology. The material, after additional fixation in formalin and embedded in paraffin, was stained with hematoxylin and eosin according to Van Gieson, and a PIC reaction was performed. Statistical processing of the results was carried out using the Student's t-test.

**Results:** The presence of small lymphocytes in the epithelial layer, and in the stroma under the epithelium of immunocompetent cells at different stages of maturation may indicate that a full-fledged immunological reaction is possible in the laryngeal mucosa.

*Secondly, confirmation of the regular nature of the distribution of lymphoid elements in the mucous membrane of the larynx is an increase in their number in areas of a thinner epithelial layer, in places of microdamage of the epithelium, as well as in the laryngeal ventricle, where, due to anatomical features, prolonged contact of exogenous antigens with the mucous membrane occurs. In addition, evidence of the role of lymphocytes in the processes of morphofunctional organization of the epithelium is the fact of an increase in the number of MELs at the border of different types of epithelium, for example, multilayer squamous non-keratinizing and "transitional" epithelium. Consequently, the mucous membrane of the larynx reacts to exogenous stimulation as a single structure, the constituent elements of which - the epithelium and the stroma - are in close interaction aimed at optimal adaptation to the stimulus.*

*Third, a manifestation of the non-randomness of the distribution of immunocompetent cells in the mucous membrane of the larynx is the absence of large differences in morphometric data in individuals. The obtained data can serve as a basis for the analysis of immunomorphometric features in CHF.*

*Conclusions: 1. The epithelium of the mucous membrane of the larynx of people who did not suffer from respiratory diseases is prone to metaplasia, accompanied by increased infiltration of the layer by lymphocytes.*

*2. Interepithelial lymphoid cells are represented by small lymphocytes located in the basal layer of the epithelium; the density of their distribution in the zones of the multilayer epithelium is higher than in the stratified squamous epithelium.*

*3. The proper lamina of the mucous membrane contains immunocompetent cells at various stages of differentiation - from blast forms to plasma cells; stromal infiltration is greatest in the laryngeal ventricle, somewhat smaller in the vestibular fold and least in the vocal fold.*

*4. The epithelial-stromal relationship can probably be regarded as a manifestation of the adaptive properties of the laryngeal mucosa.*

*Keywords: chronic hyperplastic laryngitis, lymphoid cells, intact larynx, immune system*

## **1. INTRODUCTION**

In patients with chronic hyperplastic laryngitis (CHF), an imbalance of the immune system with a predominance of suppression mechanisms was revealed, the pathogenetic role of these disorders was investigated. At the same time, the state of the immune system in patients with CHF, assessed by a combination of a number of immunological parameters of peripheral blood, is only one side of the problem of the interaction of the chronic inflammatory process in the larynx and the mechanisms of its control. The other side, and no less important, is local immunological manifestations in the pathological focus. Both aspects are interrelated and reflect different hierarchical levels of the organization of the immune system. Immunological processes at the local level cover two circles of phenomena; 1) protection of the internal environment of the body from foreign genetic information, 2) regulation of plastic processes, stimulation or restriction of growth, and differentiation of tissue elements [1,2]. It is logical to assume that the systems of local immunity of the respiratory tract (primarily those migrating to the laryngeal mucosa and associated lymphoid cells) can undergo changes in conditions of long-term productive inflammation of the mucous membrane and, in turn, affect this process.

This series of works is devoted to the study of local immunological manifestations in CHF. Since the literature data on the distribution of lymphoid tissue in the laryngeal mucosa [3,4] do not affect the morphometric aspect of the issue, the purpose of the first stage of the study was to study the quantitative patterns of epithelial-stromal relationships in the laryngeal mucosa of people who did not suffer from respiratory diseases ("intact larynx").

## 2. MATERIALS AND RESEARCH METHODS

### 2.1 Study Center

The prospective, observational, study was carried out at the Samarkand medical institute in Pathological Anatomy departments, from November 2019 to May 2020.

### 2.2. Participants

The object of the study was 21 larynx (16 male, 5 female) of people aged 20-65 who died suddenly from causes not associated with the pathology of the respiratory system (acute heart failure, car accident, etc.). The larynx was fixed in 10% neutral formalin, dissected in the longitudinal direction; for histological examination, preparations were left that did not contain macroscopic signs of pathology. From symmetrical sections of both halves of the larynx, histotopographic sections were made, including the mucous membrane of the vestibular and vocal folds, and the laryngeal ventricle. The material, after additional fixation in formalin and embedded in paraffin, was stained with hematoxylin and eosin according to Van Gieson, and a PIC reaction was performed. Areas of increased keratinization were revealed by the main brown color (13), mononuclear cells - by thionine (6). In the mucous membrane of the vestibular and vocal folds and the laryngeal ventricle, the type of integumentary epithelium, the thickness and degree of differentiation of the epithelial layer, and the state of the basement membrane were determined. Morphometric studies were carried out using an eyepiece grid 8 (ob. 40, ca. 7). Determined the number of interepithelial lymphocytes (IL) per 100 epithelial cells of the basal layer, the density of distribution of lymphoid cells (migrants) in the lamina propria of the mucous membrane per conditional unit of field area; the last value is 0.1 mm (squared). Statistical processing of the results was carried out using the Student's t-test.

## 3. RESULTS AND DISCUSSION

The qualitative and morphometric characteristics of epithelial-stromal relationships did not depend on the age and sex of the subjects and were mainly determined by histotopographic features. In different parts of the mucous membrane of the larynx, the integumentary epithelium has a different structure. The most diverse is the vestibular fold. In all preparations in this part of the larynx, there were sections of bilayer epithelium with well-defined goblet cells, a layer of fat cells. The structure of such areas resembled the structure of the transitional epithelium of the urinary tract. In 4 cases "transitional" epithelium was found on the entire surface of the fold, in 10 cases a combination of "transitional" and multi-row ciliated epithelium was noted. In this case, as a rule, the multi-row epithelium was found on the vestibular surface of the fold, and the "transitional" one - on the ventricular. In these cases, along the length of the vestibular fold, a "mosaic" alternation of various types of the epithelium was found. In all parts of the fold, areas of stratified squamous non-keratinizing epithelium were revealed; the layer was clearly differentiated into divisions - germ and functional. In the latter, in some cases, small zones of parakeratosis were determined. The

basement membrane in the vestibular fold was flat in all preparations. The epithelium very rarely (in 2 cases) formed small areas of acanthosis.

IL were located only in the basal layer of the epithelium. Their penetration into the overlying layers of the functional department took place only in 2 observations, while single ILs were detected in the layer of spiny cells. Round cell epithelial infiltrate is represented by small lymphocytes. We did not find blast forms, plasma cells, or macrophages among IL in any preparation. The density of the lymphocytic infiltrate was clearly dependent on the type of epithelium. From the table, it follows that in the areas of the stratified squamous non-keratinizing epithelium, the number of ILs is significantly lower than in the zones of multilayer and "transitional" epithelium ( $p < 0.001$ ).

The proper lamina of the mucous membrane of the vestibular fold is represented by a loose stroma with a slightly pronounced collagenization, dilated blood and lymphatic vessels. The cellular composition of the stroma is represented mainly by fibroblasts and fibrocytes. On the surface of the fold, the excretory ducts of the mucous glands, lined with multi-row epithelium, open. No cases of epidermisation of the ducts were found. Immunocompetent cells of the stroma are represented by small lymphocytes, lymphoblasts, macrophages, and plasmocytes. The density of the stromal infiltrate in the areas under the stratified squamous non-keratinizing epithelium was significantly less than the density of immunocompetent cells under the areas of the multilayered and "transitional" epithelium. It is significant that an increase in the number of lymphoid elements also took place near the zones of microdamage to the epithelium.

Epithelial-stromal relationships in the mucous membrane of the laryngeal ventricle also obeyed patterns. In all cases, this section of the larynx had a multi-row epithelium with well-defined goblet cells, a clearly traced basement membrane. In 9 cases, the multi-row epithelium was combined with the "transitional" zones (on the vestibular surface of the ventricle and in its deep parts). In 2 persons, areas of stratified squamous non-keratinizing epithelium were found; in these cases, the "transitional" epithelium was located between the zones of multilayer and stratified squamous non-keratinizing epithelium. The latter in the laryngeal ventricle also well-differentiated into the germ and functional sections had the same thickness and regularities of dystrophic changes as the multilayer squamous non-keratinizing epithelium of the vestibular fold. The number of ILs in the multi-row epithelium of the ventricle significantly exceeded the corresponding indicator in the vestibular fold ( $p < 0.005$ ). The number of ILs in the transitional epithelium was also quite high. The cellular composition of the lymphocytic infiltrate of the epithelium and stroma of the laryngeal ventricle was the same as in the vestibular fold. In a loose, well-vascularized stroma, accumulations of lymphoid tissue in the form of follicles with distinct germinal centers were found. The density of the lymphoid infiltrates significantly exceeded that in the vestibular fold ( $p < 0.001$ ).

The vocal fold epithelium was characterized by less structural diversity. In all preparations, the fold was covered with multilayer squamous epithelium (both non-keratinizing and keratinizing), which was combined with "transitional" areas only in 4 cases. Significantly more often than in other parts of the larynx, acanthosis was encountered, parakeratosis and keratinization of the surface layers of the layer were more pronounced. ILs in the vocal fold was found as single small lymphocytes. The stroma is represented by dense collagen fibers, poor in connective tissue cell elements (only fibrocytes), in some cases stromal hyalinosis took place. The density of the distribution of lymphocytes in the

epithelium and stroma of the vocal fold is significantly less than in other parts of the larynx ( $p < 0.005$ ).

Thus, the epithelial-stromal relationship in the mucous membrane of the larynx of people who did not suffer from chronic respiratory diseases is characterized by certain patterns. First, the epithelial layer in each section of the larynx is represented by a varied combination of different types of epithelium. This fact suggests that the laryngeal epithelium is subject to the process of metaplasia [5]. This is probably due to the constant exposure to the mucous membrane of the larynx of unfavorable exogenous factors - microbial antigens, household and food allergens, industrial hazards, which causes the restructuring of the epithelial layer towards the formation of the most stable type of epithelium - multilayer flat [6]. Such an active restructuring of the epithelial layer is of a compensatory and adaptive nature and, therefore, needs to be regulated by the functional systems of the body that control the processes of tissue growth and differentiation, in particular, the immunogenetic system. This regulation can be carried out by lymphoid cells migrating into the epithelium and the lamina propria. It has been shown that IL is small (mature) T-lymphocytes capable of performing a dual function; antigenic recognition and transmission of "regenerative stimulus" to epithelial cells [1, 3].

The presence of small lymphocytes in the epithelial layer, and in the stroma under the epithelium of immunocompetent cells at different stages of maturation may indicate that a full-fledged immunological reaction is possible in the laryngeal mucosa.

Secondly, confirmation of the regular nature of the distribution of lymphoid elements in the mucous membrane of the larynx is an increase in their number in areas of a thinner epithelial layer, in places of microdamage of the epithelium, as well as in the laryngeal ventricle, where, due to anatomical features, prolonged contact of exogenous antigens with the mucous membrane occurs. In addition, evidence of the role of lymphocytes in the processes of morphofunctional organization of the epithelium is the fact of an increase in the number of MELs at the border of different types of epithelium, for example, multilayer squamous non-keratinizing and "transitional" epithelium. Consequently, the mucous membrane of the larynx reacts to exogenous stimulation as a single structure, the constituent elements of which - the epithelium and the stroma - are in close interaction aimed at optimal adaptation to the stimulus.

Third, a manifestation of the non-randomness of the distribution of immunocompetent cells in the mucous membrane of the larynx is the absence of large differences in morphometric data in individuals. The obtained data can serve as a basis for the analysis of immunomorphometric features in CHF.

#### **4. CONCLUSIONS**

1. The epithelium of the mucous membrane of the larynx of people who did not suffer from respiratory diseases is prone to metaplasia, accompanied by increased infiltration of the layer by lymphocytes.
2. Interepithelial lymphoid cells are represented by small lymphocytes located in the basal layer of the epithelium; the density of their distribution in the zones of the multilayer epithelium is higher than in the stratified squamous epithelium.

3. The proper lamina of the mucous membrane contains immunocompetent cells at various stages of differentiation - from blast forms to plasma cells; stromal infiltration is greatest in the laryngeal ventricle, somewhat smaller in the vestibular fold and least in the vocal fold.

4. The epithelial-stromal relationship can probably be regarded as a manifestation of the adaptive properties of the laryngeal mucosa.

1. **Financial disclosure:** The authors have no funding to disclose.
2. **Conflict of interest:** The authors declare that they have no conflicts of interest.
3. **Author contribution:** We confirm that all of the authors participated in the preparation of the manuscript.

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