

ORIGINAL ARTICLE

FEATURES OF THE CONNECTIVE TISSUE COMPONENT OF THE PALATINE TONSILS IN PATIENTS WITH RECURRENT TONSILLITIS

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ABSTRACT

The aim: To explore the morphological changes of palatine tonsil at the levels of the epithelial layer and connective tissue; to determine the relative area of the connective tissue component in the tonsillar tissue (fibrosis) in patients with recurrent tonsillitis compared to the control.

Materials and methods: This study presents a morphological assessment of the palatine tonsils of 10 people. Tonsils' material with surrounding tissue was fixed in 10% formalin solution. The samples were dehydrated in increasing ethanol concentrations, cleared in xylol, impregnated with paraffin. Microscopy was then performed with samples stained beforehand.

Results: In the samples of patients with recurrent tonsillitis pericapsular sclerosis was noted, along with thickening of interlobular septa and pronounced subepithelial fibrosis. A ratio of the dense connective tissue surface area to the total surface area of tonsil tissue was determined. The control group showed a statistically significant decrease in the degree of sclerosis of the tonsil stroma.

Conclusions: Multiple changes were found in the tonsils of patients with recurrent tonsillitis at the level of the epithelial layer that manifested in structural alterations. Significant and irreversible changes were also observed in the connective stroma of the tonsil - pericapsular sclerosis, thickening of interlobular septa, and pronounced subepithelial fibrosis. A statistically significant increase in the relative surface area of the connective tissue component of the tonsil (fibrosis) by a factor of 1,26 was noted in patients with recurrent tonsillitis compared to the results of the control group of patients.

KEY WORDS: respiratory tract, tonsillitis, tonsils, morphology

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INTRODUCTION

Palatine tonsils belong to peripheral hematopoietic and defensive immunological organs and represent a parenchymal organ. Their parenchyma is made of myeloid and lymphoid tissues, while stroma is divided into dense and soft. Dense stroma is represented by connective tissue, while soft stroma - by reticular tissue. The connective tissue of the tonsils is dominated by loose connective tissue which is a combination of various cells, a small number of multidirectional fibres (mostly collagen), and a relatively large volume of intercellular substance [1]. Reticular tissue is no less significant, being a specialized connective tissue. It ensures a stromal foundation for lymphoid elements. Being a part of the loose connective tissue, collagen fibres form a connective capsule around the tonsil where the connective tissue strands branch along the crypts. Hypothetically the following sections of the collagen stroma can be defined: peritonsillar, subepithelial, and septal.

Palatine tonsils have a large surface contact area with the external environment, they come in contact with a large number of antigen materials originating in the air and food, and take an active part in reactions of the cell and antibody-mediated immunity. They perform their functions on the verge of normal and pathological, ensuring a high level of regional and systemic immunity. Palatine tonsils induce both antibody-mediated and cell-mediated immune responses. This can be explained by the fact that the contact with antigens in the lymphoid tissue leads to an immune reaction, and therefore - to inflammation, where lymphocytes, macrophages, and granulocytes are involved. It is considered that palatine tonsils exist in the state of so-called "physiological" inflammation at rest [2]. Thus the term "tonsillitis" is relevant only when some clinical symptoms are present, such as sore throat, patches on the tonsils, absence of cough, or increased body temperature. There is data that at the histological level a lymphocytic inflammatory reaction is observed in cases of viral inflammation, and granulocytic - in bacterial inflammation [2-4].

Presently the term "chronic tonsillitis" is not going to be used in line with new evidence-based definitions [2,5,6]. It is mentioned that the term "chronic tonsillitis" is as deceptive as the fact that the tonsils are not in a physiological inflammatory reaction. In recurrent inflammatory reactions on top of physiological one in the tonsils the process of fibrosis takes place along with the spread of inflammatory process to the surrounding tissues, particularly the peritonsillar tissue which clinically manifests as fixation of the tonsil in its bed or so-called "discoursity" of the tonsil [2,7,8].

The study of the morphological changes in the tonsil and peritonsillar space in patients with recurrent tonsillitis, particularly their connective component, could explain the development of irreversible fibrous alterations in their structure.

THE AIM

The aim of our study was to explore the morphological changes of the palatine tonsil at the level of the epithelial layer and connective tissue, to determine the relative surface area of the connective component of the tonsil tissue (fibrosis) in patients with recurrent tonsillitis compared to the control group.

MATERIALS AND METHODS

The study group included 10 people, their ages ranging from 21 to 40 years old, average age 30 years old. Seven of them were the experimental group of patients who had recurrent tonsillitis and had tonsillectomy performed, while the rest three were the control group that included intact palatine tonsils of tragically deceased patients.

Material of the tonsils along with the surrounding tissue was fixed in 10% formalin solution (pH 7,4) for no less than 24 hours at room temperature [9]. The samples were then dehydrated in the increasing concentrations of ethanol, cleared in xylol, and impregnated with paraffin. Afterward, the paraffin impregnated tissue was poured into paraffin blocks. Serial sections with a thickness of no more than 4 μm were made of the blocks using Thermo HM 355S microtome (Thermo Scientific, Germany). Sections of each tissue sample were used for general histological tissue staining using Goldner's Masson trichrome. Prior to staining the sections were deparaffinized in xylol, rehydrated in decreasing (100, 95, 70%) ethanol concentrations, and were placed in Bouin's solution (10% formalin in saturated picric acid solution) for 1 hour for additional fixation to improve nuclear staining. Following the rinsing in distilled water the samples had been stained in Weigert's iron hematoxylin (components: hematoxylin and ferrous chloride - both produced by Sigma, Germany) for 10 minutes and were then rinsed in the running water for 20 minutes. The samples were stained in acid fuchsin solution (Sigma, Germany) and Xylidine ponceau (Sigma, Germany) for 10 minutes, and processed in 2% phosphomolybdic acid solution (Sigma, Germany) for 10 minutes. Afterward, the samples were immersed in the solution of Light green SF yellowish (Sigma, Germany) for 10 minutes. They were then dehydrated in increasing ethanol concentrations, differentiated in xylol, and mounted under cover slips.

Microscopic examination of the histological sections was performed using an Axio Imager 2 (Zeiss, Germany) microscope at × 200 and × 400 magnifications.

Taking into account a small number of studied samples (n = 14 for the study group and n = 6 for the comparison group) the nonparametric Mann–Whitney U test (U) was utilized using Microsoft Excel 2010. The statistical result was considered significant when $p \leq 0,05$.

RESULTS

Histological examination of the tissues in the control group showed that the mucous membrane of the palatine tonsil was covered with mature non keratinized stratified squamous epithelium, however, over its transition to the crypt it gradually lost its maturity and became thinner. Multiple microvessels were found directly underneath the epithelium of the crypts (fig. 1). Postcapillary venules with high endothelium also known as "high endothelial venules" are specific microcirculatory structures, present only in the peripheral lymphoid organs, including palatine tonsils. They were easily identified among the other vessels of the microcirculation in the researched material due to the presence of a cuboid endothelium with a noticeable nucleus. These blood vessels reached up to 50 μm in diameter, here and there their nuclei significantly bulged in the vessel lumen. Such postcapillary veins in particular serve as a recirculation pathway for T and B cells.

Both areas of non keratinized and keratinized epithelium were noted in the superficial epithelium of the tonsil samples in the experimental group (fig. 2). Microscopically erosions of the superficial epithelium were discovered as well, especially at the level of the crypts, although such alterations were not common. In addition, moderate and in some places pronounced leukocytic infiltration of the epithelium and adjacent connective tissue was found. The cytoplasm of epitheliocytes was vacuolized, the nuclei were hyperchromic, apoptotic phenomena were occasionally observed. The basal membrane had a convoluted form across significant areas of the epithelium. In some areas of the epithelium lesions of desquamation of the epithelial layer were identified. Intense leukocytic infiltration was found in the epithelial tissue of the crypts and superficial epithelium, expressed in the epithelium of the crypts the most. Among the leukocytes lymphocytes were prevalent, but plasmocytes, individual macrophages, and tissue basophils were observed as well. Microcirculatory vessels were dilated, and in some cases, stasis of blood cells was noted. Here and there minor bleeding in subepithelial and subcapsular areas was observed which could be explained by additional injury in the course of tonsillectomy.

It should be mentioned that connective tissue of the tonsils in the control group was represented by soft reticular fibres that served as a stromal foundation for lymphoid elements, and by well-developed collagen strands that spread circularly around the tissue of the tonsil, forming a connective capsule, and descended deep within lymphoid elements making up their trabecular structures. Trabecular strands in turn followed the direction of descending

Table I. Morphometric analysis results

Groups	Specific surface area of the connective tissue ±SD (%)
Recurrent tonsillitis (n=14)	29,72±9,73*
Control (n=6)	23,6±4,1

* – $p<0,05$.

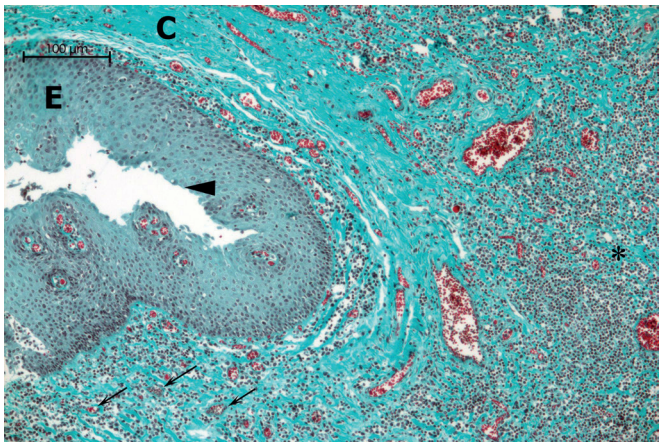

Fig. 1. Tissue of an intact tonsil with a fragment of a crypt (arrowhead) covered with stratified squamous epithelium (E) and surrounded by connective tissue stroma (C). Parafollicular lymphoid tissue with multiple microvessels - on the left (*). In the subepithelial area postcapillary venules with high epithelium are noted (arrows). ×100. Masson-Goldner trichrome staining

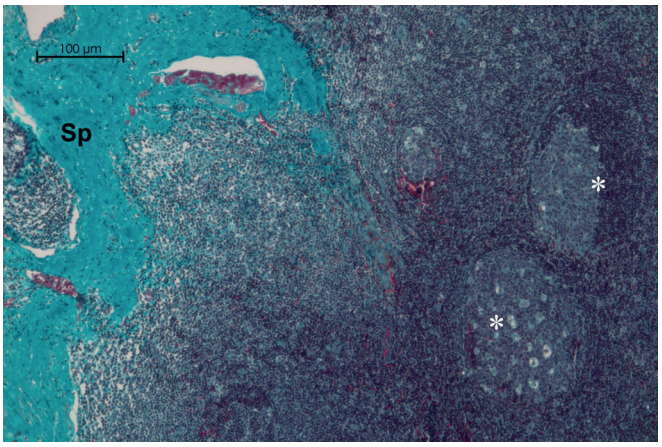

Fig. 2. Female, 33 years old, recurrent tonsillitis. Parafollicular fibrosis (F). Hyperplasia of lymphoid follicles (*), in the lacune - leukocytic infiltration of epithelium, hyperkeratosis (arrowhead). ×100. Masson-Goldner trichrome staining

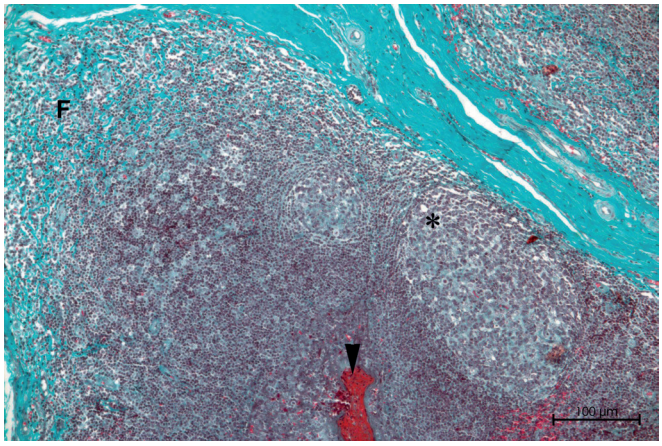

Fig. 3. Female, 30 years old, recurrent tonsillitis. Significant thickening of the connective tissue septa (Sp), follicular hyperplasia(*). ×100. Masson-Goldner trichrome staining

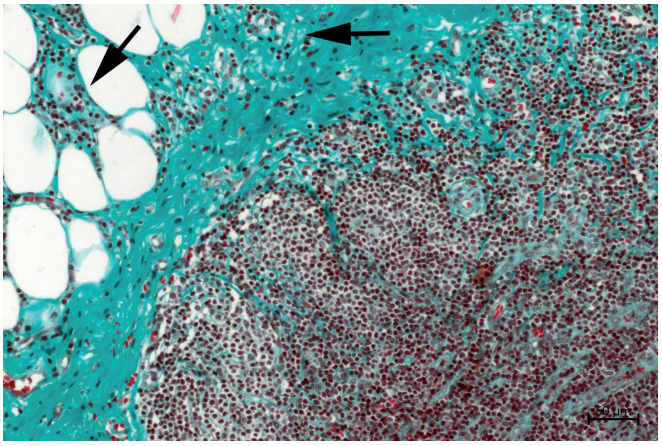

Fig. 4. Female, 33 years old, recurrent tonsillitis. Inflammatory lymphohistiocytic infiltrates are immersed in fibrous and adipose tissue (arrows). ×200. Masson-Goldner trichrome staining

crypts. The connective capsule extended into connective elements of the neck and was surrounded by striated muscles and salivary glands. Thus the following sections of tonsillar collagen stroma could be defined: peritonsillar, subepithelial, and septal (trabecular). Respectively, when some parts of the stroma were thickened, pericapsular sclerosis, thickening of interlobular septa, and marked subepithelial fibrosis were observed. Naturally, all three of these patterns were present in the samples of patients in the experimental group, compared to the control group (fig. 2, 3). Examination of the peritonsillar connective tissue of tonsils in the experimental group allows for a description of the connective stroma as collagen fibres that were often assembled in thick bundles (fig. 2) These patients were also found to have inflammatory infiltrates located separately from lymphoid tissue and immersed in fibrous tissue (fig. 4). Diffuse infiltration of adjacent muscle and adipose tissue with the chronic inflammatory infiltrate mentioned above was observed as well.

Having performed the aforementioned research the following histomorphometric parameter was measured: ratio of the surface area of the dense tissue to the total surface area of the tonsillar tissue as a percentage. The proportion of tonsillar tissue and its capsule that corresponded to the existing dense connective tissue, and which was stained using the trichromatic staining method, to the total tonsillar tissue volume that consisted of lymphoid tissue, stratified squamous epithelium, and connective tissue capsule was measured.

The extent of the collagen stroma varied significantly among the patients of the experimental group. The maximum value of the connective tissue-specific surface area was 47% of the total surface area of the section. It should also be mentioned separately that in the control group the minimum average value of connective tissue component was 19,8% of the total surface area of the section. When comparing the rates of fibrosis between the experimental group and the control (29,72±9,73% and 23,6±4,1%, re-

spectively), the latter demonstrated a significantly lower degree of sclerosis of the tonsillar stroma ($p < 0.05$).

Morphometric analysis results are presented in Table I.

DISCUSSION

Analysis of the histological examination of the palatine tonsils tissue revealed that the mucous membrane of palatine tonsils in the control group was covered with mature non keratinized stratified squamous epithelium while the tonsil samples of the patients in the experimental group showed areas of both non keratinized and keratinized epithelium. Moreover, erosions of the superficial epithelium were noted, especially at the level of the crypts, and moderate, in some places pronounced leukocytic infiltration of the epithelium and underlying connective tissue. In some parts of epithelium the lesions of desquamation of the epithelial layer were identified which were not observed in the control group. Microcirculatory vessels were dilated, and stasis of blood cells was noted in the capillaries, while these alterations were not seen in the tonsils of the control group.

Comparing the structure of connective tissue of the two studied groups, a connective tissue in the control group was represented by soft reticular fibres that combined into trabecular structures thus forming the tonsil stroma of normal thickness. The following sections of tonsillar collagen stroma were defined: peritonsillar, subepithelial, and septal (trabecular). Instead, three important features were found in the experimental group. Particularly, these include pericapsular sclerosis, thickening of interlobular septa, and pronounced subepithelial fibrosis. These samples were also found to have inflammatory infiltrates that were located separately from the lymphoid tissue and immersed in fibrous tissue. Diffuse infiltration of the adjacent muscle and adipose tissue with chronic inflammatory infiltrate was observed.

Having measured such a histomorphometric parameter as the ratio of the surface area of dense connective tissue to the total surface area of tonsil tissue, we were able to attain the results that demonstrated a significant difference in the surface area of connective tissue between the patients of the experimental group and the control, which was about 29.7% and 23.6% respectively (statistically significant difference $p < 0.05$). These results match the contemporary research that observed the tendency of an increasing fraction of connective tissue in recurrent tonsillitis [10]. Thickening of the parenchyma and scarring of the connective tissue due to chronic inflammation is one of the major changes in patients with recurrent tonsillitis [11]. Moreover, fibrosis of tonsillar tissue as a morphological manifestation of recurrent tonsillitis can lead to damage to the barrier function of this organ. As a result, dysfunction of local immunity may develop that might later lead to the perpetuation of infections [12]. At the same time, immunologic dysfunction is explained by the presence of chronic inflammation that promotes migration and proliferation of fibroblasts that as a result of their activity lead to an increase in the amount of stromal collagen, and thus occurs substitution of immunologically active elements with fibrous tissue [10,13].

CONCLUSIONS

1. In patients with recurrent tonsillitis, multiple changes were found at the level of epithelial level in the tonsils, which reflects in the structural change of the latter (emergence of keratinized epithelium, erosions, leukocytic infiltration, local desquamation).
2. Significant and irreversible changes were noted in the connective tissue stroma of the tonsil - pericapsular sclerosis thickened interlobular septa and pronounced subepithelial fibrosis.
3. Statistically significant increase by a factor of 1.26 in the surface area of connective tissue component of the tonsil tissue (fibrosis) in patients with recurrent tonsillitis compared to the results of the control group of patients ($p<0.05$), which is explained by a persistent inflammatory process that could be caused by repeated antigen stimulation.

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Conflict of interest:
The Authors declare no conflict of interest.

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