Histopathological and immunohistochemical aspects in chronic suppurative maxillary rhinosinusitis

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Abstract

Chronic rhinosinusitis is a multifactorial disease with pathophysiological mechanisms, which remain unclear, and with a high prevalence worldwide. They generate social problems due to the high number of days of leave and relatively elevated medical expenses. The histopathological and immunohistochemical study that we conducted revealed many lesional aspects of the epithelium of the sinus mucosa, which ranged from hypertrophy, hyperplasia and metaplasia, to erosion and discontinuities. In the chorion of the sinus mucosa there was an inflammatory infiltrate composed mainly of lymphocytes, plasma cells and macrophages, and also a highly developed vascular network. Among immune cells, T-cells appeared to be more numerous than B-lymphocytes and macrophages. We believe that microscopic changes are due mainly to microscopic organisms that make up the biofilm of the sinus cavity, whose virulence has been more or less influenced by exogenous or endogenous factors.

Keywords: rhinosinusitis, chronic inflammatory infiltrate, angiogenesis, macrophages.

Introduction

Chronic sinusitis is one of the most common diseases, with a considerable impact on the quality of life of patients suffering from this condition. Patients usually present with various symptoms such as facial pain, long lasting nasal obstruction, rhinorrhea and/or olfactory disorders. In the U.S., the prevalence of this disease is estimated at 14% of the population. The economic consequences are particularly serious because they represent about 73 million days of leave, with annual health costs estimated at 2.4 billion dollars [1].

The term “sinusitis” was replaced with “rhinosinusitis” because the nasal mucosa is almost entirely involved in the pathologic process [2]. The etiology of these diseases is still controversial. Most researchers believe that the etiopathogenesis of rhinosinusitis is plurfactorial. The normal morpho-functional aspect of the sinus mucosa depends on three factors: ostial permeability, ciliary function, and the consistency of sinus secretions [3, 4]. Any change in these factors can irritate the mucosa of the paranasal sinuses and, by disturbing local homeostasis, can cause inflammation, swelling, mucociliary dysfunction, reduced airflow up to complete obstruction and bacterial proliferation [5]. The exact role of infection in the pathogenesis of chronic rhinosinusitis is still subject of debate. The fact is that the paranasal sinuses, normally considered sterile, in patients with chronic rhinosinusitis contain numerous infectious agents: bacteria, especially Staphylococcus aureus, anaerobic bacteria, or different strains of viruses [6, 7]. It is considered that infections induce inflammation of the sinus mucosa that can extend to the jawbone causing osteitis, further complicating the symptoms and evolution of the disease.

In our study, we aimed to assess the histopathological and immunohistochemical changes in patients with chronic suppurative rhinosinusitis that required surgery.

Materials and Methods

The biological material studied consisted of fragments of sinus mucosa collected from a total of 43 patients with chronic suppurative maxillary sinusitis, aged between 11 and 78 years, admitted in the ENT Department, County Hospital, Târgu Jiu, who underwent radical maxillo-ethmoidal therapy with total removal of the pathological sinus mucosa. Immediately after sampling, the biological material was placed in 4%
For single immunohistochemistry, after antigen retrieval, sections were allowed to cool down to room temperature and were incubated for 30 minutes in a 1% hydrogen peroxide solution. The sections were next washed in PBS, followed by a blocking step of 30 minutes in 1% skim milk. Next, the slides were incubated with the primary antibodies overnight at 4°C, and the next day, the signal was amplified for 30 minutes using a peroxidase polymer-based secondary detection system (EnVision, Dako). Finally, the signal was detected with 3,3′-diaminobenzidine (DAB) (Dako) and the slides were coverslipped in DPX (Fluka) after Hematoxylin staining.

The sections were imaged with a Nikon Eclipse 55i microscope (Nikon, Aipidrag, Romania) equipped with a 5-megapixel cooled CCD camera. Images were captured and archived using a Nikon frame grabber and the Image ProPlus 7 AMS software (Media Cybernetics Inc., Buckinghamshire, UK).

### Results

The microscopic study of sinus mucosa fragments allowed us to note significant changes in both the lining epithelium and the underlying connective tissue. Epithelial changes varied widely between patients. The most common lesions were epithelial erosions. These extended both on the surface and in the depth, up to the basal cells or the connective-epithelial junction (Figure 1). Epithelial erosions sometimes exceeded the basement membrane placing in contact the external environment with the chorion of the sinus mucosa, opening the way for pathogens entering the internal environment. In these cases, epithelial erosions were accompanied by the presence of hemorrhagic infiltrates within the chorion or at the surface of the mucosa (Figure 2).

Other patients showed areas with epithelial hyperplasia, more common in the crypts of the sinus mucosa, areas where epithelial architecture was highly altered. Epithelial cells appeared smaller, with irregular arrangement within the thickness of the epithelium, with round hypochromatic nuclei, vacuolar cytoplasm and a few cilia or no cilia at all (Figure 3).

In the lining epithelium, rare areas of metaplasia were also identified. Metaplastic areas were of squamous type, where the covering ciliated columnar pseudostratified epithelium was gradually replaced with areas of stratified squamous epithelium, characteristic of the oral mucosa. Within these areas, in the depth of the epithelium, cubico-cylindrical type cells were identified in contact with the basement membrane, representing proliferative, regenerating cells. Cells in the superficial layers were polyhedral, with round central nuclei, nucleoli and acidophilic cytoplasm. In metaplastic areas, the connective-epithelial junction had an irregular appearance, with a tendency to curl, characteristic of the Malpighian epithelium (Figure 4). We believe that the transformation of the covering epithelium from columnar pseudostratified to a stratified squamous one is slow, time consuming, and involves a first phase of progressive reduction until disappearance of the vibratile cilia, fewer mucus-secreting cells and phenotypic changes of the proliferative cells in the basal layer of epithelium.

The subepithelial connective tissue showed a rich chronic inflammatory infiltrate composed mainly of mononuclear round cells, i.e., plasma cells, lymphocytes and macrophages. The immune system cells had a completely heterogeneous disposition, which varied from one patient to another and even from one area to another of the same histological preparations, which indicates the presence of various antigens, probably formed from bacteria, viruses or fungi which are diffusely spread either on the covering epithelium or the subepithelial connective tissue where epithelial erosions occurred, which caused a variable immune reaction. Also, a highly developed chorionic vascular system was revealed, with many congested vessels, consisting mainly of blood capillaries, arterioles and venules (Figure 5). In some samples, we found angiogenic vessels with reduced lumena, lined by turgid endothelial cells, which explains the increased number of chorionic blood vessels (Figure 6).

By immunohistochemistry, studies have sought to quantify the cellular immune response. Thus, we used the anti-CD20 antibody for B-lymphocytes, the anti-CD3 antibody for T-lymphocytes and the anti-CD68 antibody for the study of macrophages.

Within the chorion of the sinus mucosa B-lymphocytes had a completely heterogeneous distribution, being found both in the deep the superficial one. On some histological samples, B-lymphocyte number was very small, especially where the integrity of the covering epithelium was not altered. However, we identified an increased number of B-cells in abundant inflammatory infiltrates, located mainly...
around congested blood vessels or angiogenic ones (Figure 7).

The T-lymphocytes response in inflammatory infiltrate from the sinus mucosa was more intense than that of B-lymphocytes, but their arrangement was similar to that of B-lymphocytes, completely heterogeneous from sample to sample, even from one area to another within the same sample. Most T-lymphocytes were identified beneath the covering epithelium and around blood vessels, especially where the inflammatory infiltrate showed a tendency to organize into lymphoid follicles (Figures 8 and 9).

Macrophage cell reaction was less intense in chronic suppurative sinusitis compared to that of T-cells. They were more numerous immediately beneath the epithelium, i.e., at the site of penetration of foreign antigens. In the depth of the sinus mucosal chorion the macrophage response was reduced, which indicates that cell and tissue lysis are reduced in chronic sinusitis (Figure 10).

Figure 1 – Sinus mucosa with deep epithelial erosions reaching the basement membrane (HE stain, ×200).

Figure 2 – Deep epithelial erosion associated with micro-hemorrhages within the crypts (HE stain, ×100).

Figure 3 – Epithelial hyperplasia within a crypt of the sinus mucosa (HE stain, ×100).

Figure 4 – Squamous metaplasia (HE stain, ×200).

Figure 5 – Chorion with lympho-plasmocytic inflammatory infiltrate and vascular congestion (HE stain, ×400).

Figure 6 – Highly infiltrated chorion with lympho-plasmocytic cells and angiogenic capillaries (HE stain, ×200).
Discussion

Processes leading to chronic inflammation in chronic rhinosinusitis are complex and unclear. Most often, it is considered that pathogens are at the origin of sinus mucosal inflammation. Numerous studies have shown the presence of bacterial biofilms embedded in the glycocalyx on the surface of the covering epithelium [8]. They include bacterial species such as *Haemophilus*, *Staphylococcus* and *Pseudomonas*. There is growing evidence that bacterial biofilms may contribute to persistence, recurrence and severity of certain clinical forms of chronic rhinosinusitis [9, 10].

In addition to the infectious factor, many other endogenous or exogenous factors seem to be involved in the etiopathogenic mechanism of chronic sinusitis. One of the etiopathogenic factors involved in the development of chronic sinusitis is active smoking. Some researchers have shown that oral and/or nasal inhalation of cigarette smoke causes a significant reduction in mucociliary transport in vivo [11, 12]. Agius AM et al. (1995) showed that a toxic metabolite of nicotine called cotinine is able to significantly reduce the movement of cilia of epithelial cells in vitro [13]. In 2009, Cohen NA et al., working on epithelial cell cultures, have also reported that the particles in cigarette smoke reduce epithelial cilia movement. Smoking is also associated with profound changes in the mechanisms of mucus production [14]. Chronic exposure to tobacco smoke causes respiratory mucosal epithelium metaplasia with increased number and size of goblet cells and, consequently, increased mucous secretion in the upper respiratory tract [15, 16]. Other animal studies have shown that chronic or intermittent exposure to cigarette smoke causes morphological changes of respiratory epithelium, ranging from hyperplasia when exposure is intermittent, to loss of cilia and metaplasia with keratinization at higher concentrations of cigarette smoke, and also submucosal thickening by inflammation with round mononuclear cells [17].

In our study, we frequently found changes in covering epithelium covering, ranging from hyperplasia and metaplasia to deep epithelial erosions. We believe that these lesions are due primarily to mucosal pathogens that colonize the sinuses, represented by bacteria, viruses or fungi. We believe that exposure to adverse weather conditions (humidity, cold, etc.), smoking and pollution play an adjuvant role, which favors proper conditions for development of microbial pathogens. Inflammatory reaction is a non-specific, complex defensive reaction, developed in the body under conditions of exogenous aggression, which includes alterative as well as reactive, vasculo-exudative,
proliferative and repair phenomena. So, microscopically, the inflammatory reaction is characterized by the presence of vascular, leukocytic, tissue and metabolic changes.

We believe that the histological and immunohistochemical changes observed are the result of the inflammatory response as well as tissue remodeling following the inflammatory process. Other authors [18] also believe that tissue remodeling is present in all chronic rhinosinusitis and are expressed by basement membrane thickening, hyperplasia and hypertrophy of covering epithelium, glands and epithelial crypts, together with excessive deposition of modified extracelluar matrix. The exact mechanisms leading to the remodeling of sinus mucosa remains unknown, but one can speculate that repeated injury-repair cycles result in an excess of inflammatory reaction. It is also possible for the tissue remodeling process to contribute to severe forms of chronic rhinosinusitis with excessive mucus production, tissue edema with ostial obstruction and impaired mucociliary function.

Conclusions

In chronic suppured maxillary rhinosinusitis, the changes of the covering epithelium ranged from hypertrophy, hyperplasia and metaplasia, to erosion and discontinuities. The chorion of the sinus mucosa revealed the existence of an inflammatory infiltrate composed mainly of lymphocyte-like round mononuclear cells, plasma cells and macrophages. Of all immune cells, T-cells appeared to be more numerous than B-lymphocytes, plasma cells and macrophages. Of all immune cells, T-cells appeared to be more numerous than B-lymphocytes, plasma cells and macrophages. The chorion also revealed a highly developed vascular system, consisting mainly of capillaries, arterioles and venules, mostly congested, dilated and anastomosed with each other.

Acknowledgments

This paper is supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64109.

References


