ORIGINAL ARTICLE

CONTENTS

Cytomorphological characteristics of the Nasopharyngeal zone mucosa in children with Covid-19

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ABSTRACT

Aim: To investigate cytomorphological presentations of the mucous membrane damage of the nasopharyngeal zone in Coronavirus disease in children.

Materials and Methods: A epithelium scraping from the surface of the Pharyngeal tonsils was performed with the following double fixation: the samples were fixed in a 2% solution of glutaraldehyde (25% Glutaraldehyde E. M. Grade Polyscieces Inc.) in 0.1M cacodylate buffer (Cacodylic acid Sodium salt. Fluka) at pH 7.2 for 2–2.5 hours in the cold. Washing (2 times) in cacodylate buffer was performed. The next stage was additional fixation of the samples in a 1.5% solution of OsO4 (Osmium Tetroxide. SPI – CHEM USA) in 0.1 M sodium cacodylate solution at pH 7.2 for 2 hours in the cold with subsequent washing (2 times) in buffer. Sections are made on an UMTP-6M ultramicrotome using a diamond knife (DIATOM). Sections were viewed using a TEM-100 transmission electron microscope (Ukraine).

Results: Based on the electronic presentation, we can observe all the processes of inflammation and their consequences. We will consider the pathogenetic processes which were monitored in our studies. Inflammation consists of three main types of tissue disorders, which include: alteration, exudation, proliferation. Presentation of a section of the Palatine Tonsil epithelial cell is marked by increased focal hemorrhage and degenerative changes in the membrane of papillary structures, reactive-degenerative changes in the stroma and increased hemorrhages in the vessels, partial sclerosis and microcalcification of the Tonsil epithelial cell tissue, pronounced sclerotic changes in the epithelial cell and partial destruction of the cell membrane were also visualized, as a result of which the semiconductivity in the cell is disturbed.

Conclusions: Cytological changes in the studied material of Nasopharyngeal zone in the children with Coronavirus disease can be considered as nonspecific common violation. These changes were characterized by the presence of diffuse inflammatory polymorpho-cellular infiltration, impaired hemodynamics and diffuse desquamative, dystrophic, sclerotic changes in epithelial cells, which are consistent with numerous data of scientists.

KEY WORDS: Covid-19, changes in the mucous membrane of the nasopharyngeal zone, cytological examination, children

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INTRODUCTION

Mucosal tolerance is essential and provides a robust barrier and suppressive mechanism in response to respiration, food, and ingestion. Development during the neonatal period is essential for this function, when the presence of the epithelial barrier is vulnerable due to their poor development [1]. At the beginning of 2020 y., the discovery of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) marked the start of the COVID-19 pandemic [2]. The nasal cavity serves as a point of entry, and the elevated viral load observed in this area as the primary site of SARS-CoV-2 infection and subsequent immune response [3-5].

AIM

To investigate cytomorphological presentations of the mucous membrane damage of the nasopharyngeal zone in Coronavirus disease in children

MATERIALS AND METHODS

A epithelium scraping from the surface of the Pharyngeal tonsils was performed with the following double fixation: the samples were fixed in a 2% solution of glutaraldehyde (25% Glutaraldehyde E. M. Grade Polyscieces Inc.) in 0.1M cacodylate buffer (Cacodylic acid Sodium salt. Fluka) at pH 7.2 for 2–2.5 hours in the cold. Washing (2 times) in cacodylate buffer was performed. The next stage was additional fixation of the samples in a 1.5% solution of OsO4 (Osmium Tetroxide. SPI – CHEM USA) in 0.1 M sodium cacodylate solution at pH 7.2 for 2 hours in the cold with subsequent washing (2 times) in buffer. Sections are made on an UMTP-6M ultramicrotome using a diamond knife (DIATOM). Contrasting of sections is carried out in 1% uranyl acetate solution and in Reynolds lead contrast medium [6, 7]. Sections were viewed using a TEM-100 transmission electron microscope (Ukraine).

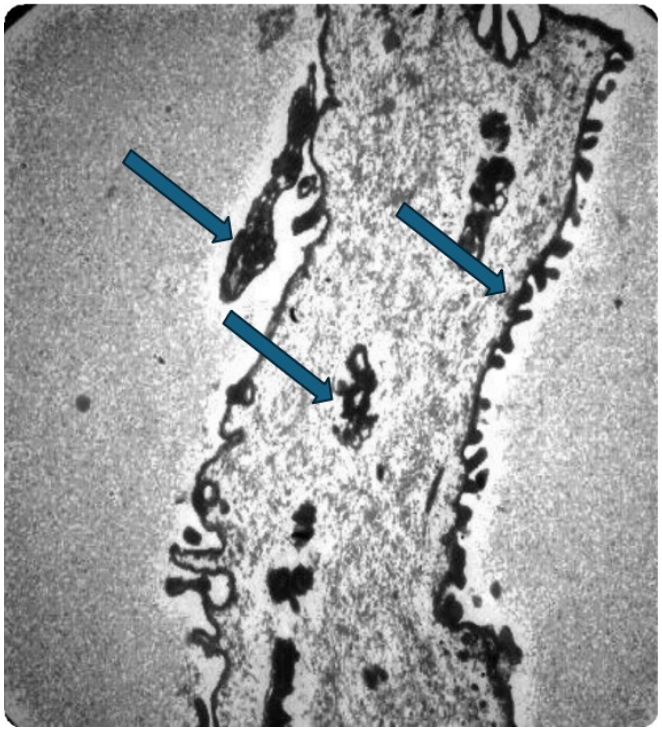

Fig. 1. Electronic photograph (magnification x10000).
Section of an Palatine Tonsil epithelial cell in which focal hemorrhage and degenerative changes in the membrane of papillary structures are observed
Picture taken by the authors

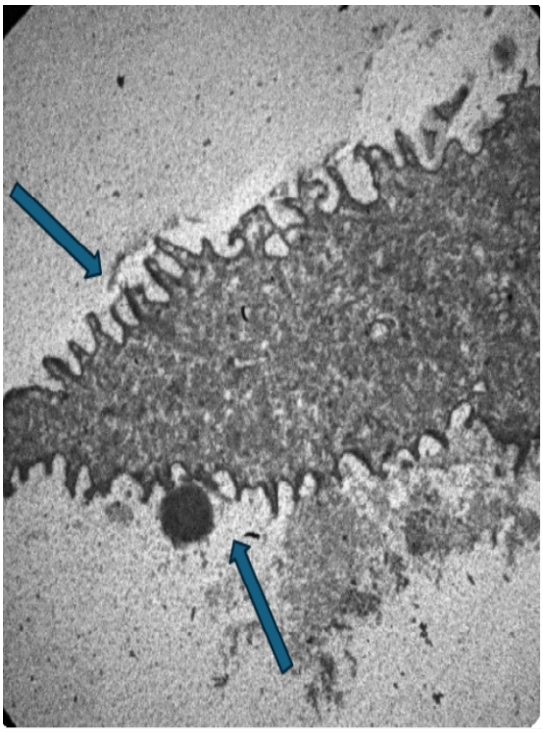

Fig. 2. Electronic photograph (magnification x10000).
Papillary structures (lacunae) located on the basement membrane during Phagocytosis (active phase of inflammation)
Picture taken by the authors

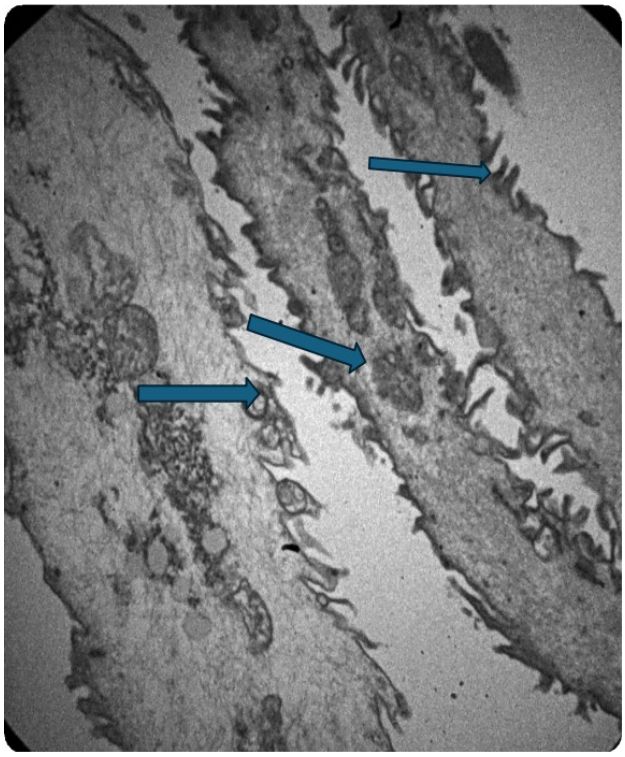

Fig. 3. Electronic photograph (magnification x10000).
Palatine Tonsil with reactive-degenerative changes in the stroma and increased hemorrhages in the vessels
Picture taken by the authors

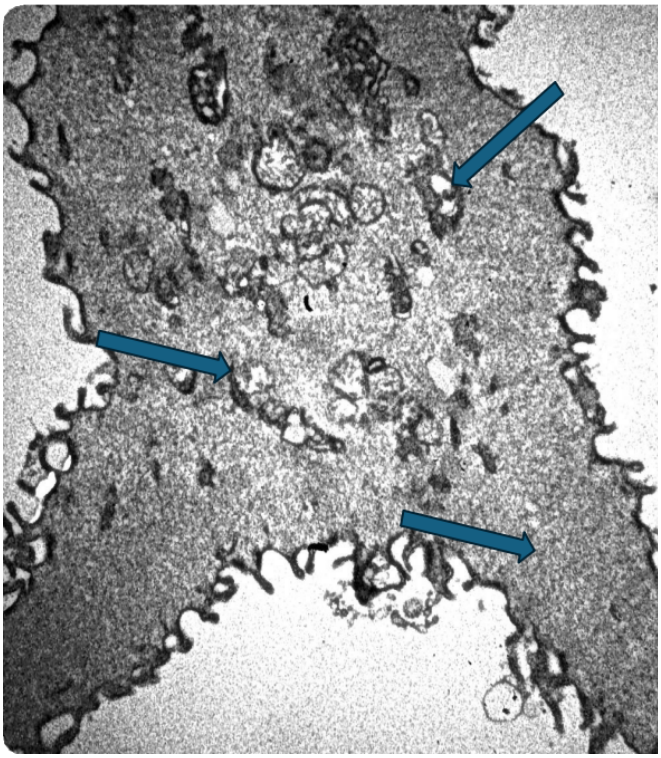

Fig. 4. Electronic photograph (magnification x10000). Partial sclerosis and microcalcification of the Tonsil epithelial tissue
Picture taken by the authors

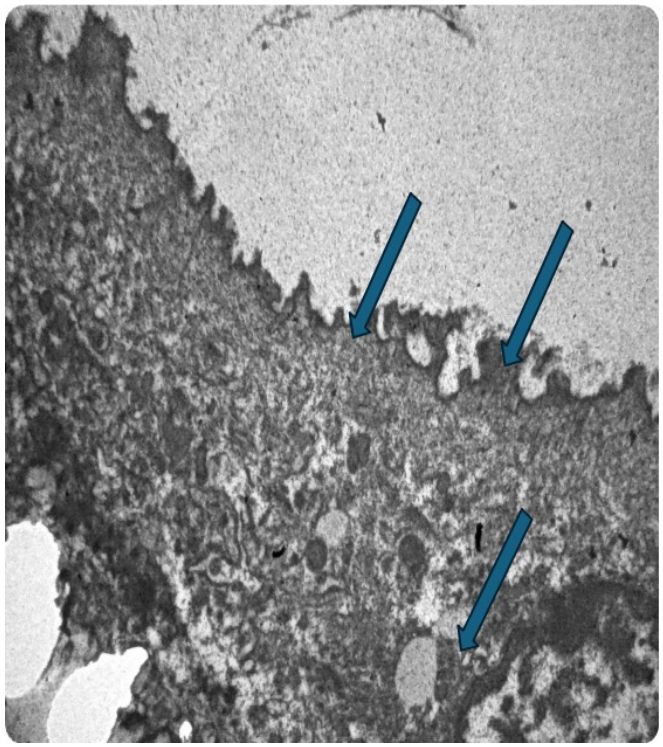

Fig. 5. Electronic photograph (magnification x10000).
Pronounced sclerotic changes in the epithelial cell and partial destruction of the cell membrane, resulting in impaired semiconductivity in the cell
Picture taken by the authors

RESULTS

The S protein of SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE-2) protease as a receptor for entry into human cells, which is facilitated by other proteases such as TMPRSS2 [8, 9]. SARS-CoV-2 has the potential to limit the innate immune response by weakening the Interferon response in epithelial cells after Coronavirus successful entry [10].

Virus-induced inflammation of the mucosa occurs, in which Neutrophils and Macrophages (CD68+ cells) circulate, with subsequent infiltration and increased Cytokine levels [11, 12]. When epithelial cells of the upper and lower Respiratory tract are affected, symptoms of Acute Rhinopharyngitis may develop at the onset of the disease. At the same time, in a significant number of cases this period may remain without manifestation, and a significant part of infected people endure this stage of the disease in an erased form, creating the main pool of latent infection [12].

The nasopharyngeal mucosa contains a moderate amount of mucosa-associated Lymphoid tissue (MALT), which is conveniently located for immunological sampling but also creates vulnerability to pathogens. In recent years, the Nasopharyngeal location has been implicated in the pathogenesis of important human diseases (COVID-19), but this tissue has never been described in detail in any species. The oral cavity is an accessible and predominant environment for the interaction of SARS-CoV-2 with the Mucosal Immune system and target cells; also, direct exposure to the virus impairs the antiviral inflammatory response and causes oral disorders, particularly in association with Immunodeficiency states and Autoimmunity [13]. The most typical electronic photographs of changes in the nasopharyngeal mucosa in children with diagnosed Coronavirus infection are presented (Fig. 1, Fig.2, Fig.3, Fig.4, Fig.5). The clinical manifestation was represented by complaints from the upper Respiratory tract on the background of fever (body temperature was 38-39^{o} C).

Pathological processes were detected in the mucous membrane of the Nasopharyngeal zone, which were characterized as polymorphic-cellular infiltration and presented by an active phase of inflammation, in the structure of the lacuna on the basement membrane of the cell during phagocytosis, according to the results of electron microscopy. Presentation of a section of the Palatine Tonsil epithelial cell is marked by increased focal hemorrhage and degenerative changes in the membrane of papillary structures. The Palatine Tonsil with reactive-degenerative changes in the stroma and increased hemorrhages in the vessels indicates degenerative changes that accompany the Acute infectious process, caused by Covid-19. Partial sclerosis and microcalcification of the Tonsil epithelial cell tissue, pronounced sclerotic changes in the epithelial cell and partial destruction of the cell membrane were also visualized, as a result of which the semiconductivity in the cell is disturbed.

Based on the electronic presentation, we can observe all the processes of inflammation and their consequences. We will consider the pathogenetic processes which were monitored in our studies. Inflammation consists of three main types of tissue disorders, which include: alteration, exudation, proliferation [14]:

1) alteration is the occurrence of damage under the influence of an inflammatory factor, in our case the Covid-19 virus and presented by the active phase of inflammation of papillary structures (lacunae) located on the basement membrane during phagocytosis;

2) changes in microcirculation, vascular wall permeability, exudation;

- a section of an epithelial cell of the Palatine Tonsil in which focal hemorrhage and degenerative changes in the membrane of papillary structures are observed.
- a Palatine Tonsil reactive-degenerative changes in the stroma and increased hemorrhages in the vessels;

3) proliferation;

- pronounced sclerotic changes in the epithelial cell and partial destruction of the cell membrane, resulting in impaired semiconductivity in the cell,

- partial sclerosis and microcalcification of the tonsil epithelial cell tissue.

DISCUSSION

Necrotic inflammation may develop in the case of predominance of alteration process, which is manifested by mucosal epithelium damage, its desquamation and exposure of the basement membrane [15], which were observed in the review of our presentations.

The membrane structure damage caused by the effects of physical and chemical factors, free radicals, the complement system, etc. This pathological process leads to cell necrosis, but local damage can be restored, however, with partially loss of membrane [15]. This date were also noted in our studies.

SARS-CoV-2 infection usually begins in the Nasopharynx and cytological and molecular correlates are not sufficiently characterized. Cytological analysis documented that glandular cells infected with SARS-CoV-2 showed marked degeneration with ciliocytophtory; viral inclusions were not detected. Co-expression analysis showed that virus-infected cells had increased apoptosis, as indicated by strong expression of activated *Caspase* [16].

Cytology studies performed in Sudan also did not reveal significant cytological and pathological changes. Examination of Nasopharyngeal swabs revealed an abnormally high number of Lymphocytes (Lymphocytosis), a large number of inflammatory cells. A thorough search failed to detect any viral inclusions due to the absence of squamous cells. This suggests that the claim of viral inclusions with COVID-19 disease may rather represent a non-specific cytopathic effect in Nasopharyngeal swabs, such as lymphocytosis [17,18].

CONCLUSIONS

Cytological changes in the studied material of the Nasopharyngeal zone with common changes in glandular cells can be considered as nonspecific. We founded the same type of morphological changes, by electron microscopy using of the mucous membrane investigation in the patients with COVID-19. These changes were characterized by the presence of diffuse inflammatory polymorpho-cellular infiltration, impaired hemodynamics and diffuse desquamative, dystrophic, sclerotic changes in epithelial cells, which are consistent with numerous data of scientists.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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