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THE PLACE OF THE ENDOTHELIAL COMPONENT IN THE PATHOGENESIS OF INFLUENZA *A*

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Abstract

Influenza A viruses are genetically diverse and the most unpredictable variable pathogens. It is a dangerous and poorly managed infection, able to reassort, with a high probability of developing urgent conditions, complications and death at any age. **The objective:** to determine the diagnostic value of CEC in influenza A. **Materials and methods:** There were 158 influenza A patients included in the survey presented. Observation period - 2015-2018. The 1st group included 32 patients with a mild flu course, the 2nd group - 62 patients with moderate flu, and the 3rd group - 64 patients with a severe flu and mild pneumonia. The severity was determined by clinical manifestations and the presence of complications. Exclusion criteria from the study: age under 18 and over 45 y.o., pregnancy and severe somatic pathology. The control group consisted of healthy volunteers - 30 persons, similar in sex and age with a patient's group. **Conclusions.** It was established that endothelium damage occurs even at mild course of influenza A. Severe influenza infection is accompanied by a massive endothelial damage with the development of endothelial dysfunction. Determination of circulating endothelial cells in blood is a simple and reliable indicator of the disease severity and its complications development.

Key words: influenza A, endothelial dysfunction, desquamated endotheliocytes.

Introduction

Influenza occupies a special place in the entire etiologically diverse group of acute respiratory viral infections (ARVI). Influenza is a dangerous and poorly managed infection with a constantly changing antigenic structure and biological properties of the pathogen, able to reassort, with a high probability of developing urgent conditions, complications and death at any age.

The first pandemic of influenza in the 21st century was the one of type A / H1N1 in 2009. In addition to mass infection of people in different regions of the world, the new virus was distinguished by an increased ability to infect the lower respiratory tract and induce rapidly progressive pneumonia, often of severe course. The high frequency of lung lesions in influenza A is due to the tropism of the virus to epithelial cells, including endothelial cells, which determines the characteristic clinical picture of influenza and influenza pneumonia [1, 3]. The presence in the lungs of a significant mass of endothelial cells in the alveolar-capillary membrane determines the development of the systemic inflammatory response syndrome in these patients. All this contributes to the release of a large number of cytokines and other biologically active substances into the bloodstream, which leads to the development of multiple organ failure with impaired microcirculation [Malyarchikov AV, Shapovalov KG].

In this regard, of particular interest is the study of the influence of influenza A virus on the development of endothelial dysfunction (ED) as a modulator of many pathological processes in the body, which plays a key role in the pathogenesis of the disease and the development of multiple organ failure [1, 5, 6]. Considering the endotheliotropic properties of the virus, the dominant point, in our opinion, is the study of a direct marker of endothelium's acute damage - circulating endothelial cells (CEC) [2].

CEC are mature, differentiated cells that are separated from the endothelium wall during its damage [8]. And therefore, they can act as a direct cellular marker for endothelial dysfunction. The number of CEC in peripheral blood of healthy persons is very small, because in the absence of pathological conditions, the process of endothelium's updating is slow, and non-viable CEC are quickly removed from the bloodstream by the reticuloendothelial system [11].

For the first time, the existence of CEC in peripheral blood was described in 1978 by J. Hladovec, who observed the phenomenon of so-called endothelialmia in rats after administering endotoxin, hyaluronidase, streptokinase and vasoactive drugs to them [10]. Since then, the CEC have attracted a lot of attention from many scientists in terms of their clinical and pathophysiological significance in the development of various diseases.

Thus, endothelium is currently regarded as a large endocrine organ that is involved in many physiological and pathological processes in the body, without which none regulatory system - nervous, endocrine, immune – can avoid. Endothelial dysfunction is accompanied by a number of changes in LP / AOD system, which in most cases constitute, in our opinion, the pathogenic basis of the disease. In the available literature, despite numerous studies, ED and its causes at influenza A, are still not well understood, which undoubtedly creates prospects for its further study [7, 12]. In addition, it is promising to develop methods for the pharmacological correction of ED dysfunction, which will improve the results of treatment of this pathology.

The objective: to determine the diagnostic value of CEC in influenza A.

Materials and methods

Dynamic survey of 158 influenza A patients treated on the basis of the Odessa National Medical University in the diagnostic departments of the Clinical Infectious Diseases Hospital in Odessa (Ukraine) in 2015-2018 was conducted.

The patients were divided into the following groups: the 1st group - 32 patients with a mild flu course, the 2nd group - 62 patients with moderate flu, and the 3rd group - 64 patients with a severe flu and mild pneumonia. The severity was determined by clinical manifestations and the presence of complications. Exclusion criteria from the study: age under 18 and over 45 y.o., pregnancy and severe somatic pathology. The control group consisted of healthy volunteers - 30 persons, similar in sex and age with a patient's group.

The diagnosis of influenza was made on the basis of epidemiological, clinical and virological research methods. The study included only patients with a positive result of swabs from the nasopharynx for influenza A by PCR. The above mentioned analyses were performed on a patient's admission to the hospital and on the 5^{th} day of observation.

Traditional methods of examination were carried out (C.B.C., common urine examination, biochemical blood tests, chest X-rays). To study the direct marker of ED, in the serum of patients the intensity of endothelial desquamation was determined by J. Hladovec modified method (1978). The method mentioned consists in determining the total number of circulating endothelial cells and their further investigation at different stages of apoptosis [10]. The calculation was performed in Goryaev's chamber by phase-contrast microscopy.

Statistical processing of the results was performed on a personal computer using the STATISTICA 10.0, MedCalc 14.8.1 and Microsoft Excel 2010 with the AtteStat 12.5 add-in, and WebPagestat Perform Statistical Calculations (http://statpages.info).

Results and discussion

For influenza A patients under study, the following was characteristic: admission to the hospital after 3 days of illness, prolonged fever, persistent severe weakness, nausea and loss of appetite, muscle pain, aching joints, nasal congestion and rhinitis, dry unproductive cough, intense intractable headache (Fig. 1).

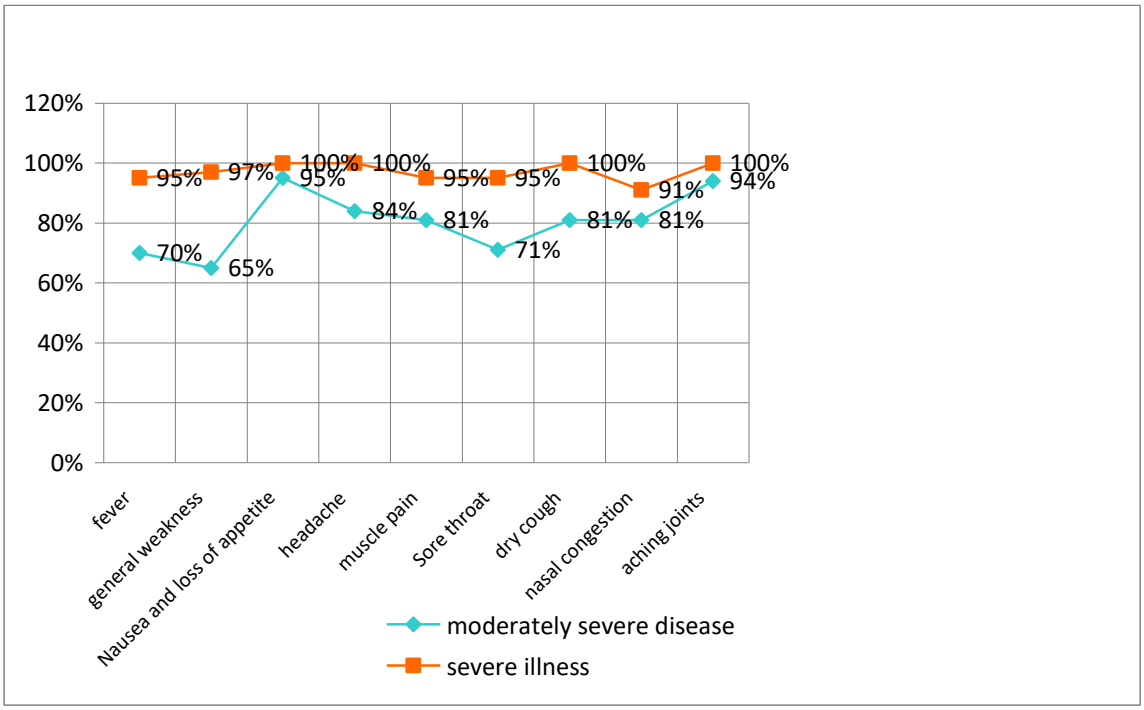

Fig. 1. Clinical characteristics of the patients with moderate and severe flu

As shown in Fig. 2, even in a mild course of influenza, an increase in the total number of CEC to 1300–1600 cells/ml (1505±161) took place. Statistically significant increase in the content of the total CEC in all the groups under study was revealed in comparison with the control (700–1300 cells / ml; 993±102). Also, the relationship of CEC morphological changes with the manifest illness stage and the severity of the disease was determined. Thus, in moderate flu patients, the total number of CEC was already 1700 - 2400 cells / ml (202762 ± 232), and in severe cases, 2500-3900 cells / ml (3120±287). In our opinion, high endothelimia in peripheral blood in the patients under study confirms the high endotheliotropic character of influenza virus toxins. Morphological study of influenza A patients CEC's plasma showed the predominance of apoptosis pronounced stage in all the groups.

Among the patients included in the study, primary viral pneumonia was diagnosticated in 64 patients of the 3rd group. Patients were treated in the diagnostic department and did not need to be transferred to the intensive care unit.

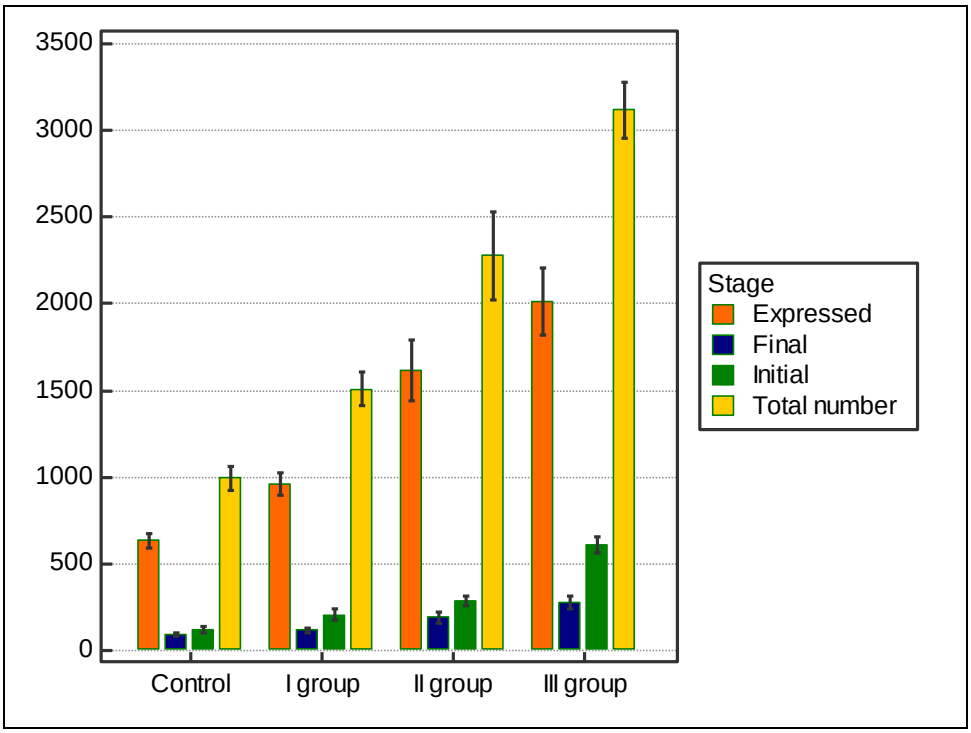

Fig. 2. The total number of CEC with distribution according to the degree of apoptosis in patients depending on the severity of the flu

In this category of patients, pneumonia developed as a rule for the 2-3 days of illness. It was noticed that in all patients of this group, the total number of CEC was high at the first examination with its increase at repeated blood tests (3120 ± 287) cells / ml (Fig. 3).

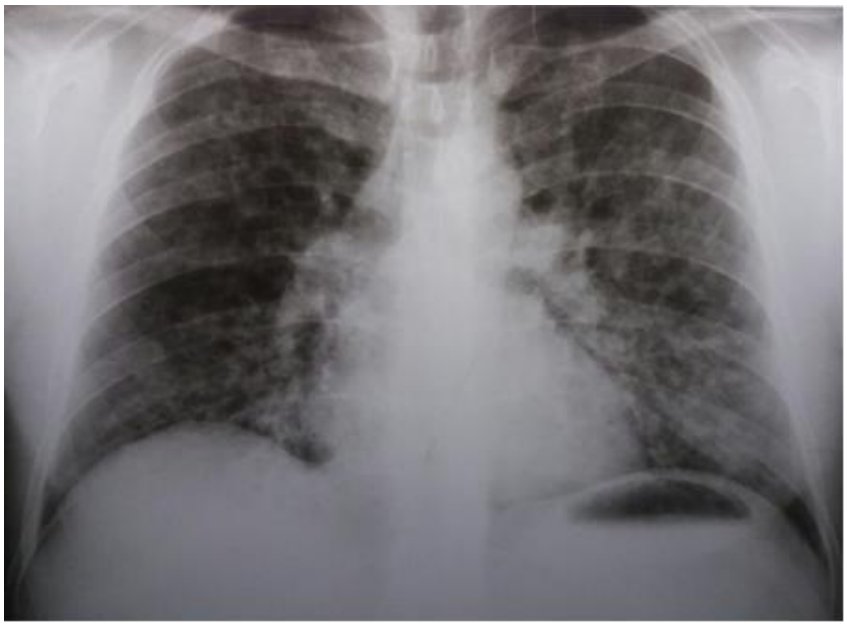

Fig. 3. X-ray picture of patient M., 23 y. o. Primary viral pneumonia

On the basis of the total number of CEC in the groups under study we determined criteria of influenza complicated course, depending on the total number of CEC increase. In the patients with uncomplicated (mild flu) course, the total amount of CEC was in the range (1505±161) cells / ml in the absence of pulmonary physical changes on the X - ray image. In moderate flu, the total cells number was (2276 ± 232) cells / ml with clinically and roentgen confirmed bronchitis symptoms. Maximizing the total amount of the CEC (3120 ± 287) cells / ml we observed in patients with primary viral pneumonia, confirmed clinically and radiographically.

Thus, the degree of endothelium desquamation has strong direct correlation with the severity of the disease (Spearman's coefficient $r = 0,72$, $p < 0,001$) that is, the severer is the course of the disease, the higher is the degree of endothelium desquamation.

Conclusions:

1. On the grounds of the investigation made, it was established that endothelium damage occurs even at mild course of influenza A.

2. Severe influenza infection is accompanied by a massive endothelial damage with the development of endothelial dysfunction.

3. Determination of circulating endothelial cells in blood is a simple and reliable indicator of the disease severity and its complications development.

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