



ASSESSMENT OF THE CELL-MEDIATED IMMUNITY AND IL-4 AND IL-6 LEVELS IN CHILDREN WITH CHRONIC TONSILLITIS

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ABSTRACT

Chronic tonsillitis has been a pressing issue for pediatricians. Firstly, it is caused by immaturity of the immune system, particularly the lymphatic system, at a young age. Secondly, possible complications caused by tonsillitis and chronic diseases of other organs and systems may occur.

The state of colonization resistance, which can be seriously affected in patients with acute or chronic tonsillitis, plays an important role to protect the tonsils, mouth cavity, and nasopharynx against incursion by pathogens. Cytokines are involved into infectious inflammation of specific immune response and non-specific effector mechanism during chronic inflammatory diseases of the respiratory tract. Besides, they determine clinical course, severity, and clinical outcome of pathologic process.

Our aim was to assess the cell-mediated immunity status and the IL-4 and IL-6 levels in children with chronic tonsillitis.

The article presents important features of the immune status of children with chronic tonsillitis. We evaluated the levels of lymphocytes, T cells, T helpers, T suppressors, and immunoregulatory balance in serum. We assessed the immune status in children by certain immunological indices including the levels of T lymphocytes, T helpers, T suppressors, B lymphocytes, phagocytal index, phagocytal number; IgG, IgA, IgM, and cytokine profile. We detected T and B lymphocytes and their subsets by the immunofluorescence technique using monoclonal antibodies. The serum cytokine (IL-4 and IL-6) levels were measured by the ELISA technique. Phagocytic activity of the neutrophils was evaluated for their capacity to absorb latex-particles using a light microscope.

Thus, the T-cell branch tended to be suppressed while there were higher levels of CD8+ in children with chronic tonsillitis in comparison with relatively healthy children. During the study of the changes in proinflammatory and anti-inflammatory cytokine levels of children with chronic tonsillitis, higher IL-4 level was observed in the period of exacerbation of the disease and after receiving the standard therapy. The level of IL-6 in patients with chronic tonsillitis increased significantly in the first days of hospitalization and decreased after the standard treatment, but these figures did not reach the levels of healthy children.

KEYWORDS: children, chronic tonsillitis, palatine tonsils, immune system, cytokines.

INTRODUCTION

Nowadays chronic tonsillitis has become a pressing issue for pediatricians. Firstly, it is caused by immaturity of the immune system, particularly the lymphatic system, at a young age. Secondly, possible complications caused by tonsillitis and chronic diseases of other organs and systems may occur [Buryak V et al., 2011; Kryuchko T et al., 2012].

The state of colonization resistance, which can be seriously affected in patients with acute or

chronic tonsillitis, plays an important role to protect the tonsils, mouth cavity, and nasopharynx against incursion by pathogens. Modern scientists consider the tonsils as the immunocompetent organ that serves as a protective barrier of the mucous membrane of the respiratory tract. Besides, they provide adequate immune response to microorganisms [Nagoev B, Nagoeva M, 2009; Shlyakhova N, 2011].

Changes in general and local responsiveness of the organism mainly determine the effect of the bacterial factor in the presence of chronic tonsillitis [Shlyakhova N, 2011].

In modern times, the study of mediators of the innate and acquired immune systems (particularly

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interleukins) to regulate the immunity of mucous membranes has become quite important. Cytokines are involved into infectious inflammation of specific immune response and non-specific effector mechanism during chronic inflammatory diseases of the respiratory tract. Besides, they determine clinical course, severity, and clinical outcome of pathologic process [Efthimiadis I et al., 2011; Kaidashev I, 2012; He L et al., 2013].

Our objective was to assess the cell-mediated immunity status and the IL-4 and IL-6 levels in children with chronic tonsillitis.

MATERIALS AND METHODS

We examined the cell-mediated immunity status of 63 children (aged 13-17 years) with chronic tonsillitis on 1st or 2nd day of the hospital admission. We also studied 33 children after they had received the standard therapy (on the 12–14th day). We assessed the IL-6 and IL-4 levels in 42 children with chronic tonsillitis (on the 1st day of hospitalization) and in 22 children after they had undergone the standard therapy (on the 12–14th day) in order to analyze the state of proinflammatory and anti-inflammatory cytokines. Twenty relatively healthy children of the appropriate age and sex served as controls.

We assessed the immune status in children by certain immunological indices including the levels of T lymphocyte, T helper, T suppressor, B lymphocyte; phagocytal index, phagocytal number, IgG, IgA, IgM, and cytokine profile (proinflammatory IL-6 and anti-inflammatory IL-4). We detected T and B lymphocytes and their subsets by the immunofluorescence technique using monoclonal antibodies. The following monoclonal antibodies were employed to identify the markers in general population of T lymphocytes – CD3+; T helpers – CD4+; T suppressors – CD8+; and B lymphocytes – CD22+. The serum cytokine (IL-4 and IL-6) levels were measured by the ELISA technique. Phagocytic activity of the neutrophils was evaluated for their capacity to absorb latex-particles using a light microscope.

Statistical analyses of the results were processed with the help of Microsoft Office Excel (2007), which was adapted for medical and biological research. Statistical analysis of the materials employed the following statistical methods of calculating the arithmetic mean (M), standard deviation (δ),

the average error arithmetic mean (m), evaluation of the reliability indices (p). Assessment of differences between mean values of independent samples was done via a parametric method for determining statistical significance difference of two sets of observations, the Student-Fischer method and the difference were considered accurate for values of $p < 0.05$.

The study was approved by the Institutional Bioethics Committee and conforms to the principles outlined in the Declaration of Helsinki (Br. Med. J. 1964; p.177).

RESULTS

The study confirmed that there were certain immune status disorders in children with chronic tonsillitis in comparison with the controls even during the first days of disease progression.

For instance, the phagocytic index of B-cell immunity reduced on the day of the hospital admission to $48.73 \pm 0.90\%$ and the phagocytic number $7.30 \pm 0.21\%$ to compare with $55.72 \pm 1.39\%$ and $9.48 \pm 0.27\%$ in the control group ($p < 0.001$). Simultaneously some changes in the cell-mediated immunity were presented as reduction of the numbers of CD3+ to $57.80 \pm 1.58\%$; CD4+ to $30.61 \pm 0.26\%$ and the immunoregulatory index to $1.60 \pm 0.03\%$ in comparison with the control indices ($p < 0.001$). However, the number of CD8+ increased to $29.50 \pm 0.87\%$ that was significantly higher than in relatively healthy children ($p < 0.05$) (Table).

When the clinical symptoms from exacerbation of chronic tonsillitis became less evident, we pointed out that the phagocytal index had significant increase to $52.06 \pm 0.96\%$ ($p < 0.05$); the phagocytal number tended to increase as well, but it was lower than in the controls ($p < 0.001$).

The analysis of the cell-mediated immunity of children with chronic tonsillitis, who had received the standard therapy, revealed that the CD3+ level significantly increased to $63.42 \pm 1.10 g/l$ ($p < 0.01$), so it almost reached the indices of the relatively healthy children ($p > 0.05$). At the same time, the levels of CD4+ and immunoregulatory index, even after treatment, changed slightly ($p > 0.05$) and they were different from the control group ($p < 0.001$). The CD8+ level tended to decrease ($27.21 \pm 0.95 g/l$) and it was returning to the normal index ($p > 0.05$).

We found out that the levels of proinflamma-

tory (IL-6) and anti-inflammatory (IL-4) interleukins increased in both groups compared with relatively healthy children during exacerbation of chronic tonsillitis.

TABLE.
Changes in the cell-mediated immunity indices in children with chronic tonsillitis for the group 1 and group 2, M±m

Immunological indices	Before treatment (n = 63)	After treatment (n=33)	Relatively healthy children (n=20)
	1	2	3
Leucocytes, 10 ⁹ /l	6.51±0.26 p ₁₋₂ >0.05 p ₁₋₃ >0.05	6.15±0.30 p ₂₋₃ >0.05	6.09±0.29
Lymphocytes	41.80±1.19 p ₁₋₂ >0.05 p ₁₋₃ >0.05	40.81±1.19 p ₂₋₃ >0.05	39.00±1.60
	2.70±0.10 p ₁₋₂ >0.05 p ₁₋₃ >0.05	2.51±0.08 p ₂₋₃ >0.05	2.38±0.07
CD3+	57.80±1.58 p ₁₋₂ <0.01 p ₁₋₃ <0.001	63.42±1.10 p ₂₋₃ >0.05	65.32±1.14
	1.56±0.05 p ₁₋₂ >0.05 p ₁₋₃ >0.05	1.59±0.05 p ₂₋₃ >0.05	1.56±0.05
CD4+	30.61±0.26 p ₁₋₂ >0.05 p ₁₋₃ <0.001	31.30±0.40 p ₂₋₃ <0.001	39.12±0.71
	0.82±0.04 p ₁₋₂ >0.05 p ₁₋₃ <0.05	0.79±0.04 p ₂₋₃ <0.05	0.93±0.05
CD8+	29.50±0.87 p ₁₋₂ >0.05 p ₁₋₃ <0.05	27.21±0.95 p ₂₋₃ >0.05	26.60±1.14
	0.80±0.10 p ₁₋₂ >0.05 p ₁₋₃ <0.05	0.68±0.09 p ₂₋₃ >0.05	0.63±0.06
CD22+	21.14±0.37 p ₁₋₂ >0.05 p ₁₋₃ >0.05	21.00±0.53 p ₂₋₃ >0.05	21.80±0.88
	0.57±0.04 p ₁₋₂ >0.05 p ₁₋₃ >0.05	0.53±0.04 p ₂₋₃ >0.05	0.51±0.04
Immunoregulatory index,%	1.60±0.03 p ₁₋₂ >0.05 p ₁₋₃ <0.001	1.62±0.04 p ₂₋₃ <0.001	2.20±0.05

Notes: p – statistical significance: p₁₋₂ – between the “before” and “after” treatment indices; p₁₋₃, p₂₋₃ – between the “before” and “after” treatment indices for children with chronic tonsillitis and the controls

Thus, on the day of the hospital admission children with chronic tonsillitis had significant increase in the IL-4 level to 85.73±3.24 pg/ml, it was 12 times higher than for the controls (p<0.001). During the recovery period, the IL-4 level tended to lower to 81.32±3.85 pg/ml (p>0.05), but it was higher than for the controls (p<0.001).

Having analyzed the changes in the IL-6 level, we concluded that it significantly increased to 32.00±1.48 pg/ml (p<0.001) in children with chronic tonsillitis on the first days after hospitalization. The IL-6 level reduced to 16.07±1.37 pg/ml (p<0.001), but it did not return to the normal index (p<0.001) even when children had received the standard therapy.

Assessment of the immune status of children with chronic tonsillitis revealed suppression of the B-cell and T-cell branches, particularly reduction in the phagocytic index and phagocytic number, CD3+ and CD4+ levels, immunoregulatory index (p<0.001), while the CD8+ level (p<0.01) increased in comparison with relatively healthy children. During the study of the changes in proinflammatory and anti-inflammatory cytokine levels of children with chronic tonsillitis, higher IL-4 level was observed in the period of exacerbation of the disease and after receiving the standard therapy. Obtained data most likely represent high level of anti-inflammatory reaction and intense immune response of the child's organism with chronic tonsillitis. During the first days of exacerbation of chronic tonsillitis the higher IL-6 level is caused by activation of both local and systemic immunoregulatory mechanisms directed at developing inflammation and immune response to pathogens. In the recovery period the IL-4 level tended to decrease (p>0.05) and the IL-6 level also dropped significantly (p<0.001), but those indices did not return to normal.

Thus, these results should be applied for well-ground and correct diagnosis of different immune disorders. They can also determine appropriate treatment for children with chronic tonsillitis considering changes in the cell-mediated immunity indices and IL-4 and IL-6 levels. These steps can prevent chronic tonsillitis decompensation and enhance life quality for such children.

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