

ORIGINAL ARTICLE

ANTIBODIES TO MICROBIAL ANTIGENS AND CYTOKINES IN THE CELLS OF THE PALATINE TONSILS AND SERUM OF CHILDREN WITH PALATINE TONSILS HYPERTROPHY AND CHRONIC TONSILLITIS

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ABSTRACT

The aim: The aim of the study is to compare the class G antibody content in serum and tissue lysate from tonsils of children with hypertrophy and chronic tonsillitis to: streptolysin-O of *Str. haemolyticus*, protein-A of *S. aureus*, proteoglycans of *Klebsiela spp.*, as well as to compare the content of interleukins 1 β , 10, TNF- α , γ -IFN and lactoferrin in serum and tissue lysate from tonsils of children with hypertrophy and chronic tonsillitis.

Materials and methods: We studied tonsils of 33 children aged 4-18 years with hypertrophy of palatine tonsils (HPT) and with chronic tonsillitis (CT). The content of interleukins 1 β , 10, TNF- α , γ -IFN and lactoferrin in tonsil lysate and serum was determined by immunofluorescence assay. Antistreptolysin O was studied by neutralization test of micromethod; class G antibodies to protein A of *S. aureus* and proteoglycans of *Klebsiela spp.* were studied by treponema pallidum hemagglutination assay. All the results were statistically processed using U-test (Mann-Whitney-Wilcoxon test) and Fisher's z-transformation.

Results: The serum and tissue lysate from tonsils of patients with HPT showed significantly high level of antibodies to streptolysin O in comparison with similar studies of substrates from patients with CT. Anti-inflammatory cytokine IL-10 was detected only in the serum of patients with CT. The TNF- α concentration in the lysates of tonsils in the group of patients with HPT was 2 times higher than in the group of patients with CT. The γ -IFN concentration was significantly lower both in the serum and in the lysates of tonsils of patients with CT. The content of lactoferrin in the lysates of patients with CT was 3 times higher ($P < 0.05$) than in the lysates of patients with HPT.

Conclusions: The results indicate a significant difference in the state of antibodies to microbial antigens and cytokines production in case of HPT and CT. In tonsils with HPT, there predominate reactions of antibody production to bacterial antigens and antiviral reactions like a high-level cytokines TNF- α and γ -IFN in tissue lysate of palatine tonsils.

KEY WORDS: Hypertrophy of palatine tonsils, chronic tonsillitis, immunity factors, interleukins, lactoferrin, antibodies

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INTRODUCTION

A very common abnormality in the clinical status of palatine tonsils is the state of their hypertrophy at an early age. Due to the clinical need, such tonsils are often the object for clinical intervention which involves partial resection of tonsillar tissue, that is tonsillotomy (partial tonsillectomy). The state of the palatine tonsil after such an intervention can hardly be defined as functionally preserved. In addition, taking into account present data on the role of this organ in the immune system, the development of the following local immune deficiency of mucous membranes can be assumed, an indicator of which is sIgA [1, 2]. The main pathological abnormalities in the state of tonsils are most often manifested as their hypertrophy or chronic inflammation.

Pathological characteristics and immunology research in recent decades have not identified significant differences between these conditions. And the development of new directions in immunology offered real possibilities for a more objective assessment of the tonsillar tissue state, the influence on the systemic and local immune response of various peptides formed in the palatine tonsils in case of hypertrophy and chronic inflammation [3, 4, 5, 6].

THE AIM

The aim of the study is to compare the antibody content in serum and tissue lysate from tonsils of children with hypertrophy and chronic tonsillitis to: streptolysin-O of *Str. haemolyticus*, protein-A of *S. aureus*, proteoglycans of *Klebsiela spp.*, as well as to compare the content of interleukins 1 β , 10, TNF- α , γ -IFN and lactoferrin in serum and tissue lysate from tonsils of children with hypertrophy and chronic tonsillitis.

MATERIALS AND METHODS

The content of cytokines and protective proteins in lysates of tonsil cells and serum of children with grade 2-3 hypertrophy according to Brodsky's classification and in those with chronic tonsillitis was studied. The study involved 15 patients with CT and 18 patients with HPT aged 4-18 years.

After surgical removal, tonsillar extracts were immersed in 199 medium containing gentamicin (100 μ g/ml), kept at 40°C, mechanically homogenized and filtered through a nylon sieve; the cell concentration was calculated, adjusted to a standard

Table I. The content of class G antibodies to microbial antigens in the lysates of the cells of tonsils and serum of children with CT and HPT.

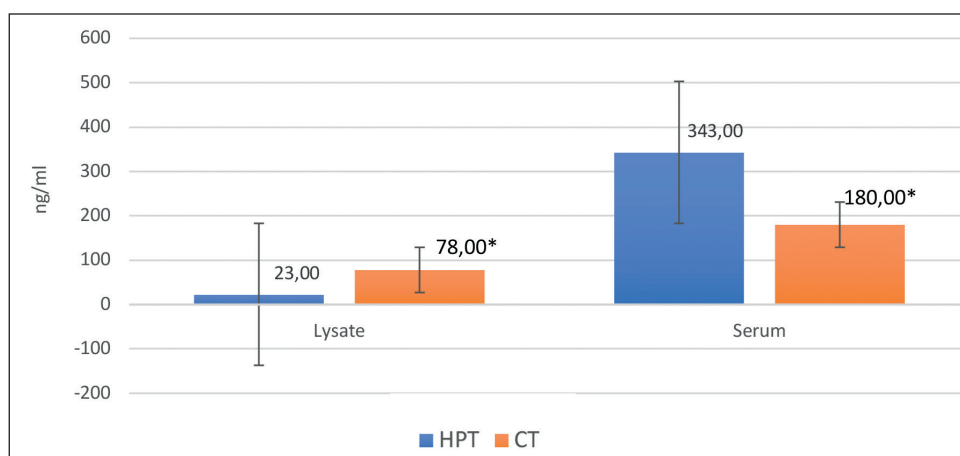
Group of patients	Lg2 antibody titers (average) to microbial antigens					
	streptolysin O of <i>Str. haemolyticus</i>		protein A of <i>S. aureus</i>		proteoglycan of <i>Klebsiela spp.</i>	
	Lysate	Serum	Lysate	Serum	Lysate	Serum
CT (n=15)	1.3	4.5	0	4.0	0	2.1
HPT (n=18)	3.5*	8.5*	0	3.25	1.2*	3.6

Note: *P<0.05

Table II. The content of cytokines in the lysates of the cells of tonsils and serum of children with CT and HPT.

Group of patients	Concentration of cytokines, pg/ml							
	IL-1 β		IL-10		TNF- α		γ -IFN	
	Lysate	Serum	Lysate	Serum	Lysate	Serum	Lysate	Serum
CT (n=15)	46.0	0.2	0	2.1	130.4	34.2	11.2	8.2
HPT (n=18)	41.6	0.2	0	0	236.0*	29.3	36.5*	19.6*

Note: *P<0.05

**Fig. 1.** The content of lactoferrin in the lysates and serum of patients with HPT (n=18) and CT (n=15). *(P<0.05).

content of 1 ml and processed in lysis buffer, followed by incubation in isotonic saline solution and centrifugation.

Supernatants were frozen and stored for 1 month at a temperature of minus (-) 20°C, after which the concentration of cytokines and other peptides and proteins was determined. Serum was obtained from venous blood the day before surgery.

The content of IL-1, IL-10, tumour necrosis factor (TNF- α), interferon gamma (γ -IFN) and lactoferrin in tonsil lysate and serum was determined by immunoenzyme method and assay kits manufactured by "Proteinovy Kontur", "Cytokin" and "Vector-Best" (RF). A Stat-Fax-2100 reader (USA) was used as an analyzer. Antistreptolysin O was studied by neutralization test of micromethod; antibodies to protein A of *S. aureus* and proteoglycans of *Klebsiela spp.* were studied by treponema pallidum hemagglutination assay using LATEST reagent kits (RF). All the results were statistically processed using U-test (Mann-Whitney-Wilcoxon test) and Fisher's z-transformation.

RESULTS

The data on the content of class G antibodies to microbial antigens in the lysates of the cells of tonsils and serum of children with CT and HPT are presented in Table I.

As can be seen from Table I, a high level of antibodies is determined in relation to streptolysin O in the serum and lysates of the cells of tonsils in patients with HPT in comparison with similar studies of substrates from patients with CT. The antibodies to protein A of *Staphylococcus aureus* and proteoglycan of *Klebsiela spp.* were almost not detected in the lysates of tonsillar cells, and in the serum, they were at a lower level than to streptolysin O, and did not differ statistically in both comparison groups.

The data on the content of cytokines in the lysates of the cells of tonsils and serum of children with CT and HPT are presented in Table II.

As can be seen from Table II, the concentration of IL-1 β in the serum of patients of both groups was minimal (<0.2 pg/ml), and in the lysates of the comparison groups it did not differ statistically. The anti-inflammatory cytokine IL-10 was detected only in the serum of patients with CT. The TNF- α concentration in the lysates of the cells of tonsils in the group of patients with HPT was 2 times higher than in the group of patients with CT; in the serum there was no significant difference between the groups. The γ -IFN concentration was significantly lower both in the serum and lysates of the cells of tonsils of patients with CT.

When studying the concentration of lactoferrin in the lysates and serum of patients with CT and HPT, the following results were obtained (Figure 1).

As can be seen from Figure 1, the content of this iron-containing prodefensin in the lysates of the cells of patients with CT was 3 times higher ($P < 0.05$) than in the lysates of patients with HPT, whereas in the serum the opposite significant dependence was determined.

DISCUSSION

The findings indicate a pronounced immunological activity of the tissue of the palatine tonsils both in case of hypertrophy and chronic inflammation. At the same time, a more intense production of antibodies in the tonsils with hypertrophy indicates a greater immunologic capacity of hypertrophied lymphoid tissue. This can also be evidenced by a pronounced inflammatory reaction in the palatine tonsils of patients with HPT, which is confirmed by the level of IL- 1β no less than in the tonsils of patients with CT.

The analysis of the data on the TNF- α content, which was within the normal range in the serum of patients of both groups and significantly higher in the lysates of tonsillar cells of patients with HPT than those with CT, indicates an inflammatory reaction in the hypertrophied palatine tonsils, but mainly to viral antigens. This is also evidenced by a reliably high level of γ -IFN both in the lysates and serum of patients with HPT compared to similar substrates of patients with CT.

The findings indicate that the processes of antibody production to microbial antigens, especially hemolytic streptococcus, have a more intense course in the tonsils in case of HPT than in case of CT. The TNF- α content in hypertrophied tonsils is at a higher level than in CT, which, together with an increased content of γ -IFN, indicates antibody response of the immunity to infectious agents.

In addition, to gain the understanding of the role of individual parts of the immune system and allergy in the immunopathogenesis of both HPT and CT, it is necessary to conduct further studies on the range of abnormalities in the content and functional activity of molecular factors that are important in the implementation of protective reactions in the tonsils in both pathological conditions.

CONCLUSIONS

1. The level of class G antibodies to *Str. haemolyticus* in the lysates of patients with HPT was 2.7 times higher ($P < 0.05$) than in those with CT, and in the serum of patients with HPT it was 1.9 times higher ($P < 0.05$) than in those with CT.
2. The level of class G antibodies to *Klebsiella spp.* in the serum of patients with HPT was 1.7 times higher ($P < 0.05$) than in those with CT.
3. The TNF- α concentration in the lysates of patients with HPT was 1.8 times higher ($P < 0.05$) than in those with CT.
4. The γ -IFN concentration was reliably higher ($P < 0.05$) in patients with HPT than in those with CT (3.2 times higher in the lysates, and 2.4 times higher in the serum).

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Conflict of interest:

The Authors declare no conflict of interest.

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