

## CLINICAL STUDY

# The immune status *in situ* of recurrent tonsillitis and idiopathic tonsillar hypertrophy

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**Abstract:** *Objective:* To analyze the immune status *in situ* of tonsils of patients with recurrent tonsillitis (RT) and idiopathic tonsillar hypertrophy (ITH) with the aim to discuss the indications of tonsillectomy (TE) and tonsillectomy (TT) in young children.

*Methods:* The histological and immunohistological study of tonsillar tissue of RT and ITH in correlation with immunological parameters in peripheral blood in 13 patients with RT and 16 patients with ITH.

*Results:* In the RT group, we found a higher degree of fibrosis with a higher density of memory lymphocytes (CD45R0<sup>+</sup>), B-lymphocytes (CD20<sup>+</sup>) and cytotoxic T-lymphocytes (CD8<sup>+</sup>) in surface epithelium of tonsils compared to the ITH group (NS). The density of immunoglobulin IgG in the crypt epithelium in RT was significantly higher than in the ITH group ( $p = 0.041$ ). We also measured a higher sera concentration of immunoglobulines (IgG, IgM, IgA) and TNF- $\alpha$  in RT compared to the ITH group (NS) and T<sub>H</sub>-1 immune response in tonsillar tissue based on differences between local cytokine concentration TNF- $\alpha$  and IL-4.

*Conclusions:* RT has a higher inflammatory reaction in tonsillar tissue as a result of persistent bacterial antigenic stimulation. In patients with RT, the tonsillectomy might be the only option for surgical treatment. In patients with ITH with mild symptoms, the tonsillectomy should be preferred (*Tab. 3, Ref. 24*). Full Text in PDF [www.elis.sk](http://www.elis.sk).  
Key words: recurrent tonsillitis, idiopathic tonsillar hypertrophy, immunohistochemistry, histology, immunity.

Epidemiological trends in the numbers of TE have increased rapidly and the surgical indications have shifted from infection to upper airway obstruction (1, 2). In younger children, the obstruction of airways without infection is the main indication for this procedure, with the age the infection becomes more prominent indication (3)

Tonsillectomy (TT) is accepted as a safe and effective method for treatment of obstructive sleep-related respiratory disturbances in children (4). In tonsillar hyperplasia even without history of infection the chronic inflammation can be found histologically (5) and there is a low risk of regrowth of tonsillar tissue after TT (4, 5).

That's the reason why the indication for these ENT procedures are being discussed among ENT clinicians and pediatricians. In the present study we have investigated the immune status *in situ* in patients with RT and ITH.

## Patients and methods

### Patients

For this study we have selected 13 patients with RT (10 boys, 3 girls in the age 2–12 years, mean 5.87 years $\pm$ 2.346) and 16

patients with ITH (13 boys, 3 girls in the age 3–8 years, mean 4.67 $\pm$ 1.779 years) who underwent surgery of tonsils under general anaesthesia at the ENT clinics in the time period November 2007 – June 2008.

All thirteen patients with RT had history of tonsillitis with more than 4 episodes per year – they underwent tonsillectomy. The patients with ITH had history of obstructive symptoms or history of sleep apnea syndrome with no history of recurrent infections. Ten patients with ITH (62.5 %) underwent TT and six of them (37.5 %) underwent TE.

The patients with the history of known allergies, the history of antibiotic or antihistaminic drugs taken at least 1 month before surgery, or patients with suspicion of malignant disease were not included in the study. All patients had no evidence of clinical infection at the time of surgery.

Before the surgery, the blood needed for immunological investigation was taken.

All procedures were performed in accordance with the ethical guidelines of the Children's University Hospital in Bratislava. A signed written consent was obtained from parents of the patients.

### Histological investigation

Each tonsil specimen was divided into 2 parts – one section was preserved in 10 % buffered formaline at room temperature for 24 hours routinely processed in automatic tissue processor and then embedded in paraffin blocks. Five  $\mu$ m thick slices were stained with hematoxylin and eosin for histological assessment. The second section was prepared for immunohistochemical investigation. Additionally, in 2 patients with RT 1 gram of tonsillar tissue was

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homogenized in 2 ml of physiological solution and then centrifuged at 3000 rpm for 10 minutes. The supernatant was kept at -20 °C until the cytokines were analyzed (Flowcytomix, Bender Medsystems, Austria).

#### Immunohistochemistry

Sections were processed using antihuman monoclonal antibodies according to the peroxidase-antiperoxidase method. Following cell surface antigens were used:

- (i) examination of memory lymphocytes CD45<sup>+</sup>R0<sup>+</sup> (DAKO, 1:150)
- (ii) examination of cytotoxic T-lymfocytes CD8<sup>+</sup> (DAKO, RTU)
- (iii) examination of B-lymfocytes CD20<sup>+</sup> (DAKO, 1:400)
- (iv) examination of immunoglobulin IgA and IgG (LAB VISION, RTU)

#### Immunological investigation

The blood samples were taken in both groups of patients before ENT surgery.

The *subpopulations of lymphocytes* were determined by flow cytometry using monoclonal antibodies (BeckmanCoulter FC 500) for total T lymphocytes (CD3<sup>+</sup>), helper T-lymphocytes (CD4<sup>+</sup>), cytotoxic T-lymphocytes (CD8<sup>+</sup>), B-lymphocytes (CD19<sup>+</sup>). *Immunoglobulines* were analyzed in serum by nephelometry.

In the RT group, the cellular immunity was evaluated in 10 patients (76.9 %), humoral immunity in 11 patients (84.6 %). In ITH group, the subpopulations of lymphocytes were stained in 14 patients (87.5 %), the immunoglobulines in 11 patients (68.7 %).

The *TNF- $\alpha$  in serum* was measured in 4 patients with RT and in 3 patients with ITH by ELISA (Human TNF- $\alpha$  instant ELISA, Bender Medsystems, Austria).

#### Histological and immunohistochemical evaluation

We evaluated histological specimens in 15 patients with ITH (93.7 %) and 12 patients with RT (92.3 %).

Each section was assessed in the light microscope with original magnification 400x. We evaluated the surface epithelium according to the pathology classification (0 – absence or 1 – presence of keratosis / parakeratosis, oedema, infiltration of bacteria, polymorphonuclear leukocytes (PMN), the degree of fibrosis 1 – light, 2 – severe), the crypts and the number of follicles per high power field.

We performed a semiquantitative analysis using the following scale for the density of cells positive for CD45R0<sup>+</sup>, CD20<sup>+</sup>, CD8<sup>+</sup> s and immunoglobulines IgG, IgA: 0 – absence, 1 – mild density (0–33 %), 2 – moderate density (33–66 %), 3 – severe density (> 66 %).

#### Statistical analysis

The chi quadrat test and Fischer exact test were performed to compare the presence or absence of histological and immunohistochemical findings in cross tables.

For 2-group comparison analyse, the Student t-test (for normal distribution) or Mann-Whitney test were performed (if the material was not normally distributed). p values less than 0.05 were considered to be significant. We used the SPSS for Windows statistical packet program.

## Results

#### Histological investigation

The patients with RT had 1.66 times more often a higher degree of fibrosis (degree 2) in the surface epithelium compared to the ITH group (33.3 % vs 20 %). The patients with ITH had 1.30 times more often oedema in the surface epithelium than the RT group (86.7 % vs 66.7 %).

There was no difference in the presence of parakeratosis or keratosis in the epithelium (66.7 % in both groups of patients), intraepithelial colonization of bacteria (26.7 % in the ITH group, 25 % in the RT group) and intraepithelial infiltration of polymorphonuclear leukocytes (60% in the ITH group, 66.7 % in the RT group). Almost in all patients of both groups, there was fibrin, lymphocytes and polymorphonuclear leukocytes observed in the crypts. The number of follicles per high power field was found to be similar in both groups – in the ITH group the mean was 12.87  $\pm$  4.955, in the RT group 13.58  $\pm$  4.144. The results were not statistically significant (Tab. 1).

#### Immunohistological investigation

In the RT group, there was 1.58 times more often higher *density of memory lymphocytes* (density 2+3) in the surface epithelium of tonsils (69.3 % vs 43.8 %) and 1.37 times higher in the crypt epithelium (76.9 % vs 56.3 %) compared to the ITH group. In the surface epithelium, there was also 6-times more often higher density of *cytotoxic T-lymphocytes* (density 2+3, 38.5 % vs 6.3 %) and 1.54 times more *B-lymphocytes* (density 2+3, 38.5 % vs 25.0 %) in RT comparing ITH group.

In both groups of patients there was a higher density of B-lymphocytes in the crypts than in surface epithelium – in the RT group 2.4 times higher (92.3 % / 38.5 %) and in the ITH group 3.5 times higher (87.5 % / 25%). The results were not significant.

The patients with RT had a statistically significant higher density of staining of immunoglobulin IgG under the crypts (p = 0.041).

**Tab. 1. Histological investigation of tonsils.**

Group		Keratosis		Oedema		Fibrosis			Bacteria		PMN	
		0	1	0	1	0	1	2	0	1	0	1
RT	N.	4	8	4	8	1	7	4	9	3	4	8
	%	33.3%	66.7%	33.3%	66.7%	8.3%	58.3%	33.3%	75.0%	25 %	33.3%	66.7%
ITH	N.	5	10	2	13	3	9	3	11	4	6	9
	%	33.3%	66.7%	13.3%	86.7%	20.0%	60.0%	20.0%	73.3%	26.7%	40.0%	60.0%
P value		1.000		0.213		0.568			1.000		1.000	

Abbreviations: N – number of patients, 0 – absense, 1– light degree, 2 – severe degree of histological changes

**Tab. 2. Immunohistochemical investigation of tonsils.**

CD45R0 <sup>+</sup> lymphocytes												
Localisation	Surface epithelium			In crypts			Extrafollicular area			Intrafollicular		
density	1	2	3	1	2	3	1	2	3	1	2	3
ITH N.	9	3	4	7	8	1	2	11	3	7	8	1
%	56.3	18.8	25.0	43.8	50.0	6.3	12.5	68.8	18.8	43.8	50.0	6.3
RT N.	4	6	3	3	7	3	1	9	3	4	8	1
%	30.8	46.2	23.1	23.1	53.8	23.1	7.7	69.2	23.1	30.8	61.5	7.7
p value	0.260			0.346			1.000			0.844		
CD8 <sup>+</sup> lymphocytes						CD20 <sup>+</sup> lymphocytes						
Localisation	Surface epithelium			Extrafollicular area			Surface epithelium			In crypts		
density	1	2	3	1	2	3	1	2	3	1	2	3
ITH N.	15	1	0	5	6	5	12	4	0	2	10	4
%	93.8	6.3	0.0	31.3	37.5	31.3	75.0	25.0	0.0	12.5	62.5	25.0
RT N.	8	3	2	4	4	5	8	4	1	1	7	5
%	61.5	23.1	15.4	30.8	30.8	38.5	61.5	30.8	7.7	7.7	53.8	38.5
p value	0.096			1.000			0.535			0.865		
immunoglobulin IgG												
Localisation	In crypts			Surface epithelium			Intrafollicular			Extrafollicular		
density	1	2	3	1	2	3	1	2	3	1	2	3
ITH N.	8	5	3	3	6	7	12	3	1	10	5	1
%	50.0	31.3	18.8	18.8	37.5	43.8	75.0	18.8	6.2	62.5	31.3	6.3
RT N.	1	9	3	4	3	6	8	2	3	7	6	0
%	7.7	69.2	23.1	30.8	23.1	46.2	61.5	15.4	23.1	53.8	46.2	0.0
p value	0.041			0.721			0.451			0.835		

Abbreviations: RT – recurrent tonsillitis, ITH – idiopathic tonsillar hypertrophy, N – number of patients, % – percentage of positive cells, 1 – mild density, 2 – moderate density, 3 – severe density.

**Tab. 3. Cytokines in tissue supernatant in RT.**

Cytokine pg/ml	IL-10	IL-8	IL-4	IL-1 β	TNF-α	TNF-β
patient 1	92.6	4.180	17.07	3.549	467.87	15.89
patient 2	172.89	4.071	0	4.561	430.47	41.25

No patient had grade zero of immunohistochemical markers (Tab. 2).

#### Immunological investigation

The patients with RT had a higher sera concentrations of immunoglobulines IgG (in the RT group the mean was  $10.16 \pm 3.655$  g/l, in the ITH group the mean was  $7.49 \pm 2.240$  g/l), IgA (in the RT group the mean was  $1.03 \pm 0.760$  g/l, in the ITH group the mean was  $0.96 \pm 0.657$  g/l), IgM (in the RT group the mean was  $1.95 \pm 2.698$  g/l, in the ITH group the mean  $0.79 \pm 0.335$  g/l).

We found no significant differences in subpopulations of lymphocytes in peripheral blood between both patient groups: CD3<sup>+</sup> lymphocytes (RT mean  $69.18 \% \pm 4.925$ , ITH mean  $63.7 \% \pm 9.857$ ), CD4<sup>+</sup> lymphocytes (RT mean  $33.96 \% \pm 3.897$ , ITH mean  $31.14 \pm 7.775$ ), CD8<sup>+</sup> lymphocytes (RT mean  $25.06 \% \pm 4.389$ , ITH mean  $26.36 \% \pm 5.678$ ) and CD19<sup>+</sup> lymphocytes (RT mean  $19.29 \% \pm 5.882$ , ITH mean  $20.86 \% \pm 7.539$ ).

The higher sera concentrations of TNF-α in patients with RT than in patients with ITH were also measured (mean  $10.03 \pm 3.489$  pg/ml in the RT group,  $6.89 \pm 3.856$  pg/ml in the ITH group).

In two patients with RT, the cytokines in tissue supernatant were analyzed. The cytokines IL-2, IL-5, IL-6, IL-12p70 were not detected in the samples. The highest concentration was measured for cytokine TNF-α (mean  $449.17$  pg/ml).

The results of immunological investigation of peripheral blood and tissue supernatant were not statistically significant (Tab. 3).

#### Discussion

The palatine tonsils as the part of Waldeyer's ring are located at the gateway of gastrointestinal and respiratory tract where they are continually being bombarded with antigens entering the respiratory and gastrointestinal tract. The epithelial area exposed to antigen is maximized by 10–30 blind-ending crypts extending deeply into tonsillar tissue. The tonsils consist of four lymphoid compartments: the reticular crypt epithelium, the extrafollicular area, the mantle zones of lymphoid follicles and the germinal centres. Within the antigen is taken up by antigen-presenting cells of the macrophage lineage in crypt epithelium, it is presented to T-cells and consequently the cell-mediated and humoral responses are induced (6).

The aetiology of ITH is not well understood. The increase of B- and T-lymphocytes was reported in recurrent tonsillitis with tonsillar hypertrophy (RTTH) compared to RT (8). There might be a deficiency of proliferating active cells in response to mitogenic stimulation in RTTH (9).

In the present study we have investigated the immune status *in situ* in patients with RT and ITH in correlation with histological and immunological investigation of peripheral blood.

In comparison of both patients groups we found a higher degree of epithelial fibrosis in the RT group. Other authors also reported fibrosis in the RT group (5, 7). The fibrosis of the tonsillar epithelium in RT lead to impaired barrier function of the tonsils with a local dysfunction of immunity what consequently caused the re-

current infections (7). These histological changes of the tonsillar epithelium as parakeratosis or keratosis can be also observed in both patient groups. Alatas reported no difference in the epithelial fibrosis between patients with RT and RTTH (9). These different findings might be due to different groups of patients, because the patients with ITH in our study didn't present with the history of recurrent infection and the higher degree of epithelial fibrosis in our RT group was a result of recurrent infections. The epithelial fibrosis causes an impaired antigen uptake and this in turn leads to the recurrence of inflammatory process.

In the ITH group, the epithelial oedema of the surface epithelium might be due to non-specific bacteria-induced release of histamin (10) or due to local allergic reaction to bacterial or food allergens (11).

Previous studies observed that ITH occurs with follicular hyperplasia (9, 12) and RT with a higher number of follicles (9, 13). We didn't measure the follicular area by histological investigation and we didn't observe any significant changes in follicular number between patients with RT and ITH.

The immunohistochemical studies differ in analyzing subpopulations of lymphocytes in patients with RT and ITH. There are not many studies evaluating cellular immunity in different lymphoid compartments in tonsillar tissue.

Rosenmann et al detected no significant changes in subpopulations of T- and B-cells in tonsils and peripheral blood between patients with ITH and RT (14). Brodsky reported that the infection and bacterial load led to increase of T- and B-lymphocytes in RTTH compared to RT (8).

We investigated in patients with RT a higher density (degree 2+3) of memory lymphocytes (CD45R0<sup>+</sup>) in surface epithelium and in the crypt epithelium compared to the ITH group. In surface epithelium, there was also a higher density of cytotoxic T-lymphocytes (CD8<sup>+</sup>) and B-lymphocytes (CD20<sup>+</sup>) in RT comparing to the ITH group. The patients with RT had a statistically significantly higher density of staining of immunoglobulin IgG under the crypts. There was a higher density of B-lymphocytes in crypts than in surface epithelium in both groups. The observed findings showed that crypt epithelium in the case of RT had more active role in specific immune response than in the ITH group. Crypt epithelium may have an epithelial barrier different from surface epithelium, the integrity of cryptal epithelium is important for immune function of palatine tonsils as a defense organ against infections (15).

Our results are only partially comparable with those of Alatas (9), but there are clear differences in the target patient groups. He observed in the RTTH group a higher density of B-lymphocytes (CD20<sup>+</sup>) in crypt epithelium and a decrease of B-lymphocytes in surface epithelium, extrafollicular area and follicles, a lower density of memory lymphocytes (CD45R0<sup>+</sup>) in extrafollicular area compared to tRT group. A higher density of B-lymphocytes in crypt epithelium in the RTTH group is due to different patients group, while all patients with RTTH presented with history of recurrent infections and in our study the patients in ITH group had mostly obstructive symptoms with no recurrent infections in a history.

Difference from our study, he didn't analyze memory lymphocytes (CD45R0<sup>+</sup>) in crypts and surface epithelium. B-lymphocytes and memory T-lymphocytes are crucial for induction of secondary immune response and they are more abundant in crypts than in surface epithelium due to repeated bacterial stimulation in the RT group.

During disease, also the dendritic cells decrease in the surface epithelium and increase in the crypt epithelium (16, 17). The ratio of dendritic cells in surface to crypt epithelium was 1 : 1 for normal tonsils, but dropped to 1 : 3 for diseased tonsils (16). Alatas found the ratio of dendritic cells in surface to crypt 0.5 : 1 in the RT group and 1 : 0.8 in the RTTH group (9).

In patients with RT we measured higher sera concentrations of immunoglobulines (IgM, IgA, IgG) together with a higher concentration of TNF- $\alpha$  in serum compared to the ITH group. There was T<sub>H</sub>-1 immune response in tonsillar tissue in patients with RT based on differences between local cytokine concentration TNF- $\alpha$  and IL-4, the local concentration of TNF- $\alpha$  was 40 times higher than in serum. This can be caused by local overproduction due to monocyte -macrophage activation caused by repeated stimulation by the pathogenic agents. The presence of high levels of these cytokines, nevertheless instead of inducing an increase in immunological efficiency may lead to activation and proliferation of endothelial cells and fibroblasts which result in replacement of immunologically active tissue with fibrotic tissue (7).

The higher levels mainly of IgG (18, 19) together with pro-inflammatory cytokines such as TNF- $\alpha$  in serum (20) in patients with RT are due to persistent bacterial antigenic stimulation. We observed no significant changes in subpopulations of lymphocytes in peripheral blood between both patient groups.

## Conclusion

Our study reported a higher inflammatory response *in situ* in patients with RT – we found a severe degree of fibrosis with a higher accumulation of lymphocytes in the surface epithelium in RT compared to the ITH group. The results were not statistically significant because of small patient group except the immunostaining of IgG under the crypts in RT ( $p = 0.041$ ).

We measured T<sub>H</sub>-1 immune response in the tonsillar tissue in RT in correlation with other studies (21–24). This observation was based on differences between local cytokine concentration TNF- $\alpha$  and IL-4. The local concentration of TNF- $\alpha$  was 40-times higher than in sera. It would be helpful to measure the local cytokine levels in ITH to understand better the aetiology of tonsillar hypertrophy.

In humoral immunity, we measured a higher sera immunoglobulin concentration (mainly IgG) in RT compared to ITH due to persistent bacterial stimulation.

These mentioned results confirmed the focal infection in tonsils of patients with RT. The tonsillectomy must be therefore the only option for surgical treatment in patients with RT. The young children with ITH can be clinically observed and if they don't have history of recurrent infections, the tonsillotomy should be preferred.

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