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Early stage impacts of tonsillectomy on immune functions of children

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Summary ***Objective:*** The purpose of this study was to investigate the changes in the humoral and cellular immunity of patients with chronic tonsillitis before and 1 month after tonsillectomy. ***Patients and methods:*** 37 patients scheduled for tonsillectomy were enrolled in this study. The levels of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD25^+$ and $CD16^+{+}56^+$ were measured for cellular immunity, and levels of IgG, IgA, IgM, C3 and C4 were measured for humoral immunity in blood samples taken from these patients before and 1 month after the operation. ***Results:*** The levels of $CD3^+$, $CD8^+$ and $CD19^+$ were reduced in post-operative period as compared to pre-operative period but this was not statistically significant ($P > 0.05$). However it was found that the level of $CD4^+$ was significantly increased while the level of $CD25^+$ was reduced ($P < 0.05$) in the post-operative period. There were statistically significant differences between pre- and post-operative levels of immunoglobulins, C3 and C4, which were decreased after tonsillectomy ($P < 0.05$), but these levels were comparable with those of the control group. ***Conclusion:*** The results from the present study indicate that humoral and cellular immunity of patients undergoing tonsillectomy were decreased in the early period and came to normal later. The cellular and humoral immune responses are stimulated in patients with tonsillitis, and tonsillectomy removes this stimulus without negatively affecting the patient's immune functions.

1. Introduction

The palatine tonsils, which are major components of the lymphoid tissue in Waldeyer's ring, appear to function as the host's first line of defense against exogenous microorganisms [1]. Inflammatory diseases of the tonsils constitute the most common cause of primary care visit to physicians, and tonsillectomy represents one of the most common operations performed in children [2].

Human tonsils are known to be immunologically reactive lymphoid organs which manifest specific antibodies and B- and T-cell activity in response to a variety of antigens, carrying out the functions of humoral and cellular immunity [3]. It is well known that inflamed human tonsils have many surface crypts showing characteristic lymphoepithelial symbiosis, which play an important role in immune response. The cryptepithelia are rich in blood vessels. This proliferation of blood vessels is

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thought to be the source of various kinds of immune response [4].

The human immune responses are divided into two types: (a) humoral immune response, which is dependent on B cells, plasma cells and antibodies, and (b) cellular immune response, which is dependent on T cells and cytokines. The lymphocytes are the most important cells in the immune system. All lymphocytes develop from a common lymphoid precursor cell in the bone marrow, from where they migrate to the peripheral lymphatic tissues [3—5].

Various investigators have reported high serum levels of IgG and IgA in patients with chronic tonsillitis [3,6]. However, many studies indicate that inflammation and/or hypertrophy of adenoids and tonsils are caused by hypofunction of local and systemic immunity [7—10].

The purpose of this study was to investigate the possible impacts of tonsillectomy on the cellular and systemic immunity of children with chronic tonsillitis.

2. Patients and methods

The present study was performed on a total of 37 children (21 girls and 16 boys aged 5—9 years, mean age 7.29 ± 2.6 years) at the ENT Clinic, at Fırat University. The design of this study has been approved by the Ethical Committee of the Fırat University. Thirty-seven children were recruited into the study from April 2002 through November 2002. Among indications for tonsillectomy were included hypertrophy with obstructive symptoms and recurrent tonsillitis.

A standard anesthetic protocol was applied to the all patients. The patients were given 0.006 mg/kg atropine, 4 mg/kg pentobarbital sodium, and 0.5 mg/kg atracurium besylate for muscular relaxation. After oral intubation, anesthesia was maintained with nitrous oxide, oxygen, and inspired isoflurane. The usual physiological parameters (respiratory rate, pulse, blood pressure, and temperature) were monitored throughout the surgery. Standard surgical techniques were used. The dissection and snare technique for tonsillectomy was performed by otolaryngology residents under the supervision of the attending surgeon.

The control group comprised of 35 healthy children (20 girls and 15 boys, aged 5—9 years, mean age 7.4 ± 3.4 years) without a history of recurrent upper tract infections and hypertrophy of tonsils. The control group consisted of patients who underwent general anesthesia, because of septoplasty and foreign body in oesophagus.

2.1. Immunologic analysis

Blood samples were taken 24 h prior to tonsillectomy (pre-operative) and 1 month after surgery (post-operative). A volume of 10 ml of venous blood was obtained. After clotting, the serum was separated and kept at −20 °C until the immunoglobulins were analysed.

Following examinations were carried out for all children: serum levels of immunoglobulins A, G and M; complements C3 and C4; percentage of $CD4^+$ (T helper), $CD8^+$ (T cytotoxic), $CD3^+$, $CD19^+$, $CD16^+{+}56^+$ and $CD25^+$.

The serum levels of immunoglobulins A, G and M and complements C3 and C4 were determined by a standard turbidometric technique, using Shaperelli biosystem (Holland). Lymphocyte subpopulations were determined by BD FACScan flow cytometry in the peripheral blood by using Becton Dickinson (BD) monoclonal $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+{+}56^+$ and $CD25^+$ antibody.

2.2. Statistical analysis

A Microsoft Excel 98 spreadsheet and Statistical Package for Social Sciences 9.5 (SPSS Inc.,USA) were used for analysis. Student's *t* test for unpaired data was used to compare the results between patients and control groups. The comparison of pre- and post-operative values was performed by Student's *t* test. A probability value of 0.05 was considered as significant.

3. Results

Table 1 shows the data obtained from the analysis of IgG, IgA, IgM, C3 and C4 values of patients, before and after tonsillectomy, and those of the control group. There were statistically significant differences between pre- and post-operative values of IgG, IgA, IgM, C3 and C4, which decreased after surgery ($P < 0.05$). One month after tonsillectomy, the serum levels of IgA, IgG and IgM significantly decreased ($P < 0.05$). Pre- and post-operative values of concentrations of complement factors C3 and C4 were significantly different ($P < 0.05$). It was observed that the mean pre-operative values of immunoglobulins and complements (IgA, IgG, IgA, C3 and C4) were significantly higher in patients before tonsillectomy than those of the control group ($P < 0.05$). After tonsillectomy, no significant differences were seen between the levels of immunoglobulins and complements (IgA, IgG, IgA, C3 and C4) when compared with those of the control group ($P > 0.05$).

Table 1 Humoral immune parameters in children

Parameters	Pre-operative ($n = 37$)	Post-operative ($n = 37$)	Controls ($n = 35$)
IgA	$2.07 \pm 0.47^{*,\dagger}$	1.75 ± 0.63	1.70 ± 0.45
IgG	$12.87 \pm 2.39^{*,\dagger}$	11.95 ± 2.65	11.45 ± 1.77
IgM	$1.17 \pm 0.35^{*,\dagger}$	0.98 ± 0.28	0.97 ± 0.12
C3	$1.78 \pm 0.22^{*,\dagger}$	1.56 ± 0.22	1.44 ± 0.14
C4	$0.28 \pm 0.4^{*,\dagger}$	0.23 ± 0.70	0.22 ± 0.02

Values are given as mean ± SD.
$^{*}P < 0.05$ (paired Student's t test); comparison of pre-operative values with post-operative values.
$^{\dagger}P < 0.05$ (unpaired Student's t test); comparison of pre-operative values with controls.

Table 2 Cellular immune parameters in children

Parameters	Pre-operative ($n = 37$)	Post-operative ($n = 37$)	Controls ($n = 35$)
CD3	$61.91 \pm 11.20^{\dagger}$	65.52 ± 8.46	67.89 ± 3.67
CD4	$34.26 \pm 6.17^{*,\dagger}$	$38.05 \pm 4.75^{\ddagger}$	45.64 ± 3.61
CD8	$32.75 \pm 8.75^{\dagger}$	29.85 ± 5.56	29.28 ± 4.91
CD4/CD8	$1.09 \pm 0.38^{*,\dagger}$	$1.32 \pm 0.34^{\ddagger}$	1.52 ± 0.29
CD19	14.01 ± 4.91	12.85 ± 3.35	12.5 ± 2.80
CD16 + 56	16.86 ± 5.57	16.58 ± 3.72	16.59 ± 5.16
CD25	$20.73 \pm 10.43^{*,\dagger}$	14.71 ± 6.90	13.51 ± 6.10

Values are given as mean ± SD.
$^{*}P < 0.05$ (paired Student's t test); comparison of pre-operative with post-operative.
$^{\dagger}P < 0.05$ (unpaired Student's t test); comparison of pre-operative with controls.
$^{\ddagger}P < 0.05$ (unpaired Student's t test); comparison of post-operative with controls.

Table 2 shows the results obtained from the analysis of T lymphocytes in the subgroup of patients before and after tonsillectomy and those of the control group. One month after tonsillectomy, we observed a statistically significant ($P < 0.05$) increase of $CD4^+$ T lymphocytes. But the percentages of $CD25^+$ T lymphocytes statistically significantly decreased ($P < 0.05$). There were no statistically significant differences between pre- and post-operative values of $CD3^+$, $CD8^+$, $CD16^+{+}56^+$ and $CD19^+$ T lymphocytes. The levels of $CD8^+$ and $CD25^+$ were higher—and the levels of $CD3^+$ and $CD4^+$ were lower—in patients before tonsillectomy compared with those of the control group ($P < 0.05$). But after tonsillectomy, when lymphocyte values for the subpopulation were compared with those for the control group, the significant difference in values of $CD4^+$ ($P < 0.05$) became insignificant ($P > 0.05$).

4. Discussion

The tonsils are situated at the entrance of the respiratory and alimentary tracts and represent the first site of contact with a variety of microorganisms and other antigenic substances present in food and inhaled air [5].

As secondary lymphatic organs, tonsils are concerned with antigen processing. Small amounts of antigen are transported trough the reticular cell epithelium by M cells and antigen-presenting cells (APC). They probably play a major role in local immunity [4,11].

In humans, $CD4^+$ and $CD25^+$ function as regulatory T lymphocytes in blood and have recently been identified in tonsils. Seven to 10% of T cells are represented by these cells, which express cell surface markers of an activated memory phenotype [12]. It is known that the tonsil of a child has a greater concentration of lympocytes than an adult's and that the helper and cytotoxic T lymphocytes are increased in sick tonsils. Also, higher levels of immunoglobulins in the pretonsillectomy period is due to constant antigenic stimulus from infected tonsils [13,14]. Moreno et al. [15] showed that the immune response was decreased after tonsillectomy and that the higher levels of immunoglobulin in the pre-operative period were due to the stimulation of the lymphocytes by bacteria.

El-Ashmawy et al. [14] reported that the levels of IgG and IgA in serums of patients with chronic tonsillitis were increased, while IgM levels were

not significantly altered, and that 2 months after tonsillectomy, these high levels of serum IgG and IgA were significantly reduced. Sainz et al. [16] measured the levels of IgG, IgM and IgA in patients with chronic tonsillitis and reported that increased pre-operative immunoglobulin levels were significantly decreased after surgery and there were no change in the levels of serum C3 and C4 before and after tonsillectomy. Zielnik-Jurkiewicz and Jurkiewicz [4] observed statistically significant higher serum levels of immunoglobulin A, G and M in patients with hypertrophy of adenoids and tonsils compared with those of the control group before operation. One month after adenotonsillectomy the serum levels of immunoglobulins statistically significantly decreased. The presented results indicate that adenotonsillectomy provokes a deficit of humoral immunity (production of immunoglobulins) in children with hypertrophy of adenoids and tonsils. They reported changes in the distribution of $CD8^+$ lymphocytes in children with hypertophy of adenoids and tonsils. There was significantly higher $CD8^+$ T lymphocytes before adenotonsillectomy. One month and 6 months after the operation the percentage of $CD8^+$ T lymphocytes significantly decreased and in the last examination increased to the level seen in the control group [4].

Lal et al. [3] reported that the mean pre-operative level of serum IgG and IgA were significantly higher in patients with chronic tonsillitis than in the control group; the values dropped significantly 1 month after tonsillectomy, the post-operative values being comparable with those of the control group. In our study, the levels of IgG, IgA and IgM were considerably decreased after tonsillectomy. This decrease maybe explained as due to the recovery of infected tissue and disappearance of continuous antigenic stimulus. By the same way the levels of $CD3^+$, $CD8^+$ and $CD19^+$ decreased in the post-operative period but not significantly. The ratio of $CD4^+/CD8^+$ was considerably higher. This variation can be explained by the increase in $CD4^+$ and decrease in $CD8^+$ T lymphocyts. The reason for the decrease in $CD8^+$ may be due to the removal of infected tonsil tissue by tonsillectomy. It was interpreted that before the operation, the activation of the classic pathway of complement system may be the indication of increases in reaction between antigen and antibody complexes in the immune system because of the increase in microorganisms in the tonsil.

Prusek et al. [17] found normal values of T and B lymphocytes in children 4—10 months after tonsillectomy for chronic tonsillitis. Böck et al. [18] reported that removal of the tonsils does not inhibit development of the immune system in childhood. Sennaroğlu et al. [19] reported that tonsillectomy improves the abnormal neutrophyl chemotaxis and has positive effect on the immune system of patients with chronic tonsillitis.

In conclusion, immonoglobulin levels (IgA, IgG and IgM), complement fractions (C3, C4), cytotoxic T lymphocytes and activated B lymphocytes are increased in patients with chronic tonsillitis as a response to chronic bacterial stimulus. And, tonsillectomy restores these parameters by removal of bacterial stimulus without negatively affecting the patient's immune functions. Although the removal of the tonsils is still controversial because of the possible effects on immunological functions, the well-established fact that elevated immune parameters in patients with chronic tonsillitis can be a useful marker of disease when a tonsillectomy is considered.

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