Original Article

Long-term impacts of tonsillectomy on children's immune functions

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Abstract

Background: There exist a wide level of discrepancy regarding the role of tonsils and its indication among pediatricians and ENT specialists. This fact sometimes causes confusion and delay in making the right decisions by parents and specialists for appropriate treatment of patients. **Objectives:** Thus, the aim of this study was to investigate the effects of long-term tonsillectomy on the immune system of patients. **Methods:** In this case-control study we measured the status of immune system in 34 children (aged 9-15 years) following 4 to 6 years of tonsillectomy. We have also enrolled 30 healthy children with similar age group. Venous blood samples were taken and the serum levels of IgG, IgA, and IgM were detected along with expression of CD4, CD8, CD10 and CD56. Data were analyzed by SPSS version 18 software and a $P < 0.05$ was considered as significant. **Results:** We found that the mean serum levels IgM, IgA, and IgG in the case group was significantly ($P < 0.0001$) lower than the control group. Whereby, the CD4, CD8 and CD56 expressions was examined, there was no significant difference in both groups while only CD10 expression was lower in tonssiloctomized patients ($P = 0.108$). **Conclusion:** Overall, according to these findings, CD10 as a marker of B lymphocytes in children undergoing tonsillectomy was significantly less than those healthy children. This may indicate a decrease in B cells and further reduced antibody production in these patients.

Keywords: Cellular immune system, humoral immune system, tonsillectomy

Introduction

Disorders related to adenoids which also known as pharyngeal tonsils or nasopharyngeal tonsils, especially before puberty, are defined among the most important issues addressed to ENT specialists.[1] A close relationship was reported between the involvement of these organs and upper respiratory tract diseases. Conditions such as ear and nose disorders, otitis and acute sinusitis account for a large share of the several countries health care budget and are associated with irreparable complications.[2] Palatine tonsils seems to serve as a member of the immune system, playing important parts against upper respiratory tract infections.[3] Lymphoid tissue in the tonsil ring likewise to the other lymphoepithelial tissues mass in the lining and intestinal tract is nominated mucosa-associated lymphoid tissue (MALT) and exists in the upper respiratory tract.[4] Like other lymphoid organs, this tissue also possesses the ability to create specific immune reactions in response to various antigens.[5] This ability is evident and the immunological interactions of the environment cause the hyperplasia of the palatine tonsils, specifically in childhood period of life.[6] Further activation phase of immune system, which is lasted up to 8-10 years of age, the importance of the lymphatic tissue of the tonsils decreases as a member of the immune system and a similar drop takes place in the volume of lymphocytes in all tonsil regions.[7] While the immunological significance of the tonsils decreases as age rises, the tonsils tissues stuck to their own immune activity even in old ages; so that this

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will not negatively affect the decision for tonsillectomy if there is a valid indication.[8] Progression of tonsillar immunity from childhood may leading severe tonsillar hyperplasia (enlarged tonsils) and further severe functional stenosis in the bottleneck leading to difficulty in swallowing and respiratory obstruction.[9] Specially during resting time, children may experience a severe respiratory disorder completed by periods of apnea. These children also experiences long-term exposure to an increased risk of Cor pulmonale.[10,11] This is important for otolaryngologists and primary care providers to know about the immunologic profile of patient before and after tonsillectomy especially in children. In an overall view, the tonsillectomy is less recommended, even in children. Multiple studies have been carried out on the short-term effects of tonsillectomy on the immune system, however, only few investigations are available on the long-term effects of tonsillectomy on the immune system of patients.[12,13] In other words, studies on the long-term effects of tonsillectomy on the patient's immune system are also very rare. According to the above-mentioned introductory background in parallel with high rate of tonsillectomy in Iran, and regarding the disagreement between the specialists and the uncertain condition of the parents about the surgical decision, the present study was designed and aimed to determine the long-term effects of tonsillectomy on the immune system components of patients undergoing tonsillectomy, specifically including IgG, IgA, IgM, CD4, CD8, CD10 and CD56.

Patients and Methodology

In the present study 34 children aged 9 to 15 years old (15 boys and 19 girls) who 4-6 years underwent tonsillectomy have been recruited. Children were tonsillectomized for various reasons such as chronic adenotonsillar hypertrophy, recurrent tonsillitis, nocturnal snoring, mouth breathing, etc., Total of 30 healthy children with same age group (7 boys and 23 girls) were selected from primary school girls and boys in Rafsanjan, south-east of Iran. Notably, children who had any signs of craniofacial abnormalities, adenotonsillar hypertrophy, tonsillitis, upper respiratory tract infection or other infectious diseases diagnosed by history taking and examination with the help of a physician were excluded from the study. All of children who were fitted in the two groups had normal growth and had no familial history of immune or atopic disorders. All information in both groups was obtained from children's the parents and with complete willingness. In this study, data were obtained based on medical records, history taking, clinical examinations and Para clinical criteria, especially peripheral blood samples. It is clear that the full explanations of the need for the study objectives were provided to individuals in both case and control groups and their families prior any intervention, and further the informed consent of these people was won. It should be noted that the subjects who were enrolled in the study were assured about the confidentiality of the names and all relevant information, and would only meet the needs of the study objectives. This study had no any financial burden to individuals. The Ethics Research Committee of Rafsanjan University of Medical Sciences reviewed and approved this study. This research was conducted at the Moradi Hospital of Rafsanjan University of Medical Sciences. About 50 tonsillectomy surgeries are performed in this center every month, which is added to this number in hot seasons and is decreased in cold seasons. Information such as age, sex and history of surgery was collected by reviewing the patients' medical records. In addition, the levels of immunological factors in the samples collected from children were measured based on laboratory findings.

Immunologic Analysis

EDTA-containing blood samples (3 mL) were obtained for detecting the CD markers and three milliliter of clotted blood in order to measure the serum levels of immunoglobulins. Subsequent to clotting, the serum was separated and kept at −80°C until the immunoglobulins were measured. The following examinations were carried out for all children: serum levels of IgM, IgA, and IgG along with expression of of CD4, CD8, CD10, and CD56. The serum levels of IgM, IgA, and IgG were measured by ELISA technique, using ELISA kit (RADIM, Italy). In order to determine leucocyte subpopulations, a quantity of 100 µl of whole blood samples were incubated in a saturated solution with monoclonal antibodies against CD4, CD8, CD10, and CD56 and related isotype controls conjugated with fluorescein isothiocyanate (FITC) or phycoerytrine (PE), purchased from Becton Dickinson (BD) for 10 min at room temperature. The suspension then was washed twice in cold PBS to remove any unbound reactive and resuspended in 200 µl of PBS. The cells were undergone CyFlow®space flow cytometry (Partec, Münster, Germany) equipped with a laser light source (50 to 200 mW), that emits at 488 nm and FlowMax® software were employed to managing the obtained data.

Statistical analysis

Data were analyzed employ SPSS version 18.0 software. Quantitative data were reported as mean ± SD and qualitative data as frequency (percentage). At first, the homogenicity of the case and control groups was evaluated in terms of age before the beginning of the study. After the homogeneity of this variable in two groups of case and control, independent *t*-test was utilized to compare the other quantitative variables between the two groups. The significance level of the tests was considered less than 0.05.

Results

This case-control study was performed on 64 venous blood samples collected from children; including 34 (53.12%) cases aged 9 to 15 years old who had undergone tonsillectomy during the past 4-6 years and 30 (46.88%) controls who were healthy children of the same age group without tonsillectomy. The mean age was 12.35 ± 1.57 years in the case group and 9.40 ± 0.49 years in the control group ($P < 0.0001$). Gender analysis in two groups showed 15 males (44.11%) and 19 females (55.89%) in case group, and 7 males (23.33%) and 23 females (76.67%) in the control group.

Whereby the serum levels of immunoglobulins in the two groups was compared. It was obvious that the mean serum IgA level was 400.21 ± 192.48 mg/mL in the case group and 1131.45 ± 221.98 mg/mL in the control group; the difference was statistically significant (P = 0.0001). The mean serum IgM level was 55.00 ± 25.89 mg/mL in the case group and 149.33 ± 66.99 mg/mL in the control group (P = 0.0001). The mean serum IgG level was 43.57 ± 24.56 mg/mL in the case group and 210.54 ± 94.39 mg/mL in the control group (P < 0.0001).

Results obtained from flow cytometric assay in the two groups showed that there was no significant differences between case and control group in the mean count of CD4, CD8, and CD56 while the mean surface expression of CD10 had a remarkable decrease in the case group in compare to the control group (P < 0.010) [Table 1].

Discussion

Adenotonsillectomy is within the most commonly used surgeries, but the subsequent immunologic complications are yet to be fully understood.[14] Several studies have been carried out on the effects of tonsillectomy on the pediatric immune system, to date.[15-17] However, the question remained unsolved that whether the removal of the tonsils and adenoid causes a defect in the local and systemic immune system of the individual. B-lymphocytes are known as the main arm of humoral immunity and their secretory products such as antibodies are active in defense against extracellular microorganisms.[18] Cellular immunity is established by T-lymphocytes and their products, such as cytokines are important for defense against intracellular microbes.[19,20]

The results of the current study showed that IgM, IgG and IgA levels in the tonsillectomized patients were significantly lower than the control group. The expression of CD4, CD8 and CD56 in children undergoing tonsillectomy did not change significantly in comparison with healthy children. However, our study revealed that the expression of CD10 as an indicator of B cells in tonsillectomized children was significantly lower than those controls. These findings indicating a decrease in B cells and, consequently, reduced antibody production in these patients (as our findings suggest) attenuating serum levels of IgM, IgG and IgA. In a similar study conducted in 1983 by Prusek *et al.*, it has been shown that the number of T-lymphocytes in children undergoing tonsillectomy was normal 4 to 10 months postoperatively. This study reported an increase in the count of B-lymphocytes and a decrease in the count of T-lymphocytes. After tonsillectomy in the same children, the counts of B and T lymphocytes were almost normal.[21] Interestingly, a study found that levels of IgG and IgA in the serum of patients with chronic tonsillitis were increased, while IgM levels had not significantly changed. According to the results of this study, tonsillectomy not only does not reduce the function of the immune system, but also can improve the immune response.[22] Conversely, our results demonstrated that serum levels of IgM, IgG and IgA were significantly reduced in children undergoing tonsillectomy. In a study, it was also shown that tonsillectomy would not inhibit the development of the patient's immune system and the incidence of upper respiratory tract infections did not increase in children with tonsillectomy compared to the healthy control. An increase in the number of CD4 cells was seen in males who had tonsillectomy. The IgA levels were lower in children with tonsillectomy,[23] which is consistent with our study. Additionally, a study in Turkey, reported an increase in the percentage of T-lymphocytes and a decrease in the percentage of B cells following a month of tonsillectomy.[24] The findings of this study obtained alignment information. Another investigation demonstrated that tonsillectomy has initially reduced both humoral and cellular immunity, however, the immunity returned to its normal state following long period. It was also concluded that these immune responses are stimulated in patients suffering tonsillitis as well as tonsillectomy without any negative effect on the immune system eliminates this stimulation.[25] Ballester *et al.* reported that in patients who underwent appendectomy and tonsillectomy, the serum IgA measures were elevated in compare received either appendectomy or tonsillectomy, alone.[26] A study conducted on Iranian patients reported which further six months of tonsillectomy, the expression of CD3, CD8 and CD20 that were amplified would be return to the control levels. The IgM level was also significantly decreased in patients after operation.[27] Kaygusuz and colleagues reported that the immune status of patients 54 months after tonsillectomy was not significantly differed, once compared with the control.[28] Santos *et al.* measured the levels of IgA, IgM, IgG CD4, and CD8 before surgery, 1-2 months and 12-14 months after surgery in two groups of patients aged less than 4 years and over 4 years. The findings of this study showed that serum IgG level as well as CD8 expression was decreased and other parameters were increased in the group less than 4 years, however, the difference was not significant. In addition, within the over 4 year's group, all parameters were increased, but a significant increase was merely observed in CD4. Based on the result of this study, adenotonsillectomy had not affected the immune system in pediatrics either in short or long term.[29] Generally, in the most studies, the percentage of lymphocytes, despite reduction in preoperative adenotonsillectomy, after surgery, is similar to that of the control group, which at least, in partial is consistent with some of the results of the our study. The novel point of our study is the significant reduction in the expression level of CD10, which to our best knowledge has not been addressed in any of the previous studies, either in the short or long term. Although, previous studies showed that the number of

Table 1: Demonstrates the flow-cytometric findings, in the control and case groups

	Control n=30	Case n=34
CD4+ (cells/mm^3)	730.33±94.1	764.41±61.2
CD8+ (cells/mm^3)	382.20±68.2	360.16±84.3
CD10+ (cells/mm^3)	15.56±3.8	10.95±2.29
CD56+ (cells/mm^3)	316.25±74.3	318.58±46.9

*Significant differences (P<0.05). CD: Cluster of differential

lymphocytes (in general) is reduced, it has not been specifically referred to B lymphocyte. Moreover, the activation of B cells leads to their proliferation and then differentiation into plasma cells which is the source of different classes of antibodies with diverse actions.[30] The response of B cells to protein antigens requires activating messages from T-cell CD4 (which is the background reason why these T cells are called, as helper cells).[31] The B cells can respond to many non-protein antigens, without the participation of other cells.[32] Reduction in the levels of antibodies that were mentioned in other studies and confirmed by our study, may possibly be due to a decreased B cell populations, as an important finding of our research. Addressing these documents, it is hypothesized that tonsillectomy may lead to a lack of differentiation of B cells.

Conclusions

In conclusion, it seems that although the high cost of the research made us unable to measure the other surface markers of B and T- lymphocytes, our results showed that the B cells in subjects undergoing tonsillectomy were significantly reduced compared to healthy individuals, which resulted in attenuated levels of IgA, IgG, and IgM antibodies as their resources. This is in turn leading to a possible impairment in the immune system. Although other organs of the secondary immune system including appendix, spleen, Peyer's plaques, and lymph nodes may also contribute to produce lymphocytes, the tonsils may also play a protective and pivotal part in the immune system. According to the findings of studies conducted in this field of research, further studies with long-term follow-up are required to determine whether the immune system maintains its normal state for a long time after surgery which for being responsive to parental concerns about their children's immune system after surgery. Therefore, given the fact that current interventions are still ongoing clinically to establish the basic principles of the need for surgery, immunologically the cautious approach on adenotonsillectomy appears to be correct, especially in children.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms from parents of children. In the form, the cases have given their consent for clinical information to be reported in the journal. They understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Authors' contribution

All authors contributed equally to the paper.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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Conflicts of interest

There are no conflicts of interest.

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