HUMORAL AND CELLULAR IMMUNITY PARAMETERS IN CHILDREN BEFORE AND AFTER ADENOTONSILLECTOMY

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Abstract- Adenoids and tonsils are active lymphoid organs and play an important role against invading antigens of upper aerodigestive tract in children. The present study analyzes the changes in cellular and humoral immunity of children six months after adenotonsillectomy. The study population consisted of 30 children whit chronic adenotonsillar hypertrophy and 30 age-matched healthy children. In all children serum level of IgM and IgG, percentage of T lymphocytes (CD3), T helper cells (CD4), T cytotoxic cells (CD8) and B lymphocytes (CD20) were measured before surgery. These parameters were remeasured in patients 6 months after adenotonsillectomy. Before the operation, a reduction in percentage of T lymphocytes (CD3),TCD4,TC8 and B CD20 was seen compared to control group. This reduction was only significant in T lymphocytes (CD3).The serum IgM and IgG levels were not different in two groups. Six months after operation, the percentage of lymphocytes T CD3, T CD8 and BCD20 was increased and reached the control group. The IgM level was also significantly decreased in patients after operation. Our results indicate that cellular and humoral immunity decreases in children with chronic adenotonsiller hypertrophy preoperatively and increases to healthy children level, six months postoperatively. It means that chronic adenotosillar hypertrophy affect some parameters of cellular and humoral immunity and adenotonsillectomy by removing chronic stimulations and reverses these changes without any negative effect on immune function of patients.

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INTRODUCTION

The adenoids and tonsils are part of the secondary lymphatic system in which B lymphocytes are predominant (1). T and B lymphocytes are the most important components of the immune system and the cellular and humoral responses depend upon their activities (2, 3).

Adenoids and tonsils are anatomically located at the entrance of the respiratory and digestive tracts and in fact are the body's first line of contact with various pathogens present in food and air (4, 5). The histological structure of these organs is closely related to their role in the immune system. The adenoids and tonsils have no afferent lymphatics, but 10-30 crypt-like invaginations in these organs that

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are lined by squamous epithelium serve as the access route for antigens. In the depths of these crypts are APC and M cells similar to Peyer's patches of the intestines which are responsible for trapping and absorbing the antigens and presenting them to the lymphoid cells underlying the epithelium (1, 6, 7). An increased number of antigens results in proliferation of B lymphocytes which are capable of migrating to adjacent tissues and producing antibodies. The T cells have been shown to be present in tonsils and adenoids (1).

Thus, these organs are involved in both local immunity and systemic immunity by production of antibodies and changes in T and B cells (7). Although adenotonsillar tissues are immunologically active in children aged between the ages of 4-10 years, B cell activity has been reported in healthy tonsillar tissue even after the age of 80 years. However in chronic adenotonsillar hypertrophic conditions, crowding of active immunological cells results in decreased antigenic transfer and gradual decrease in B cell density (1).

There has always been difference in opinion and debate about effects of the operation on the immune system of children. Reports about possible complications and immunoglobulin production after polio vaccination or increased chances of Hodgkin's lymphoma after adenotonsillectomy need more epidemiological studies (8, 9). Various studies done in this field report that the changes occurring in cellular and humoral immunity after adenotonsillectomy are not significant enough to result in increased frequency of diseases (5, 10-12). In spite of the fact that the immunological activity of chronically hypertrophied adenotonsillar tissue is low, considering their immunological role prior to the operation, especially in younger children, is important and conservative approach for removal of them is advocated (13-15). Also, certain studies have shown that adenoids play an important role in development of immunological memory in children (16). The purpose of this study is to observe the changes in humoral and cellular immunity in children with chronic adenotonsillar hypertrophy and possible effects of adenotonsillectomy on the systemic immunity of these children.

MATERIALS AND METHODS

This study was performed on 30 children (15 boys, 15 girls) aged from 4 to 10 years (mean age of 6.5 years) with chronic adenotonsillar hypertrophy undergoing surgery. The indications for operation included: at least 5-6 attacks of tonsillitis in one year or 3 attacks in the previous 2 consecutive years or snoring and mouth breathing. Diagnosis was based on history and clinical examination.

The control group included 30 children (15 boys, 15 girls) aged between 4-10 years with a mean age of 7.2 years without any history of upper respiratory tract infection or chronic adenotonsillar hypertrophy.

All of the children enrolled in the study had normal growth patterns and no familial history of immune disorders or atopy. We obtained informed consent from parents of all participants.

Venous blood samples were taken from all of the case group subjects a few hours before hospitalization, and from the control group as well. The blood samples, consisting of 2 ml blood with EDTA for studying cellular immunity parameters and 2 ml coagulated blood for studying the immunoglobulin levels, were sent to the laboratory of Yazd Blood Transfusion Center. T lymphocytes (CD3), T helper cells (CD4), T cytotoxic cells (CD8) and B lymphocytes (CD20) were counted by flow cytometry while serum IgG and IgM levels were measured by single radial immunodiffusion method (SRID). All of the above mentioned parameters were measured in the case group 6 months after the operation.

The data obtained from the two groups were compared by ANOVA and pre-and post- operative values were compared by paired *t* test. A *P* value less than 0.05 was considered significant.

RESULTS

The comparison of results of the serum IgG and IgM levels and percentage of T lymphocytes (CD3), T helper cells (CD4), T cytotoxic cells (CD8) and B lymphocytes (CD20) in the case group preoperatively and the control group are presented in table 1.

Table 1. Comparison of cellular and humoral immunity parameters in control group and patients before operation

Parameters	Before operation	Control	*P* value
CD3	55.36 ± 0.00	60.9 ± 10.17	0.03
CD4	34.39 ± 6.25	37.96 ± 9.39	0.08
CD8	22.47 ± 3.85	24.47 ± 4.46	0.06
CD4/CD8	1.56 ± 0.33	1.60 ± 0.48	0.71
CD20	16.04 ± 5.40	18.92 ± 9.33	0.14
IgG (mg/dl)	1110 ± 172.90	1093 ± 3108.05	0.65
IgM (mg/dl)	82.16 ± 20.11	84.50 ± 14.64	0.60

Data are given as mean ± SD.

The percentage of T lymphocytes (CD3), T helper cells (CD4), T cytotoxic cells (CD8), B lymphocytes (CD20) and ratio of TCD4/TCD8 in the study group were less than the control group and the difference was significant in respect to TCD3 levels (P value = 0.03). The serum levels of IgM were slightly lower while the IgG levels were slightly higher in the study group compared to the control group.

The comparison of the statistical analysis of the parameters before and after the operation is presented in table 2. The percentage of T lymphocytes (CD3), T helper cells (CD4), T cytotoxic cells (CD8) and B lymphocytes (CD20) levels were increased post-operatively and became nearly similar to the control group (Fig. l). These increases in T lymphocytes (CD3), T cytotoxic cells (CD8) and B lymphocytes (CD20) were statistically significant. The post-operative serum IgG levels were decreased significantly (P = 0.00), while the serum IgM levels did not differ significantly compared to before the operation.

DISCUSSION

The adenoids and tonsils are secondary lymphatic organs situated at the entrance of the respiratory and digestive tracts. In these organs, the antigens are transferred via the surface crypts to lymphoid cells beneath the epithelium and by producing antibodies and division of B and T cells, they play an important role in both local and systemic immunity, especially in children (l, 4, 5). Although adenotonsillectomy is one of the most common surgical procedures, its immunological effects have not been fully understood. The question of whether removal of adenoids and tonsils results in defect in local and systemic immunity has been subject of debate. This study showed that chronic adenotonsillar hypertrophy results in changes in distribution of lymphocytes and immunoglobulins, both locally and generally. The percentage of T lymphocytes (CD3), T helper cells (CD4), T cytotoxic cells (CD8) and B lymphocytes (CD20) in the study group were lower than the control group, preoperatively.

Table 2. Comparison of cellular and humoral immunity parameters in patients before and 6 months after operation

Parameters	After operation	Before operation	Difference *P* value
CD3	60.1 ± 10.3	55.36 ± 9	0.04
CD4	36.73 ± 7.43	34.39 ± 6.25	0.13
CD8	24.63 ± 4.41	22.47 ± 3.85	0.03
CD4/CD8	1.51 ± 0.29	1.56 ± 0.33	0.45
CD20	19.19 ± 5.09	16.04 ± 5.40	0.03
IgG (mg/dl)	943.33 ± 77.38	1110 ± 172.90	0.00
IgM (mg/dl)	87.00 ± 17.59	82.16 ± 20.11	0.17

Data are given as mean ± SD.

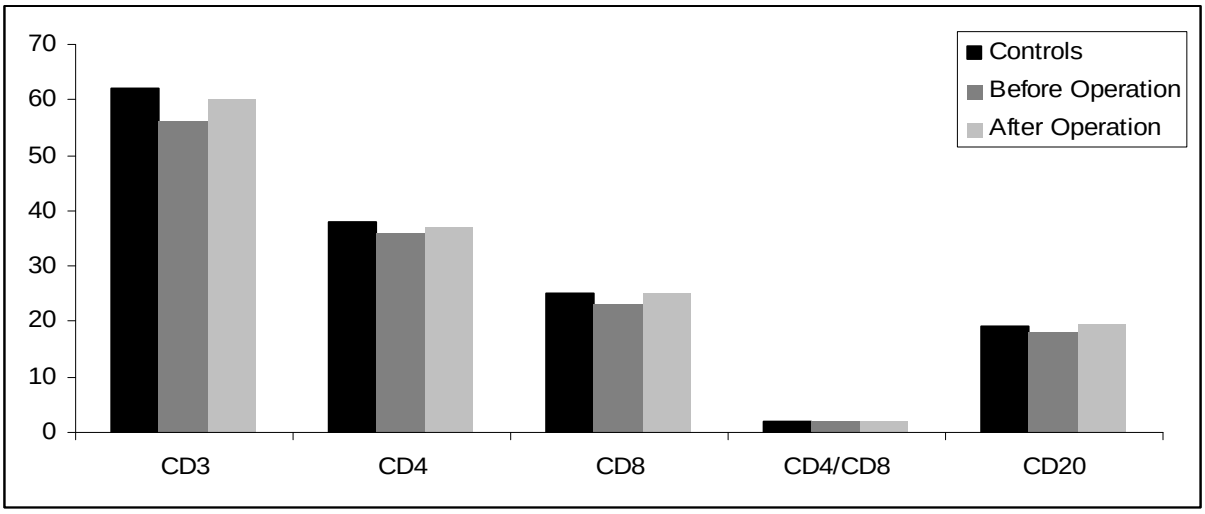

Fig. 1. Diagram of results

In a similar Turkish study in 2003, the percentage of T lymphocytes (CD3), T helper cells (CD4) and ratio of TCD4/TCD8 were lower than the control group, while the percentage of TCD8 and B lymphocytes (CD20) was higher (5). In another study in 2002, the percentage of T(CD3), T(CD4) and TCD8 lymphocytes was lower than the control group, but the percentage of BCD 19 lymphocytes was reported to be higher (4). In a study in Poland in 2002, the percentage of T(CD3) and ratio of TCD4/TCD8 were lower in the patients as compared to control group (7).

Decrease in pre-operative levels of T and B lymphocytes have also been reported by Bussi *et al.* (17), Bock *et al.* (18) and Prusek *et al.* (19). Although only decrease in the percentage of T (CD3) lymphocytes in our study group was statistically significant compared to control group, decrease in other parameters was also clinically important.

Inflammation of the reticular crypts during adenotonsillitis results in proliferation of active immunological cells that responsible for local defense. However, in the chronic inflammation, the antigenic transfer ability of the crypts decreases. The activation of local B lymphocytes is also affected and results in decrease in the number of B cells (1). The response of TCD4 which depend on B cells is also affected (20, 21). The higher levels of TCD8 in some above studies before operation is due to viral infections, but this point must be noted that increase in TCD4 and TCD8 have occurred in the initial stages of infection (22). In almost all of the above-mentioned studies, the percentages of T lymphocytes in the study group have been lower than the control group before operation.

In the present study 6 months after operation the percentage of T lymphocytes (CD3), T helper cells (CD4), T cytotoxic cells (CD8) and B lymphocytes (CD20) increased as compared to the pre-operative levels of which the rise in TCD3, TCD8 and BCD20 levels were significant. In the Turkish and Poland studies the percentage of lymphocytes after operation increased and became similar to the control group (4, 5, 7). Prusek also reported an increase in the percentage of B and T lymphocytes in children aged between 4-11 years after adenotonsillectomy (19). Bock in a follow up of patients 6 months after operation reported an increase in lymphocytes and concluded that removal of the glands does not prevent the growth of the immune system in children (18). Bussi also reported an increase in the percentage of lymphocytes 3 months after operation (17).

In our study serum IgG levels were higher than normal limit in both groups. The IgG levels decreased significantly in the study group 6 months after operation (P = 0.000) but were still within normal limits. The serum IgM levels in the study group before operation and the control group were not different and 6 months after operation its value

was still within normal limits and not significantly different from the pre-operative levels. El Ashmawy reported that the IgG levels increase in chronic tonsillitis while the IgM levels do not change significantly and 2 months after operation, the IgG levels had decreased in his study (23). Sain *et al.* reported that the increased levels of immunoglobulins decrease significantly after operation (24). Zielnik-Jurkiewicz also reported an increase in IgG, IgM and IgA levels before operation and subsequent decrease one month after operation and stated that adenotonsillectomy results in defect in humoral immunity in children suffering from chronic adenotonsillar hypertrophy (7). In both Turkish studies (4, 5), the levels of immunoglobulins were increased before operation and decreased after operation. In most of the above mentioned studies, the levels of immunoglobulins were increased before operation and decreased after operation due to removal of infected tissue and continuous antigenic stimulation (12, 14).

IgM antibodies are produced during the acute phase of infectious diseases and their serum levels decrease 1-3 months after infection. Thus IgM levels do not increase significantly during chronic infections. But IgG antibodies represent in the chronic infections and their levels are also increased in recurrent infections (25, 26). The high IgG and normal IgM levels prior to operation in our study in comparison to other studies wherein the IgM and IgG levels were above normal, shows that the course of disease in our patients was more chronic. The high levels of IgG in the control group highlight the continuous antigenic exposure in them. In other words, in contrast to certain studies where the presence of high levels of immunoglobulins together with clinical features are considered as an appropriate marker for adenotonsillectomy (7, 15), in Iranian population where high levels of immunoglobulins are present in children without clinical symptoms of adenotonsillar hypertrophy, immunoglobulin levels cannot be used as a marker of disease when operation is considered.

Certain studies have shown that chronically inflamed adenotonsillar tissue does not play an important role in defense against upper respiratory infections and in these circumstances acts as an organ responsible for decreased general immunological response and increased frequency and severity of upper respiratory infections (5,10).

In conclusion, chronic adenotonsillar hypertrophy results in changes in cellular and humoral immune parameters and adenotonsillectomy can reverse some of these changes without having a negative effect. But in order to ascertain whether these normal immunological conditions remain for a long time further studies with longer follow up periods are required. Thus, even though clinical parameters are the main basis of decisions for operations, conservative approach in cases of adenotonsillectomies, especially in children from the immunological point of view seems to be suitable.

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Conflict of interests

The authors declare that they have no competing interests.

REFERENCES

1. Waitrak BJ, Woolley AL. Pharyngitis and adenotonsillar disease. In: Cummings CW, Flint PW, editors. Cummings otolaryngology head and neck surgery. Fourth edition. Philadelphia: Elsevier Mosby; 2005. P. 4135-4139.
2. Kipps TJ. The lymphoid tissues. In: Beutler E, Lichtman MA. William's Hematology. Sixth edition, New York: McGraw Hill; 2001. P. 59-65.
3. Male D. Introduction to immune system. In: Roitt I, Brostoff J, Male D. Immunology. Sixth edition. Philadelphia: Mosby; 2002. P. 1-12.
4. Ikincioğullari A, Doğu F, ikincioğullari A, Eğin Y, Babacan E. Is immune system influenced by adenotonsillectomy in children? Int J Pediatr Otorhinolaryngol. 2002 Dec 2; 66(3):251-257.
5. Kaygusuz I, Gödekmerdan A, Karlidag T, Keleş E, Yalçin S, Aral I, Yildiz M. Early stage impacts of tonsillectomy on immune functions of children. Int J Pediatr Otorhinolaryngol. 2003 Dec; 67(12):1311-1315.

6. Dono M, Zupo S, Augliera A, Burgio VL, Massara R, Melagrana A, Costa M, Grossi CE, Chiorazzi N, Ferrarini M. Subepithelial B cells in the human palatine tonsil. II. Functional characterization. Eur J Immunol. 1996 Sep; 26(9):2043-2049.
7. Zielnik-Jurkiewicz B, Jurkiewicz D. Implication of immunological abnormalities after adenotonsillotomy. Int J Pediatr Otorhinolaryngol. 2002 Jun 17; 64(2):127-132.
8. Ogra PL. Effect of tonsillectomy and adenoidectomy on nasopharyngeal antibody response to poliovirus. N Engl J Med. 1971 Jan 14; 284(2):59-64.
9. Brodsky L, Poje C. Tonsillitis, tonsillectomy and adenoidectomy. In: Bailey BJ, editor. Otolaryngology. Third edition. Philadelphia: Lippincott Williams and Wilkins; 2001. P. 982.
10. Paulussen C, Claes J, Claes G, Jorissen M. Adenoids and tonsils, indications for surgery and immunological consequences of surgery. Acta Otorhinolaryngol Belg. 2000; 54(3):403-408.
11. Friday GA Jr, Paradise JL, Rabin BS, Colborn DK, Taylor FH. Serum immunoglobulin changes in relation to tonsil and adenoid surgery. Ann Allergy. 1992 Sep; 69(3):225-230.
12. FIlatova SV, Simonova AV, Artem'ev ME, Golubeva NM. [Immune status of patients with chronic tonsillitis before and after tonsillectomy]. Vestn Otorinolaringol. 2002;(1):18-21. Russian.
13. Brandtzaeg P. Immunology of tonsils and adenoids: everything the ENT surgeon needs to know. Int J Pediatr Otorhinolaryngol. 2003 Dec;67 Suppl 1:S69-76.
14. Moreno PM, Sanchez M, Sainz M, Gutierrez F. Changes in immunological response in tonsillectomized children. II. Decreased cellular response. Clin Otolaryngol Allied Sci. 1992 Oct;17(5):380-382.
15. Cantani A, Bellioni P, Salvinelli F, Businco L. Serum immunoglobulins and secretory IgA deficiency in tonsillectomized children. Ann Allergy. 1986 Dec; 57(6):413-416.
16. Wysocka J, Hassmann E, Lipska A, Musiatowicz M. Naive and memory T cells in hypertrophied adenoids in children according to age. Int J Pediatr Otorhinolaryngol. 2003 Mar; 67(3):237-241.
17. Bussi M, Carlevato MT, Galeazzi E, Morra B. Immunological investigations on tonsillar and peripheral blood lymphocytes after adeno-tonsillectomy. Possible suggestions for phenotypical and functional differences. Acta Otolaryngol. 1991;111(2):379-383.
18. Böck A, Popp W, Herkner KR. Tonsillectomy and the immune system: a long-term follow up comparison between tonsillectomized and non-tonsillectomized children. Eur Arch Otorhinolaryngol. 1994;251(7):423-427.
19. Prusek W, Agopsowicz T, Podwysocka M. T and B lymphocytes in peripheral blood and tonsils of children after tonsillectomy. Arch Immunol Ther Exp (Warsz). 1983;31(4):489-496.
20. Trowsdule J. Antigen presentation. In: Roitt I, Brostoff J, Male D. Immunology. Sixth edition. Philadelphia: Mosby; 2002. P. 112-113.
21. Murray PR, Rosenthal K. Medical Microbiology. Fourth edition. Philadelphia: Mosby; 2002. P. 116-139.
22. Costello M, Yungbluth M. Viral infections, clinical diagnosis and management by laboratory methods. Nineteenth edition. Philadelphia: W. B. Saunders company; 1996. P. 1109.
23. El-Ashmawy S, Taha A, Fatt-hi A, Basyouni A, Zaher S. Serum immunoglobulins in patients with chronic tonsillitis. J Laryngol Otol. 1980 Sep; 94(9):1037-1045.
24. Sainz M, Gutierrez F, Moreno PM, Muñoz C, Ciges M. Changes in immunologic response in tonsillectomized children. I. Immunosuppression in recurrent tonsillitis. Clin Otolaryngol Allied Sci. 1992 Oct; 17(5):376-379.
25. Bawa N, Tomar RH. Laboratory evaluation of imunogloboline function and humoral immunity, Clinical diagnosis and management by laboratory methods. Nineteenth edition. Philadelphia: W. B. Saunders Company; 1996. P. 917-918.
26. Turner M. Antibodies. In: Roitt I, Brostoff J, Male D. Immunology. 6th edition. Philadelphia: Mosby; 2002. P. 65-67.