

Effect of Tonsillectomy on Cellular and Humoral Immunity in Children in Qena GovernorateEssam A. Abo Elmagd¹, Mona M. Abdel Meguid², Sanaa S. Ali², Samar S. Ahmed³ and Ahlam M. Ismail⁴¹Ear, Nose and Throat (ENT) Department; ²Clinical and Chemical Pathology Department, ³Community Medicine Department and ⁴Pediatrics Department, Faculty of Medicine, South Valley University, Qena, Egypt
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Abstract: Tonsillectomy represents one of the most common operations performed in children. T and B lymphocytes are the most important components of the immune system and cellular and humoral responses depend upon their activities. The purpose of this study was to investigate the changes in the cellular and humoral immunity of children (8 to 15 years) with chronic tonsillitis before and 1 month after tonsillectomy. For this; 30 patients scheduled for tonsillectomy were enrolled in this study and also 30 healthy children as controls age and sex matched with the operated children without a history of upper respiratory tract infection or infected tonsils. The percentage of CD3, CD4, CD8 and CD19 were measured for cellular immunity and serum levels of complement fraction C3 and C4 were measured for humoral immunity in blood samples taken from these patients before and 1 month after operation. Results when comparing postoperative period with preoperative were as follow: the percentage of CD3 and CD4 were significantly increased postoperatively as compared to preoperative period (P value = 0.001). The percentage of CD8 and CD19 were reduced postoperatively when compared with preoperative with no significant reduction (P value = 0.09, 0.053 respectively). The ratio of CD4/CD8 was slightly increased but it was statistically insignificant. The serum level of C3 was reduced and the serum level of C4 was increased but both were statistically insignificant.

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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 (**Ikinciogullari et al, 2002**). 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 (**Jung et al, 1996**). The tonsils produce immune-globulins from the fetal stage, although they do not acquire important levels until birth (**Redondo et al, 2000**).

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 (**Lal et al, 1984**). T and B lymphocytes are the most important components of the immune system and the cellular and humoral responses depend upon their activities (**Kipps, 2001; Male, 2002**).

Although adenotonsillar tissues are immunologically active in children aged between 4 and 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 (**Waitrak and Woolley, 2005**). Various studies reported that the changes occurring in cellular and humoral immunity after adenotonsillectomy are not significant enough to result in increasing frequency of diseases (**Kaygusuz et al, 2003; Filatova et al, 2002**). 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, it is important and conservative approach for removal of them is advocated (**Brandtzaeg, 2003; Moreno et al, 1992**). Adding to that chronic streptococcal immune stimulus within the tonsils could act as a source for pathogenic T cells in post-streptococcal disorders; which may help to explain why eliminating this source with tonsillectomy may improve streptococcal induced squeals (**Diluvio et al, 2006**). Also, certain studies have shown that adenoids play an important role in development of immunological memory in children (**Wysocka et al, 2003**).

The objective of our study is to determine the impact of tonsillectomy on cellular and humoral immunity in children.

2. Subjects and Methods

This study was performed on 30 children 16 females and 14 males, their age ranged from 8 to 15 years with mean \pm SD of 11.5 ± 4.9 . All children presented with chronic adenotonsillar hypertrophy and undergoing surgery. Their weight ranged from 29-51 Kgs with mean \pm SD of 34.5 ± 4.9 and their height ranged from 120-153 cms with mean \pm SD of 137.35 ± 9.8 . The surgery was done at the ENT department, South Valley University in Qena governorate, Upper Egypt from January 2013 through April 2013 after pediatrics consultation and fitness to the operation. 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. Exclusion criteria for cases were unfitness to tonsillectomy or immune-comprised patients. The control group included 30 healthy controls age, and sex matched with the operated children (15 girls and 15 boys, aged from 8 to 14 years (mean age 11.0 ± 4.2 years), also their weight and height lies in the range of the operated children. Exclusion criteria for control were: a history of recurrent upper respiratory tract infections and hypertrophy of tonsils or immune-comprised children.

Five ml blood were obtained by venipuncture (from the antecubital region; 2 ml in a vacuum collection tube (Vacutainer®) with tri-potassium EDTA and the other 3 ml in a plain vacutainer) twice from each diseased child, the day before tonsillectomy (preoperative) and 1 month after the operation (postoperative), and once from the control group. The samples were subjected to the following:

- Complete blood picture (CBC) by Sysmex KX21 (Roach)
- Analysis of peripheral blood total lymphocytes populations and sub-populations by flow cytometry "figure 1" (Becton Dickinson Biosciences, San Jose, California; USA).
- Determination of serum concentration of complement factors C3 and C4 by Cobas C311 (Roach)

Preparation and analysis of peripheral blood lymphocyte populations and sub-populations by flow cytometry:

100 μ l EDTA blood was stained with 10 μ l of the monoclonal antibodies conjugated with fluorochromes (obtained from Becton Dickinson BD (Palo Alto, CA, USA), in triple combinations (CD3 PE, CD4 FITC, CD8 percb).

- CD3PE/CD4 FITC for helper lymphocytes
- CD3FITC/CD8 percb for cytotoxic lymphocytes
- In addition to CD19 FITC for B lymphocytes

The tubes were incubated for 15 minutes at room temperature in the dark. RBC lysis was done. After one wash, the cells were re-suspended in phosphate buffer saline (PBS), and analyzed by FACS Calibur flow cytometry "figure 1" with Cell Quest software (Becton Dickinson Biosciences, San Jose, California; USA). The acquisition and analysis of immunomarked cells were standardized for 10,000 events per sample and an isotype-matched negative control was used with each sample.

The laboratory results were collected for preoperative, postoperative and control cases, entered and analyzed by SPSS version 16 using statistical T-test to identify significant difference between variables. Significant difference is considered when P value is less than 0.05 and/or 0.001.

The design of this study has been approved by the Ethical Committee of South Valley University after verbal consents of the child care-takers for the included children (who undergone tonsillectomy and for the controls) on the research were taken.

3. Results

In our study the total T lymphocytes (CD3+) percentage shows a significant increase in the postoperative than the preoperative cases (table 2 and figure 2). The T helper lymphocytes (CD4+) shows a significant increase in the control group than the preoperative cases and also a significant increase in the postoperative than the preoperative cases (p value = 0.008 and 0.001 respectively (table 1, 2 and figure 2). The other cellular immune parameters including CD8+, CD4+/CD8+ and CD19+ show insignificant changes between the three groups included in the study (table 1, 2 and figure 2). Also the humoral parameters studied (C3 and C4) show insignificant changes (table 1, 2 and figure 3).

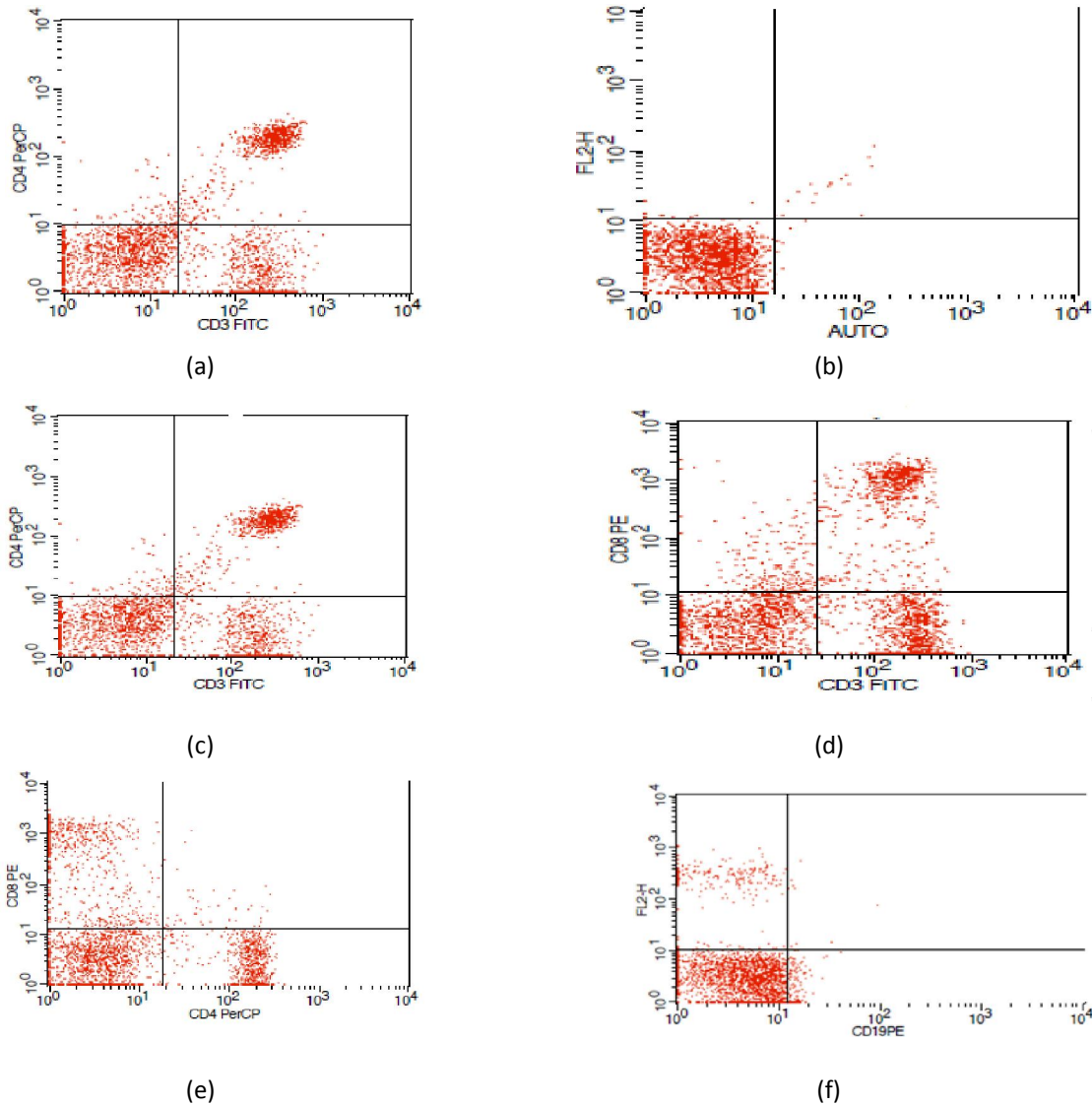


Figure (1): Flowcytometry detection of lymphocytes subpopulation: (a) Forward and side scatter histogram was used to define the total lymphocytes. (b) Isotopic control runs with each sample. (c) Expression of CD3+CD4+helper lymphocytes in total lymphocytes. (d) Expression of CD3+CD8+cytotoxic cells lymphocytes in total lymphocytes (e) Expression of CD4+/CD8+ ratio (f) Expression of CD19.

Table 1: Cellular and Humoral Immune Parameters in Preoperative, Postoperative and control groups

Parameter	Pre-Operative Cases	P 1 Value	Control	P 2 Value	Post-Operative Cases
CD3+ (%)	53.3 ± 9.0	0.124	59.7 ± 15.3	0.42	62.8 ± 6.3
CD4+ (%)	35.3 ± 4.9	0.008*	39.1 ± 3.4	0.7	38.8 ± 4.6
CD8+ (%)	21.2 ± 2.7	0.4	22.2 ± 4.4	0.08	20.1 ± 2.5
CD4+/CD8+	1.8 ± 0.2	0.5	1.8 ± 0.3	0.9	1.8 ± 0.3
CD19+ (%)	11.2 ± 3.2	0.174	9.7 ± 3.4	0.521	9.1 ± 2.6
C 3 (mg/dl)	137.0 ± 21.9	0.1	134.0 ± 23.2	0.8	135.9 ± 22.7
C 4 (mg/dl)	25.6 ± 8.2	0.194	23.1 ± 5.0	0.118	32.2 ± 24.1

P1: t- test for preoperative and control groups;
* Significant difference

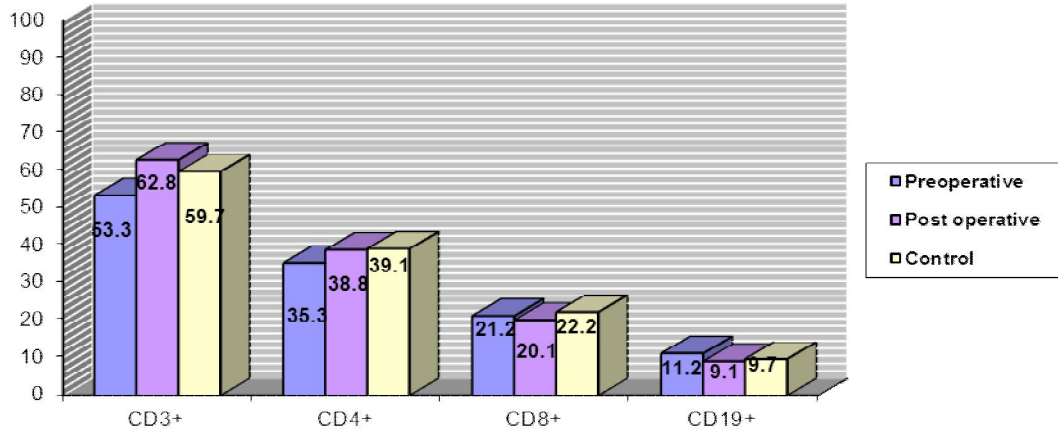
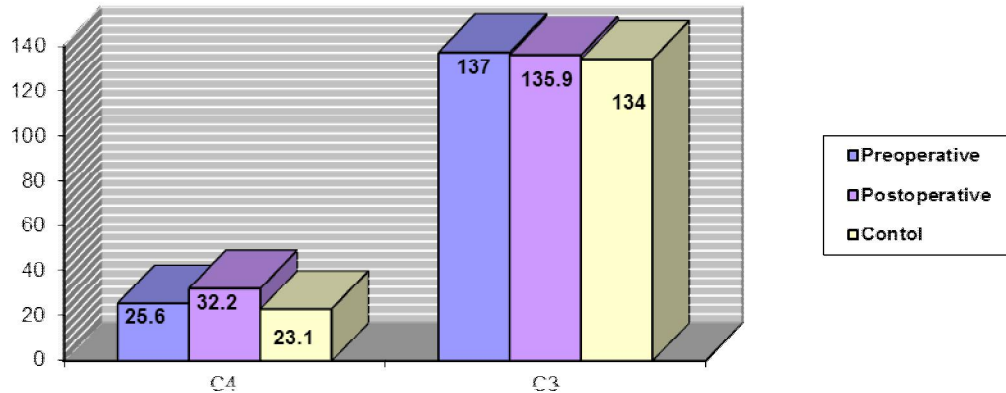
P2: t- test for postoperative and control groups

Table 2: Cellular and Humoral Immune Parameters in Preoperative and Postoperative Patients

Parameter	Pre-Operative Cases	Post-Operative Cases	P* Value
CD3+ (%)	53.3 ± 9.0	62.8 ± 6.3	0.001**
CD4+ (%)	35.3 ± 4.9	38.8 ± 4.6	0.001**
CD8+ (%)	21.2 ± 2.7	20.1 ± 2.5	0.09
CD4+/CD8+	1.8 ± 0.2	1.8 ± 0.3	0.4
CD19+ (%)	11.2 ± 3.2	9.1 ± 2.6	0.053
C 3 (mg/dl)	137.0 ± 21.9	135.9 ± 22.7	0.1
C 4 (mg/dl)	25.6 ± 8.2	32.2 ± 24.1	0.27

P* Value: Paired t- test for preoperative and postoperative groups;

** Highly Significant difference.

**Figure (2):** Cellular immune parameters in preoperative, postoperative patients and Control**Figure (3):** Humoral immune parameters in preoperative, postoperative patients and Control

4. Discussions

The function of the palatine tonsil is to process the environmental antigen and participate with the initiation and maintenance of the local and systemic immunity. The question of whether removal of tonsils and adenoid compromises the protection of the upper respiratory tract resulting in immunodeficiency continues to be the subject of debate (Kipps, 2001).

The results concerning T and B lymphocytes percentage show controversy from one research to another. Baradaranfar and colleagues, 2007 found that the percentage of T lymphocytes (CD3+), T

helper cells (CD4+), T cytotoxic cells (CD8+) and B lymphocytes (CD20+) in the study group preoperatively were lower than the control group. In a study done by Aral and Yildiz; the percentage of T lymphocytes (CD3+), T helper cells (CD4+) and ratio of CD4+/CD8+ were lower in the preoperative patients than the control group, while the percentage of T cytotoxic (CD8+) and B lymphocytes (CD20+) were found to be higher (Kaygusuz et al, 2003). In another study in 2002, the percentage of T (CD3+), T-helper and T-cytotoxic lymphocytes was lower in the preoperative group than the control group, but the

percentage of B (CD19+) lymphocytes was reported to be higher (**Ikinciogullari et al, 2002**). **Zielnik-Jurkiewicz and Jurkiewicz in 2002** found that the percentage of T (CD3+) and ratio of CD4+/CD8+ were lower in the preoperative patients as compared to control group. The inflammation of reticular crypts during tonsillitis causes proliferation of active immunological cells which is responsible for local defense. However, in chronic inflammation there is a decrease in the antigenic transfer ability of the crypts. The activation of local lymphocytes is also affected and results in decrease in the number of these immunological cells (**Waitrak and Woolley, 2005**).

In this study, we did not find significant difference in the percentage of total T lymphocytes (CD3+) and T-cytotoxic (CD8+); although they decreased in the preoperative children than the control group; while the T-helper (CD4+) show significant decrease in the preoperative group compared to the control group (P= 0.008). B lymphocytes (CD19+); Complement C3 and C4 show insignificant differences between the two groups (table 1, figure 2 and 3). This may be explained by that the patients have a more chronic course of the disease taking broad spectrum antibiotics before tonsillectomy. Also, the majority of children in Upper Egypt are living below the poverty level and it is assumed that they have naturally lowered both cellular and humoral immunity.

We have found that the percentage of CD3+ and CD4+ (which is dependent on B lymphocytes) were significantly increased (P = 0.001 for each) after tonsillectomy than before (table 2 and figure 2). B lymphocytes (CD19+) percentage show insignificant differences between the two groups which come in line with **Faramarzi et al, 2006 and Ikinciogullari et al, 2002** who did not find any statistically significant decrease in B cell numbers after surgery. **Bock et al, 1994 and Bussi et al, 1991** have shown an increase in T and B cell numbers after the surgery. The changes in cellular immunity detected in patients after adenotonsillectomy may be attributed to a modulating effect on systemic immunity of the tonsils to stabilize the activation caused by chronic tonsillitis and adenoid hypertrophy. Therefore; there is no indication that surgery hinders the development of systemic immunity and causes immunodeficiency. The increased T and B lymphocyte activation may be attributed to a struggle of the immune system to compensate for the decrease in B lymphocyte counts (**Ikinciogullari et al, 2002**).

The higher levels of CD8+ in some studies (**Trowsdole, 2002; Murray and Rosenthal, 2002**) before operation are due to viral infections, but the increase in CD4+ and CD8+ have occurred in the initial stages of infection (**Costello and Yungbluth,**

1996). In this study, there is no significant change in the percentage of CD8+ between the three groups (table 1, 2 and figure 2).

In our study, the humoral immunity as indicated by the levels of the serum complements fractions (C3 and C4) did not significantly altered in patients before and after tonsillectomy or even in the apparently healthy children group (tables1, 2 and figure 3). **Sainz et al, 1992** measured the levels of IgG, IgM and IgA in patients with chronic tonsillitis and reported that increased preoperative 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, 2002** 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. 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 increase in microorganisms in the tonsils (**Kaygusuz et al, 2003**).

In this study, we did not find any significant changes in the parameters of cellular and humoral immunity between the control and the postoperative groups which was explained by **Prusek and his colleagues, 1983** that removal of the glands does not prevent the growth of the immune system in children.

In conclusion, humoral immunity as indicated by the complement fractions (C3 and C4) is not affected by tonsillectomy. Chronic tonsillitis results in changes in cellular immune parameters as a response to chronic bacterial stimulus and removal of this stimulus by tonsillectomy can reverse some of these changes without having a negative effect. So, tonsillectomy when indicated is preferred to prevent post-streptococcal sequels. The small sample size is a limitation in our study so there is a need for further investigations on a large scale to determine whether the immune system maintains its normal status in the long-term in the post-operative period and whether the age of the patients influences the immune alterations detected after surgery.

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