Expression of CD3 and CD20 in Antistreptolysin-O Titer Seropositive and Seronegative Children with Chronic Tonsillitis

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Abstract

Background: Chronic tonsillitis (CT) is a common inflammatory illness in children, and serum antistreptolysin O titer (ASOT) is a common investigation performed for these cases and considered a perfect sign for tonsillectomy. Objective: To evaluate the expression of tonsillar T-and B-lymphocytes markers in relation to seropositive or seronegative ASOT in cases of CT. Materials and Methods: Thirty children (15 males and 15 females) aged 6–10 years were divided equally into two groups: Group A seropositive ASOT (≥400 IU) and Group B seronegative ASOT (<400 IU). Both performed bilateral tonsillectomy. Specimens from the removed tonsils were taken and prepared for light microscopic examination and immunohistochemical evaluation of CD20 and CD3 expression. Results: Seropositive ASOT group showed significant histopathologic changes in the form of hyperplasia of the stratified squamous nonkeratinized epithelium, Urgas’s abscess, and severe lymphocytic infiltration. Immunohistochemical results of seropositive ASOT group showed marked expression of CD3 and CD20, while seronegative ASOT group showed mild expression of CD3 and CD20. Conclusion: Seropositive ASOT CT, in addition to histopathological changes, is associated with significant increase in both B-lymphocytes (CD20 expression) and T-lymphocytes (CD3 expression) markers.

Keywords: Antistreptolysin-O titer, CD20, CD3, chronic tonsillitis, immunohistochemistry, tonsillectomy

INTRODUCTION

Chronic tonsillitis (CT) is a common chronic inflammatory disease of the palatine tonsils that requiring surgical removal of the diseased tonsils.[1] It can bring about multiple complications in children both nearby provincial spreads such as acute rhinitis, sinusitis, and descending respiratory infections or at the distance such as glomerulonephritis, joint rheumatism, rheumatic fever, endocarditis, and appendicitis.[2]

Palatine tonsils are placed in the oropharyngeal inlet passageway for invulnerable protection against took in and breathed pathogens. Histologically, the tonsil made of mass of lymphoid follicles supported on a connective tissue framework; the tonsillar crypts penetrate from the surface, down to the center of the tonsil follicle. The luminal surfaces of the tonsils are covered with nonkeratinizing stratified squamous epithelium, which is the same tissue of the surrounding oropharynx. Protection of this zone relies upon both inborn and acquired immune responses. As indicated by their lymphoid nature, tonsils are the introductory destinations for both humoral (B-lymphocytes) and cell-mediated (T-lymphocytes) immune reactions. T-lymphocytes, specifically, are available in plenty in palatine tonsils and are to a great extent situated in the extrafollicular zones. Tonsils have been portrayed as destinations of acceptance of oral invulnerable resistance. In spite of its visionary significance, there are high quantities of tonsillectomy medical measures done to avoid complications result from tonsillitis.[3]

Histopathologically, in CT, the involved tonsils became congested, hypertrophied, or covered with white-faint bogus membranes. The tonsillar lymphoid follicles showed up as hyperplastic and hypertropic with excessive development of the germinal clear center.[4]
The most ordinarily used investigation for CT is serological detection of antistreptolysin-O titer (ASOT), a toxic enzyme produced by Group A beta hemolytic streptococcal (GABHS). This test is especially used in school-age children who are progressively susceptible to frequent streptococci infections because of its accessibility, less price, and reasonable sensitivity. Hembrum et al. thought about the affectability of high ASOT (seropositive) with throat swab and tonsillar core culture and found that ASOT sensitivity is 100%. They considered it as a valid and dependable tool for the diagnosis of recurrent tonsillitis and a perfect indication for tonsillectomy. Seropositive ASOT was found to be associated with increased number of circulating T-lymphocytes, which can lead to serious complications. However, many cases with seronegative ASOT were forced to undergo tonsillectomy. Up to our awareness, barely any studies have been made to correlate between tonsillar histopathological changes and CD3 and CD20 markers’ expression with changes in ASOT levels.

The aim of our study was to evaluate T- and B-lymphocytes (tonsillar lymphoid nodules) CD3 and CD20 markers’ expression in relation to seropositive or seronegative ASOT.

**Materials and Methods**

This study was conducted at Ear, Nose and Throat (ENT) and Histology Departments, Faculty of Medicine, Jazan University, KSA, and Faculty of Medicine, Suez Canal University, Egypt, respectively.

**Ethical considerations**

Written informed consent was acquired from first-degree relatives of the children. The local ethics committee in both faculties approved the study.

**Selection of patients**

Thirty children (6–10 years), including 15 males and 15 females, who made tonsillectomy in the period from October 2017 to April 2018, were included in this study. The indications for tonsillectomy are as follows:

1. Recurrent attacks of acute tonsillitis: In the form of at least five attacks per year for the last two successive years.
2. Obstructive sleep apnea: when there were huge tonsils associated with mouth breathing, snoring, and history of recurrent apnea during sleep.
3. Peritonsillar abscess (quinsy): After second quinsy, tonsillectomy was recommended.
4. Unilateral tonsillar enlargement.

Medical history and ENT examination of the patients were done 1 day before surgery and routine investigations included hemoglobin level, bleeding and clotting time, partial thromboplastin time, and ASOT. Exclusion criteria were general contraindications for tonsillectomy such as bleeding tendency or acute infection.

**Clinical grade classification**

Clinical tonsillar grading was done according to Dell’Aringa et al., grading classification which depends on the percentage of air obstruction caused by enlarged tonsils as follows: Grade 0 (intravicular tonsils), Grade 1+ (25% air obstruction due to protruding tonsils out of tonsillar fossa), Grade 2+ (25%–50% air obstruction), Grade 3+ (50%–75% air obstruction), and Grade 4+ (75% air obstruction).

**Serological test**

Children were set into two equal groups according to ASOT results: Group A seropositive ASOT (≥400 IU) and Group B seronegative ASOT (<400 IU). ASO latex agglutination test was done for the measurement of antibodies to antistreptolysin-O in human serum. The presence of an ASOT of 400 IU/mL or higher in the serum gives a visible agglutination of the latex particles.

**Surgical operation**

For all patients, bilateral tonsillectomy was performed under general anesthesia. Antibiotic administration was achieved (ceftriaxone at a dose of 50 mg/kg) intraoperatively and was continued for 10 days postoperatively (amoxicillin and clavulanate 90 mg/kg).

**Histopathological studies**

The removed tonsils were identified as right and left and fixed for 24 h in 10% neutral-buffered formalin. Specimens from each tonsil including surface epithelium were taken (5 pieces/each tonsil). Sections from the right tonsil paraffin blocks of 5 µm thickness were prepared and stained with Hematoxylin and Eosin (H and E) and Masson’s trichrome stain. Samples from the left tonsil were processed for immunohistochemical staining of CD20 (Monoclonal Mouse Anti-Human CD20, Dako, USA) and CD3 (Polyclonal Rabbit Anti-Human CD3, Dako, USA) in order to reveal B-lymphocytes and T-lymphocytes. Sections were deparaffinized, rehydrated, and incubated in 0.3% H2O2 for 1 h, for prohibiting the endogenous peroxidase, and then washed with phosphate-buffered saline. Sections were incubated for 1 h in the primary antibody (dilution range 1:100–300). For negative controls, sections were run as routine with skipping the adding of primary antibody. The binding of the primary antibody was disclosed using avidin–biotin–peroxidase detection kit (Dako, USA) as described by the manufacturer’s instructions. DAB Chromogenic (1–2 min) was used and slides were counterstained by Harris’s hematoxylin, then dehydrated and coverslipped.

**Morphological and morphometric analysis**

- Hematoxylin and Eosin-stained sections were examined regarding the presence of:
  a. Abnormal proliferation of lymphoid follicles (follicular hyperplasia)
  b. Focal aggregates of lymphoid tissue (reactive lymphoid hyperplasia)
  c. Small or large intraepithelial groups of tightly aggregated lymphocytes located within a vacuole that leading to a defect in the surface epithelium (Urgas’s abscess).

- In the other stains, the following parameters were measured using the imaging software analyzer (Image-Pro Plus 2D),
Histology Department, Faculty of Medicine, Suez Canal University, Egypt:
1. The mean color area percentage of collagen fibers using Masson’s trichrome stain
2. The mean count of CD3 positive cells
3. The mean count of CD20 positive cells
4. The mean epithelial thickness of the tonsillar crypt.

All the measurements were taken at five nonoverlapping high-power fields of magnification (>400/section) in three random sections/block.

Statistical analysis
The data collected were processed using one-way analysis of variance of SPSS version 15 (SPSS Inc., Chicago, IL, USA) and post hoc significant difference. Quantitative data were expressed as means ± standard deviation. The significance of the data was determined by the P value. P < 0.05 was considered statistically significant.

Results
Clinical results
There was no statistically significant difference in the mean intensity of CT symptoms severity according to visual analog scale (VAS) before tonsillectomy between Group A and Group B [Table 1]. At the same time, most of patients were clinically in Grade 1+ and 2+ in both groups without any statistically significant difference [Table 2].

Histopathological results
H&E-stained sections of tonsils in Group A (ASOT seropositive) showed marked histopathologic changes in the form of increased thickness (hyperplasia) of the stratified squamous nonkeratinized epithelium that was resting on its basement membrane, in addition to severe lymphocytic infiltration in epithelial and subepithelial areas. The mean epithelial thickness was 174.7, which was significantly higher than that of Group B (P < 0.0001) [Figure 1a and Table 3]. Furthermore, marked intraepithelial aggregates of lymphocytes within a vacuole (Urgas’s abscess) [Figure 1a], and subepithelial lymphocytic infiltration and congested blood vessels were also seen. Some epithelial cells showed vacuolated cytoplasm and pyknotic nuclei [Figure 1b]. Tonsillar crypts lined with thick stratified squamous nonkeratinized epithelium and infiltrated with lymphocytes were detected. Many enlarged (hyperplastic) lymphoid nodules with prominent reactive pale germinal center and inflammatory infiltrate rich in lymphocytes and hyalinized stroma (enriched in collagen fibers arranged in fascicles) were seen [Figure 1c and d].

H and E-stained sections of tonsils in Group B (ASOT seronegative) showed nonkeratinized stratified squamous epithelium resting on a basement membrane. The mean epithelial thickness was 42.46 [Figure 2a and Table 3]. Lymphatic nodules showed compact aggregates of lymphocytes arranged in the form of dark peripheral ring and pale germinal center. Diffuse lymphocytes were detected between lymphatic nodules [Figure 2b]. Tonsillar crypts lined by thin stratified squamous nonkeratinized epithelium were seen [Figure 2c].

Group A tonsillar sections stained with Masson’s Trichrome revealed marked increase in the green collagen fibers, between the lymphoid nodules and around blood vessels. The mean optical density of collagen fibers (632.73) was statistically significant when compared with Group B (P < 0.0001) [Figure 3a]. While tonsillar sections in Group B revealed fine collagen fibers in between lymphoid nodules and around blood vessels. The mean optical density of collagen fibers was 206.03 [Figure 3b and Table 4].
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Figure 1: Photomicrograph of a tonsil of seropositive group, showing (a) hyperplastic stratified squamous non-keratinized epithelium (Line), Urgas’s abscess (U), lymphocytic infiltration in both epithelial & subepithelial areas are shown (L). (H&E X200). (b) Massive subepithelial inflammatory infiltrates (L), dilated congested blood vessels (D) and some epithelial cells with vaculated cytoplasm (V) and pyknotic nuclei (P) are shown. (H&E X200). (c) Tonsillar crypts (C) lined with thick epithelium (E) infiltrated with lymphocytes, diffuse Lymphocytes (DL) and lymphoid nodules with prominent germinal centers are shown (GC). (H&E X100). (d) Inflammatory infiltrate rich in lymphocytes and hyalinized stroma (enriched in collagen fibers arranged in fascicles) also shown (F). (H&E X400)

Figure 2: Photomicrograph of a tonsil of seronegative group, showing (a) non-keratinized stratified squamous epithelium with a normal thickness (Line) resting on a basement membrane. (H&E X400). (b) Lymphoid nodule containing a pale germinal center (GC) and a dark peripheral ring of lymphatic cells (Arrows) and diffuse lymphocytes are also shown (D) (H&E X200). (c) Tonsillar Cyrrts (C) lined with thin epithelium (E) and lymphoid nodules with a pale germinal centers (GC) and dark peripheries are shown. (H&E X100)

Using immunohistochemical technique, tonsillar expression of CD3 (T-lymphocytes) in Group A appeared significant increase in the brown color reaction in between the lymphoid nodules when compared to Group B ($P < 0.0001$). The mean optical density of CD3 was 522.74 [Figure 4a]. While Group B showed mild brown color reaction in between lymphoid nodules. The mean optical density of CD3 was 272.82 [Figure 4b and Table 5].

Using immunohistochemical technique, tonsillar expression of CD20 (B-lymphocytes) in Group A indicated significant rise in the brown color reaction within the lymphoid nodules when compared to Group B ($P < 0.0001$). The mean optical density of CD20 was 256.53 [Figure 5a]. Group B showed faint brown color reaction within the lymphoid nodules. The mean optical density was 43.53 [Figure 5b and Table 6].

**Discussion**

CT is one of the most frequent chronic inflammation of the palatine tonsils affecting children that requiring surgical removal. The current study was designed to assess the expression of T- and B-lymphocytes (tonsillar lymphoid nodules) in relation to ASOT seropositive or seronegative in thirty children (6–10 years) diagnosed as CT and were subjected to conventional dissection tonsillectomy in ENT Department, Faculty of Medicine, Jazan University, Kingdom of Saudi Arabia, from October 2017 to April 2018.

Antistreptolysin antibodies are produced about 7:30 days following a *GABHS* infection. The ASOT antibody titer peaks at about 3–5 weeks after the illness and then tapers off but may remain detectable for several months after the streptococcal infection has resolved.\[^{10}\] In the current study, the tonsillar histopathological changes in Group A were in the form of marked lymphoid hyperplasia and lymphocytic infiltration in epithelial and subepithelial areas and Urgas’s abscess in the surface epithelium. Tonsillar crypts lined by thick stratified squamous nonkeratinized epithelium and increased amount of collagen fibers between the lymphoid nodules and around blood vessels were observed. While; Group B showed significant decrease in epithelial thickness in surface epithelium and tonsillar crypts, diffuse lymphocytes were found in between lymphoid nodules, and significant decreased amount of collagen fibers between the lymphoid nodules and around blood vessels. These results were in accordance with...
In the current study, the serological results were in accordance with the histopathological and immunohistochemical results, in which positive ASOT in Group A indicated recent streptococcal infection, which, in turn, reflected marked increase in expression of CD 20 and CD3 in the tonsillar tissue. However, negative ASOT in Group B indicated null streptococcal infection and consequences negative expression of CD 20 and CD3 in the tonsillar tissue.

**CONCLUSION**

Seropositive ASOT CT, in addition to histopathological changes, is associated with significant increase in both B-lymphocytes (CD20 expression) and T-lymphocytes (CD3 expression) markers. Further work should be done to explain the exact mechanisms that lead to increase of CD3 and CD20 expression in seropositive ASOT CT.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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