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BACTERIAL PROFILE ASSOCIATED WITH CHRONIC TONSILLITIS AND ADENOID HYPERTROPHY IN CHILDREN. A BACTERIOLOGICAL AND HISTOPATHOLOGICAL STUDY

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ABSTRACT
This study aimed to investigate the bacterial profile of surface and core of infected tonsils and adenoid tissues and histopathological examination of these organs. A total of 122 samples represented by 63 excised tonsils and 59 excised adenoids in addition to 57 blood samples were collected from 70 patients who were referred to Hilla Teaching Hospital (Ear, Nose and Throat unit) in Hilla city within a period of four months from November 2015 to February 2016. Those patients were suffering from tonsillitis and adenoid hypertrophy. The ages of those patients ranged from 2 to 15 years. All specimens were subjected to identify the aerobic bacterial types associated with chronic tonsillitis and adenoid hypertrophy by biological, histopathological and serological tests. ASO titer was estimated as immune marker by rapid latex agglutination test. The results indicated that, the most age group being susceptible for tonsillitis and adenoid hypertrophy was the group of 7-10 years, represented 33 patients with a percentage of (47%). Among this group, males were more susceptible with percentage of (59%) compared with female patients (41%). In this study, *S. aureus* was the common isolate from tonsillar surface of 31 patients with percentage (18.7%), while *K. pneumonia* from tonsillar core 21 patients (12.7%). While the most common isolates from surface of adenoid founded that both of *S. pyogenes* and *S. aureus* were 17.8%, while *E. coli* was the most common isolate from core of adenoid 11%. The result of ASO titer showed that 6 (18%) males were had positive ASO titer, while only 2 (9%) females had positive ASO titer. In this study, the histopathology examination of tonsil and adenoid showed the presence of lymphoid hyperplasia, multiple lymphoid follicles, vascular congestion, germinal centers, fibrosis and infiltration of inflammatory cells. In conclusion, the adenoid and tonsil bacterial flora is polymicrobial in nature. *S. aureus* is the most predominant isolate of tonsillar surface, while *K. pneumoniae* is the most predominant in tonsillar core. Most predominant isolates of adenoidal surface are *S. pyogenes* and *S. aureus*, while *E. coli* is most common isolate in adenoidal core.

KEY WORDS: chronic tonsillitis, adenoid hypertrophy, ASO titer.

INTRODUCTION

Tonsillitis affects all age groups especially children (Babita *et al.*, 2014). It is mainly attributed to microorganisms represented by bacteria and viruses. Tonsillitis is acute, recurrent acute or chronic tonsillitis. Efforts have been made to manage the infectious disease of tonsils. It has been reported that the impact of tonsillar diseases may not only affect the tonsils alone but it can reach other related anatomic structures like the paranasal sinus, upper aerodigestive tract, and Eustachian tube-middle ear complex (Bista *et al.*, 2006). On the other hand, some studies have reported that bacteria causing tonsillitis inhabit not only the tonsillar surface but also the tonsillar deep tissue (Panga *et al.*, 2013). *Streptococcus pyogenes* is an important pathogen that infects tonsils (Peter and Ray, 2007). *S. pyogenes* is of a major clinical importance because it can trigger post-infection systemic complications, acute rheumatic fever, and post-streptococcal glomerulonephritis (Anjos *et al.*, 2014). The complications of tonsillitis are acute otitis media, peritonsillar abscess (quinsy), parapharyngeal abscess, retropharyngeal abscess, pulmonary infections (pneumonia), glomerulonephritis, rheumatic fever and scarlet fever (Peter and Ray, 2007). Adenoid hypertrophy (adenoid vegetation) and associated symptoms of children are a common condition in pediatrics and ENT practice that is responsible for a great clinical problem (Marseglia *et al.*, 2011). Nasopharyngeal obstruction caused by adenoid hypertrophy may lead to several other diseases and symptoms such as hyponasality, snoring, obstructive sleep apnea syndrome (OSAS), acute otitis media, otitis media with effusion (OME), middle ear atelectasia, cholesteatoma formation, slow feeding, acute sinusitis, abnormal facial development, and behavioral problems (Arens and Marcus, 2004).

MATERIALS & METHODS

The study involved investigation at excised tonsils and adenoids and serum that collected from 70 patients who suffering from tonsillitis and adenoid hypertrophy. The age of patients ranged 1- 15 years for both sexes (29 females and 41 males) who were referred to Hilla teaching hospital in Hilla city during a period of four months from (November 2015 to February 2016). Swab was collected by rotating a sterile cotton swab over the surface of the tonsil and adenoid. It was placed in transport media and

transported to microbiology lab for bacteriological evaluation. The outer surface of tonsil and adenoid was sterilized by iodine solution. The tonsil and adenoid was totally immersed in beaker contained povidone iodine solution 10% for 30 to 45 seconds. Then the tissue was sectioned by sterile scalpel and put it in sterile cap containing formalin (for histological section), and disaggregating the inner part (core) of tissue by mortar and pestle, centrifugation, and the sediment was cultured on proper culture media. A volume of 2 ml of blood was collected from vein of patient then sterile cotton was placed over the injection site as the needle was removed. The blood was immediately placed in gel tube without anticoagulant, and then transported to the laboratory for additional analysis. The specimens were subjected identify the bacterial types associated with chronic tonsillitis and adenoid hypertrophy by biochemical and serological tests.

1. Bacterial identification: The isolates were identified manually (culturing, staining, biochemical tests) and two isolates were identified by vitek technique.
2. Serological study: Antistreptolysin O Titer (ASOT) rapid latex agglutination test for the qualitative screening and semi quantitative determination of anti-streptolysin O (ASO) antibodies in human serum was employed.
3. Histological sectioning: Eight samples (4 tonsils and 4 adenoids) were selected according to severity of disease. The tonsils and adenoids were removed immediately and fixed in 10% buffered neutral formalin. The specimen was washed with normal saline to carry out through routine procedure by taking serial sections.

Ethical approval

The necessary ethical approval from ethical committee in Hilla Teaching Hospital was obtained. Moreover, all subjects involved in this work were informed and the agreement required for doing the experiments and publication of this work was obtained from each one prior the collection of samples.

RESULTS

A total of 122 samples made up 63 tonsils and 59 adenoids were collected from seventy patients who were suffering from chronic tonsillitis and/or adenoid hypertrophy.

1. Bacteriological study

Table (1) shows the number of samples revealed positive results of the bacterial growth. The surface of all tonsil and adenoid samples exhibited positive results of bacterial growth, while in the core of tonsil 44 samples (70%) were positive results of bacterial growth and 19 samples (30%) were negative results (no growth), on the other hand, the core of adenoids 32 (54%) samples were positive results and 27 (46%) samples were negative results.

TABLE 1: The number of samples according to the growth

	Tonsil		Adenoid	
	Surface n (%)	Core n (%)	Surface n (%)	Core n (%)
Growth	63 (100%)	44 (70%)	59 (100%)	32 (54%)
No growth	-	19 (30%)	-	27 (46%)
Total	63 (100%)	63 (100%)	59 (100%)	59 (100%)

As shown in Table (2), the numbers of cases included in this study were distributed according to age and sex. Among the reported age groups, maximum tonsillitis and adenoid hypertrophy cases were observed in the age group of 7-10 years 33 patients with percentage of (47%) followed by age group ranged from 2-6 years 27 patients (39%) and the least incidence of 10 patients (14%) were shown in age group 11-15 years.

TABLE 2: Distribution of tonsillitis and adenoid hypertrophy according to the age and sex

Age	No. of male (%)	No. of female (%)	Total (%)
2-6 years	14 (20%)	13 (19%)	27 (39%)
7-10 years	20 (29%)	13 (19%)	33 (47%)
11-15 years	7 (10%)	3 (4%)	10 (14%)
Total	41(59%)	29(41%)	70(100%)

TABLE 3: Bacterial types isolated from the entire cases surface and core from both tonsil and adenoid

Gram positive isolates	**Percentage**
Strept. pyogenes	61 (19%)
Staph. aureus	75 (23.6%)
Staph. epidermides	7 (2.2%)
Staph. saprophyticus	1 (0.3%)
Gram negative isolates	
Hemo. influenzae	37 (11.6%)
E. coli	57 (18%)
Kleb. pneumoniae	55 (17.3%)
Sphingo. paucimobilis	14 (4.4%)
Pseudo. aeruginosa	6 (2%)
Pseudo. stutzeri	5 (1.6%)
Total	318 (100%)

A total of ten bacterial types were identified in this study. Four types were Gram-positive and six types were Gram-negative two of them identified by vitek-2 technique as shown in table (3), all these types were described according to colonial morphology, microscopic examination and biochemical tests for each organism.

2. Serological study

Anti-streptolysin O titer (ASOT)

A total of 57 serum samples were collected from 70 patients at Hilla Teaching Hospital. The ASO titer in patients with or without group A streptococcal infection was determined by using latex agglutination test. Table (4) shows the result of ASO titer according to sex; the result showed that male was 6 (18%) had positive ASO titer, while only 2 (9%) females had positive ASO titer.

TABLE 4: The correlation between sex and Anti-Streptolysin-O (ASO) titer

Sex / ASO Titer	Male	Female	Total
Negative (< 200)	28 (49%)	21 (37%)	49 (86%)
Positive (> 200)	6 (10.5%)	2 (3.5%)	8 (14%)
Total	34 (59.5%)	23 (40.5%)	57 (100%)

3. Histopathological examination:

Eight specimens were taking for histopathological examination (4 adenoids and 4 tonsils) according to the severity of disease. In this study, the histopathology examination of tonsil showed the presence of lymphoid hyperplasia, multiple lymphoid follicles, vascular congestion, germinal centers, fibrosis and infiltration of inflammatory cells as shown in Figure (1).

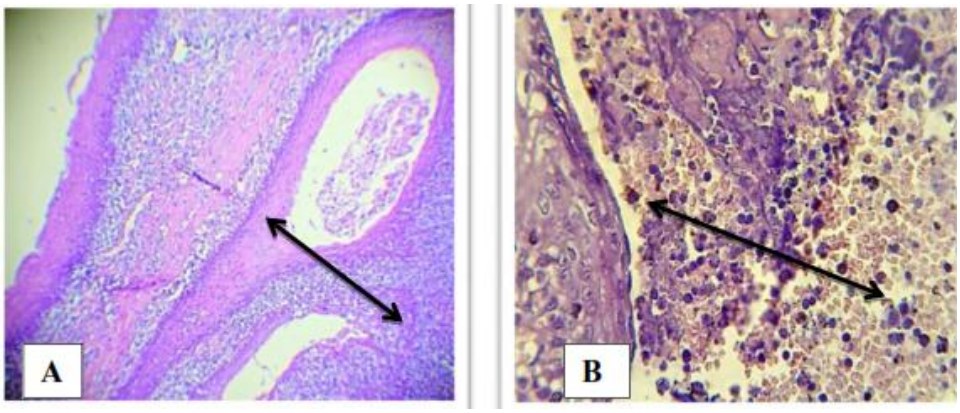

FIGURE 1: Section in the tonsil of patients shows (A) The vascular congestion and lymphoid hyperplasia. (B) Severe hemorrhage with inflammatory cells infiltration particularly neutrophils and mononuclear cells ⟷(H&E stain 100x)

In this study, the stroma of this tissue was composed of fibro collagenous tissue (fibrosis), with neutrophils and mononuclear cells infiltration and vacuolation of epithelial layer as shown in Figure (2).

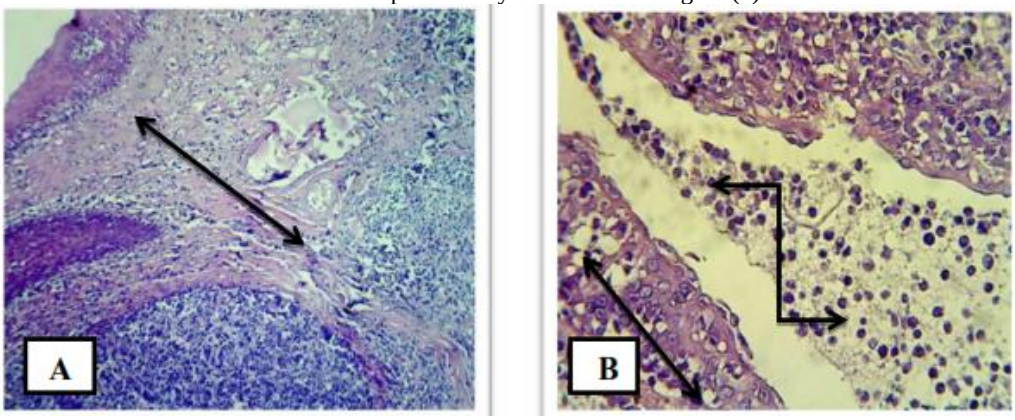

FIGURE 2: Section in the tonsil of patients shows (A) fibrosis of stroma (H&E stain 100x) (B) section in tonsil of patient shows fibrin deposition with neutrophils and mononuclear cells infiltration ⟷ vacuolation of epithelial layer ⟷(H&E stain 400X)

On histopathological examination of adenoid, the presence of lymphoid hyperplasia, numerous lymph follicles with prominent germinal centers was the chief finding in the adenoids removed from children as show in Figure (3).

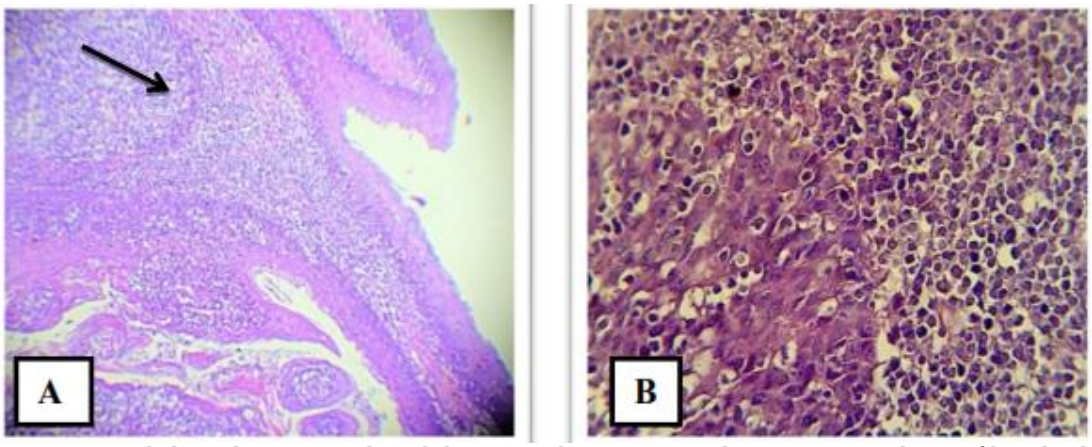

FIGURE 3: Histopathological section in adenoid shows (A) Showing germinal centers. (B) Depletion of lymphoid tissue (H&E stain 400X)

Figure (4) shows apoptosis of lymphocytic cells multiple spaces filled with cellular debris in adenoid tissue.

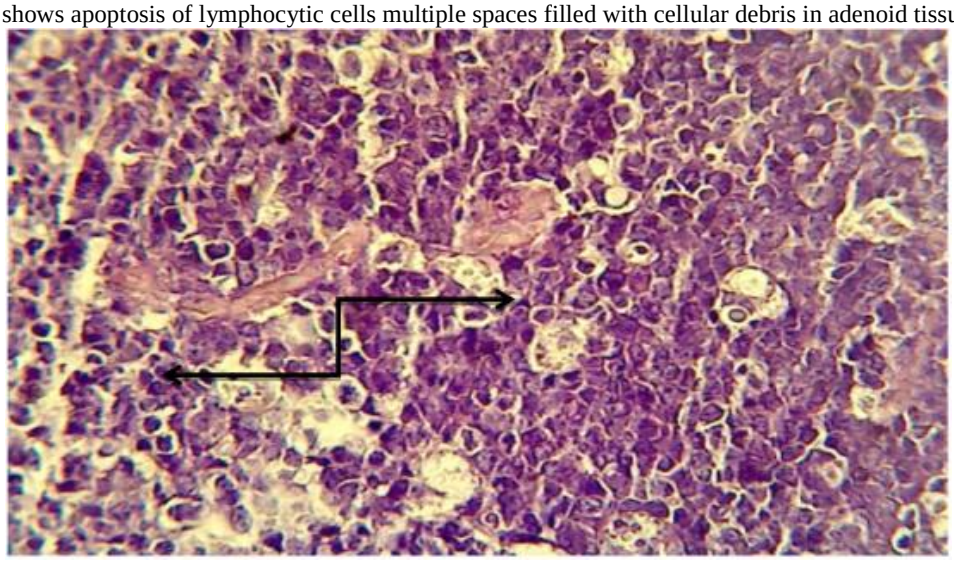

FIGURE 4: Histopathological section in adenoid shows apoptosis of lymphocytic cells left multiple spaces filled with cellular debris (H&E stain 400X)

DISCUSSION

There often is a substantial difference between the pathogens located on the surface of the tonsil or adenoid and those that reside deep inside these folds of tissue (Charles and Scott, 2000). For this reason, superficial swab and deep cultures were taken. *Staphylococcus aureus* was the commonest isolate (23.6%) because it is having antimicrobial activity of saliva and teichoiec acid in its cell wall which helps it in attaching in epithelial tissue of oral cavity and this made it most common pathogen in tonsillitis. In addition, the localization of the causative agents in superficial biofilms or inside the tonsillar tissue could contribute to functional antibiotic resistance in spite of absent specific resistance mechanisms (Swidsinski *et al.*, 2007). While an intracellular localization of *S. pyogenes* in tonsillar cells and an associated resistance to β-lactam antibiotics is generally accepted based on few *ex vivo* and numerous *in vitro* studies (Podbielski *et al.*, 2003), considerably, smaller extent knowledge about the role of intracellular *S. aureus* in the upper respiratory tract infections. Sex differences in disease have always been attributed in a verity of factors like genetic, biological, environmental or psychosocial factors.

The antibodies toward streptolysin O appears 7-10 days after the infection. The highest level of ASO is during the third week as stated by (Kumar *et al.*, 2007). The range of normal values for ASO depends upon the age, geographical area, season and environmental factor (Sethi *et al.*, 2003). The ASO titer is primarily used in determining that previous *Streptococcus* infection has caused a post-streptococcal sequel such as glomerulonephritis, rheumatic fever and bacterial endocarditis or scarlet fever (Pagana and Panga, 2009). According to this explanation about elevation in antibodies to streptolysin O while, throat culture is negative can lead to conclude that healthy person who had positive ASO test may be either received antibiotics to treat a recent streptococcal tonsillitis which inhibit the growth of group A *Streptococcus*, while ASO is not affected by antibacterial therapy or they had post-streptococcal infection. Those positive results to both group A *Streptococcus* and ASO titer ≤ 200 may indicate that those patients are carrier for *S. pyogenes.* In this study, the histopathology examination of tonsil showed the presence of lymphoid hyperplasia, multiple lymphoid follicles, vascular congestion, germinal centers, fibrosis and

infiltration of inflammatory cells. These results were similar to Rajeshwary *et al.* (2013). The presence of the germinal center indicates that the lymphoid follicle is very active in producing lymphocytes (Junqueira *et al.*, 1995). Lymphoid hyperplasia is enlargement of lymphoid organs as a consequence of hyperplasia of some or all of the cellular components, reflecting stimulation of the lymphoid cells by a variety of antigens or allergens (Ioachim and Ratech, 2002). In this study, the results show that decrease in lymphoid tissue, neutrophils and mononuclear cells infiltration and fibrosis similar to Uğraş and Kutluhan (2008) were related to frequent attacks or a long time chronic infection which eventually migration of more chronic inflammatory cells to the tissue. In chronic infection as chronic tonsillitis, the circulation of blood is poor as a result of degenerative changes causing parenchymal fibrosis. Ozdemir *et al.* (1985) found that a decrease in blood flow in chronic tonsillitis and an increase in hypertrophic tonsils compared to the normal controls. In this study, the result shows apoptosis of lymphocytic cells multiple spaces filled with cellular debris in adenoid tissue, these results similar to Uğraş and Kutluhan (2008) were found numerous lymphocytes in the lymph epithelium, degenerated cells and cellular debris in the crypts of tonsil. Some authors showed that cell proliferation and cell death by apoptosis were preponderantly produced of the level of the reactive lymphoid follicles and in a small part, at the level of the interfollicular zone and at the surface epithelium level (Kučera *et al.*, 2004).

REFERENCES

Anjos, L.M., Marcondes, M.B., Lima, M.F., Mondelli, A.L. and Okoshi, M.P. (2014) Streptococcal acute pharyngitis. Revista da Sociedade Brasileira de Medicina Tropical, 47(4):409-413.

Arens, R. and Marcus, C. (2004) Pathophysiology of upper airway obstruction: A developmental perspective. Sleep, 27(5):997–1019.

Babita Sanjeev, S., Kheya, M. and Shankar, P. (2014) Study on bacteriology of tonsillitis in a hospital. J Evol Med Dent Scie. 3(7): 1639-1641.

Bista, M., Amatya, R. and Basnet, P. (2006) Tonsillar microbial flora: A comparison of infected and non-infected tonsils. Kathmandu University Med J. 4(13):18–21.

Charles, W.G. and Scott, E.H. (2000) Tonsils and Adenoids. Pediatr Rev. 21 (3): 75-78.

Ioachim, H. and Ratech, H. (2002) Ioachim's lymph node pathology. Philadelphia. Lippincott Williams Wilkins.189-96.

Junqueira L; Carneiro J. Sistema I and Órgãos L. (1995). In: Histologia Básica. 8 ed. Rio de Janeiro: Guanabara-Koogan; p.220-40.

Kučera T; Pácová H; Veselý D; Astl J and Martínek J. (2004). Apoptosis and cell proliferation in chronic tonsillitis and oropharyngeal carcinoma: role of nitric oxide and cytokines, Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 148(2):225–227.

Kumar V; Abbas A; Fausto N and Mitchell R. (2007). Robbins Basic Pathology. 8th ed. Sanders Elsevier. 403-406.

Marseglia G; Caimmi D; Pagella F; Matti E; Labó E; Licari A; Salpietro A; Pelizzo G and Castellazzi A. (2011). Adenoids during childhood: The facts. Int J Immunopatho and Pharmacol. 24(4):1–5.

Ozdemir I; Ercan M and Kaya S. (1985). Measurement of tonsillar blood flow in normal and pathological conditions by the use of the 133Xe clearance technique. Arch Otorhinolaryngol. 242:53-6.

Pagana KD and Panga TJ. (2009). Diagnostic and Laboratory Test Reference. 9th ed. MOSBY'S.P. 38-39.

Panga A; Rani G and Kumar M. (2013). Chronic tonsillitis: A comparative study of the causative organism cultured through throat swab vs. core culture and biopsy of the tonsillectomy specimen. Int J Scie Res. 2319-7064.

Peter Bull and Ray Clarke. (2007). Disease of Ear, Nose and Throat. 10th edition.

Podbielski A; Beckert S; Schattke R; Leithauser F; Lestin F; Gossler B and Kreikemeyer B. (2003). Epidemiology and virulence gene expression of intracellular group A streptococci in tonsils of recurrently infected adults. Int J Med Microbiol. 293(2-3):179–90.

Rajeshwary A; Rai S; Somayaji G and Vidya P. (2013). Bacteriology of symptomatic adenoids in children. North American J Med Sci. 5(2): 113-118.

Sethi S; Kaushik K; Mohandas K; Sengupta C; Singh S and Sharma M., (2003). Antistreptolysin O titers in normal healthy children of 5-15 years. Indian Pediatr. 40(11): 1068-1071.

Swidsinski, A., Göktas, O., Bessler, C., Loening-Baucke V., Hale, L.P., Andree, H., Weizenegger, M., Hölzl, M. Scherer, H. and Lochs, H. (2007) Spatial organization of microbiota in quiescent adenoiditis and tonsillitis. J Clin Pathol. 60(3):253–60.

Uğraş, S. and Kutluhan, A. (2008) Chronic tonsillitis can be diagnosed with histopathologic findings. Eur J. Gen Med. 5(2):95-103.