

## Bacteriological, Serological and Histopathological Study on Tonsillectomy Specimens

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### ABSTRACT:

#### BACKGROUND:

Tonsillitis is one of the commonest infectious disease occurring in the young age group. Tonsillectomy is generally indicated when there are frequent attacks of acute tonsillitis, usually six attacks or more per year for two successive years. Other indications of tonsillectomy include obstructive sleep apnea, quinsy ( usually after the second attack of quinsy) and suspicion of malignancy

#### OBJECTIVE:

The aim of the present study is to: i) identify the types of bacteria in the tonsil core and to study the sensitivity of bacterial isolates to some antibiotics. ii) estimate serum concentration of Antistreptolysin O (ASO), C-Reactive Protein (CRP) and Epstein Barr Virus (EBV) serology in patients subjected to tonsillectomy compared to control group. iii) study the histopathological changes of routine tonsillectomy specimens.

#### METHODS:

This case series included hundred patients who underwent tonsillectomy at Al-jamhori Teaching Hospital from January 2010 to July 2012. The main indication for surgery was recurrent attacks of tonsillitis for two or more successive years. Core of the tonsil was studied regarding bacteriology and sensitivity of the cultured bacteria to some antibiotics. At the same time blood sample was obtained and analyzed for ASO, EBV and CRP. Finally the removed tonsils were sent for histological examination.

#### RESULTS:

The mean age of our patients was 10.32 years with a range of 3-35 years. The study included 60 males (60%) and 40 females (40%) with a ratio of 1.5 :1. Group A beta hemolytic *Streptococcus pyogenes* was the commonest isolate from tonsil core (21.4%) and was most sensitive to azithromycin. Serological tests for ASO, CRP and EBV were positive in 24%, 22% and 20% compared to 22%, 8% and 10% in control group respectively. CRP was significantly higher among patients than control group. Histological examination revealed chronic non-specific tonsillitis with reactive lymphoid hyperplasia.

#### CONCLUSION:

The resistance of many bacteria to penicillin and amoxicillin, and the high percentage of beta-lactamase secreting microbes in recurrent tonsillitis mandate the need to consider a combination of amoxicillin and clavulanic acid or second generation cephalosporin. ASO titer is not an accepted indication for tonsillectomy.

**KEY WORDS:** tonsillectomy, tonsillitis , core bacteria.

### INTRODUCTION:

Tonsillitis is one of the commonest infectious disease occurring in the young age group. Chronic tonsillitis results from recurrent acute

tonsillitis which is thought to result from insufficient penetration of antibiotics into the core of tonsil or inappropriate antibiotic therapy<sup>(1)</sup>.Waldeyer's ring is a lymphatic aggregate located at the wall of pharynx. It consists of six elements according to their site: palatine, lingual, nasopharyngeal (adenoids) and tubal tonsils, in addition to lateral pharyngeal bands and lymphatic aggregates at posterior pharyngeal wall. The definitive function of Waldeyer's ring

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is unknown but it is thought to have important role in the body's immune system where coming antigens may be aspirated or ingested<sup>(2)</sup>.

The palatine tonsils are two ovoid masses of lymphoid tissue situated on either side of the oropharynx. They are coated by non-keratinized stratified squamous epithelium as an extension of the oropharyngeal mucosa, including 15 deep crypts that invaginate into the parenchyma, in which B-lymphocyte are found<sup>(2)</sup>.

Tonsillectomy is generally indicated when there are frequent attacks of acute tonsillitis, usually six attacks or more per year for two successive years. Other indications of tonsillectomy include obstructive sleep apnea, quinsy (usually after the second attack of quinsy) and suspicion of malignancy<sup>(2,3)</sup>.

Causative agents of tonsillitis include viruses: Reo virus, Adenovirus, Epstein Barr Virus (EBV), Influenza virus and Echo virus, and bacteria: beta hemolytic streptococcus. In rare instances tonsillitis can be caused by Fungi or parasites<sup>(3,4)</sup>. The determination of the core bacteriology is important for two reasons. First: several studies concluded that tonsillar disease arise from the bacteria within the substance (core) of the tonsil rather than bacteria identified on the surface. Second: the surface of tonsils is consistently exposed to oral secretions with their attended flora. Tonsillar surface culture is likely to grow these organisms<sup>(5-8)</sup>.

### OBJECTIVE:

1. To identify the types of bacteria in the tonsil core and to study the sensitivity of bacterial isolates to some antibiotics.
2. To estimate the serum concentration of Antistreptolysin O (ASO), C-Reactive Protein (CRP) and Epstein Barr Virus (EBV) serology in patients subjected to tonsillectomy compared to control group.
3. To study the histopathological changes of routine tonsillectomy specimens.

### PATIENTS AND METHODS:

This case series includes analysis of 100 patients who underwent tonsillectomy (with or without adenoidectomy) at Al-jamhori Teaching Hospital for the period from January 2010 to July 2012. The results of serological tests were compared to control group (50 persons) whose ages were matched as far as possible.

**1- Selection of patients:** Patients were referred from out-patient department (OPD) of Al-Jamhori Teaching Hospital and from private clinics of the first and third authors. The indications for tonsillectomy were:

1. Recurrent attacks of acute tonsillitis: 6 attacks per year for at least two successive years.

2. Obstructive sleep apnea: this is clinical diagnosis due to unavailability of polysomnography. The condition is diagnosed when there are very large tonsils and adenoids in addition to mouth breathing, snoring and history of recurrent apnea during sleep. Size of adenoid is assessed by X-ray of postnasal space and fiberoptic nasal endoscopy.

3. Peritonsillar abscess (quinsy): tonsillectomy is advised after second quinsy.

4. Unilateral enlargement of tonsil, with clinical suspicion of malignancy.

Careful history and ENT examination of the patients were done 1-2 days before surgery to exclude active infection and to make sure that the patient is not taking antibiotics which may interfere with results of culture and sensitivity. Routine investigations included hemoglobin, bleeding and clotting time and partial thromboplastine time. Informed consent from every patient (or from parents if the patient is younger than 18 years) was obtained. Blood samples of fifty healthy volunteers were used as control group.

**2-Operative procedure:** All operations were performed under general anesthesia and endotracheal intubation. Boyl- Davis's mouth gag was inserted and gently opened and then pharyngeal pack was inserted to prevent aspiration of blood and saliva. If there is large adenoid, it was removed by curette and the postnasal space palpated to exclude presence of residual tissue, then postnasal pack was inserted. Draffin's suspension was arranged and tonsillectomy done by dissection method. The lower pole is usually ligated by silk while the upper pole is either ligated or cauterized by mono-polar cautery. The area is carefully checked for any bleeding. Finally pharyngeal and postnasal packs were removed and mouth gag was closed and removed too.

**3-Specimen Collection:** Removed tonsils were collected in sterile containers from total of 100 patients.

**4- Blood samples collection:** Two milliliters of venous blood was taken from all patients and from control group. The blood samples were collected in plain tubes and centrifuged for 10 minutes at 2500 RPM. The sera were separated and kept frozen at -20C<sup>o</sup> until used for serological tests.

**5-Culture:** Tonsils were sectioned in half and sample from the core material was cultured by using sterile cotton swab. Swab specimens were cultured on Blood agar, Chocolate agar, MacConkey agar, and Mannitol salt agar, and all plates were incubated under aerobic conditions at 37 C for 24– 48 hours. Then the swab specimens were cultured on another blood agar and chocolate agar and were incubated under anaerobic conditions using an anaerobic jar with gas PaK. Gram's staining of fixed smears was prepared from the growth and biochemical tests for the confirmation of bacterial identification was accomplished<sup>(3)</sup>.

**6- Antibiotic Sensitivity Test:** Kirby-Bauer disk method was used and reading of the plates was done by measuring the size of inhibition zone in mm .

**7-Serological tests:** Plasmatec latex agglutination slide tests were used for the qualitative and semi quantitative determination of Antistreptolysin O titer (ASO), C-Reactive Protein and Epstein Barr Virus (EPV) in non-diluted serum or plasma. A positive reaction compared by positive control is indicated by observation of agglutination in the reaction mixture.

**8-Histopathological Studies:** Tonsils were sectioned in half and fixed for few days in 10% buffered neutral formalin, and after dehydration embedded in paraffin blocks followed by trimming and sectioning. Finally they were stained using Hematoxylin and Eosin stain<sup>(2)</sup>.

### RESULTS:

There were 200 tonsils from 100 patient, so there were 200 histopathological specimens, with 100 culture and 100 serological samples. Age of our patients ranged from 3-35 years with mean age of 10.32 years. The study included 60 (60%) males and 40 (40%) females. Control group included 50 volunteers whose ages range from 9-41 years with mean age 12.2 years. There were 25 males and 25 females from whom blood samples were collected for serological tests only.

Tonsillectomy was carried out for 85 (85%) patients due to recurrent acute tonsillitis, 12% for

obstructive sleep apnea, 2% for quinsy and 1% for suspicion of malignancy. In 74 (74%) patients tonsillectomy was accompanied with adenoidectomy, and in the remaining 26% tonsillectomy alone was done.

Bacteriological analysis of core tonsils showed that all specimens gave positive bacterial growth. There were 117 bacterial isolates: 72 (61.5% ) were gram positive bacteria and the remaining 45 (38.5% ) were gram negative bacteria .

The predominant microorganisms (gram positive and gram negative) were *Group A β- hemolytic Streptococcus pyogenes* (GAβHS ) (21.4% ) followed by *Haemophilus influenza* (17.1% ) , *α- hemolytic Streptococcus pneumoniae* (15.4% ) , and *Klebsilla pneumoniae* (8.5% ). The least isolated microorganism was *Bacteroides fragilis* (3.4%) as shown in (Table 1) .

Azithromycin, Vancomycin, Ciprofloxacin and Cefotaxime were the most effective antibiotic against gram positive isolates: 90.2% , 79.1% , 76.3% and 73.6% respectively (Table 2) .

Gram negative isolates were most sensitive to Ciprofloxacin, Cefotaxime, Amoxicillin-clavulanic acid and Norfloxacin with sensitivity of 95.5%, 86.6%, 82.2% and 80% respectively (Table 3) .

The result of serological tests indicated that ASO titer was positive (>200 IU / L) in 24 (24%) patient, CRP level was positive (> 6 mg / L) in 22 (22%) patient and EBV serology was positive in 20 (20%) patients. In the control group (n=50) these tests were positive in 11, 4 and 5 patients respectively with percentage of 22%, 8% and 10% respectively. Statistical analysis using chi-square test revealed that CRP was significantly high; P-value= 0.033 whereas there was no significant difference in ASO titer (P-value= 0.785) and EBV serology (P-value= 0.121) between patients and control group (Table 4).

All tonsillectomy specimens showed almost the same histopathological findings including chronic non-specific tonsillitis with reactive follicular lymphoid hyperplasia (reactive florid germinal centers). There was no malignancy or granuloma in any of the collected specimens.

## HISTOPATHOLOGICAL STUDY ON TONSILLECTOMY

**Table 1: Types of core bacteria.**

	Types of Bacteria	No. isolates	Percentage %
	Gram Positive Bacteria		
1.	Group A $\beta$ - hemolytic Streptococcus pyogenes ( GABHS )	25	21.4
2.	$\alpha$ -hemolytic Streptococcus pneumonia	18	15.4
3.	Staphylococcus aureus	16	13.7
4.	$\alpha$ -hemolytic Streptococcus viridans	7	6
5.	Corynebacterium diphtheria	6	5.1
	Gram Negative Bacteria		
1.	Haemophilus influenza	20	17.1
2.	Kliebsiella pneumonias	10	8.5
3.	Pseudomonas aeruginosa	6	5.1
4.	Haemophilus para influenza	5	4.3
5.	Bacteriodes fragilis	4	3.4
	Total	117	100%

**Table 2: The antibiogram of gram positive bacteria.**

Antibiotic Bacteria														
	No isolates	Penicillin G	Amoxicillin	Methicillin	Oxacillin	Cefoxitin	Cefotaxime	Vancomycin	Amikacin	Ciprofloxacin	Azithromycin	Doxycyclin	Cortimoxazole	Novobiocin
Streptococcus Pyogenes	25	13/52	14/56	15/60	12/48	12/48	12/48	12/48	12/48	20/80	25/100	9/36	0/0	12/48
Streptococcus Pneumonia	18	14/77.7	14/77.7	12/66.6	12/66.6	14/77.7	18/100	18/100	9/50	17/94.4	17/94.4	5/27.7	17/94.4	9/50
Streptococcus Viridians	7	7/100	7/100	4/57.1	4/57.1	4/57.1	7/100	7/100	4/57.1	4/57.1	7/100	3/42.8	3/42.8	7/100
Staphylococcus Aureus	16	1/6.25	2/12.5	12/75	16/100	12/75	12/75	16/100	10/62.5	9/56.25	10/62.5	16/100	11/68.7	8/50
Corynebacterium diphtheria	6	3/50	2/3.33	2/33.3	3/50	3/50	4/66.6	4/66.6	3/50	5/83.3	6/100	3/50	2/33.3	3/50
Mean sensitivity %	72	38/52.7	39/54.1	45/62.5	47/65.2	45/62.5	53/73.6	57/79.1	38/52.7	55/76.3	65/90.2	36/50	33/45.8	39/54.16

**Table 3: The antibiogram of gram negative bacteria.**

Antibiotic Bacteria														
	No isolates	Ampicillin	Amox.clavunat	Carbencillin	Cefoxitin	Cefotaxime	Amikacin	Clindamycin	Ciprofloxacin	Azithromycin	Doxycyclin	Cortimoxazole	Polymyxin B	Norfloxacin
Haemophilus Influenza	20	16/80	20/100	9/45	10/50	20/100	13/65	7/35	18/90	18/90	16/80	20/100	13/65	15/75
Haemophilus Parainfluenzae	5	3/60	5/100	2/40	4/80	5/100	3/60	2/40	5/100	3/60	3/60	5/100	3/60	3/60
Klebsiella Pneumonia	10	0/0	10/100	5/50	6/60	10/100	10/100	5/50	10/100	8/80	4/40	5/50	5/50	10/100
Bacteroides fragilis	4	0/0	2/50	0/0	4/100	4/100	2/50	4/100	4/100	2/50	2/50	2/50	2/50	2/50
Pseudomonas Aeruginosa	6	0/0	0/0	0/0	0/0	0/0	6/100	6/100	6/100	4/66.6	5/83.3	3/50	6/100	6/100
Mean sensitivity %	45	19/42.2	37/82.2	16/35.5	24/53.3	39/86.6	34/75.5	24/53.3	43/95.5	35/77.7	30/66.6	35/77.7	29/64.4	36/80

Table 4: Comparison between serological tests of the two groups.

Serological tests	Patients [n = 100]		Control [n = 50]		Odd's Ratio	95% C.I	P-value*
	No.	%	No.	%			
ASO	24	24	11	22.0	1.12	0.50; 2.52	0.785
CRP	22	22	4	8.0	3.24	1.05; 10.00	0.033
EBV	20	20	5	10.0	2.25	0.79; 6.40	0.121

Chi-square test was used.

**DISCUSSION:**

Different types of gram positive and gram negative microorganisms were detected in the current study of which gram positive bacteria constituted (61.5% ) and gram negative one were (38.5% ); these findings goes with that of Kurien et al. (1).

In the current study ( GAβHS ) was proved to be the major causative microorganism of tonsillitis which constituted (21.4%) of total isolates . The result was in concinnity with the findings of Kielmovitch et al. (5), Ungkanot et al. (9), Osterlund et al. (10), and Brook and Gober (11) who found (GAβHS ) to be the most prevalent bacteria that causes tonsillitis, with almost similar percentages. However other studies reported a lower percentage of (GAβHS); Abdulrahman et al. (7) reported (18.5%) and Shah (12) reported (15.9%). Sadoh et al. reported a higher isolation rate (48.72%) (3).

Antibiotics are used to treat bacterial tonsillitis. Studies have shown that antibiotics can reduce illness time by at least one day, and also reduce the incidence of complications (13-15).

The result of our study is consistent with Fujimori et al. (16), Winn et al. (17) and Harvey et al. (18) who found *Strept. pyogenes* to be the most prevalent pathogen in tonsillitis. However, amoxicillin was found in the current study to be much less effective than in the mentioned studies, probably due to the misuse of antibiotics in some parts of our locality.

The resistance of many bacteria to pencillin and amoxicillin, and the high percentage of beta-lactamase secreting microbes in recurrent tonsillitis mandate the need to consider a combination of amoxicillin and clavulanic acid or second generation cephalosporin (11,19-22). Hadi et al. (8) and El Amin and Faidh (23) believed that the chronically inflamed tonsils and adenoids

contain more scar tissue after each infection, causing an impairment of antibiotic penetrating into their core and more resistance to antibiotics. Antistreptolysin O titer starts to increase after the second week of an acute streptococcal infection, and it usually remains high for weeks or months. High ASO titer means that the patient had history of streptococcal infection (whether tonsillitis or not),it by no means indicate surgery. In this study it was positive in 24% compared to 22% in control group (P-value=0.785 : insignificant) which is comparable to other studies (24,25).

C- Reactive Protein level –on the other hand-drops dramatically when the acute attack of tonsillitis subsides. It was positive in 22% compared to 8% in control group (P-value =0.033 : significant). Other studies found much higher levels of CRP than in our study (even 100 fold or more). The cause of such difference is that our samples were not collected during acute attack (26,4).

Many authors believe that tonsils of children can be colonized with EBV and that this virus may have direct or indirect role in recurrent tonsillitis and nasopharyngeal carcinoma (27-30,4). Shah have shown that EBV may cause tonsillitis in the absence of systematic mononucleosis and found EBV responsible for 19% of exudative tonsillitis in children (12). In our study EBV serology was positive in 20% compared to 10% in control group (P-value =0.121 : insignificant).

The result of histopathological study is consistent with Berkowitz and Mahadevan (31) and Dell'Aringa et al. (2) who found follicular lymphoid hyperplasia to be the main histologic alterations in tonsils, although tonsils are not uncommon sites for onset of head and neck neoplasia . There was no malignancy or granuloma in any of the collected specimens.

There was one patient for whom tonsillectomy was done due to recent huge asymmetric enlargement of tonsils, with clinical suspicion of malignancy. However, histopathological result showed reactive follicular lymphoid hyperplasia with foci of sub-epithelial hemorrhage but no malignancy.

### CONCLUSIONS AND RECOMMENDATIONS:

1. The resistance of many bacteria to penicillin and amoxicillin, and the high percentage of beta-lactamase secreting microbes in recurrent tonsillitis mandate the need to consider a combination of amoxicillin and clavulanic acid or second generation cephalosporin.
2. When the indication for tonsillectomy is recurrent acute tonsillitis in otherwise healthy child, it is not mandatory to send removed tonsils for histological examination, unless there is clinical suspicion of malignancy or gross inspection of the tonsils is suggestive, for example unilateral tonsillar enlargement.
3. ASO titer is not an accepted indication for tonsillectomy.

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