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| RESEARCH ARTICLE

Determination of Antibody for Streptolysin-0 (ASO) As an Indicator for Tonsillar Infection

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| ABSTRACT
This study was conducted to determine the relationship between the anti-Streptolysin O (ASO) titer and patients with history of recurrent tonsillar infection of different age and sex groups. To evaluate the validity of serum ASO in assessing cases of recurrent tonsillar infection. A cross-sectional study done on 50 patients of different ages and sex groups who were clinically diagnosed with recurrent tonsillitis and wishing to undergo a tonsillectomy at Basra Teaching Hospital over a period of 10 months from October 2023 to July 2024. Surface swabs were obtained for cultures. ASO titer was performed to estimate its relation to tonsillar infection. The percentage of patients who had high ASO titer among patients who had positive *GABHS* cultures is nearly similar to those who had negative cultures. Although ASO titer is used commonly to determine streptococcal infection, it seems that it is not a decent indicator for recurrent tonsillar infections.

| KEYWORDS

Anti- Streptolysin O, Group A *β-hemolytic streptococcus*, tonsillitis.

| ARTICLE INFORMATION

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1. Introduction

One of the most conditions that facing otolaryngologists during their routine daily work is acute or recurrent tonsillitis whether in childhood or adulthood age groups and in both sexes. It is extremely importance for the otolaryngologists and general physicians to reach a proper diagnosis of tonsillar infection and prescribe the most appropriate antimicrobial therapy to prevent the serious complications of tonsillitis particularly *Group A β-hemolytic streptococcus (GABHS)* pharyngitis or tonsillitis[1] . Usually, the cause of tonsillar infection is viral especially during winter time; however, worldwide, about 600 million of people were reported to have positive *GABHS* each year, one tenth of them developed recurrent tonsillitis. The estimated prevalence rate of recurrent tonsillitis is nearly 12% in patients with tonsillar infection [2,3,4]. Two toxic cytolysins are mainly released from *Streptococcus pyogenes,* namely Streptolysin O (SLO) and Streptolysin S (SLS). The former is an antigen produces by the majority of *Group A β-hemolytic streptococcus (GABHS)* and is able to induce macrophage apoptosis [5,6]. Anti-Streptolysin O antibody that generated as reaction to the Streptolysin O (SLO) antigen by humoral immunity appears in serum from one to five weeks after streptococcal infection [7].

2. Materials and Methods:

2.1. Study Population:

A cross-sectional study was conducted on 50 patients of different age groups who were clinically diagnosed with recurrent tonsillitis and indicated for a tonsillectomy at Basra Teaching Hospital over a period of 10 months from October 2023 to July 2024. A swab was taken from every tonsil. Patients ages ranged from (2 – 46) years old. Those who aged 16 and under were categorised as children and the older people were grouped as adults. A total of 29 patients were males and the rest 21 were

females. Any patients taking antibiotics over the last two weeks were excluded from this study. A thorough otorhinolaryngological plus general medical examinations were done for all patients.

2.2 Collection of specimens:

Blood samples were collected and sent for CBC, coagulation screen, renal function tests, FBS, HIV, HBsAg, as well as ECG and chest X-ray. Fresh serum was obtained to assess ASO titer using ELISA technique. Titer of more than 200 I.U. was considered as a positive result [8].

While patients were sitting upright, a swab from the external surface of the examined tonsil was taken by using a sterile cotton wool swab in a rotatory manner, taking care not to streak the adjacent parts of oropharynx.

These swabs specimens were immediately sent to the microbiology department to perform bacteriological cultures. Conventional culture was conducted using blood agar, chocolate-bacitracin agar and Maconkey agar. Plates were incubated overnight at 37^{o}C. Colony identification was accomplished using the standard techniques including Gram's staining, Catalase test, Oxidase test, Coagulase test and Urease test.

All of these processes were carried by the researchers themselves.

3. Results

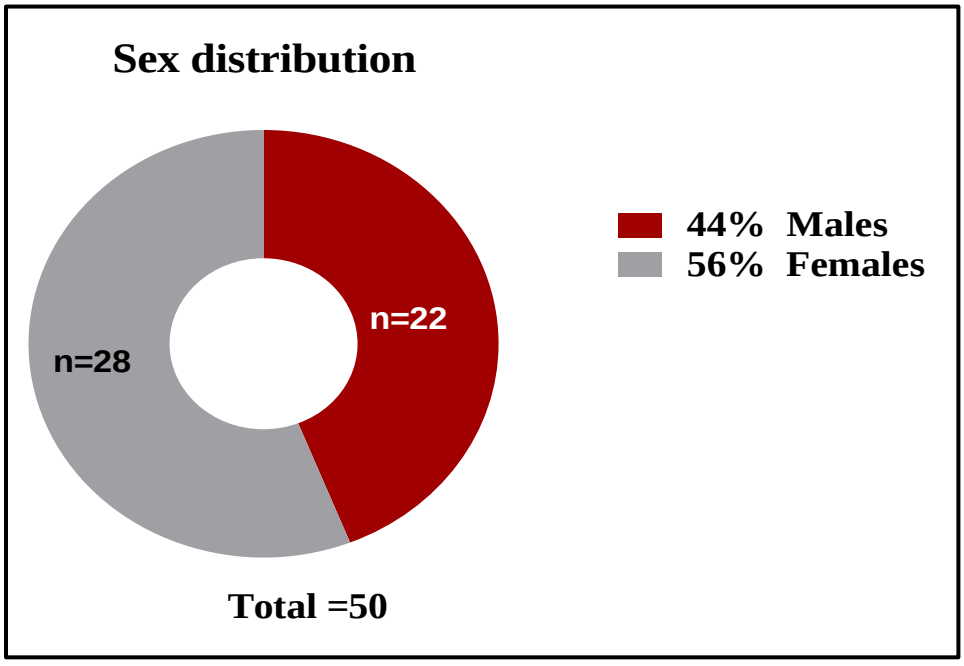
3.1 Demography of the study

As shown in table-1 and figure-1, the total number of patients enrolled in this study were 50 patients. 56% of them were females (n=28) and 44% were males (n= 22) patients. Participants of less than 14 years were considered as children. In our study, 32 patients were children, of which 18 females and 14 males. In adult groups, the number of women were also more than men, 10 compared to 8.

Sex	Age		Total
	Children	Adult	
Female	18	10	**28**
Male	14	8	**22**
Total	**32**	**18**	**50**

Table-1: Total distribution of patients according to sex and age

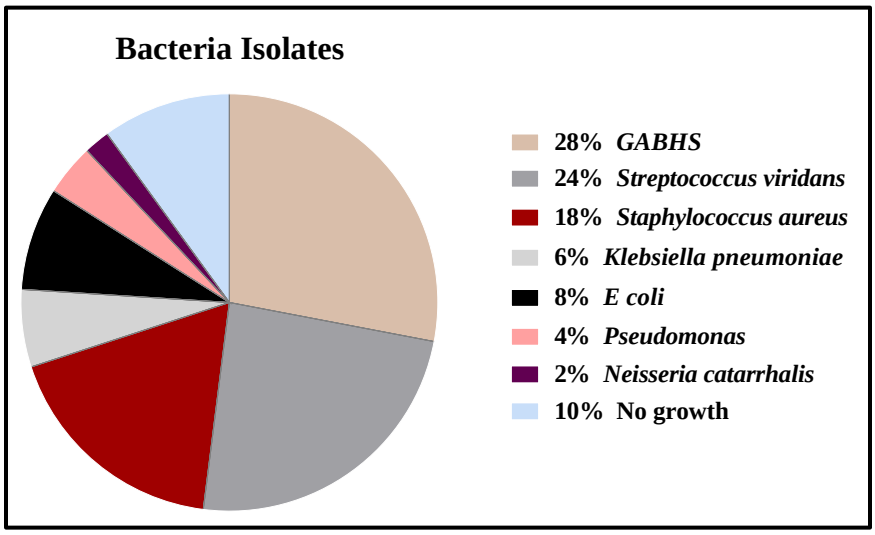
Figure-1: Distribution of patients according to sex



3.2 Bacteriological isolates from tonsillar surface

Figure-2 demonstrates the bacteria isolated from tonsillar surface swabs. It is obvious that the highest percentage of isolated bacteria was *GABHS* (28%) and the second most common bacteria was *Streptococcus viridans* (24%). *Staphylococcus aureus* also accounted for relatively high percentage at 18%. The remaining isolates include *Klebsiella pneumoniae*, *E coli*, *Pseudomonas*, and *Neisseria catarrhalis,* were collectively occupied 20% of all isolated bacteria. One tenth of all swabs did not show any pathogens or bacterial floral growth.

Figure-2: Percentages of isolated bacteria



3.3 Correlation of GAβHS culture and ASO titer

The relationship between GABHS culture results and ASO titer test is presented in table-2. Out of 14 patients who had positive culture results to *GABHS*, only 38% had positive ASO titer. Similarly, 39% of patients who had negative *GABHS* culture had positive ASO titer.

Culture results	ASO Results				Total	
	Positive ASO		Negative ASO		No.	%
Positive *GAβHS*	**5**	**38%**	**9**	**62%**	**14**	**100%**
Negative *GAβHS*	**14**	**39%**	**22**	**61%**	**36**	**100%**
Total	**19**	**38%**	**31**	**62%**	**50**	**100%**

Table – 2: Correlation of GAβHS culture and ASO titer

4. Discussion:

Tonsillar infection with *Group A β-hemolytic streptococcus (GABHS)* may carry serious complications, particularly in genetically susceptible patients, such as rheumatic fever, cardiac valvular diseases and glomerulonephritis, which might result in a considerable morbidity and high mortality [9,10,11].

The microbiological swabs of tonsils in the current study revealed that *GABHS* was the commonest isolated organism. This finding is consistent with Guntinas-Lichius et.al. [12], Rawan M. Alghamdi et.al. [13] and Angham Najah Alkhafaji [14]. However,

many researchers demonstrated that *Staphylococcus aureus* was the commonest pathogen isolated from tonsils, such as Veraluce Paolini Cavalcanti et.al.[15], Abdulrahman et al [16], Loganathan et. Al. [17] and Gustave Buname et.al. [18]. On the other hand, *E-coli* and *Klebsiella* isolates are seen only in a low percent in present study, a finding is similar to what was reported by Abdulrahman et. Al. [16].

Interestingly, 10% of cases demonstrated no growth on the media even 48 hours after incubation. This indicated that a reasonable percent of the patients didn't show any growth of pathogenic organism. Several factors could be the reason behind this result; 1. no anaerobic cultures were done to swabs at all, 2. time limit during sample delivery was not considered, 3. Length of culture time; different microorganisms require different culture time, 4. the probability of viral tonsillitis as causative organism, 5. wrong swabbing technique, and finally 6. Probability of antibiotic administration before culture being performed.

The culture and serological results in the current study stratified our cases into three categories:

First: Those with recent infection and positive culture results. They are further divided in two groups:

a. Those with very recent infection even before antibody formation, they carried 62% of *GAβHS* positive culture.

b. Those with recent infection and positive ASO test (beginning of antibody formation), they carried 38 % of *GAβHS* positive culture.

Second: Those with history of recent infection and negative culture. Patients in this category established immune response in form of formation of antibody against streptococcus (positive ASO test) and had negative culture. They represented 39% of *GAβHS* negative culture.

Third: Those with no infection. Patients in this group did not develop infection with streptococcus on the last a few months and those patients had negative ASO test and negative streptococcal. 61% of our patients were in this category.

ASO level usually start to rise in (1-3) weeks after streptococcal infection, peaks in (3-5) weeks, and then goes back to insignificant level over (6-12) months. A positive result therefore might indicate current and more recent infection with group A, C, and G streptococci [19,20]. This fact would support the above-mentioned findings of the current research. It is worth to mention that normal values for ASO titer very often change with the age group, locality, affected area and seasonal variations [21].

Another consideration that observed in this study, we detected that 62% of cases who had positive culture for *GAβHS* did not show a positive ASO titer and this finding is in agreement with Ayoub EM & Harden E [22]. Positive ASO despite negative culture could be due to technical faults (oxidation of Streptolysin O reagent as an example) or there was hepatic disease in some patients which can alter the productivity of antibodies [23]. This raises the need for another more specific test such as Anti-DNase B which can be remained elevated for longer period and can help to indicate the presence of recent streptococcal infection even if the ASO titer declined after its rise [24].

5. Conclusions:

Group A β-*haemolytic Streptococci* was the commonest isolated pathogen from the tonsillar surface swabs.

ASO test is useful in detecting Streptococcal infection after a short period of time, however, it is not considered as a marker for recurrent tonsillar infection. Therefore, this test is not useful as an indicator for tonsillectomy.

Recommendations: Unfortunately, we did not consider Anti-DNase B titer in our assessment. It is advisable to confirm the presence streptococcal antibodies and compare the results with ASO titer.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions:

All authors shared in visualization, data collection, interpretation and analysis, writing original draft and redrafting. All authors revised the final typescript and accept it.

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