Comparative study of the Antibody Responses to *Streptococcus pyogenes* between school Children carriers and patients with Tonsillitis

Fadwa .E. Abd Al-Kareem*[1], Aruba .KH. Abbas[1], Median .A. Hussein[2]
[1]Department of Microbiology, College of Medicine, Al–Mustansiriya University, Baghdad, Iraq.
[2]Specialist physician, Al Yarmouk Teaching Hospital, Baghdad, Iraq.

Abstract

This study is designed to evaluate the immune status of patients and schoolchildren with respect to *Streptococcus pyogenes*. A prospective study was conducted to investigate antibody against GAS in children patients and asymptomatic healthy carriers in serum samples with tonsillitis and compare antibody response (ASO) between patients and healthy carriers with tonsillitis.

Tonsillar swabs were obtained to detect the presence of GAS and blood samples were collected to determine elevated ASO titer in serum.

A total of 376 sample patients and asymptomatic healthy carriers were included in this study, 142 (37.7%) samples are GABHS positive, included 80 (56.3%) patients and 62 (43.6%) asymptomatic healthy carriers. The finding of a significant relationship between ASO positive and GABHS carriage and patients indicated that ASOT measurement might be used together with throat culture to identify GABHS carriers and GABHS patients.

The value of ASOT varies with the age of the subject, and the most pronounced differences are between the values of preschool age children and school age children. The upper limits of normal values for these groups were as follows: preschool age (100) and school age (170).

Recently infected children showed raised ASO antibody titer than the carrier. But this study present elevated of ASOT in asymptomatic healthy carriers indicated recent infection or past infection then this children recovery from disease but remain record titer of antibody.

Keyword: Antibody response, *Streptococcus pyogenes*, Tonsillitis.

دراسة مقارنة لاستجابة الاجسام المضادة ضد بكتريا المكورات المسبحية (*Streptococcus pyogenes*) بين اطفال المدارس الحاملين للبكتريا المصابين بالتهاب اللوزتين.

فدوى عماد عبد الكريم[1]*، عروبة خالد عباس[1]، مدين علي حسين[2].
[1]قسم الاحياء المجهرية، كلية الطب، الجامعة المستنصرية، [2]الطبيب الاختصاص، مستشفى اليرموك التعليمي، بغداد، العراق

الخلاصة:

تم تصميم هذه الدراسة لتقييم الحالة المناعية للمرضى وأطفال المدارس فيما يتعلق ببكتريالعقدية المقيحة. وقد أجريت دراسة للتحقيق من الأجسام المضادة ضد بكتريا المكورات المسبحية الم جموعة (أ) في الأطفال المرضى والحاملين للبكتريا في عينات مصل الدم مع التهاب اللوزتين ومقارنة بين استجابة الأجسام المضادة (ASO) بين المرضى والحاملين الصحيين مع التهاب اللوزتين.

*Email: fadwa-e89@yahoo.com

تم الحصول على مسحات من اللوزتين للكشف عن وجود GABHS وعينات الدم تم جمعها لتحديد ارتفاع ASO عيار في مصل الدم.

عينة الدراسة تتضمن 376 عينة من المرضى والحاملين للبكتريا صحيين في هذه الدراسة، 142 (37.7٪) كانت ايجابية GABHS ، وشملت 80 (56.3٪) من المرضى و 62 (43.6٪) حاملين للبكتريا غير عرضيين والباقي من العينة هم سلبية للبكتريا . أشارت النتائج وجود علاقة ذات دلالة إحصائية بين ASO وGABHS. وأن قياس ASOT يمكن استخدامها مع زراعة مسحة اللوزتين لتحديد الحاملين GABHS من المرضىGABHS.

قيمة ASOT يختلف مع عمر الاطفال، والاختلافات أكثر وضوحا ما بين القيم من الأطفال في سن ما قبل المدرسة والأطفال في سن المدرسة . وكانت الحدود العليا من القيم العادية لهذه المجموعات كما يلي : سن ما قبل المدرسة (100) وسن المدرسة (170).

أظهرت الاصابات المتكررة من الاطفال ارتفاع ASO عيار الأضداد في المصابين عن الحاملين . ولكن في هذه الدراسة الحالية ارتفاع ASOT في الحاملين للبكتريا نتيجة عدوى أخيرة أو عدوى في الماضي ثم شفاء الأطفال من المرض ولكن تبقى عيارة من الأجسام المضادة مسجلة.

Introduction

Tonsillitis is one of the upper respiratory diseases, a disorder in which the tonsils are inflamed. Occurring predominantly in the younger age group (5-15 year) [1]. Tonsils are active immunologic organs. All the ten toll-like receptors, components of the innate immune system which recognize pathogenic microorganisms, are expressed in human tonsil tissue and may play a vital role in the immunological outcomes of these tissues. [2]. There are three Types of tonsillitis, acute (non-specific) tonsillitis, recurrent tonsillitis and chronic (specific) tonsillitis. [3-5]. The important cause of the tonsillitis is bacterial and viral causes, the most common cause of acute tonsillitis is bacterial, include Group A ß-hemolytic streptococci (GABHS) *(streptococcus pyogenes)*. About 30% to 40% of tonsillitis cases are caused by GABHS [6].

Streptococcus pyogenes is strictly a human pathogen [7]. β-haemolysis shows on a blood agar plate as a clear zone around the bacterial colonies. School-aged children (5-15 years) are considered as the major reservoir of GABHS. The carrier state may vary by age, season, and geographical location [8].

Humoral antibodies to specific streptococcal extracellular products such as antistreptolysin O (ASO), anti-DNAse B and other can be demonstrated readily by neutralization assays. They have been particularly useful in allowing a more precise method of defining GABHS infection in clinical and epidemiologic studies and in documenting the occurrence of a preceding GAS infection in patients with a suspected non-suppurative complication. The ASO assay is the most commonly used streptococcal antibody test. Antibody titers against GABHS extracellular antigens reported by clinical immunology laboratories may vary. Upper limits of normal are higher for children than for adults and these values, even for the same age group, are higher in some populations than in others [9].

SLO is a poreforming cytolysin which has toxic effects on a variety of cells and is able to induce apoptosis of macrophages [10]. SLO is antigenic and produced by almost all GABHS strains [11]. Streptolysin O is inactivated by oxygen (Oxygen Labile). Antibodies to Streptolysin O are generated by the Humoral Immunity and can be quantified as Anti-streptolysin O Antibodies (i.e. ASO titer). Antistreptolysin O (ASO) is specific neutralizing antibody produced after infection with these organisms & it appears in serum from 1 week-1month after the onset of a streptococcal infection. The ASO antibody response raises approxiamately 1 week after initial GABHS infection, reaching a peak response 3 to 6 weeks later. ASO titer begins to decline after 6 to 8 weeks in most patients with uncomplicated infection, but may remain elevated for indefinite periods in some individuals [9]. ASO titers peak during the third week after the onset of acute symptoms of a streptococcal disease; at six months after onset, approximately 30% of patients still exhibit abnormal titers [12].The rang of normal values for ASO depend upon the age of the patient, geographical location, epidemiological setting and season of the year [13].

Hence the Aim of this study, was carried out to assess the prevalence of GABHS bacteria in throat specimens among children patients with tonsillitis compared to healthy carriers children of the same age group.

Determination the levels of Serum level of ASOT in sera of patient and healthy carriers with GABHS children among (5-15 year).

Materials and Methods

Subjects:

The study encompassed 376 subjects (126 patients and 250 carriers) age among (5-15 year).The study was carried out in ear, nose and throat department (ENT department of Al- Yarmook Teaching Hospital), Children upper Teaching Hospital, Child Central Teaching Hospital, Health Center (Karkh/2) and middle and primary schools for boys and girls (Karkh/2). During period from December 2012 until end May 2013.

These Three hundred and seventy-six subjects were classified into three groups:

Group I: Patients

Comprised 80 patients from 126 subjects who consist of 34 males and 46 females.

Group II: carriers

Comprised 62 carriers from 250 subjects which consist of 24 males and 38 females.

Group III: control

34 from 234 apparently healthy persons who matched with patients for age and gender were selected. It is ensured that they have no history or clinical evidence of tonsillitis or any chronic disease, and obvious abnormalities.

Three hundred and seventy-six subjects were classified according to age:

Group I: (5-10 year)

Group II: (11-15 year)

Swab collection

Throat swab of enriched media were obtained from healthy carriers and patients childrens age of (5-15 year) who were suspected to have bacterial upper respiratory tract infection (Tonsillitis), diagnosed by physician. throat swab samples collected according to standard clinical methods [14].

Blood collection

Blood samples were collected from patients and carriers of school student's age between (5-15 year). Blood samples of 3-5 ml were collected by venipuncture, using plastic disposable 5ml syringes, from all patients, carriers and control groups. Blood samples were allowed to clot at room temperature, and then centrifuged for 10 minutes at approximately 1500 rpm to obtain at least 0.5 ml of unhemolyzed cell-free serum. Serum samples were stored in aliquots at -20 ºC until used for the measurement of immunological parameters [15].

Swab Culture

Swabs taken from the tonsils were inoculated on blood agar plates. The plates were incubated at 37ºC overnight and examined on the following day for growth .Colonies were counted on each plate

Identification of Bacteria:

Macroscopical examination of bacteria:

When the bacteria are grown on agar that contains sheep blood, it will appear as a translucent colony with a zone of hemolysis surrounding the colony. This is due to specific enzymes produced by this type of streptococcus species, Colony morphology: Glossy, grayish-white, translucent, large zone beta hemolysis.

Streptococcal Antibody Tests:

Measurement of Antistreptolysin O titer (ASOT) [16]

Test principle

The ASO-latex reagent of Hannover Company, Germany is a suspension of polystyrene particle sensitized with streptolysin O. Slide agglutination test for the qualitative and semi-quantitative detection of anti-streptolysin O antibodies. Latex particles coated with streptolysin O are agglutinated when mixed with samples containing ASO.

Kit components

Reagents composition:	
Latex particles coated with streptolysin O PH (8.2) Sodium azide (0.95 g/L)	Latex (5 ml)
Human serum with an ASO concentration ≥400 IU/ml Sodium azid (0.95 g/L)	Control (+) (1 ml)
Animal serum Sodium azid (0.95 g/L)	Control (-) (1 ml)

Specimen

Fresh serum, stable 8 days at 2-8˚C or 3 months at – 20˚C. Samples with particles or fibrin should be centrifuged to eliminate them. haemolized samples were rejected.

Test procedure

Semi-quantitative method:

Allow the reagents and samples to reach room temperature, the sensitivity of the test may be reduced at low temperature.

Place (50µ) of the sample and one drop of each positive and negative control into separate circles on the slide test.

Make serial two fold dilutions of the sample in 9 g/L saline solution**.**

Place (50µ) from the each tube above into separate circles on the slide test and place one drop of the ASO-latex reagent.

Mix both drops with a stirrer, spreading them over the entire surface of the circle.

Rotate the slide with mechanical rotator at (80-100 r.p.m) for (2 minutes), false positive results could appear if the test is read later than two minutes.

Remain examine all the tube until the tube last is negative; the titer is defined as the highest dilution showing a positive result.

Calculations

The approximate ASO concentration in the patient sample was calculated as follows:

200 (reagent sensitivity) x ASO titer can be defined as the highest dilution showing a positive result = IU/ml

Normal value

Up to 200 IU/ml = adult

Up to 150 IU/ml = children

Results and Discussions:

Table 1- Frequency & Percentage of total sample, age and sex according to healthy carrier and patients groups.

Characteristics	**Frequency**	**Percentage (%)**
Total	376	
Patients	126	33.5%
Healthy carriers	250	66.4%
Age		
5-10 year	243	64.6%
11-15 year	133	35.3%
Sex		
Female	270	71.8%
Male	106	28.1%

This study shows frequency & percentage of total sample, age and sex according to healthy carrier and patients groups was included 376 Iraqi patients with streptococcal tonsillitis and other healthy carriers for group A streptococci, patients was 126 (33.5%) and asymptomatic healthy carriers was 250 (66.4%). And age of this sample show (5-10 years) 243 (64.6%) more than (11-15 year) 133 (35.3%). And sex of sample show female 270 (71.8%) more than male 106 (28.1%), table-1.

Table 2- Number & percentage of total sample according to positive and negative healthy carrier and patients groups.

Characteristics	**Frequency**		**Percentage (%)**	
Total	376			
Positive	142		37.7%	
	Patients	Healthy carriers	Patients	Healthy carriers
	80	62	56.3%	43.6%
Negative	234		62.2%	
Control	34		9.04%	

This study included 376 Iraqi patients with streptococcal tonsillitis and other healthy carriers for group A streptococci. They were divided into two groups, in which 80 from 126 (56.3) patients with GAβHS and 62 from 250 (43.6%) asymptomatic apparently healthy carriers for GAHS. Which included 24 (38.71%) males, and 38 (61.29%) female and there were 34 from 234 (9.04%) healthy individuals, table-2.

Anti-streptolysin O (ASOT)

Table 3- Seasonal effect and groups in ASOT (IU/ml)

LSD Value (P-Value)	Mean ± SE (IU/m l)		LSD Value (P-Value)
	Healthy carrier	Patients	
December 12	---	5680.45 ± 894.23	---
January 1	3600.00 ± 535.34	9777.78 ± 1939.8	4609.60 ** (0.01)
February 2	3800.00 ± 422.03	11840.0 ± 2008.4	4154.80 *** (0.0004)
March 3	3400.00 ± 575.24	8746.67 ± 1629.1	3911.20 * (0.0094)
April 4	2560.00 ± 530.66	9333.33 ± 2434.7	5776.50 * (0.0217)
May 5	2080.00 ± 480.00	9920.00 ± 436.42	2125.0 * (0.112)
LSD Value	1673.10 NS	6736.20 NS	---
P-Value	0.248	0.509	---

* (P<0.05), ** (P<0.01), *** (P<0.001).

This study showed the effect of month and groups in ASOT IU /ml was studied. During January that there were highly significant statistical differences between patients and healthy carriers in ASOT IU/ml, and P-value (0.01) (P<0.01). And mean of patients (9777.78±1939.8). While it was lower than that in healthy carriers was (3600.00±535.34), table-3.

During February that there were highly significant statistical differences between patients and healthy carriers in ASOT IU/ml, the LSD value (415.80) and P-value (0.0004) (P<0.001). And mean

of patients was (11840.0±2008.4). While it was lower than that in healthy carriers was (3800.00±422.03), table-3.

During March that there were significant statistical differences between patients and healthy carriers in ASOT IU/ml, the LSD value (3911.20) and P-value (0.0094) (P<0.05). And mean of patients was (8746.67±1629.1). While it was lower than that in healthy carriers was (3400.00±575.24), table-3.

During April that there were significant statistical differences between patients and healthy carriers in ASOT IU/ml, the LSD value (5776.50) and P-value (0.0217) (P<0.05). And mean of patients was (9333.33±2434.7). While it was lower than that in healthy carriers was (2560.00±530.66), table-3.

During May that there significant statistical differences between patients and healthy carriers in ASOT IU/ml, the LSD value (2125.0) and P-value (0.112) (P<0.05). And mean of patients was (9920.00 ± 436.42). While it was lower than that in healthy carriers was (2080.00±480.00), table-3.

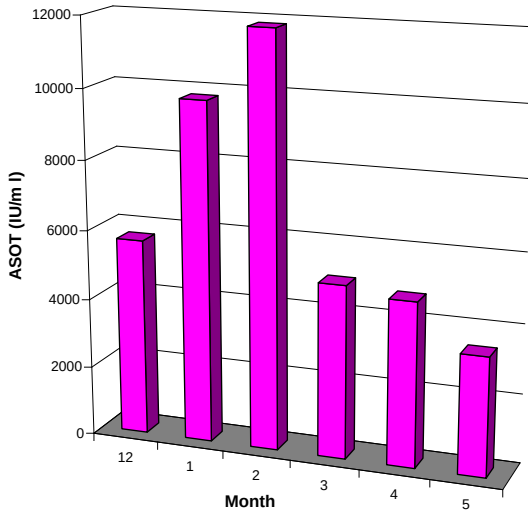

Figure 1- showed the effect of month in ASOT IU/ml in the patients. The result noticed that there was High percentage in December and Higher than that of in the January and Higher than that of in February and Begins to descend in March and Less than that in April and Less than that in May.

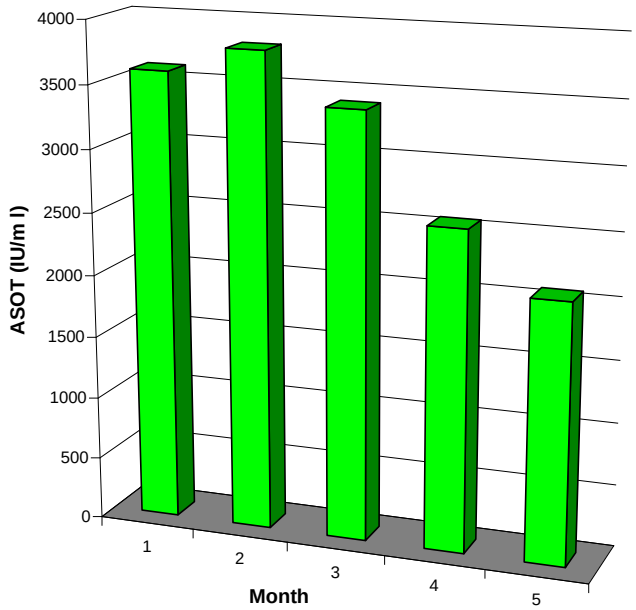

Figure 2- showed the effect of month in ASOT IU/ml in the healthy carriers. The results recorded that there was High percentage in January and Higher than that of in the February and Begins to descend in March and Less than that in April and Less than that in May.

Discussion:

Tonsillitis is a highly prevalent disease in pediatric age from 5-10 years [17]. Tonsillitis is an infection of tonsils, which are glands on either side of the back of the throat. The tonsils are part of the immune system, which protects & helps the body to fight infections. Tonsils aid the body in fighting off diseases & infection in children. The tonsil tissues can become diseased with recurrent infections. When this happens, they lose their effectiveness in helping the immune system & actually become a source of recurrent infection. Hence, timely treatment is most essential [18].

The first goal in this study was to estimate the prevalence rate of group A β-haemolytic streptococcal (GAβHS) infection among children at variable ages (from 5-15 years) who were suffering from sore throat at the time of their sampling in the pediatric hospitals and clinic in the city of Baghdad. Increasing or decreasing in the prevalence rate of GAβHS in different regions in Baghdad is a matter of extreme complexity as there are unlimited number of factors that can affect this rate including the socioeconomic states, sanitation level, endimicity of certain strains, climatic variation, crowdness condition, age, gender, technical and human errors and many others.

This study included three hundred and seventy-six sample patients with tonsillitis the number was 126 (33.5%) as well as carriers of the bacteria that cause tonsillitis the number was 250 (66.4%), which may these samples, were collected from several hospitals and health centers in several areas of Baghdad / second Karkh. The statistical results showed that there are highly significant differences between these groups as in, table-1.

The results of present study showed that the GABHS was a major cause of tonsillitis as confirmed by positive culture of these microorganisms in 142 (37.7%) of samples as can be observed in, table-2. These results were in agreement with the findings from several studies were done in many countries, [19, 20].

This study finding that no isolates of any bacteria from blood culture in patients with tonsillitis and these results was in contrast with the other reports which indicated that positive blood culture (invasive group) due to bacteremia, [21, 22]. Our study suggested that this negative result of blood culture might be due to administration of antibiotic before collecting of blood from patients who were no in febrile period in which the bacteria were shedding from blood

Serum Anti-streptolysin O (ASOT):

This study indicate elevated of anti-streptolysin O titer provide strong serological evidence of prior GAS infection Here was agreed with [23]. The rang of normal values for ASO depend upon the age of the patient, geographical location, epidemiological setting and season of the year [13].

Table-3, showed that titer of ASOT was elevated in December and reaches its peak-conscious in the both January and February and then titer of ASOT begins to decrease starting from March to end of May. This ratio for both patients and healthy carriers as in the figures [1 and 2].

ASOT highly varies due to climate change, where the cooler air starting from December And end the cold to May. In cold weather increases the incidence responsible for tonsillitis and start incidence decreases with the end of the cold. ASOT highly varies according to age and the immune status of the patients and healthy carriers. As well as overcrowding and direct contact between school students play a major role in the spread of the disease and increase ASOT titer. The finding of a significant relationship between ASO positivity and the carriage of GABHS. These results are in agreement with those reported by [24] who found the ASO titers were elevated in children who carried GABHS [25] who reported a significant difference between GA.B.H.S. Carriage ASO positivity values

ASO appears in the blood one week to one month after the onset of a streptococcus infection. A high titer (high levels of ASO antibody) is indicative that a streptococcal infection is present or may have happened in the recent past Here was agreed with [12]. ASO titers peak during the third week after the onset of acute symptoms of a streptococcal disease; at six months after onset, approximately 30% of patients still exhibit abnormal titers [12]. But the study were do not agree with [26] which show no correlation was between the culture results and the ASO results.

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