

## Bacteriological and Serological Study of Beta Hemolytic Streptococcal Throat Infection Among School Children In Najaf Governorate.

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### الخلاصة:

تستعرض هذه الدراسة نتائج الفحوصات البكتريولوجية والمصلية على 250 طفلاً يعانون من التهاب البلعوم و (350) طفلاً (كنماذج سيطرة أي لا يعانون من التهاب البلعوم). مئة وخمسة طفلاً مصابون بالتهاب البلعوم ومائة طفل غير مصابين بالتهاب البلعوم اختيروا لغرض تقدير مستوى ASO و CRP. حيث أظهرت نتائج هذه الدراسة إن مجموعة A التابعة للمسبقيات المحللة للدم نوع-بيتا عزلت وبشكل متغلب عند الأطفال المصابين بالتهاب البلعوم مقارنة بالأصحاء (43.6% مقابل 26% على التوالي) وكذلك هناك فرق معنوي ( $p < 0.005$ ) بين الأطفال المصابين بالتهاب البلعوم و الأطفال الأصحاء في معدل عزل مجموعة C التابعة للمسبقيات الحالة للدم نوع-بيتا (16.4% مقابل 8.9% على التوالي). كما وأظهرت الدراسة إن هناك فروق معنوية بين قيمة ASO فوق 200 وحدة دولية/مل بين الأطفال المصابين بالتهاب البلعوم (57.2%) والأطفال الأصحاء (4%). كما وجد إن هناك فرق معنوي بين قيمة CRP بين الأطفال المصابين بالتهاب البلعوم (94.3%) والأصحاء (نماذج السيطرة) (20%). حيث وجدت الدراسة إن حساسية وتخصصية فحص الـ ASO هي على التوالي (57% و 96%) أما بالنسبة لفحص CRP فقد وجدنا (94% و 80% على التوالي).

### Abstract:

The study presents the findings of bacteriological and immunological examinations of (250) children suffering from throat infection and (350) school children without throat infection as control. one hundred and five (105)infected children and one hundred (100) uninfected children were selected for asesment of ASO titer and CRP level. It was found that group A beta-hemolytic streptococci, a known sore throat pathogens, were

significantly detected more often among children with throat infection than

among healthy carriers as controls (43.6 % versus. 26 %) respectively. Also there was significant difference ( $p < 0.005$ ) between infected children and healthy carriers in isolation rate of group C beta-hemolytic streptococci (16.4 % vs. 8.95 %) respectively.

The study indicated that there was significant difference in ASO value 1/200 IU/ml between infected children (57.2 %) and controls (4 %) as well as there was significant relationship between ASO positivity and beta-hemolytic streptococci carriage.

There was significant difference in CRP value between infected children (94.3 %) and controls (20 %). the sensitivity and specificity for ASO test was 57 % and 96 % respectively and for CRP test was 94.5 % and 80 % respectively.

## Introduction

Pharyngitis and tonsillitis are the commonest infection of the oral cavity at childhood (**Hayes and Williamson, 2001**) in addition to viral infection, bacterial infection is the usual causes of pharyngitis and tonsillitis and the most important causative bacteria are beta-hemolytic streptococci (**Pichichero, 1998**). In Iraq it has been reported that group G beta hemolytic streptococci play an important role in pharyngotonsillitis. In addition to group A beta-hemolytic streptococci (**Al-Shabeeb, 1977; Shalash, 1994**).

The host response immunologically to streptococcal infection with appearance of antibodies against many streptococcal cellular and extracellular components. Streptolysin-O is immunogenic; stimulate the formation of anti streptolysin-O antibody (ASO), an Ab that appears following infection with any streptococci that produce streptolysin-O (**Barnum, 1986; Rouff, 1988**). and C-Reactive protein (CRP) increases whenever there is inflammation some where in the body. This protein is mainly produced by hepatocytes in the liver in response to the cytokines IL-1, IL-6, TNF $\alpha$  and IFN $\gamma$  released by activated macrophages and natural

killer cells. IL-6 appears to be the major cytokine of importance in enhancing production of (CRP) (Lydyard et al., 2000).

***Aims of Study :***

1. To determine the carrier and infection rates of beta-hemolytic streptococci among school children and patients with throat infection in Najaf Governorate.
2. To isolate and identify the predominant group of beta-hemolytic streptococci causing throat infection in this area.
3. To find the most effective antibiotic against the most important causative agent of throat infection.
4. To determine the level of (ASO) and to detect the level of (CRP) in patients with throat infection.

**Materials and methods:**

This study was carried out during the period between October 2003 to September 2004 in Najaf Governorate. Nine schools were chosen at random from two types of socioeconomic Urban and Rural background from each school 10% of school pupils and preschools children were chosen at random in each of the two areas. The final number was (350) school pupils with age (5-15) years. The study was carried out on (250) out patients with throat infection during the same period at ENT Department in Al-Sader Teaching Hospital and Al-Hakim Hospital.

Throat swabs were taken from children then each swab soaked immediately in Brain – Heart infusion broth (B.H.I.B.) and transferred quickly to the laboratory . At the same time 3 ml of venous blood was collected from (105) patient group and from (100) healthy control group these were used to determine the ASO titer and for detection of CRP,. the sera were stored in small test tube at (-20) c<sup>0</sup> until it use.

The presence of CRP in the sera of the tested individuals was evaluated by using CRP (Liner chemicals, Spain), which is a rapid test for the determination of CRP in serum by agglutination of latex particles on slide. And for the determination of ASOT in the sera of tested individuals, ASOT kit (Liner chemicals, Spain) was used.

The data were analyzed statistically using chi-square ( $\chi^2$ ) test and P-value 0.05,0.005 and sensitivity and specificity test (Daniel, 1999).

**Results and Discussion:**

The incidence of different groups of beta-hemolytic streptococci got from the culture of (600) throat swabs collected from (350) healthy children and (250) children suffering from throat infections. the results indicate that group A beta-hemolytic streptococci 200(33.3 %) and group C 72(12 %) were predominant in the throats of children in comparison with group B 25(4.2 %), group D 57(9.5 %), group G 14(6.8 %), group F 5(0.8%) and negative results for beta-hemolytic streptococci 200(33.3 %).(table.1)

**Table (1) Incidence of different groups of beta-hemolytic streptococcus isolates.**

Bacterial isolate	No.	%
Group A streptococci	200	33.3
Group B streptococci	25	4.2
Group C streptococci	72	12
Group D streptococci	57	9.5
Group G streptococci	14	6.8
Group F streptococci	5	0.8
Negative results	200	33.3
Total	600	100

Table (2) shows a comparison between the numbers and type of beta-hemolytic streptococci isolated from (250) patients with tonsillitis or pharyngitis and (350) healthy carrier. The comparison indicated that 109 isolates of Lancefield group A streptococci (*Streptococcus pyogenes*) isolated from children suffering from throat infections. Which means 43.6 % of them were harboring this bacterial species, while 91 isolate --from healthy carriers, which means only 26 % of them were harboring this bacteria. with significant difference (p<0.005). The incidence of group B B.H.S.

showed no significant difference ( $p>0.05$ ) between patients 10(4 %) and healthy controls 15(4.3 %) which reduce their importance in causing of tonsillitis or pharyngitis. Generally, these results agreed with the results reported by (Shalash, 1994 and Bisno, 2001) and contrary with the results of Al-Shabeeb (1977) who pointed out that the great majority is to group G B.H.S. as a causative agent of pharyngitis and tonsillitis in children in Iraq. And Cimolai et al., (1993) who pointed out that group C B.H.S. was not associated with throat infection. As a result, it was concluded that *Streptococcus pyogenes* is the most important group of beta-hemolytic streptococci that correlated with the incidence of throat infection in children.

**Table (2) a comparison between the number and types of beta-hemolytic streptococci isolates.**

Bacterial isolate	Patients		Carriers		P-value
	No.	%	No.	%	
Group A streptococci	109	43.6	91	26	$X^2 = 33.185, P<0.005^*$
Group B streptococci	10	4	15	4.3	$X^2 = 0.029, P>0.05^*$
Group C streptococci	41	16.4	31	8.9	$X^2 = 7.855, P<0.005$
Group D streptococci	20	8	37	10.6	$X^2 = 4.788, P<0.05$
Group G streptococci	23	9.2	18	5	$X^2 = 10.124, P<0.005$
Group F streptococci	2	0.8	3	0.9	$X^2 = 0.005, P>0.05$
Negative results	45	18	155	44.3	$X^2 = 34.106, P<0.005$
Total	250	100	350	100	$X^2 = 65.252, P<0.005$

Results of the immunological study are important to give us a clear picture of the human defense system.

in this study there were higher percentage 60(57.2 %) of patients with B.H.S. throat infection in ASO titer above 1/200 IU/ml. whereas only 4(4%) of healthy controls had ASO titer above 1/200 IU/ml. a difference was highly significant ( $p<0.005$ ) as shown in (table 3).

**Table (3) Antistreptolysin-O titer.**

ASO titer IU/ml	Patients		Controls	
	No.	%	No.	%
0-80	16	15.2	83	83
80-200	29	27.6	13	13
200-400	22	21	4	4
400-800	32	30.5	0	0
800-1600	6	5.7	0	0
1600-2300	0	0	0	0
Total	105	100	100	100
$\chi^2 = 89.9$ $df= 5$ $p < 0.005$				

These results are in agreement with those reported by **Beckman (2003)** who found approximately 80-85 % of the patients who demonstrate a group A B.H.S. throat infection will also demonstrate an elevated ASO titer.

The finding of a significant relationship between ASO positivity and the carriage of B.H.S (table 4). indicated that ASO measurement might be used together with throat culture to identify B.H.S. especially group A.B.H.S. carrier. These results are in agreement with those reported by **Okuyama et al. (1989)** who found the ASO titers were elevated in children who carried A, C,G,B,H,S also **Ozturk et al. (2004)** who reported a significant difference between GA.B.H.S. carriage ASO positivity values.

Table (4) The percentage of beta-hemolytic streptococcal throat carriage and associated serum ASO positivity among (100) healthy individual.

Individuals	Culture results		ASO titer $\geq 200$ IU/ml	
	NO.	%	NO.	%
B.H.S. carrier	26	26	10	38.4
B.H.S. non carrier	74	74	7	9.5
<b>X<sup>2</sup>=7.34</b>		<b>df=1</b>	<b>P&lt;0.05</b>	

Beta-hemolytic streptococci was isolated from 95(90.5 %) of the total 105 throat culture from children with throat infection, and other bacteria was isolated from other 10 cultures. A difference between ASO tests and B.H.S. culture results are statistically non significant ( $p>0.05$ ) as shown in (table 5).

Table (5) Comparison between the results of culture of beta-hemolytic streptococci and Antistreptolysin-O test in children with throat infection.

Results	Culture of B.H.S.		ASO test	
	No.	%	No.	%
Positive	95	90.5	89	84.8
Negative	10	9.5	16	15.2
Total	105	100	105	100
<b>X<sup>2</sup> = 1.56</b>		<b>df= 1</b>	<b>p&gt; 0.05</b>	

These results are in agreement with those reported by **Centor et al., (1989)** and **Tanz et al., (1997)** who reported there is no acceptable gold standard predicting throat infection caused by B.H.S.

The results of CRP showed a highly significant difference ( $p < 0.005$ ) between patients with throat infection and controls .

These results are in agreement with those reported by **Putto et al., (1986)** who found that mean CRP levels in a patient who presents with a clinical picture for streptococcal pharyngotonsillitis was ( $6.5 \pm 4.9$  mg/dL). However, this study confirms that B.H.S. throat infections are associated with markedly elevated CRP concentrations(table 6).

**Table (6) C-Reactive Protein Titer**

CRP titer Mg/dL	Patients		Controls	
	No.	%	No.	%
Negative	6	5.7	80	80
≥ 0.6	45	42.9	20	20
1.2	32	30.5	0	0
2.4	10	9.5	0	0
4.8	6	5.7	0	0
9.6	6	5.7	0	0
Total	105	100	100	100
$X^2 = 127.18$ $df = 5$ $p < 0.005$				

Also there is significant difference ( $p < 0.05$ ) between ASO and CRP test in predicting throat infection. (Table 7).

the present study found that the sensitivity of ASO and CRP for predicting throat infections was 57 % and 94 % respectively. And the specificity of ASO and CRP was 69 % and 80 % respectively. These findings may indicate that ASO test was more specific than CRP in predicting B.H.S. throat infection.



These results are in agreement with those reported by **Audit et al., (2002)** who found CRP rapid test was poor specificity as a diagnostic to all in of case respiratory tract infection.

**Table (7) Comparison between the results of Antistreptolysin-O and C-Reactive protein titration.**

Titer	ASO titer	Patients positive with ASO test		CRP titer	Patients positive with CRP test	
		No.	%		No.	%
first	200	29	32.6	≥ 0.6	45	45.5
second	400	22	24.7	1.2	32	32.3
third	800	32	36	2.4	10	10.1
fourth	1600	6	6.7	4.8	6	6.05
fifth	3200	0	0	9.6	6	6.05
total		89	100		99	100
$X^2 = 20.49$ $df= 4$ $p < 0.05$						

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