Comparative Study between Bacteriological and Serological tests in determining Streptococcal Throat Infection among School Children

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Abstract: Group A Streptococcus (GAS) is an important cause of morbidity and mortality among children and responsible for 20-30 % of bacterial pharyngitis. A cross sectional study was conducted on 535 children aged 4-15 years with throat infection, during the period from October 2015 to May 2016 at Ha'il Provence, Saudi Kingdom. Demographic data were collected by questionnaire. Throat swabs were collected and processed with the standard microbiological techniques to isolate GAS. Females accounted for 57.2 % more than male 37.3 %. Sixty-two percent of the infected children were in 4-10 years old. The number and percentage of group A β-hemolytic streptococci for patient and healthy were the most common. It recorded 99 (55 %) and 55 (15.49 %), respectively. Statistically, there is no significance difference between the culture and ASO titer in determining of Streptococcal throat infection ($p > 0.05$). Also, our study found that the sensitivity and specificity of ASO and CRP for predicting throat infections was 46.85, 92.72, 90.9 and 76.6 % respectively. Statistically, there was a significant correlation between ASOT and CRP test in determining of streptococcal throat infection ($r = 0.919$, $p = 0.027$).
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1. Introduction

Streptococcus pyogenes or Lancefield Group A β-hemolytic streptococci is one of the commonest bacterial pathogens that cause pharyngitis among school aged children living in lower socioeconomic conditions (Gupta *et al*., 1992; Bisno, 2001; Nandi *et al*., 2002). The prevalence of group A β-hemolytic streptococci (BHS) carriage in the throat of school children varies from 13 to 50 % depending upon the population studied, season and other factors (Gupta *et al*., 1992).

Around the world, an estimated 18 million people currently suffer from a serious GAS disease with over 1.7 million new cases per year and 500 000 deaths per year. In addition to serious diseases, there are over 100 million prevalent cases of pyoderma and over 600 million new cases of GAS pharyngitis per year (Carapetis, *et al*., 2005). The use of immunological assays such as Anti Streptolysin-O (ASO) titer would provide useful in the diagnosis of streptococcal infections and their complications, and during follow-up, as well as in evaluating the effectiveness of treatments (Mahendrappa, 2010) as well as in situations when the throat culture technique is ineffective or when the patient has commenced antibiotics therapy. Significant findings have shown that an ASO positive measurement might be used in conjunction with throat culture to identify GAS) (Manandhar *et al*., 2013).

The ASO assay is the most commonly used streptococcal antibody test. Antibodies to Streptolysin O are generated by the humoral immunity and can be quantified as Anti-Streptolysin-O Antibodies (i.e. ASO titer). Anti Streptolysin-O (ASO) titer is specific neutralizing antibody produced after infection with these organisms and it appears in serum from 1 week to 1 month after the onset of a streptococcal infection.

The ASO antibody response rises approximately 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 (Shet and Kaplan, 2002). 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 (Chernecky and Berger, 2001). The normal values for ASO depend upon the age of the patient, geographical location, epidemiological setting and season of the year (Sethi *et al*., 2003).

C-reactive protein (CRP) increases whenever there is inflammation somewhere in the body. This protein is mainly produced by hepatocytes in the liver in response to the cytokines IL-1, IL-6, TNFα and IFNγ 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). The current study aimed to determine the prevalence of beta-hemolytic streptococci among school children with throat infection at Ha'il Provence, Saudi Kingdom, as well as to compare between bacteriological and serological tests in determining streptococcal throat infection.

2. Materials And Methods

2.1. Study Population

Our study was conducted on 535 children aged 4-15 years with throat infection, during the period from October 2015 to May 2016 at Ha'il Provence, Saudi Kingdom. Children who were on antibiotic treatment or who had taken antibiotics within 7 days before sample collection were excluded from the study. Patients' demographic information was collected through a questionnaire.

2.2. Collection, transportation and processing of throat swab

Throat swabs samples were collected from posterior pharynx and tonsils using sterile cotton swabs. Necessary care was taken not to swab the cheeks, tongues, lips or other areas of the mouth. The swabs were placed immediately in Amies transport medium (Oxoid, England) and transported to Microbiology Laboratory and processed within 2 hours of collection (Carroll and Reimer, 1996; McDonald, *et al*., 2006). Then, the throat swabs were inoculated onto 5 % sheep's blood agar plates and incubated for 24 hours at 37 ^{o}C in a candle jar, which can provide an atmosphere of 5 % CO_2. Culture plates negative for β-haemolytic colonies were incubated for additional 24 hours to allow the growth of slow growers. Identification of GAS isolates was made based on the standard microbiological techniques which include β-haemolytic activity on sheep's blood agar, small colony characteristics, Gram positive cocci, catalase production negative, 0.04-U bacitracin disc susceptible and PYR positive (Hardy Diagnostics, USA) tests (Carroll and Reimer, 1996; Cheesbrough, 2002; Brahmadathan and Gladstone, 2006).

2.3. Lancefield Grouping of Streptococci

The procedure of the manufacture company (OMEGA D. Ltd.) has been employed, as following: In a clean test tube, 0.3 ml of extraction enzyme has been placed; the five colonies have been suspended by using a loop. This suspension has been incubated in a water bath at 37 ^{o}C for 10 minutes (shaking after 5 min.), then by using pasture pipette, one drop of the extract has been added to each of the circles on the test card. After that one drop one each latex reagent has been added also and mixed well by using a clean mixing stick each time. The card has been rotated gently for up to one minute to check the appearance of agglutination in corresponding group. Positive reaction was detected by red clumps of a green background (Facklam, 1980).

2.4. Serological Tests

2.4.1. Blood Samples Collection

Blood samples were collected from patients and carriers of school student's age 4 - 15 year. Blood samples of 3 - 5 ml were collected by venipuncture, using plastic disposable 5 ml 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 (Hoff, 2000).

2.4.2. Determination of ASOT

For the determination of ASOT in the sera of tested individuals, ASOT kit (Liner chemicals, Spain) was used. The latex reagent was mixed with an equal volume of serum, if the reaction took place due to the presence of Anti Streptolysin-O in the serum sample, a clear agglutination became evident. Positive results were further tested by the same procedure after preparing serial double dilutions to determine the highest dilution that was still present as a clearly visible agglutination. The approximate ASO rate present in the sample could be obtained by multiplying the titer by the limit of sensitivity (200 IU/ml) (Klein, 1976; Haffejee, 1992).

2.4.3. Determination of C-reactive protein

The presence of C-reactive protein (CRP) in the sera of the tested individuals was evaluated by using CRP kit (Liner chemicals, Spain), which is a rapid test for the determination of CRP in serum by agglutination of latex particles on slide. The analytical procedure was: added one drop (50 µl) of sample and of each one of the controls into card. Added into each circle one drop of the reactive CRP latex near to the sample to be tested, helped with small stick mix the components recovering all the surface of the circle. Rotate the slide slowly either by hand or by means of a mechanical rotator for a period of 2 minutes. Compared test result with control sera mild or strong agglutination indicates a positive reaction. The positive sample had to be diluted. To do that makes diluted series two by two in saline solution. The titer from a serum was defined as the highest dilution that gives the positive result. The approximated CRP rate present in the sample could be obtained by multiplying the titer by the limit of sensitivity (0.6 mg/dl) (Young, 1997).

2.5. Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Science (SPSS),

Version 15 for Windows. Chi-square test was used to compare categorical variables. A p-value less than 0.05 were considered significant at 95 % confidence level.

3. Results

The present study was carried out on 535 children aged 4-15 years with throat infection. Structured questionnaire was used to collect demographic information. Our study showed frequency and percentage of total sample (patient and healthy children) according to age, sex and residence was included 180 (34 %) patients with streptococcal tonsillitis and the other 335 (66 %) healthy (Fig. 1). Table 1 represents the prevalence of β-hemolytic streptococcus (BHS) with respect to socio demographic characteristics among children with throat infection. For age of this sample showed (4-10 years) 113 (62.7 %) more than (11-15 year) 67 (37.3%) and sex of sample showed female 103 (57.2 %) more than male 77 (42.8 %). Also, for residence of sample urban area recorded a higher number and percentage of patients 132 (73.4 %) more than rural area 48 (26.6 %).

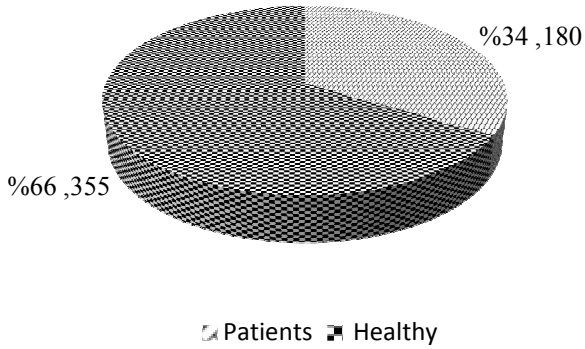


Fig. 1: The frequency and percentage of patient and healthy children.

Table 1: The prevalence of β-hemolytic streptococcus (BHS) with respect to socio demographic characteristics among children with throat infection

Characteristics	Number of BHS isolated		P- Value
	Frequency	Percentage (%)	
Age			
4-10 year	113	62.7	P = 0.00
11-15 year	67	37.3	
Sex			
Male	77	42.8	P = 0.05
Female	103	57.2	
Residence			
Urban	132	73.4	P = 0.00
Rural	48	26.6	
Total	180	**100 %**	--

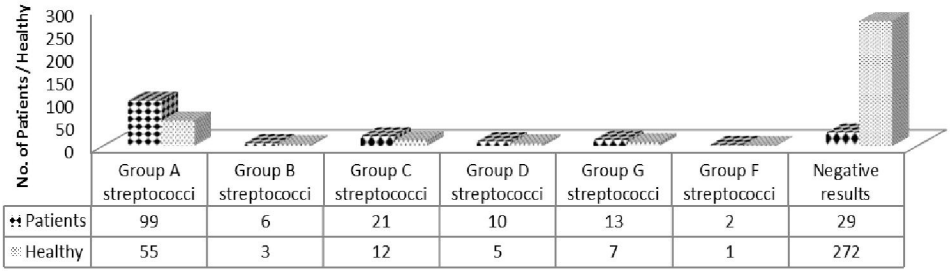


Fig. 2: A comparison between the number and types of ß-hemolytic streptococci isolates in patients and healthy children

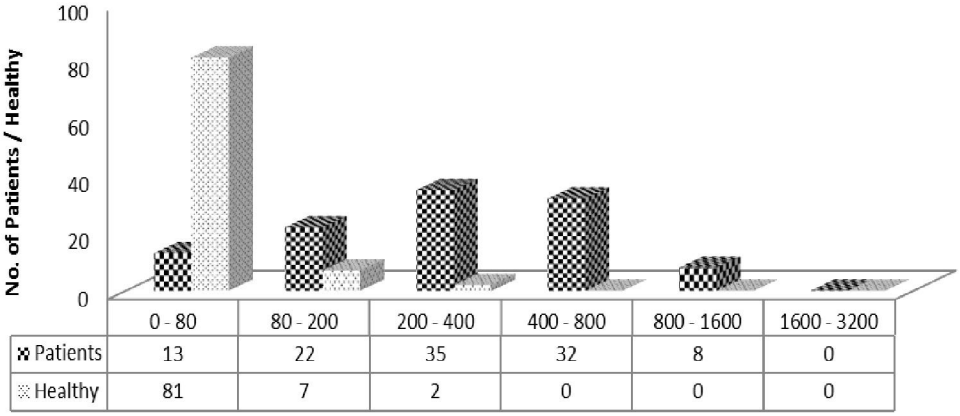
3.1. A comparison between the number and types of ß-hemolytic streptococcal isolates in patients and healthy children.

Fig. 2 represents the incidence of different groups of β-hemolytic streptococci got from the culture of (535) throat swabs collected from (355) healthy children and (180) children suffering from throat infections. The results showed that group A β-hemolytic streptococci for patient and healthy were the most common, it recorded 99 (55 %) and 55 (15.49 %), respectively, followed by group C β-hemolytic streptococci for patient and healthy, were recorded 21 (11.66 %) and 12 (3.38 %), respectively. Group A and C β-hemolytic streptococci were

predominant among the infected children (Patients) in comparison with group B β-hemolytic streptococci 6 (3.33 %), group D β-hemolytic streptococci 10 (5.55 %), group G β-hemolytic streptococci 13 (7.23 %), group F β-hemolytic streptococci 2 (1.12 %) and negative results for β-hemolytic streptococci 29 (16.11 %). Statistically, our data indicated that there was a highly significant difference between the number and percentage of Group A β-hemolytic streptococci in patients and healthy children (P = 0.000).

3.2. A comparison between the number and percentage of Antistreptolysin- O titer (ASOT) in patients and healthy children

Results of the immunological study are important to give us a clear picture of the human defense system. Our study recorded a higher number and percentage 75 (68.19 %) of patients with β-hemolytic streptococci throat infection in ASO titer above 1/200 IU/ml, whereas only 2 (2.22 %) of healthy controls had ASO titer above 1/200 IU/ml (Fig. 3). ASO titer ranged from 200-400 and 400-800 IU/ml were recorded a highest number and percentage of patients. They recorded 35 (31.8%) and 32 (29.1%), respectively. Statistically our data indicated that there was a highly significant difference between the number and percentage of Antistreptolysin- O titer (ASOT) in patients and healthy children (p = 0.000).



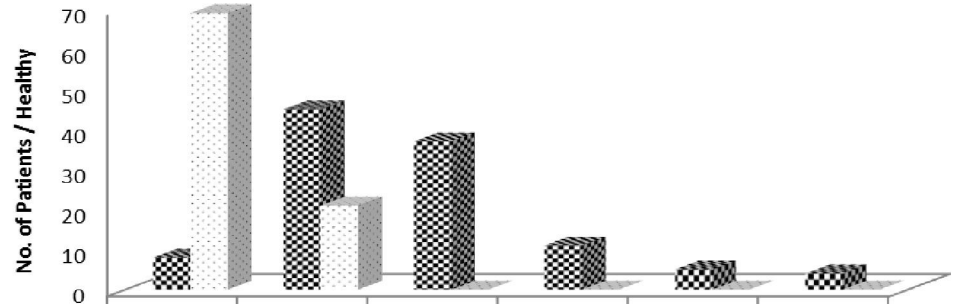
	0 - 80	80 - 200	200 - 400	400 - 800	800 - 1600	1600 - 3200
Patients	13	22	35	32	8	0
Healthy	81	7	2	0	0	0

Fig 3: A comparison between the number and percentage of Antistreptolysin- O titer (ASOT) in patients and healthy children.

3.3. A comparison between the number and percentage of C-reactive protein (CRP) in patients and healthy children.

Our data of CPR test showed a higher number and percentage of patients and healthy children at titer ≥ 0.6. It recorded 45 (41.82 %) and 21 (23.34 %), respectively. Also, the number and percentage of patients were predominant at titer ranged between (0.6 - 1.2 mg/dl).

Statistically, the results of CRP test showed a highly significant difference ($p < 0.05$) between patients with throat infection and healthy children at all titers (Fig. 4).



	Negative	≥ 0.6	1.2	2.4	4.8	9.6
Patients	8	45	37	11	5	4
Healthy	69	21	0	0	0	0

Fig 4: A comparison between the number and percentage of C - reactive protein (CRP) in patients and healthy children.

3.4. Comparison between the results of culture of β-hemolytic streptococci and Antistreptolysin-O titer in children with throat infection.

Fig. 5 represents the comparison between the results of culture of β-hemolytic streptococci and Antistreptolysin-O titer in children with throat infection. β-hemolytic streptococci was isolated from 98 (89.1 %) of the total 110 throat culture from children with throat infection, also ASOT was positive in 97 (88.2 %) of total 100 children with throat infection. Statistically, there is no significance difference between the culture and ASO titer in determining of *Streptococcus pyogenes* (P > 0.05).

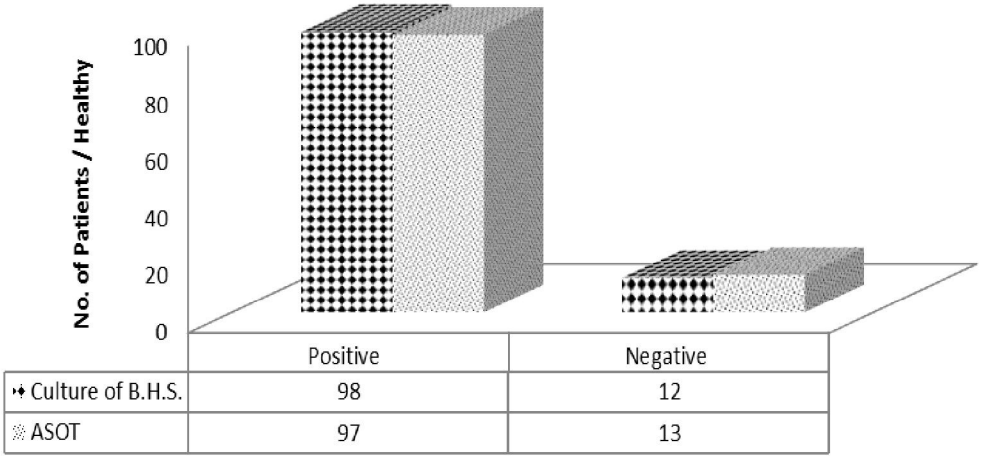


Fig. 5: Comparison between the results of culture of β-hemolytic streptococci and Antistreptolysin-O titer in children with throat infection.

3.5. Comparison between the percentage of C-reactive protein (CRP) and ASOT among children with throat infection.

Fig. 6 represents the comparison between the percentage of C-reactive protein (CRP) and ASOT among children with throat infection. First and second titer in both ASOT and CRP test were predominant, it recorded 22.68, 45.09; 36.09 and 37.26, respectively. Statistically, our data showed significant difference between ASO and CRP test in predicting throat infection (p < 0.05). Also, our study found that the sensitivity of ASO and CRP for predicting throat infections was 46.85 % and 92.72 % respectively. And the specificity of ASO and CRP was 90.9 % and 76.6 % respectively. Statistically, there was a significant correlation between ASOT and CRP test in determining of streptococcal throat infection (r = 0.919, p = 0.027) (Fig. 7 and Table 8).

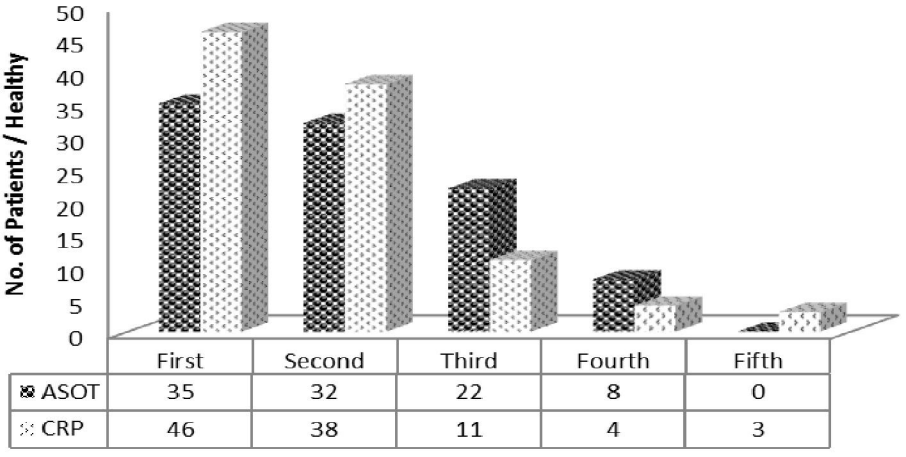


Fig. 6: Comparison between the percentage of C - reactive protein (CRP) and associated serum ASOT positivity among children with throat infection.

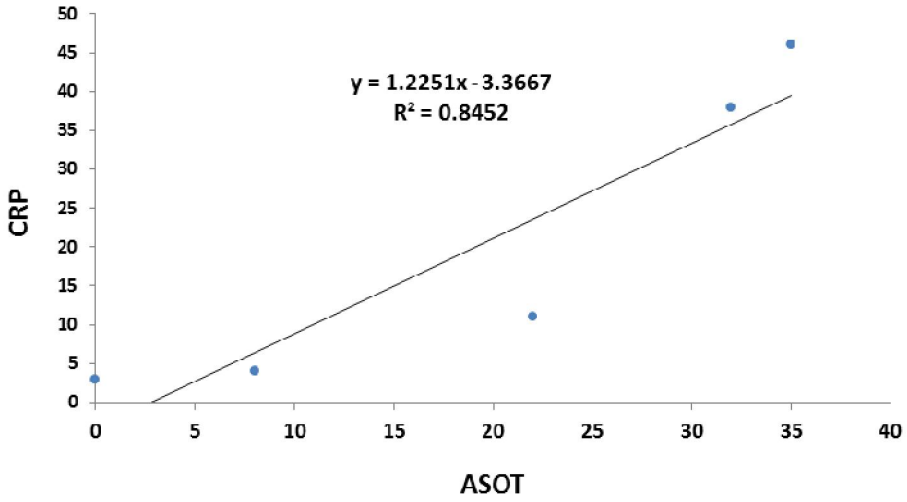


Fig. 7: Correlation between ASOT and CRP (r = 0.919, p = 0.027).

Table 8: Pearson's correlation coefficient between ASOT and C - reactive protein

	ASOT	CRP
ASOT Pearson Correlation	1	0.919*
Sig. (2-tailed)		0.027
N	5	5
CRP Pearson Correlation	0.919*	1
Sig. (2-tailed)	0.027	
N	5	5

**** Correlation is significant at 0.01 levels.**

The Pearson's correlation coefficient between ASOT and CRP (0.919*), the p-value of the correlation (0.027), this correlation is significant because the p-value is less than 0.05.

4. Discussion

Group A Streptococcus (GAS) is the most common bacterial cause of acute pharyngitis, responsible for 20-30 % of sore throat in children (Shulman *et al*., 2012). The prevalence of this type of pharyngitis varies among different age groups. It is most commonly seen in school-age children (Kim, 2015). Our study showed that group A β-hemolytic streptococci for patient and healthy were 99 (55%) and 55 (15.49%), respectively. Also, group C β-hemolytic streptococci for patient and healthy were 21 (11.66%) and 12 (3.38%), respectively.

Similar work carried out by Anitha *et al*., (2016), he found that the most commonly isolated bacteria was *Streptococcus pyogenes* (37%), followed by *Klebsiella pneumoniae* (31%), *Pseudomonas aeruginosa* (14%), *Staphylococcus aureus* (9 %) and 3% for *E.coli*, *Citrobacter koseri*, *Acinetobacter baumanii*.

Group A and C β-hemolytic streptococci were predominant among the infected children (Patients) in comparison with group B β-hemolytic streptococci 6 (3.33 %), group D β-hemolytic streptococci 10 (5.55 %), group G β-hemolytic streptococci 13 (7.23 %), group F β-hemolytic streptococci 2 (1.12 %) and negative results for β-hemolytic streptococci 29 (16.11 %). Statistically, our data indicated that there was a highly significant difference between the number and percentage of Group A β-hemolytic streptococci patients and healthy children (P= 0.000). Generally, these results were in agreement with the results reported by Shalash (1994) and Bisno (2001).

Tesfaw *et al*., (2015) studied the prevalence of group A β-haemolytic streptococcus among children with pharyngitis, he found that females accounted for 57.7 % of 355 children with pharyngitis. Similarly, our study showed that the number and percentage in female accounted for 103 (57.2 %) more than male 77 (42.8 %). Also, for age (4-10 years) our data recorded 113 (62.7 %) more than (11-15 year) 67 (37.3%), this finding was in agreement with Grace *et al*., (2015) and Suri *et al.,* (2016), they found that the prevalence of *Streptococcus pyogenes* was more in the age group 5-10 years and slightly more in females than males.

Our study recorded a higher number and percentage 75 (68.19 %) of patients with β-hemolytic streptococci throat infection in ASO titer above 1/200 IU/ml, whereas only 2 (2.22 %) of healthy controls had ASO titer above 1/200 IU/ml. this indicated highly significant relationship between ASO positivity and GAS carriage. These finding was in agreement with those reported by Beckman (2003) and Ozturk *et al*., (2004) they found approximately 80-85 % of the patients who demonstrate a group A

β-hemolytic streptococci throat infection will also demonstrate an elevated ASO titer and indicated that ASO measurement might be used together with throat culture to identify GAS carriers.

Our data showed that CRP test at titer ≥ 0.6 mg/dl in patients and healthy children recorded 45 (41.82 %) and 21 (23.34 %), respectively. Also, the number and percentage of patients were predominant at titer between (0.6 to 1.2 mg/dl). Statistically, the results of CRP test showed a highly significant difference between the number and percentage of patients and healthy children at all titers ($p < 0.05$). These results was 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 ± 4.9 mg/dl).

However, this study confirms that β-haemolytic streptococcus (B.H.S.) throat infections are associated with markedly elevated CRP concentrations. β-hemolytic streptococci was isolated from 98 (89.1 %) of the total 110 throat culture from children with throat infection, also ASOT was positive in 97 (88.2 %) of total 100 children with throat infection. Statistically, there is no significance difference between the culture and ASO titer in determining of *Streptococcus pyogenes* ($P > 0.05$).

First and second titer in both ASOT (200 and 400 IU/ml) and CRP (≥ 0.6 and 1.2 mg/dl) test were predominant, it recorded 22.68, 45.09, 36.09 and 37.26, respectively. Statistically, our data showed that there is no significant difference between ASO and CRP test in predicting throat infection ($p < 0.05$), this finding was not in agreement with Ekah and Blessing (2015); they found that the level of ASO in patients that tested positive for the presence of ASO titer ranged from 400 to 3200 IU/ml. Also, our study found that the sensitivity of ASO and CRP for predicting throat infections was 46.85 % and 92.72 %, respectively, and the specificity of ASO and CRP was 90.9 % and 76.6 % respectively. Statistically, there was a significant correlation between ASOT and CRP test in determining of *Streptococcus pyogenes* ($r = 0.919$, $p = 0.027$). These finding indicated that ASO test was more specific than CRP in predicting β-hemolytic streptococci throat infection. Similarly, our finding was in agreement with those reported by Audit *et al*., (2002) and Manandhar *et al*., (2013), they found CRP rapid test with poor specificity as a diagnostic to all in of case respiratory tract infection. Also, showed that an ASO-positive measurement might be used in conjunction with throat culture to identify Group A Streptococcus (GAS) carriers.

It was concluded that Streptococcal throat infection were predominant in age (4-10 years) more than age (11-15 year) and for sex showed female more than male. Also, for residence, urban area recorded a higher number and percentage of patients than rural area. Group A and C β-hemolytic streptococci were predominant among the infected children and there is no significance difference between the culture and ASO titer in determining of *Streptococcus pyogenes*. Also, there was a significant correlation between ASOT and CRP test in determining streptococcal throat infection.

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