RESEARCH ARTICLE

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ASSESSMENT OF SOME SEROLOGICAL TESTS FOR DIAGNOSIS OF TONSILLITIS CAUSED BY B-HEMOLYTIC STREPTOCOCCI IN RAMADI, IRAQ

ABSTRACT:
The main objective of the present study was to evaluate the performances of five serological assays Anti-Streptolysin O Titer, (ASOT), C Reactive Protein (CRP), Rheumatoid Factor (RF), Systemic Lupus Erthymatosus (SLE), Infectious Mononucleosis (IM) tests for the diagnosis of tonsillitis due to beta hemolytic streptococci in AL-Ramadi (west of Iraq). One hundred ninety one tonsillitis patients were included in this study. They were attending department of ENT in Al Ramadi General Hospital in Iraq during the period from April 2008 to February 2009. Serum samples were taken from each one. Patients were classified into three groups; first one consists of 109 patients with recurrent tonsillitis, second consists of 45 patients with chronic tonsillitis and third consists of 37 patients with recurrent acute tonsillitis. In addition, twenty serum sample of healthy control group were included. There was a high titer of antibodies against Streptolysin O (ASOT) test in patients with a recurrent, chronic, recurrent acute tonsillitis due to beta hemolytic streptococci, whereas the responses rates were markedly very low using other serological tests. The present study confirmed that the anti-Streptolysin O test (ASOT) is recommended for detection group A beta hemolytic streptococci (GBHS) among the patients with tonsillitis.

KEY WORDS:
Beta-hemolytic streptococci, Serological assays

INTRODUCTION:
Tonsillitis is a disease that frequently occurs with wide global distribution. Streptococcus pyogenes (group A) is still the most frequent cause of tonsillitis and can lead to several squeals (severe post infection sequence) including rheumatic fever and glomerulonephritis (Bisno AL. 1991; Bisno AL. 2001; Cooper et al., 2001). Many Iraqi researchers contributed to identification of group A streptococcus (group A) as an etiological agent of tonsillitis disease (Al-Mehdi, 1989; Fadhil, 1989; Abdul-Hameed, 1999; Al-Ani, 2000; Al-Gebori, 2007)

Various microorganisms may be involved in tonsillitis like viral, bacterial and other microorganisms (Gerber, 1986). Although many infectious agent may cause tonsillitis, Streptococcus pyogenes (group A) are the most significant which is responsible for 15 % of all cases , while 80-90% of cases are caused by viruses particularly adeno virus (Durand et al., 1998). The viral infection is usually self-limiting with symptoms lasting for less than five days (Rotbart, 1998), whereas the bacterial infection is considered a serious infection when complications occurs (WHO, 1989). Isolation of Streptococcus pyogenes (group A) with very low significant rise of antibodies indicates a carrier state (WHO, 1988; Koshi, 1982).

This study was conducted to evaluate the serological tests (ASOT, CRP, RF SLE, IM) for measuring antibody response for tonsillitis infection and to provide comparative data for dissemination for inquiring clinicians.

MATERIAL AND METHODS:
Three ml of venous blood were taken aseptically from each patient and transferred to clean and dry tube (without anticoagulant) for serum collection after clotting and centrifuging the blood at 2500 rpm for 5 minutes. Pooled serum was kept in Epindruf tubes at -20°C to be used. Serum samples were taken from 191 patients attending the ENT department in Al Ramadi General Hospital during the period extended from
April, 2008 to February, 2009. Control samples were taken from healthy control persons. Patients were divided into three groups, group one consists of 109 patients with recurrent tonsillitis, group two consists of 45 patients with chronic tonsillitis (tonsillectomy) and group three consists of 37 patients with recurrent acute tonsillitis.

Sero logical tests in this work included ASOT, CRP, RF, SLE, and IM tests were used in the diagnosis of infections with group A beta-hemolytic streptococci (GBHS) and other microorganisms as the causative agent for tonsillitis. These tests were carried out according to the type of test as follows; ASOT was performed as described previously (Spaun et al., 1961) using a kit of ASOT latex test (Linear, chemical, Barcelona, Spain) by standard micro titer procedure base on the intramural standard method (Bisno, 2001). RF test was performed essentially by using a kit of RF latex test (Linear, chemical, Barcelona, Spain) as described by Ball and Lawrence (Ball and Lawrence, 1963). CRP test was performed using of CRP latex test (Omega, diagnostic, Scotland, UK, according to Fisher et al. (1976). SLE test was performed by using a kit of SLE latex test (Omega, diagnostic, Scotland, UK). I.M test was performed by using a kit of monolatex test (Biokit, Barcelona, Spain).

**RESULTS:**

One hundred and nine patients with recurrent tonsillitis due to GBHS were included in this study. Fifty six (51.4%) of them were showing positive results for ASOT and CRP while thirteen (11.9%) of them were positive for RF, only four (3.7%) of them were positive for SLE and three (2.8%) for IM test (Table 1 & Fig. 1).

Table 1. Frequency of elevated Antibody (RF, ASOT, CRP, SLE, and IM) with GBHS of individuals infected with recurrent tonsillitis.

<table>
<thead>
<tr>
<th>Test type</th>
<th>RF I.U/dl</th>
<th>ASOT I.U/dl</th>
<th>CRP mg/l</th>
<th>SLE</th>
<th>Mononucleosis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Value</td>
<td>8±</td>
<td>≤16</td>
<td>200±</td>
<td>≤400</td>
<td>6±</td>
</tr>
<tr>
<td>Total Exam</td>
<td>(88.1%)</td>
<td>(11.9%)</td>
<td>(48.6%)</td>
<td>(51.4%)</td>
<td>(48.6%)</td>
</tr>
<tr>
<td>GABHS</td>
<td>14</td>
<td>1</td>
<td>5</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>GFBHS</td>
<td>32</td>
<td>4</td>
<td>16</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>GCBHS</td>
<td>17</td>
<td>0</td>
<td>12</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>GBBHS</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>GDBHS</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other M.O</td>
<td>29</td>
<td>8</td>
<td>18</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Control group</td>
<td>20</td>
<td>0</td>
<td>19</td>
<td>1</td>
<td>18</td>
</tr>
</tbody>
</table>

Fig. 1. Frequency of elevated Antibody (RF, ASOT, CRP, SLE, and IM) with GBHS of individuals infected with recurrent tonsillitis.
Forty-five patients with chronic tonsillitis due to the BHS were included in this study. Twenty-six (57.8%) of them were showing positive results for ASOT, while four (8.8%) showing positive results for CRP, two only (4.5%) of them were for positive RF and two (8.8%) of them were positive for SLE and two only (4.5%) for IM test (Table 2 & Fig. 2).

Table 2. Frequency of elevated Antibody (RF, ASOT, CRP, SLE, and IM) with GBHS and other microorganism of individuals infected with chronic tonsillitis.

<table>
<thead>
<tr>
<th>Test type</th>
<th>RF I.U/dl</th>
<th>ASOT I.U/dl</th>
<th>CRP mg/L</th>
<th>SLE</th>
<th>Mononucleosis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Value</td>
<td>8±</td>
<td>≤16</td>
<td>200±</td>
<td>≤400</td>
<td>6±</td>
</tr>
<tr>
<td>Total patients</td>
<td>(95.5%)</td>
<td>(4.5%)</td>
<td>(42.2%)</td>
<td>(57.8%)</td>
<td>(91.2%)</td>
</tr>
<tr>
<td>BHS and other O.M</td>
<td>(69.7%)</td>
<td>(100%)</td>
<td>(68.4%)</td>
<td>(73.1%)</td>
<td>(70.7%)</td>
</tr>
<tr>
<td>BHS</td>
<td>(4.6%)</td>
<td>(0.0%)</td>
<td>(5.2%)</td>
<td>(3.8%)</td>
<td>(2.5%)</td>
</tr>
<tr>
<td>Other M.O</td>
<td>(25.7%)</td>
<td>(0.0%)</td>
<td>(26.4%)</td>
<td>(23.1%)</td>
<td>(26.8%)</td>
</tr>
</tbody>
</table>

Thirty-seven with patients with recurrent tonsillitis due to BHS were included in this study. Ten (27.1%) of them were showing positive results for ASOT, while Fifteen (40.5%) of them were showing positive results for CRP. Two (5.5%) of them were for positive RF, only one (2.8%) of them were positive for SLE and IM test (Table 3 & Fig. 3).

Table 3. Frequency of elevated Antibody (RF, ASOT, CRP, SLE, and IM) with GBHS and other microorganism of individuals infected with recurrent acute tonsillitis.

<table>
<thead>
<tr>
<th>Test type</th>
<th>RF I.U/dL</th>
<th>ASOT I.U/dL</th>
<th>CRP mg/L</th>
<th>SLE</th>
<th>Mononucleosis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Value</td>
<td>8±</td>
<td>≤16</td>
<td>200±</td>
<td>≤400</td>
<td>6±</td>
</tr>
<tr>
<td>Control group</td>
<td>(100%)</td>
<td>(0.0%)</td>
<td>(95%)</td>
<td>(5%)</td>
<td>(90%)</td>
</tr>
<tr>
<td>GABHS</td>
<td>(11.5%)</td>
<td>(0.0%)</td>
<td>(7.4%)</td>
<td>(20%)</td>
<td>(9.0%)</td>
</tr>
<tr>
<td>GBBHS</td>
<td>(5.7%)</td>
<td>(0.0%)</td>
<td>(3.7%)</td>
<td>(10%)</td>
<td>(0.0%)</td>
</tr>
<tr>
<td>GCBHS</td>
<td>(45.7%)</td>
<td>(0.0%)</td>
<td>(48.1%)</td>
<td>(30%)</td>
<td>(45.5%)</td>
</tr>
<tr>
<td>BHS &amp; OM</td>
<td>(37.1%)</td>
<td>(100%)</td>
<td>(40.7%)</td>
<td>(40%)</td>
<td>(45.5%)</td>
</tr>
<tr>
<td>Total patients</td>
<td>(94.5%)</td>
<td>(5.5%)</td>
<td>(72.9%)</td>
<td>(27.1%)</td>
<td>(59.5%)</td>
</tr>
</tbody>
</table>
DISCUSSION:
Several studies have shown that the high incidence of antibodies against Streptolysin O. Streptolysin O is a toxic immunogen protein produced by Streptococcus pyogenes (Alouf, 1977). Regarding the patients with chronic and acute recurrent tonsillitis generally displayed higher titer of antistreptolysin O antibodies; these findings regarding ASOT disagree with findings reported by Chreitah (Chreitah et al., 2005), Sevinc and Enoz (2007), and Mzoughi et al. (2004) who found that only 40%, 52%, and 62.7%, respectively were positive patients for GBHS while the present study was in agreement with Koh and Kim (2008), Radosz et al. (1998), and Abbas et al. (1997) who found that 15%, 20%, and 19%, respectively positive for ASOT for patients infected with GBHS.

Unexpectedly, regarding groups F, C, B, and D beta-hemolytic streptococci isolation from recurrent tonsillitis patients, ASOT, CRP tests were positive for some of those tonsillitis patients these findings was inaccodance with the finding of WHO (2004). In this study group F streptococci was predominant bacteria among other Lancefield groups that circulate in Iraqi population, this was in accordance with previous referees in Iraq (Al-Mehdi, 1989; Fadhil, 1989; Abdul-Hameed, 1999; Al-Ani, 2000; Al-Gebori, 2007).

Regarding bacterial isolation from the patients with recurrent , chronic and acute recurrent tonsillitis we failed to isolate and detect Streptococcus pyogenes (group A) and this might be due to the long latent period between the antecedent streptococcal infection and development of tonsillitis symptoms, early administration of antibiotics before any attempt to do throat culture and throat swab culture results that can give false negative results for these reasons, throat culture is less satisfactory than streptococcal antibodies tests as supporting evidence of recent streptococcal infection. Serological specimens from those patients were showing positive for ASOT as (33.9%), (23.1%), and (40%), respectively. These findings were in agreement with results reported by Abbas et al. (1997), Chreitah (2005), Koh and Kim (2008). In addition, other Streptococcus and various microorganisms were isolated from the specimens of this study.

Decreased CRP values in the serum of tonsillitis patients infected with beta-hemolytic Streptococci and other microorganism causing tonsillitis in our study were in contrast to findings of Potter et al. (1982), Ruuskanen et al. (1985), Putto et al. (1986), and Fadhil (1989) who found that (69-83.7%) were positive for CRP test in tonsillitis patients, this higher CRP levels might be due to difference of strains. Our findings were in agreement with findings of Al-Mahdi (1989) and Nakayama et al. (1993) who found that (8-43%) was positive for CRP test in tonsillitis patients. SLE test results in the present study were in agreement with the results reported by Fadhil (1989), Zabriskie (1986) who found that 2% was positive for SLE test in tonsillitis patients. Our findings regarding RF test were consistent with previous studies reported by Sesso et al. (1986), and Fadhil (1989) who found that 14-32% of tonsillitis patients were positive for RF test , this lower RF levels might be due to the levels of RF were detected in age group 60 years and older only. The present results are in agreement with Sesso et al. (1986) who found that 30% RF were sero-positive. Low levels of IM in the present study were in contrast with Dogan et al. (2010) who reported that 75% were positive in the patients with tonsillitis. However, this result was consistent with previous studies reported by Endo et al. (2001) who found that Epstein-Barr virus (EBV) which causes infectious mononucleosis infection occurs in early childhood and can persist in palatine tonsils.
REFERENCES:


تقييم بعض المحوسبات السيرولوجيَّة الخاصة بتشخيص النوبات اللوزتين الناتجة عن الإصابة

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تهدف الدراسة الحاليَّة إلى تقييم فاعلية خمسة اختبارات مصلية

Anti-Streptolysin O Titer، (ASOT)، C Reactive Protein (CRP)، Rheumatoideal Factor (RF)،

Systemic Lupus Erthymatous (SLE)، Infectious Mononucleosis (IM)

ت langue بالبول، للنوبات اللوزتين، طب، طب

 Delaware.

المكونات:

قسم علم الحيوان، علوم الديم

قسم الميكروبيولوجي، طب، طب

أ.د. نعمة عثمان الدبي

أ.د. مية زكي طلعت

числен من 109 مرضى مصابين بالنوبات اللوزتين، و

званة من 45 مريض بألبات اللوزتين، و

المجموعة الثالثة مكونة من 37 مريض بالنوبات اللوزتين، والمنكر، إضافة إلى 20 عينة مصلية من أشخاص أصحاء

ASOT

C

RF

SLE

IM