Rapid Diagnostic Test for Streptococcal Throat Infection in Egyptian Children

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Abstract

Background: Acute streptococcal pharyngitis represents a high prevalence in developing countries responsible of morbidity and mortality. Difficulty of prompt diagnosis is responsible for either inadequate therapy and subsequent rheumatic heart disease and renal failure, or abuse of antibiotics. Group AB hemolytic streptococcus (GABHS) detection by rapid tests is useful due to its rapidity and relative low cost. However sensitivity and specificity remain debatable. In Egypt, suspicion about its accuracy remains an important obstacle for mass utilization. The objective of this study was to evaluate the accuracy of a GABHS rapid antigen detection test kit, in comparison with oropharynx swab culture.

Methods: Children aged 3 to 15 years with clinical diagnoses of non treated acute pharyngitis were recruited at public emergency health centers in a rural region eastern Cairo, with 95 patients, subjected to oropharyngeal swabs for rapid GABHS testing and conventional culture.

Results: We observed sensitivity of 80.65%, specificity of 96.88%, a positive predictive value of 92.59%, a negative predictive value of 91.18% and a positive likelihood ratio for the rapid test used here, compared with throat culture. It was found a group of any 3 of fever, sore throat, dysphagia, cervical lymphadenopathy always present in false negative cases.

Conclusions: The rapid test studied exhibited a good correlation with culture and is, therefore, of great use in clinical practice to increase the sensitivity of the test.

Key Words: Streptococcal – Pharyngo-tonsillitis – Streptatest – Culture.

Introduction

ACUTE pharyngitis (AP) is one of the commonest diagnoses in children. The group A streptococcus causing the widest range of disease in humans of all bacterial pathogens [1], represents a high prevalence in developing countries responsible, when inadequately treated, of morbidity and mortality [2,3]. The greatest burden is due to rheumatic heart disease, with a prevalence of at least 15.6 million cases, with 282,000 new cases and 233,000 deaths each year [4]. Viruses are responsible for >80% of acute pharyngitis, while Group A Streptococcus (GAS) accounts for 15% of them [5]. It is quite impossible to establish the etiology depending only on clinical features [6]. Subsequently, antibiotics are prescribed frequently and empirically despite all squeals of abuse [7,8]. Alternatively, and according to The current recommendations of: (the American Academy of Pediatrics (AAP), the Centers for Disease Control and Prevention (CDC), the American Heart Association (AHA) and the Infectious Diseases Society of America (IDSA) to confirm diagnosis of GABHS diagnosis by investigatory tests. On one side, conventional throat culture using blood agar necessitates a long time around 48h before confirming the diagnosis [9]. Associated to this, expenses and fallacies remain a handicap in rural areas. On the other side, immunologic techniques allow nowadays rapid antigen detection tests (RADT), of the specific streptococcal group A antigen from a simple throat swab on outpatient clinic basis [10]. However, results remain contradictory. GABHS rapid detection has passed through several eras from latex to immune assay in order to improve its sensitivity and specificity to be a reliable test. Depending on the revolutionary improvement in rapid diagnostic tests based on radioimmunoassay, our target is to follow a new attitude to diagnose streptococcal pharyngitis without abuse antibiotics or laboratory investigation in order to prevent complications of GABHS.

Material and Methods

This prospective observational study was conducted in the period from 14 December 2006 to 7 March 2008. The study was conducted after the
approval of the Ethics Committee at the NRC. 100 children presenting to either the outpatient clinic of health center or the school dispensary room were taken and data were registered according to a special sheet. Patients were subjected to the study after fulfilling the inclusion criteria:

1- Approval of the tutor of the child aged from 3 to 15 years presenting with acute pharyngotonsillitis.

2- Clinical diagnosis depended on the presence of at least 3 of the following: A picture starting from simple erythema, pyogenic spots or pyogenic membrane affecting pharyngeal pillar or enlarged tonsils, fever incidence, 38.5°C once or twice 38°C at 1 hour interval without antipyretic and after tight covers or clothes removal. Cervical lymphadenopathy more than 1 cm diameter and or carrying the criteria of acute inflammation: Red, hot, tender, as part of a solitary or multiple group(s), abdominal pain in the form of diffuse cramps or discomfort, toxic facies, dysphagia.

3- In order to target bacterial infection all criteria for viral infections as solitary running nose, sore throat post prolonged cough or vomiting, foreign body and previous antibiotics start within the lat 2 weeks were excluding criteria. At least 2 criteria for streptococcal pharyngitis were necessary according to Centor criteria (absence of cough, high fever >24h without antibiotics, purulent pharyngitis, enlarged and painful cervical lymph nodes) [11]. Depending on clinical findings, this study was conducted by 2 research medical doctors, where the first was responsible of filling the medical sheet according to history taking and general examination and the other responsible of the practical part of the study: A first throat sampling for both laboratory culture and a second one for the rapid test then blood sampling for further investigations.

Microbiological examination:

Each throat swab used 2 cotton sticks, firmly taken covering the anterior pillars, tonsillar crypts bilaterally the first subjected to culture by a microbiologist ignoring the results of the other swab for the rapid test: Throat swabs are plated the same day on blood agar and incubated in 5% CO2 overnight at 37°C. 1ug benzyl penicillin disc is put on the heavily inoculated well of the primary culture plate to give a first indication of the probable presence of S. pyogenes. S. pyogenes is always sensitive to benzyl penicillin. Identification of S. pyogenes on culture is based on colonial morphology, beta haemolysis, sensitivity to benzyl penicillin and gram stain microscopy showing short chains of gram positive cocci.

Streptatest: A second swab subjected to rapid test. The kits of Streptatest®, Dektra Pharm, Strasbourg, France, were kindly offered by the company. The price of one test is equivalent to the 1/4 to 1/3 of an average antibiotic course.

Principle: Strepta test is an immuno chromatographic test on membrane using sandwich capture method. An anti-streptococcus antibody is fixed on the region test of the membrane. Another anti-streptococcus antibody is conjugated to latex purple particles placed just to over the immersion zone of the membrane. At a first time, the streptococcus A antigen is extracted from the swab with the help of extracting reagents. The lower part of strip will be immersed to the extracting reagent. The streptococcus A antigen will be coupled to the anti-streptococcus A antibody fixed to the latex particles. The mixture will migrate on the membrane by chromatography and the complexes antigen antibody will be fixed on the zone test. The appearance of a purple line indicates a positive test with its absence indicates a negative one. At the control zone the appearance of a purple line indicates a succeded procedure, otherwise the test is not valid.

Materials:
1- An aluminum sachet containing a strip indicator and a drying small sachet.
2- Extraction reagent A formed of 2M sodium nitrite, 1 0ml.
3- Extraction reagent B formed of acetic acid 0, 4 M, 1 0ml.
4- Inactivated positive control streptococcus A, 1ml.
5- Inactivated negative control streptococcus C, 1ml.
6- Cotton stick.
7- Extraction plastic tube.
8- Tongue depressor.
9- Chronometer.

Procedure: Swab used 1 cotton stick targeting the pharynx and all inflammatory zones either exudative or ulcerative, firmly taken covering the anterior pillars, tonsillar crypts bilaterally with getting sure not to contaminate it with saliva by using a tongue depressor. The cotton stick was immediately immersed in the extraction tube containing a mixture of 4 drops of pink reagent A and 4 drops of colorless reagent B after being gently agitated till the pink color turns to colorless. The cotton stick was to be rotated 10 times and rested for 1 minute in the mixture before squeezing it to
the border of the tube to retain the maximum of fluid. Finally the strip indicator was placed in the tube for 5 minutes. The test was considered highly positive if appeared in the first minute. However we should wait for five minutes to give the final result, but not beyond 10 minutes. Streptococcal AP due to GABHS (case): Patients with clinical diagnosis of AP and a culture positive for GABHS. Negative (control): Patients with clinical diagnosis of AP and a culture negative for GABHS. Complete blood count was done for leucocytosis, neutrophilia as a complementary test to confirm the bacterial origin of the inflammation the same as for anti-streptolysin O titre in order to strengthen the GABHS diagnosis. It was designed to treat all patients by antibiotics whatever the result of the rapid test. The decision to discontinue antibiotics was reported to the result of the culture within 48 to 72 hours and depending on the accessibility to reach the patients for follow-up.

Statistical analysis: SPSS for Windows, version 10.0 computer program was used for statistical analysis. Data were represented as frequency, percent, median and range. Chi-Square test or Fisher’s exact test was used to compare between independent proportions. A $p$ value of less than 0.05 was considered statistically significant. In order to evaluate the accuracy of the test, sensitivity, specificity, positive predictive value, and negative predictive value were calculated. The sensitivity indicates the chance of testing positive among those with the condition, it is calculated as the percent of true positive (TP) cases in relation to true positive cases plus false negative (FN) cases. Specificity is the chance of testing negative among those without the condition. It is calculated as the percent of true negative (TN) cases in relation to true negative cases plus false positive (FP) cases. The Positive Predictive value is the chance of having the condition among those that test positive, it is calculated as the percent of the true positive cases in relation to true positive cases plus false positive cases. The negative predictive value is the chance of not having the condition among those that test negative, it is calculated as the percent of the true negative cases in relation to true negative cases plus false negative cases.

Results

100 patients were enrolled in the study, 5 were excluded due to throat culture technical problems. 95 children were eligible for analysis, of them 58 females and 37 males. The median age was 8.98 years (3.29-13.78). Patient’s characteristics are showed in Table (1) and investigations characteristics are showed in Table (2). Throat culture/streptase test relation as regard TN versus FN or TP versus FP is showed in Table (3). The final diagnosis was group AB hemolytic streptococcus (GABHS) pharyngo-tonsillitis $n=31$, while Non group AB hemolytic streptococcus (NGABHS) pharyngo-tonsillitis $n=64$. The final diagnosis of group (GABHS) was confirmed by ASOT and throat culture. And the relation between TP and ASOT was statistically significant $p=0.03$. The same was for TP and throat culture $p<0.0001$. When we compared the results of the strepta test with those of the throat culture, we found the sensitivity 80.65%, the specificity 96.88%, the positive predictive value 92.59%, and the negative predictive value 91.18%. The common symptoms and signs in false negative strepta test were fever $>38\degree C$, sore throat, dysphagia, and cervical lymphadenopathy.

![Table (1): Patient's clinical characteristics.](image-url)
Table (2): Laboratory investigations and bed site result.

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Value</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC x 10E3 (median)</td>
<td>12.386</td>
<td>4-39</td>
</tr>
<tr>
<td>ANC x 10E3 (median)</td>
<td>6</td>
<td>1-35</td>
</tr>
<tr>
<td>CRP</td>
<td>Positive</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>49</td>
</tr>
<tr>
<td>ASOT</td>
<td>Positive</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>48</td>
</tr>
<tr>
<td>Throat culture</td>
<td>Positive</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>64</td>
</tr>
<tr>
<td>Streptotest</td>
<td>Positive</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>68</td>
</tr>
</tbody>
</table>

TLC: total leucocytic count. ANC: absolute neutrophil count.

Table (3): Streptotest assessment with throat culture.

<table>
<thead>
<tr>
<th>Number</th>
<th>Percent</th>
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<tbody>
<tr>
<td>False negative</td>
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</tr>
<tr>
<td>False positive</td>
<td>2</td>
</tr>
<tr>
<td>True negative</td>
<td>62</td>
</tr>
<tr>
<td>True positive</td>
<td>25</td>
</tr>
</tbody>
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Table (4): False negative test clinical criteria.

<table>
<thead>
<tr>
<th>ID</th>
<th>Fever</th>
<th>Sore</th>
<th>Dysphagia</th>
<th>Cervical lymphadenopathy</th>
</tr>
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<tbody>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>72</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>86</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Discussion

Streptococcal pharyngo-tonsillitis is the most important upper respiratory tract organism responsible for morbidity and subsequent mortality in developing countries [2]. We conducted a prospective study of 100 patients presenting with pharyngitis. According to throat culture, 1/3 of cases presenting with acute pharyngitis proved to be GABHS. This goes with other groups [12-14]. These international studies emphasized by 2 studies of the Egyptian population: The first evaluating the effectiveness of clinical guidelines for the presumptive treatment of streptococcal pharyngitis in 450 Egyptian children. In this study when relying on clinical picture only according to the WHO Acute Respiratory Infections (ARI) guidelines, there was a high specificity but low sensitivity that limits the unnecessary use of antibiotics, but does not treat 88% of children with a positive streptococcal throat culture who were at risk of acute rheumatic fever. Even by modifying new clinical guidelines, the sensitivity would increase on the price of a decreased specificity which will lead to abuse antibiotics [6]. The second study was done on an outpatient clinic Egyptian population and the incidence of streptococcus was 22%. This study confirmed the usefulness of rapid test for early treatment of streptococcal pharyngitis [15]. On the other hand, Dos Santos and Berezin recommended a microbiological support before antibiotics prescription [14].

Between abuse of antibiotics and too lengthy procedure throat culture (although the golden test), in limited resources countries it is recommended to use a rapid diagnostic test in children with sore throat [16]. Current guidelines recommend that only tonsillopharyngitis due to group A beta-haemolytic streptococcus (GABHS) diagnosed by rapid diagnostic test should be treated with antibiotics [17]. In order to restrict antibiotics to the proper cases we had to evaluate a rapid test giving its result in 5 minutes. In our study, the test was done simultaneously with other laboratory investigations but its result was not taken into consideration. All patients received antibiotics as the worse diagnosis was retained till the results of investigations. Considering a minimum of 72h before delivering the throat culture results, other factors could be contributing in more delay as the different working hours between the lab and the health center especially in isolated districts. In our study, the sensitivity of the streptotest was 80.66% which means that in a number of 31 positive throat culture we had true 25 positive streptotest associated to 6 patients who could pass unnoticed from antibiotic prescription due to false negative result. This result remains unsatisfactory to others. The negative predictive value which calculates to what extent we can tell the test is negative and it is truly negative in relation to all negative tests whether true or false, was 91.18% which means that out of 100 patients, 91 are really not diseased and 9 are considered not to be but they are. In order to minimize this impact, antibiotics could be however prescribed if other clinical criteria are fulfilled. In our study these clinical criteria were fever, sore throat, dysphagia and cervical lymphadenitis >1 cm and at least 3 of them were present in all false negative cases. On the other hand, elements of these criteria were sporadic in the whole non GABHS group.

As regards the specificity of the test it was 96.88% which means that in a group of 64 negative
thorat cultures, we had 2 cases who received unnecessary antibiotics as they were false positive which remains satisfactory in avoiding unnecessary antibiotics as the other 62 patients. The positive predictive value which calculates to what extent we can predict the test to be true positive and it is really true positive in relation to all positive tests whether true or false positive and was 92.59% which means that over 100 patients, 93 would be really diseased and 7 considered disease while they are really not.

It exist many rapid tests where the principle depends on latex or radioimmunoassay. In previous studies, results are heterogeneous the sensitivity may range from (65.6–93.9%), the negative predictive value from (94.2–96.9%) specificity (68.7–100%) and positive predictive value from (67.4–90.4%) [13,18-23]. In a study aiming to assess the diagnostic value of a rapid streptococcal antigen test in addition to four clinical features in patients with sore throat, using throat culture and antibody titre as reference tests. Four clinical features (fever "history of" > or =38 degrees C, lack of cough, tonsillar exudate, and anterior cervical lymphadenopathy), the rapid test gave a sensitivity of 91 %, specificity of 91%, positive predictive value of 73% and a negative predictive value of 98%. For patients with three or four clinical features, however, the sensitivity was considerably higher at 97% [24].

Conclusion:

These results are interesting, with an acceptable accuracy of the test, with a special interest to clinical picture that tends to increase the sensitivity of the test in the presence of an association of fever, sore throat, dysphagia and cervical lymphadenitis. The decision making is spontaneous to prescribe antibiotic and subsequent rapid treatment of the patient. On the other hand, the reduction of antibiotic consumption is remarkable with all benefits on the medical level-related to emergence of resistant strains and side effects of the drug itself-and the economic level related to saving losses in unnecessary drug prescription especially in limited resources countries. So we recommend use encouragement of this test on wider scale at the emergency sectors in health centers.

References


