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Original Article

Rapid Antigen Diagnostic Test for Quick Detection of Streptococcal Pharyngitis in Pediatric Age Group

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ABSTRACT

Background: *Streptococcus pyogenes* is one of the leading causes of acute tonsillopharyngitis across the globe. The inappropriate routine of the antibiotics has steered towards the prevalence and growth of antibiotic resistant bacteria which are becoming virulent pathogens. Misdiagnosis of many infectious diseases, such as streptococcal pharyngitis caused by *Streptococcus pyogenes* has led to inappropriate use of antibiotics. The symptoms of streptococcal and viral pharyngitis usually similar, this makes the accurate diagnosis by clinical assessment very difficult. The standard methods used for the diagnosis of *S. pyogenes* include the clinically approved Centor-McIsaac or Modified Centor scoring, Rapid Streptococcal Antigen Detection Test (RADT) and the culture. However, these methods have not been validated in our setup.

Objective: To evaluate the efficiency of Rapid Streptococcal Antigen Detection Test (RADT) method for identification of G*roup A beta hemolytic Streptococcus (GABHS)* and comparing its results with that of throat culture.

Material and Methods: It was a cross sectional study carried out at the department of Microbiology, Army Medical College Rawalpindi (National University of Medical Sciences) Pakistan and department of pediatrics Military Hospital Rawalpindi from April 2017 to April 2018. The subject of this study was the patients of pediatric age group from 1 year to 15 year old attending Military Hospital Rawalpindi with complains of sore throat. The clinical assessment was done according to Modified Centor criteria and two throat swabs were taken; one was use for Rapid antigen detection test (RADT) assessment and other for throat culture. The results of Rapid antigen detection test (RADT) were compared with throat culture results. And sensitivity, specificity, positive and negative predictive values were determined.

Results: Two hundred and seventy children (having mean age of 5.2 years ± 3.1 SD) were considered in the research. The sensitivity and specificity of Rapid antigen detection test came out to be 41.2 % and 99.0 %, respectively. And positive predictive value was 93.3% [95% CI: 73.2%-100%], whereas negative predictive value was 83.3% [95% CI; 81.6 %-84.8 %].

Conclusion: This concluded that Rapid Antigen Detection Test although have high specificity but low sensitivity, are a significant step forward in the diagnosis and management of sore throat.

Key Words: *Streptococcus pyogenes*, diagnostic tests, pharyngitis, Sore throat, rapid antigen detection test (RADT).

Introduction

Streptococcus pyogenes which is also known as group A *Streptococcus* is an important extracellular Gram positive bacteria, colonizes the skin or throat and cause many suppurative and nonsuppurative infections.(1) To avoid host defenses they have developed many virulence mechanisms.(2, 3)They commonly cause bacterial pharyngitis and also impetigo and scarlet fever.(4)

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Streptococcus pyogenes account for up to 37% of pediatric cases and 5 to 15% of cases in adults.(5, 6) It can cause many clinical illnesses, including pharyngo-tonsillitis, skin infections, bacteremia, necrotizing fasciitis and toxic shock-like syndrome.(7) Acute rheumatic fever and post Streptococcal acute glomerulonephritis are the immunological sequelae of streptococcal infection and in developing countries it represent a significant disease burden especially in children. Although rapid results are provided by these test but still culture throat swab for *Streptococcus pyogenes* remains the gold standard. The sensitivity of tests ranges from 58% to 96%. However, a routine

back-up culture is dispensable in adult patients, due to the low incidence of Streptococcal pharyngitis and rheumatic fever in this age group. The specificity of rapid antigen tests is high, however false-positive results are seen from patients diagnosed and/or treated for *Streptococcus pyogenes* in the past.(8) or patients colonized with non-*Streptococcal pyogenes*. One of the leading causes of acute respiratory tract infection is *GABHS* (*S. pyogenes*) especially in school age group. Throat swab culture is gold standard for the diagnosis of *GABHS* pharyngitis, although it does not discriminate between colonization and acute infection and results are available after 24 to 48 hours, so rapid methods for the diagnosis of *GABHS* pharyngitis are required. Pharyngitis caused by GAS or GABHS infection share a great significance in community associated infection. Aggressive identification is justified for the prevention of suppurative and non-suppurative complications, to rapidly improve clinical complains, to decrease contagiousness early, leading to rapid resumption of the daily activities and also to minimize the adverse effects of improper antibiotic therapy. There is still a need of research to have early detection of streptococcal pharyngitis to avoid complications. For this purpose, assessment of rapid antigen detection test (RADT) for the rapid diagnosis of streptococcal sore throat is done in this study. McKesson Consult diagnostic Strep A Dipstick was used to detect the sensitivity and specificity of rapid antigen detection of *Streptococcus pyogenes* from throat swab and the results are compared with the results of throat culture.

Objective

To evaluate the efficiency of Rapid Streptococcal Antigen Detection Test (RADT) method for identification of G*roup A beta hemolytic Streptococcus (GABHS)* and comparing its results with that of throat culture.

Material & Method

It was cross sectional study. This study was carried out at the Department of Microbiology, Army Medical College, Rawalpindi, and the samples were collected from the Peadiatric Department of Military Hospital, Rawalpindi affiliated with National University of Medical Sciences. Sample size was calculated by using WHO sensitivity and specificity calculator. Sensitivity=93%9 (9) Specificity=90% 10 (10) Precision of sensitivity=7%, Prevalence =32% Minimum sample size (n) = 112 Non-probability convenience sampling technique was applied in this study. All patients of age 1 to 15 of both sexes with sudden onset of sore throat, fever, headache, with or without tonsillo-pharyngeal inflammation and exudates, tender cervical lymph nodes were included in study and Patients of more than 15 years of age, Patients with viral infection (such as herpes gingiva-stomatitis or hand foot and mouth disease). Patients who took oral antibiotics within the last one week or intramuscular benzathine penicillin within last one month were excluded from study. Commercial RADT kit with positive and negative control samples was used.

This study was approved by Ethics Review Committee of Army Medical College. The procedure was explained to the parents/guardians and an informed consent was obtained before the collection of throat swabs.

The McKesson Consult Diagnostics Strep A Dipstick (a rapid chromatographic immunoassay for the qualitative detection of Strep A carbohydrate antigen from throat swab was used as per directions in the test pack. Appearance of two distinct red lines, one line in the control region and another line in the test region show positive result. A positive result indicates that Strep A is detected in the sample.

Culture of a throat swab on blood agar plate has been the standard for the confirmation of the acute streptococcal pharyngitis. Throat swab sample was obtained from the tonsillar fossa or the surface of tonsil and the posterior pharyngeal wall. After plating, culture is incubated at 37 ^{o}C for 18-24 hours. Additional 24 hours incubation was done for the plates showing negative result. After attaining the growth, tests were done for confirmation of group A streptococcus including Gram staining (Gram positive cocci seen under microscope), catalase (streptococcus was catalase negative), bacitracin disk test and latex agglutination test.

Results

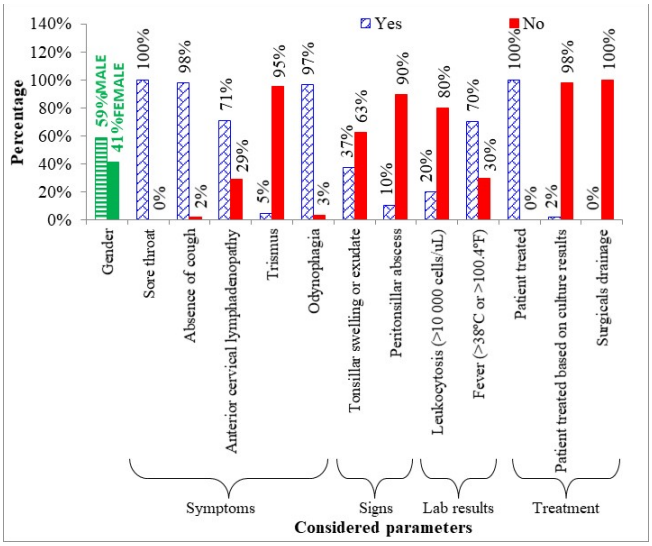
We evaluated all the patients on two aspects: age wise i.e. age of 1-6 years and 7-15 years old patients with a mean age of 3.5 years and 9.2 years, respectively, and gender wise i.e. male and female patients with a mean age of 5.4 years and 4.9 years, respectively, as shown in Table 1. Graphical presentation of clinical assessment of all patients is given in Figure 1. In group 1, odynophagia 180 (96%) and anterior cervical lymphadenopathy 131 (70%) were the most frequent features. Six (3%) patients complained of trismus. Tonsillar swelling or exudates were observed in 63 (34%) of patients and 14 (7%) had peritonsillar abscess. Leukocytosis of >10,000 was seen in 35 (19%) of these

patients. In Group 2 which comprises of 82 patients, with mean age of 9.5 years. Eighty two (100%) of the patients had odynophagia , 7 patient (9%) complaint of trismus and 61 (74%) patients had anterior cervical lymph adenopathy. Thirty six (46%) patients had tonsillar exudates and 14 (17%) had peritonsillar abscess. Leukocytosis of >10,000 cells/ul was observed in 19 (23%) patients. All of the patients were treated and none of the patients were treated on the basis of culture positive. The results of Modified Centor scores are compared in two groups. According to our calculation and analysis 4 (2%) of group 1 and 0 (0%) of group II had 0-1score, 33 (18%) of group 1 and 7 (9%) of group II had 2 score, 57 (30%) of group 1 and 24 (29%) of group II had 3 score, 55 (29%) of group I and 28 (34%) of group II had 4 score and 39 (21%) of group I and 23 (28%) of group II had 5 score. The performance of a specific laboratory test can differ across different subgroups within a population, a phenomenon called as spectrum bias or, as spectrum effect. It is recognized by the relation to the severity of disease. We assumed and hypothesized that within the paediatric population, the sensitivity of RADT for group A streptococci would show spectrum bias, which results in a higher sensitivity for the subgroup of patients with manifestations of more severe disease. If this theory is true and the sensitivity is high, then culture confirmation of negative RADT is pointless for some children. The results of this study show that spectrum bias may occur when RADT is used for diagnosis of GABHS pharyngitis among children. The sensitivities observed in this study were substantially lower than the sensitivity claimed by the manufacturer. The reason behind, RADT displaying spectrum bias is unknown. One factor may be the quantity of bacteria in the pharynx.

Table 1: Clinical assessment of patients of all patients including bifurcation based on age and gender

Parameter *	All Patients** No. (%), n = 270	Patients with 1-6 years** No. (%), n = 188	Patients with 7-15 years** No. (%), n = 82	Male Patients ** No. (%), n = 159	Female Patients ** No. (%), n = 111
Sex					
Male	159 (59%)	107 (57%)	52 (63%)	159	-
Female	111 (41%)	81 (43%)	30 (37%)	-	111
Age, y, mean range	5.2	3.5	9.2	5.4	4.9
Symptoms					
Sore throat	270 (100%)	188 (100%)	82 (100%)	159 (100%)	111 (100%)
Absence of cough	265 (98%)	185 (98%)	80 (98%)	157 (99%)	108 (97%)
Anterior cervical lymphadenopathy	192 (71%)	131 (70%)	61 (74%)	114 (72%)	78 (70%)
Trismus	13 (5%)	6 (3%)	7 (9%)	8 (5%)	5 (5%)
Odynophagia	261 (97%)	180 (96%)	81 (99%)	153 (96%)	108 (97%)
Signs					
Tonsillar swelling or exudate	101 (37%)	63 (34%)	38 (46%)	57 (36%)	44 (40%)
Peritonsillar abscess	28 (10%)	14 (7%)	14 (17%)	18 (11%)	10 (9%)
Laboratory Results					
Leukocytosis (>10 000 cells/uL)	54 (20%)	35 (19%)	19 (23%)	31 (19%)	23 (21%)
Fever (>38°C or >100.4°F)	190 (70%)	138 (73%)	52 (63%)	108 (68%)	8 (74%)
Treatment					
Patient treated	270 (100%)	188 (100%)	82 (100%)	159 (100%)	111 (100%)
Patient treated based on culture results	5 (2%)	5 (3%)	0 (0%)	2 (1%)	3 (3%)
Surgical drainage	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Modified Centor Score					
0, 1	4 (1%)	4 (2%)	0 (0%)	2 (1%)	2 (2%)
2	40 (15%)	33 (18%)	7 (9%)	22 (14%)	18 (16%)
3	81 (30%)	57 (30%)	24 (29%)	50 (31%)	31 (28%)
4	83 (31%)	55 (29%)	28 (34%)	47 (30%)	36 (32%)
5	62 (23%)	39 (21%)	23 (28%)	38 (24%)	24 (22%)

Note: All patents are being considered in MH Rawalpindi.



*(NOTE: * Solid portion in pi-chart means "YES" for that particular parameter.)*

Figure 1: Graphical presentation of clinical assessment of all patients

Distribution of the rapid antigen test (RADT) and throat culture results of all patients including bifurcation based on age and gender are given in Table 2. Accordingly, sensitivity, specificity, positive predictive value and negative predictive value of all patients including bifurcation based on age and gender are given in Table 3. For 1-6 age group, the prevalence of GABHS in this age group was calculated as 22.9% with throat culture. The sensitivity and specificity of RADT was 39.5% and 100% respectively. The positive predictive value was 100% (17/17) and negative predictive value was 84.8% (145/171). For 7-15 age group, the prevalence of *GABHS* was 30.5% with throat culture. The sensitivity and specificity of RADT was 44.0% and 96.5% respectively. The positive predictive value (the chance of culture positive if RADT is positive) was 84.6% (11/13) and negative predictive value was 79.7% (55/69). Figure 2 shows the area under the ROC curve measures the accuracy of RADT.

Table 2: Distribution of the throat culture and rapid antigen test (RADT) results of all patients including bifurcation based on age and gender

	Culture (+) n (%)	Culture (-) n (%)	Total n (%)
ALL PATIENTS			
Rapid antigen test (+)	28 (10.4%)	2 (0.7%)	30 (11.1%)
Rapid antigen test (-)	40 (14.8%)	200 (74.1%)	240 (88.9%)
Total	68 (25.2%)	202 (74.8%)	270 (100%)
PATIENTS WITH 1-6 YEARS AGE			
Rapid antigen test (+)	17 (9.0%)	0 (0.0%)	17 (9.0%)
Rapid antigen test (-)	26 (13.8%)	145 (77.1%)	171 (91.0%)
Total	43 (22.9%)	145 (77.1%)	188 (100%)
PATIENTS WITH 7-15 YEARS AGE			
Rapid antigen test (+)	11 (13.4%)	2 (2.4%)	13 (15.9%)
Rapid antigen test (-)	14 (17.1%)	55 (67.1%)	69 (84.1%)
Total	25 (30.55)	57 (69.5%)	82 (100%)
MALE PATIENTS			
Rapid antigen test (+)	15 (9.4%)	2 (1.3%)	17 (10.7%)
Rapid antigen test (-)	22 (13.8%)	120 (75.5%)	142 (89.3%)
TOTAL	**37 (23.3%)**	**122 (76.7%)**	**159 (100%)**
FEMALE PATIENTS			
Rapid antigen test (+)	13 (11.7%)	0 (0%)	13 (11.7%)
Rapid antigen test (-)	18 (16.2%)	80 (72.1%)	98 (88.3%)
TOTAL	**31 (27.9%)**	**80 (72.1%)**	**111 (100%)**

Table 3: Sensitivity and specificity of the rapid antigen test of all patients including bifurcation based on age and gender

Rapid Antigen Test*	Percentage** (%)				
	All Patients	Patients with 1-6 years	Patients with 7-15 years	Male Patients	Female Patients
Sensitivity	41.2%	39.5%	44.0%	40.5%	41.9%
Specificity	99.0%	100%	96.5%	98.4%	100%
Positive Predictive Value	93.3%	100%	84.6%	88.2%	100%
Negative Predictive Value	83.3%	84.8%	79.7%	84.5%	81.6%

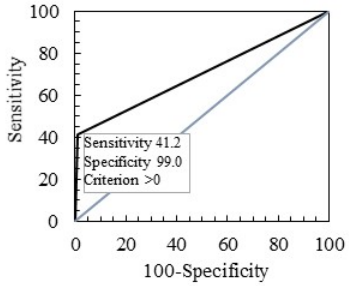


Figure 2: The area under the ROC curve measures the accuracy of RADT. (The sensitivity was 41.2%, while specificity was 99.0%. The positive predictive value was_93.3% and negative predictive value was 83.3%.)

The calibration of the Centor score was assessed across four levels (0-1, 2, 3, 4 and 5). Figure 3 shows Modified Centor Scores, ranging from 0-5, of patients for all four groups. Calibration enables visual and quantitative assessment across different levels of risk. The predicted number of patients with GABHS pharyngitis (based on the probability calculated in the derivation study were compared with the observed number of patients with GABHS pharyngitis in each validation study. The Centor score data were analyzed in groups (score 0-1, 2, 3, 4 and 5) as on the basis of these categories ACP/ASIM guidelines recommend treatment options. In maximum studies, data were available for all score categories; the results added together to form the group data (0-1, 2, 3, 4 and 5) was used to calculate the predicted score. We carried out a subgroup analysis to determine the influence of disease prevalence on the performance of the Centor Scores.

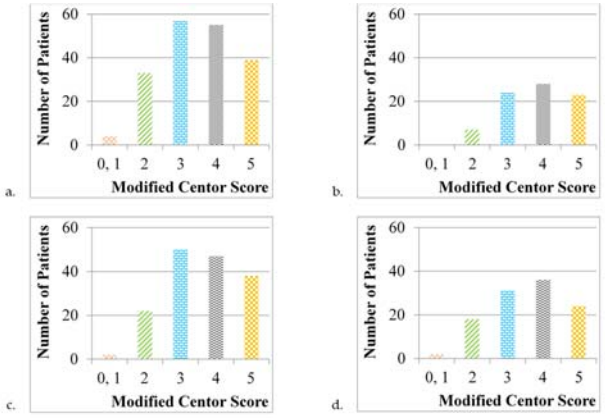


Figure 3: Modified Centor Scores, ranging from 0-4, of patients for: a. 1-6 years age group (n=188), b. 7-15 years age group (n=82)., c. male group (n=159), and d. female group (n=111)

Figure 4 shows the pi-chart of Modified Centor Scores, ranging from 0-5, of patients for all four groups. Figure 4a shows the results of 1-6 years age group. In this group n= 81 (number of patients of this age group in the study). Of these 188 patients 2%, 18%, 30%, 29%, 21% had 0-1, 2, 3, 4 and 5 score, respectively. Figure 4b shows the results of 7-15 years age group. In this group n= 82 (number of patients of this age group in current study). Of these 82 patients 0%, 9%, 29%, 34%, 28% had 0-1, 2, 3, 4, 5 score, respectively.

Figure 4: Pi-chart of Modified Centor Scores, ranging from 0-4, of patients for: a. 1-6 years age group (n=188), b. 7-15 years age group (n=82)., c. male group (n=159), and d. female group (n=111)

Discussion

In this study the McKesson consult diagnostic dipstick was used and was found to very easy to use for the detection of *S. pyogenes* in a clinical setting. The cost of the test pack of 25 was 32 $ which is around 4000 Rupees and one test cost around 150 Rupees. This is not very much when compared with throat culture which usually cost 800-1000 rupees. The time required for performing throat culture is 24-48 hours while the RADT requires only a few minutes (>15 minutes) to give results. The sensitivity in current study was 41.2% which is very less than was advertised 97% by the manufacturer. The specificity, however was higher than was claimed 99% against 95%. As the children are known to have higher rate of acute sore throat, we evaluated the accuracy of RADT for only the children (1-15 years) and adults were not included. With high prevelance rate of streptococcal pharyngitis in children the positive predictive value is also high in our study but it is likely that it may differ in adult population. This should be further investigated by application of RADT in adults in our set up. According to the results of the index study the sensitivity was 41.2% which is comparatively low when compared with other studies and only a few other studies have such sensitivities. According to one study the sensitivity was found to be 59.5% 11 (11) and in another study the sensitivity was 68.2% 12 (12). The specificity was calculated to be 99.0 % which is higher than the specificity of some other studies (68.7%-99%). The high specificity in the index study may be caused

by the usage of commercial test kit. The positive predictive value was 93.3 % and the negative predictive value was 83.3% in our study. The positive predictive value is vital in the diagnosis of GAS because it helps in the decision making for the advice of antibiotics. For the start of treatment there is no need to wait for the culture result If RADT result is positive. In the current study the positive predictive value was 93.3% while in other studies the value ranged from 65%-90%. As already asserted for the first generation of the RADTs, the newer generations have an additional value for the diagnostic management of sore throat. In children, eight observational studies along with two guidelines supported to confirm RADT negative cases with throat culture. Whereas, two observational studies, a clinical trial and one guideline were of the opinion that throat culture confirmation of RADT negative cases is not necessary.(13,14,15,16,17)

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Conclusion

The conclusions derived from this experimental study are, alone clinical presentation is not reliable to discriminate between viral and GAS pharyngitis except when overall features of viral infection, like cough, rhinorrhea, hoarsness and oral ulcers are present. So for the accurate diagnosis and management RADT and throat culture should be performed. However for detection of GABHS in children RADT is reliable. Throat culture is gold standard but it cannot give results before 24-48 hours. RADT can be used for preventing unnecessary use of antibiotics and in case of Streptococcal infection early initiation of treatment.

Conflict of interest: There is no conflict of interest declared by any author

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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion