Original Article

Role of Rapid Antigen Detection Test (RADT) and Throat Culture in the Diagnosis of Streptococcal **Pharyngotonsillitis**

1. Karam Ali Mirjat 2. Pushpa ValiRam 3. Izhar Fatima

1. Asstt. Prof. of Pathology, Dow Medical College, Karachi 2. Asstt. Prof. of Pathology, Dow Medical College, Karachi 3. Asstt. Prof. of Pathology, Dow Medical College, Karachi.

ABSTRACT

Objective: To observe the sensitivity and specificity of the rapid antigen detection test and throat culture in the diagnosis of pharyngo tonsillitis.

Study Design: An Experimental study

Place and Duration of Study: This study was conducted in microbiology department, basic medical sciences institute, Jinnah post graduate medical centre, National institute of child health, and civil hospital Karachi, from May 2003 - April 2004.

Materials and Methods: A total of 300 children attending OPD's and admitted (250 suspected and 50 Normal as control cases) of age group 5 – 15 years were included in this study and this age group was again divided into three sub groups I.e: first group from 5-8 years, second group was from 9-12 years, and the third group was from 13-15 years.

Results: Rapid antigen detection test carried out was based on immuno - chromato graphic membrane assay procedure, a total of 24 positive antigen detection test from suspected 250 cases and 5 from 50 control cases were isolated and these isolated (RADT positive) cases were again confirmed by throat culture. The Bacitracin sensitivity and catalase tests were also performed.

Conclusion: The Rapid antigen detection test (RADT) is a rapid way of diagnosing the group A, Beta hemolytic streptococci, result can be obtained within 5-10 minutes so the treatment may be started accordingly, while the throat culture is still considered as the Gold standard for the diagnosis of group A beta hemolytic streptococcal pharyngotonsillitis. The positive as well as negative RADT cases were confirmed by the culture.

Key Words: Group A, Beta hemolytic streptococci, Rapid antigen detection test, throat culture, Pharyngotonsillitis.

INTRODUCTION

Streptococcus C and G (non group A) are responsible for community and food borne causes of acute pharyngitis². Pharyngotonsillitis is one of the most common respiratory disease in the community, particularly during childhood. Approximately 28-40% of these infections estimated to be caused by Group A beta hemolytic streptococcus (GABHS) which is considered the important etiological pathogen in terms of sequelae and complications.

Since streptococcal pharyngitis can lead to rheumatic disease and its sequelae, and to suppurative complications, it is a potentially serious medical

Group A streptococcal pharyngitis is more common during the winter and rainy months, and occurs most frequently in school going children. Typically, the age group between 5-15 years old with a sudden onset of high fever and a sore throat. The symptoms may be accompanied by headache, malaise, nausea, vomiting and abdominal pain. Physical examination reveals an erythematous pharyngeal exudative tonsillitis, tender cervical lymph adenopathy. Palatal petechiae may be present and papillae of the tongue may be prominent and erythematous, giving the appearance of a

"strawberry tongue". During winter months in temperate climates, upto 20% of asymptomatic school age children may be group A streptococcal carriers. Group A beta hemolytic streptococci are ordinarily spread by direct person-to-person contact, most likely through droplets of saliva or nasal secretions, crowding increases transmission and outbreaks of pharyngitis which are common in institutional settings, the military, schools and families. Outbreaks resulting from human contamination of food during preparation have also been reported³.

If this study was compared with the study done in Eskischiv, fenleey, the organisms isolated was 13.16%, which is more than this study (Atindis et al., 2004)⁴.

We have compared the male children with the female suffering from tonsillitis and pharyngitis. Pharyngitis was seen more in male 11 (6.6%) and in female children the pharyngitis is more 6 (7.1%) than tonsillitis, which was 3 (3.6%), while among 166 male children the number of cases positive for tonsillitis were 4 (2.4%). This is again in contrast that male/female ratio is 1:1 (Thomas et al., 2002)⁵.

The American Academy of Paediatrics and the Infectious Disease Society of America recommended confirmation of negative rapid antigen detection test results with a throat culture (Bourbeau

Heiter, 2003)⁶.

Group A, Beta hemolytic streptococcal pharyngitis is an important cause of childhood morbidity and the cause of acute rheumatic fever (Aujard et al., 1995)⁷.

Culture isolation of Group A, Beta hemolytic streptococcal organisms from the pharynx is the standard method, but rapid antigen detection testing is now widely available (Hall et al., 2004)⁸.

Rapid testing has many benefits i.e. early treatment within 48 hours after onset can provide symptomatic relief (Herbeck et al., 1993)⁹.

MATERIALS AND METHODS

A total of 300 subjects (250 suspected children and 50 healthy children as control) from 5-15 years of age were included and subjects were divided into three groups; 5-8 years, 9-12 years and 13-15 years respectively.

Methods: Two throat swabs were taken from the patient one for the Rapid test and the other immersed in the transport media (Brain Heart Infusion broth) for further processing in the laboratory. Culture was performed in the laboratory on Blood agar and after inoculation, the plates were incubated at 37°C for 24-48 hours. On the next day, Gram's staining of the growth was carried out, and Microscopy was done. A bacitracin disc of 0.04 U was impregnated on the inoculum on Blood agar plate and zone of inhibition was observed on the following day. Catalase test was performed according to the standards mentioned, which was found negative, and finally the drug sensitivity was carried out.

The rapid antigen detection test was performed according to the standards mentioned. The type of the test performed was immuno-chromatographic membrane assay to detect the Streptococcus pyogenes group A antigen from throat swab.

Principles of the Procedure (RADT): To perform the test, a throat swab is inserted into the test device. Extraction reagents are added from dropper bottles. The swab is rotated three times clockwise. After a one minute incubation, the test device is closed to bring the extracted sample in contact with the test strip. Strep A antigen captured by immobilized anti-Strep A reacts to bind conjugated antibody. Immobilized rabbit anti-goat IgG captures the second visualizing conjugate. A positive test result is read visually in 5 minutes. A negative Strep A Test result, read in 5 minutes, indicates a presumptive negative for the presence of Group A Streptococcal antigen (Binax, 2004)¹⁰.

Interpretation: The test is interpreted by the presence or absence of visually detectable pink-t~purple coloured lines. A positive result will include the detection of both a Sample and a Control line, while a negative assay will produce only the Control line. Any

other test result indicates an invalid assay (Binax, 2004)¹⁰.

Limitations of the procedure: Strep A test does not differentiate between viable and non-viable organisms. Patients that have recently been treated for Strep A similar infections may give positive results for a period of time following effective treatment due to the presence of Group A strep antigen in non-viable organisms. Pharyngitis can be caused by organisms other than Group A streptococcus, further diagnostic testing, including culture, should be performed if laboratory findings are inconsistent with clinical presentation. Strep A test will not differentiate asymptomatic carriers of Group A Streptococcus from those exhibiting streptococcal infection. A negative result can be obtained if the amount of extracted antigen is below the sensitivity of the test. Culture confirmation is recommended for all Strep A test negative test results. A single swab that is used both to inoculate a culture plate and to perform the rapid test may have reduced sensitivity in the Strep A test (Bin ax. $2004)^{10}$.

Comparison Between RADT and Throat Culture\

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Name of	Type of Test	Sensitivity and			
Test		Specificity			
Throat Culture	Specimen obtained by throat swab of posterior tonsillopharyngeal area and incubated on to a 5% sheep blood agar plate to which a bacitracin (0.04U) is applied. Result in 24-48 hours.	Sensitivity 97% Specificity 99% (Results depend on the technique, medium, incubation)			
Rapid Antigen Detection Test (RADT)	Detects presence of Group A, Streptococcal carbohydrate on "a Throat swab (change in colour indicates A, positive result). Result available within minutes in office test.	Specificity 95% Sensitivity 80- 100% (Depend on the test)			

(Vincent et al., 2000)⁵.

RESULTS

Table 1 shows that the number and the percentage of the positive isolated cases were same i.e. 24 (9.6%) in suspected (250) cases and 04 (8.0%) among 50 healthy subjects, which shows the sensitivity of the test and its correlation with culture.

Table No.1: Comparison of %age between RADT and Culture cases.

and Culture cases.						
Children	RADT +ve cases	%	Culture +ve cases	%		
Suspected (n=250)	24	9.6	24	9.6		
Healthy (n=50)	04	8.0	04	8.0		

suspected³.

Table 2 shows the positive cases for group a beta hemolytic streptococci i.e. 24 (9.6%) out of 250 suspected cases and negative cases for GABHS i.e. 226 (90.4%) out of 250 suspected cases.

Table No.2: Distribution of group A and B Streptococci in Pharyngitis and Tonsillitis (n=250)

Bacterial pathogen	Cases	Percent
Positive for GABHS	24	9.6
Negative for GABHS	226	90.4

Table 3 shows the positive isolates among male children (n=166) was 15 (9.04%) and female children (n=84), 9 (10.7%) showing the distribution of GABHS according to the sex.

Table No.3: Distribution of Gabhs According to Sex by Radt

Isolates from children	Cases	Percent	
Male (n=166)	15	9.04	
Female (n=84)	09	10.70	
Total (n=250)	24	9.60	

DISCUSSION

Streptococcal pharyngotonsillitis has been a matter of medical concern over the years, particularly because of its potential for causing serious problems such as rheumatic fever and suppurative complications. The prevalence of acute pharyngotonsillitis caused by GABHS is approximately 28% to 40% worldwide; most are known to be susceptible to penicillin. This percentage also varies from region to region¹.

In countries where rapid detection tests are routinely used, a controversy exists regarding weather or not a confirmatory culture is necessary when the results are negative. Although most of the doctors do not usually follow this procedure, most of the medical societies have recommended the back up cultures¹.

The use of a rapid detection test, plus a bacterial culture for the negative results, is currently considered the most effective clinical strategy, increasing the marginal costs only slightly (it increases the initial costs, but lowers the global cost when considering the prevention of complications of the non-treated rapid test undetected cases¹.

Although several studies have shown that the rapid test, applied alone, does not have sufficient sensitivity to eliminate the need for cultures¹.

Some researchers have demonstrated that the more recently available rapid tests can be more sensitive than bacterial culture, particularty the immune assay based tests, which can give results in a few minutes¹.

Interestingly, bacterial culture has been considered by many as the "Gold Standard" method of GABHS detection, however, according to recent studies, this would be a high cost choice in relation to its effectiveness¹.

According to the American Academy of Paediatrics

and the American Heart Association, a positive rapid antigen detection test may be considered definitive evidence for the treatment of Streptococcal pharyngitis. A confirmatory throat culture should follow a negative rapid antigen detection test when the diagnosis of Group A beta hemolytic streptococcal infection is strongly

In the present study, sensitivity of the rapid test was 100% and was confirmed by throat culture. This is approximately the same as in other studies¹¹. A disadvantage of culturing a throat swab on blood agar plates is the delay (overnight or longer) in obtaining the culture results¹².

Culture of a throat swab on a sheep blood agar plate remains the standard for the documentation of the presence of Group A streptococci in the upper respiratory tract and for the confirmation of the clinical diagnosis of acute streptococcal pharyngitis. Several variables impart on the accuracy of the throat culture results, the manner in which the swab is obtained has an important impact on the yield of streptococci from the throat culture¹².

Throat swab specimen should be obtained from the surface of both the tonsils (or tonsillar fossae) and the posterior pharyngeal wall. Other areas of the oropharynx and mouth are not touched before or after the approprate areas have been sampled. In addition a false negative result may be obtained if the patient has received antibiotics shortly before or at the time the throat swab specimen is collected 12.

It has also been reported that the use of anaerobic incubation and selective culture media may increase the proportion of positive cultures. Another variable that can impart on the yield of the throat culture is the duration of incubation. Once plated, cultures should be incubated at 35-37°C for 18-24 hours before they are read. However, the period can be extended for upto 48 hours¹².

The throat culture has always been considered the "gold standard" for diagnosing the presence of Group A Streptococci. The manner in which the throat swab is obtained has an important impact on the accuracy of throat culture results¹³.

CONCLUSION

The RADT is a rapid way of diagnosing the GABHS the result can be obtained within 5-10 minutes and so the treatment may be started accordingly.

While the throat culture is still considered as the Gold Standard for the diagnosis of GABHS pharyngitis. The positive as well as the negative RADT should be confirmed by the culture.

REFERENCES

1. Santos Q, Weckx LlM, Pignatar ACC, Pignatar SSN. Detection of Group A β Hemolytic

- Streptococcus employing three different detection methods: Culture, Rapid Antigen Detection Test and Molecular Assay. Braz J Infect Dis 2003; 7:297-300.
- 2. Thomas BJ, Powers RD, Lawlor MT. Pharyngitis, Bacterial. Last Updated. J Inf Dis 2002;16; 11-14.
- 3. Hayes CS, Williamson H. Management of Group A J3-Hemolytic Streptococcal pharyngitis. Am Fam Physician 2001;63:1557-564.
- AltindisM, Aktepe OC, Kocagoz T. Comparison of Dio-Bact, Bactracin-Trimethoprim/ Slphamethoxazole and Latex Agglutination in the Diagnosis of Group Beta hemolytic Streptococci. Yonsei Med J 2004; 45(1):56-60.
- 5. Danchin MH, Rogers, Selvaraj G, Kelpie L, Rankin P. the burden of group A streptococcal pharyngitis in Melbourne Families. Indian J Med Res 2004; 119(Suppl): 144-147.
- 6. Bourbeau PP, Heiter BJ. Use of swabs without transport media for the Gen-Probe Group A Strep Direct test. J Clin Microbiol 2004; 42:3207-3211.
- 7. Aujard Y, Boucot I, Brahimi N, Chiche D, Bingen E. Comparative efficacy and safety of four day cefuroxime axetil and ten day penicillin treatment of group A Beta hemolytic streptococcal pharyngitis in children. Pediatr Infect Dis J 1995; 14:295-300.

- 8. Hall MC, kieke B, Gonzales R, Belongia EA. Spectrum bias of a rapid antigen detection test for group A, beta hemolytic streptococcal pharyngitis in pediatric population. Epidemiol Res Center 2004; 83: 132-135.
- Herbeck RJ, Teague J, Crossen GR, Maul DM, Childrens PL. Novel, rapid optical immunoassay technique for detection of group A streptococci from pharyngeal specimens: comparison with standard culture method. J Clin Microbiol 1993; 31:839-844.
- 10. Binax, 2004 URL: http://www.mendeley.com/tags/antigen+detection/-unitel states.
- 11. Vincent MT, Celestin N, Hussain A. Pharyngitis. American Family physician 2004;72; 1-4.
- 12. Bisno AL, Gerber MAt Gwaltney JM, Kaplan EL Schwartz RH. Diagnosis of management of Group A Streptococcal Pharyngitis: A practice guideline. Clin Infect Dis 1997; 25:574-83.
- 13. Shet A, Kaplan E. Addressing the burden of group A. Streptococcal disease in India. Indian J Paeds 2004; 71(1): 41-44.

Address for Corresponding Author: Dr. Karam Ali Mirjat,

Asstt. Prof. of Pathology, Dow Medical College, Karachi