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Anti-Streptolysin o Test in the Diagnosis of Group A Beta Hemolytic Streptococcal Pharyngitis in Endemic Regions: A Preliminary Study

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Abstract: Antistreptolysin O titers were determined in school children colonized or infected with beta hemolytic streptococci using a rapid commercial latex agglutination kit. Seven (16.2%) of the 43 symptomatic children and five (4.8%) of the 105 asymptomatic children were ASO positive; all were from who were GAS+ve. Of the seven symptomatic children five had a titer of 200 IU/ml, one had a titer of 400 IU/ml and another had a titer of 1200 IU/ml; of the five asymptomatic children, three had titers of 400 IU/ml, one had 600 IU/ml and another 800 IU/ml. None with non-group A streptococci were positive for ASO. The percentage positivity of ASO among symptomatic and asymptomatic children was statistically significant (<0.05). We conclude that rapid ASO test is a useful adjunct in the diagnosis of GAS pharyngitis.

Key words: Group A streptococci (GAS), Beta hemolytic streptococci (BHS), ASO test, ULN value.

INTRODUCTION

Microbiological confirmation of a 'bonafide' group A streptococcal pharyngitis is essential for instituting penicillin prophylaxis for the control and prevention of rheumatic fever / rheumatic heart disease. This is of significance in endemic countries where nearly two thirds of clinical pharyngitis cases are of viral etiology; therefore it becomes all the more important to differentiate it microbiologically from all cases of viral pharyngitis (Brahmadathan *et al*., 2006, Bisno *et al*., 1997, Anita and Kaplan, 2004). A 'bonafide' GAS pharyngitis is normally diagnosed by isolating the organism from throat culture in heavy numbers in the absence of other conventional pathogens with accompanying evidence of a four-fold rise in anti-streptococcal antibodies (Johnson et *al*., 1996, WHO, 1988). Since it is not often possible to collect two samples from a patient, most laboratories test point-serum samples for antibody determination and interpret it based on manufacturer's instructions. This can lead to over diagnosis and hence over treatment as, many asymptomatic healthy individuals in endemic countries may also possess varying titers of ASO.

During an ongoing epidemiology study on streptococcal infections, we compared the ASO test results in symptomatic and asymptomatic children. Here we present our preliminary data to show that ASO done on point serum samples can help in confirming a diagnosis of GAS pharyngitis in endemic situations.

MATERIALS AND METHODS

A total of 148 school children, who were positive for beta-hemolytic streptococci (BHS) aged 5-15 years in 5 different schools were selected for the study. This included 43 children with clinical signs and symptoms of pharyngitis and 105 asymptomatic healthy children. Informed consent was obtained from their parents or other adult legal guardians. Children were examined with regard to the presence of clinical symptoms such as sore throat, fever, chills, malaise and erythema and swelling of the pharyngeal mucosa. Those who had any such symptoms or signs were included. And also asymptomatic children were included in the study.

Two – three ml blood samples were collected in clean, sterile small test tubes aseptically; sera were separated and stored at –20ºC till further use.

Each serum sample was tested for ASO by a commercial kit using their instructions. Equal volumes (50µl) of serum and the reagent were mixed on a microscopic slide for a few minutes and observed for agglutination. Development of visible agglutination indicated a positive qualitative ASO test and represents 200 IU/ml as per the manufacturer's instructions. If positive, the final titer was determined semi-quantitatively. For this, the

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positive serum was diluted 1:2, 1:4, 1:8, 1:16, 1:32 ….etc. with sterile normal saline. Each dilution was tested as for the qualitative test. To get the final titer, the dilution factor was multiplied by 200. Thus if the serum sample is positive at 1:4 dilutions, the titer is taken as 200 IU x 4 = 800 IU/ml (Tulip Diagnostics,Goa, India).

Statistical analysis was performed using Z statistics, A P value ≤ 0.05 was considered statistically significant.

Results:

Table 1 gives the results of ASO done on 43 symptomatic and 105 asymptomatic children whose throat cultures grew BHS. Seven (16.3%) of the 43 symptomatic children were ASO positive; all were from those who were positive for GAS. Five of these had titers of 200 IU/ml, one had 400 IU/ml and another had 1200 IU/ml. Of 105 asymptomatic children, five (4.76%) were positive for ASO. their titers were 400 IU/ml (n = 3), 600 IU/ml and 800 IU/ml. All were from children who were positive for GAS. None of the children with non-GAS were positive for ASO.

Tulip diagnostic antistreptolysin-O antibodies in serum test kit instruction and information sheet

Table 1: ASO positivity among symptomatic and asymptomatic children with GAS and Non-GAS in their throat.

ASO	SYMPTOMATIC (43)		ASYMPTOMATIC (105)	
	GAS^{+ve}	Non GAS^{+ve}	GAS^{+ve}	Non GAS^{+ve}
Positive	7*	0	5**	0
Negative	10	26	32	68
TOTAL	17	26	37	68

ASO-Antistreptolysin O; GAS-Group A streptococci

The difference in proportion of ASO+ and GAS+ among symptomatic (7/17) and proportion of ASO+ and GAS+ among asymptomatic(5/37), is statistically significant with P= 0.0232 (<0.05).

Discussion:

Our study clearly shows that ASO done on point serum samples can be a useful adjunct for confirming the diagnosis of a 'bonafide' GAS infection. Normally, in the absence of a 'four-fold' rise in titer, interpretation is based on upper limit of normal value (ULN) (or cut-off) standardized with serum samples taken from normal healthy children with no history of GAS pharyngitis in the recent past (Shet and Kaplan,2002). This is very important because in endemic regions, a high streptococcal carriage in the throat results in the development of ASO responses that can be sufficiently high to confuse results from those with GAS pharyngitis (Kaplan,1980). In most situations standardization of ULN is not done and laboratories resort to cut-off values given in the kit insert. This is not desirable because such cut-off values are based on ULN standardized in western countries where prevalence of GAS infections is much less. Our study is based on a relatively small sample size and the findings need to confirmed by carrying out a study with a larger sample size.

Interestingly, our study shows that ASO responses are quite specific to GAS infections as compared to non-GAS infections. This is confirmed by the fact none of the children with non-GAS in throat (n = 148) were positive for ASO.

In the final analysis, a laboratory diagnosis of GAS pharyngitis can only be made by recovering the organism in high numbers (>100 colonies on sheep blood agar) in the presence of clinical signs and symptoms. However in endemic regions, this may also be due to a viral infection with heavy GAS colonization. If ASO test is carried out on such cases, it can act as a useful adjunct in confirming the true nature of the infection.

Conclusion:

This pilot study confirms the usefulness of ASO test as an adjunct in confirming a diagnosis of GAS infection. Its result should always be interpreted along with a culture positive for GAS.

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