

“Evaluation of ASO test and correlation with clinical request in a tertiary care teaching hospital: An insight study”

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Abstract:

Introduction: Post streptococcal diseases caused by beta haemolytic group A streptococcus usually confirmed by antistreptolysin O test. The aim of this study is evaluation of ASO titre and correlation with clinical request.

Methodology: It is a retrospective and descriptive study conducted at the Central laboratory of Department of Microbiology, Malda Medical College. We analyzed all requests during last one year. From sera, obtained from patient, we done qualitative test to determine ASO positive sera and semiquantitative test to determine ASO titre.

Results: There were 586 requests of ASO titration during the last one year of study. The mean age was 32.5 years (ranging from 5 to 60 years), with a female predominance (53.9 %). Cardiac symptoms were most common request (54.9%). Most common frequency of ASO request was found from age group 16 to 30 years (43.6 %). Out of 586 tests, 220 tests shows positive result means ASO titre level more than >200 IU/ml and 366 tests shows negative result means ASO titre level less than <200 IU/ml. Most common titre level was found >200<400 IU/ml.

Conclusion: ASO test is cheaper and easy to perform even in rural settings. The test can provide baseline information for use in diagnosis though it has not been deployed in many clinical settings in India and other developing countries. ASO test is very helpful when culture is negative and patient already has taken antibiotics.

Keywords: ASO titration, Post-streptococcal infections, Malda

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I. Introduction

Streptococcal infection have high rate of incidence and prevalence in developing countries. Humans are the natural reservoir for group A beta haemolytic streptococci and the organism is transmitted from person to person by the respiratory route. Post Streptococcal diseases caused by beta haemolytic streptococcus group A comprise cardiac involvement, joint involvement, acute renal failure, abnormal involuntary movements and other complications.^{1,2,3} The prevalence of post streptococcal diseases considerably decreased in industrialized countries but it still remains a major public health concern among developing countries like India.^{4,5} Immunological testing is necessary in order to prove that clinical symptoms are caused by post streptococcal diseases, by performing ASO test. The antistreptolysin O antibody is directed against extracellular antigen of streptococcus group A.⁶

II. Objectives

The aim of this study is evaluation of ASO titre and correlation with clinical request.

III. Methodology

It is a retrospective and descriptive study will be conduct at the Central laboratory of Department of Microbiology, Malda Medical College. We analyzed all requests during last one year. We looked at age, gender and clinical symptoms which led to the request.

All requests from both inpatients and outpatients of ASO will be include in this study and we will be exclude incomplete data, especially those in which age, clinical symptoms are missing.

3 ml. of blood was obtained by venepuncture of the forearm and then it was centrifuged with 1500 rpm for 10 minutes. Serum was obtained.

Qualitative test- The qualitative test is a screening test to determine the presence of the ASO antibody in the serum. The test was carried out by bringing test reagents at room temperature. Pipette one drop of test sample on to the identified ring of the test slide using a disposable dropper provided along with test kit. Then

add one drop of latex reagent to the drop of test sample. Positive and negative controls were set up. Mix gently using separate mixing stick uniformly within the full area of the circle. Slowly rock the slide for two minutes and observe for agglutination under a high intensity of light source. If ASO concentration in serum is 200 IU/ml. or more than 200 IU/ml. gives visible agglutination reaction.

Semi Quantitative test – For each test to be titrated, six test tubes were set up and labelled 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and to each tube 0.2 ml. of physiological saline added. To tube number one 0.2 ml of undiluted test serum was added and two fold dilutions were serially made by transferring 0.2 ml. of mixing contents to tube number two. Each serial transfers was repeated for each tube. Pipette one drop of diluted sample from each tube onto the identified circle of test slide. Add one drop of ASO latex reagent to the circle containing diluted sample and mix well using mixing stick. Slowly rock the slide for two minutes and observe for agglutination under a high intensity of light source. The highest dilution of the test sample showing agglutination is considered the endpoint titre.⁷

IV. Results

There were 586 requests of ASO titration during the last one year of study. The mean age was 32.5 years (ranging from 5 to 60 years), with a female predominance (53.9 %).

Table 1 shows, Cardiac symptoms were most common request (54.9%). Others common request were Oto- rhino laryngological cases mostly tonsillitis (18.0%), Nephrological cases mostly glomerulonephritis (5.4%) and rheumatologic cases mostly arthralgia and arthritis (4.7%).

Figure 1 shows, frequency of ASO prescription in relation with age group. Most common frequency of ASO request was found from age group 16 to 30 years (43.6 %), then 31 to 45 years (35.4%). Others chronologically >45 years (12.6 %), 6 to 15 years (4.7 %), upto 5 years (3.4 %).

Figure 2 shows, ASO titre levels relation with number of test . Out of 586 tests, 220 tests shows positive result means ASO titre level more than >200 IU/ml and 366 tests shows negative result means ASO titre level less than <200 IU/ml. Most common titre level was found >200<400 IU/ml. (21.16%). Other titre levels were >400<800 IU/ml. (13.65%), >800<1600 IU/ml. (0.022%), >1600<3200 IU/ml. (0.005%).

Table 2 shows, Total number of ASO positive test (n= 220) in relation with clinical symptoms. Most common ASO positive test was found in relation with cardiac symptoms (54.9%) and oto rhino laryngological symptoms (34.5%). Other positive test were found in rheumatological (11%), neurological (1%), nephrological (1%) and dermatological symptoms (0.5%).

V. Discussion

The beta hemolytic streptococcal infection is very common and its complications reinforces the need of a precise diagnosis, mainly an immunological proof of the infection.⁸ Our study shows that most of the ASO requests showed a low level of ASO titre, even if the level of ASO titre is high, it doesnot mean necessarily that the patient has a true post streptococcal infection because the antibody titre decrease slowly and may persist longtime after the infection.⁹ Besides this, a single ASO titre assay has some limitations, especially with regard to chronic nasopharyngeal group A beta hemolytic streptococcus carriage status. ASO is helpful when the throat culture technique is ineffective or when the patient has already taken antibiotics.⁷ The majority of patients were belong to age group 16 to 30 years, then 31 to 45 years, which is not compatible with the typical presentation of acute rheumatic fever usually occurring in children aged of 5 to 14 years.^{10,11} The clinical request below 15 years only 8.1%, it is mismatched with other studies.^{9,10,12,13} It is probably due to the fact that clinician not always wait for ASO test results of the children attending paediatric department. They start antibiotic after clinical diagnosis without delay. The evaluation of ASO titre is a part of current guidelines for the diagnosis of acute rheumatic fever.¹⁴ There is an increase of ASO titre one week after the infection with a maximal level in 3 weeks. Our study shows, most of the clinical requests related with cardiac symptoms with a female predominance found ASO test positive and most of the positive titre values lies between >200 but <400 IU/ml. This finding correlates with other study.^{12,13,15,16} About dermatological symptoms, they may be due to Streptococcus, such as erythema marginatum, cellulitis, impetigo.¹⁵ So, 0.5% positivity of dermatological cases is relevant. Recurrent tonsillitis and pharyngitis may be due to bacteria or viruses, some authors recommend an evaluation of ASO titre to distinguish both of them.^{17,18} But it is better to perform a bacteriological diagnosis. The ASO titre level may vary depending on age group of patients, site of infection and population affected in different countries.^{19,20} Finally, for confirmation of complications of Gr. A streptococcal infection, two or more serum sample should be tested to show the increase of the titre in case of a true infection.

VI. Figures and Tables

Table 1: Frequency of symptoms leading to a request of ASO

Type of symptoms	Clinical information	Frequency	%
Cardiac symptoms	Carditis, vulvulopathy, polyarteritis, arterial hypertension, angina pectoris	322	54.9%
Rheumatologic	Arthralgia, Arthritis	28	4.7%
Neurologic	Chorea, Seizure, Headache, Stroke	2	0.3%
Nephrology	Glomerulonephritis, acute renal failure, dysuria, UTI	32	5.4%
Oto- Rhino Laryngology	Tonsillitis, otitis, keratitis, keratoconjunctivitis	106	18.0%
Dermatology	Erythema marginatum, subcutaneous nodules, cellulitis, impetigo	24	4.0%
Others	Oedema, vertigo, stiff neck	4	0.6%
No clinical information		68	11.6%
Total		586	100%

Figure 1: Frequency of ASO prescription in relation with age group

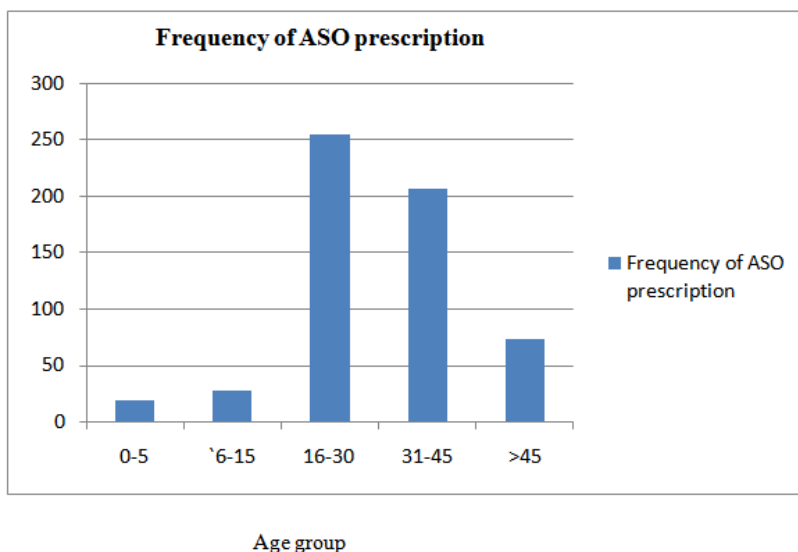
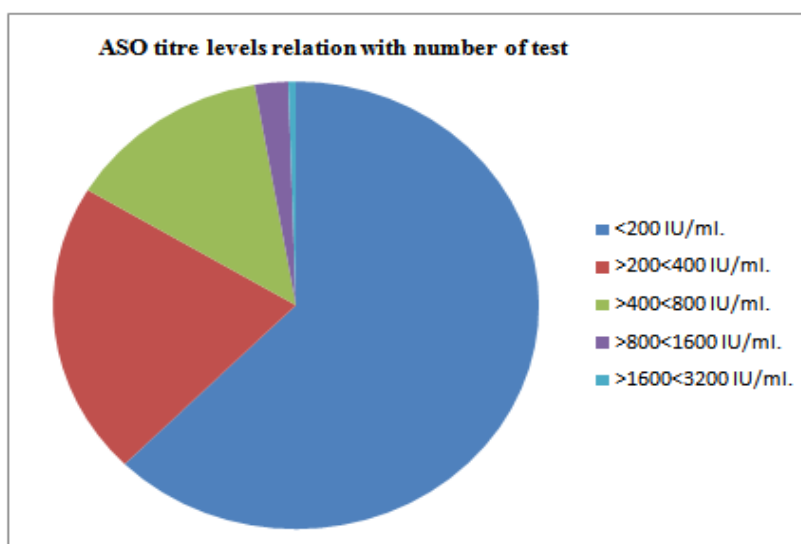


Figure 2: ASO titre levels relation with number of test



<200 IU/ml = 62.45%
 >200<400 IU/ml = 21.16%
 >400<800 IU/ml = 13.65%
 >800<1600 IU/ml = 0.022%
 >1600<3200 IU/ml = 0.005%

Table 2: Total number of ASO positive test (n= 220) in relation with clinical symptoms

Type of symptoms	ASO test Positive (%)
Cardiac symptoms	52 %
Rheumatologic	11 %
Neurologic	1 %
Nephrology	1 %
Oto- Rhino Laryngology	34.5 %
Dermatology	0.5%
Others	0 %
No clinical information	0 %
Total	100%

VII. Conclusion

ASO test is cheaper and easy to perform even in rural settings. The test can provide baseline information for use in diagnosis though it has not been deployed in many clinical settings in India and other developing countries. Immunological testing is necessary in order to prove the post streptococcal diseases, by performing ASO test. ASO test is very helpful when culture is negative and patient already has taken antibiotics.

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