

## Sero-prevalence of anti-streptolysin 'O' antibodies at tertiary care hospital: A two year of prospective study

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### Abstract

**Background:** *Streptococcus pyogenes* (Group A Streptococcus/GAS) is the most prevalent human bacterial pathogens amongst streptococcus species. It causes a wide range of suppurative infections in the respiratory tract and skin, life-threatening soft tissue infections, and certain types of toxin associated reactions. Some of these infections may, in addition, result in severe non-suppurative sequelae due to adverse immunological reactions induced by the infecting streptococci. As the diagnosis of non-suppurative infection caused *Streptococcus pyogenes* is only depends on immunological reaction, present study was carried out to see the sero-prevalence of Anti-streptolysin O antibodies in our region.

**Material & Methods:** In the present prospective study was carried out in the Department of Microbiology under serology section at a tertiary care hospital from January 2015 to December 2016. Blood samples were taken from clinical suspicion cases of streptococcal infection. All samples were tested by using qualitative and semi-quantitative antibody detection method as per the standard guidelines.

**Results:** A total of 1526 blood samples were collected and processed for the detection anti-streptolysin O antibodies. Out of which 92 (6.03%) were positive for ASO, among them 59 were from out door-patients department and 33 were from indoor-patients department. Out of 92 positive cases, 48 were males and 44 were female patients. Highest positive cases were found in the age group of 11-20 years (7.15), followed by the age group of 21-30 years where the prevalence was found to be 6.40%.

**Conclusion:** The presence of elevated streptococcal antibody titers in such population, which probably reflects a high background prevalence of streptococcal infections, should be taken into consideration when evaluating the role of the group A streptococcus in non-purulent complications of infections.

**Keywords:** Anti-streptolysin O (ASO), Sero-prevalence, Group A Streptococcus (GAS), Rheumatic diseases.

### Introduction

*Streptococcus pyogenes* (Group A Streptococcus/GAS) is the most prevalent human bacterial pathogens amongst streptococcus species.<sup>(1)</sup> It causes a wide range of suppurative infections in the respiratory tract, skin, life-threatening soft tissue infections, and certain types of toxin associated reactions.<sup>(2)</sup> Some of these infections may, in addition, result in severe non-suppurative sequelae due to adverse immunological reactions induced by the infecting streptococci.<sup>(1,2)</sup> A similar spectrum of infections may be caused by closely related group C and group G Streptococcosis. Strains of *streptococcal pyogenes* express a large arsenal of virulence factors involved in adherence, evasion of host immunity, and tissue damage.<sup>(3)</sup>

The ability of *Streptococcus pyogenes* to resist phagocytosis by polymorphonuclear leucocytes is largely due to the cell-surface exposed M-protein. Two serious diseases may develop as a sequelae to *S.pyogenes* infections that is Rheumatic fever, a potential sequel to pharyngitis (including scarlet fever) and Acute glomerulonephritis, which is primarily, but not exclusively, associated with skin infections both are caused by immune cross-reaction.<sup>(4)</sup>

Clinical correlations suggest that certain form of psoriasis may also trigger by *S.pyogenes* throat

infection. Preliminary evidence supports the hypothesis that some streptococcal superantigens cause disruption of immunological tolerance of a CD8<sup>+</sup> T cell subset that recognizes cross-reactive epitopes on M proteins and skin keratin.<sup>(1,4)</sup>

Rheumatic fever is an inflammation of the joints (arthritis), heart (carditis), central nervous system (chorea), skin (erythema marginatum) and/or subcutaneous nodules. Rheumatic fever is a major cause of acquired heart disease in young people throughout the world.<sup>(5)</sup> The incidence of rheumatic heart disease worldwide ranges from 0.5-11 per 1000 of population. The disease is autoimmune in nature and is believed to result from the production of auto-reactive antibodies and T lymphocytes induced by cross-reactive components of bacteria and host cells. Another manifestation is acute glomerulonephritis usually referred to as an immune complex mediated disease.<sup>(5)</sup>

Taking into account, the diagnosis of *Streptococcus pyogenes* infection is most important for the judicial use of the drugs and proper institution of the therapy, by which to prevent non-suppurative post streptococcal infection.<sup>(6,7)</sup>

The diagnosis of *Streptococcal pyogenes* by using culture method is the most reliable and gold standard method, but expected results have some limitations, false negative results may occur due to prior antibiotic

therapy or misdiagnosed with streptococcal infection due to a carriage state, in that case, detection streptococcal antibodies are necessary to rule out post-streptococcal infection.<sup>(8)</sup> The anti-streptolysin O (ASO) test is available and easy to interpret. ASO is not only useful in the diagnosis of streptococcal infections or complications, but also in the follow-up process and in evaluating the effectiveness of treatments. It measures the ability of serum to neutralize streptolysin O. The results of conventional assays are reported as Todd units which are the reciprocal of the highest dilution of serum that is positive. Antibodies to deoxyribonuclease B (ADB) are used less commonly and those against hyaluronidase are rarely used.<sup>(5,7)</sup>

As ASO is not always elevated after streptococcal infection or sequelae, it is necessary to add the alternative serological test. Since ADB has a longer half-life than ASO, it can be a valuable tool in the diagnosis of remote past infections.<sup>(8)</sup>

Hence present study was carried out to see the prevalence of Anti-streptolysin O antibodies in our region.

### Materials and Methods

After the approval of the institutional ethical committee, present prospective study was carried out in the Department of Microbiology under Serology section at tertiary care hospital from January 2015 to December 2016. The blood samples were collected from clinically suspicion patients of streptococcal infection attending outpatient and in-patients department.

All blood samples were left to clot for 15 minutes at room temperature and sera were separated by using micropipette. All serum samples were further subjected ASO TEST method, is a rapid latex agglutination test for the qualitative and semi-quantitative determination of anti-streptolysin-O in serum.<sup>(5,8)</sup> The ASO test kit was procured from Tulip Diagnostics Pvt. Ltd. Mumbai. Test procedure was followed as per instruction manual provided with the test kit. Results were compared with Positive control and negative control. ASO titre more than 200 IU for adult and 300 IU for children was considered to be significant. The data were analyzed by using window based excel program.

### Observations and Results

A total of 1526 blood samples were collected and processed for the detection of anti-streptolysin O antibodies. Out of which 92 (6.03%) were positive for ASO, among them 59 were from out-patients department and 33 were from indoor-patients department.

Out of 92 positive cases, 48 were males and 44 were females. Highest positive cases were found in the age group of 11-20 years (7.15), followed by the age

group of 21-30 years where the prevalence was found to be 6.40%. Details can be seen in Table 1, 2, 3.

**Table 1: Sero-prevalence of ASO among OPD & IPD patients**

Source	Anti-streptolysin 'O' test		
	Positive	Negative	Total
OPD	59	1008	1067
IPD	33	426	459
Total	92 (6.03%)	1434 (93.97%)	1526

**Table 2: Sex-wise sero-prevalence of all cases**

Sex	Anti-streptolysin 'O' test			
	Positive	Negative	Total(%)	Prevalence (%)
Male	48	695	743 (48.68)	6.46
Female	44	739	783 (51.31)	5.61
Total	92	1434	1526 (100)	6.03

**Table 3: Sero-prevalence of ASO distribution in Different Age Groups**

Age	Anti-streptolysin 'O' test			
	Positive	Negative	Total (%)	Prevalence (%)
0-10	13	255	268 (17.54)	4.85
11-20	33	428	461 (30.20)	7.15
21-30	27	405	432 (28.30)	6.40
31-40	06	148	154 (10.09)	3.89
41-50	05	107	112 (7.33)	3.24
51-60	04	93	97 (6.35)	4.12
61-70	03	63	66 (4.32)	4.54
>70	01	27	28 (1.83)	3.57
Total	92	1434	1526 (100)	6.03

### Discussion

The serological test for streptolysin O antibodies (ASO) is commonly used for the diagnosis of antecedent infections caused by the group A streptococcus (GAS) and are particularly useful for the diagnosis of acute rheumatic fever and acute post-streptococcal glomerulonephritis.<sup>(9,10)</sup> Since streptococcal infections are related to some rheumatic conditions, family physicians and rheumatologists measure ASO titers as a predisposing factor for rheumatic diseases or as an additional acute-phase reactant in order to evaluate the severity and activity of these diseases.<sup>(11)</sup>

The diagnosis of post-streptococcal infection is routinely done on the basis of the measurement of antibodies to various combinations of the extra-cellular GAS antigens: (ASO, DNase B and streptokinase antibodies).<sup>(12)</sup> However, *Blyth et al.*<sup>(13)</sup> showed that the addition of anti-streptokinase antibodies measurement did not increase the sensitivity and specificity of serological testing for the diagnosis of acute post-streptococcal disease.

Ideally, it is recommended that the ASO test is reliable only when there is evaluation of four-fold rise in antibody titer in subsequent samples. However, it is not always practicable to obtain a second sample for titer determination, particularly in developing countries, where acute rheumatic fever is the most common.<sup>(9,10)</sup>

Therefore, it is generally accepted that if only a single specimen is available, a titer greater than the upper limit of normal at the initial testing can be considered presumptive evidence of a preceding streptococcal infection. Antistreptolysin-O (ASO) is the commonest, best standardized & practical test for detecting a preceding streptococcal infection. The appearance of ASO in serum of a patient or an increase in the ASO titer is usually indicative for a recent streptococcal infection.<sup>(8,9,10)</sup> This is especially true when considering the diagnosis of non-suppurative sequelae of GAS infection.<sup>(11)</sup>

ASO titer has a utilitarian role in the diagnosis of Acute Rheumatic Fever. Even a single ASO titer is a useful investigative tool aiding in the confirmation of the diagnosis. The ASO titers are elevated in the acute phase and show a subsequent lower titer level as the disease progresses.

In our study the overall prevalence is found to be 6.03%, though the rate of positivity is low in our region as compared with other studies, but this has been shown to vary with age, geographical location and site of infection.<sup>(9,10,11)</sup>

The highest rate of positivity was seen in the age group of 11-20 years and our finding were in concordance with the studies carried out by many researchers.<sup>(1,9,10)</sup>

## Conclusion

The estimation of ASO antibodies is a simple, cost effective way for detecting antecedent streptococcal infection. However, the results should be interpreted judiciously keeping the upper normal limits of ASO titer, for the given geographical area, in mind. Increased ASO titer supports but do not prove the diagnosis of rheumatic fever.

The prevalence of Anti-streptolysin O (ASO) antibody in total cases was 6.03%. In male was 6.46% and in female was 5.61%. The highest percentage (7.15%) was found in age group 11-20 years. The presence of elevated streptococcal antibody titers in such a population, which probably reflects a high

background prevalence of streptococcal infections, should be taken into consideration when evaluating the role of the group A streptococcus in nonpurulent complications of infections.

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