Analysis of ASO Titres among Paediatric Age Group Patients Attending a Tertiary Care Hospital, Secunderabad, Telangana

Vara Prasad Konditi[1]

[1]Department of Microbiology, Gandhi medical College and Hospital, Secunderabad, Telangana, India.

ABSTRACT

BACKGROUND

Streptococcus pyogenes can affect most often in children with peak age related incidence between 11 to 15 years of age and all races of the world. ASO estimation as an early diagnosis can help to start early & accurate treatment. The objectives of this study were to determine the ASO levels among paediatric age group patients and to estimate the age and sex relation to ASO levels.

METHODS

A total of 200 patient samples were considered under study population for investigating Anti streptolysin O titres. All the serum samples were processed using Tulip Diagnostics ASO Kits. All blood samples of study population were collected and processed immediately by centrifugation at 3000 rpm for 15 minutes. Serum sample is extracted and processed by semi quantitative method for detection of Anti Streptolysin – O.

RESULTS

Majority of the patients were observed in the age group of 11 - 15 years i.e., 104 out of 200 (52 %) followed by 5-10 years makes up to 41 %. Male preponderance was noted which accounts for 56.5 % of total cases. Out of 68 male positive patients, 21 (30.8 %) had ASO titres of 200-400 IU / mL, 27 (39.7 %) had ASO titres of 400-800 IU / mL and 20 (29.4 %) had ASO titres of > 800 IU / mL. Out of 31 female ASO positive patients, 5 had (19.2 %) of ASO 200 - 400 IU / mL, 17 had (38.6 %) of ASO 400-800 IU/mL and 9 had (31.03 %) ASO titres of > 8 00 IU / mL.

CONCLUSIONS

Increase in ASO titres indicate recent infection, which can be tested easily and timely report. Many hospitals are choosing ASO test as a priority investigation to diagnose GAS infections.

KEYWORDS

Anti-Streptolysin O, Children

Corresponding Author:
Dr. Vara Prasad Konditi,
Assistant Professor,
Department of Microbiology,
Gandhi Medical College/Gandhi Hospital,
Secunderabad, Telangana, India.
E-mail: varaprsd184@gmail.com

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BACKGROUND

Streptococcus is a gram positive bacterium which can cause mild infections such as throat infections, pyogenic infections, cellulitis, impetigo to severe infections including septicaemia, rheumatic heart disease. Streptococci acts as an antigen when it enters in to the body and produce various antibodies. Antistreptolysin-O (ASO / ASLO) is one such antibody which rises in the body during streptococcal infections.

Streptococci which is a heterogeneous group of bacteria divided into several groups. ASO or ASLO is produced by most strains of group A Streptococci (GAS) and many strains of group C and G streptococcus bacteria. Group A Streptococci is also referred to as Group A Beta haemolytic Streptococci (GABHS). Streptolysin O is responsible for causing haemolysis which is an immunogenic, oxygen labile streptococcal haemolytic exotoxin, against which body produces ASO. Group A Streptococci (GAS) infections and their late sequelae like rheumatic fever (RF) and rheumatic heart disease (RHD) remain an important and major health problem in India. For these diagnosis, according to Jones Criteria require the evidence of Streptococcal infection as a minor criteria.[1]

GAS cause significant morbidity and mortality in public health especially in developing countries. The prevalence of severe GAS disease is at least 18.1 million cases with an incidence of at least 1.78 million cases per year.[2] Incidence of Group A Streptococcal infections sequelae rheumatic fever was noted as only 0.3 %. Cardiac involvement is reported to occur in 30 - 70 % of patients with their first attack of rheumatic fever and in 73 - 90 % of patients when all attacks are counted.[3] It can affect most often in children with peak age related incidence between 11 to 15 years of age and all races of the world. Epidemiological factors classically associated were lower socioeconomic status, overcrowding, under nutrition and poverty.

ASO is measured in terms of IU / ml units in blood samples. ASO levels begin to rise 1 to 3 weeks after streptococcal infection, peaks in 3 - 5 weeks & later slowly antibodies levels decrease within 6 months. ASO titre of > 200 units in adults and > 100 units in children are considered as significant values, but these values need to be correlated with clinical findings.[4] Increase in ASO titres in paired sera or appearance of antibodies to Streptococcus confirms the recent GAS infections.[5] During evaluation of Anti Streptolysin O titres microbiologists should have a thought on false positive and false negative results. False positive results can occur due to lipemic samples, tuberculosis, liver diseases mainly acute viral hepatitis, bacterial contaminated samples, haemolysed blood samples; haemolysed and lipemic blood samples should reject before processing and request clinician to send a repeat blood sample for processing. False negative results can occur due to antibiotic or steroid intake prior to sampling, streptococcal skin infection.

Objectives

ASO estimation as an early diagnosis can help to start early & accurate treatment. Hence in this study, we estimated the ASO titre among paediatric age group patients attending to OPD of a tertiary care hospital. The objectives of this study are to know the ASO levels among paediatric age group patients and to estimate the age and sex relation to ASO levels.

METHODS

This is a cross-sectional Observational study conducted on Paediatric age group patients attending "Gandhi Hospital/Gandhi Medical College" at Secunderabad, Andhra Pradesh from January 2018 to December 2019. This study conducted in the department of Microbiology, Gandhi Medical College, Secunderabad.

Inclusion Criteria

Patients with age group of 5-18 years of both sexes

Exclusion Criteria

- Known cases of Rheumatic Heart Disease and Rheumatic Fever
- Patient's aged above 18 years
- Patient's with autoimmune disorder

A total of 200 patient samples were considered under study population for investigating Anti Streptolysin O titres. Institutional ethical committee approval has been taken before conducting this study. All patient details were kept unlinked anonymously as per the protocol. All blood samples of study population were collected at serology section of department of Microbiology were processed immediately. After receiving blood samples of 3 ml which are not haemolysed and not lipemic in a clot activator vacutainer, centrifuged at 3000 rpm for 15 minutes. Serum sample is extracted and processed by semi quantitative method for detection of Anti Streptolysin-O.

All the serum samples were processed using Tulip Diagnostics ASO Kits. Principle of this test kit is ASO Slide test for detection of antibodies to Streptolysin O is based on the principle of agglutination. The test specimen is mixed with ASO latex reagent and allowed to react. If antibodies to streptolysin O are present in concentrations more than 200 IU / ml but less than 4000 IU / ml then a visible agglutination is observed. If antibodies to streptolysin O are not present or are in concentrations less than 200 IU / ml then no agglutination will be observed.

Semi Quantitative Method

Procedure

Bring reagent and samples to room temperature before testing. All the reagents should be stored at 2 - 8^0 C, should not freeze.

1. Using isotonic saline prepare serial dilutions of the serum sample positive in the qualitative method 1 : 2, 1 : 4, 1 : 8, 1 : 16 and so on.

2. Pipette the diluted specimens on the slide. Start with the 1:2 diluted test specimen.
3. Add one drop of ASO reagent to it and mix well. Spread the mixture uniformly over the entire circle.
4. Immediately start a stopwatch. Rock the slide gently, back and forth, observing for agglutination macroscopically at two minutes. Proceed similarly with each dilution as test specimen

Interpretation

Agglutination in the highest serum dilution corresponds to the amount of ASO in IU/ml present in the test specimen.
The concentration of ASO can be calculated as follows:
ASO (IU / ml) = S X D
Where S = sensitivity of the reagent i.e., 200 IU / ml
D = Highest Dilution of serum showing agglutination.

Statistical Analysis

Data received along with blood samples pertaining to patient identification number, age, sex, department, probable diagnosis were entered into spread excel sheet along with ASO Results. All Descriptive quantitative variable were expressed as numbers and percentages.

RESULTS

In the present study we have assessed ASO titres in paediatric age group patients. Majority of the patients were observed in the age group of 11 -15 years i.e., 104 out of 200 (52 %) followed by 5 - 10 years makes up to 41 %. Male preponderance was noted which accounts for 56.5 % of total cases, even in 11-15 years age group male patients suspicious of Group A beta haemolytic Streptococci (GABHS) was noted predominantly (Table 1).

Age in yrs	Male	(%)	Female	(%)	Total	(%)
5-10	38	19	44	22	82	41
11-15	66	33	38	19	104	52
16-18	9	4.5	5	2.5	14	7
Total	**113**	**56.5**	**87**	**43.5**	**200**	**100**

Table 1. Age and Sex Distribution of ASO Samples

Titre in IU / mL	5 - 10	%	11 - 15	%	16 - 18	%	Total	%
<200	47	23.5	42	21	12	6	101	50.5
200-400	8	4	16	8	2	14.2	26	13
400-800	17	8.5	27	13.5	0	0	44	22
>800	10	5	19	9.5	0	0	29	14.5
Total	**82**	**41**	**104**	**52**	**14**	**7**	**200**	**100**

Table 2. ASO Titres in Relation to Age Distribution

Out of 200 patients, 101 (50.5 %) didn't show ASO positivity and remaining 99 (49.5 %) patients showed ASO positivity i.e., >200 IU / mL of ASO. Among 99 ASO positive patients, 26 (26.6 %) had ASO titres of 200 - 400 IU / mL, 44 (44.4 %) had 400-800 IU / mL and 29 (29.2 %) had > 800 IU / mL ASO titres (Table 2). On observing titre in relation to age showed that 62.6 % (62 out of 99) of positive cases were in the age group of 11 - 15 years, 35.3 % (35 / 99) of positivity shown in 5 - 10 years age group and 2.02 % (2 / 99) of ASO positivity were in the age group of 16 - 18 years (Table 2).

ASO titres in relation to sex was observed that Out of 113 male paediatric population 68 (68.6 %) patient samples were ASO positive. Out of 87 females paediatric study population 31 (35.6 %) were ASO positive (Table 3). Out of 68 male positive patients, 21 (30.8 %) had ASO titres of 200 - 400 IU / mL, 27 (39.7 %) had ASO titres of 400-800 IU / mL and 20 (29.4 %) had ASO titres of > 800 IU / mL. Out of 31 female ASO positive patients, 5 had (19.2 %) of ASO 200 - 400 IU / mL, 17 had (38.6 %) of ASO 400-800 IU / mL and 9 had (31.03 %) ASO titres of > 800 IU / mL.

Titre in IU/mL	Male	(%)	Female	(%)	Total	(%)
<200	45	44.5	56	55.4	101	50.5
200-400	21	80.7	05	19.2	26	13
400-800	27	61.3	17	38.6	44	22
>800	20	68.9	9	31.03	29	14.5
Total	**113**	**56.5**	**87**	**43.5**	**200**	**100**

Table 3. ASO Titres in Relation to Sex Distribution

DISCUSSION

The Anti Streptolysin O (ASO) is an international standardized test, it is the assay of antibodies against (ASO). It gives better results following streptococcal throat infection than skin infection. The determination of Anti Streptolysin O is by neutralization of Streptolysin O by ASO antibodies, only reduced streptolysin O is active in this reaction. The non-neutralized antibodies can be find out by adding erythrocytes, which is demonstrated as haemolysis of those added erythrocytes.[6]

Anti-Streptolysin O typically develops 2-4 weeks after Group A Beta Haemolytic Streptococcus (GABHS) pharyngitis infection. It is most useful in during diagnostic challenging scenarios such as unable to isolate Streptococcal pathogens from throat swab culture, testing is needed after weeks of pharyngitis infection, unknown pathology of joint pains, no history of antecedent pharyngitis; in these scenarios increase in ASO titres would aid as an evidence of GABHS. The chances of detection of antibody response to Streptococcus pyogenes is high on repeated investigating the ASO titres at weekly intervals.

Isolation of GAS is uncommon, so physicians prefer Anti Streptolysin O titres even though it is influenced by age, environmental factors etc.[7] Wide range of values were observed depending on the geographical area, there is a necessity to know the upper limit of normal population and also it has to be observed periodically for may change in the value to higher or lower side.

Diagnosis of Streptococcal infections can be done by throat swab culture, other specimens culture, antigen detection tests and antibody detection tests. Culture of Streptococcus pyogenes is a gold standard investigation, need an experienced microbiologist doctor to diagnose the pathogen accurately which can avoid false positive and false negative results. False positive happens due to misdiagnosis of pathogen and false negative occurs due to inadequate specimen and patients on prior antibiotics. Disadvantage of culture is difficult in isolation in few cases and delay in culture result. Rapid antigen detection tests which works on enzyme immuno assay or agglutination test principle helps to give the result in a little time, this test also have excellent

specificity of more than 95 % in relation to culture. Even though this is an expensive, the advantage projects in terms of timely treatment to patient. Antibodies against anti DNAase or Anti Streptolysin O also aids in the detection of Streptococcus pyogenes by slide agglutination test method. ASO test is useful for acute rheumatic fever diagnosis, whereas Anti DNAase test is helpful in the diagnosis of Skin related Streptococcus pyogenes infections. Streptozyme test is a slide agglutination test used to detect antibodies against five different group A streptococcal antigens including ASO, Anti DNAase, anti-hyaluronidase. This test has higher sensitivity and specificity when compared to doing the test like ASO alone.

In this study majority of the patients were observed in the the age group of 11 - 15 years i.e., 104 out of 200 (52 %) followed by 5 - 10 years makes up to 41 %. Male preponderance was noted which accounts for 56.5 % of total cases. Khaled AA et al[8] selected paediatric patients randomly to assess the ULN in their community. They have noted that the subjects were symmetrically distributed in all age groups. 19.3 % were in age group 15 years, 13.9 % in age group 5 - 6 years, followed by 16.3 % in age group 7 - 8 years. Age group 9 - 10 years and 11 - 12 years were 16.8 % and age group 13 - 14 years was 16.8 %. Alexandre B Merlini et al[9] demonstrated the quantitative and qualitative assessment of ASO serology test association with the GABHS presence in culture and noted that ASO has best accuracy at 200 U Todd and demonstrated 100 % sensitivity and 80 % specificity in the GAS infection documentation.

Out of 200 patients, 101 (50.5 %) didn't show ASO positivity and remaining 99 (49.5 %) patients showed ASO positivity i.e., > 200 IU / mL of ASO as per this study. Seema Solanki et al[10] did a study on seroprevalence of ASO titres in children aged between 5 - 18 years in Himachal Pradesh, observed a total of 664 samples, among them 237 (35.6 %) were found to be positive for ASO titres. Kesavan S et al[11] did a study on Anti Streptolysin O titres on upper limit of normal population in relation to age and socio economic status in Tamilnadu. They observed 12.5 % of children had 201 - 250 IU / mL ASO titres, 4.5 % children had titres between 251 to 300 IU / mL and 9.5 % of children had > 300 IU / mL. They also concluded that there is no statistical correlation between lower and higher socioeconomic status in relation to upper limit of normal population (ULN).

In the present study 62.6 % (62 out of 99) of positive cases were in the age group of 11 - 15 years, 35.3 % (35 / 99) of positivity shown in 5-10 years age group and 2.02 % (2 / 99) of ASO positivity were in the age group of 16 - 18 years. Our study is supported by Seema Solanki et al[9] stated that 37.9 % were ASO positives in 5 - 9 years of age group, 54.8 % were showed ASO of > 200 IU / mL in 10 - 14 years age group and 7.1 % were noted to have ASO titres of above 200 IU / mL in 15 - 18 years of age. 54.8 % and 45.1 % of males and females respectively had high ASO titres. It has been clearly depicted that males and the age group of 10-15 years population are highly exposed to external environment, those are likely to get infected by Streptococcus pyogenes. Danchin MH et al[12] observed that 6 to 9 years of age had more Anti Streptolysin O titres when compared to 10 to 14 years age group patients. Renneberg J et al[13] also noted 9 to 12 years aged children have higher values of ASO titres than other age groups. Sunil Sethi et al[14] showed 11 to 15 years having more ASO titres than 5 to 19 years children. Rajendra Kumar conducted a study on ASO titres of paediatric population, observed 2.04 % positivity in 5 - 10 years and 15.6 % positivity in 11 - 15 years age groups. The overall positivity rate was 9 % which is lesser than our study, this variation might be due to geographical variation. They found statistically significant association of raised Anti streptolysin O titres in overcrowded and not over crowded population. Most common presentation of Streptococcus pyogenes infections occurs in 5 - 15 years age group children, the reasons behind maybe poor hygiene, vulnerability and over-crowded classrooms.

Mandor Baki et al investigated the prevalence of ASO titres and serogroups of bet haemolytic Streptococci in the throat of apparently health children in Calabar, Nigeria. A total of 437 patients specimens were examined for Streptococcus species isolation and ASO titres. They observed ASO titre of 400 IU / mL were most frequently observed levels among 3 - 15 years of age group children, which makes up to 42.5 % of subjects. Among the serogroups of beta haemolytic streptococci groups, Group C was associated with high ASO titres around 93 % of isolates. The mean ASO titres for test group and control group population were noted as 902 IU / mL and 929 IU / mL respectively. Statistical significance was associated ($r = 0.5$, $p = 0.000$) between the presence of Streptococci in the throats and high Anti Streptolysin O titres in the sera of subjects.

Usually Streptococcal pharyngitis resolves on its own, but early initiation of antibiotic therapy hastens the clinical recovery by 12 - 24 hours. Main beneficial reason for early and appropriate antibiotic therapy for streptococcal infections during the earliest diagnosis is to prevent the occurrence of acute rheumatic fever, which may lead to long term complications such as acute rheumatic diseases and acute glomerulonephritis. This ASO antibodies can help to start treatment immediately without waiting for streptococcal isolation in culture. Along with clinical inspection taking an appropriate or relevant past history of fever and sore throat attacks, household contact with documented evidence of streptococcal pharyngitis helps us to arrive at diagnosis.

Appropriate start of antibiotic therapy before the nineth day of streptococcal pharyngitis is most helpful for prevention of acute rheumatic fever. Penicillin V in a dose of 250 mg bid or tid for 10 days for children and 250 to 500 mg per dose for adults is the drug of choice, even oral amoxicillin and benzathine penicillin or benzathine - procaine penicillin G combinations can also be used. Erythromycin is the drug of choice for patients allergic to beta lactam antibiotics. Clindamycin of 20 mg / kg per day divided into 3 doses is the most effective treatment regimen for eradication of Streptococcal carriage.

Primary prevention of GABHS is by avoiding initial attacks especially in paediatric population and during intial attacks appropriate antibiotic therapy is need to be initiated before the ninth day of symptoms of GABHS infection.[15] Secondary prevention for patients who are already suffering from

GABHS infection, require antibiotic prophylaxis as soon as possible. For people who are at high risk of having carditis with recurrences should receive antibiotic prophylaxis well into adulthood and perhaps for life. Patients who have relatively low risk of carditis with recurrences antibiotic may be discontinued at early 20s and after at least 5 years have elapsed since their last episode of Acute rheumatic fever.

CONCLUSIONS

Age group of 11 - 15 years children had high seropositivity. Male predominance was noted. Anti-Streptolysin O reports limitations are false positive or false negative reports and need to know the ULN of community for reporting as positive. If we need to clarification of titres while investigating a patient sample, the same need to be confirmed by clinician before releasing the report. Clinician suggestions helps to diagnose in indeterminate cases. Difficult to isolate the Group A Streptococci pathogen sometimes and also it can be present as a carrier in few individuals. However, increase in ASO titres indicate recent infection, which can be tested easily and timely report. On basis of these many hospitals are choosing ASO test as a priority investigation to diagnose GAS infections.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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