Original Research Article

Anti streptolysin O (ASO) titers in normal healthy children aged between 5 to 15 years in Ujjain region

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Abstract

Introduction: Rheumatic fever is an inflammatory disease that may develop after an infection with *Streptococcus pyogenes* bacteria, believed to be caused by antibody cross reactivity that can involve heart, joints, skin and brain. Measurement of Anti Streptolysin O (ASO) antibodies to specific streptococcal antigens is therefore necessary for the diagnosis of the preceding Group A Streptococcal (GAS) infection**. Aims and objectives:** To determine the upper limit of the normal ASO titers in normal healthy school going children aged 5-15 years and to determined a baseline value to compare with when a single ASO titer is available. **Material and method**: A community based cross sectional study was done on normal healthy children aged 5-15 years divided into group 1 (5-10 years) and group 2 (11-15 years) after taking informed consent from parents. Blood sample were collected after thorough sterilization of the area. ASO testing from serum sample was done using TURBILYTE Antistreptolysin 'O' diagnostic reagent for quantitative in-vitro determination of ASO in serum on photometric systems. **Results:** Out of total 200 children included in the study, 100 were in group 1 and 100 in group 2, 118 (59%) were males and 82 (41%) were females. The mean ASO titer for group 1 was 105.69 IU and that of group 2 was 144.73 IU with a standard deviation of 3.675 and 5.823 respectively (*P* value <0.05). Out of total 200 students 160 were living in overcrowded conditions. The mean ASO titer of children with overcrowded living conditions was 131.41 IU with a standard deviation of 53.472, and those with non-overcrowded living condition was 100.42 IU with a standard deviation of 39.30 (*P* value < 0.05). **Conclusion**: The upper limit of normal (ULN) was greater in the children of Group 1 (*P* value <0.05, 200 Vs 135). No statistically significant difference was found in the ASO titer according to gender. Statistically significant high mean ASO titer (131.41 IU) was found in children living in overcrowded conditions as compared to those living under non-overcrowded conditions (100.41 IU).

Keywords: Antistreptolysin O, Streptococcus pyogenes, Rheumatic fever, Upper limit of normal

Introduction

Streptococcus pyogenes or Lancefield Group A beta-hemolytic streptococcus (GAS), is one of the commonest bacterial pathogens that causes acute pharyngitis among school-aged children living in lower socioeconomic conditions [1]. Acute rheumatic fever is an inflammatory disease of the heart, joints, central nervous system subcutaneous tissues that develops after a nasopharyngeal infection by one of the group A beta hemolytic streptococci [2]. Rheumatic heart disease (RHD) is the second most frequent form of acquired heart disease in children worldwide and almost all cases and deaths occur in developing countries [3]. Group A streptococcal (GAS) infection and their sequelae like acute rheumatic fever and RHD are important and major health problem in India [4]. An absolute requirement for the diagnosis of acute rheumatic fever is supporting evidence of a recent GAS infection. One third of patients with acute rheumatic fever have no history of an antecedent pharyngitis.

Therefore, evidence of an antecedent GAS infection is usually based on elevated or increasing serum Anti streptococcal antibody (ASO) titers [5]. The antibody

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produced by the human host against this toxin, ASO, is the most widely used and the most standardized of the group A streptococcal antibody tests available [6]. ASO titer has been shown to vary with geographical location, age, season and site of infection. Hence, when ASO titer from single specimen is available it is compared with the predetermined baseline values in a given geographical area [7]. Rapid, quantitative turbidimetric immunoassay for serum ASO has proven to be superior to other available methods for measuring serum ASO [8].

The incidence of rheumatic fever varies from 0.2 -0.75 per 1000 children of 5-15 years age group whereas in India it is estimated to be 1-5.04 per 1000 cases [4]. Such data have emphasized the importance of accurate clinical diagnosis, often requiring laboratory confirmation of preceding GAS infection. It is not always possible to obtain clinical history or to recover the organism moreover it is not feasible to obtain acute and convalescent sera. Positive throat culture are obtained only in about 11% at the time of presentation of acute rheumatic fever, moreover mere presence of organism in the throat can also indicate carrier state which is seen in 2.5-35.4% of the individuals. In such cases the presence of a host immune response is the only evidence of the recent infection that remains. Measurement of antibodies to specific streptococcal antigens is therefore necessary for the diagnosis of the preceding GAS infection. ASO levels rise rapidly after about 3-4 weeks post streptococcal infection and remains elevated for months. This study was planned to determine the upper limit of the normal (ULN) of the ASO titers in normal healthy school going children aged 5-15 years; to determine the role of ASO titers in the early diagnosis of antecedent infections caused by group A beta hemolytic streptococcus like acute rheumatic fever and post streptococcal glomerulo-nephritis and to determined a baseline value to compare with when a single ASO titer is available.

Material and Methods

This community based cross sectional study was done involving the students of residential school Palwa and Balakheda, District Ujjain, Madhya Pradesh during The period of November 2012 to February 2013 after written informed consent was obtained from the principal of the schools and from the parents of children. The data was collected in a predesigned and pretested proforma and contained epidemiological and clinical determinants required according to the objectives of the study. Normal healthy children belonging to 5-15 years of age group were included in the study. Children with recent history of sore throat, fever, dark colored urine, rash, joint pains were excluded.

For ASO testing, collection of blood was done under aseptic precautions. Two ml of blood was collected and serum separated after allowing the blood to clot. The test was done by using TURBILYTE Antistreptolysin 'O' diagnostic reagent – for quantitative in vitro determination of ASO in serum on photometric systems. The ASO kit was manufactured by TULIP Diagnostic Systems VOLMOLENHEIDE 13 B-2400 MOL Belgium. Data was entered in Epi Data 3.1 software and appropriate statistical methods were used for data analysis using STATA 10.0 (Stata Corp., College Station, TX, USA) statistical software.

The independent sample t-test was used for comparison of continuous (numerical) variables, after checking for a normal distribution. The Chi-square test was used for comparison of categorical values, P-value <0.05 were considered significant.

Prior to conduction of this study, ethical approval was obtained from Institutional and Ethics Committee, R D gardi Medical College Ujjain.

Results

A total of 200 students were studied and divided into two age groups, group 1 (5-10 years) and group 2 (11-15 years) each group having 100 children. A total of 118 (59%) students were males and 82 (41%) were females. The group wise distribution of sex showed that 49 (41.5%) males belonged to age group 1 and 69 (58.5%) males belonged to age group 2; while 51 (62.2%) females belonged to age group 1, 31 (37.8%) females belonged to age group 2 (Table 1 and Figure 1). Children living in over crowded house were 160 (80%). Out of these 64 (40%) children belonged to age group 1 and 96 (60%) children belonged to age group 2. The mean ASO titer for group 1 was 105.69 and that of group 2 was 144.73 with a standard deviation of 3.675 and 5.823, respectively. There was a significant difference in the two groups (*P* value <0.05). Maximum percentage of the population in both the sexes were having ASO titers on the lower side (less than 150 IU) and almost equal percentage of the population in both the sexes had ASO titers on the higher side (More than 200 IU) (Table 2). The mean ASO titer of those having over crowded living condition was 131.41 with a standard deviation of

53.472, and those with non-overcrowded living condition was 100.42 with a standard deviation of 39.30. The difference was statistically significant ($P< 0.05$) (Table 4).

Table-1: Shows the distribution of ASO titers in 200 children distributed in two groups (n=100), group 1 aged 5 to 10 years and group 2 aged 11 to 15 years.

ASO titers (IU)	Group 1(5-10 years)	Group 2(11-15 years)	Total (n= 200)
<100	59(69.4%)	26(30.6%)	85(42.5%)
100-124	12(52.1%)	11(47.9%)	23(11.5%)
125-149	12(54.5%)	10(45.5%)	22(11%)
150-174	13(43.3%)	17(56.7%)	30(15%)
175-199	1(6.2%)	15(93.8%)	16(8%)
200-224	2(12.5%)	14(87.5%)	16(8%)
224-250	1(12.5%)	7(87.5%)	8(4%)
Total	**100**	**100**	**200**

Table-2: Shows the distribution of ASO titers in 200 children distributed according to male and female sex.

	Sex		Total
ASO titer (IU)	Male	Female	
<100	41(48.2%)	44(51.8%)	85(42.5%)
100-124	16(69.5%)	7(30.5%)	23(11.5%)
125-149	17(77.2%)	5(22.8%)	22(11%)
150-174	24(80%)	6(20%)	30(15%)
175-199	8(50%)	8(50%)	16(8%)
200-224	7(43.7%)	9(56.3%)	16(8%)
225-250	5(62.5%)	3(37.5%)	8(4%)
Total	**118 (59%)**	**82 (41%)**	**200 (100%)**

Table-3: Shows the distribution of ASO titers in 200 children distributed according to presence or absence of overcrowding.

ASO titer	Living condition		
	Overcrowding	No overcrowding	Total (n=200)
<100	58(68.2%)	27(31.2%)	85(42.5%)
100-124	18(78.2%)	5(21.2%)	23(11.5%)
125-149	19(86.3%)	3(13.7%)	22(11%)
150-174	27(90%)	3(10%)	30(15%)
175-199	16(100%)	0(0%)	16(8%)
200-224	14(87.5%)	2(12.5%)	16(8%)
225-250	8(100%)	0(0%)	8(4%)
Total	**160 (80%)**	**40 (20%)**	**200(100%)**

Table-4: Shows the distribution of mean values, standard deviation and standard error of ASO titers in 200 children distributed according to presence or absence of overcrowding.

	Living	n=200	Mean	Std. Deviation	Std. Error	T	P
ASO	Overcrowding	160	131.41	53.472	4.227	3.43	0.001
	No overcrowding	40	100.42	39.300	6.214		

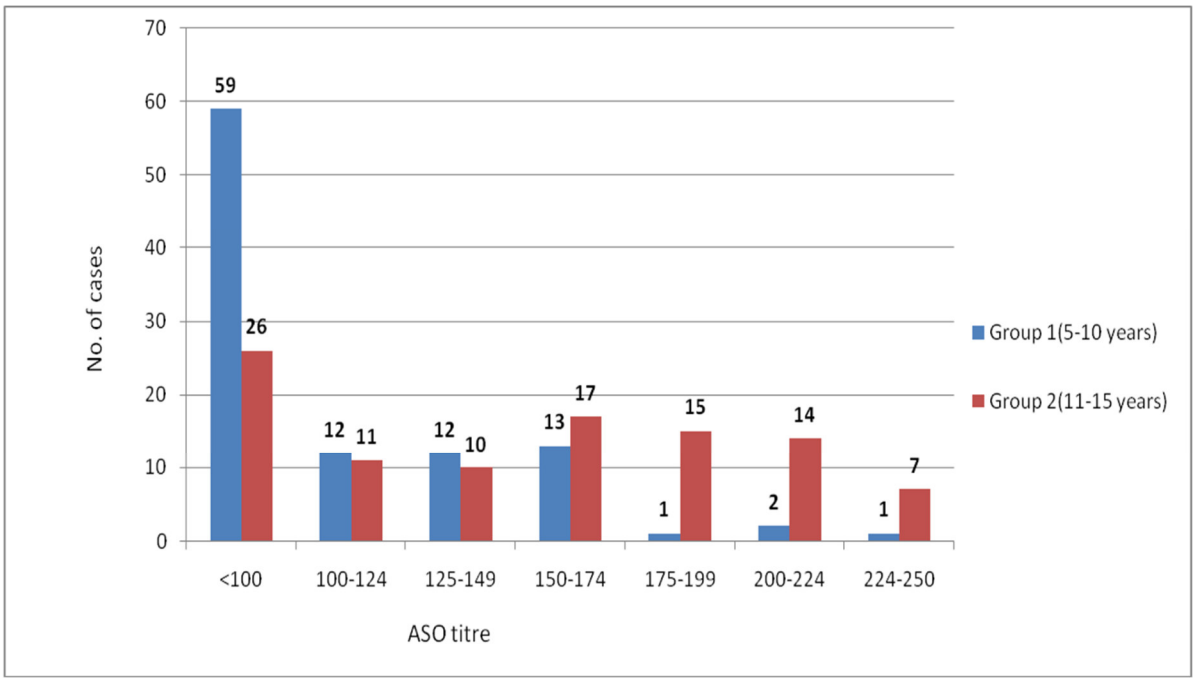

Figure-1: Bar chart showing number of children belonging to the two age groups distributed according to the ASO titers.

Discussion

An absolute requirement for the diagnosis of acute rheumatic fever is the supporting evidence of group A streptococcal infection. Evidence of antecedent group A streptococcal infection is usually based on elevated or rising serum ASO antibody titers.

Antibody titers may be elevated in the absence of clinical or bacteriological evidence of streptococcal pharyngitis. The ASO titer is the most popular antibody test. The normal level of ASO titer is defined as the highest titer exceeded by only 20% of the population. ULN is defined as that titer exceeded by 20 percent of a normal population [8]. The geometric mean titer and ULN for the ASO for the western world was determined to be 120 IU [9]. It is influenced significantly by age, geography, season and other factors. ASO titers of more than 333 are generally considered elevated in children however this was found to be 170 IU in our study. The geometric mean titer (GMT) and upper limit of normal (ULN) in our study group was greater in the children of age group 5 – 10 years (which was statistically highly significant, i.e. p value <0.05, 200 vs. 135) than the standard ASO titer quoted -250 IU for the diagnosis of acute rheumatic fever. The ULN of ASO titers in Mysore is determined to be 242 IU [7], 239 IU in Chandigarh and 305 IU in Mumbai [10]. Our study group did not have any history of repeated sore throat infections and fever. No difference was found in the ASO titer according to gender. The mean value of ASO titers in males was 128.08 IU and in the females was 121.07 IU and the difference was not statistically significant ($p = 0.353$).

Our study also showed a high mean ASO titer (131.41 IU) in children living in overcrowded conditions as compared to those living under normal conditions (100.41 IU) and this difference was statistically significant ($p < 0.05$).

Conclusion

Having established the upper limit of normal in school age children in our population, we can consider this as the baseline ASO titer (170 IU). This would help in interpreting the ASO titer in suspected acute rheumatic fever patients in population of Ujjain region. This value of 170 IU is representative of our school going population and would immensely help the Pediatricians, Epidemiologists and the Microbiologists to interpret streptococcal antibody titer correctly.

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