

TO STUDY THE UPPER LIMIT OF NORMAL ANTISTREPTOLYSIN-O TITRE IN HEALTHY SCHOOL CHILDREN AGED 5 TO 15 YEARS IN DISTRICT KANGRA, HIMACHAL PRADESH

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ABSTRACT

BACKGROUND

It is necessary to know the upper limit of normal value of ASO titre because one cannot wait for several weeks for rise of antibody titre in a patient to obtain accurate diagnosis and prescribe treatment, especially on the first visit of patient. We wanted to determine upper limit of normal of ASO titre in healthy school going children aged 5-15 years of Distt. Kangra, H. P.

METHODS

ASO titre was measured through latex agglutination test which is a qualitative cum semi-quantitative test.

RESULTS

In the age group of 5-7 years, only 3 (10.3%) out of 29 children had ASO titres more than 200 IU. The mean titre in this age group was 233.33 ± 14.434 IU. In the age group of 8-10 years, 5 (9.2%) out of 54 children had ASO titre of more than 200 IU and the mean ASO titre in children in this age was 370.00 ± 253.969 IU. In the next age group of 11-13 years, 20 (8.9%) out of 224 children had ASO more than 200 IU. The mean ASO titre in this age group was found to be 293.75 ± 90.276 IU. In children in the age group of 14-15 years, 13 (14.0%) out of 93 children had ASO titre of more than 200 IU and the mean value of ASO titre in this age group was 386.54 ± 190.015 . The overall mean ASO came out to be 328.05 IU. Difference of ASO titres between male and female children was not statistically significant. The mean ASO titre came out to be 328.05 IU in District Kangra, Himachal Pradesh.

CONCLUSIONS

This value will guide clinicians when they will consider the diagnosis of post streptococcal diseases in patients and will provide useful baseline data for future studies of intervention against group A streptococcal diseases in distt. Kangra.

KEY WORDS

ASO, Antistreptolysin O, Upper Normal Limit, Streptococcus, Rheumatic Heart Disease

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BACKGROUND

Streptococci are Gram positive aerobic organisms that cause many diseases like pharyngitis, pneumonia, wound infections and endocarditis. Symptoms vary with system involved and Non-suppurative complications occur in the form of chorea, Rheumatic heart disease, myalgia and arthritis. In acute phase, isolation of group A beta haemolytic streptococci is uncommon (<15%)¹ and confirmation of diagnosis rationally will depend upon two-fold rise of Antistreptolysin O titre from acute to convalescent phase which spans about 14 to 28 days. The importance of serial estimation of ASO titre is undermined by the fact that it is not always possible and practicable to obtain second blood sample particularly in the developing countries where the prevalence of RHD is high.

Therefore, it was suggested that only a single value of ASO titre more than upper limit of normal at initial testing can be considered a presumptive evidence of preceding streptococcal infection.

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The various factors affecting ASO titre in a given population are age, site of infection, geographical area, season of the year and use antibiotics and steroids. In western literature, the cut-off point of ASO titre are 240 IU,² 320 IU,³ 200 IU⁴ and 326 IU⁵ in USA, Australia, Sweden and Korea respectively. These values are not relevant to developing countries like India due to higher incidence of streptococcal infections, geographical variations and breakdown seasonality. So, it is prudent to suggest that every region should have its own upper limit of normal value of ASO titre in relation to local epidemiology. Keeping in view the above considerations, this study was designed to determine the upper limit of normal of ASO titre in district Kangra of Himachal Pradesh.

The word streptococcus is derived from Greek word where 'strepto' means chain and 'coccus' means berry. Although, knowledge of streptococcal diseases was known to mankind for centuries but original writings of Hippocrates, in 4th century, described erysipelas which was considered as streptococcal infection in a retrospective way. Later on Galen described streptococcus as a causative organism for uterine infections.⁶ After 16th century Adam narrated individual entity of streptococcus which was later on established by Strother in 1716 in the form of epidemics of puerperal fever and childhood fever as a major cause of mortality.⁷

The graphic representation of streptococcus was discovered with the invention of microscope by Antonie Von Leeuwenhoek (1632-1723) in eighteenth century where different shapes of streptococci were described.⁸ For almost two centuries, the link between discovery of microscopic organisms and the disease caused by them was not found. Ancient scholars wrote about sore throat, but did not describe any rash which was typical of scarlet fever.

In 17th century, Sydenham, distinguished scarlet fever from other febrile rash like illness.⁹ In 1834, Theodor Billroth first gave the description of streptococci as arranged in pairs, sometimes in chains of four to twenty.¹⁰

First time in 1879, Louis Pasteur established that streptococcus was the organism responsible for puerperal sepsis and he isolated streptococcus from blood and uterus of females suffering from puerperal sepsis.¹¹ Rosenbach in 1884 isolated the organisms from suppurative lesions and he named the species as *Streptococcus pyogenes*.¹² Differentiation of streptococci was done in 1903 by Schottmuller into haemolytic and viridans group. Further elaboration was done in 1919 by Brown into Alpha, Beta and Gamma haemolytic streptococci.¹³ Based upon differences in surface antigen, Lancefield in 1933 further subdivided streptococci into groups designated by the letters A through X. The Group A strains, *Streptococcus pyogenes*, were further subdivided according to the presence of a surface protein named M protein (Due to its matte appearance in colony formations) into different antigenic types.¹⁴

Group A streptococci produce a wide variety of secreted extracellular products namely streptolysin O, streptolysin S, streptokinase, proteinase, esterase, CAMP factor, DNase, hyaluronidase, complement inhibitor, superoxide dismutase and immunoglobulin degrading enzymes which in return determine the virulence of this organism.(Figure 1)

Keeping in view the virulence of *Streptococcus*, different tests to diagnose streptococcal infections such as ASO, Anti-DNase, Antihyaluronidase and Streptozyme tests were discovered. The diagnosis of poststreptococcal diseases can be aided by the detection of streptococcal antibodies. Detection of these antibodies is not useful in acute infections, since antibody development takes about one to two weeks after the onset of acute infection to be detectable in serum samples. Streptolysin O is an oxygen-labile haemolysin that is one of a variety of extracellular products elaborated by group A streptococci. Streptolysin O is a pore-forming, cholesterol-dependent, oxygen-labile cytotoxin.¹⁵

Similar types of haemolysins are produced by a variety of other pathogens, and the structure of Streptolysin O is similar to these other cholesterol- dependent cytolysins, but there are also some differences. The difference is in the binding of the cytolysins to cholesterol-rich membranes, where there is a structural difference in the membrane-binding interface.¹⁶ The Streptolysin O haemolysin is 69 kDa in size, which can be cleaved by the cysteine proteinase.¹⁷ The haemolysin is produced with a residue that is required for the translocation of another streptococcal product, the NAD-glycohydrolase into host cells.¹⁸ Streptolysin O pore formation occurs in many stages, including cholesterol-dependent binding to the cell membrane, later followed by oligomerization, which causes formation of pores.¹⁹ These pores cause the disruption of the host cell membranes and further lead to apoptosis.

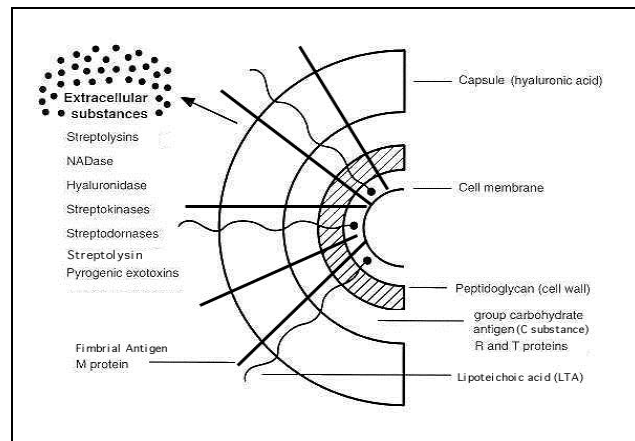


Figure 1. Structure and Extracellular Products of *S. pyogenes*

Sl. No.	Place	ULN of ASO	
1.	Minnesota, USA	333 Todd units	Wannamaker et al ²⁰
2.	USA	240 IU	Kaplan et al ²
3.	Korea	326 IU	Kim et al ⁵
4.	Yemen	276.2 IU	Khaled et al ²¹
5.	Fiji	276 IU	Steer et al ²²
6.	Australia	320 IU	Danchin et al ³
7.	Sweden	200 Todd Units	Renneberg et al ⁴
8.	Egypt	400 IU	Kotby et al ²³
9.	Chandigarh, India	239 IU	Sethi et al ²⁴
10.	Mysore, India	242 IU	Mehdrappa et al ²⁵
11.	Mumbai, India	305 IU	Karmarkar et al ²⁶

Table I. ULN of ASO of Different Geographical Areas

Age Group (Years)	Mean ASO Titres ± SD		
	Male	Female	Overall Mean
5 - 7	225.00 ± 0.000	237.50 ± 17.678	233.33 ± 14.434
8 - 10	216.67 ± 28.868	600.00 ± 282.843	370.00 ± 253.969
11 - 13	285.71 ± 98.802	298.08 ± 89.245	293.75 ± 90.276
14-15	493.75 ± 139.007	338.89 ± 196.497	386.54 ± 190.015
Total	323.33 ± 143.448	330.77 ± 162.682	328.05 ± 154.130

Table II. Mean ASO Titres Among Children in Different Age Groups (n=400)

The most widely used serologic test for diagnosis of streptococcal infection is ASO test.

In 1932 E. William Todd demonstrated the presence of ASO antibodies in the sera of patients with various streptococcal infections by neutralizing streptolysin O with serial amounts of sera of patients. The excess un-neutralized streptolysin O was then revealed by adding RBC to the system as an indicator. The end point was the highest dilution of serum having no haemolysis, with the ASO titre expressed as Todd units, which are equivalent to the reciprocal of dilution.²⁷

Different methods have been employed for detection of ASO titre. They are Haemolysin inhibition method, Latex agglutination method, Turbidimetry and Nephelometry.³ Latex Agglutination works on principle that latex particles coated with streptolysin O react with ASO antibodies in serum. Agglutination occurs when level of antibody is more than 200 IU. Its advantage is that it is a rapid test and disadvantage is that it is qualitative and semi-quantitative test. The positive ASO titre indicate recent infection with Group A, C, G streptococci. It is positive in 80-85% of infections, so negative test does not necessarily rule out infection. False positive ASO titre can be seen in lipemic samples, bacterial contaminated samples, aspecific antibodies in healthy subjects, infection with Lancefield group C & G *Streptococcus*, Tuberculosis, active viral hepatitis and monoclonal gammopathy. False negative ASO can be seen in

samples collected during latency period, samples collected late in convalescence, post-pyoderma, poststreptococcal glomerulonephritis, corticosteroids and other immunosuppressants administration and early administration of antibiotics.²⁸ ASO titre may be negative in 20% of population with acute rheumatic fever. A negative ASO titre should prompt testing for other antibodies like AntiDNase B and antihyaluronidase which lasts 6-9 months after infection. If the estimation of two antibodies is combined ninety percent of the patients will demonstrate evidence of streptococcal infections and if three antibodies are combined ninety five percent of recent streptococcal infections can be diagnosed.

The clinical application of ASO titre in diagnosing acute rheumatic fever was suggested by T. Duckett Jones in 1944, when he published original Jones's criteria. In original Jones criteria of 1944, he did not include evidence of preceding streptococcal infection as a criterion to diagnose acute rheumatic fever, but he suggested a link between raised ASO titre and acute rheumatic fever. In 1955 modified Jones criteria was given which considered evidence of preceding streptococcal infection as a 'minor criteria'. Revised Jones criteria came in 1965 which included evidence of preceding streptococcal infection as an 'essential criteria'. In 1992 apart from ASO test, Anti DNase and Antihyaluronidase tests were introduced as newer diagnostic tools.²⁹

In 2015 recent revision was made in Jones criteria which defined high risk population for acute rheumatic fever and included Doppler echocardiography as a tool to diagnose cardiac involvement in acute rheumatic fever. As per 2015 Jones criteria rising ASO titre still remains an essential criterion to diagnose acute rheumatic fever.³⁰ Along with this raised ASO titre has diagnostic implication in AGN and PANDAS.³¹

As already mentioned, the value of ASO titre varies with age, sex, region, season and use of antibiotics and steroids. So, it has been suggested that every region should have its own value of upper limit of normal of ASO titre. In literature different authors studied the upper normal limit of ASO titre in different geographical areas. The titre varied from 200 IU to 400 IU as is evident from Table I.

METHODS

The study was conducted in schools in District Kangra, Himachal Pradesh. Kangra is the largest district of Himachal Pradesh. The study was conducted for a period of one year after approval from Protocol Review Committee and Institutional Ethics Committee. This is a cross sectional analytical study. A sample size of 400 was selected for the purpose of study. Sample size was calculated using Epi Info 7 software, assuming expected frequency (Response distribution) 50%, confidence limits 5% and confidence level 95%.

All the government high schools and senior secondary schools located in district Kangra were enlisted. Schools were then selected randomly from the enlisted schools. A pre - designed and pre - tested structured questionnaire was used to collect demographic characteristics, clinical history and clinical examination of the study subjects.

Children fulfilling inclusion criteria and who were willing to participate in the study and willing for providing blood sample were included in the study. Clinical examination of

the child was conducted, and 2 ml blood sample was collected by venipuncture. Serum was separated and stored at - 20° till further use.

The ASO Latex test contains polystyrene latex particles, coated with purified and stabilized streptolysin - O (Antigen) which reacts with its corresponding Antistreptolysin - O (Antibody) in the test sample resulting in the agglutination of latex particles. The kit used was manufactured by Beacon Diagnostics Pvt. Ltd.

This is a qualitative cum semi-quantitative test. Agglutination in the highest serum dilution corresponds to the approximate amount of ASO concentration in IU/ml in test serum. The serum samples which had ASO titre more than 200 IU gave positive result by showing agglutination, and only these positive samples were further quantitatively evaluated.

Statistical Analysis

Data were analysed for percentages, arithmetic means, standard deviations and medians. Chi square test was used to compare categorical variables and independent student's t - test was used to compare continuous variables.

Ethical Considerations

Investigator and supervisors were well aware of the guidelines for ethics in biomedical research by ICMR (1994) and Helsinki Declaration (Modified 2000) and the policy of Institutional Ethics Committee of Dr. RPGMC Tanda. The following points were followed in all patients enrolled in the study-

1. The children and their parents/ guardians included in the study were informed participants. The procedure of blood sample collection was explained to them in detail before enrolling them in the study. Written consent was taken from parents/ guardians of all the children participating in the study in their local language.
2. Every precaution was taken to respect the privacy, confidentiality of the patient and to minimize the impact of the study on his/her physical and mental integrity.
3. The reports of ASO titres were provided to the parents/ guardians of the children.

RESULTS

In the age group of 5-7 years only 3 (10.3%) out of 29 children had ASO titres more than 200 IU. The mean titre in this age group was 233.33 ± 14.434 IU. In the age group of 8-10 years, 5 (9.2%) out of 54 children had ASO titre more than 200 IU and the mean ASO titre in children in this age was 370.00 ± 253.969 IU. In the next age group of 11-13 years, 20 (8.9%) out of 224 children had ASO more than 200 IU. The mean ASO titre in this age group was found to be 293.75 ± 90.276 IU. In children in the age group of 14-15 years, 13 (14.0%) out of 93 children had ASO more than 200IU and the mean value of ASO titre in this age group was 386.54 ± 190.015 . The overall mean ASO came out to be 328.05 IU. Difference of ASO titres between male and female children was not statistically significant. The mean ASO titre came out to be 328.05 IU in District Kangra, Himachal Pradesh. (Table II).

DISCUSSION

The sex distribution in these enrolled students was almost equal in order to avoid bias in results. There were 53% females and 47% males with a female to male ratio of 1.12: 1.

In our study the overall mean ASO was higher in females with a value of 330 IU as compared to 323 IU in males. Similar association was found by Khaled AA and Hassan AA in their study where slightly higher levels of ASO were reported in females (258 IU) as compared to males (252 IU).²⁶ The upper limit of normal of ASO in our study came out to be 328 IU which is higher than those reported from other parts of India.

In our study four children had ASO titres more than 450 IU. Although these children were asymptomatic and were not having any history of sore throat and skin infection in past 4 weeks, but still they had raised ASO titres. Possible explanation for this could be that these children might have suffered from streptococcal sore throat few months back and now their ASO titres were in a process to return to baseline, as the time period for returning ASO level to normal after streptococcal sore throat is not clearly defined in literature.

The latex agglutination technique used in our study is a qualitative and semi-quantitative test. This technique for determining ASO titres gives only those values which are more than 200 IU. In our study only 41 children out of 400, had ASO titres more than 200 IU. Hence, only these values were available for the calculation of mean and ULN of ASO.

The potential limitation of subject selection and testing technique has been highlighted, but in the absence of other local data this study may provide a useful guidance.

Abbreviations

1. ASO - Antistreptolysin O
2. PANDAS - Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus.
3. RHD - Rheumatic Heart Disease.
4. IU - International Unit
5. USA - United States of America
6. ULN - Upper Limit of Normal.
7. ICMR - Indian Council of Medical Research.

CONCLUSIONS

It is necessary to know the upper limit of normal value because one cannot wait several weeks for a rise of antibodies in a patient to obtain an accurate diagnosis and prescribe treatment, especially on the first visit of patient. This study provides the upper limit of normal value of ASO titre for children aged 5-15 years of district Kangra of Himachal Pradesh. This value will guide clinicians, when they will consider the diagnosis of post streptococcal diseases in patients and will provide useful baseline data for future studies of intervention against group A streptococcal diseases in district Kangra.

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