

Comparison of antistreptolysin O and anti-deoxyribonucleic B titers in healthy children to those with acute pharyngitis, acute rheumatic fever, and rheumatic heart disease aged 5–15 years

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ABSTRACT

Background : Acute rheumatic fever (ARF) affects millions of children in the third world countries like India. The diagnosis of rheumatic fever is based on the Jones criteria with serological titers, antistreptolysin O titer (ASO), and anti-deoxyribonucleic B (ADB), taken as evidence of recent streptococci infection. There is a lack of recent data available on ASO and ADB titers in children from the Delhi/NCR and thus adequate geographical area-specific cutoffs for the region are not available.

Aims and Objectives : The aim of this study is to determine and compare the ASO and ADB antibody titers in children with acute pharyngitis, ARF, rheumatic heart disease (RHD), and in healthy children of the Delhi/NCR region.

Materials and Methods : Twenty-six cases of ARF, 51 cases of RHD, 50 cases of acute pharyngitis, and 84 healthy normal children were included in the study. A single ASO and ADB titer measurement was done in these children.

Results : The ASO titers was raised in acute pharyngitis – 303 IU/ml (interquartile range [IQR], 142–520 IU/ml) and ARF – 347.5 IU/ml (IQR, 125–686 IU/ml) children in comparison to healthy controls – 163.5 IU/ml (IQR, 133–246.5 IU/ml) and RHD patients – 163 IU/ml (IQR, 98.250–324.500). The ADB titers were highest in ARF patients – 570.5 IU/ml (IQR, 276–922 IU/ml) followed with RHD – 205 IU/ml (IQR, 113.6–456.5), healthy controls – 78.25 IU/ml (IQR, 53.39–128.15 IU/ml), and acute pharyngitis – 75.12 IU/ml (IQR, 64.5–136 IU/ml). The upper limit of normal (ULN) values of ASO and ADB computed from normal healthy children were 262.4 IU/ml and 134.44 IU/ml, respectively, and these can be used as cutoff values for recent streptococcal infection in this geographical area.

Conclusions : The median ASO titers in acute pharyngitis group and ARF were significantly raised compared to that of the control group. The ADB titers were raised in ARF and RHD patients albeit the levels were higher in ARF patients. The derived ULN values can be used as cutoff reference.

Keywords : Acute pharyngitis, acute rheumatic fever, anti-deoxyribonuclease B, antistreptolysin O titer, Jones criteria, rheumatic heart disease, upper limit of normal

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INTRODUCTION

Acute rheumatic fever (ARF) and rheumatic heart disease (RHD) are the significant nonsuppurative late sequelae of Group A beta-hemolytic streptococci (GAS) infection.^[1] The incidence and prevalence of ARF and RHD still continue to be major health problem in developing countries.^[2] The WHO in a recent review on the global burden of GAS-related chronic diseases, estimated that a minimum of 15.6 million people had RHD with another 1.9 million having a history of ARF but no carditis, there were 4.7 lakh new cases of ARF and 282,000 new cases of RHD each year, and over 230,000 deaths directly related to RHD.^[3]

Pathogenesis relates to molecular mimicry between antibodies against antigens of GAS and specific human tissues.^[4] Serology is the most common test used for the confirmation of GAS infection, while establishing the diagnosis of ARF using Jones criteria. The most commonly commercially available antibody assays are ASO and ADB.^[1]

After infection, antistreptolysin O (ASO) titers begin to rise approximately by 1st week, peaks around 3rd–6th week, while anti-deoxyribonucleic B (ADB) titers are slightly late, begin to rise by 1st–2nd week and peaks by 6th–8th week. Elevated titers for both tests may persist for several months, even after uncomplicated GAS infections.^[1] The background distribution of these titers varies depending on the population, age group, geographic locations, site of infection, and seasonal variations.^[1,5] In underdeveloped countries, the high incidence of acute respiratory tract infections and impetigo subsequent to overcrowding, scabies and other skin diseases, poverty, and poor access to health care may also contribute to high background GAS antibody titers.^[6]

The upper limit of normal (ULN) for ASO and ADB titers were computed from data obtained from normal healthy children by separating the upper 20% from the lower 80% of the group distribution in a dichotomous fashion and determine the 80th percentile as the ULN for both streptococcal antibodies above which levels are considered positive for streptococcal infection.

There is no study on the ASO and ADB titers from Delhi NCR region, a major metropolis of human settlement in the northern part of India. Thus, we planned a study to determine and compare the ASO and ADB antibody titers in children with acute pharyngitis, ARF, and RHD on penicillin prophylaxis and in healthy children of the Delhi/NCR region and to evaluate their seasonal variations. We also aimed to determine ULN levels of ASO and ADB titers in normal healthy children aged 5–15 years of the Delhi/NCR region.

MATERIALS AND METHODS

This cross-sectional study was done from November 2015 to March 2017 in the Division of Pediatric Cardiology, Department of Pediatrics and Biochemistry, PGIMER, and associated Dr. RML Hospital, New Delhi.

A total of 211 children, aged 5–15 years were enrolled in the study and divided into four groups: Group A had 84 healthy controls (without a history or clinical evidence of throat infection 6–8 weeks before enrolment), Group B had 50 children with acute bacterial pharyngitis (clinically diagnosed bacterial throat infection by McIsaac scoring system), Group C had 26 children with ARF (based on the Jones criteria ACC/AHA 2015), and Group D had 51 children with RHD (clinical or subclinical mitral valve disease with or without aortic valve disease confirmed by echocardiography and on long-acting penicillin (LAP) prophylaxis every 3rd week for >1 year).

Exclusion criteria in the healthy group constituted children having a history of antibiotic treatment for any infection 4 weeks prior to enrollment and those with skin infections, while for RHD patients, a documented history of poor compliance to penicillin prophylaxis.

RESULTS

The median age of the study population was 10.4 ± 2.76 years; 94 males and 117 females with male: female ratio of 0.80, with most of the patients belonging to upper lower socioeconomic strata (modified Kuppuswamy scale). Most of the families had access to safe drinking water (64.45% using tap water) and service toilets (77.25%) with 73.93% of the cases having >4 family members per house.

ASO and ADB titers (median and interquartile range) in normal healthy control group were 163.5 IU/ml (133–246.5 IU/ml) and 78.25 IU/ml (53.39–128.15 IU/ml), respectively. In acute pharyngitis group, ASO and ADB titers were 303 IU/ml (142–520 IU/ml) and 75.12 IU/ml (64.5–136 IU/ml), respectively. ASO levels were significantly raised than that of the control group ($P < 0.001$). However, in ARF group, both ASO and ADB titers were 347.5 IU/ml (125–686 IU/ml) and 570.5 IU/ml (276–922 IU/ml), respectively, were significantly raised than that of the control group (for ASO, $P \leq 0.02$; ADB: $P < 0.0001$). In RHD group, ASO and ADB titers were 163 IU/ml (98.25–324.500) and 205 IU/ml (113.6–456.5), respectively. While ADB levels ($P < 0.0001$) were significantly raised than that of the control group, it was not the case with ASO levels ($P = 0.379$) [Table 1]. The ULN values of ASO and ADB computed from normal healthy children was 262.4 IU/ml and 134.44 IU/ml, respectively. ASO

positivity and ADB positivity were also computed to show significance of ULN value among each group [Table 2].

The receiver operating curve (ROC) of ASO in ARF shows that a cutoff of >297 IU/ml has a sensitivity of 53.8% and specificity of 95%, with positive predictive value of 77.628% and negative predictive value of 86.93%. For ADB in ARF, a cutoff of >216 IU/ml has sensitivity of 84.62% and specificity of 100% with positive predictive value of 100% and negative predictive value of 95.44%. Thus, ADB has a better diagnostic utility in ARF as compared to ASO titer. Similarly, in patients with RHD, ADB has an acceptable diagnostic utility. A cutoff >81 U/ml, has a sensitivity of 98% and a negative predictive value of 97.7845 and thus can be used for ruling out the disease with a fair degree of confidence. The ASO and ADB values have a suboptimal diagnostic value in patients with sore throat according to the ROC.

ASO and ADB titers did not reveal statistically significant seasonal variation among healthy children and those with acute pharyngitis and RHD. In ARF cases no significant seasonal variation was observed for ADB; however, ASO titers showed statistically significant variation. ASO titers in summers were seen to be far lower than that observed in other seasons in cases of ARF.

DISCUSSION

This study for the first time attempts to provide a geometric median and the ULN values for both ASO and ADB titers for the population inhabiting Delhi/NCR. Furthermore, it delineates sensitivity and specificity with positive predictive value and negative predictive value for ASO and ADB titers in ARF, RHD, and acute pharyngitis patients.

There are several factors that influence ASO and ADB titers and thus the ULN values such as age, geographical location, seasonal variation, site of infection, and antibiotic treatment.^[1] This is well evident by the reported values in different studies by Sethi *et al.*,^[7] Danchin *et al.*,^[8] Kotby *et al.*,^[9] and Delice *et al.*^[10] in Table 1.

The median value of ASO titer in normal healthy children in our study was higher 163.5 IU/ml (133–246.50 IU/ml) than that reported by Sethi *et al.*^[7] (111.63 IU/ml) and Danchin *et al.* (101 IU/ml).^[8] However, Kotby *et al.* reported higher values (245.09 IU/ml) than those observed by us.^[9]

There is a scarcity of literature on the ABD titers from India in healthy children. In normal healthy control group, median ADB titer was 78.25 IU/ml (53.395–128.150 IU/ml) in our study as against 123.6 IU/ml and 163 IU/ml, respectively, as reported by Delice *et al.*^[10] and Danchin *et al.*^[8]

ULN values has traditionally been used as a single estimated cutoff value for diagnosing recent streptococcal infection in ARF, which is late sequelae of Group A streptococcal infection a rising titer cannot be demonstrated for lack of an earlier estimation being done. ULN values of ASO and ABD in normal healthy children in our study population were 262 IU/ml and 134 IU/ml, respectively, and comparative values from other geographical areas are reported by Sethi *et al.*,^[7] Une *et al.*,^[11] Mahendrappa,^[12] and Karmarkar *et al.*^[13] [Table 2]. Lower values in our study again reflect the need to have local regional values for different populations.

We recommend that Indian clinicians use a upper limit-of-normal cutoff value for ASO and ABD titer in children aged 5–14 years (that is, the estimated 80% upper-limit values), rather than the cutoff values for subgroups for age, as recommended in other studies. This is because only minimal variability in the year-by-year values was found for children aged 5–14 years. The use of a single cutoff value for this age group also makes it far simpler for laboratory staff to report results and for clinicians to interpret them. Similar sentiments have been expressed by Steer *et al.* based on their study on Fiji islands.^[14]

The ASO response is generally brisk after a streptococcal upper respiratory tract infection but is relatively feeble after Group A streptococcal (GAS) impetigo or pyoderma. Unlike ASO, however, infection of the

Table 1: Comparison of Median Values in Normal Healthy Children among various study groups

Median value in normal healthy children	Our Study	Danchin <i>et al.</i> ^[8] (mean values)	Sethi <i>et al.</i> ^[7]	Kotby <i>et al.</i> ^[9]	Delice <i>et al.</i> ^[10]
ASO	163.5 IU/ml (133-246.50 IU/ml)	101 IU/ml	111.63 IU/ml	245.09 IU/ml	
ADB	78.25 IU/ml (53.395-128.150 IU/ml)	163 IU/ml			123.6 IU/ml

ADB: Anti-deoxyribonucleic B, ASO: Antistreptolysin O

Table 2: Comparison of ULN values in Normal Healthy Children among various study groups

ULN value in normal healthy children (mean values)	Our Study	Une <i>et al.</i> ^[11]	Sethi <i>et al.</i> ^[7]	Mahendrappa <i>et al.</i> ^[12]	Karmarkar <i>et al.</i> ^[13]
ASO	262 IU/ml	200 IU/ml	239 IU/ml	242 IU/ml	
ADB	134 IU/ml				200 IU/ml

ULN: Upper limit of normal, ASO: Antistreptolysin O, ADB: Anti-deoxyribonucleic B

Table 3: Comparison of median values in acute pharyngitis group among various study groups

Median value in acute pharyngitis	Our Study	Machado <i>et al.</i> ^[15] (mean values)	Une <i>et al.</i> ^[11]	Kotby <i>et al.</i> ^[9]
ASO	303 IU/ml (142-520 IU/ml)	77 + 30 IU/ml	12 patient >200 IU/ml to <400 IU/ml 7 patient >400 IU/ml	1138.2 IU/ml
ADB	75.12 IU/ml (64.5-136 IU/ml)			

ASO: Antistreptolysin O, ADB: Anti-deoxyribonucleic B

skin results in a brisk ADB response.^[9] In our study, the median titers for ASO in acute pharyngitis group were significantly raised than that of the control group (ASO $P = 0.001$) which was not the case with ADB (75.12 IU/ml, [64.5–136 IU/ml] $P = 0.325$). ASO rises in the 1st week of acute streptococcal infection and is the earliest serological marker of acute infection. The rise in ADB is delayed, well beyond the sampling for acute pharyngitis and hence not documented in this group of patients. Comparative data of ASO in Acute pharyngitis by Kotby *et al.*,^[9] Machado *et al.*,^[15] and Une *et al.*^[11] are shown in Table 3.

ASO and ADB titers are raised in ARF patients variably depending upon the time to presentation which can vary from acute carditis to Chorea. Usually, these values are in the variable convalescent phase of acute streptococcal infection^[16] and thus vary between different populations and geographic areas.^[1] Kotby *et al.*^[9] reported a much higher ASO titer in ARF than that of our study (347.5 IU/ml). In ARF group of our study, both the antibody titers, i.e., early phase response: ASO 347.5 IU/ml (125–686 IU/ml) and late-phase persistent response: ADB 570.5 IU/ml (276–922 IU/ml) were significantly raised than that of the control group (ASO $P = 0.02$, ADB-B $P < 0.0001$). The high levels seen in ARF may be due to the time lapse between the streptococcal infection and the occurrence of carditis which allows ASO to reach its peak level (3–6 weeks).

Kotby *et al.*^[9] reported ASO titer of 215.4 IU/ml in RHD cases which was lower than that observed in healthy controls (245.09 IU). The natural course of streptolysin response can be modified by penicillin prophylaxis, by reducing the overall number of responders and influencing the magnitude of antibody response. In our study, the ASO titer in RHD cases (163 IU/ml [98.250–324.50 IU/ml]) seems to be almost equal to normal healthy children (163.5 IU/ml [133–246.5]) [Table 4].

Prolonged penicillin prophylaxis was successful in bringing ASO titer closer to the normal healthy group; however, it did little to reduce the ADB titer (205 IU/ml [113.651–456.5 IU/ml]) in these patients. Kotby *et al.*^[9] reported that the twice-weekly LAP regimen was associated with low ASO titers, suggesting its efficacy in preventing streptococcal throat infection. Elevated ABD titers persisting for several months, even after penicillin prophylaxis in

Table 4: Comparison of median values of ARF and RHD groups with other study group

	Our Study	Kotby <i>et al.</i> ^[9]
Median value in ARF		
ASO	347.5 IU/ml (125-686 IU/ml)	1334.9 IU/ml
ADB	570.5 IU/ml (276-922 IU/ml)	
Median value in RHD		
ASO	163 IU/ml (98.250-324.50 IU/ml)	215.4 IU/ml
ADB	205 IU/ml (113.651-456.5 IU/ml)	

ARF: Acute rheumatic fever, ASO: Antistreptolysin O, ADB: Anti-deoxyribonucleic B, RHD: Rheumatic heart disease

our study could be explained by characteristics of the streptococci, host response, geographical affliction, site of infection (throat/skin pyoderma), and antibiotic treatment regimen (a thrice-weekly rather than twice-weekly LAP regime being used by us). The raised ABD levels in RHD children on penicillin prophylaxis brings to the fore concerns about understanding streptococcal disease in ARF patients in this part of the world (including role of recurrent skin pyoderma and scabies rampant in overcrowded human settlements which conspicuously elevates ABD titers, round the year availability of penicillin, quality of penicillin and its storage, injection technique, and personal training in rural area) and probably instituting an appropriate evidence-based prophylaxis recommendation.

In our study, ASO positivity at an ULN of >262 IU/ml, was significantly higher in acute pharyngitis (52%, $P = 0.0001$), ARF (57%, $P = 0.0002$), and RHD (35%, $P = 0.053$) cases when compared with normal healthy controls. Similarly, ADB positivity at an ULN of > 134 IU/ml, was significantly higher in ARF (92.31%, $P < 0.0001$) and RHD (62.7%, $P < 0.0001$) patients in comparison to normal healthy children. Mhalu and Matre^[17] reported ADB positivity in 45.9% patient with features of GAS infection, and Nair *et al.*^[18] reported ADB positivity in 82.27% in ARF. Furthermore, we observed raised ADB titers in ARF patients where ASO titers were borderline or noncommittal, suggesting that ADB can be a reliable marker for diagnosing ARF. This is in synchronization with the results obtained from the ROC of ASO and ADB in ARF, which shows that ADB has a better diagnostic utility in ARF as compared to ASO titer [Table 5].

We did not find any gender-based difference in ASO and ADB titers in healthy normal controls. This was also

Table 5: Comparison of ULN value positivity among acute pharyngitis, acute rheumatic fever and rheumatic heart disease groups with other study groups

S. no	ULN value positivity	Acute pharyngitis	Acute Rheumatic fever	Rheumatic heart disease
Our study	ASO (>262 IU/ml)	52%(<i>P</i> -0.0001)	57%(<i>P</i> -0.0002)	35% (<i>P</i> -0.053)
	ADB (>134 IU/ml)		92.31% (<i>P</i> -<0.0001)	62.7% (<i>P</i> -<0.0001)
Mhalu et al. ^[17]	ADB positivity	45.9%		
Nair et al. ^[18]	ADB positivity		82.27%	

ULN: Upper limit of normal, ADB: Anti-deoxyribonucleic B, ASO: Antistreptolysin O

true for RHD group and acute pharyngitis cases. Median values of ASO tended to be higher in females ($P = 0.053$) in ARF group which was however not the case with ADB. Madaan et al.^[19] and Sethi et al.^[7] also did not find any statistically significant gender-based difference in the ASO titer in healthy normal controls.

Shet and Kaplan^[1] reported higher prevalence of streptococcal pharyngitis and streptococcal antibody titers during winter and early spring. Kotby et al. reported^[9] significantly higher ASO titer in each studied group (normal healthy children, RHD, and chronic tonsillitis) during winter and autumn. Sethi et al.^[7] in a recent Indian study of 200 normal children with no history of recent acute pharyngitis performed in the winter to early spring, reported an ASO ULN of 239 IU/mL, which is lower than our ULN. In our study, ASO titers showed statistically significant seasonal variation in ARF where values were high in winter and rainy seasons but unexpectedly low in the summer season. In summers, the incidence of sore throat is quite less, and pyoderma and impetigo probably contribute more to the cases of ARF. Controlled epidemiologic studies have already shown that the ASO response is generally brisk after a streptococcal upper respiratory tract infection but is relatively feeble after Group A streptococcal impetigo or pyoderma.^[20] This could be a plausible explanation for the low ASO titers noted in ARF cases in summer in our study.

Limitations

The study was hospital based as we recruited all patients who visited hospital for some ailment or their siblings in the control group. The patients in the ARF and RHD group were referral based, and most of the female patients are a poor priority in our social practices. More relevant data could have been generated had it been a community-based study. More number of study patients in different categories of patients would have thrown up much more statistically significant results by eliminating the effect of false-positive results. There was difficulty in obtaining reliable “control” samples for both the ASO and ADB titers in a normal pediatric population and supports the use of the 80th percentile for the ULN instead of the more conventional 95th centile.

CONCLUSION

ASO titers in acute pharyngitis and ARF group were significantly raised substantiating evidence of a recent

streptococcal disease, whereas the raised ADB titers in ARF group ascertained carditis as a late complication of streptococcal infection. The raised ABD levels in RHD children on penicillin prophylaxis raises concerns about information gap on the streptococcal disease in this part of the world, including the interaction of the bacterium with the host (the age and immune status of the cases and the route of infection), impact of geographical factors and probably also on the availability, schedule, or the quality of drug used for prophylaxis and compliance, and reflecting an overall poor control of recurrent streptococcal (skin) infections.

In the rheumatic group (ARF and RHD), the streptolysin response was higher in children enrolled in rainy and winter season in contrast to the nonrheumatic population (normal healthy control and acute pharyngitis), whereas ADB titers were comparable in all the seasons. There was no gender-based difference in ASO and ADB titers in healthy normal controls, acute pharyngitis, ARF, and RHD groups. The reported ULN values for ASO and ADB can be used as a cutoff reference for instituting Jones criteria toward diagnosing ARF in Delhi NCR region.

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Conflicts of interest

There are no conflicts of interest.

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