Evaluation & revaluation of upper limits of normal values of anti-streptolysin O & anti-deoxyribonuclease B in Mumbai

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Received August 6, 2003

Background & objectives: For the serodiagnosis of group A streptococcal (GAS) infections and their late sequelae, a battery of tests have been suggested. However, anti-streptolysin O (ASO) and anti-deoxyribonuclease B (ADNase B) are the most widely accepted tests for the same. It is essential to evaluate the upper limits of ASO and ADNase B levels in age and sex matched normal population before using them for the detection of patients. For this study, these values were determined in the past and were reevaluated again in 2001-2002, in normal subjects.

Methods: A total of 200 healthy individuals were included in the study in 1991-1992 and same number of age and sex matched healthy individuals were included in 2001-2002. The methodology used for determination of ASO and ADNase B levels were as per the techniques recommended by the WHO.

Results: The findings show that the upper limits of normal ASO titers are 195 IU and 305 IU for adults and children respectively while the said levels for ADNase B for both adults and children were found to be 200 IU.

Interpretation & conclusion: The findings of the present study will be helpful in the follow-up, better diagnosis and prognosis of group A streptococcal infections.

Key words: ADNase B - ASO - GAS - ULN

Group A streptococcal (GAS) infections and their late sequelae like rheumatic fever (RF) and rheumatic heart disease (RHD) remain an important and major health problem in India. The incidence of RF varies from 0.2 - 0.75 per 1000 children per year in school children 5 - 15 yr of age. The prevalence of RHD varies from 1.0-5.4 per 1000 in school children. Also among the population 5 - 40 yr of age at risk for RHD, 1.4 million are presently suffering from RHD. Such data have emphasized the importance of accurate clinical diagnosis, often requiring laboratory confirmation of the preceding GAS infection. The diagnosis of GAS infections is often made by clinical observation and then confirmed by rapid antigen tests or by isolation of GAS from site of infection. However, when one is considering the diagnosis of post-streptococcal non-suppurative sequelae like acute rheumatic fever (ARF) or post-streptococcal glomerulonephritis (PSGN), it is not always possible to obtain an adequate clinical history or to recover the organism. 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 to confirm the diagnosis of the preceding GAS infection. This along with the modified Jones criteria contributes to the final confirmation of clinical diagnosis.

Because RF/PSGN are non-suppurative sequelae of GAS infection and there is a latent period between the streptococcal infection and onset of disease, serum taken at disease onset may really be convalescent; a rising titre may not be demonstrated. Hence, an upper limit of
normal or ULN value is useful when acute and convalescent sera are unavailable\(^3\). For the purpose of uniformity, ULN is defined as that titer exceeded by 20 per cent of a normal population\(^4\). ULN values are known to vary with respect to different geographical locations as also with respect to different seasons\(^3\). Hence the need was felt to evaluate and revaluate the ULN values of ASO and ADNase B levels in Mumbai.

Though diagnosis of GAS infection can be done by a whole array of tests that are serodiagnostic, anti-streptolysin O (ASO) and anti-deoxyribonuclease B (ADNase B) are the most widely accepted tests for the same. It is essential to evaluate the ULN of ASO and ADNase B levels in age and sex-matched normal population before using these for the detection of patients. For this study, these values were evaluated in normal healthy subjects in Mumbai in the year 1990-1991 and reevaluated in the year 2001-2002.

Material & Methods

Study subjects: A total of 200 normal healthy individuals (both children and adults) were included in the study in 1990-1991, and same number of age and sex matched normal, healthy individuals were enrolled in 2001-2002. These volunteers had no features suggestive of sore throat in the past three months, had no history of joint pains and had not taken any kind of antibiotic therapy for the same.

Blood samples (3-5 ml) were collected by venepuncture, serum was separated and stored at -20°C till further use.

Determination of ASO: Geometric dilutions of the patient’s serum were carried out by tube dilution method\(^5\). The dilutions used were in the range of 1:100 to 1:744; 0.5 ml of antigen i.e., streptolysin O (SLO – 2U/ml) was added to each dilution and allowed to react for 15 min at 37°C, 0.5 ml of the indicator system consisting of 5 per cent rabbit erythrocytes was added to all tubes. After a further incubation of 1 h at 37°C, the results were recorded on the basis of presence or absence of haemolysis. The highest dilution showing 50 per cent haemolysis is the end point and the corresponding reciprocal value is the ASO titre. The enzyme and buffer controls were put up along with the test.

Determination of ADNase B: Neutralization assay method\(^5\) was used to determine the ADNase B levels. Microtitre dilutions of the serum samples were carried out in 2 sets (1:50 to 1:3200 in the first set and 1:75 to 1:4800 in the second set). 25 µl of deoxyribonuclease B (DNase B – 1U/ml) was added to all tubes and the reaction was allowed to proceed for 15 min at 37°C. Next, 50 µl DNA-methyl green complex (prepared using Sigma Type V, highly polymerised DNA, from calf thymus, as per WHO Manual\(^3\)) was added and the tubes were incubated at 37°C for 24 h. Results were recorded on the basis of digestion (leading to loss of green colour) or no digestion (no loss of green colour) of substrate. The titre corresponds to the reciprocal value of serum dilution that causes an approximately 50 per cent digestion of substrate. In case of steep colour transition, the last well with total enzyme inhibition is the endpoint. The enzyme and substrate controls were put up along with the test.

All methods used in ASO and ADNase B tests are in accordance with the techniques specified in the WHO manual on laboratory diagnosis of group A streptococcal infections (1996)\(^2\).

Results

A total of 200 serum samples were available for the study, 40 of these from healthy children in the age group 5 - 15 yr of which 20 were males and 20 were females, 160 samples were obtained from healthy, adults (80 males and 80 females). In both evaluation (1991-1992) and revaluation (2001-2002), all individuals were followed up thrice on quarterly basis to check for seasonal variation.

The ULN of ASO in children during revaluation has shown a shift of one value upwards at 305 IU while the value obtained during evaluation was 244 IU. The ULN of ASO in adults remained at 195 IU for both evaluation and revaluation (Table I). The ULN of ADNase B for both children and adults remained fairly constant at 200 IU (Table II). No significant seasonal variation was noted either in evaluation or revaluation.

Discussion

Nearly two decades after declaring the year 1984 as the year of the rheumatic child, infections due to GAS are still prevalent in India. Frequent exposure to the
organism occurs due to a variety of highly predisposing factors which include overcrowding, bad hygienic practices, increased pollution leading to decreased immunity etc. The shift of ULN of ASO in children from 244 IU in 1991-1992 to 305 IU in the present scenario tells a similar story. The reason adults do not seem to show an increased ULN value may be due to the fact that GAS infections mainly affect children as they form the susceptible group. The reasons for having no change in the ULN values of ADNase B in both children as well as adults could be either a varied antigenic stimulus as compared to streptolysin O or due to limitations of the sensitivity of the test.

There is no information available in India on the ULN values, a US study shows that the ULN values in children of 2-12 yr were 240 for ASO and 640 for ADNase B, respectively as against our study which has a ULN of 305 for ASO and 200 for ADNase B in children 5-15 yr of age. The presence of a higher ULN in case of ADNase B in the study conducted in the US may be due to a higher antigenic stimulus caused by their GAS strains.

The present study opens further avenues to understand this microorganism called GAS and tells us to constantly and periodically recheck on those very same values which we ourselves may have established. Such checks may yield us results, which may serve as a foundation stone for further research on the changing antigenic stimuli of this organism.

References


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