Anti Streptolysin O; Normal Values for Healthy Children Aged from 5 to 15 Years Old in Sana’a City-Yemen

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Abstract

Backgrounds: Group-A streptococcal serology is used for the diagnosis of post-streptococcal diseases, such as acute rheumatic fever, and occasionally for the diagnosis of streptococcal pharyngitis. Experts recommend that the upper limits of normal values for streptococcal serology should be determined for each individual population because of differences in the epidemiology of group A Streptococci (GAS) between these populations.

Objectives: This study was conducted firstly to determine the normal values of the upper limit of anti-streptolysin O (ASO) titer in Sana’a city. Secondly to determine the factors that might affect the levels of ASO among the selected children.

Methods: Participants with a history of GAS disease, including pharyngitis or impetigo were excluded. A total of 404 serum samples collected from children aged 5-15 years (with a sample enriched with school-aged children) were tested for the ASO titer. Reference values, including titers that were 95% of the upper limit of normal (ULN) were obtained by the calculation of 95% prediction interval (PI 95%) using a parametric method instead of the traditional non parametric approach.

Results: Normal values for the ASO titer declined in the early childhood and rose slightly in the late childhood. The estimated ASO titers that were 95% of ULN for the total was 276.2 IU/ml.

Conclusions: This study data were similar to those reported in countries with temperate climates, suggesting that a uniform upper limit of normal for streptococcal serology may be able to apply globally.

Keywords: Group A Streptococci; Anti-streptolysin O; Upper limit of normal; Children; Sana’a city; Yemen

Introduction

Anti-streptolysin O (ASO or ASLO) is the antibody produced against streptolysin O; an immunogenic, oxygen-labile hemolytic toxin produced by most strains of group A and many other strains of group C and G Streptococci [1]. Acute rheumatic fever (ARF) is important non suppurative sequelae of Group-A streptococcal (GAS) throat infection. Diagnosis of ARF, according to the Jones criteria requires evidence of antecedent GAS infection [2]. Positive throat cultures are obtained only in about 11% at the time of ARF presentation [2]. Moreover, mere presence of the organism in the throat can also indicate a carrier state which is observed in 2.5-35.04% of individuals [3]. However, the appearance of ASO in serum of a patient or an increase in the ASO titer is usually indicative for a recent streptococcal infection [4]. This is especially true when considering the diagnosis of non-suppurative sequelae of GAS infection. Although ASO titer has provided a useful guideline to physicians, but this has been shown to vary with age [4-8], geographical location and site of infection [5-8]. Clinical microbiology laboratories often use interpretative criteria suggested by manufacturers of commercial antibody test kits. Because such ‘normal’ levels may only reflect an appropriate titer for adults, but correct interpretation of titer in children can be problematic [5-8]. Moreover, it is not often feasible to obtain
Materials and Methods

Study design and setting

This cross-sectional sero-epidemiological study was conducted among healthy children aged from 5 to 15 years in Sana’a city, Yemen. Yemen is a Middle Eastern country and part of the Arab world, is located in the south of the Arabian Peninsula. It is bordered by Saudi Arabia in the north, the Arabian Sea and Gulf of Aden in the south, Oman in the east and the Red sea in the west. The population was estimated in 2008 at 21,843,554, living in 3,058,299 households. The population structure is typical of a developing country, with the rural population comprising about 71% of the total population. The majority of the population is young, with nearly half (45%) below the age of 15 years old, while the elder age group (over 64 years) represents only about 3.4%. The literacy rate is about 47% among those of 15 years and older (males 63%, females 31%), the total fertility rate 6.2, the average household size 7.1 persons, the poverty rate about 47%, and the annual growth rate of population is 2.9%. Life expectancy at birth male/female is about 63/67 years and the probability of dying under five years in 2012 was 160/1000 live births. These and other factors contribute to Yemen’s low ranking in the development index cited in the World Human Development Report; Yemen was number 160 among the 162 countries that were rated in the year 2013 [9]. GAS disease is common in Sana’a city with high rates of invasive GAS disease, acute rheumatic fever and rheumatic heart disease [10]. In addition, there is a high prevalence (over 37%) of impetigo among schoolchildren [10] and the incidence of GAS culture-positive sore throat is similar to that in other developing countries (approximately 15 cases per 100 child years) [11]. Previous to this study, Yemen did not have any upper limit of normal (ULN) for ASO among normal healthy children between the ages 5-15 years.

Study population and sample size

Accordingly, a sample size of 404 subjects was calculated, this was selected by a systematic random method. All schools that had children aged from 5 to 15 years in Sana’a city were listed (170 schools) and then by a simple random selection, 8 of these schools (4 female schools and 4 male schools) were selected, after that by a stratified sampling method, the school classes were selected according to the age to cover all the concerned age groups (5 to 15 years); finally, every 5th child in the list of the selected class was selected (about 10% of male children and 7% of female children who refused to donate blood were excluded). All subjects were asymptomatic with normal immune systems while participating in the study.

Data collection

A full history was taken from each studied subject and the findings were recorded in a predesigned questionnaire. The collected data included name, age at the time of the study, sex, residence and history of recent sore throat, antibiotic use etc. In addition, the body weight and length were measured for each subject. All these 404 subjects were prospectively screened for current or recent GAS infection. Subjects with any of the following criteria were excluded; rheumatic heart disease proven on echocardiogram (because these subjects are at a higher risk of acute rheumatic fever at any time); recent GAS pharyngitis; and any evidence of impetigo, which included dry, crusted, or pustule lesions.

Laboratory method

For serological assessment, a venous blood sample of 2-3 ml was drawn from each subject, then serum was separated and measured for ASO titer by an ELISA technique (ASO-ELISA Kit, Norton, UK) as described previously [11]. The method provide an inexact figure for low titers (ASO titers of <60 IU/ml).

Data analysis

Epi info version 6 software (CDC, Atlanta, USA) was used for the statistical analysis of the obtained results in this study. The average value (mean), the most frequently occurring value (mode), standard deviation of the mean (on average, how much each measurement deviates from the mean), span of values over which your data set occurs (range) and the midpoint between the lowest and highest value of the set (median) for ASO serum levels among the tested subjects were calculated to determine the significance of the differences of these values among different sexes and age groups. As the standard definition of a reference range for a ASO measurement is defined as the prediction interval between which 95% of ASO values of a reference group fall into, in such a way that 2.5% of the time a sample value will be less than the lower limit of this interval and 2.5% of the time it will be larger than the upper limit of this interval whatever are the distribution of these values [12,13]. To account for these estimations, the 95% prediction interval (95% PI) is calculated as described by Sterne et al. [14] in which:

\[
95\% \text{ PI} = \text{mean} \pm t_{0.025, n-1} \times \sqrt{\frac{n+1}{n} \times \text{SD}},
\]

where \(t_{0.025, n-1}\) is the 97.5% quantile of a Student’s t-distribution with \(n-1\) degrees of freedom. This method is often acceptably accurate if the standard deviation as compared to the mean is not very large as with this study results [12-14].

Ethical approval

Ethical approval was obtained from the Faculty of Medicine and Health Sciences, Sana’a University Research Ethics Review Committee. All participants were approached for their consent, and prior to enrollment information sheets in Arabic were provided. It was required that all participants provided a written informed consent before information was collected (Children aged <10 years were enrolled only if a written consent was obtained from a parent or guardian and for children aged 10 years or older, they were insisted to give a written assent).

Results

A total of 404 children subjects, 186 males and 218 females were randomly selected and investigated for the ASO ULN. Their ages ranged from 5 to 15 years old with the Mean ± SD of age equal...
to 10.8 ± 3.3 years for the total, 10.9 ± 3.5 years for males and 10.4 ± 3.2 years for females. 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% as shown in Table 1.

Table 2 shows the Mean ± SD, Median and 95% upper-limit of normal reference values for ASO titer according to sex of enrolled subjects. The ASO ULN serum level for total subjects was 276.2 IU/ml with a median of 195 IU/ml. A slightly higher ASO ULN serum level was observed for females (258.5 IU/ml) with a median of 195 IU/ml, while a lower ASO ULN serum level was observed for males (252.5 IU/ml) with a median of 170 IU/ml.

Table 3 shows the Mean ± SD, Median, and 95% upper-limit of normal reference values for ASO titer according to age of enrolled subjects. The ASO ULN serum level for total subjects was 276.2 IU/ml with a median of 195 IU/ml. A slightly higher ASO ULN serum level was observed for the age group 9-10 years (299 IU/ml) with a median of 190 IU/ml, while a lower ASO ULN serum level was observed for the age group 5-6 years (244.8 IU/ml) with a median of 170 IU/ml, followed by 264.7 IU/ml for the age group 7-8 years. The ASO ULN values for the age group 7-8 years was 264.7 IU/ml, for the age group 11-12 years was 275 IU/ml, for the age group 13-14 years was 282.9 IU/ml and finally for the age group 15 years was 279.7 IU/ml.

**Discussion**

The normal values for streptococcal serology in this study were found to be roughly similar to those reported from other regions. In comparison with data from the USA, India, Australia and Fiji, the overall values for ASO titers were found similar to that reported in Fiji (276 IU/ml) [11] and slightly higher than that reported from the USA for 5-12 years old children (240 IU/ml) [8], but lower than that reported from India (305 IU/ml) [6] and Australia (320 IU/ml) [15]. The slightly higher ASO titers in Yemen and Australia, Fiji and India are probably due to the fact that tonsillitis and impetigo are endemic in these regions including Yemen, particularly in children [5,10]. Although children with a recent history of tonsillitis and impetigo were excluded, the ASO titers remain elevated for many months; hence, some children, whose ASO titers were in the process of returning to their baseline level after a case of tonsillitis or impetigo were included [16]. Although some have claimed that normal ranges for streptococcal antibody titers are higher in populations with endemic streptococcal infections as Yemen, this is incorrect. The studies on which these statements were based did not meticulously exclude children with recent streptococcal infections. For example, a study with an aboriginal community in Australia, in which impetigo was very common among the children of the community, found median ASO titers of 256 IU/ml respectively, but did not exclude children with current or recent impetigo [16]. The very close similarities of the titers between studies in tropical countries as India and Yemen and studies in temperate zones as USA and Australia give evidence and motivation for the concept that single upper limit of normal values for ASO titers may be able to applied globally [6,8,11,15]. By applying the simple non parametric method that has been used in previous studies to analyze the present study data, the cutoff values were found similar to those obtained by the parametric method. However, the parametric method for data analysis that was used in this study has some advantages over the non-parametric method. The non-parametric method often produces unlikely irregular patterns in the centiles with age, unless a large sample is used and wide age intervals are specified [5]. The results may be artificially affected by the choice of age groups, especially when titers have a complex pattern of change with age. In comparison, the 95% prediction interval (95% PI) which described by Sterne et al., produces smooth reference value that varies smoothly with age [14]. This study will recommend that Yemeni clinicians use the cutoff values for single upper limit of normal cutoff value for children aged from 5 to 15 years, rather than sub-age groups, such as 5 to 6 years, 7 to 8 years, 9 to 10 years, 11 to 12 years, 13 to 14 years and 15 years (Table 3) as recommended by Steer et al., Karmarker et al. and Kaplan et al. in other studies [5-7]. This is because there is minor variability in the year by year values that was found in children aged 5 to 15 years. No significant association presents between

Table 1 The age and sex distribution of children tested for ASO normal values levels, Sana’a, Yemen.

| Age groups | Male n=186 | Females n=218 | Total n=404 |
|------------|------------|--------------|-------------|
|            | No %       | No %         | NO %        |
| 5-6 years  | 32 17.2    | 24 11        | 56 13.9     |
| 7-8 years  | 30 16.1    | 36 16.5      | 66 16.3     |
| 9-10 years | 32 17.2    | 36 16.5      | 68 16.8     |
| 11-12 years| 24 12.9    | 44 20.2      | 68 16.8     |
| 13-14 years| 28 15.1    | 40 18.3      | 68 16.8     |
| 15 years   | 40 21.5    | 38 17.4      | 78 19.3     |
| Mean age   | 10.9 years | 10.4 years   | 10.8 years  |
| SD         | 3.5 years  | 3.2 years    | 3.3 years   |
| Median     | 11 years   | 11 years     | 11 years    |
| Mode       | 15 years   | 15 years     | 15 years    |
| Min        | 5 years    | 5 years      | 5 years     |
| Max        | 15 years   | 15 years     | 15 years    |

Table 2 The cut of 95% upper limit normal reference values for ASO titer by sex groups for children 5-15 years, Sana’a, Yemen.

| Sex         | ASO levels IU/ml |
|-------------|------------------|
|              | Mean ± SD        | Median   | 95% upper limit |
| Male n=186   | 182.5 ± 55       | 170      | 252.5           |
| Female n=218 | 194.5 ± 50       | 195      | 258.5           |
| The sum n=404| 188.7 ± 52.3     | 195      | 276.2           |

Table 3 The cut of 90% upper-limit-normal reference values for ASO titer by age groups for children 5-15 years, Sana’a Yemen.

| Age groups | Mean ± SD | ASO levels IU/ml | 90% upper limit |
|------------|-----------|------------------|-----------------|
|            |           | Median           |                 |
| 5-6 years  | 156.8 ± 50.9 | 170              | 244.8           |
| 7-8 years  | 175 ± 52   | 160              | 264.7           |
| 9-10 years | 199 ± 58.3 | 190              | 299             |
| 11-12 years| 194.2 ± 47 | 200              | 275             |
| 13-14 years| 193.2 ± 52.6 | 200          | 282.9           |
| 15 years   | 198.7 ± 47 | 210              | 279.7           |
| The sum    | 188.7 ± 52.3 | 195              | 276.2           |
sex and ASO concentrations, suggesting that the female cut off equal to the male cut off. This issue has not been discussed by other researchers previously.

**Conclusion**

This study provides upper limit of normal values for streptococcal serology for children aged from 5 to 15 years old in Sana’a city, Yemen determined by using the 95% prediction interval (95% PI) readily repeatable parametric statistical technique. These upper limits of normal values will guide clinicians in Yemen when they consider the diagnosis of post streptococcal diseases in their children patients and will provide useful baseline data for future studies of interventions against GAS disease in Yemen. These data could also be applied for the surrounding Yemeni cities and surrounding Arabic countries.
References

1. Blyth CC, Robertson PW (2006) Anti-streptococcal antibodies in the diagnosis of acute and post-streptococcal disease: streptokinase versus streptolysin O and deoxyribonuclease. B Pathology 38: 152-156.

2. Ferrieri P (2002) Proceedings of the Jones Criteria workshop. Circulation (Jones Criteria Working Group) 106: 2521-2523.

3. Parrillo SJ (2007) Rheumatic Fever in Emergency Medicine.

4. Saxena A (2000) Diagnosis of rheumatic fever: Current status of Jones criteria and role of echocardiography. Indian J Pediatr 67: 283-286.

5. Steer AC, Vidmar S, Ritika R, Kado J, Batzloff M, et al. (2009) Normal Ranges of Streptococcal Antibody Titers Are Similar Whether Streptococci Are Endemic to the Setting or Not. Clin Vaccine Immunol 16: 172-175.

6. Karmarkar MG, Venugopal V, Joshi L, Kamboj R (2004) Evaluation & revaluation of upper limits of normal values of anti-streptolysin O and anti-deoxyribonuclease B in Mumbai. Indian J Med Res 119: 26-28.

7. Kaplan EL, Ferrieri P, Wannamaker LW (1974) Comparison of the antibody response to streptococcal cellular and extracellular antigens in acute pharyngitis. J Pediatr 84: 21-28.

8. Kaplan EL, Rothermel CD, Johnson DR (1998) Antistreptolysin O and anti-deoxyribonuclease B titers: normal values for children ages 2 to 12 in the United States. Pediatrics 101: 86-8.

9. (2013) Statistical Year Book supply 1. Central Statistical Organization (CSO), Ministry of Planning and Development, ROY.

10. Yemen MHP (2013) News letter- supply 13. Medical Statistical Department, Ministry of Health and Population, ROY.

11. Steer AC, Carapetis JR, Nolan TM, Shann F (2002) Systematic review of rheumatic heart disease prevalence in children in developing countries: the role of environmental factors. J Paediatr Child Health 38: 229-234.

12. Hägström M (2014) Establishment and clinical use of reference ranges at Wikiversity.

13. Bangert SK, Marshall WJ (2008) Clinical Biochemistry: metabolic and clinical aspects. Philadelphia: Churchill Livingstone/Elsevier 443: 10186-10188.

14. Sterne J, Kirkwood BR (2003) Essential medical statistics. Oxford: Blackwell Science, ISBN 0-86542-871-9.

15. Danchin MH, Carlin JB, Devenish W, Nolan TM, Carapetis JR (2004) New normal ranges of antistreptolysin O and anti-deoxyribonuclease B titres for Australian children. J Paediatr Child Health 41: 583-586.

16. Nimmo GR, Tinniswood RD, Nuttall N, Baker GM, McDonald B (1992) Group A streptococcal infection in an aboriginal community. Aust Med J 157: 521-522.