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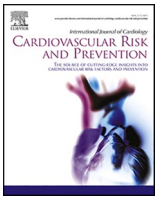

Determination of the upper limit of normal for serum anti-streptolysin-O titre in primary school children in Southern Nigeria; A model for other low resource settings

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ABSTRACT

The prevention and treatment of Rheumatic Heart disease is hinged on antibiotic administration in children with Group A *Streptococcal* (GAS) pharyngitis and Acute Rheumatic Fever (ARF). The Upper Limit of Normal (ULN) for serum Anti-streptolysin O titre (ASOT) has been employed as proof of antecedent GAS pharyngitis to fulfil the Jones' criteria for diagnosis of ARF. This value has not been generated among West African children. Generalization of values from Caucasians (200 IU/ml) is likely to result in over-diagnosis, owing to higher GAS pharyngitis incidence in Africa. We aimed to determine the serum ASOT and its ULN in apparently healthy school-aged children in Egor Local Government Area (LGA), south-south Nigeria and to determine their relationship with socio-demographic characteristics.

We recruited 384 apparently healthy school-aged pupils across eleven schools. Serum ASOT was determined by turbidimetry. Statistical analysis was done using student's t-test and Analysis of Variance (ANOVA). Level of significance was set at $p < 0.05$.

The mean age was 8.53 ± 1.97(range 6–12) years and male-female ratio was 1.1:1. The ULN and geometric mean serum ASOT were 390.76 IU/ml and 230.04 ± 1.86 IU/ml respectively. No significant correlation was found between serum ASOT and age (r-value of −4.8%). The ULN did not vary significantly with gender, socio-economic class and the presence/absence of over-crowding in homes. The ULN for serum ASOT in apparently healthy school-aged children in Egor LGA is higher than the currently used international value. Clinicians in West Africa should consider applying higher cut-off values for the diagnosis of ARF.

1. Introduction

Group A *Streptococcal* (GAS) infections are the most commonly occurring human infections worldwide accounting for up to 500,000 deaths per annum [1,2]. They cause significant soft tissue infections, particularly of the skin and throat, among children aged 5–15years [3–8]. Group A *Streptococcus* accounts for 15–30% of cases of pharyngitis among these children [2,9]. Acute Rheumatic Fever (ARF) and Rheumatic Heart Disease (RHD) are well documented sequelae of GAS throat infections [10,11]. Globally, the World Health Organization (WHO) reports 282,000 new cases of ARF annually and 233,000 deaths in sub-Saharan Africa are attributable to RHD annually [12,13]. The prevalence of RHD in Nigeria is July 0, 1000 among children aged six to twelve years [14]; making it the commonest cause of acquired heart disease and a major public health problem [2,8,15,16]. Serology is the basis for diagnosis in the post-streptococcal sequelae.

Anti-Streptolysin O (ASO) is an antibody elaborated in response to GAS [3–7] and a two-fold rise in serum ASO titre is indicative of prior GAS throat infection [6,17,18]. This rise in serum ASO titre correlates better with GAS pharyngitis than a rise in serum Anti-Dnase-B (ADB) titre, another antibody which is more readily elaborated in response to GAS skin infections [3,6,18,19]. Since it is difficult to get early and convalescent sera to demonstrate a two-fold rise, knowledge of the cut-off serum ASO titre becomes important, a rise above which would

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indicate prior GAS infection and aid fulfillment of the Jones' Criteria for diagnosis of ARF [18,20]. This cut-off is determined as the value of serum ASO titre that is exceeded by 20% of the apparently healthy population [21,22]. The currently established international cut-off value is 200IU/ml [23].

Similar cut-off titres have been generated by several researchers outside of Africa and in North Africa. A study by Kotby *et al* [24] among apparently healthy Egyptian children found that the Upper Limit of Normal (ULN) serum ASO titres was similar to values generated in Ethiopia [25] but was much higher than the values generated from studies in USA [26], India [27–29] and even Tanzania [30]. Serum ASO levels are affected by the incidence of GAS infections, which likewise varies across geographic regions and climes with varying health care capabilities and sanitary practices [24]. As such, generalization of these already determined values to Nigerian children, is not particularly useful as it would likely result in over-diagnosis of post-streptococcal sequelae, in this population who have a high burden of febrile illnesses which can mimic ARF [31]. This may have led clinicians to abandon attempts at diagnosing ARF, despite its high incidence and the growing prevalence of RHD [7,31,32]. Progression of ARF to RHD can be easily prevented with the institution of appropriate prophylactic antibiotic therapy [33,34]. Improving diagnostic capabilities for ARF is therefore the key to curbing the high prevalence of RHD [31,34].

Such a cut-off value is yet to be determined among Nigerian children and children of other west African countries. Likewise, the relationship between serum ASO concentration and socio-demographic variables had not been previously studied in Nigerian children. This study aimed to determine the ULN for serum ASO titre among apparently healthy school-aged children to facilitate the diagnosis of ARF and to identify the relationship between serum ASO titre and socio-demographic variables.

2. Methodology

This was a descriptive cross-sectional study. The subjects were apparently healthy children, aged 6–12years, attending primary schools in Egor Local Government Area (LGA) of Benin- City, Edo State in South-South Nigeria. Children with an episode or history of sore throat in the preceding 8 weeks, those who had been on a course of antibiotics or completed antibiotic therapy within 2weeks of sample collection, those with history of joint pain/swelling suggestive of arthritis, ARF/RHD, hyperaemic tonsils and pharynx, with or without exudate, a cardiac murmur, impetigo, facial swelling or haematuria on dipstick urinalysis were excluded [18,24,35,36]. Ethical approval was obtained from the Health Research and Ethical Committee of the University of Benin Teaching Hospital and written permission was obtained from the State Ministry of Education, Local Education Authority and heads of selected schools. Written informed consent (Appendix I) was obtained from Parents/Guardians of the subjects, additionally, verbal assent was obtained from those aged 12 years [37].

The formula below was used to calculate the minimum sample size required for the study.

$$n = Z_2(SD)^2 / \ d^2$$

Where: **n** = Minimum sample size.

Z = Standard normal deviation for the defined confidence level of 95% = 1.96.

SD = Standard deviation of ASO titres as determined from a prior study. Taken to be 39.3 from a study done in Ujjain, India [17].

d = Margin of error to be tolerated (fixed at 5%; 95% confidence interval).

This yielded a sample size of 238. However, to improve the power of the study and to arrive at the highest possible minimum sample size, we assumed an SD of 50.

$$\text{Hence : } \mathbf{n} = 1.96^2 \text{ x } (50)^2 \ /5^2 = 384$$

A total number of 384 subjects were recruited for the study. The participants were recruited using a multistage sampling technique to avoid bias.

First, three electoral wards were randomly selected from the ten wards in Egor LGA by balloting. Next, the number of government and private schools to be selected were assigned proportionately, based on the total number of each in the selected wards. Thereafter, the schools to be sampled were randomly selected by balloting from a list of schools in each ward which was obtained from the state Ministry of education. In each school, the classes were used as a proxy for the age of the children, as it was expected that school-aged children would be evenly distributed across the six years/classes of primary school by age. Class lists were obtained from the school authorities and the number of students to be selected per class was proportionately distributed across the classes. Finally, with the aid of the class lists, the number of participants to be selected from each class was proportionally distributed between males and females in each class.

The participants so selected were stratified into equal age brackets based on the class interval determined using the Sturge's formula [38] as follows:

$$W = R/K$$

Where:

W – class interval R – range (which is the age range between 6 and 12 years; 6)

K = 1 + 3.322 (logN) (N – the number of items (ages) in the sample population; that is 7)

$$W = 6/1 + 3.322(\log 7) = 1.57 \cong 2$$

Hence, the ages were stratified into three groups with two-year intervals 6–8 years, 9–10 years and 11–12years.

2.1. Data collection

A proforma (Appendix II) was used to collect data. The biodata of participants and their parents' occupation and level of education were used to determine their socioeconomic class (SEC), using the method described by Olusanya *et al* [39] (Appendix III). Information regarding the nature of their housing, the number of rooms and the number of occupants in the child's room, was used to determine the presence or absence of overcrowding. Overcrowding was taken to be more than two children aged 6–12years dwelling in one room [40]. The weight and height measured in standard fashion [41], were inputted into the said proforma.

2.2. Sample collection and laboratory analysis

Venous blood was collected by venipuncture. The sample was centrifuged, serum extracted and ASO determination done by the turbidimetric assay following the recommendation of the manufacturers of the Fortress® diagnostic ASO kit (lot number 202108, 202109, 202110, 202111, 202112) used for the study.

3. Results and interpretation

Serum ASO titre was determined as

$$\frac{\Delta A \text{ (Sample)}}{\Delta A \text{ (Calibrator)}} \text{ x calibrator concentration} = \text{ASO titre (IU / ml)}$$

3.1. Quality control

ASO control sera level 2 was added to each batch of samples and also run separately ten times to determine inter-assay and intra-assay precision respectively. The inter- and intra-assay co-efficient of variability were determined to be 6.13% and 5.08%, giving precision of 93.87%

and 94.92% respectively.

4. Statistical analysis

The data obtained was coded, entered and analyzed using the Statistical Package for Social Sciences (SPSS) spread sheet version 21.0. The values obtained for the primary outcome (serum Anti-Streptolysin O titre) were tested for normality using Shapiro Wilk's test and was found to be positively skewed. The data was then log transformed and attained normal distribution. Statistical tests were applied to the log transformed data. The results were then reverse-log-transformed and presented as geometric mean values and standard deviation at 95% confidence intervals (the reverse-log transformed values were presented in the results because these are the clinically relevant values). The ULN serum ASO titre was determined as the value of serum ASO exceeded by 20% of the study population [21,22] (the serum ASO titre at the 80th percentile). The categorical variables were presented as proportions using charts and tables. T statistical test of significance was used to determine the difference between the geometric mean serum anti-streptolysin O titre for the categorical variables with two groups. ANOVA was used to determine the difference between the geometric mean serum ASO titre for SEC and age groups. The level of significance was set at p value of <0.05. Spearman's rank correlation was used to determine the relationship between serum ASO titres and the age of the study participants. The level of significance was set at p value of <0.05.

5. Results

There were slightly more male than female participants with a male to female ratio of 1.1:1. Approximately half of the study participants were aged between 6 and 8years old. The greater proportion of the participants (76%) lived in over-crowded homes even though most of them (71.1%) were from families in the high SEC (Table 1).

As shown in Fig. 1, the geometric mean serum ASO titre for the study participants was 230.04 ± 1.86 (64.35–785.55) IU/ml while the median (inter-quantile range -IQR) serum ASO titre for the study population was 224.74 IU/ml (140.39–360.67) (see Fig. 1).

The children aged 9–10 years had the highest geometric mean serum ASO titre, 234.53 ± 1.75 (range: 89.18–785.55) IU/ml. However, there was no statistically significant difference in geometric mean serum ASO titre between the age groups ($p = 0.745$). Similarly, although the males had slightly higher mean serum ASO titre (231.90 ± 1.78 IU/ml) than the females (227.93 ± 1.93 IU/ml), the difference was not statistically significant ($p = 0.784$). There was no statistically significant difference noted between the SEC ($p = 0.178$) and between the over-crowded and non-overcrowded home dwellers ($p = 0.058$) (see Table 2).

The ULN for serum ASO titre (the value exceeded by 20% of the study population) [21,22] was 390.76 IU/ml (Table 3). The highest ULN (440.15IU/ml) was noted in children from the high SEC. Furthermore, Table 3 reveals that the highest ULN for age (413.37 IU/ml) was observed in children aged 11–12 years and the lowest (384.86 IU/ml) in the children aged 9–10years. Females had higher ULN for serum ASO titre (393.21 IU/ml) than their male counterparts (387.67 IU/ml).

In the scatterplot of the serum ASO titres with the age of the study population, there was a non-significant, negative correlation (r = -4.8%) between the variables ($p = 0.346$) Fig. 2.

Table 1
Socio-demographic characteristics of the study population.

Age (years)	Frequency (n = 384)	Percentage (%)
6–8	210	54.7
9–10	93	24.2
11–12	81	21.1
Socio-Economic family Class		
High	273	71.1
Middle	84	21.9
Low	27	7.0
Overcrowding		
No	92	24.0
Yes	292	76.0

6. Discussion

The geometric mean serum ASO titre was 230.04 ± 1.86 IU/ml in this study. This is comparable to that observed by Kotby and colleagues [24] in Egypt (245 IU/ml). The concord between the present study and theirs could be accounted for by the similar socio-demographic characteristics of the study participants viz; apparently healthy school-aged children mostly from the high SEC. Likewise, the median serum ASO from our study (224.74IU/ml) compared favourably with that reported by Okello and colleagues [42] (220 IU/ml) in apparently healthy Ugandan children. Both study locales are warm and humid. Regions with similar climatic conditions are known to have similar rates of GAS pharyngitis and thus comparable average serum ASO titre in the apparently healthy population [3]. Clinicians working in regions that share similar climatic conditions would thus expect similar serum ASOT and so can apply the same reference value in the evaluation of patients for non-suppurative post-streptococcal sequelae.

On the contrary, the geometric mean titre in the current study was higher than the values reported from Australia and India by Danchin *et al* [43] and Madaan *et al* [17] respectively. It would have been expected that the similarity in the climatic conditions between these countries and the current study locale should have yielded similar mean ASO titres. However, the higher values in this study might be due to poorer health seeking behavior among the study participants [44]. Poor health seeking behaviour such as delayed presentation to health care facilities would delay early commencement of appropriate antibiotic therapy for GAS, an intervention that is known to blunt the serologic response to GAS and result in lower mean serum ASO titres. The absence of this would result in significant serologic response to GAS and thus higher mean serum ASO titres, as found in this study. Clinicians in such areas can thus expect higher baseline serum values of ASO titres.

The ULN for serum ASO titre of 390.76 IU/ml in this study is similar to the values observed in Egyptian (400 IU/ml and 398.5 IU/ml) [24,45], Ugandan (389 IU/ml) [42] and Ethiopian (360 IU/ml) [25] studies. The children in the present study had similar socio-demography (over-crowding, SEC, health seeking behaviour) and probably similar GAS pharyngitis rates as the participants in the other African studies. Studies from Yemen (276.2 IU/ml) [46], Fiji Islands (276 IU/ml) [47], India (262 IU/ml, 242 IU/ml, 239 IU/ml, 305IU/ml) [27–29,48], USA (240IU/ml, 330 IU/ml) [21,26] and Australia (320IU/ml) [43], reported lower ULN in children, despite the warm weather in these areas. Further buttressing the fact that the climate alone was not significant enough to unify the serum ASO titres. GAS pharyngitis rates appeared to play a greater role viz; the incidence of GAS pharyngitis in the study locale is 48% [11] compared to 14.7% [47] and 14% [49] in Fiji Island and Australia respectively. Renneberg *et al* [50] found even lower ULN serum ASOT in Swedish children, another country renowned for its hygienic practices and low GAS pharyngitis rates. It would be safe to infer therefore that improvement in environmental sanitation and overall hygiene practices would result in a reduction in the ULN for serum ASOT and reduce the incidence of ARF and GAS pharyngitis, even in areas with tropical weather.

The current study observed higher ULN for serum ASO titre compared to studies in Dar Es Salaam (200 IU/ml),30 and Ujjain (131.41IU/ml) [17]. This observation appears unusual as these regions share similar weather and socio-demographic characteristics as the area where our study was carried out. However, seasonal variation in GAS pharyngitis might explain the observed differences. In this study, the participants were recruited during the rainy season (April to July; spring and summer months) when GAS pharyngitis incidence is usually higher, whereas in these other studies, participants were enrolled during the

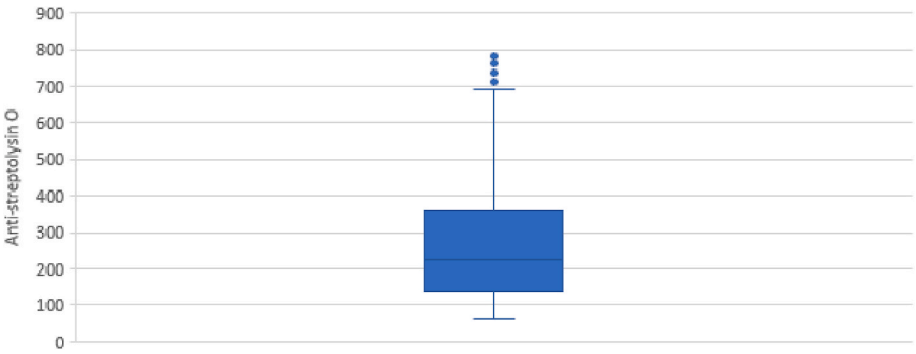

Fig. 1. Box and Whisker plot showing the median serum ASO titre of the study participants.

Table 2
Relationship between the geometric mean serum ASO titre and socio-demographic characteristics of the study population.

Variable	N (384)	Log mean ASO ± SD	Geometric Mean ASO ± SD (IU/ml)		P-value
Age				**F – test** 0.294	0.745
6–8 years	n = 210	2.36 ± 0.27	232.42 ± 1.87		
9–10	n = 93	2.37 ± 0.24	234.53 ± 1.75		
11–12	n = 81	2.34 ± 0.28	219.68 ± 1.90		
Gender				**t – test** 0.275	0.784
Male	n = 203	2.36 ± 0.25	231.90 ± 1.78		
Female	n = 181	2.35 ± 0.28	227.93 ± 1.93		
Socioeconomic class				**F – test** 1.770	0.178
High	n = 273	2.37 ± 0.28	235.45 ± 1.91		
Middle	n = 84	2.32 ± 0.20	209.36 ± 1.62		
Low	n = 27	2.38 ± 0.28	243.67 ± 1.92		
Overcrowding				**t – test** 1.911	0.058
No	n = 92	2.40 ± 0.23	253.98 ± 1.73		
Yes	n = 292	2.34 ± 0.27	222.95 ± 1.88		

The p – values of statistical significance were calculated using the log-transformed data.

dryer months of the year (September to December in Dar es Salaam and New Delhi; and December to February in Ujjain) when the incidence of GAS pharyngitis is low and viral pharyngitis predominates [43]. Gharazozloo and Ghavamian [51] and Kotby *et al* [24] also reported similar seasonal variation in serum ASOT, with higher values during the rainy season. It would be safe to assume that clinicians can expect to have more cases of ARF during the wet months. Hence, it would be advisable for Governmental and Public health interventions aimed at reducing the incidence of ARF to be deployed especially during the wet months. The administration of flu vaccines and Palivizumab, during the winter months in western countries, for the prevention of flu and respiratory syncytial virus pneumonia respectively, are examples of effective implementation of such interventions.

In the present study, it was found that the ULN for serum ASO increased with age, the highest values were found in the children aged 11–12 years. This was not surprising, owing to the higher rates of viral pharyngitis in the younger age group [52]. Similarly, older children with prior GAS infection are likely to have more pronounced serologic response to subsequent infections. As such, older children would be expected to have higher serum ASO titres. The observation in the present study is comparable to the findings of Kaplan *et al* [26], Danchin *et al* [43] and Asfaw *et al* [25]. This may represent progressively greater risk of ARF with increasing age among school-going children. There was however no statistically significant difference in the ULN for serum ASO titre with age groups. This finding supports the use of a single cut-off value of ULN for the diagnosis of ARF in Nigerian school-aged children and clinicians do not need to develop age specific ULN for serum ASO titre.

The ULN for serum ASO titre in this study was noted to be slightly higher (393.19IU/ml) in females compared to males (387.70IU/ml), this difference was however not statistically significant. This observation may suggest that irrespective of gender, a single cut-off value for serum ASO titre, as determined in this study, can be applied among Nigerian children as evidence of an antecedent GAS infection. Solanki *et al* [53] and Madaan and colleagues [17] in India and Asfaw *et al* [25] in Ethiopia have also previously reported no significant difference in the

Table 3
Distribution of serum ASO percentiles by age, gender, socio-economic class and presence or absence of over-crowding.

Percentiles	ASOT value (IU/ml)	Age			Gender		Socio-Economic class			Overcrowding	
		6–8 years n = 210	9–10 years n = 93	11–12 years n = 81	Male n = 203	Female n = 181	High n = 273	Middle n = 84	Low n = 27	Yes n = 292	No n = 92
5th	79.58	73.52	90.61	78.32	90.43	69.37	79.58	77.57	84.39	77.2	103.2
10th	96.06	95.68	102.4	87.66	110.41	87	93.03	105.78	96.05	91.26	117.98
25th	140.38	142.79	156.68	134.4	156.68	136.33	140.38	150.94	133.41	139.38	184.88
50th	224.75	232.17	244.91	200.4	218.47	244.91	243.44	208.74	256.86	208.74	250.67
75th	360.66	360.66	335.27	381.5	360.66	365.85	384.5	302.06	413.33	341.43	374.71
80th	**390.76**	**389.05**	**384.86**	**413.33**	**387.7**	**393.19**	**440.15**	**341.43**	**413.33**	**390.75**	**396.64**
90th	524.45	524.45	477.97	551.44	524.45	551.32	561.18	374.89	655.09	524.45	555.52
95th	672.36	658.72	651.18	681.55	675.93	673.6	713.35	417.93	675.46	674.37	672.05

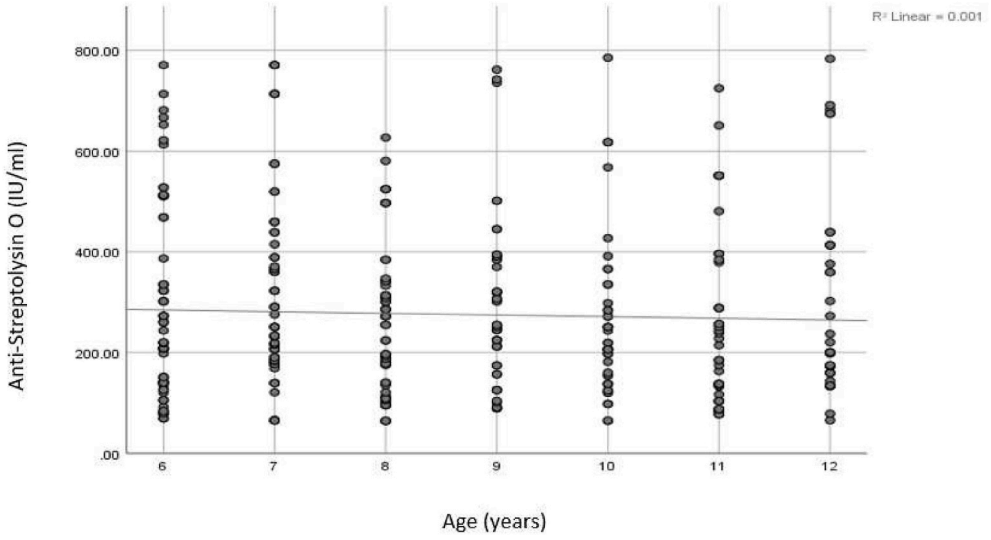

Fig. 2. Scatter plot showing the relationship between age and serum ASO titre.

ULN for serum ASO titre between males and females. The similarity in findings between the current study and those of other researchers is likely because the pupils are exposed to the same risk of GAS pharyngitis and so would acquire the infection at the same rate and respond similarly irrespective of gender. The slightly higher titre among females in the current study may be reflective of a tendency to greater immunologic response in females compared to the males. The lack of statistical significance in the difference between serum ASO titres across gender negates the need to generate gender specific ULN.

The turbidimetric assay method employed in this study is more sensitive and avoids approximation of results compared to the haemolysin inhibition and latex agglutination technique employed in previous studies [17,25,43,53]. However, results from the different methods have been shown to be comparable and thus, the serum ASOT and ULN values can be compared with the findings from other studies irrespective of the methods of estimation employed.

7. Conclusion

The observed ULN for serum ASO titre among school-aged children in the study locale is 390.76 IU/ml. This value is higher than the pre-set and currently used value of 200 IU/ml [23]. The Serum ASO titre among the school-aged children in this study locale does not vary significantly with age, gender and SEC.

Recommendation

1. Clinicians should seek to apply higher values of serum anti-streptolysin-O as proof of antecedent GAS infection.
2. A single cut-off value can be used irrespective of age, gender and socio-economic class.

Strength of the study

The turbidimetric assay employed in this study is highly sensitive and avoids approximation of serum ASO titres compared to haemolysin inhibition and latex agglutination techniques utilized in earlier studies.

Limitation of the study

Information about the prior GAS pharyngitis used to exclude subjects was obtained by recall which can be unreliable.

Line of future research

A large scale, multi-national, multi-centre study across West Africa should be carried out to determine the ULN for serum ASOT in the sub-region.

CRediT authorship contribution statement

Emmanuel U. Eyo-Ita: Conceptualisation, Data curation, Formal analysis, Funding acquisition (out of pocket), Investigation, Methodology, Project administration, Resources, Software, Visualisation, Writing – original draft, Writing – review & editing. **Wilson E. Sadoh:** Conceptualisation, Data curation, Methodology, Project administration, Supervision, Writing – review & editing. **P.O. Abiodun:** Project administration, Supervision, Writing – review & editing. **Ifueko A. Eyo-Ita:** Data curation, Funding acquisition(out of pocket), Formal analysis, Investigation, Supervision, Analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcrp.2024.200256.

References

[1] World Health Organization, WHO Preferred Product Characteristics for Group A Streptococcus Vaccines, 2018. Geneva.
[2] World Health Organization, The Current Evidence for the Burden of Group A Streptococcal Diseases, 2005. Geneva.
[3] M.W. Cunningham, Pathogenesis of group A streptococcal infections, Clin. Microbiol. Rev. 13 (3) (2000) 470–511.
[4] G.J. Worrall, Acute sore throat, Can. Fam. Physician 53 (11) (2007 Nov) 1961–1962.
[5] G.J. Worrall, Acute sore throat, Can. Fam. Physician 57 (3) (2011) 791–794.
[6] B. Spellerberg, C. Brandt, Laboratory Diagnosis of Streptococcus pyogenes (group A streptococci), in: Streptococcus Pyogenes : Basic Biology to Clinical Manifestations, 2016, pp. 1–2.
[7] World Health Organization, Rheumatic Fever and Rheumatic Heart Disease Report by the Director-General, vol. 2016, 2018. Geneva.
[8] D.A. Watkins, C.O. Johnson, S.M. Colquhoun, G. Karthikeyan, A. Beaton, G. Bukhman, et al., Global, regional, & national burden of rheumatic heart disease, 1990-2015, N. Engl. J. Med. 377 (8) (2017) 713–722.
[9] W. Sadoh, A.E. Sadoh, Need for a clinical decision rule for the management of pharyngitis in Nigeria, Niger. J. Paediatr. 40 (1) (2013) 1–5.
[10] A.C. Owobu, W.E. Sadoh, O. Oviawe, Streptococcal throat carriage in a population of nursery and primary school pupils in Benin City , Nigeria, Niger. J. Paediatr. 40 (4) (2013) 389–394.

[11] W.E. Sadoh, A.E. Sadoh, A.O. Oladipo, O.O. Okunola, Bacterial Isolates of tonsillitis and pharyngitis in a paediatric casualty setting, J. Med. Biomed. Res. 7 (1 & 2) (2008) 37–44.
[12] C. Nkoke, A. Menanga, J. Boombhi, D. Chelo, S. Kingue, A new look at acquired heart diseases in a contemporary sub-saharan african pediatric population: the case of Yaoundé, Cameroon, Cardiovasc. Diagn. Ther. 5 (6) (2015) 428–434.
[13] J.R. Carapetis, A.C. Steer, E.K. Mulholland, M. Weber, The global burden of group A streptococcal diseases, Lancet Infect. Dis. 5 (11) (2005 Nov) 685–694.
[14] O. Ogunbi, H.O. Fadahunsi, I. Ahmed, A. Animashaun, S.O. Daniel, D.U. Onuoha, et al., An epidemiological study of rheumatic fever and rheumatic heart disease in lagos, J. Epidemiol. Community Health 32 (1) (1978) 68–71.
[15] M.D. Seckeler, T.R. Hoke, The worldwide epidemiology of acute rheumatic fever and rheumatic heart disease, Clin. Epidemiol. 3 (1) (2011) 67–84.
[16] M.U. Sani, K.M. Karaye, M.M. Borodo, Prevalence and pattern of rheumatic heart disease in the Nigerian savannah: an echocardiographic study, Cardiovasc J Afr 18 (5) (2007) 295–299.
[17] R. Madaan, A. Pathak, H.L. Tiwari, M. Dhaneria, R. Gupta, A. Pathak, Anti streptolysin O (ASO) titers in normal healthy children aged between 5 to 15 years in Ujjain region, International Journal of Paediatric Research 4 (2) (2017) 122–126.
[18] A.C. Steer, S. Vidmar, R. Ritika, J. Kado, M. Batzloff, A.W.J. Jenney, et al., Normal ranges of streptococcal antibody titers are similar whether streptococci are endemic to the setting or not, Clin. Vaccine Immunol. 16 (2) (2009) 172–175.
[19] E.M. Ayoub, B. Nelson, S.T. Shulman, D.J. Barrett, J.D. Campbell, G. Armstrong, et al., Group A streptococcal antibodies in subjects with or without rheumatic fever in areas with high or low incidences of rheumatic fever, Clin. Diagn. Lab. Immunol. 10 (5) (2003) 886–890.
[20] B.A. Choby, Diagnosis and treatment of streptococcal pharyngitis, Am. Fam. Physician 79 (5) (2009) 383–390.
[21] L.W. Wannamaker, E.M. Ayoub, Antibody titers in acute rheumatic fever, Circulation 21 (April) (1960) 598–614.
[22] E.M. Ayoub, L.W. Wannamaker, Evaluation of the streptococcal deoxyribo-Nuclease B and Diphosphopyridine Nucleotidase antibody tests in acute rheumatic fever and acute glomerulonephritis, Pediatrics 29 (4) (1962 Apr 1) 527–538, cited 2021 Dec 28.
[23] A. Shet, E.L. Kaplan, Clinical use and interpretation of group A streptococcal antibody tests: a practical approach for the pediatrician or primary care physician, Pediatr. Infect. Dis. J. 21 (5) (2002) 420–426.
[24] A.A. Kotby, N.M. Habeeb, S. Ezz El Elarab, Antistreptolysin O titer in health and disease: levels and significance, Pediatr. Rep. 4 (1) (2012) 25–29.
[25] T. Asfaw, D. Shenkute, M. Tilahun, N. Zegeye, Antistreptolysin O titers : normal values for children ages 5 to 15 at debre berhan referral hospital , Ethiopia, J. Fam. Med. Health Care 4 (1) (2018) 1–4.
[26] E.L. Kaplan, C.D. Rothermel, D.R. Johnson, Antistreptolysin O and anti-deoxyribonuclease B titers: normal values for children ages 2 to 12 in the United States, Pediatrics 101 (1 I) (1998 Jan 1) 86–88.
[27] K.B. Mahendrappa, Rajendra. Upper limit of normal antistreptolysin-O titer in healthy school children, Indian Pediatr. 47 (7) (2010 Jul) 629.
[28] S. Sethi, K. Kaushik, K. Mohandas, C. Sengupta, S. Singh, M. Sharma, Anti-streptolysin O titers in normal healthy children of 5-15 years, Indian Paediatrics 40 (11) (2003) 1068–1071.
[29] M.G. Karmarkar, V. Venugopal, L. Joshi, R. Kamboj, Evaluation & revaluation of upper limits of normal values of anti-streptolysin O & anti-deoxyribonuclease B in Mumbai, Indian J. Med. Res. 119 (suppl) (2004) 26–28.
[30] F.S. Mhalu, R. Matre, Antistreptolysin O and antideoxyribonuclease B titres in blood donors and in patients with features of nonsuppurative sequelae of group A streptococcus infection in Tanzania, East Afr. Med. J. 72 (1) (1995) 33–36.
[31] A. Beaton, J. Carapetis, The 2015 revision of the Jones criteria for the diagnosis of acute rheumatic fever: implications for practice in low-income and middle-income countries, Heart Asia 7 (2) (2015) 7–11.
[32] C.O. Duru, N. Mesiobi-Anene, F.O. Akinbami, Pediatric heart failure among emergency room admissions in a tertiary health centre in southern Nigeria, Nigerian Journal of Cardiology 13 (1) (2016) 62–66.
[33] C.C. Uzodimma, F.I. Dedeke, V. Nwadike, O. Owolabi, G. Arifalo, O. Oduwole, A study of Group A streptococcal pharyngitis among 3–15-year-old children attending clinics for an acute sore throat, Nigerian Journal of Cardiology 14 (2) (2017) 97–102.
[34] M.A. Gerber, R.S. Baltimore, C.B. Eaton, M. Gewitz, A.H. Rowley, S.T. Shulman, et al., Prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis A scientific statement from the American heart association rheumatic fever, endocarditis, and kawasaki disease committee of the council on cardiovascular disease, Circulation 119 (11) (2009) 1541–1551.
[35] G.P. DeMuri, E.R. Wald, The group A streptococcal carrier state reviewed : still an enigma, Journal of Pediatric Infectious Diseases Society 3 (4) (2014) 336–342.
[36] R.H. Webb, C. Grant, A. Harnden, Acute rheumatic fever, BMJ 351 (2015 Jul 14) 3443.
[37] National Health Research Ethics Committee, Policy Statement Regarding Enrollment of Children in Research in Nigeria, 2016.
[38] H.A. Sturges, The choice of a class interval, J. Am. Stat. Assoc. 21 (153) (1926) 65–66.
[39] A.O. Olusanya, E. Okpere, M. Ezimokhai, O. Olusanya, O. Okpere, The importance of social class in fertility control in a developing country, West Afr J Med 4 (1985) 205–212.
[40] O. Makinde, K. Björkqvist, K. Österman, Overcrowding as a risk factor for domestic violence and antisocial behaviour among adolescents in Ejigbo, Lagos, Nigeria, Global Mental Health (Camb) 3 (2016) 1–9. Available from: https://www.cambridge.org/core/journals/global-mental-health/article/overcrowding-as-a-risk-factor-for-domestic-violence-and-antisocial-behaviour-among-adolescents-in-ejigbo-lagos-nigeria/F6EF87F4E2F8B18D8BB6AC15FCABB1D0. (Accessed 29 December 2021).
[41] WHO Expert Committee on Physical Status, The Use and Interpretation of Anthropometry, 1995. Geneva.
[42] E. Okello, M. Murali, J. Rwebembera, J. Atala, A.C. Bowen, N. Harik, et al., Cross-sectional study of population-specific streptococcal antibody titres in Uganda, Arch. Dis. Child. 105 (9) (2020) 825–829.
[43] M.H. Danchin, J.B. Carlin, W. Devenish, T.M. Nolan, J.R. Carapetis, New normal ranges of antistreptolysin O and antideoxyribonuclease B titres for Australian children, J. Paediatr. Child Health (41) (2005) 583–586.
[44] United Nations International Children's Emergency Fund, UNICEF Data: Monitoring the situation of children and women (2022). Available from Monitoring the Situation of Children and Women | United Nations iLibrary (un-ilibrary.org) (Accessed 19 December 2022).
[45] Khattab AhmedA, Bahbah WaelA, Abou-Elela DaliaH, Mohammed TamerS, Determine upper limit of normal range of antistreptolysin-O titer in normal school children from 5 to 15 years old, Menoufia Medical Journal 34 (1) (2021) 204–209.
[46] A.A. Khaled, A.A. Hassan, Anti streptolysin O; normal values for healthy children aged from 5 to 15 Years old in sana'a city-Yemen, Ann. Clin. Lab. Res. 3 (1) (2015) 1–5, 1.
[47] A.C. Steer, A.W.J. Jenney, J. Kado, M.F. Good, M. Batzloff, G. Magor, et al., Prospective surveillance of streptococcal sore throat in a tropical country, Pediatr. Infect. Dis. J. 28 (6) (2009) 477–482.
[48] N. Saini, D. Kumar, S. Swarnim, D. Bhatt, S. Kishore, 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, Ann. Pediatr. Cardiol. 12 (3) (2016) 195–200.
[49] M.I. McDonald, R.J. Towers, R.M. Andrews, N. Benger, B.J. Currie, J.R. Carapetis, Low rates of streptococcal pharyngitis and high rates of pyoderma in Australian aboriginal communities where acute rheumatic fever is hyperendemic, Clin. Infect. Dis. 43 (6) (2006 Sep 15) 683–689.
[50] J. Renneberg, M. Soderstrom, K. Prellner, A. Forsgren, P. Christensen, Age-related variations in anti-streptococcal antibody levels, Eur. J. Clin. Microbiol. Infect. Dis. 8 (9) (1989) 792–795.
[51] R. Gharagozloo, P. Ghavamian, The range of antistreptolysin-O titer among 3129 healthy individuals in winter and summer in Tehran, Iran, Pahlavi Med. J. 7 (3) (1976) 323–333.
[52] M. Regoli, E. Chiappini, F. Bonsignori, L. Galli, M. De Martino, Update on the management of acute pharyngitis in children, Ital. J. Pediatr. 37 (1) (2011) 10.
[53] S. Solanki, P. Purkayastha, S.K. Jaswal, S. Faujdar, A. Sharma, To study the seroprevalence of anti-streptolysin O (ASO) titers in children aged between 5 to 18 Years in rural and urban region in solan district , Himachal Pradesh : a hospital based study, International Journal of contemporary medical research 7 (10) (2020) 7–9.