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Determine upper limit of normal range of antistreptolysin-O titer in normal school children from 5 to 15 years old

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Objective
To determine upper limit of normal range of antistreptolysin-O (ASO) titer in normal school children from 5 to 15 years old in Menoufia Governorate.

Background
Although ASO titer has provided a useful guideline to physicians this has been shown to vary with age, geographical location, and site of infection.

Patients and methods
A cross-sectional study was carried out in Menoufia Governorate and the samples were collected from outpatient clinics of Menoufia university hospital other than infection (by advertising in the outpatient clinics for analysis streptococcus laboratory for free in children aged 5–15 years) in the period from July 2016 to May 2017. Two hundred children aged 5–15 years were participated in this study after consent from their parents. The children were categorized into two groups: group 1: include children aged 5–10 years and group 2: include children aged 11–15 years. Full history, routine, physical examination, and special investigations were taking.

Results
There was statistically significant difference between residence and social classes in the studied group. Also, there was statistically significant difference between age groups regarding ASO titer among rural patients. There was highly significant correlation between number of attacks of acute follicular tonsillitis with ASO titer level, age groups, and social classes.

Conclusion
The upper limit of normal ASO titer in normal Egyptian children is quite high, reaching up to 398.5 IU/ml. Therefore, an isolated high ASO titer is not sufficient to diagnose acute rheumatic fever. Basal levels of ASO titer increase with age but age does not affect the peak level during acute streptococcal infection. Additional studies will be required for establishment of standard values to avoid over diagnosis of acute rheumatic fever and complications of long-acting penicillin.

Keywords:
antistreptolysin-O titer, school children, streptococcal infection

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Introduction

Group A streptococcus (GAS) is synonymous with *Streptococcus pyogenes*, the only species within this group of β-hemolytic streptococci. GAS is one of the leading pathogenic bacteria that infects children and adolescents, and is associated with a wide spectrum of infections and disease states. Worldwide, there are estimated to be more than 600 million cases of GAS pharyngitis and more than 100 million cases of GAS pyoderma annually [1]. Acute rheumatic fever (ARF) and rheumatic heart disease are the most serious complication of group A streptococcal infection. Although ARF is relatively rare in developed economies, it is much more common in the developing world and among aboriginal populations [2]. Acute post-streptococcal glomerulonephritis is an inflammatory disease of the kidneys which occurs 2–3 weeks after skin or throat infection with a particular type of bacteria called GAS or occasionally groups C or G streptococcus. In the Northern Territory, most cases follow skin rather than throat infections because skin infections are the more common problem. Not all types of streptococcus cause kidney problems but only those caused by nephrogenic strains. Therefore, in the setting of acute post-streptococcal glomerulonephritis, obtaining and characterizing streptococcal isolates may better guide public health response [3]. Antistreptolysin-O (ASO or ASLO) is the antibody made against streptolysin-O, an immunogenic oxygen-labile hemolytic toxin produced by most strains of group A and many strains of groups C and G streptococci. The O in the name stands for oxygen-labile; the other related toxin being

oxygen-stable streptolysin-S. The main function of streptolysin-O is to cause hemolytic (the breaking open of red blood cells) in particular, beta-hemolysis [4]. The aim of this study was to determine upper limit of normal (ULN) range of ASO titer in normal school children from 5 to 15 years old in Menoufia Governorate.

Patients and methods

A cross-sectional study was carried out in Menoufia governorate and the samples were collected from outpatient clinics of Menoufia University Hospital in the period from July 2016 to May 2017. Two hundred children aged 5–15 years were participated in this study after consent from their parents. The children were categorized into two groups: group 1: include children aged 5–10 years and group 2: include children aged 11–15 years.

Ethical consideration: the study was approved by the ethical committee of Menoufia Faculty of Medicine and an informed consent obtained from all patient's guardian before the study was commenced.

Selection criteria for the patients:
The patients included in this study were selected according to inclusion and exclusion criteria.

Inclusion criteria: both sex healthy children ages from 5 to 15 years, urban and rural.

Exclusion criteria: history of ARF or rheumatic heart diseases, history in previous 14 days of sore throat, skin sore or received antibiotics, and systemic infection.

All cases were subjected to the following:
(1) Complete history: history of recent sore throat, skin sores or received antibiotics, history of temperature above 38°C and history of rheumatic fever or rheumatic heart diseases
(2) Full clinical examination: chest examination, cardiac examination to exclude congenital heart and rheumatic heart diseases, abdominal examination, temperature to exclude high fever, throat examination to exclude pharyngitis and skin examination to exclude impetigo
(3) Assess the socioeconomic status of the studied group according to El Gilany *et al.* [5], scoring system
(4) Investigations: ASO, throat culture, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

ASO
ASO are the specific antibodies to streptolysin-O, an extracellular enzyme produced by Lancefield group A, β-hemolytic streptococci (*S. pyogenes*). Serum ASO causes agglutination of latex particles coated with streptolysin-O. The agglutination of the latex particles is proportional to the concentration the streptolysin-O and can be measured by turbidimetry. Reagents: absorbance of the blank over 0.900 at 540 nm.

Reagent and instrument used in the antistreptolysin-O test

ASO antigen: a stabilized buffered suspension of polystyrene latex particles coated with streptolysin-O and 0.1% sodium azide as preservative. Shake well prior to use.

ASO positive control: human serum containing more than 200 IU/ml ASO and 0.1% sodium azide as preservative.

ASO negative control: human serum containing 0.1% sodium azide as preservative.

Sufficient disposable pipettes, glass test slide, timer, test tubes, pasture pipettes and rubber bulb, serological pipettes, and safety bulb.

Procedure for antistreptolysin-O test

Bring all test reagents and samples to room temperature, use a disposable pipette to draw up and place one free-falling drop of each undiluted sample into its identified circle of the slide. Retain each pipette for mixing in step 5, deliver one free-falling drop of positive and negative control into its identified circle, mix the ASO latex reagent by gently shaking. Add one free-falling drop of reagent to each control and sample, using the flattened end of the appropriate plastic pipette as a stirrer (step 2), thoroughly mix each sample with reagent within the full area of the circle, discard the disposable pipette, slowly rock the slide for exactly 2 min and observe for agglutination under a high-intensity light.

C-reactive protein (Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 2005)

Serum CRP causes agglutination of the latex particles coated with antihuman CRP. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidimetry.

Reagent A: glycine buffer 0.1 mol/l, sodium azide 0.95 g/l, pH 8.6. Reagent B: suspension of latex particles coated with anti-human CRP antibodies, sodium azide 0.95 g/l.

Reagent kit

8G65 CRP is supplied as a liquid, ready-to-use, two-reagent kit which contains:

3 × 38 ml.
3 × 43 ml.

Estimated tests per kit: 900. Calculation is based on the minimum reagent fill volume per kit.

Theactiveingredientsinclude Ethylenediaminetetraacetic acid disodium salt dihydrate 1.86%,Sodium azide 0.09% and Latex particle adsorbed anti-human CRP 0.15%

The instrument specific operation

The instrument the instrument-specific operations manual for information on results calculations. ARCHITECT System Operations Manual and AEROSET System Operations Manual.

Procedure: bring the working reagent to 37°C, zero the instrument with distilled water, pipette into a cuvette: (working reagent: 1 ml, standard(s) or sample: 7 μl), mix and immediately insert cuvette into the instrument, start stopwatch, record the absorbance at 540 nm after 10 s (A1) and after 2 min (A2). Reference values: serum value in child is up to 5 mg/l. This range is given for orientation only; each laboratory should establish its own reference range.

Method of erythrocyte sedimentation rate test

When anticoagulated whole blood is allowed to stand in a narrow vertical tube for a period of time, the red blood cells under the influence of gravity – settle out from the plasma. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after 1 h (mm/h).

The Westergren method requires collecting 2 ml of venous blood into a tube containing 0.5 ml of sodium citrate. It should be stored no longer than 2 h at room temperature or 6 h at 4°C. The blood is drawn into a Westergren–Katz tube to the 200 mm mark. The tube is placed in a rack in a strictly vertical position for 1 h at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The distance of fall of erythrocytes, expressed as millimeters in 1 h, is the ESR. Average values in healthy child are: less than 15 mm/h; in healthy females, they are somewhat higher: less than 20 mm. The values are slightly higher in old age, in both sexes.

Throat swab collection

Materials

Culturette tube, tongue blade, gloves (mask and eye protection, as needed), patient identification label (to be placed on Culturette), laboratory request form (properly labeled with patient identification label).

Procedure

Apply gloves, eye protection, and mask (mask as needed, depending on anticipated exposure), keeping cotton applicator sterile, remove from Culturette tube, depress tongue with tongue blade, if indicated, insert cotton tip of applicator to back of patient's throat, and swab area, place applicator in Culturette tube, label specimen with patient's name (first and last), date of birth, date and time of collection, and source of specimen, pyogenes is cultured on a growth medium called blood agar. Agar is a gel that is made from the cell walls of red algae. Blood agar is made from agar gel and sheep's blood. When the throat swab reaches the laboratory, it is wiped across a blood agar plate. The plate is allowed to incubate for 24–48 h to allow the growth of bacteria. If the organism is a group A hemolytic streptococcus, the area immediately around the bacterial colony will be cleared of red blood cells. Hemolytic streptococci dissolve (lyse) red blood cells, leaving a clear zone surrounding the colony.

Statistical analysis

Results were tabulated and statistically analyzed by using a personal computer using Microsoft Excel 2016 and SPSS, version 21 (SPSS Inc., Chicago, Illinois, USA). Statistical analysis was done using: descriptive: e.g., percentage (%), mean and SD. Analytical: that includes: χ^2 and analysis of variance F test.

Results

Results showed that there was no statistically significant difference between age and sex in the studied group. Also, there was no statistically significant difference between residence and sex in the studied group. While, there was statistically significant difference between residence and social classes in the studied group (Table 1).

The current study shows that there was statistically significant difference between age groups regarding ASO titer among rural patients. On the other hand, there was no statistically significant difference between age groups regarding ASO titer among urban patients.

Also, there was no statistically significant difference between residence regarding ASO titer (Table 2).

The present study showed that there was no statistically significant difference between social classes regarding ASO titer and ESR. On contrast, there was highly significant correlation between number of attacks of acute follicular tonsillitis and ASO titer level (Table 3).

Results indicated that there was high significant correlation between number of attacks of acute follicular tonsillitis and age groups. Also, there was a significant correlation between number of attacks of acute follicular tonsillitis and social classes (Table 4).

Table 1 Age distribution among males and females as well as frequency of social classes and sex among rural and urban

Age	Female [*n* (%)]	Male [*n* (%)]	Total [*n* (%)]	χ^2	*P*
5-10	58 (58.0)	65 (65.0)	123 (61.5)	3.125	0.373
11-15	42 (42.0)	35 (35.0)	77 (38.5)		
Total	100 (100.0)	100 (100.0)	200 (100.0)		
Social classes	Rural	Urban	Total	χ^2	*P*
High	28 (30.1)	23 (21.5)	51 (25.5)	3.493	0.0174*
Moderate	32 (34.4)	50 (46.7)	82 (41.0)		
Low	33 (35.5)	34 (31.8)	67 (33.5)		
Sex	Rural	Urban	Total	χ^2	*P*
Female	49 (52.7)	51 (47.7)	100 (50.0)	0.502	0.478
Male	44 (47.3)	56 (52.3)	100 (50.0)		

*8 P less than 0.05

Discussion

In the current study, it was found that there was no statistically significant difference between age and sex in the studied group. Children were categorized into two groups. Group I included 123 children in the age group of 5–10 years constituting 61.5%. Group II includes 77 children in the age group of 11–15 years constituting 38.5% for determination of age related ASO titer. Our study showed also nonsignificant correlations between age and ASO titer among the studied groups and among rural or urban patients. Our study showed also nonsignificant correlations between age and ASO titer among the studied groups and among rural or urban patients. The increase in the levels of ASO titer with age was demonstrated in a study done by Danchin MH *et al.* [4]. In contrast, Gerber MA, *et al.* [6] reported the results of a study of children aged 4–14 years that provides a local reference range for ASO titer for children in urban Australia. They found that the mean titer of ASO in children over 10 years of age (320 IU/ml) was significantly lower than those between 5 and 10 years, although it was higher than those below 5 years and could lead to substantial over diagnosis of recent *S. pyogenes* infections. Steer *et al.* [7] demonstrated the normal ranges of the ASO titer in all age groups in a Pacific island country. A total of 424 serum samples from people of all ages (with a sample enriched for school-aged children) were tested for their ASO titer. Reference value, including titer

Table 2 Relation between age groups and antistreptolysin-O titer among rural and urban patients

Age groups	ASO titer among rural patients [*n* (%)]						χ^2	*P*
	<100	100-124	125-149	150-174	175-199	200-478		
Group-I (5-10)						20 (64.5)	6.515	0.259
Group-II (11-15)	14 (41.2)	8 (66.7)	4 (80.0)	3 (50.0)	3 (60.0)	11 (35.5)		
Total	34 (100.0)	12 (100.0)	5 (100.0)	6 (100.0)	5 (100.0)	31 (100.0)		
	ASO titer among urban patients							
Group-I (5-10)	31 (64.6)	5 (62.5)	1 (20.0)	4 (100.0)	8 (66.7)	24 (80.0)	9.574	0.088
Group- II (11-15)	17 (35.4)	3 (37.5)	4 (80.0)	0	4 (33.3)	6 (20.0)		
Total	48 (100.0)	8 (100.0)	5 (100.0)	4 (100.0)	12 (100.0)	30 (100.0)		
Residence groups	<100	100-124	125-149	150-174	175-	200-478		
Rural	34 (41.5)	12 (60.0)	5 (50.0)	6 (60.0)	5 (29.4)	31 (50.8)	3.67	0.354
Urban	48 (58.5)	8 (40.0)	5 (50.0)	4 (40.0)	12 (70.6)	30 (49.2)		
Total	82 (100.0)	20 (100.0)	10 (100.0)	10 (100.0)	17 (100.0)	61 (100.0)		

ASO, antistreptolysin-O.

Table 3 Comparison between social classes and antistreptolysin-O titer as well as correlation of antistreptolysin-O titer with number of attacks of acute follicular tonsillitis

	Social class			*F*	*P*
	High	Mod	Low		
ASO titer					
Mean±SD	164.19±96.26	182.79±106.83	179.74±122.67	0.481	0.619
ESR titer					
Mean±SD	16.64±7.27	19.29±6.60	18.55±7.19	2.29	0.103
ASO titer	Pearson correlation			*P*	
No of attacks	0.514			0.001*	

ASO, antistreptolysin-O; ESR, erythrocyte sedimentation rate; *F*, analysis of variance *F* test. *Significant.

Table 4 Distribution of age and social classes of the studied groups according to number of attacks of acute follicular tonsillitis

Variables	Groups	5-10 years		11-15 years	Student *t* test	*P*
Number of attacks	Mean±SD	1.543±1.02		0.748±0.95	6.2	0.001*
	Range	0-4		0-4		
	Social class	High	Moderate	Low	Kruskal-Wallis test	*P*
Number of attacks	Mean±SD	1.23±1.25	1.67±1.03	2.22±0.83	2.67	0.033*
	Range	0-4	0-3	1-3		

*Significant.

that was 80% of the ULN, was obtained by regression analysis by use of a curve-fitting method instead of the traditional nonparametric approach.

Normal value for the ASO titer rose sharply during early childhood and then declined gradually with age. The estimated titer that was 80% of the upper limit or normal at age 10 years were 287 IU/ml for ASO. In the study by Kotby *et al.* [8] determined ASO titer in normal children and in those with rheumatic fever and tonsillitis and studied the variation of ASO titer with age and season. They found that ULN ASO titer was 400 IU in healthy child, 200 IU in rheumatic heart disease, and 1600 IU in healthy children with history of repeated follicular tonsillitis more than three times a year. Significantly high levels were seen in ARF first attack when compared to healthy child and acute follicular tonsillitis. ASO titer was significantly high in children over 10 years of age, during winter and in those with acute rheumatic carditis. ASO titer showed significant direct correlation with the number of attacks of tonsillitis. Egyptian children have high ULN ASO titer reaching 400 IU. In the study done by Asfaw *et al.* [9] reported that the ASO ULN for the both male and female children was 360 IU/ml with a median 200 IU/ml. The ASO ULN for both sex were 320 IU/ml with a median of 200 IU/ml. The highest ASO ULN was observed for the age group of 9–12 years (400 IU/ml with median of 200 IU/ml) followed by 360 IU/ml for the age group 5–8 years and age group 13–15 years with a median of 200 IU/ml. The ASO ULN in Asfaw *et al.* [9] study was found to be roughly similar to those reported from other regions. In comparison with data from the Minnesota, USA (333 IU/ml) Wannamaker and Ayoub [10], Mumbai, India (305 IU/ml) Karmarkar *et al.* [11], Australia (320 IU/ml) Danchin *et al.* [4], and Korea (326 IU/ml) Kim and Lee [12]. Relatively higher ASO ULN was obtained from study conducted in Sana'a, Yemen (276.2 IU/ml) Khaled *et al.* [13], Fiji (276 IU/ml) Kotby AA *et al.* [8], USA (240 IU/ml) Kaplan *et al.* [14], Tanzania (200 IU/ml) Mhalu and Matre [15], Sweden (200 IU/ml) Nimmo *et al.* [16], and in a different region of India (239 IU/ml) Sethi *et al.* [17]. However, lower result was obtained from study conducted in Egypt (400 IU/ml) Asfaw *et al.* [9]. Most of these values exceeded the normal level set by laboratories which is 200 IU/ml. The higher ASO titers in Yemen and Australia, Fiji and India are probably due to the fact that tonsillitis and impetigo are endemic particularly in children Yemen [18]. In Ethiopia, Rheumatic heart disease (RHD) is the number one cardiac problem in children with a prevalence rate of 4.6–7.1 per 1000. Shet and Kaplan [19] suggest that there is high prevalence of tonsillitis and pharyngitis. 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 might be in the process of returning to their baseline level after a case of tonsillitis or impetigo were included Yemen [18].

We expressed the value of ASO titer in terms of upper limit rather than as mean to show the highest acceptable value in this study with previous infection. Although the levels are high, they do not warrant any further investigations or treatment since these children are normal and had no complaint at the time of sampling. Re-infection usually results in sustained or continuously rising titers, and it is known from experimental studies that antibody responses are more impressive on repeated exposure. Shet and Kaplan [19] also, Bosmansky [20] reported an ASO titer of 187 IU/ml in patients with chronic RHD which was significantly lower than the titer in the normal population. The natural course of streptolysin response can be modified by the administration of drugs, especially penicillin, by reducing the overall number of responders, and influencing the magnitude of antibody response. While, Cilliers [21] demonstrated a statistically significant difference between the ASO titer in patients with ARF first attack and patients with recurrent RF. This further supports the hypothesis of an altered immune response to streptococci following long-acting penicillin prophylaxis. Although recurrence of rheumatic fever is evidence of non-compliance to long-acting penicillin, lower ASO titer reflects partial prophylaxis with a decrease in incidence of streptococcal Infection.

Conclusion

The ULN ASO titer in normal Egyptian children is quite high, reaching up to 398.5 IU/ml. Therefore, an isolated high ASO titer is not sufficient to diagnose

ARF. Basal levels of ASO titer increase with age but age does not affect the peak level during acute streptococcal infection. Additional studies will be required for establishment of standard values to avoid over diagnosis of ARF and complication of long-acting penicillin.

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Conflicts of interest
There are no conflicts of interest.

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