



Cross-sectional Study

Normal values of antistreptolysin O for adults ages 30 to 70 in Syria: A cross sectional study



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ABSTRACT

Background: Group A Streptococcus is a very common pathogen which infects a large scale of people around the world causing many symptoms such as scarlet fever, sinusitis, and pneumonia. Most strains of group A and many other strains of group C and G Streptococcus bacteria secreted antigen called Streptolysin O. Anti-streptolysin O (ASO) is an antibody produced against streptolysin O that rises after 1 week of infection by streptococcus bacteria, which helps in diagnosing this type of infectious diseases. We conducted a Cross-Sectional study to determine the Upper Limit of Normal (ULN) for healthy adult in Aleppo, Syria.

Materials and methods: A sero-epidemiological cross-sectional study was conducted from September to October in 2019. ASO titers were determined on 267 healthy patients' companions who visited Aleppo University Hospital. Geometric mean titer and Upper Limit of Normal of ASO were calculated according to sex, age and residency. Upper Limit of Normal were defined as the 80th percentile.

Results: Out of 267 participants; 126 (45.7%) were males and 150 (54.3) were females. The Upper Limit of Normal for total participants was 210.8 IU/ml. There was no significant difference (P-value > 0.05) among males (204.6 IU/ml) and females (225.8 IU/ml). In contrast to sex, there was a significant difference (P-value < 0.05) according to age groups, where the highest Upper Limit of Normal was in the 30–39 age group (256.0 IU/ml).

Conclusion: ASO test is a common, easy, cheap method, so getting enough data about it is very important in the developing countries (such as Syria). In Aleppo, Syria we found that the ULN was higher than ULN from other studies. There was no significant difference according to sex and residency. On the other hand, there was significant difference according to age groups.

1. Background

Group A Streptococcus (GAS) is one of the most common and widespread pathogenesis that affects humans. It causes acute pharyngitis, impetigo, sinusitis, otitis, peritonsillar and retropharyngeal abscess, pneumonia, scarlet fever, erysipelas, cellulitis, lymphangitis, puerperal sepsis, vaginitis, myositis, gangrene and perianal cellulitis. It associated mainly with two nonsuppurative sequelae: acute rheumatic fever and acute glomerulonephritis [1]. According to Jones Criteria, ARF diagnosis required an evidence of GAS infection [2]. Most strains of group A and many other strains of group C and G Streptococcus bacteria secreted antigen called Streptolysin O (SLO), which is an immunogenic, oxygen-labile streptococcal hemolytic exotoxin [3]. Anti-streptolysin O

(ASO) is an antibody produced against streptolysin O. Anti-streptolysin O titer (ASOT) rises during 1 week following the infection with streptococcus pyogenes and reaches a maximum titer after 3–6 weeks. It plays an important role in the diagnosis of recent streptococcal infection [4,5]. It helps to diagnose scarlet fever, rheumatic fever and post-infectious glomerulonephritis [6]. There are many factors influencing (ASOT) such as age, season and geography [7]. Therefore, countries should conduct more studies to determine the Upper Limit of Normal (ULN) for their population. This Cross-Sectional study aims to determine the ULN for healthy adults in Aleppo, Syria. Moreover, we assessed the impact of gender, age groups and residency on the results.

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2. Material and methods

2.1. Study design

A sero-epidemiological cross-sectional study was conducted from September to October in 2019. The current study purpose is to determine the normal range of ASO for healthy adults in Aleppo city, Syria. This study was conducted according to STROCSS criteria [8], and registered at RESEARCH REGISTRY with registration number: researchregistry7029 (<https://www.researchregistry.com/browse-the-registry#home/>).

2.2. Data collection and setting

ASO titers were determined on 267 healthy patients' companions who visited the following places: the central laboratory department of Aleppo University Hospital and the external-clinic of Aleppo University Hospital. We included healthy adults aged between 30 and 70 years. The exclusion criteria include those with a clinical history of a recent streptococcal infection as 1) sore throat in three months. 2) acute arthritis. 3) rheumatic heart disease. 4) invasive GAS diseases. 5) laryngitis during the previous 14 days or skin infections. 6) temperature above 38 Celsius during the test day. Demographic characteristics such as age, sex, residency were collected from included participants. We collected a sample of venous blood from each adult on a citrate tube and serum was separated and stored at -20°C . All ASO tests were measured by turbidimetry on the same kit and device. The ASO titer was expressed in international units (IU) and Log Units. Informed verbal consent was obtained from all participants. The approval for conducting this research was obtained from the Faculty of Medicine in University of Aleppo that was compatible with the Declaration of Helsinki.

2.3. Statistical analysis

The participants were divided into four age groups. Descriptive statistics were performed to calculate the number of participants, percent, mean and standard deviations for each sub-group. Geometric mean titer (GMT) and ULN of ASO were calculated according to sex, age and residency. ULN were defined as the 80th percentile. Differences in mean ASO titer level were assessed after log transformation by one-way ANOVA and independent samples test for each sub-group. $P < 0.05$ was considered significant. Statistical analysis was done using SPSS version 20.

3. Results

3.1. Demographic characteristics

Out of 267 participants; 126 (45.7%) were males and 150 (54.3%) were females. The participants were divided into four age groups and the mean of age was 49.3 ± 11.3 . 186 (68.1%) of the participants were urban and 87 (31.9%) of the participants were rural. Table 1 demonstrates participant's demographics.

3.2. Geometric mean titer and upper limits of normal of ASO

Table 2 shows the GMT and ULN of ASO categorized by epidemiologic characteristics. The ULN for total participants was 210.8 IU/ml with GMT of 134.8 ± 1.7 IU/ml. There was no significant difference (P -value > 0.05) among males (ULN 204.6 IU/ml) and females (ULN 225.8 IU/ml). In contrast to sex, there was a significant difference (P -value < 0.05) according to age groups, where the highest ULN was in the 30–39 age group (256.0 IU/ml), followed by 40–49 age group (223.0 IU/ml). For the age group 50–59 and 60–70 the ASO ULN serum level was lower than the another groups with ULN 194.0 IU/ml and 157.8 IU/ml respectively. Moreover, the outcomes show that the participants from

Table 1
Epidemiologic characteristics of participants.

	Number (%)
Total	267 (100)
Gender	
Male	126 (45.7)
Female	150 (54.3)
Age	
Mean \pm SD	49.3 \pm 11.3
30–39	60 (21.7)
40–49	77 (27.9)
50–59	79 (28.6)
60–70	60 (21.7)
Residency	
Urban	186 (68.1)
Rural	87 (31.9)

Table 2
Geometric mean titer and upper limits of normal of ASO categorized by epidemiologic characteristics.

Characteristic	Geometric Mean Titer \pm SD		P-value	ULN	
	Log Units	IU/ml		Log Units	IU/ml
Gender			0.183		
Male	2.1 \pm 0.2	129.0 \pm 1.7		2.3	204.6
Female	2.1 \pm 0.3	139.9 \pm 1.8		2.4	225.8
Age			0.001		
30–39	2.2 \pm 0.2	158.7 \pm 1.7		2.4	256.0
40–49	2.2 \pm 0.2	143.5 \pm 1.6		2.3	223.0
50–59	2.1 \pm 0.2	129.1 \pm 1.7		2.2	194.0
60–70	2.0 \pm 0.3	111.8 \pm 1.8		2.2	157.8
Residency			0.440		
Urban	2.1 \pm 0.2	131.9 \pm 1.7		2.3	207.4
Rural	2.1 \pm 0.2	138.9 \pm 1.7		2.4	228.4
Total	2.1 \pm 0.2	134.8 \pm 1.7		2.3	210.8

rural areas have a higher ULN (228.4 IU/ml) than urban areas (207.4 IU/ml), but without a significant difference (P -value > 0.05).

4. Discussion

Streptococcus group A is one of the most common and widespread pathogenesis which affects humans. The most serious complications specific to infected with GAS are acute rheumatic fever and acute glomerulonephritis. It is very important to confirm GAS infection to diagnose, manage and follow up the patients. Laboratory diagnoses is performed by three different tools: throat culture, Rapid Antigen Detection Testing (RADT), or streptococcal serology. The most common tests which are usually used to diagnose GAS infection are streptococcal serology including ASO antibodies and Anti-Deoxyribonuclease B (Anti-DNase B). ASO test is one of the most common, easier and cheaper methods for confirm GAS infection. It is very helpful when throat culture technique is unavailable or cannot be applied. Therefore, it plays an important role in the developing countries [9]. Syria with its location in southwestern Asia on the eastern coast of Mediterranean Sea make it an endemic region with streptococcal infections. Because there is no previous data about the ULN serological values of ASO in Syria for adult, a cross-sectional sero-epidemiological study was conducted among healthy adults aged 30–70 years in Aleppo, Syria. Mosby's Diagnostic and Laboratory Test Reference 2019 book determined the normal findings of ASO titer for Adults and elderly less than 160 Todd units/ml [10]. The ASO ULN serum level for total adult population in Aleppo is 210, this finding is higher than the results of studies in Georgia [11], Korea [12], India [13], Australia [14] and Uganda [15] (Table 3). The reason for the marked rise of the ULN may be due to environmental and genetic

Table 3
ASO titer values for healthy adult.

	ULN (Adult)	Number of populations	Age range	Year	Country
Our study	210.8	267	30–70	2020	Syria
Okello E [15]	130	48	30–50	2020	Uganda
Steer AC [14]	127	93	<35	2009	Australia
Karmarkar M [13]	195	160	NM	2004	India
Kim SJ [12]	136	231	20–70	1998	Korea
Klein GC [11]	85	220	NM	1971	Georgia

*NM: not mentioned.

factors. The date and sample's size varied among studies (Table 3), 267 adults sample were included in our study and it is the highest number among studies, that makes our results more reliable. There was a significant difference according to age groups, where the highest ASO ULN serum level in the age groups was of the age groups 30–39 years 256.0 IU/ml, followed by 223.0 IU/ml. ASO titer decreasing with increasing age (Table 2), that also matched with J Renneberg J et al. study that aimed to determine the upper 95% CL of ASO and Anti-DNase B [16]. The difference among age groups is higher than Blyth C et al. which presented the ULN of three age groups 30–39.9 years, 40–59.9 years and older than 60 is 100 IU/ml [3]. All studies determined ASO ULN of children because they are the most exposed group to GAS infections, ASO ULN of children group is higher than adults group [11–15]. As we mentioned previously ADB is a good test which used to confirm GAS infection, Anti-DNase B ULN titers were determined in many studies [11–15,17]. This method was not available in our laboratory due to the international and economic sanctions in our country, so we could not determine Anti-DNase B normal values. In addition, this method was absent in Uganda which lead to diagnostic limitations in the developing country [15].

4.1. Limitations, strengths and recommendations

This study is the first in Syria to determine the normal range for ASO. We recommend to conduct future studies on higher sample's number to get more realistic results. Moreover, we recommend to conduct similar studies on younger population because they are the most targeted group of streptococcal infection. The limitation of our study is that we did not perform throat culture to exclude streptococcal infection.

5. Conclusion

It is necessary to confirm streptococcus group A infection to diagnose acute rheumatic fever and acute glomerulonephritis, which we could do it using ASO test. ASO test is a common, easy, cheap test, so getting enough data about it is very important in the developing countries (like Syria). In Aleppo, Syria we found that the ULN was higher than ULN form other studies. There was no significant difference according to sex and residency. On the other hand, there was significant difference according to age groups.

Ethical approval

Ethical approval has been granted by the Institutional review board (IRB) of the Faculty of Medicine at Aleppo University (reference number 1535) that was compatible with the Declaration of Helsinki. Informed verbal consent was obtained from all participants which was compatible with our institutional approval.

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Author contribution

MZBA: wrote the manuscript, data collection, conducted the literature review, MNS: wrote the manuscript, data collection, study design and corresponding author, MSA: wrote the manuscript, data collection, reviewing the manuscript, MNK: wrote the manuscript, data analysis and data interpretation, AA: prepared the questionnaire, data analysis and data interpretation, AZ and RZ: wrote the manuscript, data collection, prepared the questionnaire and entering data, SHK: contributed to supervision, data interpretation and planning, All authors read and approved the final manuscript.

Registration of research studies

Name of the registry: Research Registry.

Unique Identifying number or registration ID: researchregistry7029.

Hyperlink to your specific registration (must be publicly accessible and will be checked): <https://www.researchregistry.com/browse-the-registry/#home/>

Guarantor

Mohammad Nour Shashaa.

Consent

Verbal consent was taken from participants, which was compatible with our institutional approval.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Declaration of competing interest

The authors declare that they have conflicts of interest.

List of abbreviations

ASO	Anti-streptolysin O
ULN	Upper Limit of Normal
GAS	Group A Streptococcus
SLO	Streptolysin O
ASOT	Anti-streptolysin O titer
GMT	Geometric mean titer
RADT	Rapid Antigen Detection Testing
Anti-DNase B	Anti-Deoxyribonuclease B

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.amsu.2021.103015>.

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