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Evaluation of Antistreptolysin O (ASO) Titer in Rheumatoid Heart Disease Debre Berhan Referral Hospital, Ethiopia

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Abstract: Introduction: Rheumatic heart disease (RHD) remain significant causes of cardiovascular disease and mortality in children particularly in the developing countries. This study aims to evaluate ASO titer in rheumatic heart disease patients who are on secondary prophylaxis. Methods: A cross sectional study method was employed to collect blood sample from RHD patients (children) who are on secondary prophylaxis at pediatric clinic of Debre Brehan referral hospital. The blood sample was collected, transported and processed based on standard operating procedures (SOPs) for ASO titer determination. And finally, the data was analyzed using SPSS version 21and P-value less than or equal to 0.05 was taken as statistically significant. Result: A total of 123 children on secondary prophylaxis for RHD; in the age range of 5-15 years are included. Of these, 65 (52.8) were male and 58 (47.2%) were female; 69 (56.1%) were from rural and 54 (43.9%) were from urban area. The highest frequency of participant 52 (42.3%) were in the age group of 9-12 years. Among participants 74 (60.2%) had history of pharyngitis. The result of ASO titer shows 78 (63.4%) were positive in antistreptolysin O slide agglutination test; 30 (24.4%) patients have 400IU/ml ASO titer, 19 (15.4%) patients have 600 IU/ml ASO titer, 17 (13.8%) patients have ASO titer 800 IU/ml and 12 (9.8%) patients have ASO titer ≥1000 IU/ml. Conclusion: This study suggest that in most children admitted with RHD have recent streptococcal infection as evident by raised ASO titer.

Keywords: Children, Rheumatic Heart Disease, ASO Titers, Risk Factors, Ethiopia

1. Introduction

RHD is the long term consequence of rheumatic fever, an autoimmune response to Group A streptococcal pharyngitis [1]. Without prophylaxis, patients with RHD are at risk of recurrent attacks of rheumatic fever resulting in ongoing inflammation and fibrosis with consequent valvular damage [2, 3]. Although its incidence is decreasing in industrialized countries, RHD remains a major challenge in the rest of the world. It is a leading cause of morbidity and mortality among young adults in the developing world, accounting for at least 345,000 deaths annually [4, 5]. The highest prevalence is in sub-Saharan Africa with a rate of 5.7 per 1000, compared to 1.8 per 1000 in North Africa, and 0.3 per 1000 in economically advanced countries [6]. In Ethiopia, RHD is the number one cardiac problem in children with a prevalence rate of 4.6–7.1 per 1000 [7, 8]. Particularly frustrating has been the fact that RHD is theoretically preventable but still remain a problem in developing countries. Reason most advanced is that these countries cannot afford the economic and social cost involved [9]. RHD is an autoimmune disease that follows infection with GAS; however, the isolation of GAS is uncommon (<15%), and so confirmation of the diagnosis often relies on streptococcal antibody tests [10]; the most frequently performed tests are anti-streptolysin O (ASO) titer [11]. Therefore, this study aims to evaluate ASO titers in rheumatic heart disease patients who are on secondary prophylaxis.

2. Method

2.1. Study Design and Setting

Cross sectional study was conducted among 123

confirmed RHD children (5-15 years) who are on secondary antibiotic prophylaxis at pediatric clinic of Debre Berhan referral hospital from 2016- September, 2017. Debre Berhan referral hospital is located in the Semen Shewa Zone of the Amhara Region, 130 Kms Northeast of Addis Abeba.

2.2. Sample Collection, Handling, Transport and Analysis

Blood was collected from each study participant by trained nurse. A total of 3ml of venous blood was collected using sterile 5ml syringe and immediately transferred to a glass tube without anticoagulant. It was transported to Debre Berhan university medical microbiology laboratory and then serum was separated at 3000 RPM for 10 minutes. The demographic data was collected using a questionnaire. All expected factors were included in the questionnaire and clinical information was obtained from all patients by reviewing medical records. Patients with incomplete data were excluded.

2.3. Determination of Anti-Streptolysin-O (ASO) Titer

Anti-Streptolysin O latex reagents (LiNEAR Chemicals. s. L, Spain) were used to determine ASO titer. All test reagents and serum samples were brought to room temperature before the test was done. Normal saline (0.85% NaCl) was used for dilution purpose. Fifty micro liter of serum and one drop of the ASLO-Latex antigen were mixed using disposable stirrer on a circle on the card and rotated on the mechanical rotator (100 RPM) for 2 minutes. Both positive and negative controls were run in parallel. It was observed under visible light source for any degree of agglutination. When the test becomes reactive, 50 µL of the serum diluted with 50, 100, 150,200 µL of saline etc to make it simple dilution (undiluted, 1/2, 1/3, 1/4, 1/5 etc) until the result became non-reactive. The serum ASO concentration could then be calculated approximately by multiplying the dilution factor (*i.e.* 2, 3, 4 or 5) by the detection limit. This method was chosen because it is the widely used technique for ASO titer measurement in Ethiopia.

2.4. Data Quality Control Measures

Standard Operational Procedures were strictly followed during collection, preservation and transportation of blood samples. Socio-demographic data was obtained using structured questionnaire.

2.5. Data Entry, Management and Analysis

Data obtained from each sample was entered and cleared into Microsoft Excel then summarized and analyzed using SPSS version 21 software. The average value (mean), standard deviation of the mean (on average, how much each measurement deviates from the mean), 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. A p-value of less than or equal to 0.05 was considered as statistically significant difference.

2.6. Ethical Considerations

Ethical approval was obtained by Debre Brehan university research and review committee and official permission was obtained from head department of Debre Brehan referral hospital. Written informed consent was obtained from each child's parent or guardian and assent from study participants older than 12 year.

3. Results

A total of 123 children on secondary prophylaxis for RHD; in the age range of 5-15 years are included in this study. Of these, 65 (52.8) were male and 58 (47.2%) were female; 69 (56.1%) were from rural and 54 (43.9%) were from urban area. The highest frequency of participant 52 (42.3%) were the age group of 9-12 years. Among participants 74 (60.2%) had history of pharyngitis. The result of ASO titer shows 78 (63.4%) were positive in anti-streptolyasin O slide agglutination test (Table 1).

Table 1 also shows Risk factors associated with RHD. Among participant from rural area 47 (68.1%) were positive for ASO titer >200 IU/ml and have significant correlation p-value = 0.003. Among participants who have history of pharyngitis 56 (75.7%) were positive for ASO titer >200 IU/ml and have significant correlation p-value = 0.002. Among student participants with the age of 5-15 years 57 (64.1%) were positive for ASO titer >200 IU/ml and have significant correlation p-value = 0.046 and there was also a significant correlation between ASO titer and age of the participants.

Table 1. *Demographic characteristics of the RHD patients, result of ASO Titer and Risk factors associated with RHD.*

Risk factors		Frequency	ASO titer		
			Positive	Negative	P-value
Gender	Male	65 (52.8)	41 (63.1)	24 (36.9)	0.065
	Female	58 (47.2)	38 (65.5)	20 (34.5)	
Age groups (years)	5-8	40 (32.5)	24 (60)	16 (40)	0.004
	9-12	52 (42.3)	31 (59.6)	21 (40.4)	
	13-15	31 (25.2)	24 (77.4)	7 (22.6)	
Residence	Rural	69 (56.1)	47 (68.1)	22 (31.9)	0.003
	Urban	54 (43.9)	33 (61.1)	21 (38.9)	
Family size	≤5	57 (46.3)	39 (68.4)	18 (31.6)	0.087
	>5	66 (53.7)	41 (62.1)	25 (37.9)	
Family educational status	Literate	52 (42.3)	31 (59.6)	21 (40.4)	0.406
	Illiterate	71 (57.7)	49 (69)	22 (31)	

Risk factors		Frequency	ASO titer		
			Positive	Negative	P-value
Participant educational condition	Student	89 (72.4)	57 (64.1)	32 (35.9)	0.046
	Not student	34 (27.6)	24 (70.6)	10 (29.4)	
History of pharyngitis	Yes	74 (60.2)	56 (75.7)	18 (24.3)	0.002
	No	49 (39.8)	27 (55.1)	22 (44.9)	
ASO titer IU/ml (category)	>200	78 (63.4)			
	≤ 200	45 (36.6)			
Duration Of treatment for RHD	≤ 3 month	68 (55.3)			
	> 3month	55 (44.7)			

Table 2 shows level of ASO titer in ASO Latex slide positive Patients. 30 (24.4%) patients have 400IU/ml ASO titer, 19 (15.4%) patients have 600 IU/ml ASO titer, 17 (13.8%) patients have ASO titer 800 IU/ml and 12 (9.8%) patients have ASO titer ≥1000 IU/ml and the total frequency of ASO positive patients were 78 (63.4%). P-value =0.003.

Table 2. *Level of ASO titer in ASO Latex slide positive Patients.*

ASO Test	ASOT (IU/ml)				Total	P- value
	400	600	800	≥1000		
Positive	30 (24.4%)	19 (15.4%)	17 (13.8%)	12 (9.8%)	78 (63.4%)	0.003

Table 3 shows the Mean ± SD, Median, and 80% upper limit of normal reference values for ASO titer according to sex and age of enrolled subjects. The ASO titer Upper Limit of Normal (ASO ULN) reference for the total subjects was 800 IU/ml with a median 400 IU/ml. The ASO ULN for both male and female children were 800 IU/ml with a median of 400 IU/ml. The ASO ULN was 800 IU/ml with a median of 400 IU/ml for all age groups.

Table 3. *The cut of 80 percentile upper-limit of normal reference values for ASO titer by sex and age groups for children 5-15 years.*

Characteristics	No (%) of subjects	ASO (IU/ml)		
		Mean ± SD	Median	80% upper limit of normal
Sex				
Male	65 (52.8)	492.3±329.9	400	800
Female	58 (47.2)	500±337.7	400	800
Age				
5-8	40 (32.5)	470±305.7	400	800
9-12	52 (42.3)	447.1±290.1	400	800
13-15	31 (25.2)	606.3±404.7	600	800
Total	123 (100)	495.9±332.2	400	800

4. Discussion

The diagnostic criteria of rheumatoid heart disease is ASO level greater than 200 IU/ml. Anti-streptolyasin O (ASO) serum titer in excess of 200 IU/ml is considered abnormally high and suggest either recent infection with streptococci or persistently high antibody level due to earlier exposure in hypersensitive persons [12]. Therefore, this study was carried out to evaluate ASO titer in rheumatic heart disease patients who are on secondary prophylaxis. A total of 123 children on secondary prophylaxis for RHD are included. Among these 78 (63.4%) patients were positive in anti-streptolyasin O slide agglutination test. 30 (24.4%) patients have 400IU/ml ASO titer, 19 (15.4%) patients have 600 IU/ml ASO titer, 17 (13.8%) patients have ASO titer 800 IU/ml and 12 (9.8%) patients have ASO titer ≥1000 IU/ml and the total frequency of ASO positive patients were 78 (63.4%). These is comparable result from the study conducted at Punjab Institute of Cardiology Lahore, Pakistan [13].

The ASO ULN for the total subjects was 800 IU/ml with a median 400 IU/ml. The ASO ULN for both male and female children was 800 IU/ml with a median of 400 IU/ml. The ASO ULN was 800 IU/ml with a median of 400 IU/ml for all age groups. Different study were indicated that in chronic RHD patients, ASO ULN was less than standard (200 IU/ml) due to the effect of penicillin on the immune response to streptococci [14], But in this study ASO ULN was higher (800 IU/ml) than standard (200 IU/ml). This might be attributed to the recent streptococcal infection in which (68 (55.3%) patients had ≤ 3 months duration of treatment.

The present study also shows risk factor associated with RHD. Among participant from rural area 47 (68.1%) were positive while from urban area 33 (61.1%) were positive for ASO titer >200 IU/ml and have significant correlation p-value = 0.003. the same study from Punjab Institute of Cardiology Lahore indicate that RHD most commonly occur in rural area possibly due to poor sanitation and poor nutrition or it may be related to higher percentage of total population in rural area 13]. Among participants who have history of pharyngitis 56 (75.7%) were positive for ASO titer >200 IU/ml and have significant correlation p-value = 0.002; the same study conducted in Egypt showed significant correlation between the antistreptolysin O titer and number of attacks of tonsillitis [15]. Among student participants with the age of 5-15 years 57 (64.1%) were positive for ASO

titer >200 IU/ml and have significant correlation p-value = 0.046 these may be due to high numbers of students per class in underdeveloped countries like Ethiopia risks the student for respiratory infection of *streptococcus pyogens;* here in our study we found that the average number of student per class was more than 50. There was also a significant correlation between ASO titer and age of the participants. In general there was a significant correlation between socioeconomic level and the prevalence RHD. The high prevalence of RHD in low socioeconomic group could be attributed largely to a low standard of living. The decline in the prevalence of RHD in industrialized countries has been attributed mainly to improvement in living standards and areas in which it persists, it is associated with low social circumstances and poverty [16].

5. Conclusion

This study suggest that most children admitted with RHD have recent streptococcal infection as evident by raised ASO titer. The upper limit of normal reference value for the total subjects was also 800 IU/ml, indicating high titer value.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contributions

TA-performed the laboratory activities. TA- analyzed the data and wrote the manuscript. DS, MT and NZ reviewed the manuscript. TA and NZ participated in its design. All authors read and approved the final manuscript.

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