Alternate Pathway Haemolytic Complement (AH_{50}) Activity among under 12 Years Children with some Bacterial and Malaria Parasite Infections

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ABSTRACT

Complement system is an essential part of our body's defense against infections. It helps the cells in our immune system to better recognize and capture microbes and can also lead to direct elimination of microbes by destroying their cell membrane. There is increase in the incidence of complement deficiencies globally. There is paucity of data on complement activity among children especially in our locality. This study was set to evaluate the AH_{50} Complement activity among children with some bacterial and malaria parasite infections. This is a cross sectional study in which71 children with some bacterial and malaria parasite infections attending Usmanu Danfodiyo University, Sokoto, were recruited. Blood sample was collected from the children then processed to obtain a serum. The serum AH_{50} was analysed using sandwich enzyme linked Immunosorbent assay (ELISA).The AH_{50} activity in the children was found to be between 71.3 U/ml-176.9 U/ml. Children whose ages fall between 9-12 years have higher AH_{50} activity (94.63 U/ml) compared with other age groups, but statistically not significant. Children diagnosed with malaria parasite have higher AH_{50} activity (87.49 U/ml) compared with those diagnosed with bacterial infection (p=0.05). Among the causative organisms, children with Staphylococcus aureus infection have higher AH_{50} activity (88.17 U/ml) compared with other infectious agent, but the differences is not statistically significant. AH_{50} activity fall within the assay range this indicate normal activity. There is significant difference in AH_{50} activity between children diagnosed with malaria parasites and bacterial infections. Therefore we recommend further research to identify the reasons behind the differences.

Keywords: Alternate Complement Pathway, Enzyme-Linked Immunosorbent Assay, Immunity, Immune System, Innate

INTRODUCTION

Complement protein plays a role as a central part of innate immunity and constitutes the first line of defence in the detection and removal of pathogens that have breached the host's protective barriers.[1] It serve as a link between innate and acquired immune responses that allow an integrated host responses to pathogenic challenges.[2] The Complement system is a component of the innate immune system that defends the host against microbial infections by promoting inflammatory response and opsonization.[3]

Severe Primary Immuno deficiencies (PID) are increasingly becoming appreciated as a relevant health problem, indeed diagnostic procedures and screening profiles to allow earliest possible diagnosis on a population scale have already been developed in the USA and few European countries.[4] In addition, complement deficiencies has been shown to be between 1 and 10% of all primary immunodeficiencies.[5] Deficiencies of complement system is more likely among young children that have had recurrent and persistent infections[6] and affect both sexes equally.[7]

A study show that complement cascade is activated during malaria infection.[8] Among the infections, Malaria remains one of the world's most important infectious diseases and is responsible for over 1 million deaths each year globally.[9] Furthermore deficiencies in the complement cascade can lead to overwhelming infection and sepsis[5]. Patients with deficiency of either classical or alternative pathway suffer recurrent pyogenic infections with *Streptococcus pneumoniae, Haemophilusinfluenzae* or *Neisseria meningitidis* infections which may later manifest as meningitis, sepsis, or arthritis.[10,11]

Early detection of Complement deficiencies will enable aggressive treatment of microbial infections with appropriate

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antimicrobial therapy.[12] We realized that there is paucity of data regarding alternative pathway haemolytic complement (AH_{50}) activity in children especially in our locality. Therefore this study is set to give an insight on AH_{50} activity among children and find out whether there is differences between the children with some bacteria and malaria parasite (MP) infections. This could serve as baseline information for Clinical and Laboratory practice.

MATERIALS AND METHODS

Study Area

This research was conducted at Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto. The Hospital is a tertiary institution located within the Sokoto metropolis. Sokoto is the capital city of Sokoto State located in the extreme North-west geopolitical region of Nigeria.

Study Design

This is a cross sectional study set to determine the AH_{50} complement activity in children with some bacterial and MP infections attending Paediatric unit of Usmanu Danfodiyo University Teaching Hospital (UDUTH)

Study Population

A total of 71 children diagnosed with bacterial or MP infections attending paediatric unit of UDUTH, Sokoto were randomly recruited for this study. The recruitment was based on Clinical and Laboratory diagnosis. Children who did not assent or whose parent(s) or guardians did not give consent and children suffering from any other disease ormalnourished as well as children co-infected with bacteria and MP were excluded from this study. A semi structured interviewer questionnaire was administered to all consenting parent(s)/guardians or assenting children to obtain information on their socio-demographic and medical history.

Ethics Statement

Approval for this study was obtained from Ethics and Research Committee of Usmanu Danfodiyo University Teaching Hospital, Sokoto with reference number UDUTH/HREC/2017/No. 616. Informed consent for inclusion into the study was obtained from the child's parent/guardian using a standard informed consent form; assent was obtained from children greater than seven years using an assent form.

Sample Collection and Processing

Three millilitres (3ml) of venous blood sample was collected aseptically from each participant and dispensed into a plain Vacutainer. The sample was allowed to clot, and then centrifuged at 3000 revolution per minute for 5 minutes. The serum was transferred into pre-labelled 2ml plain container and stored at -20ºC until assay.

Determination of alternate pathway haemolytic complement Activity (AH_{50})

The Complement activity was determined quantitatively using human AH_{50} Complement ELISA kit (Sunlong Biotech, Co., Ltd, China). The assay uses sandwich ELISA technique. The procedure was performed according to the manufacturer's instructions. The concentrations of AH_{50} in the samples were calculated by comparing the OD of the samples to the standard curve. The assay range was 6.25 U/ml- 200 U/ml. Sensitivity is 1.0 U/ml.

Statistical Analysis

The results obtained were entered into SPSS version 21 for analysis. Test for normality was carried out to ascertain normal distribution of the variables. Data was not normally distributed based on tests of normality results. Categorical variables were expressed in percentage whereas continuous variables were expressed in Median. Mann Whitney U test and Kruskal Wallis test were carried out to compare between two non-parametric variables. The p-value of ≤ 0.05 was used to determine the level of statistical significance.

RESULTS

Demographic Characteristics of the Children

As depicted from Table 1, seventy one (71) children, aged <1-12 years participated in this study, 45 (63.4%) were males and 26 (36.6%) were females. Based on age group of the children, the age group 1-4 years has the highest frequency 26 (36.6%) whereas <1 year had least frequency of 7 (9.9%). More than half of the children 47 (66.4%) resides in the urban area. Based on infections, 36 (50.7%) of the children were

suffering from MP infection whereas 35 (49.29%) had bacterial infections.

AH_{50} Complement Activity of the Children

The range of AH_{50} Complement activity in the children was between 71.3 U/ml-176.9 U/ml. As depicted from Table 2, the comparison of children's AH_{50} complement activity based on their age range shows that children between the ages of 9-12 years have the highest median concentration of Ah_{50} complement (94.63 U/ml) compare to other age groups. While children within the age range of 1-4 years have the lowest (82.90 U/ml) AH_{50} complement activity ($p>0.05$).

From Table 3, the result indicated that Female children have higher median concentration of AH_{50} complement (86.56 U/ml) compared with that of male children (84.61 U/ml)($p>0.05$). The result in Table 4 shows that Children that had MP infection have higher (87.49 U/ml) AH_{50} complement activity compared to children that had bacterial infection (83.45 U/ml) ($p=0.05$). Among the causative organisms, children diagnosed with *Staphylococcus aureus* infection have high concentration of AH_{50} complement (88.17 U/ml) whereas those with *Escherichia coli* have the lowest AH_{50} complement concentration (78.1 U/ml) ($p>0.05$) as shown in Table 5.

Table 1: Socio-Demographic characteristics of the children

Characteristics	Frequency (N)	Percentage (%)
Gender		
Male	45	63.4
Female	26	36.6
Total	71	100
Age group (years)		
<1	7	9.9
1-4	26	36.6
5-8	21	29.6
9-12	17	23.9
Total	71	100
Residence		
Urban	47	66.2
Rural	24	33.8
Total	71	100
Infections		
Bacterial	35	49.29
Malaria parasite	36	50.7
Total	71	100

N= number of children

Table 2: Relationships between age ranges and AH_{50} complement activity

Age group (years)	Frequency N (%)	AH_{50} Median concentration (U/ml)	p-value
<1	7(9.9)	90.64	
1-4	26(36.6)	82.90	
5-8	21(29.6)	84.19	0.19
9-12	17(23.9)	94.63	
Total	71(100)		

Kruskal Wallis test: $\chi^2= 4.734$.

Table 3: Comparison between genders on AH_{50} complement activity

Gender	Frequency N (%)	AH 50 Median Concentration (U/ml)	p-value
Male	45 (63.4)	84.61	
Female	26 (36.6)	86.56	0.73
Total	71 (100)		

Mann Whitney U Test: 557.000.

Table 4: Comparison between Bacterial and MP infections on AH_{50} complement

Infection	Frequency N (%)	AH 50 median concentration (U/ml)	p-value
Bacterial	35 (49.29)	83.45	
MP	36 (50.70)	87.49	0.05
Total	71 (100)		

MP = Malaria Parasite, Mann Whitney U Test: 462.5

Table 5: Relationship between different causative organisms and AH_{50} complement

Causative organism	Frequency N (%)	AH 50 median concentration (U/ml)	p-value
Escherichia coli	20 (28.2)	78.10	
Salmonella spp	2 (2.8)	87.62	
Streptococcus spp	6 (8.5)	86.91	
Staphylococcus aureus	5 (7.0)	88.17	0.23
Neisseria spp	2 (2.8)	87.77	
Plasmodium falciparum	36 (50.7)	87.49	
Total	71 (100)		

Kruskal Wallis: $\chi^2 = 6.768$

DISCUSSION

The complement system is part of innate immunity, which can be activated quickly whenever it recognise a sterile and nonsterile stimuli as threat to the host.[13] The alternative pathway has a wide range of binding site provided by variety of polysaccharides and glycoproteins, including Lipopolysaccharides from Gram-negative, teichoic acids from Gram-positive and viral membrane glycoproteins.[14]

The range of AH_{50} Complement activity in the children was between 71.3 U/ml-176.9 U/ml. Therefore it falls within the assay range this possibly indicate the normal activity of the alternative complement pathway. The AH_{50} is the primary screening test for alternative complement deficiencies. It measures functional activity of the pathway. Of note classical complement pathway is activated by pathogen-bound antibody whereas the alternative pathway activation is due to spontaneous C3 hydrolysis or immunogen.

Out of 71 children who participated in this study, 36 (50.7%) were infected by MP while 35 (49.2%) were infected by bacteria. *Plasmodium falciparum* malaria is a major cause of mortality in sub-Saharan Africa.[13] In this study, although not significant statistically, we observed that children at the age range between 9-12 years have higher AH_{50} complement activity on comparison with other age groups, this is in line with report which indicates that the level of

complement protein is significantly lower in elderly and middle age group and slightly higher in children of 10-15 years old.[15] However, it has been reported that concentrations of complement proteins are lower in children at early infancy but return to normal level within weeks after birth.[16]

Between the two infections this study shows higher AH_{50} complement activity (87.49 U/ml) among children diagnosed with MP than those with other bacterial infections. This finding support the notion that indicate complement is activated during malaria infection.[8,17] However, the complement level differs depending on the severity of malaria. For instance severe malaria is associated with lower complement haemolytic activity as well as decreased activity of alternative pathway and other two complement pathways.[18,19]

Based on causative organisms, in this study we observed high AH_{50} complement activity (88.17 U/ml) in children infected with *Staphylococcus aureus*than other infectious agent such as *Plasmodium falciparum, Streptococcus spp* etc. However, children infected with *E. coli* tend to have lowest AH_{50} complement activity (78.10 U/ml). Our finding highlights normal complement activation in response to infections as such it complements the health status of the children. Moreover, peptidoglycan layer of some bacteria such as *S. aureus*and others were shown to activate complement.[20] Specifically these organisms can activate the alternative complement pathway.[21] Therefore the degree of complement activation varies among organisms. In addition the plasma proteins of the complement system are essential in the innate immune response against bacteria.[22] The disparity between our result and other study may be associated to sample collection, processing, subject and techniques used which likely dictate the result.

CONCLUSION

This study shows that the AH_{50} activity among children with some bacterial and malaria parasite infections fall within the normal assay range which indicate normal activity. It revealed that there is significant difference in AH_{50} activity between children diagnosed with malarial parasite and bacterial infections. Therefore we recommend further investigation that will lead to identify the possible causes of the difference.

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