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Classical Complement (CH₅₀) activity among children with some bacterial and parasitic infections in Sokoto, NigeriaKalgo, M.U.*¹, Muhammad, T.B.¹, Yeldu, M.H.¹, Garba, B.I.², Hamid, K.M.¹, Isiyaku, A.¹, Raji, H.Y.¹, Bello, M.³, Bunza, N.M.³, Garba, M.R.¹,*Department of Immunology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, P.M.B 2346, Sokoto, Nigeria*¹, *Department of Paediatrics, Usmanu Danfodiyo University/Usmanu Danfodiyo Teaching Hospital, Sokoto, Nigeria*², *Department of Medical Microbiology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria*³.Author for Correspondence *: umustapha50@gmail.com/+234-803-962-8476**Abstract**

Complement system is a biochemical cascade that helps, or “complements” the ability of antibodies to clear pathogens from the body of an infected individual. Deficiency of any of the complement proteins may lead to recurrent infection and sepsis. This study evaluated the activity of the complement protein (CH₅₀) among children with some bacterial and parasitic infections, attending Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria. A total of 71 children, comprising of 45 males and 26 females suffering from either parasitic or bacterial infection, were recruited from the Emergency Paediatric Unit (EPU) of UDUTH. Socio-demographic characteristics of the study subjects were obtained with the aid of structured interviewer-administered questionnaire, while the presence of parasitic and bacterial infection was confirmed using microscopy and culture techniques respectively. Blood samples were collected from the children and processed to obtain serum. The serum CH₅₀ activity was assayed using sandwich Enzyme-linked Immunosorbent Assay (ELISA). The result indicated no significant ($p = 0.294$) differences between the assayed CH₅₀ activity and different microbial infections. Similarly, age, gender, and residence of the participants appeared not to be a factor affecting classical complement activity of children with microbial infections. Further studies are hereby suggested to explore the various mechanisms of complement CH₅₀ activity in the defense against microbial infections.

Keywords: Complement activity, Children, ELISA, Microbial Infection

Introduction

The Complement System is an essential part of innate defense against infections (Wen *et al.*, 2004). It helps the cells in our immune system to better recognize and capture pathogens, and can also lead to direct elimination of bacteria, fungi and parasite by destroying their cell membrane (Wen *et al.*, 2004; Jason and Wenchao, 2010). There are more than fifty known complement proteins that can be activated in three distinct pathways (Judith *et al.*, 2013). Complement proteins are synthesized by the liver hepatocyte, monocyte (macrophages), epithelial cells of gastrointestinal and genitourinary tract (Suzan, 2001). Complement activation, regardless of the pathway, converges on generation of four broad effector molecules that enable the complement proteins to fulfill their physiological function, which include opsonization, lysis/rupturing of cells, clearance of circulating immune complexes, and activation of memory B cells (Winkelstein, 2004). Complement deficiency is an immunodeficiency characterized by the absent or suboptimal functioning of one of the complement proteins (Sarma *et al.*, 2011). Deficiency in any of the complement proteins can lead to recurrent and difficult-to-control infections, the deficiency can be inherited or acquired and complete or partial (Kolev and Kemper, 2017). Acquired immunodeficiency may occur as a result of infection while genetic deficiency of early components of the classical pathway (C1q, C1r/s, C2 and C4) may be linked with autoimmune diseases (Bryan and Wu, 2014). Most of the genetically determined deficiencies of the complement proteins are inherited as autosomal recessive traits (Michael *et al.*, 2016). Severe primary immunodeficiency

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 USA and few European countries (Borte and Reichenbach, 2015). There is lack of awareness in complement deficiencies especially in African countries (Orren, 2007). Early detection of complement deficiencies will enable aggressive treatment of microbial infections with appropriate antimicrobial therapy (Michael *et al.*, 2016).

Materials and Methods

Study Design

This is a cross sectional study set to determine the haemolytic activity of the classical complement CH₅₀ pathway in children with bacterial or parasitic infection attending EPU of UDUTH, Sokoto-Nigeria.

Study Area

This study was conducted in Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, North-Western Nigeria. UDUTH is a tertiary institution located within Sokoto metropolis. Sokoto is the capital city of Sokoto State located in the extreme North-West of Nigeria, near the confluence of the Sokoto River and the Rima River. The State is in the dry Sahel, surrounded by sandy savannah and isolated hills, with an annual average temperature of 28.3. Sokoto is on the whole, a very hot area. However, maximum daytime temperatures are for most of the year generally under 40 and the dryness makes the heat bearable. The rainy season is from June to October during which shower are of daily occurrence. The projected population of Sokoto stands at around 4.9 million (NPC, 2016).

Study Subjects

A total of 71 children attending EPU of UDUTH Sokoto were randomly recruited for this study. Written informed consent was obtained from the parents of the children before collecting sample. Children with bacterial or parasitic infections were confirmed by culture and microscopy respectively. The study subjects were grouped based on socio-demographic characteristics and presence of bacterial or parasitic infection.

Ethical Considerations

Approval for the study was obtained from Ethics and Research Committee, UDUTH (Ref. No: UDUTH/HREC/2017/No.616).

Sample Collection and Processing

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

Determination of CH₅₀ Complement Activity

The Complement activity was determined quantitatively using human classical complement (CH₅₀) ELISA kit (Sunlong Biotech, China). The assay uses sandwich ELISA technique. The procedure was performed according to the manufacturer's instructions.

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. A p-value of less than or equal to 0.05 ($p \leq 0.05$) was considered as statistically significant.

Results

The result in Table 1 shows the socio-demographic characteristics of the study subjects. Seventy-one (71) children, aged ¹⁻¹² years participated in this study, 45 (63.4%) were males and 26 (36.6%) were females. More than half of the participants 47 (66.4%) reside in urban areas; out of which 36 (50.7%) had parasitic infection and 35 (49.29%) had bacterial infection. The relationship between age range and complement CH₅₀ activity is presented in Table 2. The children within the age range of 9 – 12 years had higher CH₅₀ activity (71.42 U/ml)

compared to those in the other age groups. Also, the children with the age range of < 1 – 4 years had lower CH₅₀ activity (61.25 U/ml).

Table 3 shows the relationship between gender and complement CH₅₀ activity. The complement CH₅₀ activity in female subjects was not significantly ($p = 0.414$, $U = 516.50$) different compared to similar values of complement CH₅₀ activity observed in their male counterparts (66.14 U/ml versus 62.34 U/ml respectively).

The comparison between median CH₅₀ activities based on bacterial or parasitic infection of the

study subjects is presented in Table 4. The result indicated that the median CH₅₀ activity of children with bacterial infection (71.42 U/ml) was not significantly ($p = 0.194$, $U = 517.00$) different compared to children with parasitic infection (61.25 U/ml).

The result on the relationship between isolates and complement CH₅₀ activities is shown in Table 5. When the result was delineated according to different isolates of bacterial or parasitic infection, the median CH₅₀ activities of children were not significantly ($\chi^2 = 6.128$; $p = 0.294$) related to the type of bacterial or parasitic infection.

Table 1: Socio-demographic characteristics of the study subjects

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

Key: N= Number of children in each category

Table 2: Relationship between age range and complement CH₅₀ activity

Age group (years)	Frequency N (%)	Median CH ₅₀ activity (U/ml)
<1	7 (9.9)	36.38
1-4	26 (36.6)	61.25
5-8	21 (29.6)	60.00
9-12	17 (23.9)	71.42
Total	71 (100%)	

Kruskal Wallis test: $\chi^2 = 2.077$, $p = 0.55$

Table 3: Relationship between gender and complement CH₅₀ activity

Gender	Frequency N (%)	Median CH ₅₀ activity (U/ml)
Male	45 (63.4)	62.34
Female	26 (36.6)	66.14
Total	71 (100%)	

Mann Whitney U Test: 516.500, p = 0.414

Table 4: Comparison between median CH₅₀ activities based on bacterial or parasitic infection of the study subjects

Infection	Frequency N (%)	Median CH ₅₀ activity (U/ml)
Bacterial	35 (49.29%)	71.42
Parasitic	36 (50.70%)	61.25
Total	71 (100%)	

Mann Whitney U Test: 517.000, p = 0.194

Table 5: Shows relationship between isolates and complement CH₅₀ activity

Isolate	Number of subjects	Median CH ₅₀ activity (U/ml)
<i>Escherichia coli</i>	20 (28.2)	66.04
<i>Salmonella spp</i>	2 (2.8)	120.13
<i>Streptococcus spp</i>	6 (8.5)	83.79
<i>Staphylococcus aureus</i>	5 (7.0)	51.75
<i>Neisseria spp</i>	2 (2.8)	64.44
Malaria parasite	36 (50.7)	87.49
Total	71 (100%)	

Kruskal Wallis: $\chi^2 = 6.128, p = 0.294. spp = Species$

Discussion

The complement system plays a critical role in inflammation and defense against some bacterial, parasitic and fungal infections. Binding of antibody to its homologous antigen in circulation may initiate the activation of classical complement pathway, which helps in opsonization and destruction of pathogen. Deficiency of individual complement components or inhibitors of the system can lead to a variety of diseases (Behmanesh, 2010). The range of CH₅₀ Complement activity in the children is between 2 U/ml – 150 U/ml. Therefore, it falls within the assay range, this possibly indicate the normal activity of the

classical complement pathway. The CH₅₀ is the primary screening test for classical complement deficiencies. It measures functional activity of the pathway. Out of 71 children that participated in this study, 36 (50.7%) were infected by malaria parasite while 35 (49.2%) were infected by bacteria. In this study we observed that children at the age range between 9-12 years have higher CH₅₀ complement activity on comparison with other age groups, this is in line with earlier report by Davis *et al.* (1979) who reported that, the level of complement protein is significantly lower in the elderly and middle age group and slightly higher in children of 10-15years old.

In this study, the classical complement (CH₅₀) activity among the female subjects was not significantly different when compared with their male counterparts. This finding is in agreement with previous report by Norman *et al.* (1975) who reported similar values of complement activities between the male and female children indicating that gender is not a determinant of complement activity in the serum of children with bacterial or parasitic infection.

Our findings revealed that, the classical complement activity among children diagnosed with bacterial infections was similar to the values obtained among children diagnosed with malarial parasitic infection. This support the notion that massive classical complement pathway activation occurs in bacterial infection especially pyogenic bacteria like *Neisseria meningitides*, *Streptococcus species* and *Haemophilus influenza* (Ram *et al.*, 2010). However, this finding contradicts earlier report by Geng (2010), who used radial immunodiffusion techniques in detecting the classical complement activity and reported a significant decrease of CH₅₀ activity among children infected with bacterial agents when compared with the children that suffers other microbial infection. This difference may be related to different techniques and or environment factor or season of the research.

Based on causative organisms, in this study we observed high CH₅₀ complement activity (120 U/ml) in children infected with *Salmonella spp.* than other infectious agents such as *Streptococcus spp.*, *Escherichia coli* etc. However, children infected with *Staph. aureus* tend to have lowest CH₅₀ complement activity (51.75 U/ml). Our finding highlights normal complement activation in response to bacterial and parasitic infections as such it complements the health status of the children. Peptidoglycan layer of some bacteria such as *Salmonella spp.* and others were shown to activate classical complement pathway. Therefore, the degree of complement activation varies among organisms. The disparity between our result and other study may be associated to sample collection, processing, subjects and techniques used which may likely dictate the result.

Conclusion

This study reveals that having recurrent bacterial or parasitic infection does not necessarily implies decrease in complement activity or immunodeficiency. Hence advocacy for routine complement proteins investigation should be encouraged. This will help the clinicians in the improvement of patient's management.

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