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Serum Immunoglobulin Levels in Nigerian Patients with Sickle Cell Anaemia in Bone Pain Crisis and Steady State

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Abstract

Background: Sickle cell patients are prone to recurrent episodes of vaso-occlusive crisis. Bone pain crises, a form of vaso-occlusive crisis are frequently precipitated by infection. Immunoglobulin are effectors of humoral immunity produced by plasma cells and help in the fight of infection.

We determine serum immunoglobulin levels (IgG, IgA & IgM) in sickle cell patients during bone pain crisis and steady as well as correlate the immunoglobulin levels with total leucocyte count, a known prognostic factor for disease severity.

Methods: 100 participants including 50 sickle cell patients in bone pain crisis, 35 of whom were seen again 4 weeks post crisis (steady state) and 50 healthy Hb AA individuals as controls were recruited. Socio-demographic information of participants were documented. Blood samples were taken for haematological and serum immunoglobulin determination.

Results: The mean level of serum IgG, and IgA during bone pain crisis were significantly higher that of the HbAA controls (p= 0.001 respectively). Serum IgG and IgA during bone pain crisis were also significantly higher than during steady state (p=0.01 & 0.034 respectively). Furthermore, the mean leucocyte count though significantly higher during bone pain crisis than during steady state (p=0.001), however it did not correlate with the serum immunoglobulin levels (p =0.395, 0.810, 0.746 for IgG, IgA and IgM respectively).

Conclusion: Serum immunoglobulin especially IgG and IgA was significantly higher during bone pain compared with Hb AA controls as well as during steady state. However, the immunoglobulin levels did not correlate with total leucocyte count, a known prognostic factor for disease severity.

Keywords: Sickle cell anaemia; Vaso-occlusive crisis; Bone pain crisis; Steady state; Immunoglobulin

Abbreviations

SCA; SCD; VOC; BPC; IgG; IgA; IgM; WBC; CBC; ELISA; EDTA; ADVIA; HDCU; MOP; ICAM; VCAM; PAF; HLA; TNF; IL

Introduction

Sickle Cell Disease (SCD) is a group of haemoglobin disorder resulting from the inheritance of the sickle β-globin gene. The homozygous state (Hb SS or sickle cell anaemia) is the most common form of the disease, however the interaction of the abnormal haemoglobin S (HbS) with thalassemia and other haemoglobin variants also result in sickling [1,2].

SCD is a major public health problem and the most common genetic disease disorder that affect mankind [3]. The disease has a global distribution, but certain regions of the world have high prevalence for the sickle cell gene including Equatorial Africa, Sicily in Southern Italy, Middle East and Central India [3,4].

In Nigeria, the magnitude of the problem is much more pronounced given that 25% of the population carry the sickle cell gene [5]. The World Health Organization (WHO) estimates the prevalence of SCA to be 20 per 1000 live birth annually which translate to about 150,000 children born annually with SCA in Nigeria, thus making Nigeria the country with the highest disease burden globally [5,6].

The common and most distressing clinical manifestation of SCA is the episodic and unpredictable sickle cell vaso-occlusive crisis (VOC) [7]. The crisis occurs unpredictably over a life-time and account for most common cause of emergency room visits and hospitalization. The most common acute VOC is the Bone Pain Crisis (BPC). BPC is defined as painful episodes involving one or more sites of bones in the absence of preceding trauma or obvious infection [5]. The most common sites are the long bones of the limbs, lumbar spine, and thoracic cage.

Malaria bacterial and viral infection, are the most common precipitating factors for VOC and leading cause of mortality in SCD [8]. SCA patients are prone to infection because of a defect in the alternative pathway of complement activation and opsonisation [9,10] reduced ability of neutrophils to kill pathogens [9,11] and functional hyposplenism further decreasing macrophage function and immune response to organisms in the blood [9,12].

Humoral immunity is unimpaired in SCA and serum immunoglobulin levels tend to be high in response to frequent

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Table 1: Age and gender distribution of subjects and controls.

Variables	Subjects (SCA)		Controls		Chi-Square	p-Value
Age	Frequency	%	Frequency	%		
15-29	38	76.0	20	40.0		
30-45	7	14.0	26	52.0	10.390	0.001
46-60	5	18.0	4	8.0		
Gender						
Male	24	48.0	43.0	86.0		
Female	26	52.0	7.0	14.0	16.327	0.001

Table 2: Comparison of the mean serum immunoglobulin levels between subjects and controls.

Variables	Subjects (SCA in BPC) Mean (SD) n=50	Controls (HbA) Mean (SD) n=50	t-test	p-value
IgG (g/L)	19.81(2.13)	11.28(6.17)	8.475	0.001
IgA(g/L)	5.23 (1.46)	3.04 (1.43)	7.463	0.001
IgM(g/L)	2.27 (0.16)	1.54 (0.44)	0.097	0.097

infection [9,13,14]. Infection precipitates VOC by increasing the interaction of leucocytes with vascular endothelium [15]. Acute inflammatory reaction to infection or tissue injury increases local and circulating levels of tissue necrotic factor alpha (TNF-α) and interleukin-Iβ (IL-Iβ) which activates leucocytes to express more adhesion molecules (α2Mβ2 & L-selectin) and vascular endothelial cells express more ligands (intercellular adhesion molecule –ICAM, vascular adhesion molecule-VCAM-1) for the adhesion molecule of erythrocytes and leucocytes [15]. This increases aggregation of blood cells to each other and also their aggregation to the vascular endothelium leading to vaso-occlusion.

Cytokines (IL-Iβ, TNF-α) and cell adhesion molecules VCAM-I, selectin, fibrinogen, vaso-active substances, Platelet Activating Factor (PAF) and endothelin-I are elevated during state and higher during VOC [16,17]. Immunoreactive substances (IgA, IgG, and IgM, HLA class-1 heterodimer) have also been observed to be elevated in steady state and during VOC [13,14,18]. The abnormal levels of these acute phase reactants cytokines and other mediators of inflammation may play a role in the development of acute complications and chronic organ damage in SCA.

Immunoglobulins are glycoproteins molecules that are produced by plasma cells in response to immunogen and function as antibodies [13]. They are important effectors of specific humeral immunity. Elevation of one or more of the different sub-classes of immunoglobulin have been reported in SCD [13,14,18]. These have been attributed to reticuloendothelial stimulation from haematopoietic stress and infection.

Considering the unpredictable nature of VOC, effort are being made to identify the prodromal phase of sickle cell crises by determining the levels of various acute phase reactants, immunoreactive substances and others makers of inflammation and perhaps use these markers as predictors of clinical severity of the disease.

Therefore, the objectives of this study was to determine the serum levels of immunoglobulins (IgG, IgA, IgM) in SCA patients during bone pain crisis and steady state, ascertain if serum immunoglobulin levels have any correlation on the severity of SCA when compared with other known prognostic factor such as leucocytosis.

Materials and Methods

Study design

This was a prospective, cross-sectional, hospital based study designed to achieve the above set objectives.

Study population

This consists of adult SCA patients. Fifty SCA patients in BPC were recruited for the study. The patients were recruited consecutively from the Haematology Day Care Unit (HDCU), Medical Out-Patient Clinic (MOP) and Accident and Emergency Unit of the University College Hospital, Ibadan, between May – July 2010. Thirty –five of the sickle cell patients were seen again in steady state 4-weeks after the initial BPC. This group served as auto-control, while 50 healthy non- sickle cell persons served as the control group. Steady state is that period whereby sickle cell patients are symptoms free for at least 2 weeks [5].

Inclusion criteria

SCA patients who presented in BPC and healthy controls were enrolled in the study after a written informed consent had been obtained from them.

Exclusion criteria

SCA Patients who had other forms of sickle cell crisis such as acute chest syndrome, abdominal crisis, priapism among others and those with other forms of sickle cell variant such as HbSC, HbSD, HbS/β-thalassemia were excluded from the study. Also patients and control subjects who did not give written informed consent were excluded from the study.

Data collection

Data on socio-demographic information were collected directly from the patients by interviewee administered questionnaire designed for the study

Specimen collection/Analytical procedure

After obtaining an informed consent, 8ml of venous blood was obtained from each patient and control subjects from the ante-cubital fossa.

Three millilitres of this blood was dispensed into Ethylene Diamine Tetra Acetic Acid (EDTA) specimen bottle. This was used to determine the complete blood count (Haematocrit, leucocyte and platelet count) of the subjects and controls. CBC was determined using an Automated Haematology System Cell Counter (ADVIA) manufactured by Bayer cooperation, New York, United State of America. Analysis was done within 2hours of sample collection.

The remaining 5ml of blood of blood was dispensed into sterile plain bottle for assay of serum immunoglobulin. This was determined by ELISA technique using Total Human Immunoglobulin (IgG, IgA, IgM) immunoperoxidase assay kits manufactured by Immunolohy Consultant Laboratory, INC. 141 North Elliot Road, Newberg, USA, with LOT numbers as follows: Human IgG ELISA-E-80G LOT #13, Human IgA ELISA-E-80A LOT#5 and Human IgM ELISA-E-80M LOT#7.

Data analysis

The data obtained were subjected to statistical analysis using SPSS version 16. Results were presented in simple tables and inferential statistics (Chi-Square, t-test and Correlation) were used as appropriate at a significant levels of $p<0.05$.

Results

The mean age of the SCA patients was 27.10 (±8.75) years with a range of between 15-56 years while that of the control group was 33.50 (±8.53) years. There were more female subjects than males (52% and 48% respectively). Conversely, there were more males than females in the control group (86% and 14% respectively) Table 1.

The mean levels of serum immunoglobulins (IgG and IgA) during BPC among the subjects was significantly higher (19.81g/L and 5.23g/L respectively) than that of the HB AA controls (11.28g/L and 3.04g/L respectively). These values were statistically significant (P=0.001). The mean serum IgM (2.27g/L) though higher than that of the controls (1.54g/L), however, the difference was not statistically significant. (p=0.097) Table 2.

The mean levels of serum immunoglobulin (IgG and IgA) were significantly higher among SCA subjects during BPC (19.81g/L and 6.12g/L respectively) than that of subjects during steady state (15.92g/L and 5.19g/L respectively) p = 0.01 and 0.034 respectively. Also, there was statistical significant difference in the total WBC count of subjects in the BPC and steady state Table 3.

However, there was no correlation between the serum immunoglobulin levels during steady state and total leucocyte count Table 4.

Discussion

Bone pain crisis is the most common acute form of vaso occlusive crisis experienced by SCA patients. Its occurrence is often episodic and unpredictable. Infection is the most common precipitating factor for BPC. Immunoglobulins are serum glycoproteins produced by plasma cells that help the body to combat infection. They are important effectors of specific humoral immunity [13].

Several studies have reported an increase in the serum immunoglobulin levels in SCA patients both in steady state and in VOC [13,19,20]. The data from this study showed a significantly elevated IgG and IgA levels compared with that of the controls and was in agreement with findings from previous studies above.

IgG is the most abundant serum immunoglobulin in the body release in response to bacterial infection as well as neutralization of toxins and viruses [21]. Significantly high levels of IgG have been attributed to chronic stimulation of reticuloendothelial cells secondary to the chronic haemolytic state in SCA as well as recurrent infections and malaria [13,20,22]. Hedo et al, [13] further demonstrated an increase in the IgG subclass i.e. IgG1 and IgG3 among SCA patients with frequent bacterial infections. Susceptibility to infection in SCA is related to abnormalities in the alternative pathway of complement activation and opsonisation, reduced ability of neutrophils to kill pathogenic organisms and functional hyposplenism. Functional hyposplenism (94%) was a common feature among our index subjects.

Table 3: Comparison of mean serum immunoglobulin levels, total WBC and neutrophil count during bone pain crisis and steady state.

Variables	BPC Mean (SD) n=50	Steady State Mean (SD) n=35	t-test	p-value
IgG (g/L)	19.81 (1.95)	15.92 (0.01)	12.102	0.01
IgA(g/L)	6.12 (0.32)	5.19 (2.13)	2.232	0.034
IgM(g/L)	2.26 (0.16)	1.53 (0.59)	5.103	0.276
WBC/cmm	12,097 (3070)	9,097 (1,760)	8.896	0.001
Neutrophil count ($x10^9$/l)	5.5 (3.30)	4.5 (2.25)	4.294	0.062

Table 4: Correlations between the Serum Immunoglobulin and the Leucocyte Count of SCA in Bone Pain Crisis.

Total WBC	Total WBC
IgG	r =0.1509 p =0.395
IgA	r =0.0428 p =0.810
IgM	r = 0.0576 p =0.746

Similarly, a significantly elevated serum IgA levels was found among our cohort of SCA patients compared to the controls. (p=0.001) This finding corroborates with report by other investigators [13,14,23] who also worked with Nigerian SCA patients. The primary role of IgA is mucosal immunity [24]. Respiratory infection was a common feature of some of our patients in BPC. In addition, high levels of IgA have also been associated with hepatic dysfunction, cholelithiasis, chronic haemolysis and hyposplenism/post-splenectomy [25]. These features in addition to the respiratory infection are common in SCA patients and may perhaps be responsible for the high serum IgA seen in our patients.

IgM is the predominant immunoglobulin class formed during primary immune response [26]. Reports from previous studies have shown that serum IgM is only increased when there is demonstrable functional splenic tissue [27,28]. Adekile et al, [19] in their study among a cohort of SCA patients in Saudi-Arabia reported low serum IgM levels in patients with no splenic visualization on splenic-scintigraph. Similar finding was reported by Gavrilis et al. [29]. These reports contrast with the findings from this study in which the serum IgM levels was numerically higher than that of the controls despite the fact that majority of our subjects (94%) had already suffered autosplenectomy. However, it agrees with the study by Adedolu et al. who also reported a higher serum IgM levels in SCA patients than controls. The rise in IgM levels in this study may be attributed largely to environmental factors of which recurrent malaria infection is topmost given the endemic nature of this infection in our environment. Malaria infection has been shown to be a known mitogen that can stimulate the B-Cell proliferation [30]. Furthermore, Adekile et al, [31] demonstrated a direct correlation between malaria antibody titre and serum immunoglobulin levels in Nigerian SCA patients.

Comparing the serum immunoglobulin levels (IgA, IgG, and IgM) during BPC and steady state, it was interesting to note that the mean serum IgG and IgA (19.96g/L. 6.12g/L respectively) during BPC was significantly higher than steady state level (15.92g/L, 5.16g/L). p=0.01. Also, the steady state levels of IgG and IgA were significantly higher than that of the controls (1.28g/L and 3.04g/L respectively). This would suggest a persistent state of low grade inflammation,

haemolysis and on-going microvascular injury during steady state in SCD. In fact studies have shown that the levels of immunoreactive substances and acute phase reactant are high in steady state and significantly higher during VOC in SCD [32-34].

Reports from previous studies have shown that SCA patients experience marked neutrophilic leucocytosis during episodes of acute painful crisis which persist during the steady state with chronic granulocytic activation thus reflecting an on-going state of inflammation [33,35]. Some authors have suggested that the baseline neutrophil count is the single most important predictor of VOC and overall prognosis in SCA [35] while others have proposed a WBC count of 10,000/cmm and 15,000/cmm as a measure of clinical severity in SCA [36]. In a study among Afro-Caribbean SCA patients, the authors reported that a higher WBC count correlate with increased hospital admission, lower HbF and haemoglobin concentration and may be used as an independent predictors in the assessment of clinical severity in patients with SCA [36]. The statistically significant difference in the mean WBC counts of SCA patients in BPC and steady state observed in this study agrees in part with reports from study above. However, correlation between the mean serum immunoglobulins, total WBC and neutrophils counts could not be established in this study. This perhaps may be due to the small sample size of the study. It is possible that studies with larger sample size may demonstrate a relationship between serum immunoglobulin levels and total WBC and neutrophils count.

Conclusion

The serum level of immunoglobulin particularly IgA and IgG among Nigerian SCA patients either in the steady state or BPC was significantly higher than that of the Hb AA controls. Although, there was statistically significant difference in total leucocyte count (in BPC and steady state) a known prognostic factor of VOC in SCA patients; correlation between total leucocyte count and serum immunoglobulins did not show any significant difference perhaps due to the small sample size of the study population.

Limitation of Study

Some of the SCA patients (15) in steady state (auto-control) dropped out of the study. They did not return for repeat sampling 4 weeks post initial BPC. Also, using blood donors as controls did not allow for proper matching of subjects and controls because majority of donors in our environment are males in their mid-twenties and thirties.

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