SHORT COMMUNICATION

Immunoglobulin Expressions in Patients with Homozygous Sickle Cell Disease

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Abstract:

Aim: The serum immunoglobulin (IgA, IgG, IgM) levels of sickle cell patients attending General Hospital Owerri, Nigeria were evaluated to determine whether or not the serum levels of these immunoglobulins were normal. *Materials and Methods*: One hundred confirmed sickle cell patients (HbSS), and thirty sickle cell patients in crisis age 5–30 years were selected. One hundred normal subjects (HbAA) age 5–30 years were used as control. *Results*: The levels of immunoglobulins (IgA and IgG) were significantly increased in sickle cell anaemia ($p<0.05$), except immunoglobulin M, when compared with the control (HbAA). However, the levels of immunoglobulins (IgA and IgG) were significantly increased in sickle cell crisis compared with sickle disease in steady state. *Conclusion*: The result suggests that alteration in immune response is associated with Sickle cell disease.

Keywords: Immunoglobulins, Homozygous Sickle Cell Disease

Introduction:

Immunoglobulins are glycoprotein molecules produced by white blood cells or plasma cells. They are also known as antibodies [1]. They act as an important part of the immune response by recognizing and binding to particular antigens, such as bacteria or viruses and enhancing in their destruction. The antibody immune response is highly complex and exceedingly specific [2]. The various immunoglobulin classes differ in their biological features, structure, target specificity and distribution. Therefore, the assessment of the immunoglobulin class can provide useful insight into complex humoral immune response. The various antibodies produced by plasma cell are classified by isotype that differ in function and antigen responses primarily due to structure variability. Five major classes include: IgA, IgD, IgE, IgG,and IgM

Antibody isotypes are categorized according to differences in their amino acid sequence in the constant region (Fc) of the antibody heavy chains [3].The Immunoglobulins IgG and IgA are further

grouped into subclasses (e.g. in Human IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) based on additional small differences in the amino acid heavy chain sequences [4].The evaluation of individual classes can be relevant in assessing primary immune responses in sickle cell anaemia, especially if the total IgG or IgA concentration is not altered or elevated [5].

These immunoglobulins are found in different concentration in the body. Immunoglobulin A (IgA) is found in high concentrations in the mucous membranes, particularly those lining the respiratory passages and gastrointestinal tract, as well as in saliva and tears. Immunoglobulin G (IgG), the most abundant type of antibody and is found in all body fluids and protects against bacterial and viral infections [6, 7]. Also, Immunoglobulin M (IgM), which is found primarily in the blood and lymph fluid, is the first antibody to be made by the body to fight a new infection. Immunoglobulin E (IgE) is associated with allergic reactions, when the immune system overreacts to environmental antigens such as pollen or pet dander. It is found in the lungs, skin, and mucous membranes. Immunoglobulin D (IgD) exists in small amounts in the blood is the least understood antibody [8, 9].

IgA, IgG, and IgM are often measured together. That way, they can give important information about immune system functioning, especially relating to infection or autoimmune disease [10, 11]. Once an antibody is produced against a specific antigen, the next time that antigen enters the body; the immune system "remembers" its response and produces more of the same antibodies. In that way, checking for the presence of specific immunoglobulins in the blood can be helpful in diagnosing or ruling out infections or certain other illnesses or diseases. The immunoglobulin test is one of the tools to help diagnose immunodeficiencies that are when the immune system isn't working effectively. A person can be born with an immunodeficiency or acquire it through infection, disease, malnutrition, burns, or as a side effect of medicines [12].

Immunoglobulin levels are also used as part of an evaluation for sickle cell anaemia. Sickle cell anemia is an inherited blood disorder that affects hemoglobin which is the protein found in red blood cells (RBCs) that helps carry oxygen throughout the body [13]. Sickle cell anemia occurs when a person inherits two abnormal genes that cause their RBCs to change shape. Instead of being flexible and disc-shaped, these cells are more stiff and curved in the shape of the old farm tool known as a sickle [14].

Sickle cell disease usually begins in early childhood. It is characterized by features of this disorder including a low number of red blood cells (anemia), repeated infections, and periodic episodes of pain. The severity of symptoms varies from person to person. Some people have mild symptoms, while others are frequently hospitalized for more serious complications [13].

The signs and symptoms of sickle cell disease are caused by the sickling of red blood cells. When red blood cells sickle, they break down prematurely, which can lead to anemia. Anemia can cause

shortness of breath, fatigue, and delayed growth and development in children. The rapid breakdown of red blood cells may also cause yellowing of the eyes and skin, which are signs of jaundice. Painful episodes can occur when sickled red blood cells, which are stiff and inflexible, get stuck in small blood vessels. These episodes deprive tissues and organs of oxygen-rich blood and can lead to organ damage, especially in the lungs, kidneys, spleen, and brain. A particularly serious complication of sickle cell disease is high blood pressure in the blood vessels that supply the lungs (pulmonary hypertension). Pulmonary hypertension occurs in about one-third of adults with sickle cell disease and can lead to heart failure [15].

In sickle cell anaemia, the levels of IgA, IgG and IgM were determined as an index of immune response. This study was embarked upon to evaluate status of immunoglobulins in sickle cell disease patients. Also, this study was equally undertaken so that the knowledge gained from the research work could provide information for better understanding of immune response in sickle cell disease.

Materials and Methods:

One hundred HbSS diagnosed by haemoglobin electrophoresis, aged 5-30 years were selected for the study. One hundred HbAA normal subjects were used as control. Also, thirty sickle cell patients in crisis were also involved. Both male and female were equal.

Blood sample: In all subjects, 5ml of venous blood was collected into a non- anticoagulated tubes. Samples were spun in a Wisterfuge (model 684), centrifuge at 1000g for 10 minutes and the serum collected into a clean dry bijou bottle. Immunoglobulins were estimated. Informed consent of the participants was obtained and was conducted in line with the ethical approval of the hospital.

Biochemical Assay:

Immunoglobulin A, G and M levels were determined by use of immunoglobulin AGAPPE kits for IgA, IgG and IgM. The purpose is to quantitate serum levels of immunoglobulins (IgG, IgA, IgM). These measurements aid in the clinical diagnosis, assessment of disease activity, response to treatment, and follow-up in patients with various clinical conditions. These tests are measured by nephelometry technique. This is based on measurement of the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction [16].

Statistical Analysis:

The results were expressed as mean ± standard deviation. The statistical evaluation of data was performed by using student's t- test. The level of significance was calculated at $P<0.05$.

Results:

The level of IgA, and IgG, were significantly increased in HbSS and HbSS-crisis when compared with the control while IgM was not significantly decreased when compared with the control ($p<0.05$) (Table 1).

Table 1: Immunoglobulin Levels in Sickle Cell Anaemia and Control

Parameters	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)
HbAA	362.41± 81.2	1316.82±96.4	91. 22 ±31.2
HbSS	492.91±124.8	1628.7±294.8*	89 .89 ±3 1.4
HbSS -crisis	52144 ±142 .6*	179.8±253.6*	88.11±3 7.9

**Significantly different from control at p<0.05.*

Discussion:

Sickle cell disease is a genetic disease for which no cure has been available. It is characterized by inflammation, anaemia demand and vaso-occlusion [17]. Haemolysis leads to loss of hemoglobin which in turn leads to immune alteration. Detection of particular immuno globulin is a very common form of medical diagnosis, and applications such as serology depend on these methods. The levels of individual classes of immunoglobulins are measured by turbidimetry to characterize the immunoglobulin profile of patient. Elevations in different classes of immunoglobulins are sometimes useful in interpreting the immune response in sickle cell patients.

In this study, it was observed that some IgA and IgG in sickle cell disease subjects were significantly increased when compared with HbAA. .The significantly high level of IgA is consistent with the work of Olsen *et al* [18] who related IgA elevation in sickle cell disease to manifestations such as growth retardation and cell mediated immune disorder. It is linked with impaired T-helper functions, cell mediated immunity and reduced interleukin-2 production as well as increase of bacterial infection, vaso-occlusive crises, frequently hospital admissions and growth retardation [19] Immunoglobulin A is an antibody that plays a critical role in mucosal immunity. More IgA is produced in mucosal linings than all other types of antibody combined. The high level of IgA in mucosal areas is a result of cooperation between plasma cells that produce polymeric IgA (pIgA), and mucosal epithelial cells that make an immunoglobulin receptor called the polymeric Ig receptor (pIgR). pIgA is produced from the nearby activated plasma cells and binds to pIgR. This leads to movement of IgA across mucosal epithelial cells and its cleavage from pIgR for release into external secretions [20].

In the blood, IgA associate with an Fc receptor called FcαRI, which is expressed on immune effector cells, to produce inflammatory reactions.Also, the high concentration of IgG has been noted in sickle cell disease subject when compared with the control ($p< 0.05$). IgG is the main components of humoral immunity. IgG is the major type of antibody found in blood and

extracellular fluid allowing it to control infection of body tissues. Through binding varieties of pathogens such as viruses, bacteria, and fungi, IgG protects the body from infection. This is achieved through many mechanisms: IgG-mediated binding of pathogens causes their immobilization and binding together through agglutination; IgG coating of pathogen surfaces permits their recognition and ingestion by phagocytic immune cells; IgG activates the classical pathway of the complement system, a cascade of immune protein production that leads to pathogen elimination; IgG also binds and neutralizes toxins. IgG is important in antibody-dependent cell-mediated cytotoxicity to red blood cell dehydration and a concomitant increase in the symptoms of sickle cell disease. This study is in agreement with the work of Ebringer and Doyle [21].

Furthermore, the level of IgM among SCD subjects was not significantly decreased when compared with the control ($P< 0.05$). The involvement of IgM in SCD subjects is not clearly defined but Immunoglobulin M, is a basic antibody that is produced by B cells. It is the first antibody to appear in response to initial exposure to an antigen. IgM is often binds to specific antigens, even in the absence of prior immunization [18]. Hence, IgM has sometimes been referred as natural antibody. This could probably due to the high avidity of IgM that allows it to bind detectably even to weakly cross-reacting antigens that are naturally occurring [22]. Immunoglbulin M antibodies that bind to the red blood cell A and B antigens might be formed in early life as a result of exposure to A and B like substances that are present on bacteria. This is in line with the work of Kristensen [23].

Conclusion:

It is quite obvious that the levels of IgG and IgA are increased in homozygous sickle cell hence, affecting individual immunity in sickle cell disease.

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