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Comparison of some Haematological profiles in genotype AA, AS and AC persons amongst IMO state University students

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Plateletes,
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Abstract

Some haematological profiles were studied in one hundred (100) students of Imo State University with haemoglobin genotypes AA, AS and AC . The aim of the study was to evaluate their haemoglobin genotypes and compare some haematological profiles in students with haemoglobin genotype AA, AS and AC. The mean results obtained were Hb (male) 14.6±0.2 and 12.4±1.4 for AA and AS respectively, Hb (female) 13.01±1.2, 12.4±1.4 and 12.4±2.5 for AA, AS and AC. PCV (male) 43.7±0.9 and 36.6±4.6 for AA and AS, PCV (female) 38.42±3.4, 31.8±1.2 and 34.3±3.1 for AA, AS and AC. WBC 4.3±1.6, 4.0±1.2 and 4.2±1.3 for AA, AS and AC respectively and Platelet count 198±18, 164.3±12.2 and 162.3±11.7 for AA, AS and AC. The values calculated when compared with the control showed statistical and significant difference (P< 0.05). From the research, there is a significant and statistical difference in the haematological profiles of the three haemoglobin genotypes.

Introduction

Haemoglobin is the oxygen-carrying pigment of the Red Blood Cells (RBCs). It is a chromoprotein that contains four haeme groups, which are the; pigment-containing part and globin, the protein part (Akhigbe et al., 2009). Haemoglobin is formed in the developing erythrocyte in the bone marrow. The iifespan of haemoglobin is the same as the lifespan of the red cell that accommodates it, that is 120 days. In adults, the concentration of Haemoglobin in the blood is 2.5-17g/100ml. A healthy adult loses and produces 6.25g of haemoglobin per day. A litre of normal blood is capable of carrying 200ml of oxygen and 1.0g of haemoglobin. The iron content of each haemoglobin molecule is 3.4nanograms (Ochei and Kolhatkar, 2008). A molecule of haemoglobin contains four polypeptides globin chains: α, β, γ and δ chains. Genotype, by definition is the hereditary constitution of an organism. In genetics, this is usually represented by symbols which stand for the gene of the character under consideration (Okeke et al., 2002).

Haemoglobin genotypes are inherited characters determined by different combinations: α, β, γ and δ chains. They include Hb AA, Hb AS, Hb AC, Hb SC, Hb SD, Hb SE and Hb SS. Hb A is known as the adult haemoglobin which consists of two alpha and two beta chains, 97% of haemoglobin of normal adult is of this type, it has an increased delivery power of oxygen and electrophorectically at pH 8.9 it moves ahead of Hb S and Hb C (Bunn, 1997). Hb S is the most symptomatic haemoglobinopathy in the world in which the beta chain is affected, at the 6 amino acid in the A3 position. Abnormality is a change from glutamine to valine. In the deoxygenated form, Hb S becomes insoluble and forms polymers that aggregate into tubular forms, this distorts the red blood cell

making it rigid and this produces sickle cell (Ashley-Koch et al., 2000). Hb C is the second abnormal haemoglobin to be characterized and it differs from Hb A by the substitution of lysine for glutamine at position 6 of the beta chain. It usually causes a minor amount of haemolytic anaemia (Weatheral, 2001). In the normal human, from about 6 months of age, 95-97% of the total haemoglobin is haemoglobin A (HbA). The two pairs of globin chains in HbA are called the alpha and beta chains. The remaining haemoglobin consists of HbA2 (two alpha and two delta globin chains) comprising some 2% of the total, and fetal haemoglobin (HbF, with two alpha and two gamma globin chains) less than 1.5%. The amino acid sequence of these four different polypeptide chains have been determined; the alpha chain (identical in each of these three types of haemoglobin molecule) has 141 amino acid residues and its genetic locus is located on chromosome 16, whereas the: β, γ and δ chains each has 146 residues and their genetic loci reside on chromosomel 1 (Akhigbe et al., 2009).

Haematological profiles are a group of non-specific tests used basically to determine the haematological status of a patient. Cheesborough (2000) stated the haematological profiles as; Haemoglobin estimation, packed cell volume, White blood cell count, Platelet count, Erythrocyte sedimentation rate, Reticulocyte count, etc.

I. Packed cell volume (PCV) is the percentage of a total volume of blood occupied by red cells, when a known volume of whole blood is centrifuged at a constant speed for a constant period of time (Dacie and Lewis, 2007). The value thus obtained is used in the determination of the red cell indices. It is the treatment of choice in chronically anaemic patients to correct anaemia and to raise the oxygen transportation of the red cells.

II. White blood cell (leucocytes) count is used to investigate conditions related to leukocytosis or leucopenia such as HIV/AIDS and infections. Leucocytes are cells of the immune system defending the body against both infectious diseases and foreign materials, but they are all produced and derived from a multipotent cell in the bone marrow known as haematopoietic stem cell. Leucocytes are found throughout the body including blood and lymphatic system. It measures 8µm in diameter. Normal blood value varies from 4000-11000 per cubic millimeter (Cheesborough, 2000).

Platelet count is investigated when thrornbocytopenia is suspected and also to investigate abnormal skin bleeding (Cheesborough, 2000). Platelet is the smallest type of blood cell that has a diameter of 2-4cm. platelets are fragments of the cytoplasm of megakaryocytes, they are non-nucleated and formed in the bone marrow. They play an important role in blood clotting. In the circulating blood, platelets are an essential part of blood coagulating mechanism. They act as plugs around the opening of a wound and .release certain factors that are necessary for the blood clot to prevent blood loss. They also help to maintain the integrity of the blood vessels by plugging the gap in the endothelial lining (Ochei and Kolhatkar, 2008). The normal platelet count is approximately 250x109/L ranging from 150-400 x109/L and the normal platelet lifespan is 7-10 days. Up to one third of the marrow output of platelets may be trapped at any time in the normal spleen but rises to 90% in cases of massive splenomegaly (Dacie and Lewis, 2007).

Hb AA is the most predominant haemoglobin genotype in Nigeria, although there is a significant amount of other abnormal haemoglobin genotypes (Umoh et al., 2010). In a study on a small population from the Niger Delta region of Nigeria, a prevalence of 69.1%, 29.4% and 1.5% for HbAA, HbAS, and HbSS, respectively was observed (Erhabor et al., 2010).

A study carried out by Umoh in 2010 in Uyo, Akwa Iborn state in Nigeria over a period of five years gave the following result: 78.7% for HbAA, 19.6% for HbAS, 1.5% for HbSS, while 0.2% for HbAC and 0.04% for HbSC (Umoh et a!., 2010). In a study of HbC in Akwa Ibom State in 1996, Usanga et al found an incidence of 0.4% and 0.07% for Hb SC and HbCC respectively among a fishing settlement in the state (Usanga et al.., 1996). Similar results were reported where the incidence of AA, AS, AC, SS, SC and CC were 69.35, 36.94, 0.12, 3.54, 0.02 and 0.01%, respectively (Uzoegwu and Onwurah, 2003). Also in a study by Okpara et al in 2010 in Cross River State of Nigeria (a South-South state which at the time included Akwa Ibom state) the Hb genotype prevalence reported were 72.8%, 20.9%, 5.2%, 0.3% and 0.4% for HbAA, HbAS, HbSS.HbAC and HbSC respectively (Okpara et al., 2010).

Aim and objectives

1 To evaluate some haemoglobin genotypes among Imo State University students.
2 To compare haematological profiles in Imo State University students with Hb AA, Hb AS and Hb AC and determine if there is any significant difference.

Materials and Methods

Study Area

The study was conducted in Imo State University, Owerri.

Study Population

The population for this study was drawn from Irno State University, Owerri. These included 100 apparently healthy students of both sexes within the ages of 20-29 years.

Sample Collection

5mls of blood was collected with a plastic syringe by clean venipuncture. The blood was carefully emptied into bottles containing 0.04ml of 10% solution of EDTA previously dried. The blood was immediately mixed with the blood to ensure efficient anticoagulation of the blood. The samples were taken to the Imo State University Laboratory where the tests were performed.

Laboratory methods and procedures

Haemoglobin Genotype

Electrophoretic method using cellulose acetate paper and tris buffer of pH8.6. (Dacie and Lewis, 2007).

Principle

At alkaline pH, haemoglobin is a negatively charged protein and when subjected to electrophoresis will migrate towards the anode (+), haemoglobin variants separates at different rate due to difference in their electrical charge as determined by their amino acid structure.

Procedure

The electrophoretic tank was filled upto one quarter level with Tris buffer of pH 8.6, the samples which were washed with normal saline were lysed with distilled water. A drop of each haetnolysate was then placed on an electrophoretic set with Pasteur pipettes to correspond with the number on each tube. These test samples were then placed on partially dried paper with the aid of electrophoretic applicator set and placed on the tank. In each test run, a known control of genotype AS was applied along with the test. The power switch was on and the separation of the samples and controls in their different genotype bands occurred. It took 15mins at 20volts for good separation to be observed.

Haemoglobin Estimation using cyanmethaemoglobin method (Dacie and Lewis,2007)

Principle

Whole blood was diluted 1 in 200 in a modified Drabkin's solution containing potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate.The red blood cells were haemolysed and the haemoglobin was oxidized by ferricyanide to methaemoglobin.This was converted by the cyanide to stable haemoglobin (HiCN).The absorbance of the HiCN solution was read in a calorimeter at wavelength 540nm.

Procedure

Exactly 0.02ml of well mixed anticoagulated blood was measured and pipetted into 4ml of Drabkins solution in a test tube (1:200 dilutions) It was mixed and allowed to stand for 10minutes at room temperature. The absorbance was read colorimetrically at 540nm (green filter) after zeroing colorimeter with Drabkin's solution. The haemoglobin level was read from the calibration graph already prepared.

This method has the advantage of being able to convert all forms of haemoglobin likely to be found in blood.

Female: 12.5-16.0g/dl

Packed Cell Volume: (Microhaematocrit method) (Dacie and Lewis, 2007)

Principle:

The packed cell volume is that proportion of whole blood occupied by red cells, expressed as a ratio litre/litre or in percentage (Cheeseborough, 2000). Anticoagulated blood in a glass capillary of specified length bore size and thickness is centrifuged in a microhaematocrit centrifuge at 1500g revolution per minute for 5 minutes to obtain constant packing of the red cell A small amount of plasma remains trapped between the packed cells, - PCV value is read from the scale of a microhaematocrit reader or calculated by the height of the Total volume of blood.

Procedure

A plain capillary tube was three quarter filled with well mixed EDTA anticoagulated blood. The unfilled part of the tube was filled using a sealant plastercine). The filled capillary was carefully located in one of the numbered slots of the microhaematocrit rotor with the sealed end against the rim gasket to prevent breakage. This was centrifuged at 1500g for 5 minutes, after centrifuging the PCV was read using a microhaematocrit reader.

Reference value: male 45-53%
Female 35-49%

White Blood Cell Count (Cheesebrough, 2000)
Principle

Whole blood is diluted appropriately using a diluent which haemolyses red cells, leaving all the nucleated cells intact. The number of white cells in a known volume and known dilution are counted using a counting chamber.

Procedure

0.38ml of diluting fluid was dispensed in a tube. To that tube 0.02ml of well mixed EDTA anticoagulated blood was added. The improved neubauer counting chamber was charged with the well mixed diluted blood. The chamber was left undisturbed for 2minutes for the white cells to settle. The underside was dried and then using x10 objective with the condenser Iris closed sufficiently to give good contrast. The four large corner squares were located and counted.

Calculation:

No of cells x dilution x109 Volume counted.
Reference volume; 4.0 -11.0 X109/L

Platelet Count (Cheesebrough, 2000)
Principle

Blood is diluted 1 in 20 in filtered solution of ammonium oxalate reagent which lyses the red cells. Platelets are counted microscopically using an improved neubauer ruled counting chamber.

Procedure

In a tube, 0.38ml of filtered ammonium oxalate diluting fluid was measured and dispensed. To the tube 0.02ml of well mixed anticoagulated blood was added using a Pasteur pipette. The chamber was then left undisturbed for 20 minutes; the underside of the chamber was wiped dry and then mounted on the microscope stage. It was then viewed using x10 and x40 objective. They are counted in the small squares.

Calculation

No of cells counted x dilution x 109
Volume counted
Reference value; 150-400 x109/L

Results

Table1: Mean level of haematologicai profiles of IMSU students with haemoglobin genotype AA

Parameters	X±SD	Reference	P. level
		value	
PCV(%) for female PCV (%) for male	38.42±3.3 43.7+0.9	35-49 45-53	P<0.05 P<0.05
Hb (g/dl) for female Hb(g/dl) for male	13.01±1.2 14.6±0.2	12.5-16.0 13.0-18.0	P<0.05 P<0.05
WBC(x109/L)	4.31±1.6	4.0-11.0	P<0.05
LATELETS(x109/L)	198±18	150-400	P<0.05

Table2: Mean fevel of haematologicaf profiles of IMSU students with haemoglobin genotype AS

Parameters tested	X+SD	Reference value
PCV(%)for female PCV(%) for male	31.8±1.2 36.6±1.3	37-45 47-53
Hb(g/dl)for female Hb(g/dl)formale	10.6±0.9 12.4±1.4	12.5-19.0
WBC(x1Q9/L)	4.0±4.3	4.0-11.0
PLATELETS(x109/L)	164.3±12.2	150-400

Table 3: Mean level of haematoiogical profiles of IMSU students haemoglobin genotype AC

Parameters tested	X±SD	Reference value	P. level
PCV(%)for female	34.3±3.1	37-52	P<0.05
Hb(g/dl)for female	12.4±2.5	12.5-18.0	PO.05
WBC(x109/L)	4.1±1.3	4.0-11.0	P<0,05
PLATELETS(x109/L)	162.3±11.7	150-400	P<0.05

Table4: Mean level of haematological profiles for AA, AS and AC

Parameters tested	X ± S D	Ref. value	P. level
PCV (%)for AA PCV(%)for AS PCV (%)for AC	40.23±4.1 32.9±6.8 34.3±3.1	37-52	P<0.05
Hb(g/dl)for AA Hb(g/dl)for AS Hb(g/dl)for AC	13.4±1.2 11.1+1.2 12.4±2.5	12.5-18.0	P<0.05
WBC(x10^9/L)for AA WBC(x10^9/L)for AS WBC(x10^9/L)for AC	4.31±1.6 4.04+1.2 4.1±1.3	4.0-11.0	P<0.05
PLATELETS(x1 0^9/L)for AA PLATELETS(X1 0^9/L)for AS PLATELETS(x1 0^9/L)for AC	198±18 164.3±12.2 162.3±11.7	150-400	P<0.05

Discussion

Haemoglobin genotypes are inherited characters determined by different combinations: α, β, γ and δ chains. They include Hb AA, Hb AS, Hb AC, Hb SC, Hb SD, Hb SE and Hb SS, and haematologicai profiles are a group of non-specific tests used basically to determine the haematologicai status of a patient.

In this study on a small population of Imo State University students, 52% had Haemoglobin genotype AA, 42% had AS and 6% had AC. From the result obtained for male haemoglobin level, the mean level for AA 14.6±0.2 while that of AS was 12.4 ±1.4. The mean level for females was found to be 13.0±1.2, 10.7±0.9 and 12.4±2.5. When these results were compared it showed that there was a significant difference ($P<0.05$) and there was a statistical difference in the results. The value for Haemoglobin genotype AA however, was within the normal reference value of the World Health organization which is 12.5-16.0g/dL for females and 13.0-18.0g/dL for males and this confirms the work of Bakare et al in 2006 that a significant and statistical difference occurs when haemoglobin genotype AA is compared-with AS and AC (Bakare et al., 2006).

Packed cell volume for both male and female, the mean value was found to be 38.42±3.3, 31.8±1.2, 34.3±3.1 for AA, AS and AC respectively for females and 36.6±1.3 and 43.7±0.9 for male AS and AC respectively which confirms in the haemoglobin levels, according to Cheesebrough in 2000, the AS contains sickled cells that are destroyed by the reticuloendothelia system of the spleen before the normal 120 days which is the lifespan of a normal red blood cell and this causes a low level of haemoglobin that can lead to anaemia and a low level of oxygen.carrying capacity in the blood (Cheesebrough, 2000).

From the result obtained for white blood cell count, there was a significant difference between the three haemoglobin genotypes, the mean levels were 4.31 ± 1.6, 4.04±1.2, and 4.1±1.3 for AA, AS and AC although the result was in the reference range set by World Health Organisation. These results show that a high number of students were free from infections. An abnormal increase in the level of white blood cell count in the body indicates the presence of an infection in the body.

Results obtained showed that platelet level for the three genotypes was between the normal reference values of World Health Organization. The platelet level was 198±18, 164.3±12.2 and 162.3±11.7 for AA, AS and AC respectively and this shows that the three results fell within the normal World Health Organization value and this confirms that majority of the students did not have any injury because platelets play an important role in blood clotting and is increased when there is a wound according to Fleming et al. (1979).

Conclusion

At the end of the research, it was observed that a sharp difference exist in the packed cell volume and haemoglobin estimation of the three genotypes and this is as a result of the presence of sickled cells in Hb AS and AC which brought about a decrease in the number of circulating red blood cells.

The white blood cell count and platelet count showed a slight difference which is because haemoglobin which is the oxygen carrying pigment is found in red cells and not in platelets or white blood cells and because most of the students that were tested were free from infections or injuries.

It is recommended that students with haemoglobin genotype AS and AC increase their intake of food containing folate and iron which can help them increase their body's level of haemoglobin concentration and this is because a high haemoglobin level is very important in the transport of oxygen from the lungs to the tissues which aids physical performance and resistance to fatigue.

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