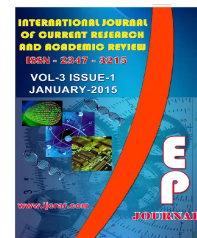




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Determination of the haemoglobin, genotype and ABO blood group pattern of some students of Imo State University, Owerri, Nigeria

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A B S T R A C T

The frequency of ABO, rhesus blood group and haemoglobin, genotype of 122 randomly selected Imo State University students were studied. The aims were to determine the ABO rhesus blood group and genotype distribution among the study group and gender. Also to find out if any relationship exists between a person's blood group and genotype. The blood groups were determined using principle of antigen antibody reaction in serum and cell grouping, while the genotypes were determined using electrophoretic method. The result analysis was done using simple percentage. Result obtained shows that the genotype AA had the highest frequency of (74.6%) 91 out of 122 and AS 31 out of 122 (25.4%), SS, SC, CC were not isolated. It was equally observed that blood group O+ Had the highest frequency of 58 out of 122 (47.5%), followed by group A+ 31 out of 122 (25.4%) and closely followed by B+ with (15.0%) 22 out of 122. No relationship between the blood groups and haemoglobin genotype was observed and there was no gender relationship. The frequency of rhesus blood group was very low. Conclusively, one's genotype does not determine the blood group.

Introduction

Blood is a complex fluid tissue responsible for the supply of oxygen and food to the other tissues of the body as well as removes carbon-dioxide and other waste products

from the body. It is made up of cells suspended in a protein salt solution known as plasma. The cellular elements are erythrocytes, leucocytes and thrombocytes.

The fluid plasma consists of various proteins such as thrombin, fibrinogen and globulin as well as electrolytes. The red cells are small, non nucleated biconcave disc which contain haemoglobin.

Dominant blood group genes express themselves through minute protein-polysaccharide substances on the surface of the red cell membrane. These substances are the product of specific genes at allelic-loci are identical, the gene is said to be expressed in double dose, but when they are different or heterozygous, the gene is said to be expressed in single dose. Each particular gene is responsible for the production of antigen receptor on the red cell membrane.

As humans differ in their facial and physical appearances, so they differ in character. The genetic makeup of an individual is known as the genotype whereas the observable characteristic is known as the phenotype or blood group. The genotype is determined by the type of haemoglobin present on the red cells, while the phenotype is determined by the antigens present on the red cells, leucocytes and platelets or antibodies present on the plasma.

There are three basic types of persons when classified genetically, these are those who inherited the normal haemoglobin, those who inherited both the normal and abnormal and those who is inherited only the abnormal. The inheritance of the abnormal haemoglobin in double dose as seen in sickle cell SS exposes one to various kinds of genetic diseases. The phenotypes are also inherited characteristics, the knowledge of which helps in compatible blood transfusion, compatible marital union and management of pregnancies resulting from incompatible marital union. The knowledge of blood group antigens is equally applied in organ transplant, forensic and anthropologic investigation.

The frequency or distribution of genotype differs in places; some are most common in a certain area while others are rare. The phenotype and genotypes are identified in the laboratory using the properties nature have endowed them with, such as the antigen and antibody present on the surface of the red cells and antibodies in the plasma for the phenotype and charge present on the chromoprotein haemoglobin for genotype.

The aim of this study is to determine the spread of haemoglobin genotype of some student of Imo State University as well as their phenotype and to see if there is correlation between the genotype and phenotype of these students.

The main objectives of this study includes, to determine the genotype of some student of Imo state university. Determine their ABO blood group. And to determine the frequency of each genotype and blood group. Compare the genotype with the phenotypes to see if there is correlation.

Materials and Methods

Study area

This study was carried at Imo State University in Owerri. Imo state is located within the South Eastern part of Nigeria. It lies latitude $50^{\circ} 27' - 50^{\circ} 31' N$ and longitude $6^{\circ} 55' - 7^{\circ} 05' E$. Imo state University has about Eight Faculties and a number of departments. The population is over five million students. It accommodates people from different state and ethnic.

Study population

The populations studied were One hundred and twenty two (122) randomly selected students irrespective of faculty, department and course.

Advocacy

A letter of introduction was obtained from the head of department of Medical Laboratory; with which informed consent was obtain from the students used for the test.

Ethical consideration

The study was approved by the department of Medical Laboratory Science, Imo State University. Informed consent was obtained from all the subjects before sample collection.

Sample collection

2mls of blood was collected from each subject, using the standard vein puncture method. The blood sample was emptied into an EDTA (Ethylene diamine tetra acetic acid) container and was properly mixed to avoid clotting and was used to determine haemoglobin genotype.

Method for determining haemoglobin

Haemoglobin Electrophoresis

Principle

Different types of haemoglobin (Hb) have different migration rate because Hb's are proteins. They can be made to run to either anode or cathode depending on the charge carried by the Hb. The buffer used determines the charge carried by the Haemoglobin (Hb).

Here alkaline cellulose acetate is used in an alkaline buffer at pH 8.4, this tends to make the Hb to migrate to the anode where there is negative charge.

Procedure

Step 1: Preparation of Haemolysate

The anticoagulant blood was centrifuge at 2500rpm for five minutes. The supernatant plasma was discarded and packed cells were washed with large volume of saline three times. After the final washing the red cells were lysed by adding equal volume of distilled water, one quarter (1/4) volume of toluene and one drop of 3% potassium cyanide and was mixed properly (Ochei and Kolhatkar, 2008).

Step 2: Electrophoresis

The buffer was poured into the electrophoresis chamber. Two wicks were soaked in the buffer and positioned such that they made contact with the buffer. The cellulose acetate paper was re-soaked in the buffer and left to soak for 20minutes. Excess buffer was removed by keeping the plate between absorbent papers.

Using an applicator stick 0.5ml of the haemolysate samples test and control were applied approximately 3cm away from the cathode. The cellulose acetate membrane plate was immediately placed in the electrophoretic chamber (tank). The chamber was connected to the power supply and the plate was electrophoreses for 20 minutes at approximately 350. After 20 minutes, the power supply was disconnected and the result was read (Ochei and Kolhatkar, 2008).

Statistical analysis

The computation analysis was done using percentage (%).

ABO blood grouping

Cell and serum grouping using both tile and tube methods

For the tile method: A tile was divided into five space to contain 1 volume of anti A serum and 1 volume of 20% patient's red cell for space 1. 1 volume of anti B and 1 volume of same red cells for space 2. Space 3 contained 1 volume of patient's serum and 20% of type A cells.

Space 4 contained 1 volume of patient's serum and 20% B cells. Tube 5 contained patient serum and cell to serve as control.

The contents of each division were mixed using applicator stick for each. The tile was titled gently and agglutination was recorded after 2 minutes

The tube method was carried out as for tile using 3% cells and the result recorded as for tile grouping

Results and Discussion

The ABO and genotype of 122 students of Imo State University were determined to ascertain the distribution and to find out if the pattern of blood group has any correlation with the genotype the following results were obtained.

The result shows that out of 122 subjects screened 91 had AA genotype, 31 had AS genotype. The ABO blood group was distributed as follows:-

A+ 31, B+ 22, O+ 58, AB+ 05, A- 02, B- 02 and O- 02. This is as represented in Table 4.1.

The genotype patterns in relationship with ABO blood group were assessed and the following were observed.

Out of the 31 subjects who were A+, 24 had AA genotype and 7 AS. Out of the 22 B+ i4 had AA genotype and 8 AS. The same goes with O+ which out of 58, 44 had AA genotype, 14 AS. Out of 5 AB+ 4 had AA genotype only 1 was AS. Out of the 2 A- subjects 1 had AA and 1 AS, the same were observed in O- 1 AA and 1 AS but all the 2 B- subjects had AA genotype. This result is represented in Table 4.1.

The gender distribution of the genotype and blood group were equally determined out of the 122 subjects, 66 were male and 56 were female.

Out of 66 male 48 had AA genotype and 18 AS.

The blood groups were as follows:

Blood group A+16 Male subjects B+ 10, O+ 34, AB+1, A- 1, B- 2.

Out of the 56 female subjects 43 had AA genotype, 13 had AS, 3 were A+, 12 B+, 24 O+, 4 AB, 1 A- and 2 O- This is represented in various table

Table 3 shows that the subjects who had AA genotype were more in number than the AS counterpart. It also shows that blood group A is much more prevalent than any other group studied.

Table 4 shows that in all the blood groups AA had the highest except in A and O negative where they AA and AS had equal distribution.

The following were observed in the table 5. In both male and female subjects, AA genotype and blood group O+ were more in number.

The genotype and blood group pattern of some students of Imo State University

Owerri were determined. The aims were to determine the frequency of distribution of their genotype, ABO and Rhesus blood group, to determine the gender distribution and to find out if any relationship exists between a person's genotype and the blood group.

The results obtained from this study have the following information as represented in the respective tables. Haemoglobin AA had the highest prevalence of 74.6% (91 out of 122) as against AS which had the prevalence of 25.4 (31 out of 122). No other abnormal haemoglobin was isolated. This result is in line with other studies on the distribution of haemoglobin genotype in different Ethnic group, such as the study by Rando (1998) who recorded highest frequency of haemoglobin genotype AA.

A study by Sinou, (2003) reported low incidence of haemoglobin S as between 15-30% and reported haemoglobin AS in West Africa and Nigeria as 5% and 40.5% respectively. Akigbe *et al.* (2009) reported frequency of haemoglobin genotype AA, AS, AC as 71.03, 22.19 and 5.26 respectively. In a study by Uzoegwu and Onwurah (2005), the incidence of AA, AS, AC, SS, SC, and CC as 69.35, 36.94%, 0.12%, 3.34%, 0.02% and 0.01% respectively.

The low frequency of abnormal haemoglobin observed in the study area and indeed in other studies confirms the high frequency of haemoglobin genotype AA, haemoglobin A as the most common haemoglobin.

The non isolation of haemoglobin SS and other abnormal haemoglobin could be associated with increase in awareness of people knowing their genotype before getting married. This has encouraged compatible marriage in terms of couples

knowing their genotype before marriage so as to avoid producing children with haemoglobinopathies. With this awareness the issue of sickle cell disease phase out with time.

The result obtained also revealed that blood group O Rhesus positive was the most frequency 58 out of 122 47.5%. this was followed by group A Rhesus positive 31 out of 122 (25.4%) and closely followed by blood group B Rhesus positive and the list was blood group AB which had 5 out of 122 (41%) Wallace and Gibb, 1986 recorded the same pattern of distribution in his study. Bakare *et al.* (2006) and Wallace and Gibbs, (1986) recorded the same pattern of ABO blood groups differ in populations and Ethnic groups. Group B is found more among African Americans and Asians.

The reason why group O is the most frequent in almost all the population and ethnic groups is beyond this study but part of the reason may be because of the fact that couples of genotype AO and Bo can produce a child of O blood group and since these genotype are common, the likely hood of producing O off spring is equally high.

In this study, no relationship was observed between the blood groups of subjects and their genotype. This is to say that one's blood type does not determine the genotype rather as the frequency of the group increases so also the genotype. Group O subjects had the highest genotype of AA, true it was observed that all the B negative subjects had AA negative but that does not in any way show that blood group B produces the genotype AA. There was equally no significance difference in the ABO and genotype among the sexes. The frequency was in line with the sample size of each sex, though more of the AB blood groups were found among the females.

Finally, the frequency of rhesus negative was very low 4.92 (6 out of 122). This is in line with several past studies which show that the frequency of rhesus negative is very low in this part of the world.

In the study, it could be concluded that the frequency of haemoglobin genotype AA had the highest frequency followed by AS. Abnormal haemoglobin such as SS, SC, CC

and others were not isolated probably because of the study group and sample size. The blood group O rhesus positive had the highest frequency and AB the lowest. No relationship was observed between the subject's blood group and genotype. No gender relationship was observed in both the genotype and blood group. Finally rhesus negative are very rare among the subjects studied.

Table.1 Cell grouping serum grouping

| Tube1 Anti A | Tube2 ANTI B | Tube 3 A CELL | Tube4 B CELL | Tube5 CONTROL | GROUP |
|-------------------------|-------------------------|--------------------------|-------------------------|--------------------------|--------------|
| + | - | - | + | - | A |
| - | + | + | - | - | B |
| + | + | - | - | - | AB |
| - | - | + | + | - | O |

Table.2 Cell grouping serum grouping

| Tube1 Anti A | Tube2 ANTI B | Tube 3 A CELL | Tube4 B CELL | Tube5 CONTROL | GROUP |
|-------------------------|-------------------------|--------------------------|-------------------------|--------------------------|--------------|
| + | - | - | + | - | A |
| - | + | + | - | - | B |
| + | + | - | - | - | AB |
| - | - | + | + | - | O |

(Cheesbrough, 2004).

Table.3 Genotype and blood group pattern of the subjects studied

| No of subjects | Genotype | | | blood group | | | | | |
|-----------------------|-----------------|------|------|--------------------|------|-----|-----|-----|-----|
| | AA | AS | A+ | B+ | O+ | AB | A- | O- | AB- |
| 122 | 91 | 31 | 31 | 22 | 58 | 05 | 02 | 02 | - |
| % | 74.6 | 22.4 | 25.4 | 18.0 | 47.5 | 4.1 | 1.6 | 1.6 | - |

Table.4 The genotype pattern in relation to blood groups of the subjects studied

| Blood group | genotype | | |
|-------------|----------|-----------|-----------|
| | N | AA | AS |
| A+ | 31 | 24 (77.4) | 7 (22.6) |
| B+ | 22 | 14 (63.6) | 8 (36.4) |
| O+ | 58 | 44 (75.9) | 14 (24.1) |
| AB+ | 05 | 4 (80) | 1(20) |
| A- | 2 | 1 (50) | 1 (50) |
| B- | 2 | 2 (100) | - |
| O- | 2 | 1 (50) | 1 (50) |

Table.5 Gender distribution of blood groups and genotype of the subjects studied

| Gender | N | AA | AS | O+ | A+ | B+ | AB+ | O- | B- | A- | AB |
|--------|----|----|----|----|----|----|-----|----|----|----|----|
| MALE | 66 | 48 | 16 | 14 | 18 | 10 | 1 | 2 | 2 | 1 | - |
| FEMALE | 56 | 43 | 13 | 24 | 13 | 12 | 4 | 2 | - | 1 | - |

Table.6 The frequency of rhesus blood group among the subject studied

| No of subjects | Rhesus positive | Rhesus negative |
|----------------|-----------------|-----------------|
| 122 | 116 | O6 |
| | 95.08 | 4.92 |

Recommendation

It is suggested that more work be done on blood groups other than ABO and Rhesus to determine their frequency in a mixed community like the University which is a representative of people from different ethnic groups.

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