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## Distribution of ABO/Rhesus Blood Groups and Haemoglobin Genotypes in Relation to Fasting Blood Glucose Levels Among Pregnant Women in Owerri

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### Abstract

**Background:** This study examined the relationship between ABO, Rhesus D blood groups and haemoglobin phenotypes with fasting capillary blood glucose level in pregnant women in Owerri, Nigeria.

**Method:** The method used was the analytical cross sectional study with 84 pregnant women as subjects. Capillary glucose level was determined by a glucose meter, ABO blood group and Rhesus D typing by agglutination and haemoglobin phenotypes by electrophoresis. Descriptive statistics and one-way analysis of variance at  $p < 0.05$  were used to analyse the data.

**Method:** The method used was the analytical cross sectional study with 84 pregnant women as subjects. Capillary glucose level was determined by a glucose meter, ABO blood group and Rhesus D typing by agglutination and haemoglobin phenotypes by electrophoresis. Descriptive statistics and one-way analysis of variance at  $p < 0.05$  were used to analyse the data.

**Results:** The age group 25-29 years old comprised most participants (32.1%) and 48.8% were in their third trimester. 13.1% reported having a previous history of gestational diabetes and 34.5% had a family history of diabetes. HbAA predominated (71.4%), followed by HbAS (26.2%) and HbAC (2.4%). The most common blood group was O positive (61.9%) and 95.2% were Rhesus D positive. Mean fasting glucose was  $172.20 \pm 23.60$  mg/dL, with a median of 165.50 mg/dL and range of 140–248 mg/dL. The level of all participants was  $\geq 126$  mg/dL. There was no significant difference between ABO/Rhesus D groups ( $F = 1.132$ ,  $p = 0.348$ ) or haemoglobin phenotypes ( $F = 1.786$ ,  $p = 0.174$ ) with regard to the fasting glucose.

**Conclusion:** The predominant ones were HbAA, O positive and Rhesus D positive. There was no significant association between ABO/Rhesus D blood group and haemoglobin phenotype with fasting capillary glucose. The consistently high results should be confirmed by standard laboratory methods.

**Keywords:** ABO blood group, Fasting glucose, Haemoglobin phenotype, Pregnancy, Rhesus D.

## 1.0 BACKGROUND OF THE STUDY

During pregnancy, there are significant metabolic changes that occur that help ensure adequate nutrients reach the developing fetus. In early pregnancy, there may be a slight decrease or unchanged in insulin sensitivity, leading to increased storage of energy. However, with the progression of gestation, the insulin sensitivity decreases with the increasing availability of glucose for fetal development, driven by placental and maternal hormones. In healthy mothers, pancreatic  $\beta$ -cells adjust their insulin output to this physiological insulin resistance. However, if this compensatory mechanism fails, the blood glucose levels go up before and after eating, creating hyperglycaemia in pregnancy (Szlapinski & Hill, 2021; Salazar-Petres et al., 2022).

Hyperglycaemia detected during pregnancy is a spectrum that encompasses gestational diabetes mellitus (GDM) and diabetes mellitus diagnosed in pregnancy. According to the World Health Organization, gestational diabetes mellitus (GDM) is an occurrence of hyperglycaemia in pregnancy with levels of glucose that are not in the range for diagnostic diabetes. The WHO criterion is to consider a fasting plasma glucose of 5.1–6.9 mmol/L as gestational diabetes (125–140 mg/dL) and a level of 7.0 mmol/L or 126 mg/dL as diabetes mellitus in pregnancy. The distinction is of clinical significance as this impacts surveillance, treatment and pregnancy outcomes (World Health Organization, 2013).

Gestational diabetes mellitus is now an important maternal and public-health problem due to its prevalence as well as the implications it has for both mother and fetus. The prevalence rate is reported to vary widely based on population characteristics, maternal age, obesity status, ethnicity, screening practices and the definition of the diagnosis. Nigerian studies involving over 46,000 pregnant women were systematically reviewed, and the prevalence rates ranged from 0.5% to 38.0% with an overall pooled prevalence of around 11%. The most frequently cited factors were advanced maternal age, obesity, family history of diabetes, delivery of a macrosomic infant and history of miscarriage. However, more recent studies have confirmed a link between overweight, obesity, history of macrosomia, stillbirth, and having diabetes in the family and gestational diabetes in sub-Saharan Africa (Azeez et al., 2021; Abera et al., 2024).

Pregnancy complications such as excessive fetal growth, operative delivery, birth defects, and neonatal metabolic disturbances are

linked to poor control of maternal hyperglycaemia. Poor maternal hyperglycaemia is linked to pregnancy complications including excessive fetal growth, operative delivery, birth complications, and neonatal metabolic disturbances. In addition to having increased risk during the pregnancy, women with GD are at an increased risk of subsequent episodes of gestational diabetes and type 2 diabetes, and their children are more vulnerable to obesity and impaired glucose regulation. Such consequences warrant the identification of easily available maternal characteristics that could be associated with glucose regulation, especially in scenarios where universal oral glucose-tolerance testing is both financially prohibitive and technically challenging due to low antenatal attendance (Adeoye et al., 2025).

The blood group ABO and Rhesus systems are two of the inherited characteristics gaining increasing research interest. People are classified into A, B, AB or O group based on the presence or absence of the A and B carbohydrate antigens, and are usually Rhesus-positive or Rhesus-negative based on whether the D antigen is present or absent. These systems are mostly used in transfusion medicine and obstetrics, but ABO antigens are also found in some tissues and have been linked to the activity of inflammatory proteins and endothelial function, as well as coagulation pathways and susceptibility to selected metabolic diseases. In a large umbrella review, Liu et al. (2024) identified 21 associations between blood groups and other health outcomes, among which, the ABO/Rhesus blood group and type 2 diabetes was consistently supported by strong evidence.

The relationship of ABO blood types to gestational diabetes is still emerging and not very consistent. There have been some observational studies reporting differences in the risk for gestational diabetes among maternal blood groups, and others reporting small or clinically questionable associations. A large study of singleton pregnancies found a slightly higher prevalence of GDM among women with blood group B, while a recent birth-cohort study indicated an increased risk in women with blood group AB. These observations do not prove causality but strengthen the need for more study in a specific population as the frequency of each ABO group varies from ethnic and geographic group to group and background metabolic risks are different (Fernández-Buhigas et al., 2023; Xie et al., 2025).

Another characteristic which is inherited that is of much importance in Nigeria is haemoglobin genotype. Normal adult haemoglobin is present mainly as haemoglobin AA, whilst genotypes that include AS and AC refer to the presence of haemoglobin S or haemoglobin C, as well as the normal haemoglobin  $\beta$ -globin gene. These variants are clinically significant as they can have an impact on red-cell physiology, pregnancy management, genetic counselling and the risk of haemoglobinopathy in offspring. The study of haemoglobin in Nigeria is invariably consistent with AA as the most common genotype and AS and AC are present at low but significant frequencies. Other blood groups such as O and Rhesus positive also widely predominate in pregnant populations of Nigeria, but their distribution is dependent on area of occurrence and ethnic groups (Wekere et al., 2023).

The potential association between haemoglobin genotype and blood glucose level is not as well defined as the association between conventional metabolic risk factors and GDM. Haemoglobin variants can influence the lifespan of red-cells and can influence the results of certain glycated-haemoglobin assays, but the level of plasma glucose is not affected by haemoglobin genotype in a similar way to the measurement of glycated-haemoglobin. Inconsistencies in available studies in pregnancy outcomes among women with sickle-cell trait suggest further research is required to consider haemoglobin genotype as a potential population characteristic and not as an established determinant of maternal hyperglycaemia (Wellenstein et al., 2019; Ziechmann et al., 2024).

However, there is a lack of information in Owerri about maternal fasting blood glucose, ABO Rhesus blood groups and haemoglobin genotypes. Examining these parameters in the same population may give locally relevant information on their distribution and also whether fasting blood glucose is different in the different inherent blood group/haemoglobin categories. This information could help to support antenatal screening, guide the interpretation of maternal laboratory results, aid in genetic counselling and serve as preliminary data for larger analytical studies. This study therefore studied the distribution of the ABO/Rhesus blood group and haemoglobin genotype of pregnant women in Owerri in relation to the level of blood glucose after an overnight fast.

## 2.0 MATERIALS AND METHODS

### Research Design and Research Settings

This was a hospital analytical cross sectional study conducted at the Federal University Teaching Hospital (FUTO), Owerri, Imo State Nigeria in February 2024 with pregnant women attending the antenatal clinic of the hospital. The hospital is a tertiary referral hospital that offers obstetric, antenatal, diagnostic and specialized laboratory services to women of Owerri and its environs in South-Eastern Nigeria.

The blood group distribution and haemoglobin phenotypes were evaluated as well as the variations in fasting capillary blood glucose levels between the blood group and haemoglobin-phenotype categories. The recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology guidelines for reporting cross-sectional studies (von Elm et al., 2007) were used to guide reporting.

### Study population and participants' recruitment

The study population was pregnant women who attended routine antenatal clinic at the study facility during the time of recruitment. The participants were recruited until the necessary sample size was reached.

Women were eligible when they were aged 18–45 years, had confirmed pregnancy, had fasted overnight for at least eight hours and provided written informed consent. Women were excluded if they had been diagnosed with type 1 or 2 diabetes mellitus prior to pregnancy, if they were on insulin or oral glucose-lowering drugs, if they had eaten food or caloric drinks during the fasting period or if they had chronic renal, hepatic or thyroid disease that was known to affect glucose metabolism. Women who were severely ill, had incomplete records or unsuitable blood specimens were also excluded. Previous history of gestational diabetes was recorded but was not used as an exclusion factor as it is a clinically relevant factor for the mother.

### Sample-size determination

The minimum sample size was determined with the single-population proportion formula:  $n = Z^2P(1-P)/d^2$ , where  $n$  is the minimum sample size needed to estimate the prevalence of the single population,  $P$  is the expected prevalence,  $Z$  is the standard normal deviate with 95% confidence level, and  $d$  is the level of precision desired. This method can be used for estimating a population proportion in a cross-sectional study (Charan & Biswas, 2013).

The prevalence used was 4.8% which was obtained from the study conducted in South East Nigeria among 250 pregnant women as reported in a systematic review and meta analysis of gestational diabetes in Nigeria. Data from 36 studies and 46,210 participants were included in the review with a pooled prevalence of 11.0% in Nigeria, although there was considerable variation across the different studies due to the differences in population, screening and diagnostic criteria (Azeez et al., 2021).

Using  $Z = 1.96$ ,  $P = 0.048$  and  $d = 0.05$ :

$$n = [(1.96)^2 \times 0.048 \times (1 - 0.048)] / (0.05)^2 = 70.22.$$

The number of participants in the minimum sample size was about 70. A 20% contingency was added in as a non-response, incomplete data and unusable specimens would be  $84.26 = 70.22 + (0.20 \times 70.22)$ . This final sampling size was 84 pregnant women.

### Data collection

Participants' sociodemographic, obstetric and clinical information were collected using a structured interviewer administered questionnaire and laboratory data form. The variables included were gestational age, maternal age, parity, previous gestational diabetes, family history of diabetes mellitus, pre-existing medical conditions, use of medication and recent blood-transfusion history.

A participant ID number was assigned to each participant. The identification number was entered on the questionnaire, specimen container and laboratory worksheet to allow participants to be matched with the laboratory results and not include personal identifiers in the analytical dataset.

## Specimen Collecting and Handling

Participants were asked to fast for the night for a minimum of 8 hours with water only being provided. Standard aseptic and safety procedures were followed when collecting blood in the morning.

The selected finger tip was cleaned with 70% alcohol and let air dry for measurement of fasting glucose. The lateral surface of the finger-tip was pierced with a sterile, single use lancet. The first drop was removed and another free-flowing drop was used to measure glucose. There was no over-squeezing of the fingertip.

About 2 mL of venous blood was drawn from antecubital vein into labelled ethylenediaminetetraacetic acid tube for ABO/Rhesus blood-group and haemoglobin-phenotype determination. To avoid clot formation the specimen was gently inverted and analysed immediately. Blood collection and specimen handling procedures were according to recommended safe phlebotomy accepted by WHO (2010).

## Laboratory analyses

### Fasting capillary blood glucose

Capillary blood glucose was measured using a portable glucose monitor and corresponding test strip as per the manufacturer's instructions after a fasting period. The meter was checked prior to use and test strips were checked for correct storage, complete packaging and expiry date.

An appropriate test strip was inserted into the meter and sufficient capillary blood sample placed on the sample area. The glucose concentration seen was recorded immediately and in mg/dL along with the participant's study ID.

The fasting capillary blood glucose was analysed mainly as a continuous variable. Portable glucose meters may be used to monitor glucose at the point of care but a definitive diagnosis of diabetes should be made based on glucose measured in venous plasma by an accredited laboratory method (Sacks et al., 2023).

The World Health Organization definition of hyperglycaemia, as early as in pregnancy, was taken into account for descriptive interpretation. In terms of these criteria, a fasting plasma glucose between 92–125 mg/dL is in the diagnostic range for gestational diabetes mellitus, while fasting plasma glucose of 126 mg/dL or higher is in the diagnostic range for diabetes mellitus in pregnancy (World Health Organization, 2013).

### Blood grouping (ABO and Rhesus D)

The ABO and Rhesus D blood groups were determined by slide agglutination using commercially prepared monoclonal anti-A, anti-B, anti-AB and anti-D antisera as per reagent manufacturer's instructions.

A single drop of each antiserum was added to a distinct part of a clean dry blood-grouping tile, each labelled separately. Blood of the participants was well-mixed and 1 drop was placed into each reagent and mixed with different applicator sticks to avoid cross-contamination. The tile was gently rocked and agglutination was observed within the time specified by the manufacturer for the reaction.

If the reaction occurred when anti-A was used, the blood group was interpreted as A and if the reaction occurred when anti-B was used, it was interpreted as B; if the reaction occurred with both anti-A and anti-B, it was interpreted as AB; if the reaction did not

occur with either anti-A or anti-B, then it was interpreted as O (Harmening, 2019).

### Haemoglobin phenotype determination

Alkaline cellulose acetate electrophoresis was used to determine haemoglobin phenotypes. The technique is based on the principle of separation of haemoglobin fractions based on the differences in their electrical charge and their mobility in an applied electric field.

Each blood sample was collected in EDTA and a haemolysate was prepared. The haemolysates of the participants were placed on a cellulose acetate membrane with known haemoglobin controls and electrophoresed in alkaline buffer following the operating instructions of the laboratory system available.

After electrophoretic separation the separated migration bands were observed and compared with the control samples. Presumptive haemoglobin phenotypes (HbAA, HbAS, HbAC or others) were reported (Kotila, 2010; Bain, 2020).

### Quality assurance

Quality-assurance procedures were put in place before, during and after the analysis to comply with the overall principles of ISO 15189:2022 for medical laboratory quality and competence (International Organization for Standardization, 2022).

The identities of all participants and their fasting status were established prior to the collection of specimens. Venous specimens were examined for sufficient volume, clotting and gross haemolysis and specimen containers and laboratory forms were checked for proper labelling. The specimens were rejected if they were unsuitable or incorrectly labelled and recollected where possible.

The glucose meter was checked prior to use and test strips were kept in the test strip container and stored away from heat, moisture and contamination. The dates of test strips were checked for expiration before analysis. Where a manufacturer's instructions was available, meter control materials were analysed as per the manufacturer's directions.

Antisera for blood-grouping were tested to confirm correct labelling, storage, validity of expiry dates and signs of deterioration. Each reaction was performed using separate applicator sticks and any questionable agglutination patterns were repeated.

Haemoglobin controls were added to every electrophoretic run. A run was only accepted if the controls showed the desired migration and good band separation. Samples showing weak, unclear or ambiguous bands were rerun.

Laboratory values were entered as they were obtained and double entered with participant identification numbers. Original questionnaires and laboratory records were compared before analysis to validate electronic data entries.

### Study variables

Fasting capillary blood glucose concentration in mg/dl was the main outcome.

The most important explanatory variables were the ABO blood group, the Rhesus D status and the haemoglobin phenotype. ABO/Rhesus blood groups were classified as O positive, O negative, A positive, A negative, B positive, B negative, AB negative or AB positive. Haemoglobin phenotypes were classified

as HbAA, HbAS, HbAC , maternal age, gestational age, parity, previous gestational diabetes and family history of diabetes mellitus were all considered as potential covariates.

### Statistical analysis

The data were analysed in Stata version 15.0. The categorical variables such as ABO/Rhesus blood groups and haemoglobin phenotypes were presented in terms of frequencies and percentages. Normality of fasting blood glucose level was determined and presented as mean  $\pm$  SD or median and interquartile range. One-way analysis of variance, Welch's analysis of variance was used to compare fasting glucose by the adequately represented ABO/Rhesus blood-group and haemoglobin-phenotype categories depending on the distribution of the data and equality of the variances. Only when the overall-test was statistically significant a post hoc comparison was carried out. All categories with less than two observations were descriptively reported, and were not included in analyses that required within group variance. Categorical associations were performed using Fisher's exact test when the cell counts were small. No inferential test of association between glucose classification and blood group or haemoglobin phenotype was conducted as all participants were in the same glucose classification. A p value of less than 0.05 was considered statistically significant.

### Ethical considerations

Prior to recruitment of the participants, ethical approval was obtained from the Research Ethics Committee of the Federal University Teaching Hospital, Owerri. Permission was sought from the Department of Medical Laboratory Science, Imo State University, Owerri and the appropriate hospital authorities.

A description of the aims, methodology, potential discomfort and anticipated benefits of the study were provided to each participant. Informed consent was obtained in writing prior to Administration of questionnaire and collection of specimens. The participation was voluntary and the refusal of a participant and his/her withdrawal did not compromise the healthcare services provided.

Participants' names were replaced by unique identification codes and study information was only available to authorised members of the research team. Blood samples were drawn using sterile equipment and precautionary measures for biosafety. Those who had significantly high glucose levels on the test were referred to the clinical team for follow up laboratory tests and management.

## 3.0 RESULTS

### The socio-demographic and clinical characteristics of the study participants

A total of 84 pregnant women participated in the study. Most participants were aged 25–29 years (32.1%), followed by those aged 30–34 years (27.4%). Almost half (48.8%) were in the third trimester of pregnancy and 34.5% were multiparous. Previous history of gestational diabetes mellitus was reported by 13.1% of the participants and family history of diabetes mellitus was reported by 34.5% of participants. Of those who participated, 73.8% did not have any pre-existing medical condition and 70.2% were only receiving routine antenatal medications. Only 7.1% had a history of blood transfusion in the past year.

**Table 1. Socio-demographic and Clinical Characteristics of the Study Participants (N = 84)**

| Variable                            | Category                           | Frequency (n) | Percentage (%) |
|-------------------------------------|------------------------------------|---------------|----------------|
| Maternal age (years)                | 18–24                              | 18            | 21.4           |
|                                     | 25–29                              | 27            | 32.1           |
|                                     | 30–34                              | 23            | 27.4           |
|                                     | 35–39                              | 12            | 14.3           |
|                                     | $\geq 40$                          | 4             | 4.8            |
|                                     | <b>Total</b>                       | <b>84</b>     | <b>100.0</b>   |
| Gestational age (weeks)             | First trimester ( $\leq 13$ )      | 8             | 9.5            |
|                                     | Second trimester (14–27)           | 35            | 41.7           |
|                                     | Third trimester (28–40)            | 41            | 48.8           |
|                                     | <b>Total</b>                       | <b>84</b>     | <b>100.0</b>   |
| Parity                              | Nulliparous                        | 26            | 31.0           |
|                                     | Primiparous                        | 24            | 28.6           |
|                                     | Multiparous (2–4)                  | 29            | 34.5           |
|                                     | Grand multiparous ( $\geq 5$ )     | 5             | 6.0            |
|                                     | <b>Total</b>                       | <b>84</b>     | <b>100.0</b>   |
| Previous gestational diabetes       | Yes                                | 11            | 13.1           |
|                                     | No                                 | 73            | 86.9           |
|                                     | <b>Total</b>                       | <b>84</b>     | <b>100.0</b>   |
| Family history of diabetes mellitus | Yes                                | 29            | 34.5           |
|                                     | No                                 | 55            | 65.5           |
|                                     | <b>Total</b>                       | <b>84</b>     | <b>100.0</b>   |
| Pre-existing medical conditions     | None                               | 62            | 73.8           |
|                                     | Hypertension                       | 9             | 10.7           |
|                                     | Asthma                             | 5             | 6.0            |
|                                     | Sickle cell disease                | 4             | 4.8            |
|                                     | HIV infection                      | 4             | 4.8            |
|                                     | <b>Total</b>                       | <b>84</b>     | <b>100.0</b>   |
| Medication use during pregnancy     | Routine antenatal medications only | 59            | 70.2           |
|                                     | Antihypertensive                   | 8             | 9.5            |

|                                         |                              |           |              |
|-----------------------------------------|------------------------------|-----------|--------------|
|                                         | drugs                        |           |              |
|                                         | Insulin therapy              | 5         | 6.0          |
|                                         | Oral hypoglycaemic agents    | 3         | 3.6          |
|                                         | Other prescribed medications | 9         | 10.7         |
|                                         | <b>Total</b>                 | <b>84</b> | <b>100.0</b> |
| <b>Recent blood transfusion history</b> | Yes                          | 6         | 7.1          |
|                                         | No                           | 78        | 92.9         |
|                                         | <b>Total</b>                 | <b>84</b> | <b>100.0</b> |

### The distribution of haemoglobin phenotypes

There were 84 pregnant women analysed. The distributions of haemoglobin phenotypes, ABO and Rhesus D blood group and fasting capillary blood glucose concentration of the participants are shown in table 2.

**Table 2. Distribution of haemoglobin phenotypes among the study participants**

| Hemoglobin variants | Number of participants | Percentage (%) Distribution |
|---------------------|------------------------|-----------------------------|
| AA                  | 60                     | 71.4                        |
| AC                  | 2                      | 2.4                         |
| AS                  | 22                     | 26.2                        |
| Total               | 84                     | 100                         |

### Blood group distribution pattern

The most common blood group was blood group O positive type with 52 participants (61.9%). This was followed by A positive (21.4%), B positive (9.5%), O negative (4.8%) and AB positive (2.4%). None of the participants had A negative, B negative or AB negative blood groups. The total number of participants was 80 (95.2%) Rhesus D positive and 4 (4.8%) Rhesus D negative.

**Table 3 Distribution of ABO and Rhesus D blood groups among the study participants**

| Blood Group       | Number of participants | Percentage (%) Distribution |
|-------------------|------------------------|-----------------------------|
| O <sup>+</sup>    | 52                     | 61.9                        |
| O <sup>-ve</sup>  | 4                      | 4.8                         |
| A <sup>+</sup>    | 18                     | 21.4                        |
| A <sup>-ve</sup>  | 0                      | 0.0                         |
| B <sup>+</sup>    | 8                      | 9.5                         |
| B <sup>-ve</sup>  | 0                      | 0.0                         |
| AB <sup>+</sup>   | 2                      | 2.4                         |
| AB <sup>-ve</sup> | 0                      | 0                           |
| Total             | 84                     | 100                         |

### Capillary blood glucose level (fasting)

The mean fasting capillary blood glucose level of the subjects was 172.20 ± 23.60 mg/dL, median 165.50 mg/dL, and range 140-248 mg/dL.

**Table 4 Summary of fasting blood glucose concentrations among the study participants**

| Variable              | n  | Mean ± SD (mg/dL) | Median (mg/dL) | Range (mg/dL) |
|-----------------------|----|-------------------|----------------|---------------|
| Fasting blood glucose | 84 | 172.20 ± 23.60    | 165.50         | 140–248       |

### Fasting blood glucose classification-based distribution

The fasting blood glucose level was ≥ 126 mg/dL for all 84 participants.

**Table 5. Distribution of participants according to fasting blood glucose category**

| Fasting blood glucose category | Frequency, n | Percentage, % |
|--------------------------------|--------------|---------------|
| ≥126 mg/dL                     | 84           | 100.0         |
| <126 mg/dL                     | 0            | 0.0           |
| <b>Total</b>                   | <b>84</b>    | <b>100.0</b>  |

### Fasting Blood Glucose based on ABO/Rhesus D blood group

The mean fasting blood glucose concentration of the participants with AB-positive blood group was the highest at 190.50 ± 2.12 mg/dL, whereas the mean fasting blood glucose concentration of the participants with B-positive blood group was the lowest at 157.50 ± 14.47 mg/dL. The difference in fasting blood glucose level between the ABO/Rhesus D blood groups was however, not statistically significant (F=1.132), (p=0.348).

**Table 6. Comparison of fasting blood glucose concentrations across ABO/Rhesus D blood groups**

| Blood group               | n         | Mean ± SD (mg/dL) | Median (mg/dL) | Range (mg/dL) |
|---------------------------|-----------|-------------------|----------------|---------------|
| O positive                | 52        | 173.12 ± 26.38    | 161.50         | 142–248       |
| O negative                | 4         | 175.25 ± 36.69    | 175.50         | 142–208       |
| A positive                | 18        | 173.39 ± 12.63    | 173.50         | 154–193       |
| B positive                | 8         | 157.50 ± 14.47    | 156.00         | 140–178       |
| AB positive               | 2         | 190.50 ± 2.12     | 190.50         | 189–192       |
| <b>Overall comparison</b> | <b>84</b> |                   |                |               |
| <b>ANOVA statistic</b>    |           | <b>F = 1.132</b>  |                |               |
| <b>p-value</b>            |           | <b>0.348</b>      |                |               |

Haemoglobin phenotype blood glucose level

The mean fasting blood glucose concentration was highest in the HbAA group (174.87 ± 25.48 mg/dL), and lowest in the HbAS group (166.86 ± 16.86 mg/dL). The mean fasting blood glucose level was lowest in participants with HbAC at 151.00 ± 1.41 mg/dL.

There was no significant difference in FBG levels between the haemoglobin phenotypes (F = 1.786; p = 0.174). Only two individuals were of this phenotype and results must be treated with caution.

Table 7 Comparison of fasting blood glucose concentrations across haemoglobin phenotypes

| Haemoglobin phenotype | n  | Mean ± SD, mg/dL | Median, mg/dL | Range, mg/dL |
|-----------------------|----|------------------|---------------|--------------|
| HbAA                  | 60 | 174.87 ± 25.48   | 169.00        | 140–248      |
| HbAS                  | 22 | 166.86 ± 16.86   | 157.50        | 142–191      |
| HbAC                  | 2  | 151.00 ± 1.41    | 151.00        | 150–152      |
| Overall comparison    | 84 |                  |               |              |
| ANOVA statistic       |    | F = 1.786        |               |              |
| p-value               |    | 0.174            |               |              |

4.0 DISCUSSION

The present study examined the relationship between ABO/Rhesus D blood groups and haemoglobin phenotypes with fasting capillary blood glucose level in pregnant women of Owerri. HbAA and O-positive were the predominant haemoglobin phenotype and blood group, respectively. Fasting glucose was different between each blood-group and haemoglobin-phenotype category, but this difference was not statistically significant, indicating that these inherited traits did not have a significant impact on fasting glucose in the studied population (Rom et al., 2022; Bahkali et al., 2022; Lemaitre et al., 2022).

The median age of the participants was 25–34 years, and about 1/5 were 35 or more years old. The trend was similar as described by Wekere et al. (2023) amongst pregnant women in Southern Nigeria. Increased maternal ageing is a risk factor for decreased insulin sensitivity and decreased pancreatic β-cell compensation, and hence may be linked with hyperglycaemia during pregnancy (Azeez et al. 2021, Wekere et al. 2023).

The majority of participants were in the second and third trimester and after this time, placental hormones are released, causing a progressive decrease in maternal insulin sensitivity. In this case, a physiological resistance to insulin may lead to hyperglycaemia if the pancreas does not compensate for this effect. Several other factors, such as multiparity and family history of diabetes, may also have affected the metabolic risk of the participants, but this is not consistently observed as an independent variable after adjustment for other factors including age, BMI and previous metabolic history (Salazar-Petres et al., 2022; Szlapinski & Hill, 2021; Azeez et al., 2021; Abera et al., 2024).

Some of the women who received insulin and/or oral glucose-lowering drugs had shown an awareness of their hyperglycemia, but not necessarily a new diagnosis. Including them may have caused an increase in the number of metabolically high-risk women, but treatment may have affected the glucose measurements recorded. Azeez et al., 2021; Sacks et al., 2023 therefore the findings should be regarded as representing an identified antenatal population and not as an estimate of the prevalence of newly diagnosed gestational diabetes in Owerri.

There were 71.4% of participants in the HbAA group, 26.2% in the HbAS group and 2.4% in the HbAC group. Wekere et al (2023)

also reported that the major phenotype among pregnant women is HbAA but at a higher frequencies with lower frequencies of HbAS and HbAC in Rivers State. The differences might be due to geographical variation or ethnic variation and may be due to the smaller population size in the present study. The relatively high frequency of HbAS remains significant for partner testing and reproductive counselling, whereas the estimate of HbAC should be taken with a grain of salt, as it is based on a small number of women (Kotila, 2010; Wekere et al., 2023).

O-positive was the most common blood group, followed by A-positive, B-positive, O-negative and AB-positive. This distribution is in conformity with the report of Wekere et al., (2023) which also reported blood group O as the most predominant among the pregnant women of Rivers State. The prevalence of Rhesus D positivity was 95.2%, similar to what was reported in that study. Despite a small number of Rhesus D-negative women, this was important as it is recognised that maternal alloimmunisation and haemolytic disease of the fetus or newborn are risks (Wekere et al., 2023).

The mean fasting capillary blood glucose level was 172.20 ± 23.60 mg/dL with all subjects obtained a fasting capillary blood glucose level of at least 126 mg/dL. This was significantly higher than the pooled Nigerian GDM prevalence reported by Azeez et al. (2021) of 11.0%. This difference could be due to the presence of previous GDM, family history of diabetes, hypertension, glucose-lowering drug treatment, or the predominance of late gestational stages over the earlier ones in the study as compared to past studies (Azeez et al., 2021; Abera et al., 2024).

Use of a portable meter might also have affected the glucose results. Calibration, storage of the strips, environmental conditions, haematocrit and operator technique may be a source of variation for point of care glucose results. The consistently high capillary readings should be confirmed by appropriate laboratory measurement of VPG and review of the compliance of the fasts and quality control procedures (Sacks et al., 2023).

The mean levels of fasting glucose were highest for the AB positive group, and lowest for the B positive group, but the difference was not statistically significant. The mean value of AB positive needs to be treated with caution as there were a limited number of participants involved (n = 2). The non-significant finding was in accordance with Bahkali et al. (2022) who reported

that there was no association between ABO/Rhesus blood group and GDM and with Huidobro et al. (2017) who reported that there was no significant relationship between Rhesus status and GDM.

Also, similar frequencies of GDM across the blood groups A, B and O were reported by Rom et al. (2022) with a slight decrease in the risk of GDM in group AB. Lemaitre et al. (2022) observed the contrary, higher odds of GDM for French women with blood group AB, and especially for those who were AB Rhesus positive. Similarly, Xie et al (2025) and Azeez & AlKatib (2025) found associations with blood group AB and Sapanont et al. (2021) found blood group O to be a risk factor. The conflicting findings might be caused by ethnicity, sample size, diagnostic criteria, participant selection and adjustment for maternal risk factors (Rom et al., 2022; Lemaitre et al., 2022; Xie et al., 2025; Azeez & AlKatib, 2025; Sapanont et al., 2021).

In general, there is no single blood group that has been proven to be the highest risk group. In conclusion, the current result lends support to the notion that ABO/Rhesus blood group does not have a significant role in predicting pregnancy-related hyperglycaemia and cannot be a substitute for the established metabolic risk assessment and biochemical testing (Bahkali et al., 2022; Rom et al., 2022; Lemaitre et al., 2022).

There was no significant difference between the mean fasting glucose of the HbAA, HbAS, and HbAC participants. As only two women had this phenotype, the lower mean for HbAC should be taken with a grain of salt, and is not viewed as a protective effect. The lack of any significant HbAA–HbAS difference was consistent with the results of Wellenstein et al. (2019), and Alayed et al. (2022) which reported that there was no strong evidence of an independent association between sickle-cell trait and risk of gestational diabetes.

Previous studies have found increased rates of unadjusted GDM among women with sickle-cell trait, however, other maternal factors and BMI were confounded, making this finding difficult to interpret. Thus, any apparent association may be due to maternal age, adiposity, ethnicity, or other factors (Stamilio et al., 2003; Wilson et al., 2020).

The non-significant haemoglobin comparison could have been due to the small and unbalanced phenotype group. Precision and statistical power were limited by only two women with HbAC and 22 women with HbAS. Haemoglobin phenotype's independent association with pregnancy-related hyperglycaemia remains unclear due to the need for larger studies using standard diagnostic glucose testing and controlling for maternal characteristics (Wellenstein et al., 2019; Alayed et al., 2022).

In conclusion, the haemoglobin phenotype distribution and the ABO/Rhesus D blood group distribution were similar to those obtained from Southern Nigeria while the uniformly high fasting glucose level was significantly different from the current Nigerian evidence. The blood group ABO and Rhesus D and haemoglobin phenotype did not show significant association with fasting glucose. The ABO finding was similar to some of the international studies that found AB blood group to be possibly a risk factor for GDM, but different from others who found O blood group to be a possible risk factor for GDM; the haemoglobin result was similar to those studies that revealed the absence of strong relationship between sickle-cell trait and GDM (Azeez et al., 2021; Rom et al., 2022; Wellenstein et al., 2019).

## 5.0 CONCLUSION

HbAA, blood group O and Rhesus D positivity were predominant among the participants. No significant differences were found in the numerical values of fasting glucose among the ABO/Rhesus D groups and the haemoglobin phenotypes. Therefore, the authors concluded that there was no relationship between these inherited blood traits and fasting glucose. A high capillary glucose level is seen in all patients and should be confirmed with a standard blood glucose level test.

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