



**ORIGINAL ARTICLE**

## **Duffy Red Cell Antigen Phenotype among Indigenous Pregnant Women attending Antenatal Clinic at Federal Teaching Hospital Gombe, Nigeria.**

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### **Abstract**

**Background and Objectives:** Duffy (FY) blood group system is implicated in transfusion incompatibilities and haemolytic disease of the foetus and newborn. The primary objective was to determine the Duffy phenotype among the indigenous pregnant women in Gombe, Gombe State, Nigeria.

**Materials and Methods:** This was a cross sectional study where simple random sampling was employed on consented participants. Two hundred and fifty nine pregnant women attending antenatal clinic at Federal Teaching Hospital Gombe were randomly recruited into the study. Three milliliters (3mls) of blood was taken, and Duffy antigens typed by standard tube technique (LORNE LABORATORY UK).

**Results:** Among the Indigenous tribe, the percentage of Fy(a+b+) was seen in 2.2% of Fulani and 3.4% of Tangale, Fy(a+b-) phenotype was seen in 4.3% of Tangale, 6.8% of Fulani, 9.5% of Tera, 10.3% of Hausa and 10.5% of Waja. Fy(a-b+) phenotype was seen in 5.3% of Waja, 7.6% of Fulani, 8.7% of Tangale, 9.5% of Tera and 12.5% of Bolawa. Fy(a-b-) phenotype was seen in 2.4% of Tula, 6.4% of Bolawa, 7.3% of Waja, 7.8% of Tera, 17.8% of Tangale, 11.8% of Hausa and 46.5% of Fulani. 84.6% of the study population had the null Duffy phenotype.

**Conclusion:** The research showed the phenotypic distribution of Duffy blood group among the study participants with relatively high percentage of null Duffy phenotype hence possible risk of alloimmunisation.

**Key words:** Duffy phenotype, Haemolytic disease of foetus and newborn, plasmodiasis.

## Introduction

The Duffy (Fy) blood group system is important in clinical medicine due to transfusion incompatibilities and haemolytic disease of the foetus and newborn (HDFN). (1) However, many causes of acute and delayed haemolytic transfusion reaction and haemolytic disease of the foetus and neonate are under reported due to poor obstetric and neonatal screening of clinically important rare blood group system. (2) The Duffy (Fy) antigen is a glycoprotein found on red cell membrane, endothelium, and epithelial cells of alveoli and collecting tubules of the kidneys. (2) The antigen act as receptor for chemokines and plasmodium vivax. (2) Globally, Haemolytic disease of the foetus and newborn affects an estimated 80 in 100,000 patients annually. (3) It could lead to bilirubin encephalopathy & late anaemia of infancy. (4) Though more commonly associated with Rhesus and ABO blood group, severe haemolytic disease of foetus and newborn has been reported with minor blood groups like the Duffy and Kell blood group system. (4) Approximately 4% of pregnant women with anti-Fya have been reported to have foetus with severe anaemia, some requiring intrauterine transfusion. (4) Knowing the distribution of blood group antigens in a specific population, helps to determine the risk of alloimmunization, and among women, the risk of haemolytic disease of foetus and newborn. The distribution of Duffy phenotype among pregnant women in Gombe state is not known, hence the need to determine the primary data.

## Materials and Methods

The study took place at Federal Teaching Hospital Gombe (F.T.H.G). This was a cross sectional study. Gombe state is located in northeastern part of Nigeria. It lies in the wooded savanna lands of the Gongola River basin. It is mainly inhabited by the Fulani, Bolewa, Tera (Terawa), Tangale, Hausa, Kanuri, Waja (Wajawa), and Tula peoples. (5) A total of 259 pregnant women attending the antenatal clinic at the Federal Teaching Hospital Gombe, who consented, were recruited. A simple random

sampling (balloting) technique was used to recruit indigenous pregnant women. Those who picked "yes" were included, whereas those who picked "no" were excluded. Socio-demographic information such as age, religion; tribe, marital status, educational level, occupation and previous obstetric history were obtained from consented participants. Three milliliters (3ml) of venous blood was aseptically collected into EDTA sample bottle from each participant and transported to the lab for processing.

Samples were analyzed in batches for Duffy antigen using standard tube technique (LORNE LABORATORY UK) (6), ABO and Rhesus blood grouping by standard tube technique (7) and identification for Plasmodium species using thin blood film by WHO MM-SOP-08 (8). Data was compiled into an excel spread sheet and analyzed using IBM SPSS Version 25 (9). Analyzed variables were presented in the form of frequency and percentage. Level of significance was set at  $p < 0.05$ .

Ethical approval (NHREC/25/10/2013) was obtained from Research and Ethics Committee of F.T.H.G.

## Determination of Duffy Antigen phenotype (LOARN LABORATORY UK)

Test procedure (standard tube technique)

1. 3-5% suspension of test red cells were prepared using isotonic saline
2. One drop of reagent anti-Fya and Fyb were made for each test and control tube. Each tube labeled appropriately.
3. A drop of the test red cell suspension was added to each labeled test and control tubes, mixed and incubated at 37°C for 30 minutes.
4. The test and control red cells tubes were washed three times in isotonic saline and the supernatant completely decanted.
5. Two drops of AHG were added to each washed test and control red cells and centrifuged at 1500rpm for 1 minute.
6. The test and control red cells gently dislodged from the bottom and examined for agglutination both visually and under a light microscope.

7. Results were recorded respectively  
**N.B.** IgG coated red cells (check cells) was used to validate negative results.

### Determination of ABO and Rh Blood group (Standard tube Technique)

1. About 3-5% suspension of test red cells were prepared using isotonic saline
2. One drop of the reagent anti-A, anti-B, anti-AB and anti- D were dispensed into clean test tubes for each test and control tubes. Each tube labeled appropriately.
3. Drops of test and control red cell suspension were added into the respective test and control tubes, mixed and spun at 1500rpm for 1 minute.
4. The red cells in the centrifuged test tubes were re-suspended and observed for agglutination and or haemolysis.
5. Results were interpreted and reported accordingly.

### Microscopic examination of thin blood film for identification of Plasmodium Species.

#### Procedure:

1. To confirm the Plasmodium species or mixed infestation, one drop of immersion oil was made on the feathered edge of the Giemsa-stained thin blood film.
2. The objective lens was moved from x10 to x100 oil immersion objective.
3. The feathery end or edge of the thin film where the red cells lied side by side, where there was minimal overlap was examined.
4. A systematic pattern of movement along the edge of the film which included; slide movement outwards by one field, inwards by one field, returning in a lateral movement were employed during examination.
5. The pattern was followed respectively, until the presence and specie of malaria parasites was confirmed, Identified and recorded for each specie in the malaria microscopy blood register.

### Quality Control

To ensure reliability of the examination, a quality control check was done for each prepared batch of the Giemsa stain used in staining the Blood smears.

### Results

All the women were married, 96.5% had secondary school education and above. Majority (91.5%) were between the ages of 18 and 35 years, with a mean age of 26.71 + 4.58years. Two- hundred and forty-eight (95.7%) were Rhesus positive and 114(44.1%) were blood group O positive.

The major indigenous tribes in this study were Fulani's (45.6%), Tangale's (17.8%), and Hausa's (11.2%). Tera's, Waja's, Bolewa's and Tula's make up the rest (23.5%) of the population. Tula's were only 5 out of 259 (1.9%) participants.

The most predominant Duffy phenotype among the study population was Fy(a-b-). This was observed in 84.6% of the women (Table 3). The Fy(a-b-) was seen in 82.2% of Fulani's, 89.7% of Hausa's and 84.8% of Tangale's, 81.0% of Tera's. 84.2% of Waja's. The least proportion (68.75%) was seen among the Bolewa's (Table 4). The Fy (a+b+) was the least common phenotype seen in only 1.9% of the study population. It was not seen in the Hausas', Tera's and the Waja's. The highest proportion for the Fy(a+ b+) was seen among the Bolewa's. (Table 4). Thirty-four percent (34%) had a history of miscarriage, 23.6% had history of neonatal jaundice and 8.1% had a history of still births. However the proportion with this bad obstetric history did not vary across the different Duffy phenotypes (Table 5). A hundred and thirty-five (51.1%) pregnant women had malaria parasite by microscopy; 65.2% had P. falciparum infestation, 12.6% had P.vivax, 11.2% had P.malariae and P.ovale (Table 6).

P falciparum was the predominant malaria specie seen among participant with Fy (a-b-) (74.52%) and Fy(a+b-) phenotypes (60%), while P.vivax was the predominant specie among those with the Fy(a-b+) (71.43%) and Fy(a+b+) (80%) phenotypes. None of the participants with the null Duffy phenotype has P vivax infestation.

**Table 1.** Socio-demographic data of the study population.

| Variable             | Frequency (n=259) | Percent (%) |
|----------------------|-------------------|-------------|
| Age group            |                   |             |
| 18-25 years          | 110               | 42.5        |
| 26-35 years          | 127               | 49.0        |
| 36-45 years          | 22                | 8.5         |
| Religion             |                   |             |
| Islam                | 193               | 74.5        |
| Christianity         | 66                | 25.5        |
| Marital status       |                   |             |
| Married              | 259               | 100         |
| Unmarried            | 0                 | 0           |
| Ethnicity            |                   |             |
| Fulani               | 118               | 45.6        |
| Tangale              | 46                | 17.8        |
| Hausa                | 29                | 11.2        |
| Tera                 | 21                | 8.1         |
| Waja                 | 19                | 7.3         |
| Bolewa               | 16                | 6.2         |
| Tula                 | 5                 | 1.9         |
| Others               | 5                 | 1.9         |
| Educational level    |                   |             |
| Tertiary             | 152               | 58.7        |
| Secondary            | 98                | 37.8        |
| Primary              | 6                 | 2.3         |
| None                 | 3                 | 1.2         |
| Occupation           |                   |             |
| Full time house wife | 138               | 53.3        |
| Civil servant        | 58                | 22.8        |
| Student              | 24                | 9.3         |
| Entrepreneur         | 23                | 8.9         |
| Applicant            | 15                | 5.8         |

**Note:** Mean age of the participants =  $26.71 \pm 4.58$  years, Median age = 26 years (IQR: 20-30), Others; Yoruba and Igbo, Shuwa.

**Table 2.** Distribution of ABO, Rhesus blood group system and Obstetric History.

| Variable                     | Frequency (=259) | Percent (%) |
|------------------------------|------------------|-------------|
| ABO group                    |                  |             |
| A                            | 78               | 30.1        |
| B                            | 56               | 21.6        |
| AB                           | 11               | 4.2         |
| O                            | 114              | 44.1        |
| Rh group                     |                  |             |
| Positive                     | 248              | 95.7        |
| Negative                     | 11               | 4.3         |
| Miscarriages                 |                  |             |
| Yes                          | 88               | 34          |
| No                           | 171              | 66          |
| Still births                 |                  |             |
| Yes                          | 21               | 8.1         |
| No                           | 238              | 91.9        |
| History of neonatal Jaundice |                  |             |
| Yes                          | 61               | 23.6        |
| No                           | 198              | 76.4        |

**Note.** N=Sample size, Rh=Rhesus blood group

**Table3.** Distribution of Duffy blood group phenotype among participants

|                  | Frequency (n=259) | Percent (%) |
|------------------|-------------------|-------------|
| Duffy phenotypes |                   |             |
| Fy (a+b-)        | 17                | 6.6         |
| Fy (a-b+)        | 18                | 6.9         |
| Fy (a+b+)        | 5                 | 1.9         |
| Fy (a-b-)        | 219               | 84.6        |
| Total            | 259               | 100         |

Fy; Duffy antigen, n; sample size.

**Table 4:** Distribution of Duffy blood group amongst the ethnic groups

| Ethnic groups | Frequency (n=259) | Percent (%) |
|---------------|-------------------|-------------|
| Fulani        |                   |             |
| Fy (a+b-)     | 8                 | 6.8         |
| Fy (a-b+)     | 9                 | 7.6         |
| Fy (a+b+)     | 4                 | 3.4         |
| Fy (a-b-)     | 97                | 82.2        |
| Hausa         |                   |             |
| Fy (a+b-)     | 3                 | 10.3        |
| Fy (a-b-)     | 26                | 89.7        |
| Tangale       |                   |             |
| Fy (a+b-)     | 2                 | 4.3         |
| Fy (a-b+)     | 4                 | 8.7         |
| Fy (a+b+)     | 1                 | 2.2         |
| Fy (a-b-)     | 39                | 84.8        |
| Tera          |                   |             |
| Fy (a+b-)     | 2                 | 9.5         |
| Fy (a-b+)     | 2                 | 9.5         |
| Fy (a-b-)     | 17                | 81.0        |
| Waja          |                   |             |
| Fy (a+b-)     | 2                 | 10.5        |
| Fy (a-b+)     | 1                 | 5.3         |
| Fy (a-b-)     | 16                | 84.2        |
| Bolewa        |                   |             |
| Fy (a+b-)     | 2                 | 12.5        |
| Fy (a-b+)     | 1                 | 6.25        |
| Fy (a+b+)     | 2                 | 12.5        |
| Fy (a-b-)     | 11                | 68.75       |
| Tula          |                   |             |
| Fy (a-b-)     | 5                 | 100         |
| Others        |                   |             |
| Fy (a-b-)     | 5                 | 100         |
| Total n (%)   | 259               | 100         |

Note: Others; Yoruba, Igbo, Shuwa

**Table 5.** Distribution of Duffy phenotype and negative obstetric history

|                          | Fy(a+b-)       | Fy(a-b+)      | Fy(a+b+)      | Fy(a-b-)       | Total          | P value |
|--------------------------|----------------|---------------|---------------|----------------|----------------|---------|
|                          | n (%)          | n (%)         | n (%)         | n (%)          | n (%)          |         |
| <b>Miscarriages</b>      |                |               |               |                |                |         |
| Yes                      | 3<br>(17.65)   | 8<br>(44.44)  | 0<br>(0.00)   | 77<br>(34.16)  | 88<br>(33.98)  | 0.52    |
| No                       | 14<br>(82.35)  | 10<br>(55.56) | 5<br>(100.00) | 142<br>(64.64) | 171<br>(66.02) |         |
| <b>Still birth</b>       |                |               |               |                |                |         |
| Yes                      | 0<br>(0.00)    | 2<br>(11.11)  | 0<br>(0.00)   | 19<br>(8.68)   | 21<br>(8.11)   | 0.07    |
| No                       | 17<br>(100.00) | 16<br>(88.89) | 5<br>(100.00) | 200<br>(91.32) | 238<br>(91.89) |         |
| <b>Neonatal jaundice</b> |                |               |               |                |                |         |
| Yes                      | 8<br>(7.06)    | 5<br>(27.78)  | 1<br>(20.00)  | 47<br>(21.26)  | 61<br>(23.55)  | 0.52    |
| No                       | 9<br>(52.94)   | 13<br>(72.22) | 4<br>(80.00)  | 172<br>(78.54) | 198<br>(76.45) |         |

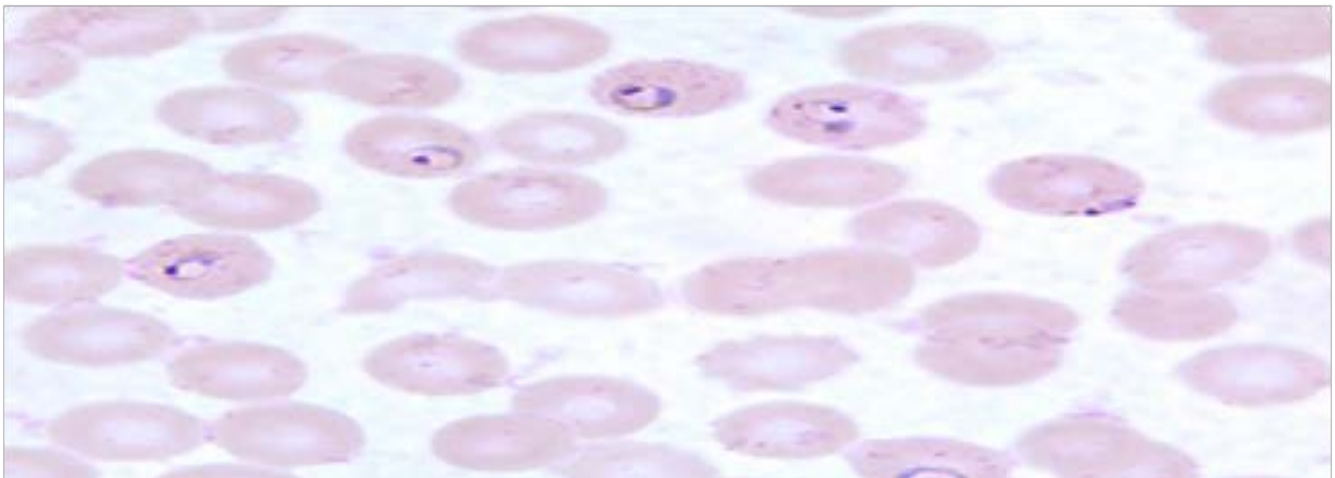
**Table 6.** Prevalence of malaria parasitaemia among participants and type of plasmodium species.

|                                     | Frequency | Percent (%) |
|-------------------------------------|-----------|-------------|
| <b>Malaria parasitaemia (n=259)</b> |           |             |
| Not seen                            | 124       | 47.9        |
| Seen                                | 135       | 51.1        |
| <b>Plasmodium species (n=135)</b>   |           |             |
| <i>P. falciparum</i>                | 88        | 65.2        |
| <i>P. vivax</i>                     | 17        | 12.6        |
| <i>P. malariae</i>                  | 15        | 11.1        |
| <i>P. ovale</i>                     | 15        | 11.1        |

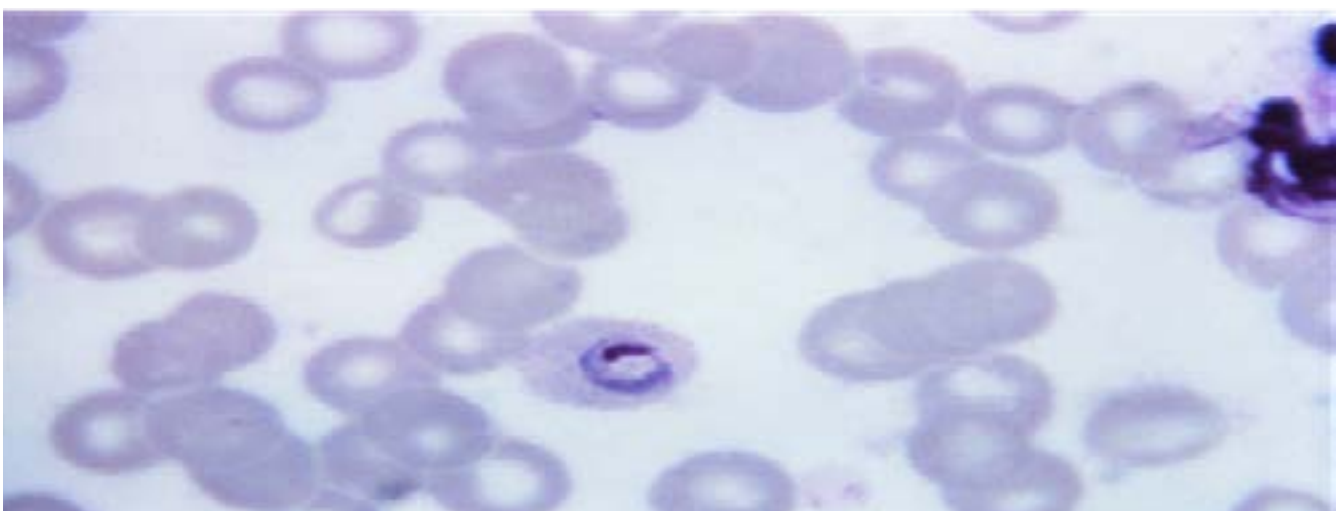
**Table 7:** Distribution of plasmodium species based on Duffy antigen status of participants.

|                     | Fy(a+b-)    | Fy(a-b+)    | Fy(a+b+)   | Fy(a-b-)     | *P value |
|---------------------|-------------|-------------|------------|--------------|----------|
|                     | n (%)       | n (%)       | n (%)      | n (%)        |          |
| <i>P.falciparum</i> | 6 (60.00)   | 2 (14.29)   | 1 (20.00)  | 79 (74.53)   | 0.001    |
| <i>P.malariae</i>   | 0 (0.00)    | 2 (14.29)   | 0 (0.00)   | 13 (12.26)   |          |
| <i>P.vivax</i>      | 3 (30.00)   | 10 (71.43)  | 4 (80.00)  | 0 (0.00)     |          |
| <i>P.ovale</i>      | 1 (10.00)   | 0 (0.00)    | 0 (0.00)   | 14 (13.21)   |          |
| Total               | 10 (100.00) | 14 (100.00) | 5 (100.00) | 106 (100.00) |          |

\*Pearson's chi square

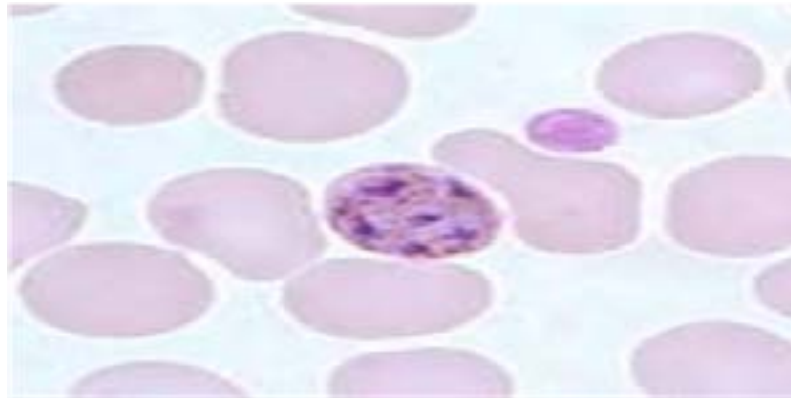


**Figure 1:** Morphologic characteristics of Trophozooid of *P. falciparum* in thin Blood film of the study participants (Light Microscopy)

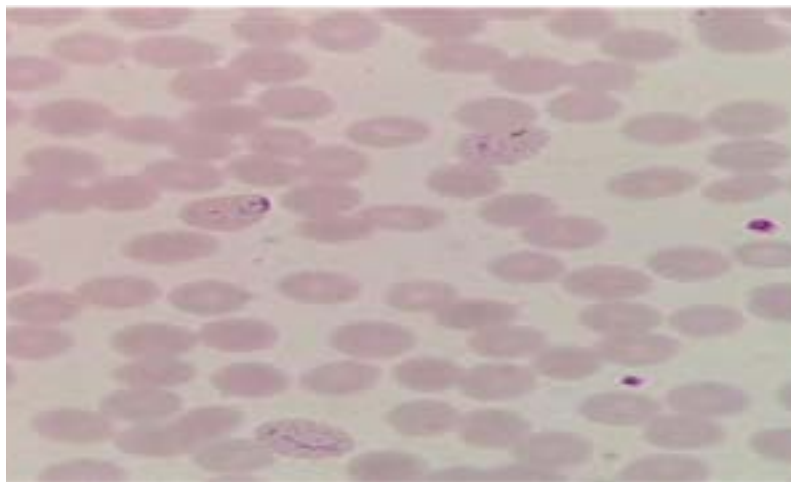


**Figure 2:** Morphologic characteristics of Trophozooid of *P. ovale* in thin Blood film of the study participants (Light Microscopy)





**Figure 3:** Morphologic characteristics of Trophozoite of *P. malariae* in thin Blood film of the study participants. (Light Microscopy)



**Figure 4:** Morphologic characteristics of Trophozoite of *P. vivax* in thin Blood film of the study participants (Light Microscopy)

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## Discussion

The Duffy blood group antibodies are IgG antibodies and have the ability to cross the placental barrier and hence are associated with risk of haemolytic disease of foetus and new born. We investigated the prevalence and distribution of the Duffy blood group antigen among pregnant women in Gombe State, North - East Nigeria.

In this study, the null phenotype was the most prevalent phenotype seen in 80- 86% of pregnant women from all the major ethnic groups in Gombe, except among the Bolewa, where it accounted for 69% of the pregnant

women. This agrees with previous study among blacks in sub Saharan Africa.(10) In a related study in Sokoto, a state in North West Nigeria, all the pregnant Fulani's women (20/162) in the study lacked both the Fya and Fyb antigen, implying they also had a null phenotype. Kulkarni et al., (11) Erhabor et al.,(12) also reported 98.8% of Duffy negativity among Hausa's in North West Nigeria. The prevalence rate of Fy (a+b-), Fy (a-b+) and Fy (a+b+) phenotype disagree with the research carried out in north western Nigeria(12), where prevalence rate of 4.3%, 5.6% and 0.61% were reported respectively.

In another research carried out at donor

clinic of Aminu Kano teaching hospital, using potent antisera, Duffy antigens were not detected among the blood donors.(13)

The Duffy antibody is an immune antibody produced in response to previous exposure and has the ability to cause immediate and delayed transfusion reaction and haemolytic disease of the foetus and newborn. In this study, out of 259 participants, 88 (33.98%) had history of miscarriage, 21 (8.11%) still births and 61 (23.55%) had neonatal jaundice. Contrary to expectations, the proportion of pregnant women with bad obstetric history was higher among the pregnant women with null Fy(a-b-) than those with Fy(a-b+). . The pregnant women with null Fy (a-b-) phenotype had the highest history of miscarriages, still births, and neonatal jaundice. The antibody against the Fya antigen (anti-Fya) is 20 times more potent than the anti-Fyb and is associated with mild-to-severe HDFN, than the anti-Fyb. The anti-Fyb is rarely associated with mild HDFN. (14) Moreover, studies have shown that blacks with the F (a-b-) phenotype who develop Duffy antibodies, do not produce anti-Fyb but usually produce anti-Fya and anti Fya3 or Fy5. (15) This is because the Fy(a-b-) phenotype in blacks is associated with a mutation (a single amino acid substitution at position 46) which impairs the erythroid promoter, GATA-1 binding motif. The mutation prevents the transcription of the FY gene in the RBCs only while the Duffy Fyb antigen is expressed on the other tissues.(16)

These and the dosage effect observed in the Duffy blood group though not statistically significant, may explain the differences observed in the obstetric history across the various phenotypes.

The Duffy antigens serve as receptor for diverse group of chemokines and *P. vivax*. The Duffy antigen receptor plays a vital role as an immune regulator where it served as a chemokine sink during inflammatory processes. In this study, out of 259 participants, 135 had plasmodiasis, out of which 11.1% were infested with *P. malariae* and *P. ovale* respectively, 12.6% *P. vivax*, and

65.2% *P. falciparum*. The prevalence rates of plasmodiasis among participants with null Duffy phenotype Fy (a-b-) was 0.0% for *P. vivax*, 12.26 % *P. malariae*, 74.53 % *P. falciparum* and 13.21 % *P. ovale*. These findings supported previous studies which stated that red cells of null Duffy phenotype individuals resist invasion by *P. vivax* which was due to non-expression of Duffy antigen on the red cell surface, hence the resistivity.(17) This study did not screen for allo-antibodies among the pregnant women with the null phenotype. Doing this will have provided more data on the risk of alloimmunization among pregnant women with the null phenotype.

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## Conclusions

The prevalence of the null Duffy phenotype is high among pregnant women in Gombe

and hence there may be a high risk of alloimmunization and obstetric complications. The least seen phenotype is the F(a+b+) phenotype.

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All authors substantially contributed to components of this study which included; Study proposal and research design, data acquisition, analysis and interpretation of findings.

**Co-author's contribution;**

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