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# Comparative analysis of the hematological Profile and alloantibodies of pregnant women in a tertiary institution – North-Central Nigeria

Chinedu Nnawike Okeke [1], Ezra Danjuma Jatau [2], Timothy Olugbenga Ogundeko [3,*], Olusegun Godwin Adeniyi [1] and Amandy Odinakachi Okeke [4]

[1] *Department of Haematology and Blood Transfusion, College of Medicine and Allied Health Sciences, Bingham University, Jos Campus, Nigeria.*
[2] *Department of Haematology and Blood Transfusion, Jos University Teaching Hospital, Nigeria.*
[3] *Department of Pharmacology and Therapeutics, College of Medicine and Allied Health Sciences, Bingham University, Jos Campus, Nigeria.*
[4] *Department of Nursing Services, Jos University Teaching Hospital, Nigeria.*

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## Abstract

The third world countries have suffered challenges relating to public health, ranging from high cost of healthcare, poverty and lack of diagnostic equipment and accessories. One of the salient issues is universal antenatal antibody screening. This study aimed to appraise the alloantibodies in comparison with hematological profiles of pregnant women accessing ante-natal care at a tertiary Institution – in Jos, North-Central, Nigeria. A total of 200 consenting pregnant women receiving ante-natal care in the Jos University Teaching Hospital, Jos, Nigeria, were recruited into this study for a period of one month. Each participant was interviewed with a pretested, structured, interviewer-administered questionnaire. Blood sample was collected from each participant with minimal stasis from the antecubital vein via sterile syringe and needle into (EDTA) bottles. Full blood count and antibody screening were done by standard methods. Results showed that there was a decrease in the RBC, PCV, Hb, MCH, MCHC, RDW, WBC, and LymP WBC and increase in MCV, NeuP, MonP, EosP, and BasP values of participants with alloantibodies compared to those without, also with no significant ($p > 0.05$) between the groups. There was no difference in the platelet counts of both groups. Alloantibodies have effect on the hematological profiles (RBC, PCV, Hb, MCH, MCHC, RDW, WBC, and LymP WBC, MCV, NeuP, MonP, EosP, and BasP) of pregnant women accessing ante-natal care at in JUTH, Nigeria.

**Keywords:** Alloantibodies; Haematological profile; Ante-natal; JUTH; North-central Nigeria

## 1. Introduction

Blood transfusion and pregnancy remain the major risk factors for development of alloimmunization to RBCs in affected individuals. Other implicated factors include organ transplantation or injection with immunogenic materials [1]. With the advent of many interventions to improve maternal and child health, pregnant women have become the focus of many health programs. However, few data exist regarding this important population. Although pregnancy induced changes occur in hematological values, very few laboratories provide specific reference ranges for pregnant women [2]. Blood volume increases in pregnancy by more than 50%, leading to hemodilution [3]. This increase is greater than the associated increase in the red blood cell mass resulting in physiological anemia (associated with low RBC, Hb, Hct. MCH, MCHC levels) [4]. The increase in blood volume during gestation is attributed to reduced atrial-natriuretic peptide levels and increased plasma renin activity [5]. With the advent of many interventions to improve maternal and child health, pregnant women have become the focus of many health programs [6, 7]. There are approximately 270 antibodies

* Corresponding author: Timothy Olugbenga Ogundeko

against red blood cell antigens [8]. The anti-A and anti-B antibodies against red blood cells are part of every adult immune system and thus are natural antibodies [9]. Any antibodies against red cell antigens other than anti-A and anti-B are considered unexpected and can be either alloantibodies or autoantibodies. In pregnant women, such antibodies may cross the placenta and cause HDFN [8, 10]. Timely detection of such antibodies in antenatal women is essential for the early and better management of HDFN, and for the transfusion safety of the mother [11]. The alloimmunization rate varies from 0.4% to 2.7% among pregnant women worldwide [12]. Immunization to RBC antigens may result from pregnancy, transfusion, transplantation, or from injection with immunogenic material. In a special care baby unit in Port Harcourt, Nigeria, the overall incidence of neonatal jaundice was 21.4%, 27.8% in outborn and 16.4% in inborn babies [13]. Although there has been increasing antenatal care attendance and administration of routine supplements among pregnant Nigerian women [14], the prevalence of anaemia in pregnancy remains high as much as 61% prevalence. Another documentation in a 2016 World Bank Report had shown a 58.5% prevalence [15]. Reduced ANC visits and increasing age where significant factors associated with anaemia in pregnancy. Prevalence of anaemia amongst pregnant women in BHUTH was 43.5%. The most common type of anaemia in this study is mild anaemia [16]. Similarly, a higher prevalence of greater than 40% has been reported in the West African subregion and in other developing nations of the world like India [17, 18]. The haematologic system adapts to make provision for foetal hematopoiesis, ensuring adequate blood supply to the enlarged uterus and its content thereby protecting both mother and foetus against the effects of impaired venous return in both the supine and erect positions in addition to safeguarding against bleeding at delivery [19].

Though less studied than in transfusion or pregnancy settings, RBC alloantibodies can also potentially be clinically significant in hematopoietic stem cell transplantation settings. Just as isohemagglutinins are significant in major ABO mismatched transplant settings (eg, blood group O recipient, blood group A donor) [20], RBC alloantibodies may have implications for the product infusion or donor RBC engraftment in situations in which the donor expresses the cognate RBC antigen in question [21]. Pregnancy is associated with the coagulation cascade being in an activated state with about 20-200% increase in fibrinogen and factors II, VII, VIII, X and XII while the level of factors XI and XIII decrease [22].

This study aimed to appraise the alloantibodies in comparison with hematological profiles of pregnant women accessing ante-natal care at a tertiary Institution – in Jos, North-Central, Nigeria.


**Figure 1** Google map of Jos University Teaching Hospital [23]

## 2. Material and methods

A total of 200 consenting pregnant women receiving ante-natal care in the Jos University Teaching Hospital, Jos, Nigeria, were recruited into this study for a period of one month. Each participant was interviewed with a pretested, structured, interviewer-administered questionnaire.

### 2.1 Sample size determination

The minimum sample size was arrived at using the formula [24]. Below:

$$\text{Sample size (n)} = \frac{z^2pq}{d^2}$$

Where:
n = the desired sample size

z = the standard error. This is usually set at 1.96 which corresponds to the 95% confidence level.
p = Rate of RBCs alloimmunization in pregnancy

$$q = (1 - p)$$

d = degree of error tolerable in this study, usually set at 0.05 (5%)

$$p = 0.048.^{22}$$

$$\text{Sample size (n)} = \frac{(1.96)^2 \times 0.048 \times 0.952}{0.05 \times 0.05}$$

$$= \underline{0.1755}$$

$$0.0025$$

$$= 70$$

The minimum sample size was 70. To get a better representation of the population, a sample size of 200 was used.

### 2.1. Inclusive Criteria

Healthy pregnant women that had not received any blood components in the preceding 4 months, as well as those that had not received passive immunization with Anti-D IgG or intravenous immunoglobulin in the preceding 3 months.

### 2.2. Exclusive criteria

Pregnant women that received any blood components in the preceding 4 months were excluded to prevent false reactions from exogenous antibodies. Furthermore, pregnant woman that had received passive immunization with Anti-D IgG or intravenous immunoglobulin in the preceding 3 months were excluded to prevent false negative reactions.

### 2.3. Collection of blood sample

A blood sample (4 mL) was withdrawn from each participant with minimal stasis from the antecubital vein using a dry, sterile disposable syringe and needle. The blood was dispensed into tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). The specimens were labeled with the subject's age, and identification number. The EDTA samples were kept at room temperature until processing, which occurred within 4 hours of collection.

### 2.4. Full blood count determination

This was performed using a Mindray BC 5000 Hematology Analyzer, a five-part auto analyzer able to test parameters per sample including Hb concentration, PCV, RBC concentration, MCH, MCV, MCHC, WBC count, and Platelet count. Standardization, calibration of the instrument, and processing of the samples were done according to the manufacturer's instructions. Each blood sample was mixed well and then approximately 20 μL was aspirated by allowing the analyzer's sampling probe into the blood sample and depressing the start button. Results of the analysis were displayed, after which the analyzer generated a paper copy of the results on thermal printing paper.

### 2.5. Antibody screening

The sera were screened alloantibodies using antibody screening panels- 3 cells (Diamed, Switzerland). The serum with alloantibodies was further tested to determine the specificity of the antibodies using identification panel- 11 cells (Diamed, Switzerland) by tube method in low ionic strength solution, and anti- human globulin. To all tests that were negative at the AHG phase, was added 1 drop of IgG-sensitized RBCs (Coombs' Control Cells). This was centrifuged for 15-20 seconds on high revolution and examined macroscopically and microscopically for agglutination or haemolysis. The Coombs' Control Cells yielded a positive reaction. If no agglutination was observed, the result for that panel cell was read as invalid and the test repeated beginning using that cell.

### 2.6. Statistical analysis

The data collected was analyzed using Epi Info version 7.2.5.0. Continuous data presented as mean and standard deviation (SD). Student's t-test was used to assess the significance between means of two groups and ANOVA used to

compare the means of multiple groups. Chi-square (X2) was used to compare categorical data. A p-value of < 0.05 was considered statistically significant. The results were reported in tables.

## 3. Result and Discussion

The ages of the studied population ranged between 18 and 44 years (mean ± SD = 29.57 ± 5.99 years). Most (73.5%) of the participants were aged between 20 and 35 years.

All the participants were married. One hundred and eighteen (59%) had obtained tertiary education, sixty-six (33%) had secondary education, twelve (6%) had primary education, while four (2%) had no formal education (*Table 1*).

**Table 1** Socio- demographic characteristics of participants

| Characteristic | Frequency (%) |
|---|---|
| Age | |
| ≤20 | 17 (8.5) |
| 21-25 | 34 (17.0) |
| 26-30 | 64 (32.0) |
| 31-35 | 49 (24.5) |
| 36-40 | 30 (15.0) |
| 41-45 | 6 (3.0) |
| Total | 200 (100.0) |
| Occupation | |
| Artisan | 16 (8.0) |
| Civil servants | 58 (29.0) |
| Housewife | 57 (28.5) |
| Student | 27 (13.5) |
| Trader | 42 (21.0) |
| Total | 200 (100.0) |
| Educational qualification | |
| No formal education | 4 (2.0) |
| Primary education | 12 (6.0) |
| Secondary education | 66 (6.0) |
| Tertiary education | 118 (59.0) |
| Total | 200 (100.0) |

In addition, most of the participants were multigravida, 144 (72.0%), while the remaining fifty-eight (28.0%) were primigravida. Ninety-five (47.5%) were in the third trimester of pregnancy, eighty-four (42.0%) in the second trimester, while twenty-one (10.5%) were in the first trimester.

**Table 2** Frequency of alloantibodies among participants

| Alloantibody | Frequency (%) |
|---|---|
| Present | 24 (12.0) |
| Absent | 176 (88.0) |
| Total | 200 (100.0) |

Alloantibodies were identified in the serum of 24 (12.0%) of the pregnant women – Table 2. This finding correlate, though with higher value with earlier reports of 28/344 (8.1%) [11] and 384 (2.4%) [25]. These peculiar high values of alloantibodies occurrence in the pregnant women reiterates public health challenges especially in pregnant women and neonates.

**Table 3** Haematological parameters of participants

| **Parameters** | **Mean ± Std n = 200** |
|---|---|
| WBC: White blood cells count x $10^9$/L | 7.05 ± 1.96 |
| RBC: Red blood cells count x $10^{12}$/L | 4.24 ± 0.49 |
| Haemoglobin concentration g/dl | 10.59 ± 0.98 |
| Packed cell volume PCV L/L | 0.38 ± 0.04 |
| Mean corpuscular volume Fl | 91.06 ± 8.97 |
| Mean corpuscular haemoglobin pg | 25.28 ± 2.67 |
| Mean corpuscular haemoglobin concentration g/dl | 28.35 ± 2.97 |
| Platelet count x $10^9$/L | 264 ± 94 |
| Neutrophil percentage % | 64.5 ± 10.3 |
| Lymphocyte percentage % | 29.5 ± 8.4 |
| Monocyte Percentage % | 3.0 ± 1.4 |
| Eosinophil percentage% | 2.2 ± 2.4 |
| Basophil percentage % | 0.6 ± 0.4 |
| Red cell distribution weight | 14.99 ± 8.98 |
| Mean platelet volume | 9.65 ± 1.06 |

**Key:** Hb: Haemoglobin concentration, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, Platelet: Platelet count, NeuP: Neutrophil percentage, LymP: Lymphocyte percentage, EosP: Eosinophil percentage, BasP: Basophil percentage, RDW: Red cell distribution weight, MPV: Mean platelet volume

### 3.1. Comparison of Complete blood counts between participants with or without alloantibodies

Despite the so-called inconsequential concern of the hematologist to the occurring haematological changes during pregnancy as compared to the physiological changes and puerperium that are principally influenced by changes in the hormonal milie [26], these haematological parameters become a thing of utmost concern with the presence of alloantibodies. Comparative analysis of the foregoing has become imperative. Furthermore, pregnant women should be monitored, and their hematological parameters properly interpreted to recognize and avoid pregnancy complications early, as this will be of paramount importance in line with meeting the SDGs target related to maternal and child health [27].

Our study showed that there was a decrease in the RBC, PCV, Hb, MCH, MCHC, RDW, WBC, and LymP WBC values of participants with alloantibodies when compared with those without alloantibodies. However, these differences were not statistically significant ($p > 0.05$). There was an increase in MCV, NeuP, MonP, EosP, and BasP values among participants with alloantibodies compared to those without. These differences were not significant ($p > 0.05$). There was no difference in the platelet counts of those with or without alloantibodies – *Table 4*. According to Doig & Zhang [28], reported the below the reference interval of MCHC hypochromia (hypochromic rbcs); MCV microcytosis (microcytic rbcs); Hb (anemia) and the RDW as a parameter that can only rise, nonetheless, occasional patient will have an RDW value that is slightly below the reference interval, but will not indicate either an instrument error or a pathological condition.

**Table 4** Comparison of Complete Blood Count between participants with or without alloantibodies

| Parameters | Mean ± SD n = 200 | Alloantibodies Positive Negative Mean ± SD Mean ± SD n = 24 n = 176 | | Df | P value |
|---|---|---|---|---|---|
| WBC x $10^9$/L | 7.05 ± 1.96 | 6.40 ± 1.67 | 7.20 ± 2.04 | 133 | 0.1169 |
| RBC x $10^{12}$/L | 4.24 ± 0.49 | 4.14 ± 0.29 | 4.24 ± 0.52 | 117 | 0.9448 |
| Hb g/dl | 10.59 ± 0.98 | 10.43 ± 0.99 | 10.62 ± 0.98 | 44 | 0.8549 |
| PCV L/L | 0.38 ± 0.04 | 0.36 ± 0.08 | 0.38 ± 0.04 | 112 | 0.539 |
| MCV fL | 91.06 ± 8.97 | 90.97 ± 5.15 | 89.40 ± 7.42 | 123 | 0.7662 |
| MCH pg | 25.28 ± 2.67 | 25.22 ± 2.23 | 25.63 ± 4.88 | 95 | 0.4613 |
| MCHC g/dl | 28.35 ± 2.97 | 27.74 ± 2.57 | 28.13 ± 2.42 | 77 | 0.7338 |
| Platelet x $10^9$/L | 264 ± 94 | 264 ± 84 | 264 ± 94 | 151 | 0.7223 |
| NeuP % | 64.5 ± 10.3 | 64.5 ± 7.9 | 64.4 ± 10.6 | 146 | 0.7879 |
| LymP % | 29.5 ± 8.4 | 28.9 ±8.6 | 29.6 ± 8.4 | 136 | 0.5011 |
| MonP % | 3.0 ± 1.4 | 3.0 ± 1.2 | 3.0 ± 1.4 | 53 | 0.5839 |
| EosP % | 2.2 ± 2.4 | 2.9 ± 2.5 | 2.1 ± 2.4 | 56 | 0.0448 |
| BasP % | 0.6 ± 0.4 | 0.60 ± 0.36 | 0.52 ± 0.34 | 18 | 0.8928 |
| RDW | 14.99 ± 8.98 | 14.13 ± 0.86 | 14.45 ± 1.16 | 47 | 0.7689 |
| MPV | 9.65 ± 1.06 | 9.46 ± 0.94 | 9.68 ± 1.07 | 46 | 0.4119 |

**Key:** Hb: Haemoglobin concentration, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, Platelet: Platelet count, NeuP: Neutrophil percentage, LymP: Lymphocyte percentage, EosP: Eosinophil percentage, BasP: Basophil percentage, RDW: Red cell distribution weight, MPV: Mean platelet volume

Monitoring hematological profiles is essential to diagnose or monitor illness in pregnant woman [29]. Various studies from different parts of the globe have reported prevalence of anemia in pregnancy with varying figures due to different factors including multi-factorial causes of anemia, socio-economic, educational status, malaria prevalence etc., [30, [31 ,32] this study however points out high occurrence of alloantibodies as another factor. This is highlighted in *Table 4*, as there was general decrease in the haematological parameters (RBC, PCV, Hb, MCH, MCHC, RDW, WBC, and LymP WBC) of the pregnant women with alloantibodies when compared with those without alloantibodies. Furthermore, the increase in MCV, NeuP, MonP, EosP, and BasP values among the pregnant women with alloantibodies compared to those without (*Table 4*), though also statistically insignificant suggests a risk of pre-eclampsia. Pre-eclampsia (PE) is a common complication during pregnancy, affecting 3–5% pregnant women. Epidemiological studies have shown that PE is a risk factor contributing to both maternal and fetal morbidity and mortality [33, 34], including maternal renal insufficiency, liver disorder, neurological complications and fetal growth restriction.

Although predisposing factors of PE includes pre-eclampsia in a previous pregnancy, being pregnant with more than one baby, Chronic high blood pressure (hypertension), Type 1 or type 2 diabetes before pregnancy, kidney disease, autoimmune disorders, use of in vitro fertilization etc., the elevated WBC count often due to an increase inflammatory response may also have been triggered by the alloantibodies in the pregnant women from our study.

## 4. Conclusion

Alloantibodies have effect on the hematological profiles (RBC, PCV, Hb, MCH, MCHC, RDW, WBC, and LymP WBC, MCV, NeuP, MonP, EosP, and BasP) of pregnant women accessing ante-natal care at in JUTH, Nigeria. Every trimester Immuno-hematological assay for pregnant women for detection of the presence of antibodies is thus suggested.

## Compliance with ethical standards

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*Disclosure of conflict of interest*

All the authors hereby declare no conflicting interest.

*Statement of ethical approval*

Ethical approval (ref; JUTH/DCS/ADM/127/XIX/6587) was obtained from JUTH Research and Ethics Committee prior to commencement of the study.

*Statement of informed consent*

A written informed consent was obtained from each study participant.

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