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RESEARCH ARTICLE

Hematological Profile and Prevalence of Bloodborne Viruses Among Pregnant Women Attending Antenatal Clinic in a Tertiary Military Hospital in Lagos, Southwest of Nigeria

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Abstract: Pregnancy outcome is determined by a host of factors, including the mother's hematological profile and the concurrent presence of blood-borne viral infections. This study aimed to determine the hematological profile and prevalence of blood-borne viruses among pregnant women who attended our facility's outpatient obstetrics department over four years. This was a retrospective cohort study using data from the clinical records of 499 pregnant women who attended the antenatal clinic at the 68 Nigerian Army Reference Hospital, Yaba, Lagos, Nigeria, over a four-year period. Demographic information, full blood count parameters and results of screening for blood-borne viruses were collected into a predesigned proforma. The Hematological parameters and demographic variables were analyzed using SPSS version 29.0, values expressed in mean, frequency and percentages. Categorical variables were compared using chi-square test with statistical significance at $p < 0.05$. The results were presented in tables, pie and bar charts using percentages and mean ± standard deviation. The mean age of the study participants was 36±2.3 years, with the blood group O being the most prevalent (49.7%). Hematocrit was lowest in the third trimester 31.21±4.1%, $p<0.001$). Anemia was significantly associated with one parous experience ($p=0.04$), the presence of HIV infection ($p=0.03$) and the AS genotype ($p=0.01$). The prevalence of HIV infection (6.0%) was the highest of the three blood-borne viruses tested. The findings from this cohort study contribute more evidence toward the creation of standardized hematological reference values for pregnant women in Nigeria. Proper supplementation and implementation of routine screening for blood-borne viruses can lead to better pregnancy outcomes for Nigerian women.

Keywords: Hematology Indices, Bloodborne Viruses; Normal Pregnancy; Haemoglobinopathies

1. INTRODUCTION

Historically, it is a common knowledge that several physiologic changes occur in a pregnant woman to accommodate the increased metabolic requirement of the pregnancy and the developing fetoplacental unit. Some of these changes start immediately after the implantation of the developing embryo and continue until the third trimester [1]. This physiological adaptation to pregnancy occurs in every organ system but becomes more pronounced earlier in the cardiovascular and hematological systems [2]. Some of these changes include an increase in maternal blood volume, decreased peripheral vascular resistance, marginal increase in heart rate, decrease in blood pressure, physiologic anemia, mild leukocytosis (mostly neutrophils) without evidence of infection, thrombocytopenia, decreased fibrinolysis and changes in procoagulant-anticoagulant balance [3, 4]. Based on these changes, it is possible to misinterpret maternal hematology parameters during pregnancy and puerperium if one is unaware of these hemodynamic adaptations. It also helps to predict fetal wellbeing and proper development of fetoplacental units.

Therefore, the changes in hematological parameters of women during pregnancy may have a significant effect on pregnancy outcome especially in our environment where women may have undiagnosed health problems or poor iron storage prior to becoming pregnant [5]. This further emphasizes the urgent need to better understand what is normal in order to avoid unnecessary intervention in pregnancy.

Just as important, in developing countries, Bloodborne viruses (Human immunodeficiency virus-HIV, Hepatitis B and C) during pregnancy contribute a significant proportion to maternal and perinatal morbidity and mortality [6-8]. These viruses invade the immunological paradox of the placenta and can be vertically transmitted to the fetus [8]. It has been hypothesized that the physiological changes in pregnancy particularly the variations in the endocrine and immune systems may cause increased predisposition of the mother to acquire these viruses or her ability to transfer it to her fetus in utero [8,9]. It even becomes more worrisome as the safety of many vaccines against these viruses and antiviral medications are still being evaluated in many studies [8-10]. This therefore increases the vulnerability of the mother and the fetus to these highly infectious agents.[8] Additionally, the concern about the effects of BBVs is not only because of the danger it poses to the mother and developing fetus via vertical transmission, but also, because of the danger of horizontal transmission to the attending Obstetricians and Midwives [6].

According to several reports in literature *Hepatitis* C virus (HCV), is speculated to have been discovered over thirty years ago [3,6]. It is a bloodborne virus and has been implicated in chronic liver disease globally [6,7]. Most infected people worldwide are asymptomatic but have the potential of progressing to chronic liver disease [7]. Owing to its asymptomatic nature, the true global prevalence is hard to determine. This is even more worrisome as most facilities in Nigeria does not incorporate HCV as part of routine antenatal screening [6].

HCV is transmitted from person-to-person via parenteral route of infected blood or its products. Several risk factors have been proposed to enhance HCV transmission during pregnancy. These risk factors include maternal HCV viral load, co-infection with HIV, maternal intravenous drug misuse, invasive prenatal testing such as chorionic villus sampling, amniocentesis and percutaneous umbilical cord blood sampling [6-8]. Others include prolonged rupture of fetal membranes, chorioamnionitis, maternal infection during pregnancy, transfusion of unscreened blood, and other Obstetric procedures such as fetal blood sampling and the use of fetal scalp electrodes and other harmful traditional practices in Africa such female genital mutilation (such as re-infibulation) [6]. These notwithstanding, opinion is divided on the risk of HCV vertical transmission and mode of delivery, with some studies suggesting that Caesarean section or vaginal delivery have a limited role on the risk of transmission [6]. These authors argue that monoinfection with HCV only may not significantly impact the route of delivery of the newborn. HCV genotype, and breastfeeding in a woman with intact nipple also does not increase the risk of vertical transmission [8, 9, 10-14].

Undoubtedly, there is a paucity of data on the normal reference values of the hematological indices of pregnant mothers in our environment and prevalence of BBVs hence the need for this study.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

It was a retrospective cohort study conducted at 68 Nigerian Army Reference Hospital Yaba (68 NARHY), Lagos, from January 2020 to December 2023. The 68 Nigerian Army Reference Hospital Yaba, Lagos is a foremost tertiary military hospital in the mega city of Lagos with about 25 million citizens. It is 500-bed capacity military hospital that serves about 7000 in-and-out patients per month and provides referral services to Nigerian service men and civilians alike. The 68 NARHY is located in Yaba Local Government Area of Lagos State. Besides antenatal and infectious disease services, it also provides other ancillary services such as efficient central laboratories, advanced radiology center and 24 hours emergency and ambulance services to support our growing obstetrics population. It runs daily adult HIV clinics at its Centre for Infectious Disease Clinic (CID) with average daily clinic attendance of 200 patients. It also runs two specialist led antenatal clinics weekly with average clinic attendance of 150 pregnant women. The hospital offers specialized treatment to all categories of pregnant women, it also has a unit dedicated to the care of HIV-positive pregnant women. There is also a pediatrics unit with neonatal intensive care unit to cater for our neonates. This unit is always open 24 hours per week with a specialist on call. Routine screening for triple viral infection and syphilis is also offered routinely as a policy in the hospital. All pregnant mothers are also screened for haemoglobinopathies at booking and

counselled appropriately. The hospital prides itself with a multidisciplinary team of consultants comprising of nurses, midwives, anesthetists, gynecologists, physicians, and pediatricians to carter for the daily need of our patients [6].

Using the findings from the study by Wang et al,14 a minimum sample size of 400 pregnant women was required to participate in the study achieve a power of 80%, a type I error rate of 5% and a nonresponse rate of 20%.

2.2 Participants

These were pregnant mothers who sought care in our facility between January 2020 and December 2023. The study included healthy pregnant women with no known chronic medical conditions. Eligibility required that all pregnant women at booking undergo both full blood count and BBV testing as part of their routine antenatal care. Women with incomplete records or those who did not complete both tests were excluded. Additionally, women with chronic medical conditions such as diabetes, hypertension, hypo- or hyperthyroidism, renal disease, asthma, or those on anticoagulants were excluded. The study also excluded women with gestational trophoblastic disease, miscarriage, ectopic pregnancy, or multiple pregnancies.

2.3 Specimen Collection and Determination of Variables of Interest

2.3.1 Determination of Hematological Indices

The standard operational procedure for sample collection for hematological indices was followed strictly. This includes collecting ten milliliters of venous blood from the pregnant mother's median cubital vein in the cubital fossa while she was made to sit in the upright position using aseptic procedure. These samples were collected using vacutainer needles. The blood sample was collected into a specialized vacutainers sample container containing the anticoagulant Na/K3- EDTA (ethylenediaminetetraacetic acid) and transported from the point of collection (antenatal clinics) immediately to the central laboratory of the 68 Nigerian Army Reference hospital by the ward orderly. Hemoglobin concentration, Packed cell volume, WBC and platelets were determined using an automated hematology analyzer Sysmex (KX 21) machine in 68 NARHY laboratory.

2.3.2 Determination of ABO and Rh Blood Groups

This was done using forward and reverse cell typing using test tube agglutination method. Antiglobulin technique was applied to confirm Rh negativity [14].

2.3.2.1 Determination of Bloodborne Viruses

A volume of 10 mls venous blood sample was obtained by venipuncture from each participant at booking and collected in a labelled plain universal specimen bottle. At the Central research laboratory of the NARHY, each clotted sample was centrifuged at 3000 rpm for 5 min. The sera collected was tested for HBsAg, and anti HCV using multiplex latex rapid agglutination slide test kits manufactured by Grand Medical Diagnostic Limited, USA. Reactive samples for HBsAg and anti- HCV were further confirmed using the enzyme linked immunosorbent assay (Bio Rad, France). Following Nigerian National HIV counselling and testing guidelines, the HIV status of the women who screened positive are further confirmed at the Human Virology Laboratory (HVL) of NARHY with Western blot.

2.3.2.2 Variables of Interest

Outcome of interest were Hemoglobin levels (Hb), packed cell volume (PCV), White blood cell count (WBC), Platelets, Blood group, HB genotype, Rhesus factor, Hepatitis B surface antigen, Hepatitis C antibody and HIV.

2.4 Data Collection and Analysis

All data sets were collected using a well-designed purpose driven proforma. The patients' demographics such as age, parity, hematological indices and BBVs were retrieved from patients' case notes. The data set was stored and later analyzed using the IBM Statistical Package for Social Sciences (SPSS Statistics) for Windows, version 29.0 Armonk, NY: IBM Corp. hematological and demographic parameters were expressed in mean, frequency and percentages. Categorical variables were compared using chi-square test with statistical significance at $p < 0.05$. The results were presented in tables, pie and bar charts using percentages and mean ± standard deviation. Associations between qualitative variables such as proportions were determined using Chi-square while continuous variables such as age, WBC, PCV, HB were summarized as means and standard deviations according to trimester.

2.5 Primary Outcome Measures

The primary outcome measures were the proportion of women that tested positive to either of the BBVs, trimester specific mean values of platelets, WBC, Hb, PCV, Rhesus status and HB genotype.

3 RESULTS

Five hundred and fifteen case notes were retrieved during the study period. Four hundred and ninety-nine had complete records and were included in the final data analysis (with a retrieval rate of 96.9%). As Figure 1 shows, the mean age of the participants was 36±2.3 (age range of 18-45). The age range of 30-34 accounted for a greater percentage (38.3%) of our participants. Figure 2 shows the parity distribution of the participants.

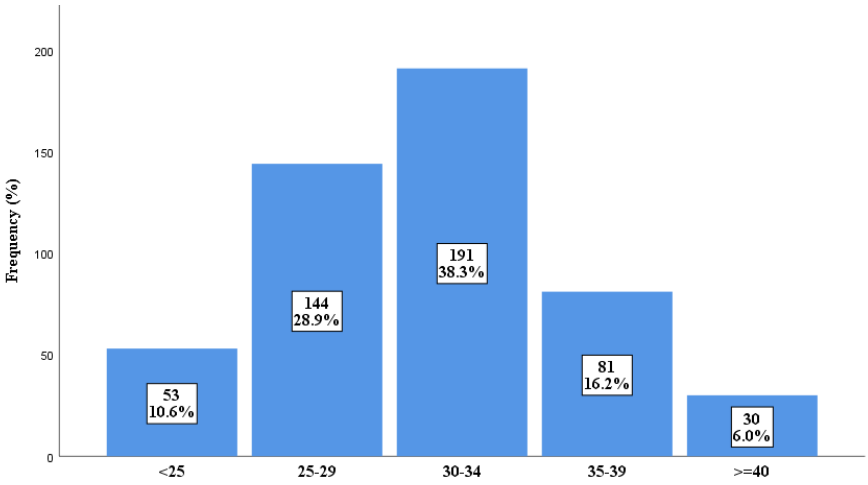

Figure 1. Age group of participants.

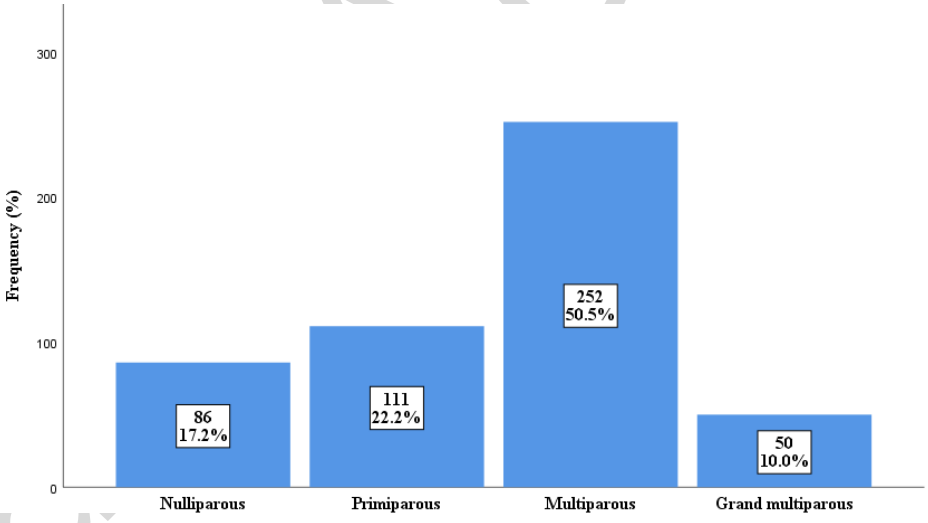

Figure 2. Parity distribution of the participants.

Figure 3 shows the distribution of Blood Hb genotypes among participants: the majority, 450 individuals (90.2%), had genotype AA, while 17 individuals (3.4%) had genotype SS, and 7 individuals (1.4%) had genotype AC.

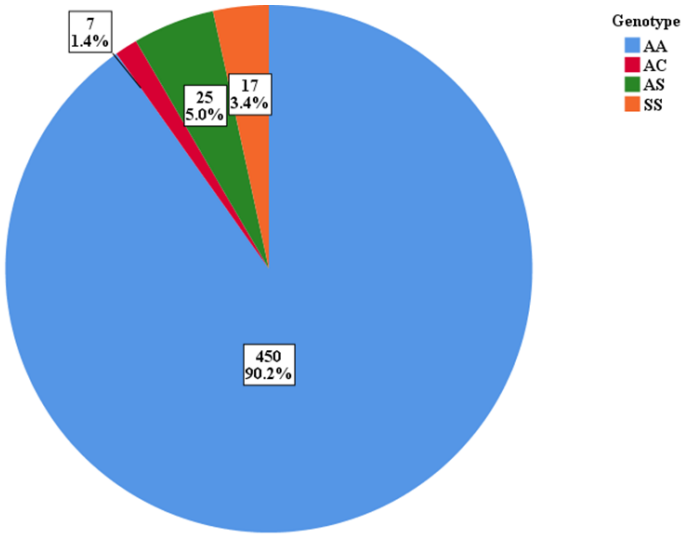

Figure 3. HB Genotype of our participants.

Table 1 highlights the distribution of blood groups among the participants. Blood group O was the most prevalent, observed in 248 individuals (49.7%), followed by blood group A, found in 142 individuals (28.5%). The least common blood group was AB, with a total of 48 occurrences (9.6%). Among the participants, 81 individuals (16%) lacked the Rhesus antigen. Notably, the absence of the Rhesus antigen was most frequently associated with blood group O, accounting for 44 individuals (54.32%), while the least common association was with blood group B, observed in 7 individuals (8.6%).

TABLE 1. ASSOCIATION BETWEEN RHESUS FACTORS AND BLOOD GROUP

	n(%)	**Rhesus positive (n=418)**	**Rhesus negative (n=81)**	**p-value**
Blood group				
O	245(49.1)	201(48.10)	44(54.32)	
A	142(28.5)	121(28.9)	21(25.9)	0.513
B	64(12.8)	57(13.6)	7(8.6)	
AB	48(9.6)	39(9.3)	9(11.1)	

The mean values of hematological parameters across the trimesters were as follows: First trimester – Hemoglobin (Hb) 10.70±1.2, hematocrit (Hct) 33.49±3.4, white blood cell (WBC) count 7.60±1.9, and platelet count 246.70±55.5. Second trimester – Hb 10.40±1.1, Hct 31.73±3.1, WBC 8.70±2.4, and platelet count 245.31±60.0. Third trimester – Hb 10.32±1.1, Hct 31.21±4.1, WBC 8.16±2.2, and platelet count 241.59±57.8 (Table 2).

TABLE 2. MEAN COMPARISON OF HEMATOLOGICAL INDICES ACCORDING TO TRIMESTER

	First trimester (Mean± SD)	**Second trimester (Mean ±SD)**	**Third trimester (Mean ±SD)**	**F-value**	**p-value**
Hb	10.70±1.2	10.40±1.1	10.32±1.1	3.221	0.04*
HCT	33.49±3.4	31.73±3.1	31.21±4.1	12.840	<0.001*
Platelet count	246.70±55.5	245.31±60.0	241.59±57.8	4.845	0.008*
WBC	7.60±1.9	8.70±2.4	8.16±2.2	11.937	<0.001*

Note. F-value= Analysis of variance

TABLE 3. POST HOC ANALYSIS

	First vs second	**First vs third**	**Second vs third**
Hb	0.014	0.087	0.701
HCT	<0.001*	<0.001*	0.459

Platelet count	0.814	0.005*	0.005*
WBC	<0.001*	0.037*	0.034*

Table 4 presents the findings on bloodborne viruses among the participants. A total of 30 women (6%) tested positive for HIV, while 5.4% and 1.4% tested positive for HBV and HCV, respectively. Additionally, three women (0.6%) in the cohort tested positive for co-infection with HBV, HCV, and HIV. Seven women (1.4%) were found to have HBV-HIV co-infection, and one woman (0.2%) tested positive for HCV-HIV co-infection.

TABLE 4. PREVALENCE OF BLOOD BORNE VIRUSES

Variable	Frequency (n=499)	Percentage
HIV		
Positive	30	6.0
Negative	469	94.0
HBV		
Positive	27	5.4
Negative	472	94.6
HCV		
Positive	7	1.4
Negative	492	98.6
HBV and HCV co-infection	3	0.6
HIV and HBV co-infection	7	1.4
HIV and HCV co-infection	1	0.2
HIV, HCV, HBV co-infection	3	0.6

Note. HBV- hepatitis B- virus; HCV- Hepatitis C virus; HIV- Human Immunodeficiency virus

Table 5 presents the multivariable analysis of parity, maternal age, HIV status, blood group, and Hb genotype. The analysis indicates that maternal HIV status, high parity, and abnormal Hb genotype (Hb SS) are significant predictors of maternal anemia.

TABLE 5- ASSOCIATION BETWEEN ANEMIA AND SELECTED CHARACTERISTICS

	Anaemia (n=326)	Normal (n=173)	$\chi 2$	p-value
Age group				
<25	32(60.4)	21(39.6)	2.129	0.712
25-29	90(62.5)	54(37.5)		
30-34	127(66.5)	64(33.5)		
35-39	57(70.4)	24(29.6)		
≥40	20(66.7)	10(33.3)		
Parity				
0	55(64.0)	31(36.0)	0.201	0.04
1	74(66.7)	37(33.3)		
2-4	165(65.5)	87(34.5)		
>4	32(64.0)	18(36.0)		
HIV				
Positive	21(70.0)	9(30.0)	0.307	0.03
Negative	305(65.0)	164(35.0)		
HBV				
Positive	14(51.9)	13(48.1)	2.290	0.130
Negative	312(66.1)	160(33.9)		
HCV				
Positive	5(71.4)	2(28.6)	0.117	0.733
Negative	321(65.2)	171(34.8)		
Blood group				
A	172(70.2)	73(29.8)	6.347	0.096
B	82(57.7)	60(42.3)		
AB	42(65.6)	22(34.4)		
O	30(62.5)	18(37.5)		
Rhesus				
Positive	272(65.1)	146(34.9)	0.076	0.783
Negative	54(66.7)	27(33.3)		
Genotype				

AA	293(65.1)	157(34.9)	5.650	0.01
AC	2(28.6)	5(71.4)		
AS	19(76.0)	6(24.0)		
SS	12(70.6)	5(29.4)		

4 DISCUSSION

Our study was comprised of participants aged mostly 30-34 years with fewer participants <25 and >40 years, which mirrors findings from literature that suggest poor social supports to attend formal healthcare facilities [15-17]. Our sample also consisted of mostly multiparous participants and less grand multiparous or nulliparous participants. This finding could be a result of poor awareness among nulliparous mothers and a false sense of security from previous pregnancies among grand multiparas. Literature has reported that other explanatory factors include lack of access to healthcare, poverty, limited knowledge about the increased risks associated with nulli and high parity, complacency due to previous normal deliveries, and cultural beliefs that may downplay the importance of prenatal care [18-20]. An alternative explanation could be a confirmation that modern family planning practices are being better adopted in Nigeria [21]. Among our participants, 450 (90.2%) had Blood Hb genotype AA- much more than reported in other literature [17], (3.4%) were SS and 7(1.4%) were AC- similar to prior studies [22]. Group O was found to be the most abundant blood group, 248 (49.7%). This was followed by blood group A, 142 (28.5) %. The least abundant blood group was AB with a total occurrence of 48 (9.6%). All of which concur with multiple reports among the different ethnic groups in Nigeria [23]. There are abundances of autosomal recessive haemoglobinopathies in Sub-Saharan Africa. Sickle cell disease is a public health importance globally because of myriads of crises that is associated with it and other pregnancy related feto-maternal complications. It is a common knowledge that a sizeable proportion of people living with Sickle cell disease reside in Sub-Saharan Africa with estimate over 50 million in Nigeria alone. Therefore, screening for this abnormal hemoglobin in pregnancy is of paramount importance [23].

Our study showed anemia at every trimester with mean values as follow -- First trimester: Hemoglobin (Hb) 10.70±1.2, hematocrit (hct) 33.49±3.4; Second trimester: Hb 10.40±1.1, hct 31.73±3.1; and the Third trimester: Hb 10.32±1.1, hct 31.21±4.1. Our study had an anemia prevalence of 65% which is similar to reported prior studies [24].In Nigeria, anemia in pregnancy results from multiple causes, including nutritional deficiency; malaria and hookworm infestation; chronic infections, such as HIV; taboos against certain diet, chronic medical conditions, sociocultural practice and hemoglobinopathies [25-27]. Additional obstetrics factors such as teenage pregnancy and short birth intervals commonly seen in Nigeria have been implicated 28. It is also important to note that anemia in pregnancy not only affect the health of the mother and the fetus but can lead to several adverse outcome including postpartum hemorrhage (PPH). Some authors have hypothesized several mechanisms through which anemia during pregnancy could lead to PPH. These mechanisms include but are not limited by increasing blood flow from bleeding vessels due to the physiological changes associated with pregnancy such as increased heart rate and cardiac output and by decreasing blood viscosity [29, 30].

As regards Bloodborne viruses, our study showed that 30 women (6%) tested positive for HIV, 5.4% and 1.4% tested positive for HBV and HCV respectively. Three of the 499 (0.6%) women in the cohort tested positive for HBV, HCV and HIV co-infection; seven (1.4) women had HBV-HIV co-infection; one (0.2%) woman had HCV-HIV co-infection. In a similar study from this sample, we emphasized the public health importance of HBV, HCV and HIV screening in our antenatal clinics and set an epidemiological baseline for future studies 6Screening of these viruses in pregnant mothers helps in reducing vertical (mother-to-child) and horizontal transmission. It is generally incorporated as part of the Worldwide efforts to decrease the burden of these pathogens in obstetric population [31]. As regards HBV, this is even more important as mother-to-child transmission accounts for more than fifty percent of global HBV burden. Additionally, it has also been established that the chronicity of HBV infection is indirectly related to the gestational age at the time of infection [6,31]. However, this vertical transmission can be reduced significantly by immunization (active and passive).

We further conducted a multivariable analysis of parity, maternal age, HIV status, blood group and Hb genotype which shows that, maternal HIV status, high parity, and abnormal Hb genotype (Hb SS) are predictors of maternal anemia. This fits in with the earlier listed predictors of anemia and further confirms the impact these conditions have on the prevalence of anemia in our sample and in Nigeria. The findings of the association between anemia in pregnancy and HIV in this study also agrees with the work of Methazia and colleagues who also suggested the multifactorial effects on the relationship between anemia in pregnancy ang advanced HIV. They opined that in HIV infected pregnant mother, that the pathophysiology of anemia could be as a result of direct effect of the virus on the bone marrow, effects of the antiviral medications, recurrent and or opportunistic infections from various pathogens as result of immunosuppression, or from immune dysregulation [32]. They further stated that in these women, that there is also subtle increased risk of both lower and upper gastrointestinal hemorrhage, reduced erythropoietin production because of HIV associated nephropathy, and possible increase in bone marrow infections which synergistically could also worsen the anemia in these women [32,33]. On the other hand, other authors also agree that increasing parity is directly proportional to the increased incidence of anemia in pregnancy. This has been argued to be possibly to decrease iron stores especially in

developing countries with predominant of women with short inter-pregnancy intervals. It has also been hypothesized that high parity is also associated with increased risk of hemorrhage, which will also invariably predispose these women to having anemia during pregnancy or immediate postpartum period [34,35].

We were limited by having only data for pregnant women without matched controls as this was a retrospective study, however this study tries to establish the distribution of hematological parameters among pregnant women in our facility.

Also, with reference to maternal age and anemia. This study also agrees with similar studies which suggested that there is an increasing risk of anemia with extremes of maternal age. Kwak and co-workers hypothesized that there an increasing risk of anemia with advancing maternal age, they argued this risk tend to worsen after the age of 40 years. In middle- and low-income countries, with increasing poverty and poor access to specialist led prenatal care, some of the factors that have been suggested to aggravate anemia also include Low family income, lack or low education status, teenage/unplanned pregnancy, advanced maternal age, intestinal parasites and low pre-pregnancy body mass index [36].

4. Conclusion and Recommendation

In conclusion, the findings from this cohort study provide further evidence supporting the development of standardized hematological reference values for pregnant women in Nigeria. Ensuring adequate nutritional supplementation and implementing routine screening for bloodborne viruses could significantly improve pregnancy outcomes among Nigerian women. To build on these findings, we recommend conducting a multicenter study involving both pregnant and non-pregnant women, matched for age and parity, to establish a comprehensive nomogram for hematological indices specific to our population. Such a study would also aid in defining gestational age- or trimester-specific hemoglobin levels, thereby enhancing maternal healthcare strategies.

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Ethical Statement

Ethical approval was obtained from the Health Research Ethics Committee (HREC) of 68 NARHY (68NARHY/EC/218). Ethical principles according to Helsinki's declaration were observed throughout the study duration. Written informed consent was not required for this study as it was retrospective in design and authors used data already on the patients' case files.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Author Contribution Statement

All authors were involved in the conceptualization and design of the study, data collection/ acquisition, statistical analysis and interpretation of the data. All authors were involved in writing and revising the manuscript for intellectual content. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

Data Availability Statement

Not applicable.

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