

# Consequences of Haemolytic Disease of the Fetus and Newborn (HDFN) and the Clinical Significance of Antibody Screening in Prenatal Diagnosis: A Study of Multigravida and Primigravida Women in Port Harcourt, Niger Delta

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## Abstract

**Background:** Haemolytic disease of the fetus and new-born involves the destruction of fetal red cells (haemolysis) by maternal antibodies. Maternal sensitization occur mainly when paternal inherited antigens of the fetus that differ from the mother crosses the placental barrier due to foetal-maternal trans-placental bleeding like in traumatic cases during pregnancy and obstetric procedures. Also, cases that are not related to pregnancy like transfusion, environmental factor and contamination of surgical tools. The consequence of this haemolysis is hyperbilirubinaemia (jaundice). Complications include kernicterus, mental damage in the newborn or hydrops and death of the foetus. HDFN is a public health issue and a major obstetric problem that has a large impact on both foetal and maternal morbidity/mortality outcome.

**Aim/Objective:** The aim of the study is to evaluate the consequences of HDFN and the clinical significance of antibody screening in prenatal diagnosis among multigravida and primigravida women with/without a history of blood transfusion in order to make the findings available for timely prevention as well as management of HDFN using prenatal antibody screening as a routine diagnostic tool.

**Method:** Observational cross sectional study, which lasted for a period of six months only focused on those accessing care in the antenatal clinic of Braithwaite Memorial Specialist Hospital in Port Harcourt, Rivers State, Niger Delta Region of Nigeria who also met the inclusion criteria. Sample size was determined using Winpepi version 11.44 and selection of the subjects was by systematic random sampling. SPSS statistical package version 21 was used for Mean, standard deviations, Spearman correlation and odd ratio and risk ratio.

**Results:** All rhesus negative mothers, 66 (65.3%), 17 (16.8%), 16 (15.8%) and 2 (2%) were blood types O, A, B and AB respectively. Maternal mean age stood at  $30.26 \pm 4.14$  and mean gestational age  $26.18 \pm 8.19$  weeks. Indirect coombs results revealed that 31 (30.7%) positive and 70 (69.3%) negative. Mean haemoglobin of  $10.82 \pm 1.16$  g/dl; direct coombs test revealed 15 (45.5%) positive and 18 (54.5%) negative. Correlation analysis between indirect coombs test with ABO blood group types, direct coombs test, maternal exposure (blood transfusion, multiparity, abortion, invasive medical procedures) and gestational age revealed spearman rho values of 0.473,  $p=0.00$ ;  $-0.332$ ,  $p=0.02$ ;  $-0.765$ ,  $p=0.02$  and  $0.560$ ;  $p=0.000$  respectively. While gestational age in relation to ABO and direct coombs test showed  $0.236$ ;  $p=0.02$  and  $0.01$ ;  $p=0.92$  respectively. Odd ratio and risk ratio (Relative risk) estimates (OR= $9.56 \pm 2.317$ ; RR= $5.21 \pm 0.875$ ,  $p=0.03$ ).

**Conclusion:** Based on the foregoing empirical evidence from this present study, ABO distribution is similar to those reported by previous studies, sensitization is bound to occur because of incompatibility and exposure to other factors. ICT and DCT are tools for diagnostic screening alongside blood typing.

**Keywords:** Haemolytic disease of the newborn; Prenatal diagnosis; Port harcourt; Health consequences

## Introduction

Hemolytic disease of the foetus and newborn (HDFN) is caused by maternal all o-antibodies directed against antigens present in foetal red cells. Paternal inherited antigens of the ABO and Rh systems which differ to those from the mother are present on foetal red cells and when the maternal immune system comes in contact with these cells, an immune response reaction is expressed. This may be due to foetal-maternal trans-placental bleeding like in traumatic cases during pregnancy, obstetric procedures, labour, caesarean section or cases unrelated with pregnancy like transfusion, environmental factor and contamination of surgical tools. In which case, maternal antibodies (IgG) can cross the placenta to activate macrophages in the fetal spleen which cause foetal haemolysis resulting from the build-up of bilirubin (jaundice). Nonetheless, complications

include kernicterus, mental damage in the newborn or hydrops and death of the foetus. HDFN is a public health issue and a major obstetric problem that has a large impact on both foetal and maternal morbidity/mortality outcome. In Nigeria, lack of appropriate measure and inefficient prophylactics program contribute massively to the development of HDFN thus, promoting crucial public health problems. Also, in spite of the fact that the frequency of Rh negative distribution is low among blacks and that the HDFN due to ABO blood type is uncommon and when present it is less severe, HDFN is still problematic due to the morbidity and mortality of both mother and child as result of lack or under-utilization of the basic prenatal diagnostic screening tests; which could probably be linked to lack of funding as health care service within the region as at present is basically out of pocket expenditure and accessibility remains a critical challenge [1] especially among the poor in the rural communities of Niger Delta.

However, it is worrisome to state at this juncture that routine screening for incompatibility assay is presently not performed in the location of this present study. Thus, the majority of the populace possess the Rhesus D antigen and a few do not (Rh D negative), in this case it is possibly believed that foetus is likely to be Rh D positive especially in a place like this where the blood type of the father is neither performed nor considered; neglecting the fact that incompatibility results from the paternal phenotype could present another health challenge with huge public health implication. Also, there is no test that is of high predictive value for severe HDFN. It can however, be suggested that haemolysis from Rhesus and ABO HDFN could be more severe amongst Nigerian neonates whose mothers tend to have higher prevalence and titres of immune anti-A and anti-B antibodies as well as mothers lacking the Rh D antigen (Rh D negative) as reported in several studies [2-4]. Thus, there may be a need for urgent routine immune antibodies screening in pregnant blood group O women to monitor foetuses that may be at risk. Furthermore, the available routine screening for ABO incompatibility between mother and foetus in most cases is neither performed nor performed according to Han et al. [5]. Nonetheless, the prevalence of immune antibodies, the racial distribution and gene frequencies of the various blood groups (ABO and Rhesus) are valuable indicators in estimating children born by blood group O mother as well as Rh D negative mothers with a non-group O and Rh D positive fathers who are at risk of developing HDFN.

In practice, it is not possible to type all the Rh antigens, but because of the maternal and child morbidity/mortality increase immunogenic Rh D, c and their variants should be typed. The D antigens are classically identified by testing the RHCs with suitable characterized polyclonal anti-D made by other people with partial D phenotypes also, by testing the patient's anti-D against RBCs with known partial D antigens. However, routine typing of this red cell is performed using monoclonal antibodies (i.e., expressed epitopes) whereas in developed countries there is provision for differential typing. Because of the challenge of classifying and identifying partial D individuals the anti-D reagents are selected to deliberately type categorize partial D mothers as D negative there by, ensuring that such mothers would automatically receive prophylactic Rh immunoglobulin following pregnancy. The weak D phenotype is often mistyped as Rh negative in which most, if not all, weak D phenotypes carry altered or abnormal Rh protein. Thus, RHCs with some weak D antigens may not be agglutinated by all monoclonal anti-D thus; differential typing is imperative and when it is not present the available antibody test becomes an alternative. In developed countries, the most significant development has been made in disease prevention. Although there is no cure for HDFN, the introduction of antibody immunosuppression was developed to prevent Rh immunization however, timely diagnosis of the condition is required as well as right investigation to be able to handle its health crises [6,7] and its attending clinical and public health consequences; therefore, the need for routine prenatal screening is paramount.

In Niger Delta communities, there is an increasing trend of paucity of information on the above subject matter, hence this present study would attempt to evaluate the consequences of HDFN and the clinical significance of antibody screening in prenatal diagnosis among multigravida and primigravida women with or without a history of blood transfusion specifically, utilizing these diagnostic tools by assessing the individual blood groups (ABO and Rhesus), determination of sensitized mothers using indirect antihuman globulin test (indirect coombs) and estimation of the direct antihuman globulin test (direct coombs) to identify babies at risk of HDFN. It is strongly believed that data generated from this present study could be useful in risk stratification these would further help to straighten the already existing weak prevention strategy as well as improve management outcome of HDFN using prenatal antibody screening as a routine diagnostic tool mechanism approach.

## Background

There are several forms of HDFN; nevertheless, serological classification of HDFN varies based on the antigens of the blood group system and other predisposing factors however, for the purpose of this present study we considered ABO haemolytic disease of the newborn and Rhesus Haemolytic Disease of the Newborn (particularly Rhesus D).

The ABO blood group system has a decreasing frequency (O, A, B and AB) in terms of distribution as reported by several studies in Nigeria with type O as the most predominant [8-11]. ABO haemolytic disease of the newborn is one of the common haemolytic consequences of maternal-fetal blood group incompatibility limited predominantly to blood group A and blood group B (nongroup O) babies born to blood group O mothers. Blood group O individuals possess immune antibodies (anti A and anti B) which belong to gamma immunoglobulin class i.e. IgG. These immune antibodies IgG have the capacity to cross the placenta barrier unlike the other pentameric immunoglobulin present in non-group O individuals (IgM). Contrasting to the rhesus disease, haemolytic disease due to ABO is usually a dilemma of the neonate rather than the fetus. A high level of these maternal immune antibodies may not present with adverse effects in-utero as A and B antigens are present on cells of all other tissues and body fluid and not only on red cells. The presence of these antigens confers a form of defence through a mechanism of neutralization a situation where the antigen present on the fetal red cells inhibits/neutralize the transferred maternal immune antibodies. However, small amounts of the maternal antibodies coat the fetal red cells, resulting to sensitization of the fetal red cells [12]. The sensitized red cells are destroyed by macrophages in the fetal reticular endothelial system particularly the spleen resulting to hyperbilirubinaemia [13]. ABO HDFN in literature is described as a condition having a very low incidence in the population and characterized by a benign evolution because of a mild degree of haemolysis [14,15] anaemia not commonly seen but hyperbilirubinaemia (jaundice) as its major clinical implication. Severe haemolysis and anaemia requiring exchange blood transfusion have however been reported [16]. Studies have revealed that statistically, in every five pregnancies there is a likelihood of one incompatibility between mother and infant among Caucasians [17,18]. In the United Kingdom, the incidence of haemolytic disease of newborn due to ABO blood group incompatibility is about 2% of all births, nevertheless a severe hemolytic disease occurs in only 0.03% of births [19]. It has been estimated that 14.3% of deliveries will result in a blood group O mother giving birth to a child who is non group O. About 4.3% of deliveries are likely to suffer ABO HDFN with 2.7% prone to suffer from moderately severe haemolysis [20].

ABO haemolytic disease occurrence have been attributed to a racial factor as some race are susceptible than other. The incidence of ABO HDFN in Blacks [21] is said to be higher than in Caucasians [22-24]. This is due to the higher prevalence and IgG titres of immune anti-A and anti-B antibodies in the Black population [3,25]. A 1 in 5 chance of ABO incompatibility between fetal red cells and maternal serum exists, but the incidence of ABO HDFN elsewhere, is said to be uncommon occurring in 2% of all births [16,19]. Race has however, been shown to have an effect on the incidence and severity of ABO HDFN with a higher incidence and severity being observed among Blacks [21] and Latin Americans [17]. Thus, we can expect the incidence and severity of ABO HDFN to be higher in Nigeria. Other studies have found that the incidence of ABO HDFN is higher in Blacks than in Caucasians [19,22,24-26] and is double that of the figures obtained for the United Kingdom [19].

The pathophysiology of ABO HDFN report that haemolysis associated with ABO incompatibility exclusively occurs in blood type O mothers with fetuses who have type A or type B blood, though it has exceptionally been seen in blood type A mothers with blood type B infants with a high level of the immune antibody (anti-B IgG). In mothers with type A or type

B, naturally occurring antibodies (IgM) do not cross the placenta, whereas 1% of type-O mothers have a high titer of the antibodies of IgG directed against both A and B. IgG cross the placenta and cause destruction of fetal red cells (haemolysis in fetus). Uncommon is haemolysis due to anti-B as opposed to haemolysis due to anti-A. Nonetheless an occurrence, it can be severe and can lead to exchange transfusion. Because A and B antigens are widely expressed in various tissues alongside red cells, only a small portion of antibodies crossing the placenta are available to bind to fetal RBCs. Current analysis of IgG subclasses in ABO incompatible direct coombs positive neonates showed IgG2 was the predominant antibody which is poorly transferred across placenta and less efficient in causing haemolysis while IgG1 was noted in 22% of neonates and as a group had similar rate of haemolysis and severity of hyperbilirubinemia [27].

In addition, fetal erythrocytes seem to have few expression of A or B antigen on their surface unlike the adult thus, resulting in few reactive sites and hence, reduced incidence of significant haemolysis in affected neonates. This defines the rationale behind hyperbilirubinemia as a predominant manifestation of incompatibility (rather than anaemia) and peripheral blood film frequently reveals a large number of spherocytes and few erythroblasts, unlike the case of Rh incompatibility (erythroblastosis fetalis), in which blood film reveals a large number of nucleated RBCs and few spherocytes [28].

The Rhesus blood group system is highly polymorphic with several antigens though Rhesus antigens D, c, E, e commonly seen. Rh gene complex consists of three genetic loci each with two major alleles. They code for five major antigens C, c, E, e, and D. Fisher and Race put forward an inheritance pattern for Rhesus blood group system, it states that these antigens form three pairs of antigen, Dd, Cc and Ee. The presence of Rh (D) indicates Rh positivity while the absence of Rh(D) indicates Rh negativity. The D antigen is known with its high immunogenicity followed by the c-antigen [29]. Approximately 95 percent of the entire cases of Rh sensitization involve the antigen D(Rh0). According to Wagner and colleagues [30], they reported molecular basis of weak D thus, stated that Rhesus protein (RhD/ RhCE which are found on a homologous gene pair) was responsible in carrying the Rhesus antigen. The expression of these Rh proteins is mediated by the presence of Rhesus associated glycoprotein (RhAG) via the cell membrane [31,32]. The Rh blood group antigen D is the most important cause of a family of inherited antigens [33]. Most Caucasians who are RhD negative have a complete deletion of RHD gene where as only 18% of African blacks and 54% of African Americans who are RhD negative have complete deletion of the gene; the rest have above non functional variants of the RHD gene [37-39]. Some studies have revealed that these D variants are seen among blacks more [10,34] compared to the Caucasians [32,35]. The frequency of weak D in the place of this current study (Port Harcourt) is about 0.95% amongst Rh negative female adults in Port Harcourt [10]. Generally, the frequency distribution of Rh negativity is higher in whites (15%) than in blacks (5%) and Hispanics (8%) and is rare in Eskimos, Native Americans, Japanese, and Asians, especially in Chinese individuals. The paternal heterozygosity determines the likelihood of an Rh-positive child being born to an Rh-negative mother [39].

Pathophysiology of Rh HDFN began with the exposure of the Rh-negative mother to Rh-positive red cells occurs as a result of asymptomatic fetomaternal haemorrhage during pregnancy. Incidence and degree of such haemorrhage appears to increase with gestation. Fetomaternal haemorrhage has been documented in 7%, 16%, and 29% of mothers during their first, second and third trimesters, respectively. Because the transplacental haemorrhage is less than 0.1 mL in most pregnancies, most women are sensitized as a result of small, undetectable fetomaternal haemorrhage. Subsequent to the initial exposure to a foreign antigen, maternal B cells recognize the red cell antigen stimulates the production

of IgM antibodies at this initial stage of primary immune response but the IgM do not cross the placenta thus, later produces IgG antibodies that pass through the placental barrier. Predominantly, IgG1 subclass is present in one third of individuals, whereas, a combination of IgG1 and IgG3 subclasses are seen in the remaining individuals. IgG3 is more capable in binding to cells of the reticulo-endothelial system, the consequence of this is haemolysis because of its longer hinge region and it is called the primary response. It is dose dependent, secondary immune response can be induced with as little as 0.03 mL of Rh-positive RBCs [38,39].

The risk of Rh immunization after the delivery of the first child to an Rh-negative mother is 16% if the Rh-positive fetus is ABO compatible with its mother, 2% if the fetus is ABO incompatible, and 2-5% after an abortion. Following sensitization, maternal anti-D antibodies cross the placenta into fetal circulation and attach to Rh antigen on fetal RBCs. Reticulocytosis, haemolysis, severe anaemia, tissue hypoxia increased umbilical arterial and venous lactate, pleural effusions et-care consequences of HDFN [40]. Furthermore, the process of increased production of erythrocytes causes the haemopoietic organs to vary in size and functionality like hepato-splenomegaly. Erythroblastosis fetalis ensues because excess erythropoiesis causes immature red cells (erythroblasts) to leak into the circulation. Moreover, fetal tissues become swollen (edematous) causing hydrops fetalis, a complication of severe HDFN. This condition is usually fatal, either in utero or soon after birth [36]. Hyperbilirubinemia becomes obvious only in the delivered newborn because the placenta effectively metabolizes bilirubin in-utero [28].

## Study design and Methods

The study recruited multigravida and primigravida women with/without a history of blood transfusion accessing care in the antenatal clinic of Braithwaite Memorial Specialist Hospital in Port Harcourt, Rivers State, Niger Delta Region of Nigeria. Braithwaite Memorial Specialist Hospital is one of the major tertiary health institutions in the Oil-rich Niger Delta Region of Nigeria. This is an apex health institution in Port-Harcourt, which is the headquarters of the oil-industry and the second most industrialized city in Nigeria. As such, it caters for a large cosmopolitan population of indigenous and expatriate oil-sector employees, who are largely in the upper socio-economic strata and therefore require advanced modern medical care as obtainable elsewhere in developed countries. The hospital has over five hundred (500) bed capacities in an ultra-modern site with a three tier managerial structure comprising of the hospital management board, hospital management committee and the departments.

## Study location

Port Harcourt is the capital of Rivers state and head quarter of all Niger Delta States in Nigeria which are also known as oil producing states in Nigeria. It lies along the Bonny River and is located in the Niger Delta region. According to the 2006 Nigerian population census Port Harcourt has a population of 1,382,592. The city is a major industrial center as it has a large number of multi-national firms as well as other industrial activities, particularly business related to the petroleum industry. Port Harcourt features a tropical monsoon climate with long and heavy rains and very short dry season across the year. All subjects recruited for this study were willing to participate and gave their consents. All subjects were pregnant Rh negative with/without a history of transfusion. Also, only those attending antenatal clinic of Braithwaite Memorial Specialist Hospital in Port Harcourt, Rivers State, Niger Delta Region of Nigeria. However, non-pregnant women and those pregnant, who did not give their consents, Smokers and patients with severe malaria, were excluded from the study.

Study design employed in this study was an observational cross sectional approach, which lasted for a period of six months (From March through August 2015). Sample size determined using Winpepi version



11.44 sample size calculator. The Study participants were selected by systematic random sampling with the use of the routine clinic attendance as a sampling frame. The systematic random sampling used here for the study involved equal selection probabilities and the start point was chosen at random using the table of random numbers. Data collection involved the use of structured questionnaires on socio-demographic data, family history, pregnancy, etc. Both primary and secondary data were collected. Laboratory diagnoses of coombs tests, blood grouping and haemoglobin estimation were performed according to Dacie et al.[41] ICT and DCT were done according to Cheesbrough et al.[42] using the maternal and neonatal blood respectively after the pre-analytic procedure of sample collection, 4ml of blood were collected from the subjects via vene-puncture from the medial cubital vein using a vacutainer and needle for K2 Ethylene Diamine Tetra Acetic Acid (EDTA) and plain containers (BD USA). The SPSS statistical package version 21 was used for the statistical analysis. Statistical analysis involved comparison between groups. Mean, standard deviations were obtained for descriptive statistics whereas; inferences were made using Spearman correlation to explore and analyze categorical data as well as to show an association. In addition, odd ratio and risk ratio were also estimated. Two tailed test was used to avoid the consequences of missing an effect in the untested direction and circumvent the conclusion that they are insignificant also; there was no specific hypothesis about the direction of the association and the asymmetrical distribution of the data. Informed written consent was obtained from the subjects after a detailed information and procedural protocol of the research were duly explained to them; they consented by endorsing on the consent form. The act of unwillingness of some patients to participate, improper documentation of some vital information, affected the study to some extent.

## Results

The present study included a total of one hundred and one pregnant women assessing care at the antenatal clinic of Braithwaite Memorial Specialist Hospital in Port Harcourt Metropolis, Rivers state of Nigeria. All study participants were rhesus negative mothers, 66 (65.3%) were blood type O individuals, 17 (16.8%), 16 (15.8%) and 2 (2%) were blood types A, B and AB respectively. Maternal age groups for <25 years, 25-29 years, 30-34 years and 35-40 years includes 9 (9.8%), 31 (30.7%), 46 (45.5%) and 15 (14.9%) respectively. The participants had a mean age of 30.26 ± 4.14. In addition, gestational age showed that 50 (49.5%) were <28 weeks (Extremely preterm), 12 (11.9%) were between 28 to <32 weeks (Very preterm) and 39 (38.6%) were between 32 to <37 weeks (Moderate to late preterm). Although a mean gestational age of 26.18 ± 8.19 weeks was reported.

Investigation of the maternal sample with indirect antihuman globulin test (Indirect coombs) revealed that about 31 (30.7%) were reactive (positive) and 70 (69.3%) were non-reactive (negative). Further investigation of anaemia status using haemoglobin level estimation reported 1 (1%), 13 (12.9%), 34 (33.7%) and 53 (52.5%) for Lower than 7.0 g/dl (severe anaemia), 7.0-9.9 g/dl (Mild anaemia), 10.0-10.9 g/dl (moderate anaemia) and 11.0 g/dl or higher (Non anaemic/Normal) respectively. A mean of 10.82 ± 1.16 haemoglobin levels was recorded in this study.

In addition, a total of thirty-three newborn infants were examined using the direct antihuman globulin test (direct coombs), 15 (45.5%) were reactive (positive) and 18 (54.5%) were non reactive (negative). See table 1 for detail.

Spearman rank order correlation was used to measure for association between the variables of ABO blood group, indirect coombs test (ICT), direct coombs test (DCT) and gestational age at significant levels of 0.01 and 0.05 for a two tailed test. The result showed a spearman rho value of 0.473 which is significant (p=0.00) at 0.01 for ABO blood type and

indirect coombs test (ICT). At a significant level of 0.05, ABO blood group and gestational age (weeks) reported a correlation value of 0.236 (p=0.02). However, no evidence of correlation existed between ABO blood group and direct coombs test (DCT). correlation analysis between indirect coombs test with direct coombs test, maternal exposure (blood transfusion, multiparity, abortion, invasive medical procedures etc) and gestational age in weeks revealed spearman rho values of -0.332, p=0.02; -0.765, p=0.02 and 0.560; p=0.000 respectively. While gestational age in relation to ABO and direct coombs test showed 0.236; p=0.02 and 0.01; p=0.92 respectively (Table 2).

Odd ratio and risk ratio (Relative risk) estimates revealed an evidence of statistical significant for mothers exposed to transfusion, abortions, multiparity, medical procedures (OR=9.56 ± 2.317; RR=5.21 ± 0.875, p=0.03).

	Frequency (%)	Mean	SD	Remark
<b>ABO Blood Types (N=101)</b>				
A	17 (16.8%)			
AB	2 (2%)			
B	16 (15.8%)			
O	66 (65.3%)			
<b>Maternal Age (N=101)</b>				
<25 Years	9 (8.9%)			
25-29 Years	31 (30.7%)	30.26	4.14	
30-34 Years	46 (45.5%)			
35-40 Years	15 (14.9%)			
<b>Indirect Coombs Test (N=101)</b>				
Non Reactive	70 (69.3%)			
Reactive	31 (30.7%)			
<b>Direct Coombs Test of Infants (N=33)</b>				
Non Reactive	18 (54.5%)			
Reactive	15 (45.5%)			
<b>Hb Level (Classification of Anaemia) N=101</b>				
Lower than 7.0 g/dl (severe anaemia)	1 (1%)			
7.0-9.9 g/dl (Mild anaemia)	13 (12.9%)			
10.0-10.9 g/dl (moderate anaemia)	34 (33.7%)		10.82	1.16
11.0 g/dl or higher (Nonanaemic/Normal)	53 (52.5%)			
<b>Gestational Age (Weeks) (N=101)</b>				
<28 weeks (Extremely preterm)	50 (49.5%)			
28 to <32 weeks (Very preterm)	12 (11.9%)		26.18	8.19
32 to <37 weeks (Moderate to late preterm)	39 (38.6%)			

**Table 1:** Frequency, Mean and standard Deviation of ABO Types, Maternal Age Group, ICT, DCT and Gestational Age

Variables Correlation	Coefficient/P-value	ABO	ICT	DCT	Gestational Age (Weeks)
ABO	Correlation Coefficient	1.000	0.473**	-0.137	0.236*
	P-value	.	0.000	0.334	0.017
ICT	Correlation Coefficient	0.473**	1.000	-0.332*	0.560**
	P-value	0.000	.	0.016	0.000
Gestational Age (Weeks)	Correlation Coefficient	0.236*	0.560**	0.014	1.000
	P-value	0.017	0.000	0.924	.

**Table 2:** Spearman Correlation for ABO, ICT, DCT and Gestational Age

\*\* Correlation significant at 0.01 (Two tailed)

\* Correlation significant at 0.05 (Two tailed)

## Discussion

The present study included a total of one hundred and one pregnant women accessing care at the antenatal clinic of Braithwaite Memorial Specialist Hospital in Port Harcourt Metropolis, Rivers state of Nigeria.

The study revealed ABO blood type distribution in a decreasing frequency of blood types O, A, B and AB whereas, the rhesus blood type in this study were all rhesus negative mothers. ABO blood group O had been reported to have the highest frequency distribution in Nigeria as revealed by this study; this is in agreement with previous studies [8-11]. Maternal age as obtained in this study was within the reproductive age bracket so, the issue of age as a cofounder was ruled out. In addition, gestational age showed almost fifty percent of the study population had extremely preterm newborns, about twelve percent had very preterm babies and about thirty-nine percent had moderate to late preterm babies however, no delivery was full term as reported in this study.

Maternal sample tested using indirect antihuman globulin test (Indirect coombs), result from the study revealed that over thirty percent were reactive (positive), this is a strong indication of immune antibody (IgG) present which can possibly cross the placenta and sensitized the foetal red cells and thus promoting a typical case of HDFN. This percentage of positive ICT and DCT reported is evident and this study agrees with the fact that HDFN is found within this population of study because the population of the study were only those who had access to the health care facility excluding the percentage of those who cannot access care health. Thus, inspite of the fact that the frequency of Rh negative distribution is low among blacks and that the HDFN due to ABO blood type is uncommon and when present it is less severe. HDFN is still problematic due to the morbidity and mortality of both mother and child as result of lack or under utilization of the basic prenatal diagnostic screening test which could be as result of lack of funding as health within the region of this present study is basically out of pocket expenditure and accessibility to functional health facility remains a huge challenge.

In addition, a total of thirty-three newborn infants examined using the direct antihuman globulin test (direct coombs) revealed that about forty-six percent of the new-borns were reactive (positive) this is usually seen in HDFN. The DCT result from this study is in contrast with Johnson et al. [8] which reported that only five percent test subjects were positive to Direct coombs test (DCT). On the other hand, the non-reactive DCT reported in this study could be lack of prior sensitization as this appeared in primigravida women, those without history of invasive medical procedures and those with no history of therapeutic transfusion whereas, the reactive subjects have maternal history of multiparity, transfusion or have had an invasive medical procedure at one point in their life. Similarly, Heddle and colleagues reported three cases of non-reactive DCT in Rh haemolytic anaemia in newborn [43]. Also, Kaplan et al. [27] reported positive coombs test in neonates in their analysis of IgG subclasses in ABO incompatibility; they further revealed that IgG2 was the predominant immune antibody which is poorly transferred across placenta and less efficient in causing haemolysis while IgG1 was noted in 22% of neonates. Nonetheless, this study did not include antibody specificity as to determine the classes (IgG, IgM) and subclasses (IgG1, IgG2-IgG2a or IgGb) of antibodies rather a mere general antibody screening was used as an indicator. However, this opens another road map of research investigation that would anchor on the specificity of the above stated antibodies.

Causality was not hypothetically determined however, measures of association were used to establish whether a relationship exists between some variables. The study revealed an evidence of statistical significance between ABO blood type, Indirect coombs test (ICT), Direct coombs test

(DCT) and gestational age. This means that the maternal blood type is associated with isoimmunisation as well as gestational age; this is similar to the ABO incompatibility reported by Kaplan et al. [27]. This is in line with the previous study done in South-west Nigeria (Lagos) which estimated that 14.3% of deliveries will result in a blood group O mother giving birth to a child who is non-group O and 4.3% of deliveries are likely to suffer ABO HDN with 2.7% probably prone to suffer from moderately severe to severe haemolysis [20]. On the other hand, there was no evidence of correlation between maternal ABO blood type and Direct coombs test (DCT) of the foetal blood sample.

A correlation analysis between indirect coombs test with direct coombs test, maternal exposure (blood transfusion, multiparity, abortion, invasive medical procedures) and gestational age in weeks demonstrated an indication of statistical significance which means an inverse relationship exist between ICT and maternal exposure as well as gestational age. However, gestational age in relation to direct coombs test was not evident of an association in this study. Also, the risk estimates of odd ratio and relative risk (risk ratio) revealed an evidence of statistical significant that the mothers exposed to transfusion, abortions, multiparity, medical procedures have over five times chances of isoimmunisation (sensitization) than those not exposed.

Based on the foregoing empirical evidence from this present study, ABO distribution is similar to those reported by previous studies, sensitization is bound to occur as a result of incompatibility and exposure to other factors mentioned earlier thus, ICT and DCT are tools for diagnostic screening alongside blood typing thus, this study showed an indication of statistical significance thus, the study which hypothesized no significant difference as well as no association reject the null hypothesis.

## Conclusion

The ABO and Rh blood group systems account for the majority of the cases of sensitization during pregnancy although almost all the other known blood factors have been reported to cause this condition. In the Rh system, D (Rh0) is not only the most common but also, the most potent in terms of antigenicity. Thus, the more severely affected cases of erythroblastosis are usually those of an Rh negative mother who has developed anti-D. In the ABO system, most cases occur in infants of group A or B with group O mothers. HDFN resulting from immune anti-A is usually more severe than that due to immune anti-B. In addition, there are significant differences in the response to ABO and Rh sensitization and the subsequent development of haemolysis, hyperbilirubinaemia and erythroblastosis as consequences of HDFN. Rhesus sensitization does not usually produce clinically recognizable HDFN until after two or more pregnancies. It is usually of a more severe variety and the severity of the disease progressively increases in the subsequent affected infants. HDFN due to ABO sensitization on the other hand may occur in the first-born infant is usually less severe, subsequent pregnancies are not necessarily affected and the direct antihuman globulin test is usually weakly positive or may even be negative.

The clinical significance of antibody screening in prenatal diagnosis among multigravida and primigravida women with or without a history of blood transfusion is paramount in order to provide accessible basis for prevention as well as management of HDFN using blood typing, ICT and DCT antibody screening as diagnostic tools routinely.

## Ethical Consideration

The ethical approval for this study was sought and approval gotten from the ethical committee of Medical laboratory Science Department of the Rivers State University of Science and Technology, Port Harcourt and the Braithwaite Memorial Specialist Hospital ethical committee.

## Conflict of Interest

No conflict of interest among authors.

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