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Research Article

Examining Blood Group Antigens as Potential Predictors of Preeclampsia Among Pregnant Women

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Abstract

Background: Preeclampsia is a significant cause of maternal and perinatal morbidity and mortality worldwide. While its etiology remains multifactorial, emerging evidence suggests a possible association between red cell antigens and susceptibility to preeclampsia. This study aimed to determine the distribution of ABO, Rh, Kell, Kidd, and Duffy blood group antigens among preeclamptic and non-preeclamptic pregnant women in South-eastern Nigeria, and to assess any potential association with preeclampsia.

Materials and Methods: This cross-sectional analytical study was conducted at the University of Nigeria Teaching Hospital (UNTH) and Enugu State University Teaching Hospital (ESUTH). The study population included pregnant women diagnosed with preeclampsia (n=78) and healthy pregnant controls (n=78) at ≥20 weeks gestation. Blood samples were collected, and red cell antigen typing was performed using standard serological methods. Statistical analyses, including chi-square tests and binary logistic regression, were conducted using SPSS version 21, with significance set at $p<0.05$.

Results: No statistically significant differences were observed in the distribution of Kell (p=0.32), Kidd (p=0.56), and Duffy (p=0.16) antigens between the two groups. Regression analysis indicated no significant predictive association between blood group antigens and preeclampsia ($p>0.05$).

Conclusion: The study found no significant association between ABO, Rh, Kell, Kidd, or Duffy blood group antigens and preeclampsia in the study population. These findings suggest that red cell antigens are unlikely to serve as independent predictors of preeclampsia among pregnant women in Enugu, Nigeria. Further large-scale and multi-center studies may provide additional insights into potential immunogenetic influences on preeclampsia.

Keywords: preeclampsia; red cell antigens; abo blood group; rh blood group

Introduction

Preeclampsia is a hypertensive disorder unique to pregnancy, characterized by the onset of elevated blood pressure and proteinuria after 20 weeks of gestation [1]. It remains a leading cause of maternal and perinatal morbidity and mortality worldwide, particularly in low- and middle-income countries like Nigeria. The aetiology of preeclampsia is multifactorial, involving genetic, immunological, and environmental factors. Recent studies have explored the potential association between maternal blood group antigens and the risk of developing preeclampsia, suggesting that certain blood groups may predispose individuals to this condition.

The ABO blood group system, first described by Karl Landsteiner in 1901, classifies human blood into four main types: A, B, AB, and O, based on the presence or absence of specific antigens on the surface of red blood cells [2]. These antigens are oligosaccharides expressed not only on erythrocytes but also on various epithelial and endothelial cells throughout the body. The distribution of ABO blood groups varies among

different populations and has been implicated in susceptibility to various diseases, including cardiovascular disorders and certain infections.

Several studies have investigated the relationship between ABO blood groups and the risk of preeclampsia. A systematic review and meta-analysis by Alpoim et al. [2] concluded that non-O blood groups, particularly AB, are associated with an increased risk of developing preeclampsia. Similarly, a study conducted in India by Ainani et al. [3] found that women with AB blood group had the highest risk, while those with O blood group had the lowest risk for preeclampsia. These findings suggest a potential link between ABO blood group antigens and the pathogenesis of preeclampsia, possibly through mechanisms involving coagulation and immune response.

In Nigeria, preeclampsia and eclampsia contribute significantly to maternal and perinatal morbidity and mortality. A systematic review and meta-analysis by Kokori et al. [4] reported a prevalence of preeclampsia/eclampsia among pregnant women in Nigeria, highlighting the substantial health burden posed by these conditions. Specifically, in Enugu, South-Eastern Nigeria, studies have documented the prevalence and outcomes of severe preeclampsia. Ugwu et al. [5] reported a prevalence of 3.3% for severe preeclampsia at the University of Nigeria Teaching Hospital, with significant maternal and perinatal complications observed.

Despite the recognized burden of preeclampsia in Nigeria, there is a paucity of research examining the association between ABO blood group antigens and the risk of preeclampsia among Nigerian women. Understanding this relationship could provide insights into the pathophysiology of preeclampsia and inform strategies for risk assessment and management in this population.

This study aims to investigate the association between ABO blood group antigens and the occurrence of preeclampsia among pregnant women attending teaching hospitals in Enugu, Nigeria. By conducting a cross-sectional analysis, we seek to determine whether specific blood groups are associated with an increased risk of preeclampsia in this population. The findings of this study could have implications for prenatal care, including the potential for blood group screening to identify women at higher risk for preeclampsia, thereby facilitating early interventions to improve maternal and fetal outcomes.

Materials And Methods

Study Design

The study was a cross-sectional analytical study involving pregnant women and 24-48 hours post-delivery women with preeclampsia (cases) and those without the disease (controls) receiving antenatal care at the University of Nigeria Teaching Hospital (UNTH) and Enugu State University Teaching Hospital (ESUTH), Enugu.

Study Area

The study was conducted in the Obstetrics and Gynaecology Departments of the UNTH Ituku-Ozala, and ESUTH Park lane, GRA, both in Enugu state. These are the two Teaching Hospitals in Enugu state, Nigeria. The participants' blood samples were analysed in the Haematology laboratory and the blood bank unit of UNTH, Enugu.

Study Population

Participants were pregnant women diagnosed of preeclampsia as defined by WHO25 (BP ≥ 140/90mmhg and proteinuria of ≥ 2+ in pregnant women of 20 weeks gestation and above as well as those 24-48 hours post-delivery) and those with normal pregnancy recruited at the antenatal clinics and wards (antenatal, postnatal and labour wards) of UNTH and ESUTH.

Inclusion Criteria

Women who were pregnant or 24-48 hours post-delivery with an established diagnosis of preeclampsia were recruited as the study group, while women who did not have preeclampsia or any other hypertensive and medical diseases, and who were above 20 weeks of gestation or 24-48 hours post-delivery were recruited as the control group.

Exclusion Criteria

Pregnant women with hypertensive disorders of pregnancy other than preeclampsia or other diagnosed morbidities and pregnant women below 20 weeks of gestation were excluded from the study. Previously transfused pregnant women were also excluded from the study.

Sampling Method

The cases and controls were recruited in a non-randomized fashion after confirming from the participants that they had not been earlier recruited for this study. The preeclamptic group was first recruited, and subsequently, the non-preeclamptic group was recruited. The non-preeclamptic group was matched with the preeclamptic group for the age range and parity group.

Sample Size Determination

The sample size was determined using the formula for comparison of two proportions:57

$n= 2(Z\alpha/2+ Z\beta)2\ P(1-P)/(P1-P2)2$, where:

n= minimum sample size

$Z\alpha/2$ = critical value of the normal distribution at $\alpha/2$

$Z\beta$ = critical value of the normal distribution at β

P= pooled prevalence (prevalence in case group, P1 + prevalence in control group, P2)

P1-P2 = difference in the proportion of events in two groups

To calculate the sample size (using the Z table), $Z\alpha/2 = 1.64$ at 90% confidence interval and $Z\beta = 0.84$ for a power of 80%. Taking P1 to be 28% (0.28) and P2 to be 12% (0.12) from the findings of Mital et al. [6] on the presence of AB blood group among preeclamptic (case) and non-preeclamptic (control) pregnant women in India, the sample size is calculated thus:

$n= 2(1.64 + 0.84)2\ 0.2(1-0.2)/(0.16)2$

$n= 12.3 \times 0.16/0.0256 = 76.88$

The calculated minimum sample size was approximately 77 for each group of the study.

Clinical and Demographic Data

Detailed clinical, pertinent personal history and other relevant information were collected by the researcher/assistant from clinical/ward records and from the patients or their caregivers using a well-structured questionnaire specifically designed for this study.

Biological Specimen Collection and Analyses

Five millilitres (5mls) of venous whole blood samples were collected from the study and control groups at recruitment. This was collected in EDTA-containing test tubes labelled with the subject's identification

number and placed in racks for storage in the refrigerator at 4 oC, for the serologic analysis of the red cell antigens (ABO, Rh, Kell, Kidd and Duffy) in batches at the Blood Bank/Blood Transfusion Unit of the Department of Haematology and Immunology, UNTH, Enugu.

Procedure for ABO blood grouping

The forward and reverse grouping methods were employed.

1. Seven clean glass tubes numbered 1-7 and appropriately labelled were placed in the test tube rack and the test sample and reagents were mixed as below:

Tube 1: 1 volume of anti-A antisera + 1 volume of 5% test cell solution (forward grouping)

Tube 2: 1 volume of anti-B antisera + 1 volume of 5% test cell solution (forward grouping)

Tube 3: 1 volume of anti-AB antisera + 1 volume of 5% test cell solution (forward grouping)

Tube 4: 1 volume of test plasma + 1 volume of 5% known A cell solution (reverse grouping)

Tube 5: 1 volume of test plasma + 1 volume of 5% known B cell solution (reverse grouping)

Tube 6: 1 volume of test plasma + 1 volume of 5% known O cell solution (reverse grouping)

Tube 7: 1 volume of test plasma + 1 volume of 5% test cell solution (own serum/auto-control)

2. Each tube was tapped gently at the base to mix the contents. These were left in the rack for 5 minutes at room temperature and then checked for macroscopic agglutination in the absence of which they were centrifuged at 1500rpm for 1 minute.

3. Each tube was tapped gently at the base and examined for macroscopic agglutination or haemolysis.

4. The contents of each tube were then examined for microscopic agglutination or haemolysis in the absence of macroscopic forms using glass slides and a light microscope.

Procedure for Rh Grouping

The forward grouping method was carried out.

1. Four clean glass slides numbered 1-4 and appropriately labelled were placed in the test tube rack and the test samples and reagents were mixed as follows:

Tubes 1-3: 1 volume of anti-D, anti-c and anti-E antisera each per test tube + 1 volume of 5% test cell solution in each test tube.

Tube 4: 1 volume of test plasma + 1 volume of 5% test cell solution (own serum/auto control).

2. The contents of each tube were mixed by gently tapping the base of each tube and then left in the rack for 5 minutes at room temperature and then checked for agglutination macroscopically.

3. In the absence of agglutination above, the test tubes were then centrifuged at 1500rpm for 1 minute and examined for macroscopic and microscopic agglutination or haemolysis following the same protocol as for ABO grouping above.

Procedure for Kell grouping

The forward grouping method was carried out.

1. Three clean glass tubes numbered 1-3 and appropriately labelled were placed in the test tube rack and the test samples and reagents were mixed as follows:

Tubes 1 & 2: 1 volume of anti-K and anti-k antisera each per test tube + 1 volume of 5% test cell solution in each test tube.

Tube 3: 1 volume of test plasma + 1 volume of 5% test cell solution (auto control)

2. The contents of each test tube were mixed by gentle taps at the base of each test tube and incubated in the rack at room temperature for 5 minutes and then checked for macroscopic agglutination.

3. In the absence of agglutination above, the solutions were centrifuged at 1500rpm for 1 minute and then examined for macroscopic and microscopic agglutination or haemolysis following the same protocol as for ABO grouping above.

Procedure for Kidd grouping

The forward grouping method was carried out.

1. Three clean glass tubes numbered 1-3 and appropriately labelled were placed in the test tube rack and the test samples and reagents were mixed as follows:

Tubes 1-2: 1 volume of anti-Jka and anti-Jkb antisera each per test tube + 1 volume of 5% test cell solution in each test tube (forward grouping)

Tube 3: 1 volume of test plasma + 1 volume of 5% test cell solution (auto control)

2. The contents of each test tube were mixed by gentle taps at the base of each test tube and incubated in the rack at room temperature for 5 minutes and then checked for macroscopic agglutination.

3. In the absence of agglutination above, the solutions were centrifuged at 1500g for 1 minute and then examined for macroscopic and microscopic agglutination or haemolysis following the same protocol as for ABO grouping above.

Procedure for Duffy blood grouping

The forward grouping method was carried out.

1. One drop of anti-Fya antisera was placed in a clean labelled glass tube and placed on the test tube rack.

2. One drop of 5% test red cell solution was added to the tube above and incubated at 370C for 30 minutes.

3. 0.3ml of the mixture was mixed with 3mls of saline and centrifuged at 1500rpm for 2-3minutes.

4. The supernatant was discarded and another 3mls of saline was added to the red cell sediment and centrifuged again as above and the supernatant was discarded.

5. The procedure above was repeated once more and one volume of the red cell sediment was mixed with two volumes of AHG in a fresh labelled glass tube.

6. The mixture was then mixed by a gentle tap at the base of the tube and centrifuged at 1500rpm for 1 minute.

7. The mixture was examined for macroscopic and microscopic agglutination following the same protocols as for the ABO grouping above.

8. The procedure above was repeated with anti-Fyb antisera.

9. An auto-control test was also carried out using the test cells and test plasma following the same protocol as above.

Interpretation of results

For each of the blood grouping procedures, the presence of agglutination or haemolysis signifies a positive reaction, indicating the presence of the tested antigen in the test cells. The absence of agglutination or haemolysis on the other hand indicates a negative reaction and the absence of the tested antigen on the test cells.

Data Analysis

All data collected from the study were cleaned, coded, entered into the computer on a pre-designed Excel spreadsheet and analysed using the statistical package for Social Sciences (SPSS) computer software version 21. Also, quantitative data were summarized using mean ± standard deviation (SD), median, mode and range; and outcomes were presented in tables. Quantitative data were presented in proportions. Differences in mean between the cases and the control were analysed using the student t-test, while differences in proportions and test of association were done using the chi-squared test. Binary logistic regression was done to ascertain the blood group that can independently predict preeclampsia. All tests were two-sided and the statistical significance was considered to be at a probability (p) value of <0.05.

Results

The socio-demographic characteristics of pre-eclamptic and non-pre-eclamptic women including age, education, occupation, religion and ethnicity were comparable (Table 1).

Table 2 summarized the ABO blood group distribution, with blood group O being the most prevalent (88.5%), followed by A (5.1%), B (4.5%), and AB (1.9%). No significant association between ABO blood groups and pre-eclampsia was found (p=0.80). Table 3 showed the Rh blood group distribution, where Rh D positive individuals constituted 82.1% of the population, while Rh D negative individuals comprised 17.9%. Although there was a higher proportion of Rh D-negative individuals among pre-eclamptic women (23.1%) compared to non-pre-eclamptic women (12.8%), the association was not statistically significant (p=0.09). However, Rh c negativity was significantly associated with pre-eclampsia (p<0.01), with 12.8% of pre-eclamptic women being Rh c-negative compared to 0% among non-pre-eclamptic women.

Table 4 indicated that the Kell K antigen was found in only one non-pre-eclamptic woman (1.3%), while all other participants were Kell K-negative. All women were Kell k-positive. Similarly, Table 5 showed that all participants were Kidd Jka-negative, while Kidd Jkb-positive individuals constituted 1.9% of the total sample, with no significant difference between the groups (p=0.56). Table 6 showed that Duffy Fya antigen was absent in all non-pre-eclamptic women but present in 2.6% of pre-eclamptic women, though this difference was not statistically significant (p=0.16). All participants were Duffy Fyb-negative.

Table 7 presents the logistic regression results for predicting pre-eclampsia. None of the ABO blood groups showed a significant predictive association with pre-eclampsia (p=0.75-0.98). Similarly, RhD positivity did not significantly predict pre-eclampsia (OR=1.96, p=0.15). RhE (p=0.49), Rhc (p=0.99), and Kidd (p=0.99) blood groups also did not emerge as significant predictors.

Characteristic	Pre-eclamptic women n=78 (%)	Non-pre-eclamptic women n=78 (%)	All Women n=156	χ^2	p-value
Age (in years)					
21-30	34 (43.6)	34 (43.6)	68 (43.6)		
31-40	36 (46.2)	36 (46.2)	72 (46.2)	0.00	1.00
>40	8 (10.3)	8 (10.3)	16 (10.3)		
Educational Status					
None	2 (2.6)	1 (1.3)	3 (1.9)		
Primary	5 (6.4)	6 (7.7)	11 (7.1)	0.93	0.82
Secondary	26 (33.3)	22 (28.2)	48 (30.8)		
Tertiary	45 (57.7)	49 (62.8)	94 (60.3)		
Occupation					
Civil Servant	30 (38.5)	26 (33.3)	56 (35.9)		
Trader	16 (20.5)	20 (25.6)	36 (23.1)		
Work in private firm	10 (12.8)	6 (7.7)	16 (10.3)		
Farmer	6 (7.7)	4 (5.1)	10 (6.4)	3.08	0.55
Others	16 (20.5)	22 (28.2)	38 (24.4)		
Religion					
Christian	72 (92.3)	74 (94.9)	146 (93.6)		
Muslim	6 (7.7)	4(5.1)	10 ((6.4)	0.43	0.51
Tribe/ethnicity					
Igbo	75 (96.2)	75 (96.2)	150 (96.2)		
Hausa	2 (2.6)	3 (3.8)	5 (3.2)	1.58	0.45
Yoruba	1 (1.3)	0 (0.0)	1 (0.6)	df=1	

Table 1: Sociodemographic Characteristics of the study participants

Blood group	Pre-eclamptic women n=78 (%)	Non-pre-eclamptic women	All Women n=156 (%)	χ^2	p-value

		n=78 (%)			
A	5 (6.4)	3 (3.8)	8 (5.1)		
AB	1 (1.3)	2 (2.6)	3 (1.9)		
B	4 (5.1)	3 (3.8)	7 (4.5)	1.01	0.80
O	68 (87.2)	70 (89.7)	138 (88.5)		

Table 2: ABO Blood Group Distribution

Blood group	Pre-eclamptic women n=78 (%)	Non-pre-eclamptic women n=78 (%)	All Women n=156 (%)	χ^2	p-value
Rh D					
-	18 (23.1)	10(12.8)	28 (17.9)	2.79	0.09
+	60 (76.9)	68 (87.2)	128 (82.1)		
Rh c					
-	10 (12.8)	0 (0.0)	10 (6.4)	10.68	<0.01
+	68 (87.2)	78 (100.0)	146 (93.6)		
Rh E					
-	14 (17.9)	16 (20.5)	30(19.2)	0.17	0.69
+	64 (82.1)	62 (79.5)	126 (80.8)		

Table 3: Rh Blood Group Distribution

Blood group	Pre-eclamptic women n=78 (%)	Non-pre-eclamptic women n=78 (%)	All Women n=156 (%)	χ^2	p-value
Kell K					
-	78 (100.0)	77 (98.7)	155 (99.4)	1.01	0.32
+	0 (0.0)	1 (1.3)			
Kell k					
-	78 (100.0)	78 (100.0)	156 (100.0)		
+	0 (0.0)	0 (0.0)	0 (0.0)		

Table 4: Kell Blood Group Distribution

Blood group	Pre-eclamptic women n=78 (%)	Non pre-eclamptic women n=78 (%)	All Women n=156 (%)	χ^2	p-value
Kidd Jka					
-	78 (100.0)	78 (100.0)	156 (100.0)		
+	0 (0.0)	0 (0.0)	0 (0.0)		
Kidd Jkb					
-	77 (98.7)	76 (97.4)	153 (98.1)	0.34	0.56
+	1 (1.3)	2 (2.6)	3 (1.9)		

Table 5: Kidd Blood Group Distribution

Blood Group	Pre-eclamptic women	Non-pre-eclamptic women	All Women	χ^2	p-value
Duffy Fya					
_	76 (97.4)	78 (100.0)	154 (98.7)	2.03	0.16
+	2 (2.6)	0	2 (1.3)		
Duffy Fyb					
-	78 (100.0)	78 (100.0)	156 (100.0)		
+	0 (0.0)	0 (0.0)	0 (0.0)		

Table 6: Duffy Blood Group Distribution

Predictor	OR (95% CI)	P value
ABO		0.98
ABO	.84 (0.13-5.24)	0.85
ABO	.74 (0.06-8.61)	0.79
ABO	1.31 (0.25 -6.88)	0.75
RhD	1.96 (0.79-4.85)	0.15

RhE	.73 (0.31-1.76)	0.49
Rhc	24.49 $x10^{14}$ (0.00-0.00)	0.99
Kidd	18.20x 10^{8} (0.00-0.00)	0.99

Table 7: Predictors of Preeclampsia

Discussion

In this cross-sectional analytic study of pregnant women in South-eastern Nigeria, blood group O was predominant, accounting for 89.7% of non-preeclamptic and 87.2% of preeclamptic women. Blood groups A, B, and AB were less prevalent, with no significant differences observed between the two groups. These findings align with a study by Okoye et al. [7] in Nigeria, which reported no significant association between ABO blood types and the risk of preeclampsia. Similarly, a systematic review by Li et al. [8] concluded that there was no effect of ABO blood types on the risk of preeclampsia in several studies, including those conducted in Nigeria and Thailand. However, other studies have reported varying associations. For instance, a meta-analysis by Franchini et al. [9] suggested that non-O blood groups might be associated with an increased risk of preeclampsia, possibly due to their impact on hemostatic balance and thrombus formation. These discrepancies highlight the need for further research to elucidate the relationship between ABO blood groups and preeclampsia.

Regarding the Rh blood group system, 87.2% of non-preeclamptic and 76.9% of preeclamptic women were Rh D positive, while 12.8% and 23.1%, respectively, were Rh D negative. The Rh c antigen was universally present in non-preeclamptic women (100%) but less so in preeclamptic women (87.2%), with a statistically significant difference ($p<0.01$). No significant difference was observed in the distribution of the Rh E antigen between the groups. The significant difference in Rh c antigen distribution suggests a potential association with preeclampsia, warranting further investigation. However, literature on the association between Rh antigens and preeclampsia is limited. A study by Okoye et al. [7] did not find a significant association between Rh blood groups and preeclampsia. The limited and inconsistent findings in the literature underscore the need for more comprehensive studies to determine the role of Rh antigens in the pathogenesis of preeclampsia.

In the present study, we also examined the distribution of specific red cell antigens—namely, Kell, Kidd, and Duffy blood group systems—among preeclamptic and non-preeclamptic pregnant women in teaching hospitals in Enugu, Nigeria. Our findings revealed no statistically significant differences in the distribution of these antigens between the two groups. Specifically, the Kell K antigen was present in 1.3% of non-preeclamptic women and absent in preeclamptic women ($\chi^2=1.01$, $p=0.32$). The Kidd antigen was found in 2.6% of non-preeclamptic women and 1.3% of preeclamptic women ($\chi^2=0.34$, $p=0.56$). The Duffy Fya antigen was absent in non-preeclamptic women but present in 2.6% of preeclamptic women ($\chi^2=2.03$, $p=0.16$).

These results align with several studies that have explored the association between red cell antigens and preeclampsia. For instance, a study conducted in Japan found no significant association between maternal ABO blood group and the risk of preeclampsia, suggesting that ABO blood group antigens may not play a pivotal role in the development of this condition [10]. Similarly, research in Nigeria reported no significant correlation between maternal ABO blood group and preeclampsia risk, reinforcing the notion that these antigens may not be critical determinants in the pathogenesis of preeclampsia [11].

However, contrasting evidence exists. Studies in Finland [12] and Italy [13] reported a higher risk of preeclampsia among women with AB blood type compared to those with O blood type, indicating a potential association between certain ABO blood groups and increased preeclampsia risk. Additionally, research in Thailand found that women with A and AB blood groups had a 1.7-fold higher risk of developing preeclampsia compared to those with O blood group [14]. These discrepancies suggest that the relationship between red cell antigens and preeclampsia may vary across different populations and ethnic groups.

Study Limitations

The lack of significant association between Kell, Kidd, and Duffy antigens and preeclampsia in our study may be attributed to several factors. Firstly, the prevalence of these antigens in the studied population may be low, limiting the power to detect significant differences. Secondly, the pathogenesis of preeclampsia is multifactorial, and red cell antigens may play a minor role compared to other genetic, immunological, or environmental factors. Furthermore, variations in study design, sample size, and population characteristics across different studies may contribute to inconsistent findings.

Conclusion

This study's findings indicate no significant association between ABO blood groups and preeclampsia, consistent with some previous studies, while contrasting with others that suggest a potential link between non-O blood groups and increased risk. The significant difference in Rh c antigen distribution between preeclamptic and non-preeclamptic women is a novel finding that requires further research. Further large-scale and multi-center studies may provide additional insights into potential immunogenetic influences on preeclampsia.

Authors' contributions: KC, TN, SO and EOU conceptualized and designed the study. KC, OA and CJM were involved in data collection/acquisition and statistical analysis. KC, TN, SO, and EOU interpreted the results and together with CLU, AIA, CON and OAU, were involved in the writing and revising of the manuscript for intellectual content. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work. All authors have accepted responsibility for the entire content of this manuscript and consented to its submission to the journal reviewed all the results and approved the final version of the manuscript.

Ethical Approval

Ethical Considerations: Ethical approval was obtained from the Health Research and Ethics committees of UNTH and ESUTH with reference numbers NHREC/05/01/2008B and ESUTHP/C-MAC/RA/034/vol-2163 respectively.

Informed Consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Declaration of Helsinki: The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Availability of research data: Authors are available and ready to supply the data upon any request through the corresponding author.

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