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ASSOCIATION OF *ANGIOTENSINOGEN RS699* SINGLE NUCLEOTIDE POLYMORPHISM AND PRE-ECLAMPSIA IN LAGOS, NIGERIA: A CASE-CONTROL STUDY

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ABSTRACT

Preeclampsia is a pregnancy disorder that endangers the lives of bpth mother and child. Gene polymorphisms of Angiotensinogen have been shown to be associated with hypertension and since pre-eclampsia is basically hypertension in pregnancy, there might be a genetic correlation. Angiotensinogen rs699 (AGT rs699) polymorphism affects spiral arteries in the uterus and controls blood pressure. Various association studies of SNP for pre-eclampsia have yielded conflicting results. This study investigated the association of AGT rs699 and ascertain the socio-medical factors with pre-eclampsia amongst gestating women in Lagos, Nigeria. Ethical approval and informed consent were obtained from Health Research and Ethical committees. Thirty-eight (38) preeclamptic (P) and 39 normotensive (N) pregnant women from a selected maternity hospital were recruited for this study. Demographic characteristics, medical history, and present medical conditions were assessed using questionnaires. Blood samples were collected and AGT rs699 was genotyped. Descriptive statistics, independent sample t-test, chi-square, and multiple regression analysis were used to analyze the data obtained ($p < 0.05$). The mean age and weight of women with pre-eclampsia were higher in preeclamptic women with no significant difference (P = 32.43 ± 5.76, N = 30.97 ± 4.54, and P =79.04 ± 10.28, N = 76.82 ± 15.35 respectively). Diastolic blood pressure (DBP), Systolic blood pressure (SBP), and Proteinuria were significantly higher in preeclamptic patients. Surprisingly, the incidence of AGT rs699 was significantly higher in normotensive (82%) women than in preeclamptic women (17%) (X^2=29.150, p = 0.00001). A significant correlation was observed between AGT rs699 and SBP in normotensive patients (r2 = 0.201, p=0.003) and a negative correlation in preeclamptic patients. In contrast, AGT rs699 was negatively related to DBP with no statistical difference in both the normotensive and preeclamptic groups. In addition, no correlation nor significant differences were observed between AGT rs699 and the weights of the preeclampsic and normatensive women. In conclusion, this study revealed a negative significant association between pre-eclampsia and AGT rs699 among cases and the control population studied.

Keywords: Pre-eclampsia, Normotensive, *AGT rs699*, Single nucleotide polymorphism, Demographic characteristics, Underlying sickness.

INTRODUCTION

Pre-eclampsia is a life-threatening disorder characterized by hypertension and proteinuria which leads to devastating effects in mothers and neonates (Michita *et al.*, 2018 and Apicella *et al.*, 2019 and Rana *et al.*, 2019). It is a significant public health concern that varies geographically, socially, and ethnically which reveals frightening healthcare in developing countries with an effect of 5-10% in Nigeria (Duarte *et al.*, 2014 and Musa *et al.*, 2018). Placenta is regarded as the cause of pre-eclampsia and the only effective treatment is the delivery of the foetus and removal of the placenta (Lumbers *et al.*, 2019). Pre-eclampsia when left untreated leads to maternal complications such as seizure, oedema, pulmonary, liver, metabolic, cardiovascular, renal malfunction, eclampsia, stroke, and postpartum depression (Behrens *et al.*, 2017 and Bernstein *et al.*, 2017). It also predisposes newborns to intrauterine growth restriction, premature birth, stillbirth, low birth weight, autism, cerebral palsy, bronchopulmonary dysplasia, and endocrine, nutritional and metabolic diseases (Magnussen *et al.*, 2007, Laresgoiti-Servitje *et al.*, 2010 and North *et al.*, 2011). Clinical manifestation of pre-eclampsia ranges from mild hypertension with proteinuria to severe hypertension associated with convulsions, multiple organ damage and even death (Mustafa *et al.*, 2012). It is influenced by race, age, weight, family history of pre-eclampsia, obesity, nulliparity, multiple gestations and underlying medical conditions (Ye *et al.*, 2017; Mayrink *et al.*, 2019).

Pre-eclampsia is a complex genetic disorder that

results in variants contributing to disease susceptibility (Skjaerven *et al*., 2005). It follows Mendelian rules of inheriting rare deleterious genetic variants and studies revealed there is a genetic association of maternal, paternal and foetal genes (Fong *et a*l., 2014). Familial predisposition to pre-eclampsia was reported by (Lardoeyt *et al*., 2013). Lie *et al.* (1998) carried out a study between 1967 and 1992 and reported that daughters born of preeclamptic pregnant mothers have a triple risk of developing it and a double risk for their sons. It also reported that daughters born of preeclamptic pregnancy may inherit susceptibility genes maternally and this predisposes them to pre-eclampsia at a later age. Maternal genetic makeup has been shown to predispose individuals to pre-eclampsia and renin-angiotensin is one of the first endocrine systems to recognize pregnancy (Mistry and Williams, 2011; Triche *et al*., 2014). Renin-angiotensin-aldosterone system deviation plays an important role in the pathogenesis of pre-eclampsia (Chengalvala *et al*., 2017). Production of angiotensinogen is stimulated by the secretion of estrogens from the fetoplacental unit and it is associated with an increased prevalence of pre-eclampsia (Ni *et al*., 2012). It also provides instruction for the Angiotensinogen gene in the regulation of blood pressure and electrolyte balance (Afshariani *et al*., 2014).

The Angiotensinogen gene is located on chromosome 1q42-43 and is present in hormonal cycles and still maintained when conception occurs (Charoen *et a*l., 2019). It however increases during pregnancy and is a central player in pre-eclampsia (Shah, 2005; Irani and Xia, 2008). In pre-eclampsia, abnormal placentation is accompanied by increased levels of angiotensinogen, and circulating angiotensinogen levels are higher in mothers with previous preeclamptic pregnancies (Sykes *et al*., 2014). Angiotensinogen rs699 was first identified in 1992 with T to C base substitution of methionine 235 with threonine and is associated with a rise in serum levels in patients with hypertension in pre-eclampsia (Powe *et al*., 2015; Heidari *et al*., 2019).

Gene polymorphisms of Angiotensinogen have been shown to be associated with hypertension and since pre-eclampsia is basically hypertension in pregnancy, there might be a genetic correlation.. Angiotensinogen rs699 (AGT rs699) polymorphism affects spiral arteries in the uterus and controls blood pressure. Various association studies of SNP for pre-eclampsia have yielded conflicting results. This study aimed to determine the association of AGT rs699 in preeclamptic and normotensive pregnant women and compare the differences in social-demographic characteristics.

MATERIALS AND METHODS

Study design

This is a case-control study of patients that registered for antenatal checks at the Ifako Ijaiye General Hospital, Lagos. The hospital was selected for its high number of registered pregnant women. Sixty-nine (69) patients were recruited for this study including 30 preeclamptic and 39 normotensive pregnant women. A researcher-designed questionnaire - Pre-eclampsia Phenotype Questionnaire was designed to collect data on social-demographic, medical history and present medical conditions. Informed consent forms were filled out before sample collection. Systolic blood pressure greater than 130 mmHg, diastolic blood pressure greater than 90 mmHg and proteinuria $\geqslant$1+ on dipstick was recorded as preeclamptic while systolic blood pressure lesser than 130 mmHg and diastolic blood pressure lesser than 90 mmHg was recorded as normotensive. Patients that presented with chronic hypertension, autoimmune diseases, collagen vascular diseases, asthma, haematological disorder, and urinary tract infection were not included in the study.

Ethical Approval

The participants gave informed consent and this study was conducted with approval from health research and ethical committees of Lagos State University Teaching Hospital (LASUTH) - ADM/DCST/HREC/018).

Sample Collection and DNA Analysis for gene polymorphism:

Five millilitres (5 mL) of whole blood were collected from individuals selected for the study into EDTA bottles via venipuncture during the antepartum period in labelled tubes for genetic analysis. Whole genomic DNA was extracted using Qiagen QIAamp Genomic DNA Kit

according to the manufacturer's instructions. The Angiotensinogen gene rs699 (M235T) was analyzed using Allele Specific PCR (ASPCR) using specific primers. - Forward: 5'-CAgggTgCTgTC CAC ACT ggA CCC C-3' and Reverse: 5'- CCg TTT gTgCAgggCCTggCT CTC T-3' in a 20 µl total reaction volume comprising of 3 µl of DNA template, 4 µl ready-to-load Mastermix (SolisBioDyne 5X FIREPol PCR Mastermix), 0.2 µl each of the forward and reverse primers, and 12.6 µl of DNase-free water. The reaction mixture was subjected to a thermocycling program of initial denaturation at 95 °C for 5 mins, followed by 35 cycles at 95 °C for 30 s, 64.5 °C for 45 s, 72 °C for 30 s with a final extension at 72 °C for 10 min and then held at 4 °C till further analysis. PCR products were electrophoresed on a 2% agarose gel and stained with red-safe. Gel pictures were taken under ultraviolet light.

Data Analysis

Data were captured in a Microsoft Excel spreadsheet and transferred to Statistical Package for Social Sciences (SPSS) version 28.0 for statistical analysis. The results are presented in frequency tables and graphs. Categorical variables were compared using Chi-square and f-test while numerical variables were expressed as means ± standard deviation and the groups were compared using a t-test. Statistical significance was set at a p-value less than 0.05 for all values of the test statistic.

RESULTS

The clinical parameters of the study are given in Table 1. The mean age for the preeclamptic women was 32.33 ± 5.76 and 30.97 ± 4.54 for the normotensive women. The mean SBP and DBP readings were 140.03 ± 8.06 and 81.39 ± 7.01 for preeclamptic women and 115.48 ± 12.93 and 68.02 ± 7.83 for normotensive women respectively. Analysis using the 2 tailed t-tests showed significant differences between the means of the preeclamptic women and normotensive women in SBP and DBP and showed no significant difference for age and weight. The mean weight of pre-eclampsia was 79.04 ± 10.28 and 76.82 ± 15.35 for normotensive.

Table 1: Clinical parameters of Pre-eclampsia and Normotensive Women.

Parameters	Preeclampsia Mean ± SD	Normotensive Mean ± SD	T-test	P-value
DBP	81.39 ± 7.01	68.02 ± 7.83	7.36	0.000001
SBP	140.03 ± 8.06	115.48 ± 12.93	9.118	0.000001
Weight	79.04 ± 10.28	76.82 ± 15.35	0.651	0.518
Maternal age	32.43 ± 5.76	30.97 ± 4.54	1.106	0.273

SBP - Systolic Blood Pressure, DBP - Diastolic Blood Pressure, SD - Standard deviation.

Proteinuria and current medical conditions were expressed in Figure 1. Few proteins were observed in the urine of the normotensive women while the mean protein in the preeclamptic women was significantly higher. Rapid weight gain, dizziness, vision changes and severe headaches were higher in preeclamptic women with no significant differences and excess vomiting were higher in normotensive women with no statistical difference. Two preeclamptic women recorded positive smoking status and no normotensive woman recorded smoking.

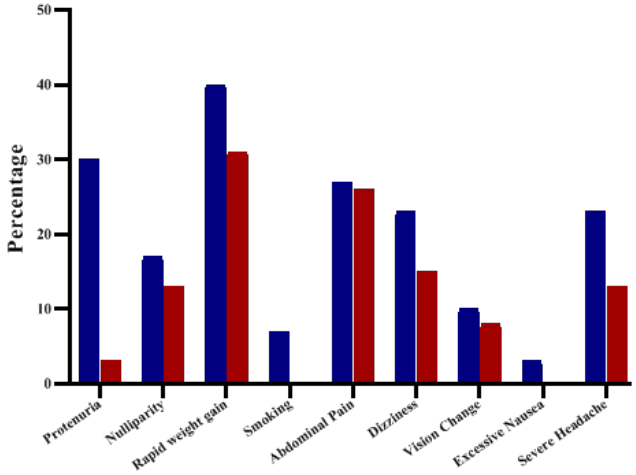

Figure 1: Current medical conditions of preeclamptic and normotensive women.

There was no significant difference in the medical history of preeclamptic and normotensive patients (Figure 2). Hypertension was higher in preeclamptic women while kidney disease and renal disease were higher in normotensive women. No preeclamptic patient recorded a family history of preeclampsia and previous history of diabetes, sickle cell disease and lupus.

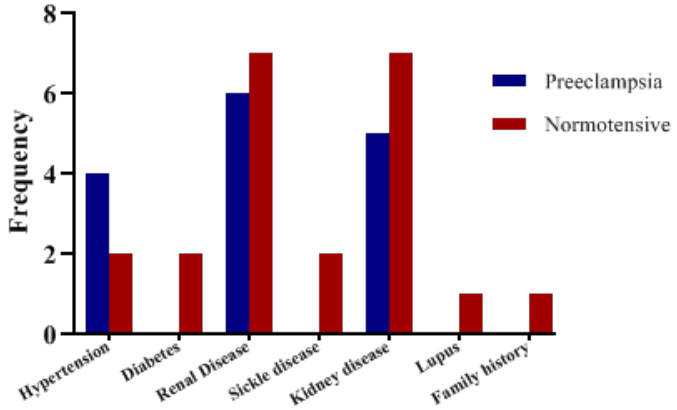

Figure 2: Medical history of preeclamptic and normotensive women

There were no significant differences in the demographic characteristics of the participants (Figure 3). The majority (49.35% - 38) of the women (preeclamptic and normotensive) were of the Yoruba tribe and most of the women were Christians and self-employed.

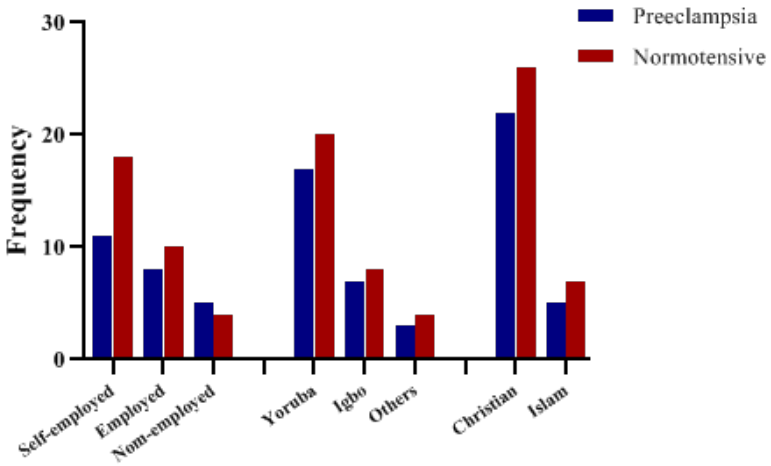

Figure 3: Demographic and social characteristics of preeclamptic and normotensive women.

Association of *Angiotensinogen rs699* with Pre-eclampsia141 bpThe incidence of *AGT rs6999* was higher in normotensive (82%) than in preeclamptic women (17%) (Figure 4). Using the chi-square test for association, AGT rs699 was significantly assocaited with normatrnsive (X2=29.150, p = 0.00001) (Table 2) and invariably negatively associated with preeclampsia. Plates 1 and 2 showed amplification for *AGT rs699* with the expected amplicon size of 141 bp.

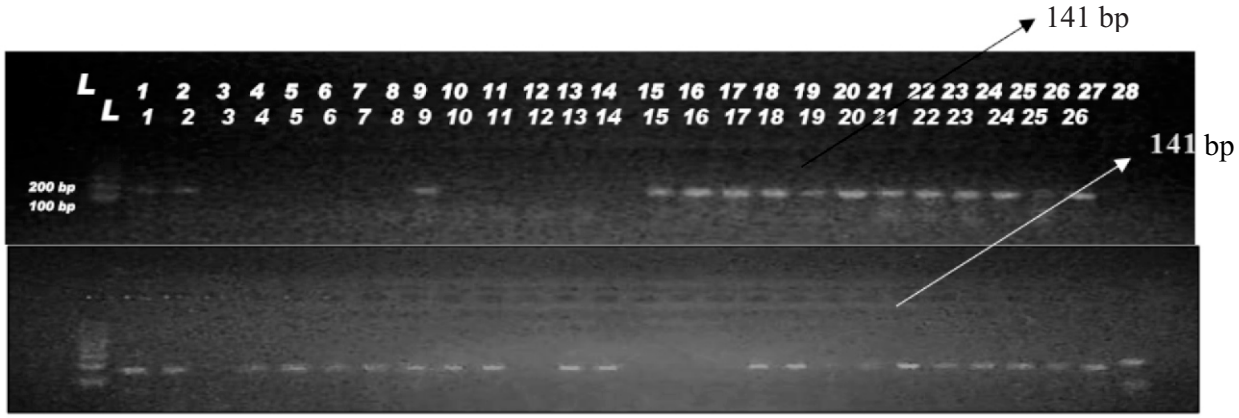

Plate 1 and 2: Gel electrophoresis result of PCR products showing the presence of the *rs699* SNP of the Angiotensinogen gene.

Table 2: Association of *AGT rs699* with Pre-eclampsia.

Chi-Square Tests	Value	df	Sig. (2-sided)
Pearson Chi-Square	29.150a	1	0.00001
Continuity Correction	26.58	1	0.00001
Likelihood Ratio	31.55	1	0.00001
N of Valid Cases	77		

a 0 cells (0.0%) have expected count less than 5. The minimum expected count is 13.91.

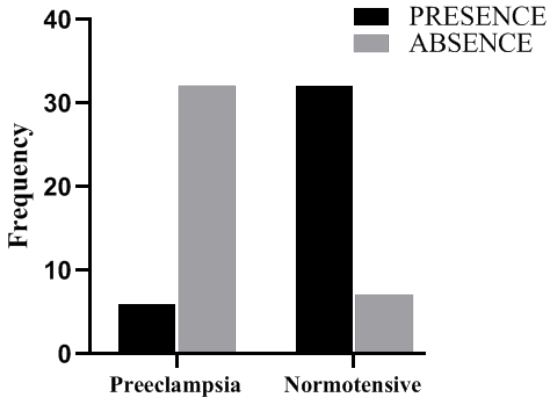

Figure 4: Frequency of *AGT rs699* in Preeclamptic and Normotensive women.

Table 3 shows the correlation between AGT rs699 with SBP, DBP and weight. Using a multivariate linear regression, AGT rs699 was inversely proportional to DBP and SBP in preeclamptic patients with no significant association but significantly associated with SBP in normotensive pregnant women (p = 0.037). There was also no significant correlation between AGT rs699 and weight in both preeclamptic and normotensive patients.

Table 3: Correlation of AGT rs699 with DBP, SBP and weight.

	Diastolic Blood Pressure		Systolic Blood Pressure		Weight	
	P	N	P	N	P	N
AGT rs699	-0.084	-0.081	-0.219	0.201	0.062	0.279
P value	0.659	0.625	0.245	0.037	0.748	1.36

P - Preeclamptic, N - Normotensive, SNP- Single Nucleotide Polymorphism

DISCUSSION

In this study, there were significant differences in the mean values of both the diastolic and systolic blood pressure readings and this is expected as preeclamptic women have a higher blood pressure than normotensive pregnant women. Similar results of significantly higher blood pressure have been reported in preeclamptic women due to the hypertension comorbidity (Al-Jameil *et al.*, 2014 and Steinthorsdottir *et al.*, 2020). Women with pre-eclampsia presented a lower mean of weight when compared to the control group which agrees with a Swedish study that came to a conclusion of normotensive pregnant women develop more weight than preeclamptic women suggesting that pre-eclampsia is more pronounced in underweight women (Johansson *et al.*, 2016). It however disagrees with Poorolajal and Jenab (2016) who showed that increased weight is associated with the risk of pre-eclampsia because of alteration in endothelial cell dysfunction due to hormonal differences in adipose tissue. Normotensive pregnant women recorded higher nulliparity than women with pre-eclampsia which is inconsistent with studies that described pre-eclampsia as a disorder of first pregnancies but well-being in a woman's first pregnancy strongly predicts risks of pre-eclampsia in subsequent pregnancies (Hernandez-Diaz *et al.*, 2009).

From this study, low prevalence of smoking was recorded in the preeclamptic group which correlates with various studies that concluded that pregnant smokers have a lower risk of pre-eclampsia than non-smokers but their offspring often weigh less than nonsmokers because of the interaction of tobacco with the transfer of placental nutrients (Xiong *et al.*, 2000 and Jeyabalan *et al.*, 2008). Maternal age and family history were higher in the pre-eclampsia group which correlated with the conclusions from Ayorinde and Bhattacharya (2017). A family history of pre-eclampsia was found higher in normotensive pregnant women which disagreed with a previous study of preeclamptic women having a three-fold higher family history (Hassanein and Mokhtar, 2007). This suggests additional factors to pre-eclampsia asides from the genetic factor. Most of the patients in this study were of Christianity background and the dominant tribe was Yoruba which was expected due to the location of sample collection where individuals living in the community are mostly Yoruba. A higher percentage of women were self-employed which may be attributed to the religious and cultural influence that encourages women to create jobs that provide available time to care for their offspring.

Numerous studies have been published on this SNP with controversial results in different populations. Using the disease association panel, the normotensive group recorded a higher frequency of SNPs compared to the preeclamptic group. AGT rs699 showed a negative significant association with pre-eclampsia which was inconsistent with studies from the Tunisian population, Caucasians, and Mongolians (Lin *et al.*, 2012 and Zitouni *et al.*, 2018). However, similar results have been reported in studies with participants of Black South Africans, Indians, African Americans, and Asian populations (Aung *et al.*, 2017; Chengalvala *et al.*, 2017; El-Garawani *et al.*, 2021).

AGT rs699 was inversely proportional to DBP in preeclamptic and normotensive patients with no significant association which agrees with the study of Charoen *et a*l. (2019) as no association was observed between the gene and SBP in the Thai population. His study also showed no evidence of an association between AGT rs699 with SBP. It however contradicts our findings as SBP was significantly associated with AGT rs699 in normotensive patients but inversely proportional in preeclamptic patients.

The occurrence of the AGT rs699 molecular

variant is twice higher in the Negroid and Mongoloids, than in the Caucasian population which is responsible for the racial predisposition difference (Rotimi *et al.*, 1994). AGT rs699 is a wild type that is predominant in individuals of West African origin with 93% in the Nigerian population and its frequency makes it difficult to yield positive association results (Bloem, 1995; Corvol and Jeunemaitre, 1997).

Other studies that associated AGT rs699 with pre-eclampsia were inconsistent in different populations and our findings confirmed pre-eclampsia is a polygenic trait. Future studies should focus on investigating the genetic and epigenetic mechanisms of pre-eclampsia.

CONCLUSION

This study showed that *AGT rs699* was significantly lower in the preeclamptic group compared to the normotensive group showing a negative significant association with pre-eclampsia in this population. There is a need to conduct studies that involve a larger population to establish an estimate of the association of this SNP with pre-eclampsia. This will help in better diagnosis and novel therapeutics.

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CONFLICT OF INTEREST

On behalf of the authors, the corresponding authors state that there is no conflict of interest or competing financial interest.

AUTHOR CONTRIBUTIONS

FTA - Conceptualization, Methodology, Investigation, Formal analysis, Writing - Review & Editing, Supervision, Project administration, Resources. **SAO** - Writing - Original Draft, Investigation, Formal analysis, Visualization, Resources. **AAK** - Conceptualization, Writing - Review & Editing, Resources. **SOG** - Investigation, Resources

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QUESTIONAIRE USED FOR THE CASE STUDY PREECLAMPSIA AMONG PREGNANT WOMEN

UNIVERSITY OF LAGOS

FACULTY OF SCIENCE

PROJECT TITLE: MOLECULAR GENETIC STUDIES OF PREECLAMPSIA NIGERIA AS A CASE STUDY

SECTION 1

Maternal parameters

Age: ..... (Years)

Ethnicity: (a) Yoruba (b) Igbo (c) Hausa (d) others

Occupation: (a) Employed (b) Self-employed (c) Non-employed

Initial weight at booking ..... (kg)

Religion .....

Height ..... (cm)

Maternal Exposure

Smoking (a) Yes ( ) (b) No ( )

Alcoholism (a) Yes ( ) (b) No ( )

SECTION 2

Medical History

Hypertension	(a) Yes ( )	(b) No ( )
Diabetes mellitus	(a) Yes ( )	(b) No ( )
Chronic Renal disease	(a) Yes ( )	(b) No ( )
Sickle cell disease	(a) Yes ( )	(b) No ( )
Rheumatoid arthritis	(a) Yes ( )	(b) No ( )
Kidney disease	(a) Yes ( )	(b) No ( )
Lupus	(a) Yes ( )	(b) No ( )
Family history of preeclampsia	(a) Yes ( )	(b) No ( )

SECTION 3

Current Medical condition

• Rapid weight gain	(a) Yes ( )	(b) No ( )
• Abdominal pain	(a) Yes ( )	(b) No ( )
• Severe headache	(a) Yes ( )	(b) No ( )
• Change in reflexes	(a) Yes ( )	(b) No ( )
• Reduced urine or no urine output	(a) Yes ( )	(b) No ( )
• Dizziness	(a) Yes ( )	(b) No ( )
• Excessive vomiting and nausea	(a) Yes ( )	(b) No ( )
• Vision changes	(a) Yes ( )	(b) No ( )
• Nulliparity	(a) Yes ( )	(b) No ( )

SECTION 4

Sample collection

Would you like to donate your blood for the study (a) Yes ( ) (b) No ( )

If no, kindly state the reason

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Thank you for participating in this study

Dr. Fagbayi

Project supervisor