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Association of IL4-VNTR and eNOS G894T Polymorphisms with Preeclampsia in Nigerian Pregnant Women: A Case–Control Study

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Abstract:

Introduction: Preeclampsia (PE) remains a major contributor to maternal and fetal illness and death. Its development is complex, involving contributions from genetic and environmental factors, immune dysregulation, and impaired endothelial function. Meanwhile, there is a paucity of information on genetic predisposition to preeclampsia in pregnant Nigerian women.

Aims: This is a preliminary study that examined the association between IL4-VNTR and eNOS polymorphisms and the risk of preeclampsia in pregnant Nigerian women, along with the contribution of plasma nitric oxide levels.

Materials and Methods: A case–control design was employed, involving blood samples from 73 participants (8 with preeclampsia and 65 controls) for DNA genotyping and plasma NO (nitric oxide) evaluation. IL4-VNTR and eNOS G894T genetic polymorphisms were analysed using the PCR and PCR-RFLP technique.

Results: Using unconditional logistic regression analysis, we found no statistically significant difference between the odds ratio (OR) and 95% confidence interval (CI) for both genes. Observing (B2/B1 versus B1/B1, OR, 0.65 [95% CI, 0.11 to 3.73]; *P* = .63 and B2/B2 versus B1/B1; *P* = .18) for IL-4 VNTR polymorphism and (GT versus GG, OR, 2.17 [95% CI, 0.00 to 0.00]; *P* = .99 and TT versus GG; *P* = .99) for eNOS G894T polymorphism. Additionally, no associations were observed between these polymorphisms and key clinical parameters. Plasma nitric oxide levels (total nitrates and nitrites) also did not differ significantly between preeclamptic women and controls (425.22 ± 184.87 vs. 450.72 ± 139.28 μM; *P* = .543).

Conclusion: We observed no significant association between IL4-VNTR and eNOS gene variants examined in this study to preeclampsia risk among pregnant Nigerian women; however, the small sample size limits definitive conclusions. Larger studies are recommended to validate these observations.

Keywords: preeclampsia, interleukin-4, endothelial nitric oxide synthase, polymorphisms.

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Preeclampsia is a hypertensive disorder of pregnancy that typically develops after 20 weeks of gestation and is characterised by hypertension and proteinuria. It represents a significant contributor to perinatal morbidity globally, affecting approximately 2–8% of pregnancies and accounting for up to 15% of maternal deaths related to obstetric complications [1, 2]. According to the World Health Organisation (WHO), preeclampsia occurs in about 2.8% of live births in developing countries, which is approximately seven times higher than the 0.4% reported in developed nations [3]. Approximately 12-25% of fetal growth restriction (FGR), as well as 15-20% of premature births, are attributed to preeclampsia [4]. Its pathogenesis is associated with several underlying pathophysiological abnormalities, including impaired trophoblast differentiation and invasion, placental and endothelial dysfunction, immune maladaptation, and an amplified systemic inflammatory response [5, 6]. The risk of developing the condition is further elevated in cases of primiparity, exposure to work-related psychosocial stress during pregnancy, adverse socioeconomic circumstances, certain ethnic backgrounds, prematurity, and younger maternal age [7, 8]. Despite extensive scientific investigation, the exact aetiology of preeclampsia remains unknown [9, 10].

Emerging evidence indicates that immune mechanisms play a central role in the pathogenesis of preeclampsia [11]. The condition is linked to an imbalance between pro-inflammatory and anti-inflammatory cytokines, characterised by a shift from a T-helper (Th) 2 response toward a Th1 immune response [12]. Interleukin signalling is a major regulator of this immune balance, as these cytokines are crucial for the activation, proliferation, and differentiation of various inflammatory cells [13]. Studies indicate that both innate and adaptive immune pathways contribute to the pathogenesis of preeclampsia, and plasma levels of anti-inflammatory cytokines such as Interleukin 4 (IL-4), a key Th2 cytokine, are reduced in affected patients [6, 14, 15].

IL-4 is the principal cytokine produced by Th2 lymphocytes and is essential for the regulation of humoral immune responses. It is typically upregulated during pregnancy [16]. This cytokine promotes the differentiation of naive T cells into Th2 and T follicular helper cells and enhances B-cell antibody production, including IgE class switching [17]. IL-4 suppresses the production of pro-inflammatory cytokines, including IL-1, IL-6, and tumour necrosis factor (TNF), thereby strongly downregulating macrophage activity [16]. Variations in the IL-4 gene have been associated with several immune-mediated disorders, including preeclampsia. It was reported that CD4+ T cells secreting IL-4 were significantly reduced in preeclampsia [18, 19]. IL-4 has progressed from a biomarker to a therapeutic potential in preeclampsia, as this cytokine treatment restored spiral artery damage and symptoms of preeclampsia in pregnant mice [20, 21]. The IL-4 gene is situated within the cytokine gene cluster on chromosome 5q31.1 [21]. A functionally significant polymorphism in this gene is a 70-base pair variable number of tandem repeats (VNTR) within its third intron, which may influence IL-4 gene expression [22]. Moreover, some past studies have positively associated genetic variants of IL-4 VNTR with preeclampsia susceptibility and increased risk of preterm birth [19, 23].

Endothelial nitric oxide synthase (eNOS) is an enzyme responsible for producing nitric oxide (NO) in the vascular endothelium. Nitric oxide is a derivative of L-arginine that functions as a vasodilator, inhibits platelet aggregation, and relaxes smooth muscle [24]. NO is essential for maintaining homeostasis and regulating endothelial function [25, 26]. The eNOS enzyme is encoded by the NOS3 gene, located on the long arm of chromosome 7 (7q35–q36), and comprises 26 exons [27, 28]. The G894T polymorphism, which leads to the substitution of aspartate for glutamate at amino acid position 298 of the NOS3 gene, is the most extensively studied and commonly observed eNOS gene variant [7]. Glu298Asp is widely recognised for its functional impact, as it affects posttranslational modifications, ultimately leading to eNOS susceptibility to proteolytic cleavage and decreased production of NO [29]. It is established that this polymorphism in the endothelial NOS3 gene affects the localisation of the enzyme to the caveolar membrane [30]. Decreased eNOS activity has been associated with a range of conditions, including preeclampsia, essential hypertension, diabetes mellitus, ischemic stroke, coronary heart disease, and erectile dysfunction [31]. NO levels are upregulated in normal pregnancy to maintain blood flow without increasing systemic vascular resistance [32].

Several studies have investigated the association of polymorphisms in various genes, including IL-4 and eNOS, with the pathogenesis of preeclampsia or related cardiovascular disorders. While most of these studies have reported positive relationships, others have found no association, especially across different populations [19, 33-37]. In our previous study, we investigated the polymorphisms in vitamin D-binding protein and cystathionine-γ-lyase genes in pregnant Nigerian women at a Lagos state general hospital and found no association with preeclampsia development [33]. Another study reported the increased risk of preeclampsia with polymorphisms in eNOS, vascular endothelial growth factor (VEGF), angiotensin-converting enzyme (ACE), and tumour necrosis factor-alpha (TNFα) genes among pregnant women at Central Hospital, Auchi, Nigeria [38]. Till date, only these mentioned studies have attempted to examine the association of genetic polymorphisms with the pathogenesis of preeclampsia in a population of pregnant Nigerian women, underscoring the scarcity of data on the contribution of genetic polymorphisms to increased preeclampsia risks in pregnant Nigerian women.

Given that preeclampsia is characterised by a significant shift toward a pro-inflammatory Th1 environment and impaired nitric oxide-mediated vasodilation, investigating the genetic determinants of these processes is vital for understanding disease susceptibility. In this study, we focus on IL-4 VNTR and eNOS G894T polymorphisms, as these specific variants are known to modulate cytokine expression and enzymatic activity, yet their roles remain poorly defined and inconsistent across different genomic backgrounds. To our knowledge, no previous study has examined IL-4 VNTR polymorphism in relation to preeclampsia development in pregnant Nigerian women; although eNOS polymorphism has been previously examined in one study, it is an established fact that genetic risk factors are usually population-specific. Hence, this study aims to investigate IL-4 VNTR and eNOS genetic polymorphisms in relation to the risk of preeclampsia in pregnant Nigerian women in a Lagos general hospital, using a case-control study. This will contribute to the growing understanding of how genetic factors predispose to preeclampsia development and help in laying the background for knowledge-based clinical management of the disorder.

2. MATERIALS AND METHODS

2.1 Subjects

This study involved pregnant women receiving antenatal care at Ifako-Ijaiye General Hospital in Agege area of Lagos State, Nigeria. This is a case-controlled study that included 8 diagnosed preeclamptic women and 65 normotensive pregnant women (controls). It is noteworthy that the small sample size, especially for cases, is partly due to time-related constraints during the study period. Following appropriate informed consent, blood samples were collected from all enrolled participants (both cases and controls) for germline DNA genotyping before the start of any new therapy. A total of 4 mL of blood was drawn into a lithium-heparinised tube, ensuring minimal hemolysis. The diagnostic criteria for preeclampsia were defined as a systolic blood pressure ≥140 mmHg and a diastolic blood pressure ≥90 mmHg. Demographic and clinical data were obtained via a standardised questionnaire, combined with a review of their medical records, which were used to clinically annotate the samples. We excluded patients with a history of intrauterine fetal deaths from this study. Blood samples were conveyed in ice packs to the Molecular Biology Laboratory at Covenant University (CU), Otta, Ogun State, where DNA extraction and subsequent molecular analyses were performed. All samples were processed for recovery within 96 hours of collection. Ethical approval was obtained from the Ethics Committee of the Lagos State University Teaching Hospital before the commencement of the study.

2.2 DNA Extraction

Genomic DNA was extracted from blood samples using a DNeasy kit (Qiagen, Inc., Valencia, CA), strictly adhering to the protocol. The extracted DNA samples were kept frozen at -80°C until required for genotyping. Before genotyping, the quantity and purity of DNA samples were evaluated using a microvolume spectrophotometer (NanoDrop™ 2000, Thermo Scientific™, Waltham, MA, USA). Samples with an A260/280 ratio < 1 were removed from further analysis. Aliquots of DNA extracts were diluted to a DNA concentration of 10 ng/μL with distilled water, resulting in a final volume of 200 μL.

2.3 Genotyping

Polymerase chain reaction (PCR) assay was used to detect the VNTR polymorphism of the IL-4 gene in the isolated genomic DNA samples as previously described [39]. eNOS G894T polymorphisms analysis was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), employing a previously described procedure with slight modification [39]. PCR was performed with a 25 µl reaction mixture, each containing 10 µL (100 ng) DNA template, 1 µL (0.8 µM) of each primer, 2 µL (200 µM of each) dNTPs, 0.2 µL (0.5 U) Taq polymerase, 2.5 µL buffer and 9.3 µL RNase-free H_2O (Qiagen, Inc., Valencia, CA). The primers and conditions for DNA amplification are presented in **Table 1** below. PCR was performed using a thermal cycler (C1000 Touch™, Bio-Rad, California, USA). Twenty (20) samples were randomly selected and subjected to agarose gel electrophoresis (PowerPac™, Bio-Rad, California, USA) to ascertain the quality of the amplicons after the completion of PCR. Electrophoresis products were evaluated by visualisation on 2% agarose gel stained with ethidium bromide under UV light (UVP, M-26V Transilluminator of BioDoc-It® 220 Imaging System). The IL-4 VNTR polymorphism analysis was completed after visualising all PCR products. The PCR product was 183 bp for the B1 allele and 253 bp for the B2 allele (Table 2). For the eNOS G894T polymorphism, PCR products were subsequently digested with a restriction enzyme.

Table 1: Primers and amplification conditions for IL-4 and eNOS genes

Primer sequences	Melting temp./time	Annealing temp./time	Elongation temp./time	No. of cycles
IL-4 Forward: 5'-AGGCTGAAAGGGGGAAAGC-3' Reverse: 5'-CTGTTCACCTCAACTGCTCC-3'	Initial: 94^{0}C, 5 mins. Subsequent: 94^{0}C, 50 s	61^{0}C, 30 s	Initial: 72^{0}C, 45 s Final: 72^{0}C, 5 mins	30
eNOS Forward: 5'-TCCCTGAGGGCATGAGGCT-3' Reverse: 5'-TGACGGTCACACAGGTTCCT-3'	Initial: 94^{0}C, 1 min. Subsequent: 94^{0}C, 30 s	61^{0}C, 1 min	Initial: 72^{0}C, 1 min. Final: 72^{0}C, 7 mins	30

For restriction enzyme digestion, the PCR amplicons of the eNOS gene were digested with BanII (New England BioLabs). The obtained fragment lengths following the restriction enzyme digestion are presented in **Table 2**. The restriction products were then separated by electrophoresis (PowerPac™, Bio-Rad, California, USA) using 2% agarose gel stained with ethidium bromide to determine the polymorphic variants of the eNOS G894T. Products of the electrophoresis were evaluated by visualisation under UV light using a Transilluminator (UVP M-26V, BioDoc-It® 220 Imaging System, Upland, CA, USA).

Table 2: Restriction enzyme and hydrolysis conditions for eNOS G894T SNP, and expected

Polymorphism	Restriction enzyme	Time/temperature of incubation	Time/temperature of deactivation	Size of amplicons/fragments (bp)
IL-4 VNTR	N/A	N/A	N/A	B1B1 (183) B1B2 (183, 253) B2B2 (253)
eNOS G894T	BanII	20 hrs, 37^{0}C	20 mins, 80^{0}C	GG (320, 137) GT (457, 320, 137) TT (457)

N/A = not applicable

2.4 Nitric Oxide (NO) Assay

Plasma NO concentration was determined by an indirect method, measuring blood nitrite (a nitric oxide metabolite) level using a slight modification of previously described procedures [40]. 50 µL of plasma was mixed with 75 µL of diH_2O for initial dilution. Subsequently, 125 µL of 0.3N NaOH was added, and the mixture was incubated at room temperature for 5 minutes. 62.5 µL of 10% $ZnSO_4$ was added to deproteinize the mixture, followed by centrifugation at 8,000 g for 15 minutes. 200 µL of the supernatant was taken and added to 200 µL of Griess reagent. Absorbance was read at 540 nm after 30 min of incubation. Concentration in the solution was calculated against a calibration curve derived from various concentrations (0 – 150 µM) of sodium nitrite standard (Figure 1). Results show plasma NO concentration in micromolar.

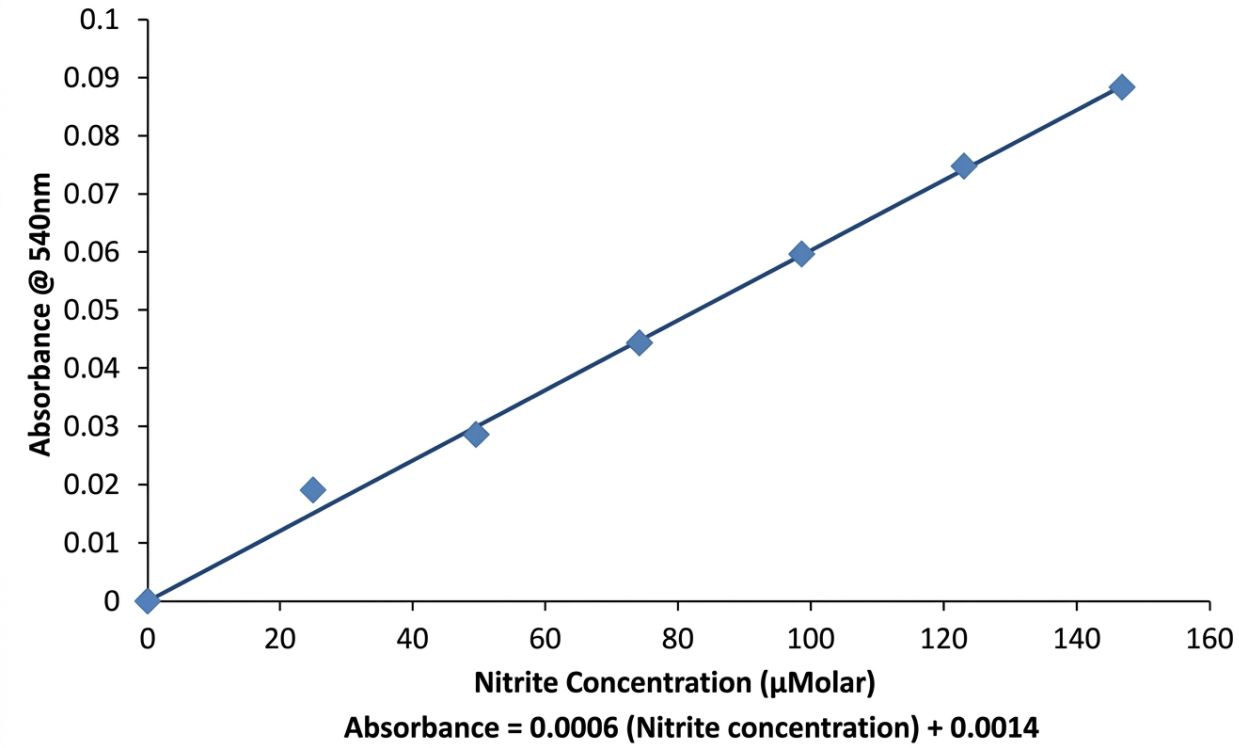

Figure 1*:* Standard curve for plasma NO concentration determination

2.5 Statistical Analysis

Data were evaluated using the independent samples t-test and the chi-square (χ^2) test. The relationship between the studied gene polymorphisms and preeclampsia risk was assessed by calculating odds ratios (ORs) along with their corresponding 95% confidence intervals (95% CIs) through binary logistic regression analysis, adjusting for potential confounding factors. The Hardy–Weinberg equilibrium (HWE) was assessed for each polymorphism. The statistical procedures were conducted with SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). A two-tailed $p < .05$ was regarded as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Demographic and Clinical Parameters

Selected demographic and clinical variables were compared between women with preeclampsia and normotensive pregnant controls (Table 3). Women with preeclampsia exhibited markedly elevated systolic blood pressure (147.73 ± 16.69 mmHg) compared with controls (105.32 ± 10.81 mmHg; $p < .001$), as well as significantly higher diastolic blood pressure (96.73 ± 12.70 mmHg vs. 66.29 ± 7.96 mmHg; $P < .001$). Additionally, preeclamptic participants demonstrated substantially greater body weight during pregnancy (80.00 ± 17.63 kg) than the control group (66.99 ± 10.22 kg; $P < .001$) and a higher mean body mass index (BMI) (30.25 ± 6.59 kg/m² vs. 25.75 ± 4.07 kg/m²; $P = .001$).

Table 3: Demographic and clinical parameters of patients with preeclampsia and control subjects

Parameter	Study group (PE) n = 15	Control group n = 72	P value
Age (years)			
mean±SD	33.07±5.29	31.24±5.34	.230
range	25 – 44	17 – 43	
median	32	31	
Gestational age (weeks)			
mean±SD	25.40±9.38	24.12±5.59	.483
range	12 – 40	8 – 38	
median	24	24	
Number of gestations			
mean±SD	2.60±1.06	2.22±1.06	.214
range	1 – 5	1 – 6	
median	3	2	
Number of parturitions			
mean±SD	0.87±0.74	0.85±0.94	.940
range	0 – 2	0 – 3	
median	1	1	
Body weight (kg)			
mean±SD	80.00±17.63	66.99±10.22	<.001
range	62 – 123	51 – 100	
median	73	67	

Body Mass Index (kg/m^2)			
mean±SD	30.25±6.59	25.75±4.07	.001
range	21.44 – 43.32	19.96 – 40.31	
median	27.943	24.912	
Systolic blood pressure (mmHg)			
mean±SD	147.73±16.69	105.32±10.81	<.001
range	127 – 180	74 – 138	
median	145	104	
Diastolic blood pressure (mmHg)			
mean±SD	96.73±12.70	66.29±7.96	<.001
range	84 – 129	48 – 86	
median	92.00	68.50	

**Value with P = .05 is considered significant.*

3.2 Genotype Distribution and Allele Frequency

Genotype and allele frequencies of the IL-4 VNTR polymorphism are shown in Table 4, and those of the eNOS 894G>T polymorphism are presented in Table 5.

The study group has 3 (37.50%), 3 (37.50%), and 2 (25.00%) of the *B1B1*, *B1B2*, and *B2B2* genotype, respectively, while the control group has 13 (20.00%), 20 (30.80%), and 32 (49.20%) displayed the *B1B1*, *B1B2*, and *B2B2* genotype respectively. Chi-square analysis revealed no significant difference in genotype distribution between the PE cases and controls (χ^2 = 1.996, *P* = .369). Allele frequencies were also comparable between groups. The B1 allele was present in 56.30% of alleles in the PE group compared to 33.80% in controls, while the mutated B2 allele occurred in 43.80% of alleles in PE cases compared with 66.20% in controls (χ^2 = 3.092, *P* = .079).

Table 4: Genotype and Alleles Frequency of the IL4-VNTR Polymorphism in Preeclampsia Patients and Control

Polymorphism of IL-4 VNTR	**Study group (PE)**		**Control group**		**χ^2 test**	**P value**
	Observed n (%)	**Expected (%)**	**Observed n (%)**	**Expected n (%)**		
B1B1	3(37.50)	22.5	13(20.00)	21.84		
BIB2	3(37.50)	31.3	20(30.80)	31.54	1.996	.369
B2B2	2 (25.00)	46.2	32 (49.20)	46.62		
Total	**8 (100.00)**	**100.00**	**65 (100.00)**	**100.00**		
B1	9 (56.30)		44(33.80)			
B2	7(43.80)		86 (66.20)		3.092	.079
Total	**16 (100.00)**		**130 (100.00)**			

**Value with P = .05 is considered significant.*

The eNOS 894G>T polymorphism showed the following genotype frequencies in preeclamptic women: the study group had GG in 0 (0.0%), GT in 7 (100.0%), and TT in 0 (0.0%). In the control group, the corresponding frequencies were GG in 4 (6.9%), GT in 52 (89.7%), and TT in 2 (3.4%). Chi-square analysis showed no significant difference in genotype distribution between preeclamptic cases and controls (χ^2 = 0.79, *P* = .67). Allele frequencies were also comparable between the two groups. The G

allele was present in 50.0% of alleles in the PE group and 51.7% in controls, while the mutated T allele occurred in 50.0% of alleles in PE cases versus 48.3% in controls (c^2 = 0.02, *P* = .90) (Table 3).

Table 5: Frequency of genotypes of 894 *G>T* polymorphism of the eNOS gene in women with preeclampsia and the control group

Polymorphism of 894 G>T	Study group (PE)		Control group		χ^2 test	P value
	Observed n (%)	Expected (%)	Observed n (%)	Expected (%)		
GG	0 (0.0)	5.7	4 (6.9)	6.2	0.79	.67
GT	7 (100.0)	91.4	52 (89.7)	90.7		
TT	0 (0.0)	2.9	2 (3.4)	3.1		
Total	**7 (100.0)**	**100.0**	**58 (100.0)**	**100.0**		
G	7 (50.0)		60 (51.7)		0.02	.90
T	7 (50.0)		56 (48.3)			
Total	**14 (100.0)**		**116 (100.0)**			

**Value with P = .05 is considered significant.*

3.3 Association of Genotype and Allele Frequency with Preeclampsia

The odds ratios (ORs) with 95% confidence intervals (CIs) assessing the relationship of the IL-4 VNTR and eNOS G894T polymorphisms with preeclampsia are shown in Table 6 – 7.

Unconditional logistic regression analysis showed no statistically significant associations between the IL-4 VNTR polymorphism and preeclampsia susceptibility. The heterozygous B1B2 genotype (OR = 0.65, 95% CI: 0.11–3.73, *P* = 0.63) and the homozygous B2B2 genotype (OR = 0.27, 95% CI: 0.04–1.81, *P* = 0.18) were not significantly linked to an increased or decreased risk of developing preeclampsia during pregnancy compared to those having the B1B1 homozygote wild-type genotype.

At the allelic level, individuals with the B2 variant allele did not demonstrate a significantly different risk of preeclampsia compared to those with the B1 allele (OR = 0.40, 95% CI = 0.14 – 1.14, *P* = 0.09), see (Table 6).

Table 6: Association between IL4-VNTR genetic polymorphism and development of preeclampsia.

Polymorphism of IL4-VNTR	OR	95% CI	P value
B1B1	1.00	Ref.	-
B1B2	0.65	0.11 – 3.73	.63
B2B2	0.27	0.04 – 1.81	.18
B1	0.40	Ref	-
B2	0.40	0.14-1.14	.09

**Ref. = Reference; Value with P = .05 is considered significant.*

Unconditional logistic regression analysis indicated no statistically significant difference between the eNOS 894G>T polymorphism and the risk of preeclampsia. Neither the GT heterozygote (OR = 2.17, 95% CI = 0.00, *P* = .99) nor TT mutated homozygote (OR = 1.00, 95% CI = 0.00, *P* = .99) genotypes showed a significant likelihood of preeclampsia development during pregnancy as compared to those having the GG homozygote wild-type genotype. At the allelic level, the presence of the mutated T variant allele was not associated with a risk of preeclampsia compared to the G allele (OR = 1.07, 95% CI = 0.35 – 3.25, *P* = 0.90), see Table 7.

Table 7: Association between eNOS gene *894 G>T* genetic polymorphism and development of preeclampsia

Polymorphism 894 G>T	OR	95% CI	P value
GG	1.00	Ref.	-
GT	2.17	0.00	.99
TT	1.00	0.00	.99
G	1.00	Ref.	-
T	1.07	0.35 – 3.25	.90

**Ref. = Reference; Value with P = .05 is considered significant.*

3.4 Plasma Nitric Oxide Concentration

Independent sample T-test comparison of plasma NO levels of the preeclamptic pregnant women with the normotensive pregnant women is presented below. There was no statistically significant difference between the two patient categories (Table 8).

Table 8: Plasma NO concentration of women with preeclampsia and the control group

Parameter	Study group (PE) n = 8	Control group n = 65	P value
NO concentration (µM)			
mean±SD	425.22±184.87	450.72±139.28	.543
range	164.33 – 609.33	164.33 – 811.00	
median	484.333	486.833	

**Value with P = .05 is considered significant.*

4.0 DISCUSSION

The current study investigated the association of IL4-VNTR and eNOS gene G894T polymorphisms with the risk of preeclampsia in the Nigerian population. This study demonstrates that there is no relationship between IL4-VNTR and G894T polymorphism and preeclampsia. Also, the preeclamptic patients and normotensive pregnant women show no significant difference in their plasma NO levels.

The present findings correspond with a previous investigation conducted among Taiwanese women, which similarly reported no evidence of an association between IL-4 gene polymorphisms and preeclampsia risk [41, 42]. Conversely, a UK-based study examined only one polymorphism, the IL-4 -590 C/T polymorphism and observed a notable trend toward increased preeclampsia prevalence among TT homozygotes [43]. Another study evaluated the possible association between IL-4 VNTR

polymorphism and susceptibility to PE in the Iranian population and found that the carriage of the RP2 allele has a positive association with preeclampsia susceptibility [19]. Also, Mingxuan et al. reported a significant correlation of NLRP3 and IL-4 VNTR polymorphisms with PE susceptibility in the female Han Chinese population [19]. Although numerous reports demonstrated the association of IL-4 VNTR genetic polymorphisms with preeclampsia risks, this study and one other found no relationship.

Consistent with the present findings, numerous studies have reported no association between preeclampsia risk and genetic polymorphism in the eNOS gene. A study on a Greek Caucasian population examined three common variants (4b/a, T-786C, and G894T) in the eNOS gene. The researchers found no significant association between any of these single loci and clinical preeclampsia status [44]. Also, in a large study of 844 pregnant women, no increase in the risk of preeclampsia was observed for the intron-4 or -786T>C polymorphisms of the eNOS gene under any model of inheritance [45]. Similarly, in another investigation in an Algerian cohort, researchers found no significant difference in the frequencies of the -786 T>C polymorphism between preeclamptic cases and healthy controls [46]. However, contrary to our findings, several studies have implicated the eNOS G894T polymorphism in the pathogenesis of various vascular disorders, including stroke, myocardial infarction, coronary atherosclerosis, venous thromboembolism, and preeclampsia, with the 894TT genotype suggested to confer heightened risk, particularly for coronary atherosclerosis [47-52]. Evidence of population-specific variability in genotype frequencies has also been reported. Yoshimura *et al*. [50] reported a significantly elevated frequency of the Glu298Asp variant in women with severe preeclampsia compared with milder cases or controls and therefore proposed it as a potential marker for heightened severe preeclampsia risk. Similarly, another study conducted in the Auchi area of Edo State, Nigeria, examined the relationship between eNOS, VEGF, ACE, and TNF-α gene polymorphisms and the risk of preeclampsia and reported positive associations for all the polymorphisms investigated [38]. These divergent results may reflect differences in the specific polymorphisms evaluated, sample characteristics, or the genetic makeup of the different populations.

In addition to the genetic analyses, we measured plasma nitric oxide (NO) concentrations in both groups and observed comparable levels between preeclamptic pregnant women and normotensive controls. This finding contrasts with another study where plasma NO concentrations were reported to be significantly lower in women with preeclampsia [6]. The discrepancy between our results may be attributable to ethnic and population differences.

Studies have also highlighted the importance of gene–gene interactions, where the combined effects of multiple polymorphisms may exert a stronger influence on preeclampsia risk than individual variants alone [38, 53]. However, in our study, we did not detect any gene–gene or gene–molecule interactions between the IL-4 VNTR and eNOS G894T polymorphisms, nor between the eNOS variant and plasma nitric oxide levels, as none of these factors showed an association with increased preeclampsia risk.

We are cautious in interpreting our findings due to noticeable limitations. First, the sample size of this study is relatively small in comparison to other genetic studies. Second, we focused on one polymorphism, though there are several variations within the eNOS gene that could influence phenotypic outcome. Third is the ethnic mix of the population evaluated in this study. This observation raises consideration of whether the lack of association between the eNOS 894G>T polymorphism and preeclampsia in our study may be influenced by ethnic confounding due to population stratification. Therefore, in future studies, ethnicity residual confounding could be assessed by genotyping highly polymorphic ancestry-informative markers (AIMs) whose allele frequencies vary across ethnic groups. Furthermore, the clinical and biological significance of these observations requires further assessment in larger study populations. We anticipate that the present dataset will prove valuable for use in future meta-analyses aimed at confirming or refuting the role of this polymorphism in preeclampsia susceptibility.

4. CONCLUSION

In conclusion, the present study found no statistically significant associations between the IL4-VNTR and G894T eNOS gene polymorphisms and preeclampsia among the Nigerian pregnant women studied.

Furthermore, plasma NO levels were comparable between preeclamptic women and normotensive women, suggesting that these polymorphisms do not contribute to the altered NO levels in this population. These findings should be regarded as preliminary and not as definitive evidence of no true association due to the small sample size in this study. Despite this limitation, this study contributes to the limited genetic data on preeclampsia in Nigeria and African populations. However, future research prioritising larger populations combined with meta-analyses separated by ethnicity will be important to clarify the role, if any, of IL4-VNTR and G894T eNOS gene polymorphisms in preeclampsia susceptibility across diverse groups.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

ADW designed the study, wrote parts of the protocol and concise the first draft, JOI, AFO and AOA collected, processed and analyzed samples, JOI conducted the statistical analysis of the data, JOI and JOA wrote the first draft of the manuscript, as assisted with sample collection and protocol, SOR assisted with study design and sample analyses, and the final copy was proofread by all authors.

ETHICAL APPROVAL

All consents and ethical approval for this research, with reference number LREC.06/10/830, were approved by the Health Research and Ethics Committee of Lagos State University Teaching Hospital

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