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Vitamin D Status and the Determinants of Preeclampsia in Pregnant Women in Goma (Democratic Republic of the Congo)

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Abstract

Background: Preeclampsia (PE) is a common condition, causing maternal and perinatal morbidity and mortality worldwide. In the absence of fully satisfactory treatment, screening remains one of the pillars of management. Low vitamin D status has been identified as a risk factor for PE. But, data on vitamin D status and risk factors for PE in the Democratic Republic of the Congo (DRC) is scanty. The aim of this study is to determine the level of Vitamin D and risk factors in preeclamptic patients in our environment. **Methods**: To fill this gap, we conducted a multicenter incident case control study on 190 pregnant women, 95 cases and 95 controls, receiving care from seven hospitals in Goma, in the eastern DRC, from April 1 to December 31, 2019. Socioeconomic, diet habits, clinical data, urinalysis and serum 25-hydroxy vitamin D [25(OH)D] levels were analyzed. Vitamin D deficiency was defined as serum 25(OH)D < 30 ng/ml. Bivariate and multivariate analysis were used to assess risk factors of PE. **Results**: The median vitamin D level in preeclamptic women was lower than in the control group (21.7 [Interquartile Range (IQR) = 19.2 - 24.1] ng/ml versus 28.5 [IQR = 24.9 - 31.4] ng/ml; (p < 0.001). PE was associated with: 1) vitamin D deficiency, Odds Ratio (OR) = 2.77 at 95% Confidence Interval-CI of [1.22 - 6.31]; p = 0.015; 2) previous history of PE (OR = 12.30; 95% CI [1.92 - 18.98]; *p* = 0.008) and 3) high BMI (OR = 2.82; 95% CI [1.28 - 6.21]; *p* = 0.010). Smoking (OR = 0.33; 95% CI [0.22 - 0.98]; *p* = 0.015) and consumption of dairy products (OR = 0.39; 95% CI [0.17 - 0.92]; *p* = 0.032) were protective. **Conclusion**: The odds of PE were 3-fold in preg-

nant women with vitamin D deficiency. Vitamin D supplementation during pregnancy might reduce the risk of developing PE and ultimately reduce the consequences on maternal and perinatal advert outcomes.

Keywords

Democratic Republic of the Congo, Vitamin D Deficiency, Preeclampsia Determinants

1. Introduction

Preeclampsia (PE) is a clinical syndrome characterized by the new onset of hypertension plus proteinuria, end-organ dysfunction, or both after 20 weeks of gestation in a previously normotensive woman [1]. PE affects globally 4.6% of pregnant women [2]. The highest prevalence occurs in developing countries, reaching 18% [3]. PE is the second leading cause of maternal death and significant maternal and fetal morbidity and mortality [4].

The pathophysiology of PE is complex. Multiple etiological factors and pathways have been cited, including: abnormal development of the placenta (abnormal remodeling of spiral arteries, defective trophoblast differentiation and placental hypoperfusion, hypoxia, ischemia); immunologic, genetic and environmental factors (High body mass index, low calcium intake), inflammation (attributed to circulating syncytiotrophoblast debris); increased sensitivity to angiotensin II; complement activation and systemic response to generalized endothelial dysfunction [5] [6] [7].

Currently, there is no PE curative treatment other than delivery, none of the interventions targeting one or another factor above have shown effectiveness in PE prevention in the general obstetric population [8] [9]. In recent years, various studies suggested the association between poor vitamin D status and PE [10] [11]. In addition, vitamin D supplementation demonstrated some promising results [12].

However, in the Democratic Republic of the Congo, data on vitamin D status and the risk factors of PE are scarce. The objective of this study was to assess the association between vitamin D status and the determinants of PE, prior to vitamin D supplementation trial in Goma.

2. Materials and Methods

2.1. Study Location

The study was conducted in Goma, a city in the eastern Democratic Republic of the Congo, with a total population of over a million, an average annual temperature of 20.5°C and an annual rainfall of 1144.6 mm (from 1971 to 2016). The soil is essentially volcanic. There are no rivers or streams in the town due to volcanic eruptions, but rather a large lake (Lake Kivu) rich in methane gas [13].

Like all over the country, Goma has permanent sunshine throughout the year.

2.2. Study Design and Sample Size

It was a multicenter, incident, unmatched, case control study. The sample size was calculated using the formula:

$$n = t^2 \times p \times (1-p) / m^2$$

(n = minimum sample size required; t = critical value for a confidence level of 95 %, *i.e.* 1.96; p = estimated proportion of the population with the characteristic. (p = 0.085) [14]; m = margin of error, *i.e.* 0.05). So, n was equal to $1.96^2 \times 0.085 \times (1-0.085) / 0.05^2 = 119.51$ pregnant women.

2.3. Ethics and Consent

The purpose of the study was communicated in the local language to eligible women. Oral and written informed consents were obtained from all participants. The research project was approved by the Institutional Review Board of the University of Lubumbashi in its February 8, 2019 session (Reference N˚ UNILU/CEM/125/2019). In addition, the study was authorized by the provincial health division of North Kivu.

2.4. Eligibility Criteria

Inclusion criteria for cases were: pregnant women diagnosed or followed up for PE in one of the seven hospitals selected. PE was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg, taken twice at 4-hour intervals, at rest after 20 weeks of amenorrhea with proteinuria ≥ 2 crosses or 30 mg/dL [8]. A woman attending ANC (antenatal care) visits with normal pregnancy evolution and no apparent chronic or debilitating conditions, immediately seen after an identified case in the same facility, was considered as a control, until we reached the desired sample size. Women were excluded if they refused to consent for the study or refused to provide a blood sample, or if they had hepato-renal disease, multiple pregnancy, or fetal death in utero.

2.5. Data Collection

Before data collection, experienced midwifes and doctors received training on all aspects of the study procedures, from how to complete the study questionnaire, to blood pressure and anthropometric measurements. Phlebotomists were trained on good practice of urine and blood collection techniques, labelling, storage, cold chain maintenance, and transportation procedures.

To standardize the perceptions of pregnant women and minimize biases when collecting data, we have adopted the following operational definitions:

- Regular consumption of milk or milk product derivatives corresponded to the pregnant woman's statement that she consumed one of these products at least once every two days.
- Previous use of modern contraceptive methods meant the use of either a

condom or an estrogen-progestin contraceptive during the period prior to the current pregnancy.

- ANC follow-up meant having received prenatal care from a nurse or physician for the current pregnancy prior to selection for the study.
- Blood pressure was taken at heart level in the arm by a calibrated OMRON JP N1 Intellisensa (Omron Healthcare Co, Japan) electronic blood pressure monitor.

2.6. Laboratory Sample Collection, Processing and Analysis

Blood was collected by venipuncture into 5 mL tubes (Becton Dickinson, Franklin Lakes, NJ, USA) that contained no anti-coagulant. The sample was left at room temperature for 30 min to clot and then placed in a cool box containing ice, for transport to Provincial Hospital of North Kivu Laboratory for processing, between 2 and 4 h. Samples were centrifuged at 3000 rpm for ten minutes to separate the serum, which was then aliquoted into 2 mL cryotubes (Sarstedt, CryoPure®), stored at – 80˚C. Series of 50 samples were analyzed for serum 25(OH)D, using iChroma II analyser (Boditech Med Inc, South Korea).

Proteinuria was diagnosed at the ANC facility making using dipstick test (GEN 10SLG, URISCAN®).

2.7. Data Management and Statistical Analysis

Data was recorded using Microsoft Excel (Microsoft Corporation, Redmond, WA), Office 2010. Data was checked for normality (Kolmogorov-Smirnov test) and changed into logarithmic scale if necessary. Continuous variables were summarized as mean and standard deviation (SD), or as median and interquartile range for non-normal variables, and as number or percentages for categorical variables. Then the following statistical tests were used: Chi-square test, Fisher's exact test, Student's t-test, Mann-Whitney, and Kruskal-Wallis tests. The ANOVA test or test of variance was used for multiple comparisons with post hoc use of the Bonferroni test. Logistic regression model after stepwise backward removal was used to examine the independent association between different factors and PE, the dependent variable. Statistical analyses were performed using IBM SPSS 23 statistical software, and the statistical significance level was fixed at $p < 0.05$.

Preeclampsia was considered severe if SBP was >160 mmHg and/or DBP > 110 mmHg and moderate if SBP was 140 - 160 mmHg and/or DBP 90 - 110 mmHg. Preeclampsia was considered late when diagnosed after 34 weeks of pregnancy [15].

3. Results

3.1. Participants Characteristics

With respect to the socio-demographic characteristics considered, there were no significant differences between preeclamptic women and controls in our study population (Table 1).

Table 1. Distribution of patients according to socio-demographic characteristics.

Characteristics		Pregnant women			
		Control	Case	*p*-value	OR [IC 95%]
Age (years)		28.0 ± 5.8	29.7 ± 6.5	0.075**	
	<18	3 (3.2)	3 (3.2)		0.60 [0.10 - 3.46]
	18 - 34	79 (83.2)	68 (71.6)		0.54 [0.10 - 3.08]
	≥35	13 (13.7)	24 (25.3)		2.85 [0.325 - 10.49]
Residence				0.362*	
	Urban	73 (76.8)	76 (80.0)		1.21 [0.60 - 2.41]
	Rural	22 (23.2)	19 (20.0)		0.83 [0.42 - 1.66]
Educational status				0.513***	
	Illiterate. read and write	13 (13.7)	8 (8.4)		0.56 [0.19 - 1.62]
	Elementary	17 (17.9)	13 (13.7)		1.24 [0.40 - 3.88]
	Secondary	44 (46.3)	51 (53.7)		1.88 [0.72 - 4.96]
	university	21 (22.1)	23 (24.2)		1.78 [0.62 - 5.14]
Marital status				0.240***	
	Single	4 (4.2)	6 (6.3)		0.73 [0.24 - 2.20]
	Married	89 (93.7)	89 (93.7)		1.36 [0.45 - 4.09]
	Divorced	2 (2.1)	0 (0.0)		-
Occupational status				0.096***	
	housewife	56 (58.9)	46 (48.4)		0.41 [0.12 - 1.45]
	Employee	4 (4.2)	15 (15.8)		1.75 [0.34 - 8.98]
	Pupil/student	5 (5.3)	5 (5.3)		0.50 [0.09 - 2.81]
	Merchant	6 (6.3)	4 (4.2)		0.33 [0.06 - 1.91]
	Resourcefulness	20 (21.1)	17 (17.9)		0.43 [0.11 - 1.66]
	Others	4 (4.2)	8 (8.4)		1.22 [0.33 - 4.47]
Total		**95**	**95**		

** Student's t-test; *** Pearson Chi-square test; *Fisher's Exact Test.

Information related to lifestyle, obstetrical, clinical and biochemical characteristics is given below (Table 2).

IQR = InterQuartile Range (25 & 75); DBP = Diastolic Blood Pressure; SBP = Systolic Blood Pressure; BMI = Body Mass Index.

Serum Vitamin D levels in preeclamptic patients displayed the following profile: deficient (9.5%), insufficient (61.1%), and normal values (only 20%).

Overall, there was a statistically significant relationship between PE and the following clinical parameters: personal PE history, primipaternity, personal history of hypertension, family history of hypertension, family history of PE on the spousal side, family history of PE on the maternal side, BMI, and serum vitamin

Table 2. Distribution of patients according to lifestyle, obstetrical, clinical and biochemical characteristics.

Characteristics		Pregnant women			
		Control	Case	*p*-value*	OR [IC 95%]
Mode of admission				0.381	
	Not referred	63 (66.3)	60 (63.2)		0.87 [0.48 - 1.58]
	Referred	32 (33.7)	35 (36.8)		1.15 [0.63 - 2.08]
History of HBP		4 (4.2)	17 (17.9)	**0.002**	4.96 [1.60 - 15.36]
History of prior preeclampsia		2 (2.1)	19 (20.0)	**<0.001**	11.63 [2.63 - 51.49]
Contraceptive history		17 (17.9)	25 (26.3)	**0.011**	1.64 [0.82 - 3.29]
Alcohol intake		25 (26.3)	23 (24.2)	0.434	0.89 [0.47 - 1.72]
Consumption of milk and derivatives		72 (75.8)	59 (62.1)	**0.030**	0.52 [0.28 - 0.98]
Smoking status		5 (5.3)	1 (1.1)	**0.016**	0.19 [0.02 - 0.67]
Family history of HBP		28 (29.5)	42 (44.2)	**0.025**	1.90 [1.04 - 3.45]
Family history of diabetes		17 (17.9)	17 (17.9)	0.575	1.00 [0.48 - 2.10]
Spouse's family history of PE (mother. sisters. aunt)		2 (2.1)	9 (9.5)	**0.029**	4.87 [1.02 - 23.19]
Maternal family history of PE (Mother. sisters. aunt..)		6 (6.3)	19 (20.0)	**0.004**	3.71 [1.41 - 9.76]
Primipaternity		4 (4.2)	8 (8.4)	**0.019**	2.09 [1.61 - 7.20]
		2.0 (1.6 - 3.1)	2.0 (1.9 - 4.1)	0.945	
Parity (median and IQR)	*Nulliparity*	26 (27.4)	28 (29.5)		0.77 [0.36 - 1.64]
	Gravida (1 - 3)	29 (30.5)	24 (25.3)		0.96 [0.38 - 2.39]
	Gravida (4 - 6)	25 (26.3)	30 (31.6)		1.24 [0.50 - 3.10]
	Gravida (>6)	15 (15.8)	13 (13.7)		1.39 [0.56 - 3.45]
History of prior abortion				0.103	
	Non	71 (74.7)	62 (65.3)		0.64 [0.34 - 1.19]
	Oui	24 (25.3)	33 (34.7)		1.31 [0.90 - 1.91]
Antenatal care follow up (ANC)		88 (92.6)	81 (85.3)	**0.048**	0.46 [0.18 - 1.2]
BMI (kg/m²)		25.4 ± 3.1	27.4 ± 5.2	**0.002**	
	18.5 - 24.9	37 (49.3)	24 (30.8)	**0.030**	0.30 [0.11 - 0.84]
	25 - 29.9	32 (42.7)	34 (43.6)	**0.009**	1.52 [0.75 - 3.09]
	30 - 35	6 (8.0)	20 (25.6)	**0.002**	5.07 [1.77 - 14.54]
SBP		111.8 ± 11.3	167.9 ± 24.2	**<0.001**	
DBP		68.2 ± 9.6	106.7 ± 18.4	**<0.001**	
Gestational age at screening (weeks)		34.5 ± 13.4	34.1 ± 4.4	0.897	1.36 [0.21 - 1.52]
25[OH] D (D₂ et D₃) Vitamin D Median and IQR		28.5 (24.9 - 31.4)	21.7 (19.2 - 24.1)	**<0.001**	
	Deficiency	5 (5.3)	9 (9.5)	**0.005**	3.60 [1.06 - 5.12]
	Insufficiency	52 (54.7)	67 (70.5)	**0.040**	2.96 [1.33 - 4.97]
	Normal	38 (40.0)	19 (20.0)	**0.004**	0.38 [0.2 - 0.72]
Total		**95**	**95**		

*Fisher's Exact Test. IQR = Inter Quartile Range (2 5 & 75); DBP = Diastolic Blood Pressure; SBP = Systolic Blood Pressure; BMI = Body Mass Index.

D level, with respective odds ratios ranged from 1.64 to 11.63 times higher than in controls. The BMI mean value in preeclamptic patients was significantly higher than in controls; BMI normal values represented a protective factor (OR = 0.30; 95% CI: [0.11 - 0.84]), while obesity was a real risk factor (OR = 5.07; 95% CI: [1.77 - 14.54]). As expected, mean values for SBP and DBP were higher in preeclamptic patients than in controls. As for vitamin D, whose median concentration was significantly lower in preeclamptic patients, concentrations below the normal value (deficit and insufficiency levels) were significantly more associated with preeclampsia (OR = 3.60; 95% IC: [1.06 - 5.12] and 2.67; 95% CI: [1.39 - 5.10], respectively), whereas normal concentrations showed a significant protective effect (OR = 0.38; 95% CI: [0.2 - 0.72]). Furthermore, consumption of milk and its derivatives (OR = 0.52; 95% CI: [0.28 - 0.98]), smoking (OR = 0.19; 95% CI: [(0.02 - 0.67]) and follow-up of ANC (OR = 0.46; 95% CI: [0.18 - 0.97]) showed a significantly more protective association with regard to preeclampsia.

As for the mode of admission to hospital, only 36.8% of patients were regularly transferred.

The median gravidity was the same for preeclamptic patients (4.0; IQR: 3.0 - 5.0) and controls (4.0; IQR: 3.0 - 4.4); besides, there was no difference of average gestational age at screening between them (34.1 ± 4.4 versus 34.5 ± 13.4 weeks).

Proteinuria ≥ 3 crosses was registered in 48.4 % of preeclamptic patients, and 70.5 % of these presented a clinical picture of severe PE. Based on the gestational age at screening for PE, 61.1% of patients had developed early PE.

The most common complaints reported by preeclamptic patients were headache (57.9%), visual abnormalities (37.9%) and tinnitus (30.5%).

With regard to the clinical course in preeclamptic patients, 5.3% of the cases progressed to eclampsia.

3.2. Risk Factors Associated with Preeclampsia

Table 3. Risk factors associated with preeclampsia.

Characteristics		Bivariate analysis		Multivariate analysis	
		p-value*	OR (IC95)	*p*-value*	OR (IC95)
Primipaternity					
	No		1		1
	Yes	0.024	2.09 [1.61 - 7.20]	0.339	1.99 [0.48 - 8.27]
History of HBP					
	No		1		1
	Yes	0.006	4.96 [1.60 - 15.36]	0.009	4.02 [1.89 - 8.05]
Past history of preeclampsia					
	No		1		1
	Yes	0.001	11.63 [2.63 - 15.49]	0.008	12.30 [1.92 - 18.98]
Consumption of milk and derivatives					

Continued

	No		1		1
	Yes	**0.043**	0.52 [0.28 - 0.98]	**0.032**	0.39 [0.17 - 0.92]
Smoking status					
	No		1		1
	Yes	**0.013**	0.19 [0.02 - 0.67]	**0.015**	0.33 [0.22 - 0.98]
Family history of HBP					
	No		1		1
	Yes	**0.036**	1.90 [1.04 - 3.45]	0.920	1.04 [0.46 - 2.38]
Spouse's family history of PE (mother, sisters, aunt)					
	No		1		1
	Yes	**0.047**	4.87 [1.02 - 7.57]	**0.028**	5.53 [1.35 - 8.69]
Maternal family history of PE (Mother, sisters, aunt..)					
	No		1		1
	Yes	**0.008**	3.71 [1.41 - 9.76]	0.994	1.01 [0.24 - 4.18]
Antenatal care follow up (ANC)					
	No		1		1
	Yes	**0.011**	2.17 [1.84 - 5.65]	0.137	1.89 [0.37 - 4.14]
BMI (Kg/m²)					
	<25				
	≥25	**0.019**	2.22 [1.14 - 4.34]	**0.010**	2.82 [1.28 - 6.21]
Vitamin D 25[*OH*] *D* (*D₂ et D₃*)					
	Normal		1 (bivariate)		1 (multivariate)
	Deficiency	**0.003**	2.67 [1.39 - 5.10]	**0.015**	2.77 [1.22 - 6.31]

*Logistic regression.

4. Discussion

To the best of our knowledge, this is the first time that vitamin D status and risk factors for PE are jointly assed in the DR Congo.

4.1. Socio-Demographic and Clinical Characteristics

From the analysis of the socio-demographic parameters (Table 1), an overall observation has been made: there was no statistically significant difference between the two groups, *i.e.* PE pregnant cases and controls, for the traits investigated.

Although a significant proportion of preeclamptic patients (71.6%) was found

in the normal reproductive age group, our study did not show a statistically significant difference. However, both extreme age groups, <18 year (3.2%) and >35 year (25.3%), were risk factors. Similar findings with higher values were reported by Essam *et al.* in Cairo for both [18 - 21 year] (32.0%) and [26 - 30 year] (41.0%) age groups [16]. In Kampala, the lowest proportion of preeclamptic women (17.9%) was aged 30 year old or older (OR = 1.58; 95% CI: 0.94 - 2.62) [17]. There was no statistically significant difference between the mean ages of preeclamptic cases and controls ($P = 0.075$). For preeclamptic women, the mean age was 29.7 ± 6.5 years and more or less similar to that reported by Kiondo *et al.* (24.07 ± 5.15 years), Maereg *et al.* (26.75 ± 5.08 years), and Merviel *et al.* (28.6 ± 1.5 years) [17] [18] [19]. Our findings are in line with reports by Beaufils *et al.* who argued that the classic double-hump distribution with a peak in very young women under 20 years and a second peak in women over 37 - 40 years of age was no longer observed in several studies except in some developing countries [20].

As regards the relationship between PE and the residence of pregnant women in this study, the majority of PE women were urban residents, 80% of whom were from the city of Goma. Significantly, similar rates of 79.0% were found in Cairo and Addis Ababa [16] [18]. Urban residence appeared to be a protective risk factor in Cairo, and the risk of PE was 4-fold higher among pregnant women living in rural areas [16]. Lack of financial and logistical resources for evacuation of PE women from rural areas may account for the absence of a similar risk profile, then supporting the lower admission rate (limited accessibility) of these rural pregnant women in this study, in comparison with the observations made in Cairo [16].

The majority of the PE pregnant women (77.9%) in our sample had a high school or university education level, while Essam *et al.* [16] reported 48.0% illiteracy in their Cairo study. The difference remained insignificant between levels of education in preeclamptic women and controls in Goma ($p = 0.513$). In Kampala [17], the risk of PE was about 1.31-fold higher for the merged group of primary education and illiterates categories, whereas in Goma it was 1.24-fold higher for the primary level and 0.56-fold lower for the illiterates with no significant difference. It should be noted for our study that the risk of PE was about 2-fold higher for the high school and university education levels, taken separately but with no significant difference. The high proportion of ANC attendance in both groups (preeclamptic and control) was indubitably due to the influence of level of education. A pregnant woman with higher education level is more likely to see a doctor if her health status is not balanced. The results observed elsewhere would probably be a consequence either of poverty or of gender-based religious discrimination commonly observed in certain states under religious influence, affecting the female gender mainly in the area of formal education.

The difference about occupations observed in preeclamptic women and controls in Goma was found to be insignificant ($p = 0.096$), whereas in Cairo [16] housewives were at greater risk than those in Goma. It is generally admitted that professional qualification is related to the level of education, and education en-

sures adequate socio-economic status with a lower potential risk of unemployment. In Goma, 48.4% of preeclamptic women were housewives (unemployed) versus 56% in Cairo [16] with a similar risk of about 1.75 for both sites, whereas the proportion of illiterates (48%) in Cairo was higher than in our study (8.4%). Unemployment-related lack of income may perpetuate early marriages that lead to accelerated reproductive activity with negative impact on socio-economic level. Pregnant women living alone, in this study, were not at risk of PE ($p = 0.240$) compared to the findings in Kampala ($p = 0.025$) [17]. As for the onset of PE, 61.1% of PE cases were early vs. approximately 10% by Cunningham *et al.* [21] who reported that high proportions were observed beyond 34th week and even during delivery or postpartum.

The mean gestational age at screening was 34.1 ± 4.4 weeks compared with 35.83 ± 3.7 weeks found in Addis Ababa [18].

PE clinical manifestations of the disease were highly variable. However, considering order of frequency in the literature, headache and visual disturbances followed by abdominal pain, altered consciousness and dyspnea have been noted. Like Sibai *et al.* [22], we found the same order of frequency with headache and then visual disturbances.

Bivariate analysis (Table 2) revealed the following risk factors for PE in our study: primipaternity, personal history of hypertension, personal history of PE, history of hypertension in the immediate family, history of PE in the immediate family (husband and/or pregnant side), lack of ANC follow-up, BMI ≥ 25 Kg/m² and vitamin D deficiency and insufficiency, while dairy consumption and smoking had emerged as protective factors against PE. After adjusting by multivariate analysis, personal history of hypertension, PE and family history of PE (husband's side), and vitamin D deficiency and insufficiency (<30 ng/mL) persisted as independent risk factors for PE (Table 3). In contrast, dairy consumption and smoking also independently reduced the risk of PE. Therefore, all of the above factors should be considered as determinants of PE for this study.

Regarding the history of PE (Table 2 & Table 3) and except the study conducted in Cairo [16] with a calculated risk estimated at 2.85-fold higher, many authors have found similar results ranging from 5.08 [23] to 8.4 [24], 8.12 [19], and 12.30-fold higher for our sample. Genetic factors are involved in the etiology of PE. Studies of candidate genes have provided evidence, although controversial, of links to several genes, including the gene coding for angiotensinogen located on 1q42-43 and eNOS (Endothelial NOS or nitric oxide synthase 3) on 7q36. The authors note that the locus related to Preeclampsia/Eclampsia is located on chromosome 2p [25] [26].

The influence of personal history of HBP (Table 2 & Table 3) seems to be unanimously reported in the literature regarding its predictive power as highlighted in studies conducted in Kampala [17], Amiens [19], California [27], Goma (this study) and even in the systematic review by Duckitt K. *et al.* [34]. The OR values observed by these authors vary from 2.3 to 5.1.

Family history of PE on the pregnant woman's side and more on the husband's side are significant determinants; Merviel P. *et al.* [19] and Duckitt K. *et al.* [24] confirmed this risk in terms of overall family history with OR values of 1.04 and 2.90, respectively. In the present study, the husband's family displayed a 5-fold higher risk.

These results support the hypothesis that the genotype of the fetus contributes to the overall risk of preeclampsia. Our results confirm studies that have suggested a paternal component in the genetic predisposition to PE. Some genes involved in the development of PE, such as the T235 allele of the angiotensinogen, the Factor V Leiden mutation and variants of the methylenetetrahydrofolate reductase gene, may be paternal in origin. There is evidence of increased incidence of PE as a result of maternal and/or paternal genetic predisposition [28].

4.2. BMI, Tobacco and Diet

Consistent results have been reported regarding the association between overweight and obesity on the one hand and PE on the other hand: A 2-fold increased risk was reported for obesity in Kampala [17], and a 3-fold increased risk by Duckitt [24]. Like obesity, overweight also confers a risk of 2.5 for Merviel [19], 2.1 for Duckitt [24] and 2.82 in Goma. O'Brien reports that the risk of preeclampsia has generally doubled with each 5 to 7 kg/m^2 increase in body mass index before pregnancy [29]. In Maya females, women with a higher prepregnancy BMI have a higher risk of PE than those with a normal pregnancy (RR = 2.82; p = 0.008 for overweight and RR = 4.22; p = 0.001 for obesity) [30].

Obesity would probably increase the risk of PE by inducing chronic inflammation along with endothelial dysfunction which together may mutualize with placental antiangiogenic factors to induce microangiopathic pathology, phenomena that characterize PE [31] [32].

Tobacco use and dairy consumption were characterized by a seemingly protective potential with regard to the results of our study (**Table 3**). Similar findings for tobacco alone were found in Amiens by Merviel [19], in Cairo [16] and in Kampala [17].

The debate triggered by the protective role of smoking remains topical due to various mechanisms that are contradictory in some respects. This role is controversial: on one hand, some authors consider the carbon monoxide (CO) role in cigarette smoking. Indeed, CO increases trophoblastic invasion and remodelling of the uterine arteries, decreases the local inflammatory response, increases placental blood flow through a vasodilator effect, decreases apoptosis phenomena at the level of the syncytiotrophoblast and finally could bind to nitrogen monoxide (NO) receptors. In addition, it has recently been described that smoking induces a higher serum PlGF (Placenta Growth Factor) level, which would promote good placental development and thus effectively counteract predisposition to PE [33]. This hypothesis seems interesting since it explains the involvement of CO (carbon monoxide) from the placental level before the

onset of endothelial disorders. However, it is weakened by the deleterious effects of tobacco, which is reputed to be a risk factor for cardiovascular disease, type II diabetes and numerous unfavourable pregnancy outcomes such as prematurity, low birth weight, intrauterine growth retardation, placental abruption, retro-placental hematomas, perinatal and maternal mortality [34] [35] [36].

Regular consumption of milk or its declared derivatives would act through the proven richness of milk in calcium, *i.e.* 117 mg/100g. It has been shown that providing women with a calcium supplementation during the second half of pregnancy reduces their risk of high blood pressure, proteinuria, and other related problems such as seizures, stroke, clotting disorders, pulmonary edema, kidney failure, and even death. As the proposed biological mechanism for the relationship between calcium and blood pressure is not yet fully confirmed, the vascular and immunological effects of calcium are suspected [37] [38].

4.3. Vitamin D

Taken separately, the 3 categories of vitamin D concentration (*i.e.* deficiency, insufficiency and normal conditions) showed an increasing risk from the condition of insufficiency (OR = 2.96) to that of deficiency (OR = 3.60) with 70.5% and 9.5 % of PE patients, respectively. The group referred to as "low level", in the present study, is a combination of these two categories. Patients in this group were three times more likely to have PE than patients with normal vitamin D levels in our study population (AOR = 2.77; 95% CI [1.22 - 6.31]).

It should be noted, however, that the results on vitamin D levels (in other studies) remain divergent in the literature: some authors have found no relationship between vitamin D deficiency measured in early pregnancy and PE [39] [40] [41]; for others there is a clear relationship when measured in late pregnancy [42] [43] [44]. The first aforementioned authors [43] [44] [45], were convinced that the risk of PE appears to depend on the time (at the beginning or at the end of pregnancy depending on the season) for vitamin D assay. Considering this hypothesis to be true, what would be the results observed in regions such as ours with almost permanent sunny periods?

It has been observed that the level of enzymes involved in the synthesis of vitamin D (24-hydroxylase and 1-alpha-hydroxylase) is not influenced by the season even though vitamin D levels were found to be low in winter when PE occurred in summer or spring [46] [47] [48].

Limitations

Many other risk factors found in the literature were not incorporated into our study due to uncertainty about the sincerity and accuracy of the responses to be given by the couple and the lack of locally Assisted Human Reproduction units.

As the vitamin D dosage may vary with the gestational age according to some authors, our assessment only took into account the moment of inclusion of the pregnant woman in the study. We did not standardize the timing of dosing. Be-

sides, we have not had white pregnant women or other racial groups in our study population. However, the study has the advantage of having covered all categories of pregnant women in our environment and to have done the dosage according to the procedure.

Authors' Contributions

Kabuyanga Kabuseba contributed to the preparation of the manuscript of this article from the outset as principal investigator. Dr. Balungwe participated in the data collection. Professors Lundimu, Elongi, Kalenga and Kakoma critically reviewed the manuscript, edited and corrected the text from the proposal to the development of this manuscript. All authors have read and approved the final manuscript.

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Conflicts of Interest

There is no conflict of interest.

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