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# Calcemia, Vitamin D and seasonal influences in preeclampsia in Goma

Richard Kabuyanga Kabuseba [1, *], Pierrot Lundimu Tugirimana [2], Jean Pierre Elongi Moyene [3], Xavier Kinenkinda Kalume [4] and Jean-Baptiste Kakoma Sakatolo Zambèze [5]

[1] *Department of Gynecology-Obstetrics, University of Goma, Goma, DR Congo.*

[2] *Department of Laboratory & Basic Sciences, University of Goma, Goma, DR Congo.*

[3] *Department of Gynecology-Obstetrics, General Hospital of Kinshasa, Kinshasa, DR Congo.*

[4] *Department of Gynecology-Obstetrics, University of Lubumbashi, Lubumbashi, DR Congo.*

[5] *Department of Gynecology-Obstetrics and School of Public Health, University of Lubumbashi, Lubumbashi, RDC.*

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## Abstract

**Background**: The etiology of preeclampsia remains less well known. It is noted that low vitamin D levels are associated with a high risk of preeclampsia (PE). Calcium ($Ca^{2+}$) levels during pregnancy appear to be involved in pregnancy-induced hypertension. Recent studies indicate that serum calcium levels may have a role in preeclampsia. Vitamin D promotes absorption of proper concentration of calcium in the blood which helps to lower blood pressure. The complications associated with calcium deficiency during a normal pregnancy are numerous and have not been extensively studied in Goma.

**Objective**: To assess blood calcium levels (ionic and total) in preeclamptic women and to analyse the seasonal influence on preeclampsia in Goma.

**Method**: A prospective case-control study (without matching) of 190 pregnant women without cardiovascular or endocrine diseases for a case-control ratio of 1:1 was conducted in six hospitals in Goma. Blood ionogram was performed by an automated system directly after blood sampling and vitamin D was measured using enzyme-linked immunosorbent method.

**Results**: The mean ionised calcium level in preeclamptic woman was 1.24±0.16 mmol/L (0.48-1.59) compared to 1.27±0.17 mmol/L (0.88-2.30) in normal pregnant woman (p=0.214). A slight negative correlation between blood calcium and blood pressure was observed in pregnant women. Low vitamin D levels were associated with preeclampsia. Hypovitaminosis D in the preeclamptic group was more observed during the rainy season than during the dry season. Pregnancies complicated by PE were from fertilisations occurring during the rainy season while the dry season was characterised by a high admission of preeclamptics.

**Conclusion**: The study found that preeclamptic women in Goma had hypocalcemia. There was also a weak negative correlation between blood pressure and serum calcium levels. The majority of preeclamptics were diagnosed during the dry season, while conception with a PE complication occurred during the rainy season. As this is a first study in this area for the Great Lakes region of Africa, a more in-depth study with a larger sample size is desired.

**Keywords:** Preeclampsia; Calcium; Vitamin D; Season; North Kivu

* Corresponding author: Richard Kabuyanga Kabuseba

Department of Gynecology-Obstetrics, University of Goma, Goma, DR Congo.

## 1. Introduction

Preeclampsia (PE) is one of the hypertensive disorders of pregnancy that affects 10% of pregnant women [1]. The prevalence of the condition is thought to be higher in developing countries where malnutrition is prevalent. Although its etiology remains partially unknown, the results of 2 meta-analyses [2,3] show that maternal vitamin D deficiency is associated with a high risk of PE [4-13]The observed relationship between PE and disruption of the metabolism of essential micronutrients such as calcium and magnesium has been investigated [14-22].

Reports of clinical studies on the benefits of vitamin D [13] and micronutrients [14, 22-26] in pregnant women are still controversial and some have shown that nutritional interventions can reduce the risk of preeclampsia. In addition to its established role in bone (phospho-calcium) metabolism, vitamin D is also endowed with extra-skeletal properties. The pleiotropic nature of its receptors is accompanied by potential actions in the pathophysiological mechanisms evoked in the onset and maintenance of preeclampsia. However, hypovitaminosis D appears to be a global public health problem with a prevalence of 18-84% in a population, depending on the country and other determinants considered [27, 28]. Hypovitaminosis D is estimated to affect 40% of pregnant women and is very frequent during breastfeeding [8,27-33]. The determinants of vitamin D are mainly represented by season, latitude, surface area covered by clothing, time of day of sun exposure, use of sunscreen, skin colour, increased urbanisation, air pollution and vegetarian diet [34]. Thus, hypovitaminosis D prevalence remains dependent on the aforementioned factors and the seasonal influence on PE also remains debated [35-41]. Researchers have turned to the analysis of weather variations, generally temperature or precipitation [42-44]. Studies have also looked at the possible influence of the time of conception on PE [45]. With the exception of smoking, no significant environmental risk factors have been identified, but evidence suggests a relationship between PE and the season of conception or the season of birth [43, 46]. Hypovitaminosis D is more prevalent in the winter months than in the summer months in some countries [13, 47, 48]. Vitamin D is involved in phospho-calcium metabolism and its supplementation in pregnant women is being investigated worldwide. It promotes calcium absorption. However, epidemiological and clinical data on calcium show an inverse relationship between calcium intake and the development of hypertension in pregnancy [49-52] and the data also demonstrate the benefit of supplementation in pregnant women living in calcium deficient areas [50,53]. Calcium plays a role in reducing hypertensive disorders in pregnancy [49,54]. Its impact is also related to pre-existing risk factors and the contributory role of diet type [54]. Vitamin D deficiency also affects the calcium balance of the maternal-fetal unit and is a risk factor for PE [55]. Calcium is involved in the control of hypertension by inhibiting the release of Parathyroid hormone (indirect regulation of blood pressure) and Renin (control of angiotensin I production) [56]. Calcium may enhance diuresis (sodium excretion) and regulate blood volume and cardiac output via regulation of the sympathetic nervous system [57]. The change in plasma calcium concentration leads to an alteration in blood pressure and its low level will induce vasoconstriction as a result of calcium accumulation in vascular smooth muscle [58]. This massive entry of calcium into the cell causes an increase in blood pressure. Nutritional calcium deficiency is implicated in preeclampsia, eclampsia, intrauterine growth retardation and even preterm delivery [50]. Evidence demonstrating the inverse relationship between hypertension and calcium intake is provided in several systematic reviews [59,60]. Simultaneous supplementation with calcium and vitamin D has been shown to have better hypotensive activity than calcium or vitamin D alone [61].

In Goma, we have no data on blood calcium levels, their relationship with vitamin D and the seasonal influence on PE. The objective of this study is to evaluate the level of this prohormone in preeclamptic women and the profiles of blood calcium-two parameters involved in hypertensive disorders of pregnancy.

## 2. Patients and Methods

This is a multicentric case-control study (incident cases) conducted in six hospitals in Goma from 1 April to 31 December 2019, during which the usual climatic seasons (dry and rainy) are recorded. Goma is a city in the east of the Democratic Republic of Congo with an estimated population of 829,761. It has a humid tropical climate softened by the wind blowing from Lake Kivu and the volcanic mountains located in the Virunga Park. As for the seasons, there are two short dry seasons (15 December to 15 February and 15 May to 15 August), a short rainy season (15 February to 15 May) and a long rainy season (15 August to 15 December). Meteorological data were provided by the weather station at Goma International Airport (N° 64 184) located at latitude 1'41'S, longitude 29°14'E and altitude 1551m [62].

We included 190 unmatched pregnancies (95 preeclamptic and 95 non-preeclamptic). Allocations to the 2 groups (normal pregnancy and pregnancy with preeclampsia) were made by the reasoned choice method and recruitment for a case-control ratio of 1:1.

Preeclampsia was defined according to the criteria of the National High Blood Pressure Education Program Working Group [63].

Pregnant women with hepato-renal disease, thyroid and other endocrine disorders, multiple pregnancies, molar pregnancies, death in utero, or pregnant women on vitamin D and/or calcium supplementation were excluded from the study.

Preeclampsia was considered severe if the SBP was >160 mmHg and/or DBP > 110 mmHg and moderate if the SBP was 140 - 160 mmHg and/or DBP 90 - 110 mmHg. Preeclampsia was said to be late when it was diagnosed after 34 weeks gestational age [64].

Venous blood was collected in the morning before 12 noon in an EDTA tube without anticoagulant. The sample was centrifuged at 3000 rpm for 15 minutes and the serum collected in a cryotube for assay by the iChromα Vitamin D test supplied by Boditech Med Incorporated (www.boditech.co.kr) and Obelis S.A of Belgium / Lot N° VDOYA78. Quality control of the assay was performed during each assay run. The plasma 25(OH) $D_2/D_3$ assay was performed by immunofluorescence with iCHROMA II and the GenruiGE300 was used for the blood ionogram. Total blood calcium consists of ions in the forms: ionic/free (50-65%), protein-bound (30-45%) and anion-complexed calcium (5-10%), mainly bicarbonate, citrate and lactate [65]. The form of calcium readily available to cells is the ionised form which therefore reflects a specific physiological state of calcium.

The following categories of variables were selected:

- Biochemical parameters: ionised calcium, total calcium, Vitamin D;
- Environmental (climatic) parameters: rainfall rate, humidity rate, seasons (dry and rainy season);
- The period of conception was determined by taking into account the date of the last menstrual period declared by the pregnant woman at the time of inclusion to which we added 14 days on average.

The data obtained were stored in the Microsoft® Access 2010 database and analyses were carried out with SPSS® statistical software version 23. The results were calculated as a percentage. Pearson's correlation and chi-square were also used. The significance level was set at $p< 0.05$.

This study was approved by the Ethics Committee of the University of Lubumbashi (N° UNILU/CEM/125/2019) as well as by the North Kivu Provincial Health Division (N° 251/281/DPS-NK/2019). Informed consent was a condition for the inclusion of pregnant women. The same was true for laboratory technicians

## 3. Results

The intrahospital prevalence of preeclampsia is 3.01%.

### 3.1. Blood calcium in the study population

The mean ionised calcium level in the preeclamptic woman was 1.24±0.16 mmol/L (0.48-1.59) compared to 1.27±0.17 mmol/L (0.88-2.30) in the normal pregnant woman. The difference was not statistically significant (p=0.214).

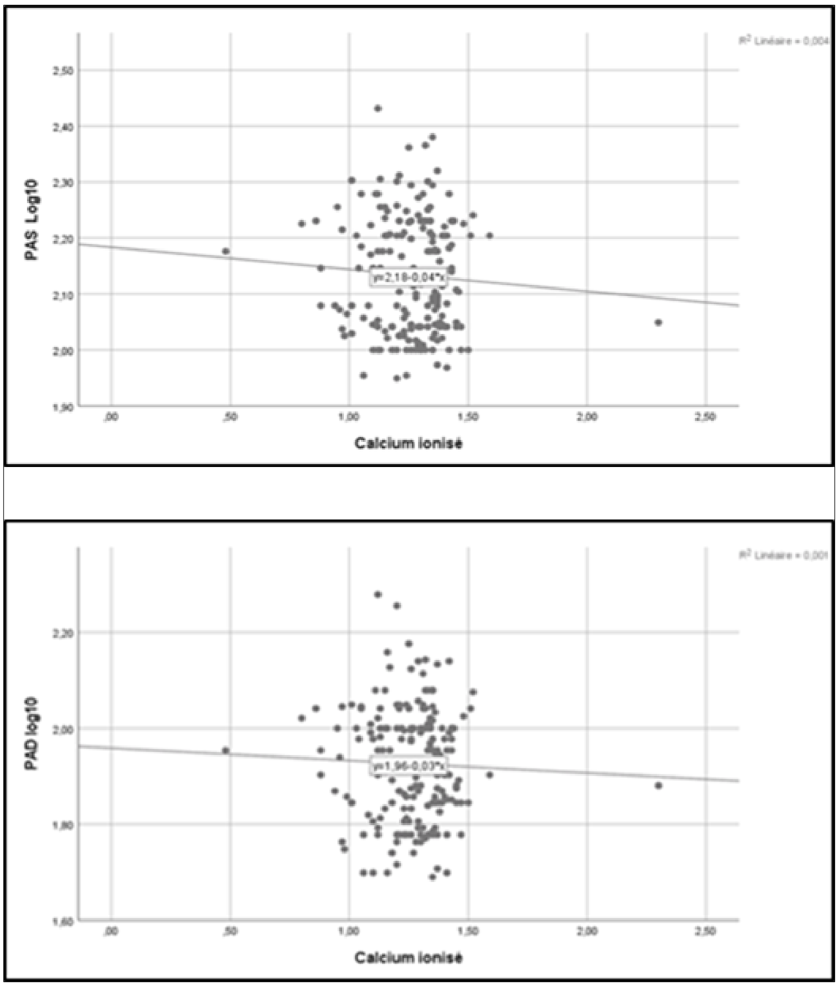
The mean total blood calcium level was 2.50±0.83 mmol/L (0.94-9.82) in the preeclamptic woman and 2.56±0.90 mmol/L (1.13-9.04) in the normal pregnant woman. The difference was not statistically significant (p=0.621).

Considering the distribution of pregnancies according to ionised calcium levels, the highest frequency of hypocalcaemia was in preeclamptic pregnancies compared to the control group (54.2% versus 45.80%). The same was true for total calcium levels (53.85% versus 46.15%). However, no statistically significant difference was observed (Table 1).

**Table 1** Distribution of pregnant women according to serum calcium level

| | | Survey population | | Total | OR | p |
|---|---|---|---|---|---|---|
| | | PE (%) | Control (%) | | | |
| Ionized calcium | Low | 13(54.20) | 11(45.80) | 24 | 1.30[0.52-3.27] | 0.564 |
| | Normal | 37(47.40) | 41(52.60) | 78 | 1 | |
| | High | 45(51.10) | 43(48.90) | 88 | 1.15[0.63-2.13] | 0.634 |
| Total | | 95 | 95 | 190 | | |
| Total Calcium | Low | 14 (53.85) | 12(46.15) | 26 | 1.21[0.48-2.61] | 0.650 |
| | Normal | 77 (49.00) | 80(51.00) | 157 | 1 | |
| | High | 4(57.10) | 3(42.90) | 7 | 1.38[0.30-6.39] | 0.674 |
| Total | | 95 | 95 | 190 | | |

## 3.2. Ionised calcium and blood pressure



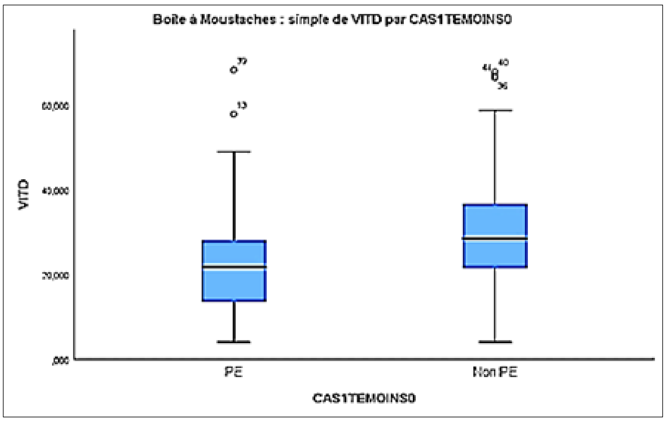

**Figures 1 & 2** Correlation between SBP (1), DBP (2) and ionised calcium

There was a negative, albeit discrete, correlation between ionised calcium and systolic blood pressure (Y = 2.18 - 0.04X; r = - 0.069; p = 0.366) and between ionised calcium and diastolic blood pressure (Y=1.96 - 0.03X; r = -0.051; p = 0.659), with a decrease in serum calcium accompanied by an increase in blood pressure (Figures 1 and 2).

### 3.3. Climatic aspects, Vitamin D and preeclampsia

*3.3.1. Vitamin D levels and preeclampsia*

The mean vitamin D level (22.2±11.4ng/ml) in preeclamptic pregnant women (PE) was lower than that (29.4±13.2) in non-preeclamptic pregnant women (Non PE) with a statistically significant difference (p<0.001)




The mean vitamin D level (22.2±11.4ng/ml) in preeclamptic pregnant women (PE) was lower than that (29.4±13.2) in non-preeclamptic pregnant women (Non PE) with a statistically significant difference (p<0.001)

**Figure 3** Mean serum 25(OH) D levels in the study population (plot box)

*3.3.2. Precipitation rates, preeclampsia and humidity levels*

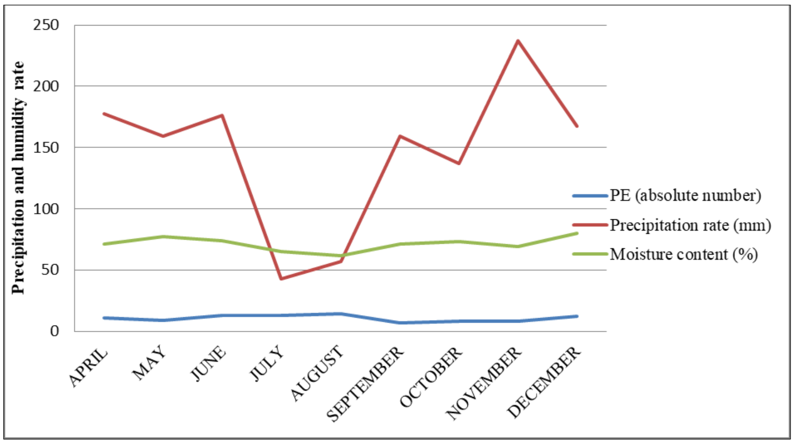



**Figure 4a** Absolute monthly distribution of preeclamptics according to weather parameters at the time of diagnosis

An increase in preeclampsia was observed when the rainfall rate dropped after June, i.e. with the onset of the dry season. The same was true for humidity levels

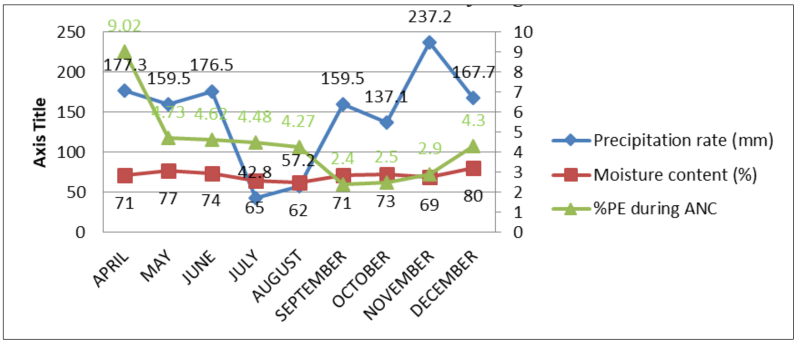
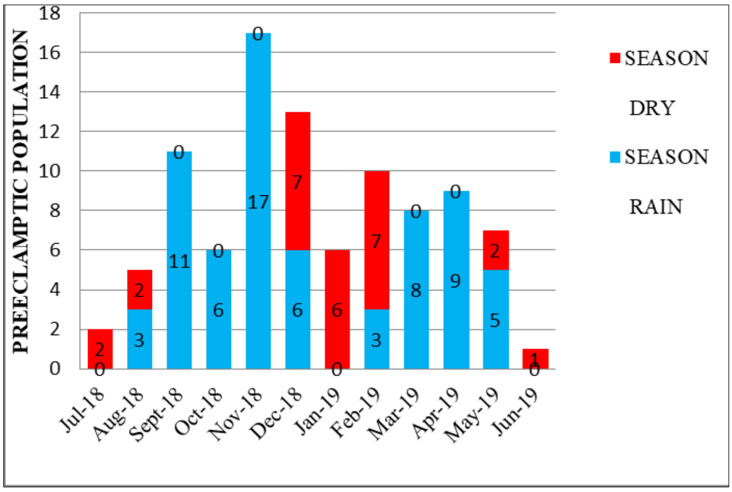



**Figure 4b** Monthly distribution of preeclamptics according to weather data at the time of diagnosis

Looking at the prevalence of preeclampsia in relation to the number of pregnant women seen each month in antenatal care (ANC), there was a dip towards the end of the short rainy season (February - May). This drop in prevalence was maintained during the dry season and even worsened at the beginning of the main rainy season (August - December) before beginning a timid recovery towards the end of the main rainy season and before the next short dry season from December to February. The explanation in relation to the humidity rate is roughly the same as the evolution of this rate is almost superimposable on that of the rainfall rate (Figure 4b).

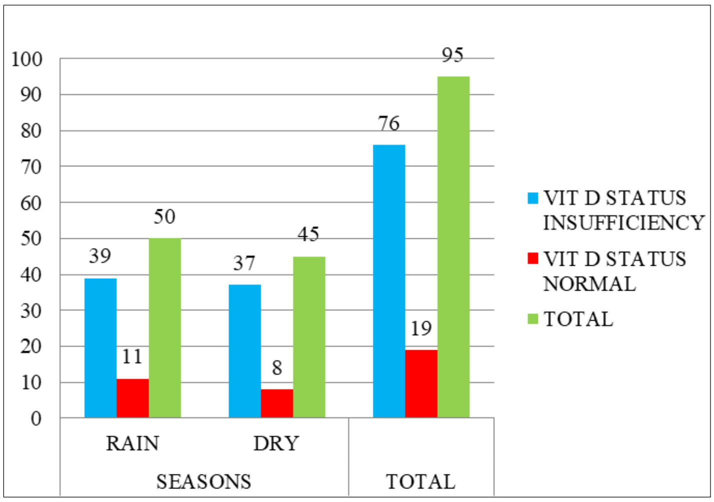
*3.3.3. Preeclampsia and climate data at conception*




Conceptions that occurred during the rainy season (March, April, May, September, October and November) were more likely to be complicated by preeclampsia. The observed frequency of conception recorded by season is 71.6% in favour of the rainy season.

**Figure 5** Monthly distribution of preeclamptics by presumed time of conception

*3.3.4. Preeclampsia, seasons and vitamin D status*




There were 52.6% of preeclamptics during the rainy season and 47.4% during the dry season (Figure 6). However, the prevalence of hypovitaminosis D among preeclamptics, depending on the season, was 82.22% during the rainy season and 47.4% during the dry season (Figure 6).

**Figure 6** Distribution of preeclamptics by season according to vitamin D status

## 4. Discussion

In our study, the serum calcium level remained low in preeclamptic women as observed by many other authors [16, 66-70]. Some have noted a statistically significant difference as in India [71], while elsewhere observations have not found it [72, 73].

Furthermore, the prevalence of hypocalcaemia in preeclamptic women was 54.20% with no statistically significant difference observed compared to normal pregnant women (45.80%). Vitamin D and calcium deficiency has been reported as one of the causes of preeclampsia and in one study it was found that hypocalcaemia could increase the risk of preeclampsia by up to 8.5 times [55]. Our observations are in line with those of Darkwa et al. in Ghana (74) [74] and Ugwuja et al. in Nigeria [75]. In Iran, Vafaei et al. [22] found hypocalcaemia in mild PE in contrast to severe PE where it was high. However, Kanagal et al. found that serum calcium levels were equivalent in normal pregnant and non-pregnant women [71].

In our series, there was a negative correlation between ionised calcium and blood pressure figures (r = -0.069; p=0.366 for SBP and r = -0.051; p = p=0.659 for DBP) although without a significant statistical difference in contrast to Darkwa et al. [74] who noted a weak non-significant positive correlation between blood pressure and serum total calcium levels in preeclamptic women (r=0.047; p = 0.806). Our observation would support the impact of maintaining good blood calcium levels to minimise the risk of preeclampsia. The roles of calcium in blood pressure regulation have been discussed above. However, physiological changes during pregnancy are characterised by an increasing need for calcium for maternal-fetal homeostasis, especially in the last trimester, to the benefit of the fetus. There is also increased glomerular filtration resulting in calciuria [50].

The differences in the results of the various authors may be related to factors that influence calcemia such as the age of the pregnant woman, gestational age, accurate identification of the diet and genetic factors. Poverty in our developing countries conditions nutritional intakes which are characterised by a significant deficit in mineral salts and other vitamins [76, 77]. Thus, an association between reduced calcium intake and preeclampsia has been frequently observed in our developing countries [16, 68], justifying the recommendation of calcium supplementation in some contexts [78-81].

Vitamin D level has been identified as a risk factor for preeclampsia in Goma [82], but there are several other factors that determine vitamin D concentration, including the environmental context [83]. Vitamin D is involved in calcium absorption. The climatic disturbances observed in recent times around the world are impacting on health well-being, with incidences of respiratory and perinatal diseases confirming this observation [84]. The seasonal distribution of preeclampsia has been noted in numerous studies [41, 44, 46, 85-87]. Climatic disturbances are accompanied by disruption of the meteorological parameters as used to formally define a given season and therefore some climatic elements could be taken into consideration.

The increase in cases of preeclampsia coincides with the fall in rainfall which is a reflection of the dry season in our environment (Figure 4). Indeed, in Kinshasa [35] and Bangkok [40], preeclampsia showed a high prevalence during the dry season while in Zimbabwe [88] it was more likely to occur during the rainy season. In South Africa [36], Brazil [89], Texas [86], Norway [85] and Kuwait [87], preeclampsia is likely to occur during winter while in Iran the highest frequencies are observed during summer and early spring [37]. Multiple justifications are put forward: some refer to the change in the type of diet imposed by the seasons [85], hence the recommendation of antioxidants; others argue that weather conditions influence the physiology of the blood vessels. Indeed, lower temperatures are likely to generate vasospasm leading to eclampsia [87].

The consideration of the *presumed time of conception* in our study was deduced by taking into account that ovulation is usually physiologically fixed around the fourteenth day of the menstrual cycle. Thus, the high proportions of preeclamptic women in our series were observed overall in pregnant women who conceived in March, April, May, September, October and November respectively (Figure 5) when low temperatures (below 20°C) corresponding to the rainy season were also recorded. These results are similar to those observed in China by Xiong et al. [84].

Regarding the relationship between the onset of preeclampsia symptoms, seasons and vitamin D levels (Figure 6), 82.22% of cases of hypovitaminosis D in preeclamptics were observed during the dry season compared to 78% during the rainy season. One might suspect dietary deficiencies related to the food available.

## 5. Conclusion

A non-significant decrease in ionised calcium and total calcium levels was found in the preeclamptic women in our series in the city of Goma. A non-significant negative correlation between serum calcium and blood pressure figures characterises our sample. These facts would reinforce the hypothesis that hypocalcaemia could play a role in the aetiology of preeclampsia. Similarly, hypovitaminosis D was globally associated with preeclampsia in the pregnant women in our study. The seasonal influence on preeclampsia would be largely tilted towards periods of low rainfall. The majority of preeclampsia cases were diagnosed in pregnancies where conception occurred during the rainy season, whereas the symptomatological expression was more apparent during the dry season. Clinical trials with early double supplementation of vitamin D and calcium during pregnancy for evaluation of benefits would be strongly recommended.

*Limitations*

Consideration of the detailed diet of pregnant women should be thoroughly investigated. A detailed analysis of weather and climate parameters taking into account daily aspects with a large sample size at this time of climate change would be desirable in order to fully establish their relationship with preeclampsia and vitamin D

## Compliance with ethical standards

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*Authors' Contributions*

Kabuyanga Kabuseba contributed to the preparation of the manuscript of this article from the outset as principal investigator. Professors Lundimu, Elongi, Kinenkinda 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.

*Disclosure of conflict of interest*

The authors declare that they have no conflict of interest and that funding was not received for performing the study.

*Statement of informed 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.

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