

## Bone degradation among elderly women in Douala, Cameroon: Biochemical markers, prevalence and associated factors

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World Journal of Biology Pharmacy and Health Sciences, 2022, 11(03), 043-051

Publication history: Received on 15 August 2022; revised on 19 September 2022; accepted on 21 September 2022

Article DOI: <https://doi.org/10.30574/wjbphs.2022.11.3.0136>

### Abstract

Bone degradation increases in women over 60 years old. It is an emerging public health problem, especially in Cameroon. In this context, this study was designed to evaluate biochemical parameters which can be helpful to early evaluation of bone degradation. A cross-sectional study was conducted from November 2018 to July 2019 at two hospitals in Douala. A pre-tested and structured questionnaire was used to collect data of each participant. Blood samples were collected to determine serum levels of calcium, phosphorus, and PTH (Parathyroid hormone). Data were analysed using SPSS v16 and GraphPad v5.03 software. A total of 92 women mostly aged 60-65 years old with a mean age of  $69 \pm 8$  years old were finally included. No statistically significant association between age and biochemical markers was found, even though calcium and PTH levels were higher in women over 75 years old ( $94.6 \pm 8.4$  mg/L and  $47.6 \pm 38.2$  pg/mL, respectively). A positive correlation was found between PTH and phosphorus ( $p = 0.02$ ). The prevalence of women at risk of bone degradation was 15.2% (95% CI 9.4-24.2%). Sensibility of calcemia to identify women at risk of bone degradation was 14.3% (95% CI 4.0 - 39.9%). Advanced age (OR = 1.23, 95% CI 1.02 - 9.15,  $p = 0.002$ ) and history of fracture (OR = 1.83, 95% CI 1.01 - 15.06,  $p = 0.0001$ ) were risk factors of bone degradation. This study outlined that bone degradation is present at significant prevalence among elderly women. PTH could be a potentially helpful biomarker for early detection of elderly women at risk of bone degradation.

**Keywords:** Bone degradation; Elderly women; Parathyroid hormone; Calcium; Phosphorus

### 1. Introduction

In humans, processes of resorption and formation occur simultaneously at similar rates in bones of young adults. This bone remodelling balance becomes negative during aging, resulting in a reduction/degradation of bone mass [1, 2]. Aging is an irrevocable biological process accompanying with morphological, functional and biochemical disturbances of different body systems, especially musculoskeletal system which gradually changes and acquires new characteristics including of muscle and bone loss [3, 4]. If untreated, bone loss may become severe and predispose individuals to fractures, particularly elderly population whose mobility is reduced and expose them to severe motor disorders [5].

World statistics from the International Osteoporosis Foundation outline that one in three women over 50 years old suffer from osteoporotic fractures in their lifetime [6]. Women are more frequently affected by bone degradation; and

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during the course of their life they can lose 30-50% of bone mass while men lose 13% of their bone mass [7, 8]. The extent of bone loss increases and reaches equilibrium ~10 years after menopause [9]. A study conducted among apparently healthy Australian postmenopausal women showed a reduction by 30% in calcium absorption while high PTH levels were found [10]. Carrivick et al. reported increasing PTH levels with age, but not correlated with renal function, ionized calcium, phosphates and 25-hydroxyvitamin D [11]. Kim et al. pointed out that high PTH levels had detrimental effects on bone mineral density of Korean postmenopausal women even though with sufficient vitamin D levels [12]. In this context, PTH levels could be helpful for identifying persons at risk of osteoporosis, especially postmenopausal women.

In Cameroon, women over 50 years old account for 17.9% of osteoporosis cases as compared to their younger counterparts (0.6%). Beyond the age of 70, osteoporosis cases were found in 55.8% of women [13]. The present study was designed to evaluate biochemical parameters which can be helpful for early evaluation of bone degradation among postmenopausal women attending two hospitals in the town of Douala, Cameroon.

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## 2. Material and methods

### 2.1. Study design and sites

A cross-sectional study was conducted from November 2018 to July 2019 at two hospitals in the town of Douala (Cameroon) namely Douala Gyneco-Obstetric and Paediatric Hospital (DGOPH) and Nylon District Hospital (NDH).

Women aged  $\geq 60$  years old, fasting and having signed an informed consent form were included in the study. Women, having undergone a thyroidectomy and/or parathyroidectomy, diagnosed with digestive cancer, under hormone therapy, with poor absorption syndrome, and chronic renal failure were excluded from the study. Additionally, we excluded women whose blood sample was haemolysed.

Ethical and administrative authorizations were obtained prior to implementation of the study. During the study, women were approached at waiting rooms of consultation and laboratory services of hospitals. They were informed about study objectives in one of the national languages (French and English). After obtaining signed informed consent, a questionnaire was administered to each eligible woman to collect data of interest. Thereafter, blood samples were collected for determining biochemical markers.

A pre-tested and structured questionnaire was used to collect data of each participant through 20-minute individual interviews. The questionnaire has three sections namely i) sociodemographic information, ii) clinical and paraclinical data, and iii) biochemical markers.

Five millilitres of blood were collected by venipuncture into dry tubes with strict respect to aseptic conditions. After coagulation and clot retraction, tubes were centrifuged at 3000 rpm for 5 minutes, and serum obtained was stored at  $-20^{\circ}\text{C}$  until biochemical tests. Calcium and phosphorus levels were determined by colorimetric method in Cobas c111 while PTH level was determined by electrochemiluminescence immunoassay on an Elecsys 2010 (Cobas e411 Roche Diagnostics, France), with a normal range of 15-65 pg/mL. Women with PTH level  $> 65$  pg/mL was considered at risk of bone degradation.

### 2.2. Ethical considerations

This study was approved by the institutional review board of the University of Douala (no. 1793-CEI-UD<sub>0</sub>/04/2019/M). In addition, authorizations were issued by ethical committees of DGOPH (no. 2019/0025/DGOPH/DG/CEI) and NDH (no. 2019/AR/MSP/DRSPL/NDH/NDH). Each woman was informed about the objectives of the study in the language she understood best. An informed consent form was signed by each woman before their enrolment in the study. We also guaranteed each woman on voluntary aspect of this study and respect of the confidentiality of their data.

### 2.3. Statistical analysis

Data were keyed in an Excel spreadsheet (Microsoft Office 2016, USA), coded, checked for consistency, and then exported to the Statistical package for social sciences, SPSS, v16 for Windows (SPSS, Inc., Chicago, IL, USA) and GraphPad v5.03 for Windows (GraphPad PRISM, Inc., San Diego, CA, USA) for statistical analysis. Data were presented as percentages and mean  $\pm$  standard deviation in figures and tables where appropriate. Quantitative variables were tested for normal distribution using Shapiro-Wilk test. Pearson's chi-square and Fisher's exact tests were used to compare percentages while analysis of variance (ANOVA), Student's, Mann-Whitney, and Kruskal-Wallis tests were used to compare mean values between groups where appropriate. Pearson correlation analysis was used to determine the

association between the different biochemical markers. Univariate logistic regression was used to identify factors associated with bone loss. The association between dependant variable (i.e., bone degradation) and independent variables was assessed through computing odds ratio (OR), their confidence interval at 95% (95% CI), and statistical significance. Using PTH as reference, the sensitivity of calcium to identify patients with bone degradation was computed as follows: Sensitivity =  $TP \times 100 / (TP + FP)$ , where TP is the number of true positive and FP is the number of false positive. A  $p$ -value < 0.05 was considered statistically significant.

### 3. Results

One hundred and thirty-five women were approached, and 92 of them were finally included in the study.

#### 3.1. Sociodemographic characteristics of the participants

Of the 92 women included in the study, nearly 40% of them were aged 60-65 years old with a mean age  $\pm$  SD of  $69 \pm 8$  years old. Women had mostly no formal level of education (48.8%), were coming from the West Region of Cameroon, and were housewives (69.3%).

Twenty-nine women (32.2%) were under food supplementation, especially with food supplement, vitamin, and calcium tablet as presented in Table 1. History of pathological fracture was reported in 16.3% of women while gastritis and myalgia/arthritis were reported at proportion of 60.4% and 92.2%, respectively.

**Table 1** Details on nutritional characteristics of the participants

Variables	n	%
<b>Calcium supplementation#</b>		
No	61	67.8
Yes	29	32.2
<b>Nature of the supplementation#</b>		
Food supplements	11	42.0
Vitamin	7	27.0
Calcium tablet	6	23.0
Food supplements + Vitamin	2	8.0
<b>Supplementation frequency#</b>		
Once/daily	14	60.9
Weekly	6	26.1
Twice/daily	2	8.7
Twice/weekly	1	4.3

#Missing data

Biochemical parameters were measured in all patients included in the study. Mean value  $\pm$  SD of PTH was  $42.4 \pm 25.2$  pg/mL, with median value of 36.2 pg/mL (Table 2).

**Table 2** Overall variation of calcium, phosphorus, and PTH levels

Parameters	n	Mean $\pm$ SD	Median	Range
Calcemia (mg/L)	92	$92.8 \pm 5.8$	92.5	80.7 - 113.8
Phosphoremia (mg/L)	92	$37.6 \pm 10.4$	37.1	17.1 - 108.8
PTH (pg/mL)	92	$42.4 \pm 25.2$	36.2	7.5 - 162.4

Data are presented as mean  $\pm$  standard deviation (SD)

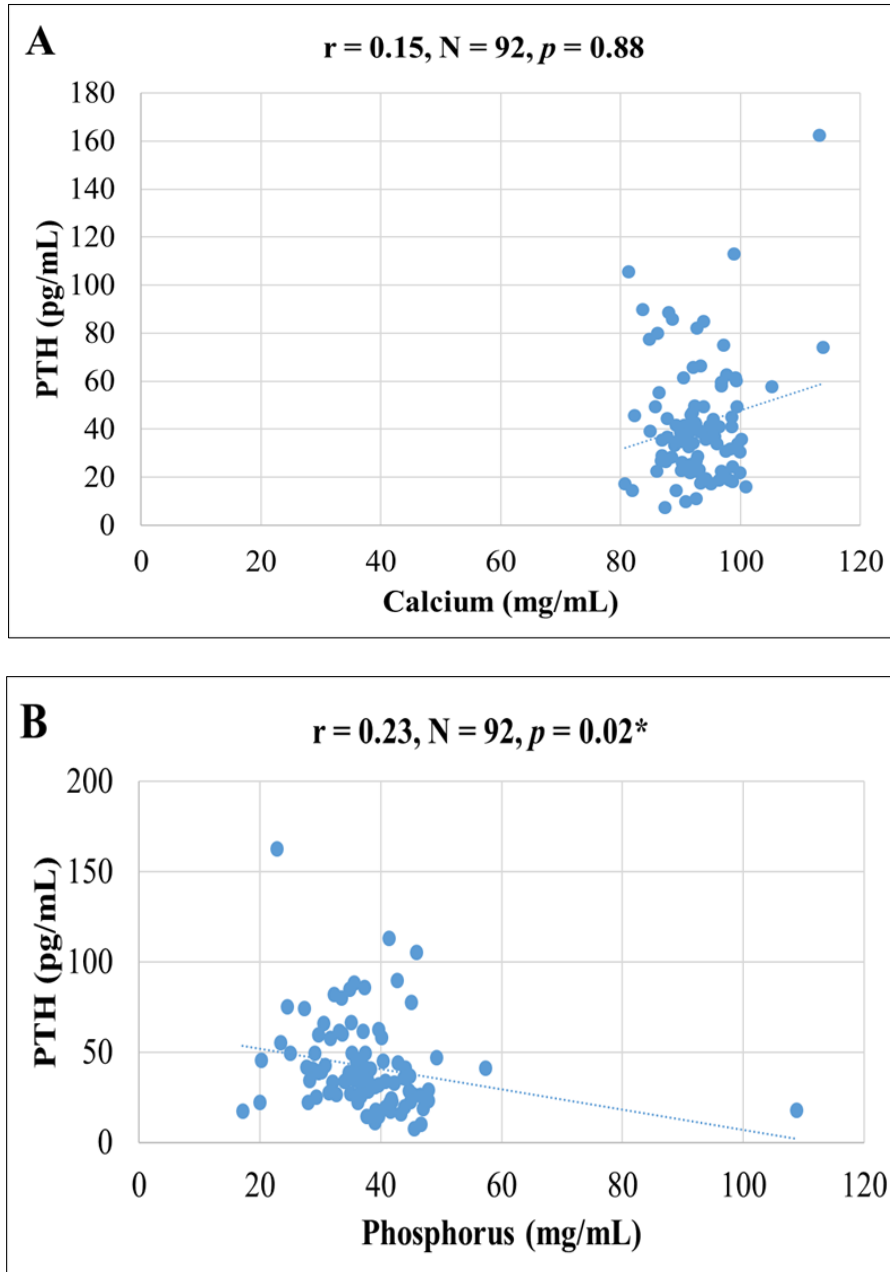
**Table 3** Variation of calcium, phosphorus and PTH according to sociodemographic and clinical characteristics

Variables	Total calcium (mg/mL)				Phosphoremia (mg/mL)				PTH (pg/mL)			
	< 82	82 - 102	> 102	<i>p</i>	< 25	25 - 45	> 45	<i>p</i>	< 15	15 - 65	> 65	<i>p</i>
<b>Age (years)</b>												
[60 – 65]	1 (1.1%)	34 (37.0%)	0 (0%)	0.22	1 (1.1%)	31 (33.7%)	3 (3.3%)	0.22	1 (1.1%)	29 (31.5%)	5 (5.4%)	0.77
[65 – 70]	2 (2.2%)	20 (21.7%)	0 (0%)		1 (1.1%)	18 (19.6%)	3 (3.3%)		1 (1.1%)	17 (18.5%)	4 (4.4%)	
[70 – 75]	0 (0%)	15 (16.3%)	1 (1.1%)		1 (1.1%)	12 (13.0%)	5 (5.4%)		1 (1.1%)	14 (15.2%)	1 (1.1%)	
≥ 75	0 (0%)	17 (18.5%)	2 (2.2%)		3 (3.3%)	12 (13.0%)	2 (2.2%)		2 (2.2%)	13 (14.1%)	4 (4.4%)	
<b>Education level*</b>												
None	1 (1.1%)	40 (42.6%)	1 (1.1%)	0.94	3 (3.3%)	33 (35.1%)	6 (6.4%)	0.16	5 (5.7%)	31 (35.2%)	7 (8.0%)	0.16
Primary	1 (1.1%)	24 (25.5%)	1 (1.1%)		3 (3.3%)	18 (19.1%)	5 (5.5%)		0 (0%)	23 (26.1%)	3 (3.4%)	
Secondary	1 (1.1%)	17 (18.3%)	1 (1.1%)		0 (0%)	17 (18.3%)	2 (2.2%)		0 (0%)	15 (17.0%)	4 (4.5%)	
<b>Region of origin</b>												
West	2 (2.2%)	57 (62.0%)	2 (2.2%)	0.63	4 (4.3%)	50 (54.3%)	7 (7.6%)	0.58	3 (3.3%)	48 (52.2%)	10 (10.9%)	0.98
Littoral	0 (0%)	17 (18.5%)	0 (0%)		2 (2.2%)	12 (13.0%)	3 (3.3%)		1 (1.1%)	14 (15.2%)	2 (2.2%)	
Other	1 (1.1%)	12 (13.0%)	1 (1.1%)		0 (0%)	11 (12.0%)	3 (3.3%)		1 (1.1%)	11 (12.0%)	2 (2.2%)	
<b>Gastritis</b>												
No	3 (3.3%)	33 (35.9%)	2 (2.2%)	0.06	3 (3.3%)	27 (29.3%)	8 (8.7%)	0.22	2 (2.2%)	31 (33.7%)	5 (5.4%)	0.85
Yes	0 (0%)	53 (57.6%)	1 (1.1%)		3 (3.3%)	46 (50.0%)	5 (5.4%)		2 (2.2%)	43 (46.7%)	9 (9.8%)	
<b>Myalgia</b>												
No	1 (1.1%)	6 (6.5%)	0 (0%)	0.21	1 (1.1%)	5 (5.4%)	1 (1.1%)	0.68	0 (0%)	6 (6.5%)	1 (1.1%)	0.79
Yes	2 (2.2%)	80 (87.0%)	3 (3.3%)		5 (5.4%)	68 (73.9%)	12 (13.0%)		5 (5.4%)	67 (72.8%)	13 (14.1%)	

Data are presented as frequency and percentage; \*Missing data; Pearson's chi-square test was used to compare groups; Statistical significance was set at *p*-value < 0.05

No statistically significant difference between different age groups was found in serum levels of biochemical parameters. Also, we did not find any statistically significant difference between range of calcium, phosphorus, PTH, and patients' characteristics (Table 3).

The relationship between biochemical parameters was evaluated, and no statistically significant association between Calcemia and PTH ( $p = 0.88$ ) was found (Figure 1A). In contrast, a statistically significant and positive correlation was found between phosphorus and PTH ( $r = 0.23$ ,  $p = 0.02$ ) (Figure 1B).



Data were log-transformed and Pearson's correlation test was used; \*Statistically significant at  $p$ -value  $< 0.05$

**Figure 1** Correlation of between (A) calcium and PTH, and (B) phosphorus and PTH

Concordant results between calcium and PTH were obtained for 74 of the 92 women, thus giving an agreement rate of 80.4% (95% CI 71.2 - 87.3%). Only 2 of the 14 women at risk of bone degradation were correctly classified as at risk of bone degradation using calcemia. Thus, sensibility of calcemia to identify women at risk of bone degradation was 14.3% (95% CI 4.0 - 39.9%) (Table 4).

**Table 4** Agreement between calcium and PTH

Calcium (mg/L)	PTH (pg/mL)			Total
	Low (<15 pg/mL)	Normal (15 - 65 pg/mL)	High (> 65 pg/mL)	
Low (< 82 mg/L)	1	1	1	3
Normal (82 - 102 mg/L)	4	71	11	86
High (> 102 mg/L)	0	1	2	3
Total	5	73	14	92 (100%)

**Table 5** Univariate analysis of factors associated with bone degradation

Factors	N	n (%)	OR (95%CI)	p-value
<b>Age (years)</b>				
[60 - 65]	35	5 (14.3)	1	
[65 - 70]	21	4 (19.0)	2.59 (0.38 - 15.10)	0.43
[70 - 75]	18	1 (5.6)	0.33 (0.02 - 6.11)	0.67
≥ 75	18	4 (22.2)	1.23 (1.02 - 9.15)	0.02*
<b>Region of origin</b>				
Others	9	1 (11.1)	1	
Littoral	16	2 (12.5)	8.09 (0.17 - 394.65)	0.29
West	62	10 (16.1)	4.23 (0.15 - 117.49)	0.41
<b>Calcium supplementation</b>				
No	61	10 (16.4)	1	
Yes	29	3 (10.3)	0.43 (0.07 - 2.64)	0.41
<b>History of fracture</b>				
No	77	11 (14.3)	1	
Yes	15	3 (20.0)	1.85 (1.01 - 15.11)	0.001*
<b>Epigastralgia</b>				
No	36	5 (13.9)	1	
Yes	55	9 (16.4)	3.64 (0.61 - 20.52)	0.21
<b>Calcemia</b>				
Low	3	1 (33.3)	1	
Normal	86	11 (12.8)	0.08 (1.60E <sup>-3</sup> - 3.93)	0.71
High	3	2 (66.7)	9.03 (0.04 - 247.08)	0.31
<b>Phosphoremia</b>				
Low	6	2 (33.3)	1	
Normal	73	10 (13.7)	1.24 (0.05 - 18.76)	0.36
High	13	2 (15.4)	0.55 (0.02 - 15.18)	0.50

OR: Odds ratio, 95%CI: Confidence interval at 95%; Univariate logistic regression was used; \*Statistically significant at p-value < 0.05

Based on data on serum PTH levels, a total of 14 women had high values (i.e., > 65 pg/mL). Thus, the prevalence of women at risk of bone degradation was 15.2 % (95% CI 9.4-24.2%). Based on univariate logistic regression, two factors (age and history of bone fracture) were found to be significantly associated with increased risk of bone degradation among women. The risk of bone degradation was 1.23 times (OR = 1.23, 95% CI 1.02 – 9.15,  $p = 0.002$ ) higher in women aged  $\geq 75$  years as compared to those aged 60-65 years. Likewise, the odds of risk of bone degradation was ~2 times higher (OR = 1.85, 95% CI 1.01 – 15.11,  $p = 0.0001$ ) in women with a history of bone fracture as compared to their counterparts without history (Table 5).

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#### 4. Discussion

This study aimed at determining variation of calcium, phosphorus and PTH, in relation with risk of bone degradation and its associated factors among elderly women attending two health facilities in Douala, Cameroon.

Most of women included in the study were aged below 70 years, and a low proportion of them were under calcium supplementation. This finding is in line with that of previous studies conducted in Brazil [10] and with what is known in Cameroon about national policies on calcium intake.

No statistical association was found between calcemia and PTH, and this is consistent with a retrospective study from Saudi Arabia [14]. In contrast, phosphoremia were negatively correlated with PTH. This is physiologically due to the fact that the secretion of PTH leads to a decrease renal tubular reabsorption of phosphorus. However, in some pathophysiological situations, this negative relationship between PTH and phosphoremia can be disturbed. For example, a positive relationship between these two parameters was found in India among patients diagnosed with renal failure [15].

Of the 14 women at risk of bone degradation, 11 had normal calcemia. PTH promotes the absorption of calcium in all regulatory sites (kidney, intestine and bone) during an imbalance of calcium homeostasis (hypocalcaemia). In this context, an increase in calcemia is expected with increasing PTH levels, but it was not the case for these 11 women. The absence of an increase in calcium could be associated with a low intake, constituting an overall deficit in the bloodstream. Bone resorption compensates this deficit to balance blood calcium level. Calcemia is a physiological parameter commonly determined in clinical practice. This discordance found in this study highlights the need to adjunct PTH to calcemia to have a better picture of bone status of elderly women.

PTH levels were higher in women aged  $\geq 75$  years even though no statistically significant association between PTH and age was found in the study. This finding does not support those of previous studies that reported increasing PTH levels with age [16, 17]. Indeed, Oluboyo and colleagues found significantly increased levels of PTH along with calcium and phosphorus among Nigerian postmenopausal women [17].

Advanced age and previous fractures were found to be associated with increased risk of bone degradation. There are several aetiologies of bone mass loss including those related to genetics, nutrition and lifestyle, but aging is the main cause [18]. Our findings are consistent with those reported previously in China and Brazil where authors identified several risk factors including advanced age and previous fracture [1, 19]. Other studies reported different risk factors of bone degradation [20, 21].

This study shows the difficulty to evaluate bone degradation in population, thereby outlining the need for more exploration of bone metabolism via others parameters or procedures to early identify disturbance. This study has some limitations. First, results were obtained from two hospitals which cannot be generalizable to elderly population in Douala. Second, we are not able to do multivariate logistic analysis given the small sample size of the study population.

#### Abbreviations

- 95%CI: Confidence interval at 95%
- ANOVA: Analysis of variance
- DGOPH: Douala Gyneco-Obstetric and Paediatric Hospital
- FP: False positive
- NDH: Nylon District Hospital
- OR: Odds ratio
- PTH: Parathyroid hormone
- TP: True positive

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## 5. Conclusion

The present study pointed out association between calcium, phosphorus, and PTH. The prevalence of bone degradation among elderly women was 15.3 %, with advanced age and previous fractures as risk factors. Thus, bone disturbance is present at significant level among elderly women, and PTH could be an interesting biomarker for early identification of risk of bone disturbance. More studies are required to confirm the possible utility of this biomarker in Cameroon.

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## Compliance with ethical standards

### *Acknowledgments*

The authors are grateful to women who accepted to take part to the study. We also thank Directors and medical staff of the Douala Gyneco-Obstetric and Paediatric Hospital, and Nylon District hospital for allowed us to conduct the study and technical assistance.

### *Disclosure of conflict of interest*

The authors have no conflicts of interest.

### *Statement of ethical approval*

Ethical clearances were issued by institutional review board of the University of Douala (no. 1793-CEI-UDO/04/2019/M), and ethics committees of health facilities - DGOPH (no. 2019/0025/DGOPH/DG/CEI) and NDH (no. 2019/AR/MSP/DRSPL/NDH/NDH).

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

### *Authors' Contributions*

Conception and design: VNE, JPNM, DDA; Data collection: VNE, NDMN, CMM; Data analysis and interpretation: VNE, JPNM, LPKF, DDA; Write-up and revision of the manuscript: VNE, JPNM, LPKF, DDA; Supervision: JPNM, DDA; Approval of final version of the manuscript: All authors.

### *Funding*

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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