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Association between serum hypovitaminosis D and preeclampsia: A nested case-control study

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Abstract

Background: Preeclampsia is a major complication of pregnancy and a major cause of perinatal and maternal morbidity and mortality. Vitamin D deficiency has been implicated in the aetiology and pathophysiology of preeclampsia. However, there is no uniformity in the findings of previous studies on the association between vitamin D and preeclampsia.

Aims and Objectives: The study is aimed at determining the association between preeclampsia and maternal vitamin D deficiency.

Materials and Methods: This nested case-control study was conducted among 158 pregnant women (78 preeclamptic women and 80 controls) with singleton pregnancies. Case participants were women with preeclampsia. The controls were matched pregnant women without preeclampsia. Their serum vitamin D levels were determined.

Statistics: Continuous data was analysed using T-test. The statistical significance was inferred at p- value ≤0.05.

Results: The prevalence of hypovitaminosis D in our study was 7.0% overall. The proportion of women with hypovitaminosis D was not significantly different between preeclampsia group and control group (7.7% vs 6.3% respectively; p=0.76). The mean serum concentration of vitamin D in the preeclamptic group was lower than that in the control group, however, the difference was not statistically significant (118.8±17.4nmol/L vs 129.0±19.7nmol/L, p=0.17). There was a weak association between gestational age and the level of serum vitamin D in both groups (r= 0.062 and r= -0.13 respectively).

Conclusions: Hypovitaminosis D is not significantly associated with preeclampsia when compared with control. However, there was a weak association between gestational age and the level of serum vitamin D in both groups.

Keywords: Cholecalciferol; Vitamin D; Hypovitaminosis D; Vitamin D deficiency; Preeclampsia; Pregnancy

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1. Introduction

Preeclampsia is a disorder associated with pregnancy consisting of hypertension and significant proteinuria that presents, in most cases, after the 20th week of pregnancy.[1,2]It accounts for about 25% of maternal mortality and is a major cause of perinatal and maternal morbidity and mortality.[3–5] A previous Nigerian study by Obiechina et al,[6]has put maternal mortality from preeclampsia/eclampsia at 27%. In another Nigerian study on hypertensive disorders of pregnancy, with severe preeclampsia as the leading cause, the reported stillbirth rate was 17.4% and the perinatal mortality rate was 20.9%.[7]

Recognized risk factors for preeclampsia include primigravidity/nulliparity, previous history of preeclampsia, change of partner, family history of preeclampsia in mother or sister, black race, pre-existing diabetes mellitus, multiple pregnancy and pre pregnancy obesity etc.[1,8] several theories, some of which are interlinked, have attempted to explain the aetiology and pathophysiology of preeclampsia. Recent epidemiological studies have emphasized the role of vitamin D deficiency in the development of preeclampsia.[9] The pathogenesis of preeclampsia involves biological processes that may be directly or indirectly affected by vitamin D, including immunologic dysfunction, abnormal placental implantation, abnormal angiogenesis, excessive inflammation, and hypertension.[9] Vitamin D is emerging as a promising agent for preeclampsia prevention.[5]

Vitamin D is a fat soluble vitamin and its deficiency during pregnancy is caused by poor exposure to sunlight needed to synthesize vitamin D3 (cholecalciferol) in the skin, coupled with dietary intakes that are too low to meet the increased demands of pregnancy and dark skin colour. [10,11] Indeed, black women typically have lower serum vitamin D concentrations and have a higher chance of vitamin D deficiency, compared with their white counterparts.[12] This may be due to high amount of melanin in the skin of black women which reduces the amount of sunlight that get to the skin for production of vitamin D.[13]Magnesium sulphate which is used in the treatment of preeclampsia is known to increase the levels of parathyroid hormone which in turn promotes the conversion of 25-hydroxycalciferol to 1,25-dihydroxycalciferol and also increases the production of 1,25-dihydroxycalciferol from the placenta.[3] Seasonal patterns in preeclampsia also suggest a role for vitamin D and sunlight. The incidence of preeclampsia is higher during winter months, when sunlight-dependent 25hydroxyvitamin D production is lower.[14] The concurrent disproportionate higher prevalence of hypovitaminosis D and preeclampsia in black women when compared with their white counterparts suggest vitamin D may be relevant in the pathogenesis of both diseases.[12,15,16]

According to Sassan et al.[5], supplemental vitamin D during pregnancy reduced the risk for preeclampsia by 27%. Further, among women who received vitamin D supplementation during their first year of life, later development of preeclampsia was reduced by half[4] underscoring the importance of adequate vitamin D status during critical developmental windows extending beyond the antenatal period.[17]these observations suggest that a reduced level of serum vitamin D may be an important risk factor for preeclampsia.

The existing researches as documented by Gbadegesin et al[18]in Nigeria, Ullah et al[19] in Bangladesh, Robinson et al,[20] in USA and Hashemipour et al[21] in Iran investigating the correlation between serum vitamin D levels and preeclampsia have shown conflicting findings. Moreover, such findings may not directly apply to African-Blacks due to the difference in vitamin D profile accounted for by the reduced dietary intake as well as the decreased absorption of ultraviolet rays by the melanin-rich black skin.[22] In Africa and Nigeria where the burden of vitamin D deficiency is high,[23] there is paucity of literature on this subject. It is therefore imperative that more studies be carried out in an environment where the impact and incidence of preeclampsia may be more profound. Thus, the objective of the present nested case-control study was to determine whether maternal vitamin D deficiency is associated with higher incidence of preeclampsia.

2. Participants, Materials and methods

The participants in the study were pregnant women with singleton gestation aged 18 to 45 years who presented to the ante natal clinic or labour ward of Nnamdi Azikiwe University Teaching Hospital, Nnewi, South-east Nigeria within the 6 months of the study (September 2018 to February 2019). The cases were women who had preeclampsia while the controls were pregnant women without preeclampsia. The controls were matched for age (±2 years), parity (±1) and gestational age (±2 weeks) with the cases. Pregnant women with one or more of the following conditions were excluded: chronic hypertension, diabetes mellitus (pregestational or gestational), multiple gestation, retroviral disease, chronic renal diseases. Other exclusion criteria included current or past history of the following within the last 6 months: lactation, smoking, vitamin D supplementation, malignancy. The ethical approval was obtained from the Nnamdi Azikiwe University Teaching Hospital Ethics Committee before the commencement of the study (Reference Number:

NAUTH/CS/66/VOL.10/221/2017/ 135; Approval date: 12th January, 2018).Prospective participants were counselled and written informed consent was obtained from each eligible woman before participation in the study. On each arm of the study, 80 women were recruited though 78 were used for final analysis on the preeclamptic arm.(See Fig 1).

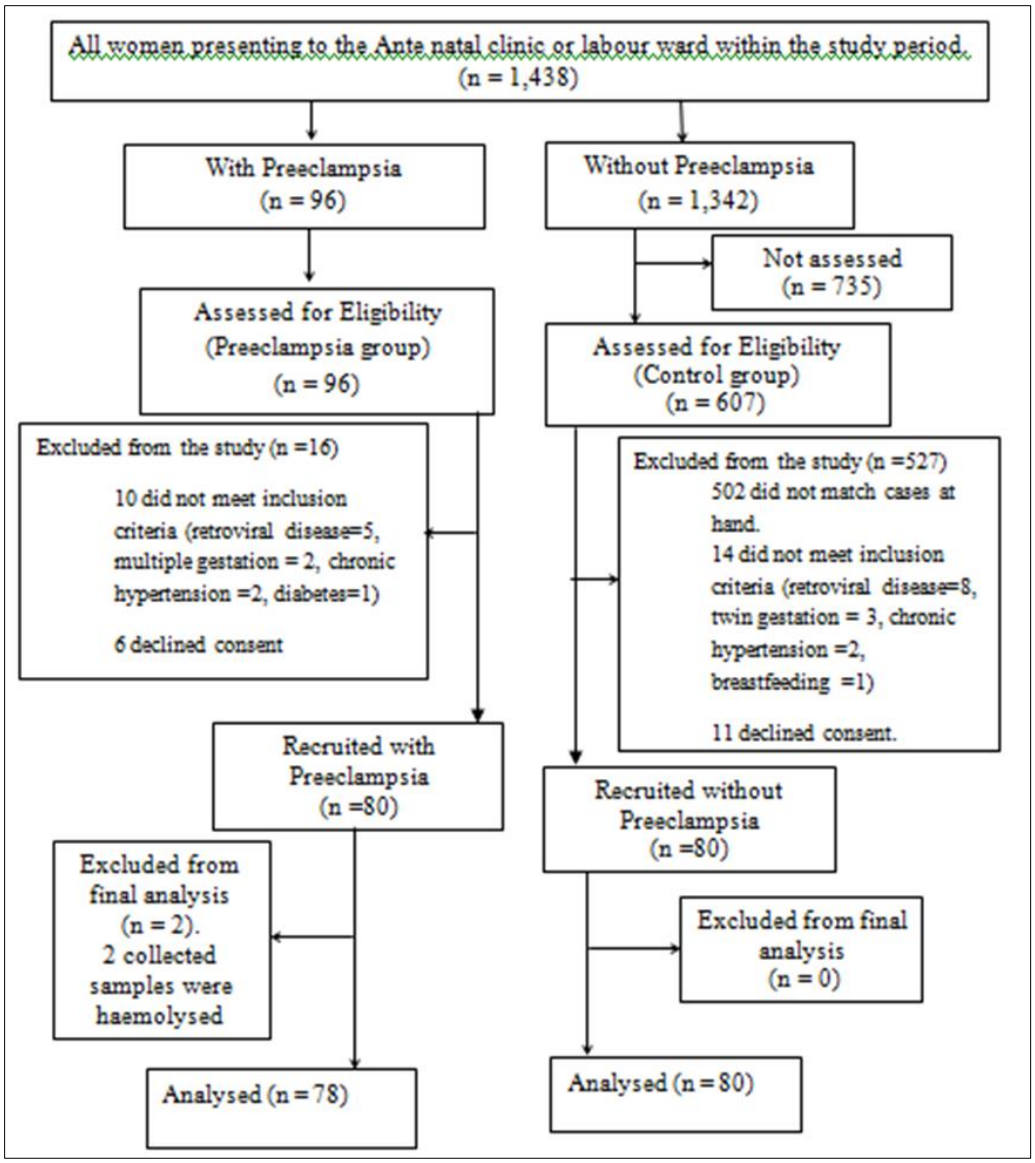

Figure 1 Flow chart for study

The demographic details, brief medical history and findings on physical examination were obtained from the participants using proformas. The age, parity, gestational age, blood pressure and urinalysis result were recorded. The gestational age (GA) was calculated from the first day of the last menstrual period (LMP), but for those who were unsure of their LMP, the GA was based on the earliest ultrasound report. The measurement of the blood pressure was done using a mercury-in-glass sphygmomanometer with a standard adult cuff size. Each participant rested for at least 5 minutes and the blood pressure was measured with the participant sitting comfortably on a chair with a back rest and resting her left arm on a desk at the same level with her heart. Clean catch mid-stream urine were collected for urinalysis using a Medi Test combi 2 (Neumann-Neonder Str.68952355 Duren) dipstick. For those on urinary catheter, urine specimen from the catheter was used. Venous blood samples were collected, allowed to clot and subsequently centrifuged at 2500 rpm for 10 minutes. The serum was separated and frozen at -20 degree centigrade until analysed using a commercially available kit produced by The Calbiotech®, Inc (Calbiotech Inc., 10461 Austin Dr, Spring Valley, CA, 91978 Tel (619) 660-6162, Fax (619) 660-6970, www.calbiotech.com). The kit is a solid phase enzyme-linked immunoassay (ELISA). The principle of the test is based on competitive binding.[24] The women were grouped into 3 different groups with respect to vitamin D status: 25-Hydroxyvitamin D3 deficiency is levels less than 50nmol/L (or 20

ng/ml), insufficiency is levels from 50nmol/L to less than 75nmol/L (or 30 ng/ml), while sufficiency is levels of 75nmol/L and greater.[25]

We estimated that a sample size of 136 with a 1:1 case to control ratio (68 cases and 68 controls) would allow us to accept a two-tailed alpha error of 0.05 with 80% power using mean of 48.9nmol/L and standard deviation of 16.8nmol/L for cases and mean of 57.0 nmol/L for controls as reported in a previous study by Wei et al.[26] However, we recruited 80 participants in the case and control groups each to account for possible loss of sample and withdrawal of consent.

The data was checked for completeness and tabulated. Analysis was done using the statistical package for social sciences (SPSS) version 23 (IBM Corp 2015). Data was presented in tables and charts, while continuous data was presented in mean and standard deviation. Continuous data was analysed using T-test. The statistical significance was inferred at p-value ≤0.05. Serum levels of 25-Hydroxyvitamin D3 were analysed as both continuous and categorical variable.

3. Results

A total of 1,438 women presented to the antenatal clinic and labour ward out of which 96 (6.7%) women had a diagnosis of preeclampsia while 1342 (93.3%) women were without preeclampsia. Of the seventy eight (78) preeclamptic women which were used for final analysis, 71(91.0%) had severe preeclampsia while 7 (9.0%) had mild preeclampsia. See Table 1.

The age of women who participated in the study ranged from 20 to 40 years for the preeclamptic group with a mean age of 30.4±5.4 and 21 to 40 years for the control group with a mean age of 31.8±5.1. There was no statistically significant difference in the mean ages of the two groups of women in this study (t= -1.68, p=0.096) as shown in Table 1. The mean parity of women in the preeclamptic group was 1.3± 1.4. This is comparable to the mean parity of 1.6±1.4 among the women in the control group. There was no statistically significant difference in parity between the two groups (t= -0.99, p=0.32) as shown in Table 1. Nulliparous women were highest among both the preeclamptic group and the control where they constituted 38.5% and 32.5% respectively. The mean gestational ages of women in the preeclamptic and control group were 35.3±3.1 weeks and 35.7±3.1 weeks respectively. There was no statistically significant difference in gestational age between the two groups (t= -0.89, p=0.37). The characteristics of the women in the two groups were similar.

Other socio-demographic characteristics of the women were also shown in Table 1

Overall, the serum vitamin D levels in the participants ranged from 34.3nmol/L to 265nmol/L with a mean of 124.0±18.7nmo/L. The mean serum concentration of vitamin D in the preeclamptic group was 118.8±17.4nmol/L while the mean serum concentration of vitamin D in the control group was 129.0±19.7nmol/L. This was lower in the preeclamptic group than in the control group but was not statistically significant (t = -1.4, p=0.17) as was shown in Table 2. There was a weak association between gestational age and the level of serum vitamin D in both the preeclampsia and the control groups (r= 0.062 and r= -0.13 respectively). This was not statistically significant (p=0.59 and p=0.25 respectively). In the control, there was negative correlation. Most, 82.9% (n=131) of the participants had sufficient vitamin D status, 10.1% (n=16) had insufficient status while only 7.0% (n=11) had vitamin D deficient status. Within the preeclampsia group, 64 (82.0%), 8 (10.3%) and 6 (7.7%) and in the control group, 67 (83.7%), 8 (10.0%) and 5 (6.3%) had sufficient, insufficient and deficient vitamin D status respectively. There was no statistically significant difference in the vitamin D status in the preeclampsia and the control groups (p=0.94). The prevalence of hypovitaminosis D in our study was 7.0% overall, 7.7% for the preeclampsia group and 6.3% for the control groups respectively. The difference in the prevalence between the case and control groups was not statistically significant (p=0.76).

Subgroup analysis of the case participants showed that the mean serum concentration of vitamin D was 118.8±19.4nmol/L in women with severe preeclampsia and 120.3±15.2nmol/L in women with mild preeclampsia. This difference, however, was not statistically significant (p=0.18) as was shown in Table 3. Among women with severe preeclampsia, 58 (81.6%), 7 (9.9%) and 6 (8.5%) had sufficient, insufficient and deficient vitamin D status respectively while in the subgroup of women with mild preeclampsia, 6 (85.7%) and 1 (14.3%) had sufficient and insufficient vitamin D status respectively. There was no woman with deficient vitamin D status among the subgroup of mild preeclampsia. There is no statistically significant difference in the vitamin D status in the severe preeclampsia and the mild preeclampsia sub groups (p=0.57). Table 3

Table 1 Socio-demographic characteristics of the women

	Preeclampsia	**Control**	**Total**	**p-value**
Number	78(71 severe, 7 mild)	80	158	
Maternal Age	30.4±5.4	31.8±5.1	31.1±5.3	0.096
Parity	1.3± 1.4	1.6±1.4	1.4±1.4	0.41
Marital Status				
Married	73 (93.6%)	80 (100.0%)	153 (96.8%)	
Single	5 (6.4%)	0 (0.0%)	5 (3.2%)	
Total	78 (100.0%)	80 (100.0%)	158 (100.0%)	
Residence				
Rural	34 (43.6%)	38 (47.5%)	72 (45.6%)	
Urban	44 (56.4%)	42 (52.5%)	86 (54.4%)	
Total	78 (100.0%)	80 (100.0%)	158 (100.0%)	
Educational level				
Primary	4 (5.1%)	0 (0.0%)	4 (2.5%)	
Secondary	30 (38.5%)	28 (35.0%)	58 (36.7%)	
Tertiary	44 (56.4%)	52 (65.0%)	96 (60.8%)	
Total	78 (100.0%)	80 (100.0%)	158 (100.0%)	
Occupation				
Artisan	4 (5.1%)	9 (11.3%)	13 (8.2%)	
Civil servant	10 (12.8%)	12 (15.0%)	22 (13.9%)	
Housewife	13 (16.7%)	16 (20.0%)	29 (18.4%)	
Public servant	11 (14.1%)	12 (15.0%)	23 (14.6%)	
Student	3 (3.8%)	0 (0.0%)	3 (1.9%)	
Trader	37 (47.4%)	31 (38.8%)	68 (43.0%)	
Total	78 (100.0%)	80 (100.0%)	158 (100.0%)	
Gestational age (wk)	35.3±3.1	35.7±3.1	35.5±3.1	0.37

Table 2 Vitamin D levels and status of the women

	Preeclampsia	**Control**	**Total**	**p-value**
Number	78(71 severe, 7 mild)	80	158	
Vitamin D levels (nmol/L)	47.5±17.4	51.6±19.7	49.6±18.7	0.17
Vitamin D status				
Sufficient (≥75nmol/L)	64 (82.0%)	67 (83.7%)	131 (82.9%)	
Insufficient (50- 74.9nmol/L)	8 (10.3%)	8 (10.0%)	16 (10.1%)	
Deficient (<50nmol/L)	6 (7.7%)	5 (6.3%)	11 (7.0%)	0.76
Total	78 (100.0%)	80 (100.0%)	158 (100.0%)	

Table 3 Vitamin D levels, vitamin D status and the severity of Preeclampsia

	Severe	Mild	p-value
Number	71	7	
Mean vitamin D levels (nmol/L)	118.8±19.4	120.25±15.2	0.18
Vitamin D status			
Sufficient (≥75nmol/L)	58 (81.6%)	6 (85.7%)	
Insufficient (50 - 74.9nmol/L)	7 (9.9%)	1 (14.3%)	
Deficient (<50nmol/L)	6 (8.5%)	0 (0.0%)	0.57
Total	71 (100.0%)	7 (100.0%)	

4. Discussion

In this nested case-control study, we identified that the prevalence of hypovitaminosis D in our study population was 7.0% overall. This is at variance with previous studies that reported higher prevalence of vitamin D deficiency state during pregnancy. In India, Sahu et al.[27] reported a prevalence of 90%, Ullah et al.[19] reported a prevalence of 78.19 % while Umar et al.[28] in Lahore Pakistan, reported that 95% of study population, had serum 25(OH)D concentrations indicative of vitamin D deficiency. Lesser values of 18.9% and 29% respectively were reported by Zhou et al.[29] in Asia and Gbadegesin et al.[18] in Nigeria, both from similar tropical environment with abundant sunshine. Vitamin D deficiency may not really be prevalent in Nigeria as initially thought.

The possible reasons for the varied prevalence of vitamin D deficiency all over the world include differences in nutritional habits, exposure to sunlight, mode of dressing, colour of skin, seasonal variations and body mass.[30] Apart from the above, another possible reason is sensitivity of the method used in determining the serum vitamin D level. Vitamin D deficiency has a very wide prevalence that ranges from 18% to 84% depending on the place of residence, ethnicity, local clothing customs and dietary intake as well as the cut off used.[5] While some authors regard vitamin D levels less than 20ng/ml (or 50nmol/L) as deficient, others put the cut off at less than 30ng/ml (or 75nmol/L).[8,25] For example, just like in our study, the study by Gbadegesin et al., defined vitamin D deficiency as serum vitamin D levels less than 20ng/ml (or 50nmol/L) while it was defined as serum vitamin D levels less than 30ng/ml (or 75nmol/L) in the study done by Ullah et al.[18,19]

The mean serum concentration of vitamin D in the preeclamptic group of 118.8±17.4nmol/L (or 47.5ng/ml) was lower than 129±19.7nmol/L (or 51.6ng/ml) reported in the control group, though the difference was not statistically significant (t= -1.4, p=0.17). Sahu et al.[27] (9.06±5.20 ng/ml vs 13.67±7.24ng/ml), Bakacak et al.[9] (19.3 ± 4.31ng/ml vs 23.7 ± 5.93ng/ml) and Ullah et al.[19] (23.96 ± 1.31 ng/ml vs 24.86 ± 1.02 ng/ml) all reported significantly lower serum vitamin D levels among women with preeclampsia when compared with controls. Umar et al.[28] reported insignificantly lower vitamin D levels in preeclamptic than healthy controls. The finding of this work that there was no statistically significant difference in the mean serum concentration of vitamin D in women with severe preeclampsia when compared with those with mild preeclampsia is consistent with the finding of Hashemipour et al[21] in Iran. Hashemipour et al even reported higher vitamin D levels among the severe preeeclamptic subgroup.

Most of the women in both the preeclamptic and control groups have sufficient vitamin D status - 64 (82.1%) in the preeclampsia group and 67 (83.8%) in the control group. Eight (8) women in each group (10.3% in preeclampsia group and 10.0% in the control group) had insufficient vitamin D status while 6 (7.7%) in the preeclampsia group and 5 (6.3%) in the control group had deficient vitamin D status. There was no statistically significant difference in the vitamin D status in the preeclampsia and the control groups (p=0.94). Similarly, Singh et al.[31] in India, reported from their published work that about 82.8% of preeclamptic and 31.25% of non preeclamptic patients were found deficient in vitamin D. Vitamin D levels were sufficient in only 17.2% of preeclamptic and 68.75% of non preeclamptic patients. However, they did not make any comment if this difference is statistically significant. Again, these differences could be attributed to differences in nutritional habits, exposure to sunlight, mode of dressing, colour of skin, seasonal variations and body mass.[30]

One of the strengths of this study is that the study participants in the two groups were matched. The participants were recruited strictly complying with the inclusion and exclusion criteria. The chemical pathologist who was helpful in

analysis of the samples was blinded to which group each of the samples belonged to. A limitation of the study was that it did not assess the serum vitamin D levels of the case subjects prior to the onset of preeclampsia. It is therefore difficult to make inference on causality, it is possible that any difference in vitamin D concentration may be a consequence rather than a cause of the disease.

5. Conclusion

Hypovitaminosis D is not significantly associated with preeclampsia when compared with healthy women (without preeclampsia). There is also a weak association between gestational age and the level of serum vitamin D in both the preeclampsia and healthy women (without preeclampsia). Considering the low prevalence of vitamin D deficiency in the study population and the insignificant difference in serum levels of vitamin D between preeclamptic women and healthy control, it may not be justified to routinely offer women vitamin D supplementation in pregnancy for the purpose of preventing or treating preeclampsia. It does appear that vitamin D deficiency may be just one of the factors involved in the interplay that leads to occurrence of the disease. It is important that effort should be made in the search for other possible factors implicated in the aetiopathogenesis of the disease. Nevertheless, this present study may not be generalizable but will serve as an impetus and also a foundation for a more robust, multi-centered longitudinal study, which will be of immense benefit in elucidating strongly whether or not vitamin D plays a role in the pathogenesis of preeclampsia.

Compliance with ethical standards

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Data Availability

The data used to support the findings of this study are available from the site publicly.

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Contribution to Authorship

C.C.Okoro, G.O.Udigwe and G.U. Eleje contributed to the conception of the study, design and manuscript writing and revision. O.C. Ikpeze, C.I. Enechukwu, J.I Ikechebelu, A.D. Okoro, E.C. Ezema, C.O. Ezeigwe and A.M. Ibekwe contributed to data analysis, manuscript writing and revision. C.C.Okoro, R.O.Egeonu and C.G. Okafor contributed to data collection, analysis and manuscript writing. All authors gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure of conflict of interest.

The authors declare that they have no conflict of interest.

Statement of ethical approval

Approval was obtained from Nnamdi Azikiwe University Teaching Hospital Ethics Committee (Approval number: NAUTH/CS/66/VOL.10/221/2017/ 135).

Statement of informed consent

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

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