Original Article

Markers of Lipid and Protein Peroxidation among Nigerian University Students with Dysmenorrhea

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ABSTRACT

Introduction: Oxidative stress has been associated with primary dysmenorrhea, but studies that have assessed multiple markers of peroxidation are scarce. This study investigated malondialdehyde (MDA), nitrotyrosine (3-NT), and protein carbonyls (PrCarb) as markers of oxidative stress and antioxidant status by serum alpha tocopherol level in young Nigerian women with dysmenorrhea. **Materials and Methods:** In a case–control design, 45 female undergraduates who had had regular menses for at least six previous cycles were recruited consecutively from a university clinic as cases and 45 apparently healthy age-matched counterparts in their hall of residences as controls. Serum levels of MDA, 3-NT, and PrCarb were determined using standard methods, and the values were compared between cases and controls using Mann–Whitney *U*-test and graphs. **Results:** Study participants' ages range from 16 to 29 years (mean = 22.0 ± 3.1 years). Serum level of 3-NT (45.89 ± 37.11 vs 21.27 ± 13.94 ng/mL) and MDA (0.75 ± 0.19 vs 0.45 ± 0.11 nmol/mL) was significantly higher in cases than controls. Plasma alpha tocopherol was significantly lower in cases (7.51 ± 1.95 μmol/L) than controls (8.98 ± 1.95 μmol/L). Conversely, PrCarb levels were not significantly difference between cases and controls. There were significant correlations between alpha tocopherol and 3-NT (r = −0.285; P = 0.007) and MDA (r = −0.321; P = 0.002), whereas this relationship was not shown with PrCarb (r = −0.073; P = 0.496). **Conclusion:** Remarkable lipid and protein peroxidation observed in young Nigerian women with dysmenorrhea was accompanied by correspondingly low level of serum alpha tocopherol suggesting potential need for vitamin E supplementation.

KEYWORDS: *Alpha tocopherol, malondialdehyde, nitrotyrosine, primary dysmenorrhea, protein carbonyls, undergraduates*

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INTRODUCTION

Primary dysmenorrhea, defined as painful menstrual cramps without obvious pathology, is a common gynecologic disorder in young female adults (18–25 years) worldwide.[1] It refers to any degree of cramping pain during menstruation which may last for 2 or more days associated with nausea, diarrhea, headache, and flushing.[1] Menstrual pain may be accompanied by various symptoms that can disrupt the life of women at school, work, and home and interfere with their social interactions, resulting in isolation. Primary dysmenorrhea has significant public and occupational health impact, but its occurrence varies remarkably, with studies performed in different settings reporting prevalence ranging from 20% to 94%.[2-6] These wide differences in prevalence may be attributed to the diversity of ethnic, sociocultural, or biological factors of the study populations and variation in the definitions of dysmenorrhea adopted by researchers. In

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Nigeria, a study in a university setting revealed that the prevalence of dysmenorrhea among students could be as high as 53.3% and it affects school performance and attendance.[7]

Although menstruation without pain depends on coordinated interaction among sex hormones, the pathogenesis of dysmenorrhea remains less understood and an area of research interest.[8] Although Guliaeva *et al.*[9] had suggested that inflammation and endothelia dysfunction which occur during dysmenorrhea are characterized by lipid peroxidation, an indication of oxidative stress as early as 1988, evidence in support of its significant roles in the pathophysiology of primary dysmenorrhea remains controversial.[10] One of the convincing mechanisms is that the uterus suffers vasoconstriction from sustained contractions mediated by prostaglandins "PGF2 and PGF2α" from the secretory endometrium during menstruation.[11] In most women with primary dysmenorrhea, there is increased endometrial secretion of PGF2 and leukotrienes during the menstrual phase.[11] However, literature suggests that reactive oxygen species (ROS) attack lipid, protein, and nucleic acid simultaneously in living cells during injury and inflammation.[12] It is, therefore, conceivable that peroxidation of lipids and proteins, which occurs as part of cellular injury in human, can be used as an indicator of oxidative stress in individuals suffering from primary dysmenorrhea.[13,14]

The measurement of malondialdehyde (MDA) is widely used as an indicator of lipid peroxidation, and an increase in its plasma level is indicative of a variety of pathophysiological processes in human.[15] Other important and reliable biomarkers that reflect oxidative stress are protein carbonyls (PrCarb), plasma proteins containing oxidized residues such as aldehydes and ketones, as well as 3-nitrotyrosine (3NT), a marker for nitrated proteins.[16]

Recently, the end products of oxidative stress and its related metabolites are increasingly becoming the target for therapeutic interventions in diseases associated with excessive inflammation and endothelia dysfunction.[17-19] Although anecdotal evidence suggests that biochemical markers of oxidative stress could be higher in individuals with dysmenorrhea than those with pain-free menstruation, data on their roles are scarce. It is therefore conceivable that if significant oxidative stress occurs in individuals with dysmenorrhea, cheap and available scavengers of free radical and antioxidants could constitute potential prevention and intervention. Given the debilitating effects menstrual pain may have on young women and the associated decrease in quality of life, it is important to investigate whether oxidative stress markers are higher among individuals with dysmenorrhea than their counterparts with painless menstruation. This is important because targeting reduction of oxidative stress may be a possible approach to ameliorating painful menstruation.

The only study that has reported oxidative stress among Nigerian women measured only the level of MDA in relatively small sample; it used descriptive study design and protein peroxidation markers were not explored.[20] It is doubtful whether measuring only MDA level, as an indicator of oxidative stress, is sufficient,[21] as oxygen radicals produced during dysmenorrhea have the potential to damage lipid and protein concurrently.[22] Therefore, it is necessary to investigate plasma levels of PrCarb and 3-NT, which are markers of protein oxidation in man.[23] A diligent search for literature investigating multiple markers of oxidative stress including protein oxidation in dysmenorrhea revealed that only one study was carried out among Turkish women age 20–34 years.[24] This study[24] found increased MDA among women suffering from dysmenorrhea, but 3-NT levels were not significantly different. To our knowledge, there is no study on combined markers of oxidative stress or that investigated antioxidant status by real antioxidant markers in patients with dysmenorrhea among Nigerians. This study was, therefore, carried out to investigate lipid and protein peroxidation markers in young Nigerian women with dysmenorrhea.

Materials and Methods

Study design and setting

In this study, a case–control design was adopted to investigate a convenient sample of female students who presented with dysmenorrhea at the University of Ibadan Health Centre (Jaja Clinic) and individuals with no history of painful menstruation from female halls of residence. The Jaja Clinic runs general medical care and weekly specialized clinics for gynecological care. Anecdotal observation from the University of Ibadan Health Centre (Jaja Clinic) suggests that about 20% of female undergraduates present with dysmenorrhea.

Study population and sampling

Female students of the University of Ibadan were the target population for the research. All potential study participants were invited to the University of Ibadan Health Centre (Jaja Clinic) and interviewed by the consultant gynecologist to establish the history of dysmenorrhea. Eligibility for cases (dysmenorrhea) was based on self-reported experience of lower abdominal/pelvic pain within 6–12 h of menstruation associated with onset of menses and lasted 8–72 h in the 6-month period preceding the study.[24] Female students

who had history of recent alcohol consumption, cigarettes smoking, and those with body mass index (BMI) greater than 25 kg/m^2 were excluded from the study as these factors are known to alter oxidative stress markers.[25,26] Students were eligible to be recruited as controls if they had never experienced lower abdominal/pelvic pain within 6–12 h of menstruation associated with onset of menses and lasted 8–72 h as well as history of illness or clinic consultation in 2 weeks preceding contact with the investigator. Cases were consecutively recruited as identified during the weekly clinic session, whereas age-matched individuals were purposively sought for at the corresponding hall of residence for each case and recruited as control. Cases and control were matched by selecting a control whose age equals age of the case plus or minus 1 year.

Sample size determination

Given that the mean serum MDA levels were 1.32 ± 0.46 and 0.91 ± 0.26 nmol/mL for the dysmenorrhea and control groups in a similar study, respectively,[24] recruiting 18 cases and 18 controls at 95% level of confidence will achieve a study power of 90%. Thus, a minimum of 36 participants were estimated as the required sample size. This estimate was obtained using the sample size formula for comparison of two mean estimates in online OpenEpi.[27]

Data collection and laboratory analyses

A validated questionnaire was used to record information on sociodemographic characteristics, anthropometrics, and clinical examination findings by the attending physician. Venous blood samples were collected into anticoagulant bottles. To ensure the chemical stability of alpha tocopherol and peroxidation markers, the supernatant obtained, after centrifugation at 1000*g* for 15 min at 4°C, was aliquoted, transported, and stored away from the reach of sunlight within 30 min of collection at −20°C till laboratory analyses. Plasma 3-NT levels were measured by enzyme-linked immunosorbent assay (ELISA) using test kits from Elabscience Biotechnology Co., Ltd (Houston, TX, USA). The levels of PrCarb ware determined using the OxiSelect Protein Carbonyl ELISA kit from Cell Biolabs, Inc. (San Diego, CA, USA). Plasma MDA levels were determined using the double-heating thiobarbituric acid assay method described by Jentzsch *et al.*,[28] and alpha tocopherol (vitamin E) was measured by spectrophotometric method as modified by Rutkowski and Grzegorczyk.[29]

Data analysis

Plasma levels of oxidative stress markers, namely, MDA, PrCarb, and 3-NT, as well as plasma level of alpha tocopherol were the dependent variables, whereas history of dysmenorrhea or no dysmenorrhea and other variables constituted the independent variables. The mean values with corresponding 95% confidence intervals were calculated for each of the markers of oxidative stress and alpha tocopherol. These values were compared between cases and control using Mann–Whitney *U*-test for comparison of mean of nonparametric data. In addition, values and differences were graphically displayed using box plot. All data were analyzed using Statistical Package for Social Scientists (SPSS) 20.0 for Windows (SPSS Inc., Chicago, IL, USA) at $P = 0.05$.

Ethical consideration

The study protocol was reviewed and approved by the University of Ibadan and University College Hospital Ethics Review Committee (UI/UCH ERC; approval number UI/EC/15/0395). All ethical principles as stipulated in the Declaration of Helsinki were strictly observed. All participants needing medical attention received appropriate care according to standard treatment protocol of the university clinic.

Results

The study participants comprised female undergraduates 16–29 years of age (mean age = 22.0 ± 3.1 years). Distribution of participants by other characteristics is as shown in Table 1. Almost three-quarter of the participants were from families in the middle social class (74.4%), and only 16 (17.8%) of 90 were menstruating at the time of their participation in the study. Comparing cases with controls, there were no statistically significant differences in the mean ages ($P = 0.121$), weight ($P = 0.111$), height ($P = 0.789$), waist circumference ($P = 0.122$), and hip circumference ($P = 0.127$) as well as distribution by social

Table 1: Characteristics of study participants

	All participants, *n*=90	Cases, *n*=45	Controls, *n*=45	*P*
Mean age (years)	22.0±3.1	22.5±3.3	21.8±2.8	0.121
Mean weight (kg)	58.7±9.1	57.1±8.2	60.2±9.6	0.111
Mean height (m)	1.6±0.1	1.6±0.1	1.6±0.1	0.789
Mean BMI (kg/m^2)	22.7±3.3	22.2±3.2	23.3±3.5	0.026
Mean waist circumference (cm)	71.7±6.7	70.1±6.1	73.3±7.1	0.122
Mean hip circumference (cm)	93.6±7.6	92.4±7.6	94.9±7.5	0.127
Social class, *n* (%)				
Low	18 (20.0)	10 (22.2)	8 (17.8)	0.757
Middle	67 (74.4)	32 (71.1)	35 (77.8)	
High	5 (5.6)	3 (6.7)	2 (4.4)	
On-going menstruation				
Yes	16 (17.8)	8 (17.8)	8 (17.8)	1.000
No	74 (82.2)	37 (82.1)	37 (82.1)	

BMI=Body mass index

Table 2: Mean values of markers of peroxidation in the blood samples of participants

Indicators of peroxidation	Cases			Controls			*P
	Range	Mean±SD	95% CI	Range	Mean±SD	95% CI	
All participants in each group							
Protein carbonyl (nmol/mg)	1.96-2.28	1.53±0.27	1.45, 1.61	1.02-1.60	1.50±0.47	1.36, 1.64	0.187
Nitrotyrosine (ng/mL)	11.44-148.10	45.89±37.11	34.74, 57.04	7.10-77.95	21.27±13.94	17.08, 25.45	<0.001
Malondialdehyde (nmol/mL)	0.26-1.27	0.75±0.19	0.69, 0.80	0.21-0.70	0.45±0.11	0.42, 0.48	<0.001
α-Tocopherol (µmol/L)	2.35-13.2	7.51±1.95	6.93, 8.10	4.55-13.59	8.98±1.95	8.39, 9.56	<0.001
Participants with ongoing menstrual flow							
Protein carbonyl (nmol/mg)	1.24-1.68	1.42±0.19	1.27, 1.59	1.12-3.77	1.81±0.89	1.07, 2.56	0.254
Nitrotyrosine (ng/mL)	21.68-117.37	44.82±32.49	17.68, 72.0	9.83-34.75	18.93±8.43	11.88, 25.98	0.047
Malondialdehyde (nmol/mL)	0.48-0.93	0.71±0.16	0.58, 0.85	0.43-0.70	0.56±0.79	0.49, 0.62	0.030
α-Tocopherol (µmol/L)	4.94-8.81	7.41±1.49	6.16, 8.66	6.92-13.59	9.44±2.0	7.76, 11.11	0.038
Participants without ongoing menstrual flow							
Protein carbonyl (nmol/mg)	1.18-2.28	1.54±0.28	1.45, 1.64	1.02-2.25	1.42±0.29	1.33, 1.52	0.071
Nitrotyrosine (ng/mL)	11.44-148.11	46.11±38.44	33.29, 118.54	7.10-77.94	21.77±14.90	16.80, 26.74	0.001
Malondialdehyde (nmol/mL)	0.26-1.27	0.75±0.20	0.69, 0.82	0.21-0.61	0.43±0.20	0.69, 0.82	<0.001
α-tocopherol (µmol/L)	2.35-13.12	7.53±2.05	6.85, 8.21	4.54-12.09	8.88±1.95	8.22, 9.52	0.005

SD=Standard deviation; CI=Confidence interval. *Derived from "Mann-Whitney *U*-test" used to compare mean values of cases and control

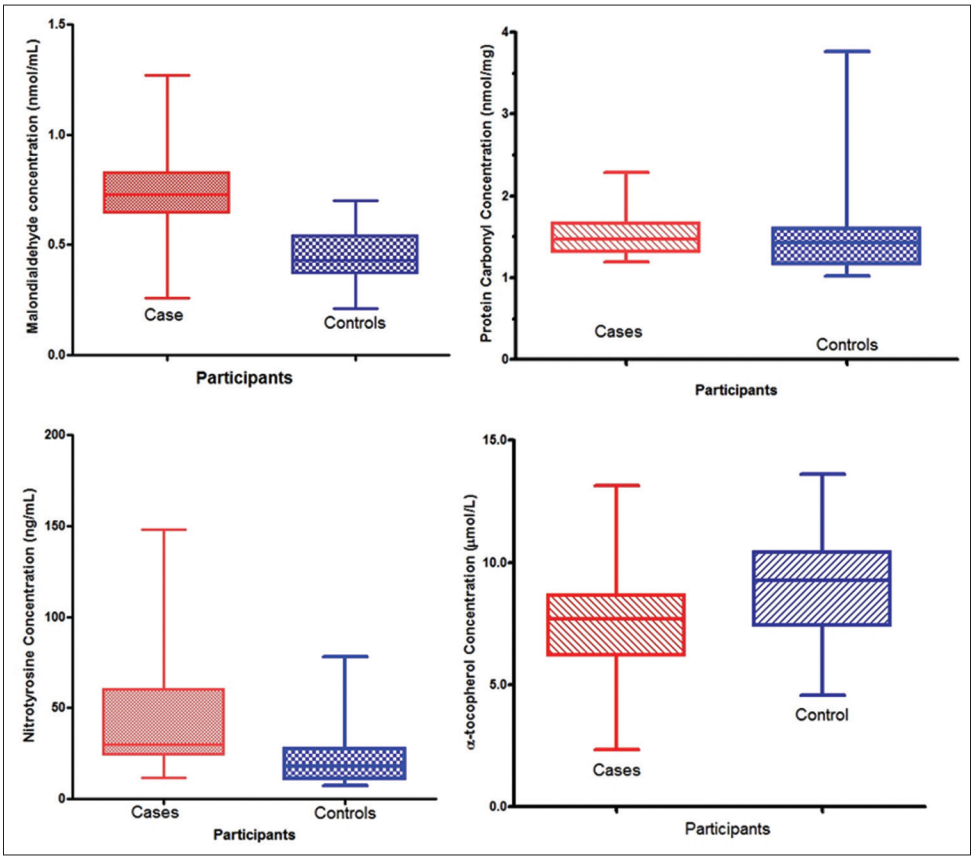

Figure 1: Box plots showing median values and interquartile ranges of plasma peroxidation markers

class ($P = 0.757$) and whether they were menstruating at the time or not ($P = 1.000$) [Table 1]. However, the participants who experienced dysmenorrhea (cases) had significantly lower BMI than those in the control group ($P = 0.026$).

The mean plasma levels of PrCarb, 3-NT, MDA, and alpha tocopherol for cases and control are displayed in Table 2. Notably, the mean values of 3-NT (45.89 ± 37.11 vs 21.27 ± 13.94 ng/mL) and MDA (0.75 ± 0.19 vs 0.45 ± 0.11 nmol/mL) were significantly higher in cases than controls. Conversely, the mean value of plasma levels of alpha tocopherol was significantly lower in cases (7.51 ± 1.95 µmol/L) than

controls (8.98 ± 1.95 μmol/L). Although the mean value of PrCarb was relatively higher in cases than controls, the difference was not statistically significant. Figure 1 graphically presents the median values (middle line of the box plot) and interquartile range (upper and lower margins of the box) of the markers of peroxidation, further illustrating the spread and differences expressed in Table 2.

Further analysis, by stratification of participants into those who had on-going menstruation and those without, showed that 3-NT and MDA levels were consistently higher among participants with dysmenorrhea than controls [Table 2]. When participants were not having menstrual flow, all the three markers of peroxidation (PrCarb, 3-NT, and MDA) were significantly higher among those who had history of dysmenorrhea than controls. Also, the levels of alpha tocopherol were consistently lower among individuals with dysmenorrhea than controls whether they were having menstrual flow or not [Table 2].

There were significant correlations between alpha tocopherol and 3-NT ($r = -0.285$; $P = 0.007$) and MDA ($r = -0.321$; $P = 0.002$), whereas this relationship was not shown with PrCarb ($r = -0.073$; $P = 0.496$).

Discussion

This study revealed that plasma levels of 3-NT and MDA, which are markers of protein and lipid peroxidation respectively, were elevated in Nigerian young women who experienced dysmenorrhea. There was also a concurrently lower plasma level of alpha tocopherol in those women. There is evidence in literature to suggest that a wide range of disruptions of major biological molecules occur during oxidative stress.[30] Notably, some of the molecules frequently affected include lipids, proteins, nucleic acids, and carbohydrates.[31] Most of the previous studies[24,32-35] that investigated oxidative stress in women with dysmenorrhea used MDA levels as the only biomarker, but this study also determined plasma levels of 3-NT and PrCarb to assess plausible protein peroxidation.

The fact that young women who experienced dysmenorrhea in this study were similar to those who had never had painful menstruation in demographic, social, and biophysical characteristics, except BMI, provides the logical basis for further comparison. This observation suggests that age, social class, or any of the anthropometrics could not explain the observed differences in levels of markers of oxidative stress. However, the observed difference in BMI could have occurred as a result of chance, but its effect on the differences in markers of oxidative stress was not explored, and therefore cannot be explained from our data. Nevertheless, earlier studies have demonstrated a direct relationship between oxidative stress markers and BMI, indicating an increase in plasma levels of markers of oxidative stress, including MDA, as BMI increases.[32,33] This potential adverse relationship could possibly be ameliorated by moderate to rigorous exercises which has been demonstrated to cause a remarkable decrease in ROS production in phosphorylating state.[36,37]

The remarkably higher level of MDA in women with dysmenorrhea than control observed in this study agrees with previous reports by Turhan *et al.*,[24] Yeh *et al.*,[34] and Dikensoy *et al.*[35] In attempts to explain the high level of MDA in dysmenorrhea, these studies[24,34,35] consistently referred to previously hypothesized pathogenesis of dysmenorrhea which linked serious inflammatory disruption and massive release of oxygen radical as observed in typical tissue and endothelial injuries with dysmenorrhea. In dysmenorrhea, frequent and prolonged prostaglandin-induced uterine contraction that reduces blood flow to myometrium has been reported.[38,39] Available data also suggest that hypoxia-ischemia which occurs during uterine contraction activates phospholipase A2, which hydrolyses the acylglycerolipids and produces free fatty acids, especially arachidonic acid. When perfusion is re-established during myometrium relaxation and oxygen supply improved, arachidonic acid is acted upon by three enzymes, namely, cyclooxygenase, lipoxygenase, and cytochrome P450 leading to eicosanoid formation and the release of activated oxygen species.[38,39] Thus, the released activated oxygen species are the possible cause of lipid and protein peroxidation.

The observed elevated levels of plasma 3-NT in this study are contrary to the report by Turhan *et al.*[24] who reported no significant difference in the plasma level of 3-NT of women with dysmenorrhea and healthy control. While Turhan *et al.*[24] used ELISA method for determination of plasma 3-NT as in this study, the number of study participants was relatively fewer. Also, the number of cases of dysmenorrhea ($n = 25$) was not the same as control ($n = 33$) in that study.[24] These two methodical issues may partly explain the difference. To our knowledge, no other study on plasma 3-NT in dysmenorrhea cases is accessible in public domain as at the time of this report. Therefore, further research with larger sample size is required to support or refute protein oxidation during dysmenorrhea using 3-NT as indicator.

On the other hand, the lack of statistically significant difference in the level of PrCarb between women with dysmenorrhea and the control group suggests the absence

of remarkable protein peroxidation in individuals experiencing dysmenorrhea. A review article published earlier showed that the early formation of PrCarb group and relative stability of carbonylated proteins make the usage of PrCarb the most reliable biomarkers of oxidative stress compared with the other measures of oxidation products including MDA.[40] It is conceivable that the slightly high level of PrCarb in women with dysmenorrhea, despite lack of statistically significant difference from the mean value obtained in control, may be biologically important given the indicative supremacy of this biomarker for protein peroxidation.

Another remarkable finding from our study is that the level of alpha tocopherol (vitamin E) was considerably lower in women who experience dysmenorrhea than controls suggesting relative deficiency of this antioxidant. However, it was observed that all the women (both cases and control) had relatively low serum level of alpha tocopherol, below the expected normal reference interval of 12–42 μmol/L.[41] A number of factors including storage condition, dietary intake, plasma albumin, and lipids at the time of sample collection are known to influence serum alpha tocopherol levels.[41] While precaution was taken during sample collection and optimal condition was carefully considered for stored blood samples, plasma albumin and lipids levels were not determined as part of this study. However, it is plausible that the lower level of alpha tocopherol depicts a relative deficiency among women with dysmenorrhea compared with controls. This apparent deficiency of serum alpha tocopherol among women with dysmenorrhea, a known antioxidant, could partly explain the observed higher level of oxidative stress markers than in the control group.

Further examination of the effect of menstrual flow at the time of blood sample collection on oxidative stress markers showed that the elevated levels observed among women who had dysmenorrhea are maintained whether the participants were currently menstruating or not. These results suggest that the high levels of oxidative stress markers could be a constant experience in Nigerian women who experience dysmenorrhea. Moreover, our results agree with the report by Akande and Akinyinka which earlier demonstrated consistently high levels of MDA during luteal phase of menstruation among Nigerian women.[20]

Undeniably, a number of factors that could confound the interpretation of results of this study were not investigated. For instance, the effect of dietary intake, plasma lipids, and albumin remains unknown because no data were collected to examine it. Participants with dysmenorrhea in this study were recruited from a specialist clinic indicating the possibility of having recruited those who were suffering from serious or severe forms of dysmenorrhea. Thus, it is not clear whether the observed result will be the same among women who have less serious forms of menstrual pain. Notwithstanding, the choice of study design allowed a reasonable measure of multiple exposures for dysmenorrhea which is a single outcome with adequate statistical power. Therefore, our findings are not only representative but also justifiably been generalized to population of women in the same age group.

Conclusion

Remarkable lipid and protein peroxidation, indicated by elevated plasma MDA and 3-NT, was observed in young Nigerian women with dysmenorrhea. These indicators of oxidative stress were accompanied by correspondingly low level of serum alpha tocopherol suggesting potential need for vitamin E supplementation. However, further investigation on a larger number of women would be required to prove or refute our findings. A future study to investigate whether short-term vitamin E supplementation ameliorates elevated oxidative stress markers or not may provide data that can support its use in management of primary amenorrhea.

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Conflicts of interest

There are no conflicts of interest.

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