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Research Paper

OXIDATIVE STRESS MARKERS PROFILE IN PRIMARY DYSMENORRHEA PATIENTS DURING MENSTRUATION IN MENOUA DIVISION-CAMEROON

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Abstract

Aim: The study was carried out to investigate the level of oxidative stress in primary dysmenorrhea patients during menstruation in Menoua Division-Cameroon by multiple serum makers including malondialdehyde (MDA), catalase, superoxide dismutase (SOD) and nitric oxide (NO). **Methods**: 423 women aged between 17 and 38 years, who had regular menses for at least six previous cycles, no infectious diseases (hepatitis B and C, Typhoid and Malaria) any gynecological disease, rheumatic, renal, cardiovascular, endocrine and metabolic disorders, were involved. Blood samples were obtained from each patient on the first or the second days of her menstrual cycles. The studies groups consisted of 299 subjects with primary dysmenorrhea and the control group consisted of 124 healthy subjects (without primary dysmenorrhea). **Results**: The serum levels of MDA, NO, SOD and catalase were higher in dysmenorrhea group compared to the control group; nevertheless, only catalase level showed a significant (p0.05) increase (16.39±4.05 µM/min/ml) in dysmenorrhea group with reference to the control group (12.53±3.80 µM/min/ml). Regarding the duration of

primary dysmenorrhea, results show a significant increases level of MDA with duration which is more pronounced and significant ($p<0.05$) at more than seven (7) years of dysmenorrhea. **Conclusion**: oxidative stress markers are increased with primary dysmenorrhea opens new avenues in the monitoring and also in the prevention of this disease.
Key words: Catalase, Malondialdehyde, Menoua Division, Nitric oxide, primary dysmenorrhea, Superoxide dismutase.

INTRODUCTION

Menstruation is a periodic and temporary genital bleeding, lasting from menarche to menopause [1].It is characteristic of primates and is defined as cyclic uterine hemorrhage, dependent on endometrial disintegration and exfoliation, which occurs approximately in a normal cycle of 21 to 45 days, with 2 to 6 days of flow and mean blood loss of 20 to 60 ml, in general lasting 40 years [2].

Dysmenorrhea, on the other hand, is pelvic or lower abdominal cyclic or recurrent pain, associated to menstruation. It is the most common gynecological complaint among young women, with prevalence between 43 and 93% [3] ·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. According to symptoms' intensity, it is also a major cause of school or work absenteeism in women [4]. Although menstruation without pain depends on coordinated interaction among sex hormones, the pathogenesis of dysmenorrhea remains less understood, and is an area of research interest [5,6].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 role in the pathophysiology of primary dysmenorrhea remains controversial [7].

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 [8]. In most women with primary dysmenorrhea, there is an increased endometrial secretion of PGF2 and leukotrienes during the menstrual phase [8]. However, literature suggests that reactive oxygen species (ROS) attack lipid, protein, and nucleic acid simultaneously in living cells during injury and inflammation [9] . It is therefore conceivable that lipids peroxidation, which

occurs as part of cellular injury in human, can be used as an indicator of oxidative stress in individuals suffering from primary dysmenorrhea [10,11] .

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 humans [12]. Other important and reliable biomarkers that reflect oxidative stress are SOD, catalase (antioxidants) and NO (anti -oxidant) [13,14,15] which are antioxidant. The purpose of the present study was to evaluate the profile of MAD,SOD, NO and catalase in primary dysmenorrhea and comparing the same with healthy controls

MATERIAL AND METHODS

- **Study site and population**

This study was carried out in Dschang District Hospital between March 2017 and December 2018. 299 patients with primary dysmenorrhea and 124 healthy women as control were involved. They all signed an informed consent. The study protocol was approved by the local Institutional Ethics Committee (No:10023 00040 0041 30000 35 ;swift :UCMACMCX). The eligibility criteria for participants were as follows: non-smoking, non-drinking women aged between 14 and 38, who used an acceptable method of barrier contraception, but who do not use an intra-uterine contraceptive device or an oral contraceptive, no infectious diseases like Hepatitis B and C, Typhoid and Malaria. Participants having any gynaecological disease, rheumatic, renal, cardiovascular, endocrine and metabolic disorders, inflammatory bowel disease, fibromyalgia which may cause dysmenorrhea were excluded.

- **Procedures of collecting dada**

After. taking into consideration the inclusion criteria (absence of pelvic pathology, problems of kidney and liver, none usage of intra-uterine device and pain-killers for other reasons, no pregnancy) , they were invited into the laboratory for blood collection during menstruation (first or second day of menstruation) ;After clotting and centrifugation 400 rpm for 10 min, serum samples were separated in Eppendorf tubes and frozen immediately at-4 °C until analysis. Parameters were measured by automated colorimetric methods (auto analyzer–dialab).

- **Assessment of dysmenorrhea**

All participants were questioned about their menstrual status during the three previous months. From the baseline-questionnaire, participants' dysmenorrhea was defined using the OLDCART (onset, location, duration, characteristics, aggravating factors, relief, and treatment) mnemonic as abdominal pain or lower back pain during menstrual bleeding, and as two or more days of primary dysmenorrhea during menstrual bleeding [16].

- **Evaluation of parameters related to oxidative stress**

✓ **Determination of the serum superoxide dismutase (SOD) activity**

Principle: The presence of superoxide dismutase (SOD) in a sample inhibits the oxidation of adrenaline to adrenochrome. The increase in absorbance read at 480 nm is proportional to the activity of superoxide dismutase.

Procedure: It was determined by the method of Misra and Fridovich [17].with some modifications. To do this, to 150 µl of serum were added 500 µl of carbonate-bicarbonate buffer (pH 10.2. 0.3M, pKa 10.3), then 250 µl of an EDTA solution (0.6 mM), and 350 µl distilled water. The mixture obtained was homogenized and 250 µl of adrenaline (4.5 µm) was added in order to initiate the reaction. Autooxidation of adrenaline was measured by reading the OD at 480 nm 30 seconds and 180 seconds after adding epinephrine.

SOD activity expressed as percentage inhibition was calculated as follows:

% inhibition = [100- (ΔOD sample/ΔOD blank)] x 100

Knowing that 50% inhibition corresponds to one unit of activity

✓ **Determination of serum catalase level**

Principle: le: When the dichromate is brought into contact with acetic acid, it is reduced to chromic acetate which can be measured colorimetrically. The intensity of chromic acid staining is proportional to the amount of non-decomposed H2O2 by the catalase present in the tissue homogenates and therefore reflects the inverse of the activity of the latter..

Procedure: It was determined by the method of Dimo and collaborators [18] .To do this, 375 µl of phosphate buffer pH 7.4 was added to 25 µl of serum, then 100 µl of H_2O2 (50 mM). One minute later, 1 ml of potassium dichromate (5%) prepared in 1% acetic

acid was introduced into the reaction medium. The mixture obtained was incubated for 10 minutes in a boiling water bath and then cooled in an ice bath. The reading of the optical densities was made at 570 nm against the blank (the extract was replaced by distilled water in the blank tubes). The enzymatic activity of catalase was deduced by the Beer-Lambert law.

- ✓ **Determination of nitric oxide (NO)**

Principle: NO content in serum is measured by Griess reagent. Absorption of the chromophore during ionization of nitrite with sulfanilamide coupled with naphthylethylenediamine (NED) is read at 520 nm [19].

Procedure: 340 µl of 1% sulfanilamide (prepared in 5% orthophosphoric acid) was introduced into 340 µl of serum and homogenates. The resulting mixture was homogenized and left in the dark for 5 minutes at room temperature. Next, 340 µl of naphthylethylenediamine (0.1% NED) was added to the reaction medium and the whole was left once more in the dark for 5 minutes. The optical densities were read at 520 nm against the blank. The NO content was determined from a calibration curve derived from the different Na2NO concentrations.

- ✓ **Détermination of malondialdehyde (MDA)**

Principle: Lipid peroxidation was evaluated by the determination of malondialdehyde (MDA) according to the method by Oyedemi and collaborators with certain modifications [20]. MDA is one of the final products of the decomposition of polyunsaturated fatty acids (PUFAs) under the effect of free radicals released during stress. In acidic and hot medium (pH 2 to 3. 100 °C), an MDA molecule condenses with two thiobarbituric molecules (TBA) to form a pink colored complex (reading at 532 nm) [20].

Procedure: Five hundred microliters of 1% orthophosphoric acid and 500 µl of precipitation mixture (1% thiobarbituric acid in 1% acetic acid) were added to 100 µl of sera. The resulting reaction mixture was homogenized and incubated for 15 minutes in a boiling water bath. After quenching in an ice bath, the mixture was centrifuged at 3500 rpm for 10 min. The absorbance of the supernatants was read at 532 nm against the blank. Lipid peroxidation was calculated on the basis of the molaldehyde (MDA) molar extinction coefficient and expressed in micromoles of MDA

- **Statistical analyses**

Data were analyzed using statistical software SPSS, version 16.0 (SPSS Inc, Chicago, IL, USA). The normally distributed data were presented as mean ± standard deviation (SD) and compared with one-way analysis of variance (ANOVA). The limit of significance was 5%.

RESULTS

The biological data for both groups are summarized in Table 1. In both groups, there were no significant ($p>0.05$) difference in body mass index and age. Meanwhile, a significant ($p<0.05$) difference was registered among dysmenorrhea duration.

Table 1: biological data for the studies groups

Factors	Modalities	Prevalence of dysmenorrhea (%)	P.value
Age of patients(years) (n=299)	[15-16] (n=61)	20.40	
	[17-18] (n=74)	24.70	
	[19-20] (n=73)	24.40	0.106
	[21-38] (n=91)	30.40	
Body weight index of patients (n=299)	[12-18[(n=58)	19.40	
	[18-20[(n=49)	16.40	0.088
	[20-22[(n=60)	20.10	
	[22-25[(n=78)	26.10	
	[25-42[(n=54)	18.10	
Duration of dysmenorrhea (years) (n=299)	[1-2] (n=85)	28.40[a]	
	[3-4] (n=95)	31.80[a]	0.000
	[5-6] (n=73)	24.40[a]	
	[7-12] (n=46)	15.40[b]	

The values in the table are presented as means ± standard errors of the means. Assigned values with different letters are significantly different by comparing the values of different groups are significantly different by comparing the values of the groups with the reference probability level of 5% (Waller Duncan's Test) . n = number of participants per group. p = probability.

The table 2 shows the variation of oxidative stress markers depending on the presence or absence of dysmenorrhea. The serum activities of SOD and catalase, the serum concentrations of MDA and NO increase with dysmenorrhea, but only the catalase activity revealed a statistical significant ($p<0.05$) increase with dysmenorrhea.

Table 2: Oxidative stress parameters depending on the presence or absence of dysmenorrhea.

Oxidative stress indicators	**Presence of dysmenorrhea (n=271)**	**Absence of dysmenorrhea (n=110)**	**P. value**
SOD (µM/min/ml)	0.65±0.24	0.60±0.27	0.301
Catalase (µM/min/ml)	16.39±4.05[a]	12.53±3.85[b]	0.002
MDA (µM/ml)	0.87±0.30	0.84±0.21	0.557
NO (µM/ml)	20.98±5.83	20.20±4.43	0.484

The values in the table are presented as means ± standard errors of the means. Assigned values with different letters are significantly different by comparing the values of different groups are significantly different by comparing the values of the groups with the reference probability level of 5% (Waller Duncan's Test) . n = number of participants per group. p = probability

The impact of the dysmenorrhea length on the oxidative satress markers is represented in table3.we observe the concentration of sod and catalase which are antioxidant with the duration of up six years dysmenorrhea then decreases at more than six years .However, only sod is significantly increased ($p<0.05$) at five to six years of

dysmenorrhea .Concerning MDA, its level increases with duration but more pronounced and 4significant($p<0.05$) at more than seven years of dysmenorrhea.

Table 3: The impact of the dysmenorrhea length on the oxidative stress markers.

Oxidative stress markers	**Duration of dysmenorrhea (years)**				**P. Value**
	[1-2] (n=85)	**[3-4] (n=95)**	**[5-6] (n=73)**	**[7-12] (n=46)**	
SOD (µM/min/ml)	0.60 ± 0.11^{ab}	0.67 ± 0.14^{a}	0.68 ± 0.16^{a}	0.56 ± 0.12^{b}	0.047
Catalase (µM/min/ml)	15.05±4.31	15.86±4.18	16.97±6.53	15.79±5.57	0.846
MDA (µM/ml)	0.85 ± 0.21^{ab}	0.76 ± 0.19^{b}	0.88 ± 0.33^{ab}	1.00 ± 0.38^{a}	0.044
NO (µM/ml)	21.04±5.41	21.44±4.88	21.93±5.84	21.31±6.02	0.979

The values in the table are presented as means ± standard errors of the means. Assigned values with different letters are significantly different by comparing the values of different groups are significantly different by comparing the values of the groups with the reference probability level of 5% (Waller Duncan's Test) . n = number of participants per group. p = probability

DISCUSSION

Oxidative stress is an imbalance of free radicals and antioxidants in favor of free radicals in the body which can lead to cell and tissue damage through several mechanism like lipid peroxidation witnessed by the increase in Malondialdehyde [21]. Our study results show increases level of MDA with duration which is more pronounced and significant ($p<0.05$) at more than seven years of dysmenorrhea. The higher level of MDA in women with dysmenorrhea than the control observed in this study agrees with previous reports by Turhan *et al.*[22] Yeh *et al.* [23], Orimadegun *et al.*[24]and Dikensoy *et al.*[25]. In attempting to explain the high level of MDA in dysmenorrhea, these studies [22, 23,24,25,26] consistently referred to previously hypothesized pathogenesis of

dysmenorrhea which were linked to serious inflammatory disruptions and massive release of oxygen radicals, 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 [27,28] .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[27,28]. Thus, the released activated oxygen species are the possible cause of lipid peroxidation.

CONCLUSION

Coming at the end of the present study, which aimed to investigate the level of oxidative stress in the patients with dysmenorrhea by multiple serum makers namely malondialdehyde, catalase, superoxide dismutase and nitric oxide .The observation that serum oxidative stress markers are increased with the presence of dysmenorrhea and more pronounced when participant had dysmenorrhea for more than seven years, opens new avenues in the monitoring and also in the prevention of this disease (dysmenorrhea).

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