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Biochemistry Section

# Oxidative Stress and Antioxidant Status in Primary Dysmenorrhea

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## ABSTRACT

**Objectives:** Primary dysmenorrhea is the menstrual pain associated with ovulatory cycles in the absence of pathological findings common in adolescents. Oxygen free radicals and the formation of primary dysmenorrhea is closely related to atrial contraction of uterine smooth muscle. The present study was to investigate the oxidative stress and antioxidants status in primary dysmenorrhea.

**Methods:** A 45 cases of primary dysmenorrhea and 25 age and sex matched controls were included in the study. The lipid peroxidation status was measured by estimating malondialdehyde. The antioxidant status was measured by estimating reduced glutathione, superoxide dismutase, vitamin E and vitamin C respectively.

**Reslults:** The malondialdehyde level was increased in cases than controls ($p = 0.001$). The antioxidant parameters studied like reduced glutathione, superoxide dismutase, vitamin E and vitamin C, all were decreased individually in cases than controls respectively ($p = 0.001$).

**Conclusions:** Our study showed a significant increased lipid per oxidation and decreased anti oxidants levels in primary dysmenorrhea cases than controls. This study reveals increased oxidative stress and decreased antioxidants as one of the important contributing factor in the pathogenesis of primary dysmenorrhea.


**Key Words:** Lipid Peroxidation. Antioxidant, Dysmenorrhea

## INTRODUCTION

Primary dysmenorrhea is defined as painful menstrual cramps without any evident pathology to account for them. It refers to any degree of perceived cramping pain during menstruation. 5–10% of girls in their late teens or early 20 years are severely incapacitates by primary dysmenorrhea for short hours each month [1]. The pain is a low, midline, wave like, cramping pelvic pain, radiating to back or inner thighs. Cramps last for one or more days with nausea, diarrhea, headache and flushing's. Uterus suffers with vasoconstriction, anorexia, sustained contractions mediated by prostaglandins "PGF2 and PGF2α". Oxidative stress was studied during such attacks of pain.

In most but not all women with primary dysmenorrhea, there is increased endometrial secretion of menstrual prostaglandin F2 (PGF2) during the menstrual phase [2],[3].The release of prostaglandins into the menstrual fluid is a continuous discontinuous process [4] that is, the amount of menstrual fluid and prostaglandins varies throughout any window of time. The intensity of the menstrual cramps and associated symptoms of dysmenorrhea are directly proportional to the amount of PGF2 released [5].

The other factors are circulating vasopressin levels are higher in women who have dysmenorrhea. Vasopressin stimulates uterine contractility. Leukotrienes and endothelins also increase myometrial contractility but their role in dysmenorrhea is unclear [6].

Oxygen free radicals and the formation of primary dysmenorrhea is closely related to artrial contraction of uterine smooth muscle, when the muscle between the blood vessels leading to the emergence of uterine compression, transient ischemic muscle and endometrial cells to the uterus as ischemia-reperfusion and produce more oxygen free radical scavenging chlorine superoxide dismutase will correspondingly reduce the psychosocial factors and primary dysmenorrhea [7].

The purpose of the present study was to evaluate oxidative stress and antioxidant status in primary dysmenorrhea and comparing the same with healthy controls.

## MATERIAL AND METHODS

A 45 women of primary dysmenorrhea who attended the Department of Obstetrics and Gynaecology of Mamata Medical College, Khammam were included in the study. A 25 healthy, age and sex matched controls were included in the study. The lipid peroxidation status was assessed by estimating MDA and antioxidant status was assessed by estimating superoxide dismutase (SOD), reduced glutathione (GSH), vitamin E and vitamin C in all the subjects respectively. Fasting venous blood samples were collected for the study of various parameters and taken in EDTA vial, in plain vial (without anticoagulant) and in heparinized vials. Samples were used for the estimations of plasma vitamin C, Plasma vitamin E, whole blood reduced glutathione, red cell SOD activity and Serum MDA.

MDA was estimated in terms of thiobarbutric reacting species (TBARS) by the method of M .Sasikala and et al [8].The thiobarbituric acid reaction is used to measure serum MDA. In this test, the chromogen is formed by the reaction of one molecule of MDA with two molecules of TBA. The method involves heating the sample (serum) with trichloroacetic acid and thiobarbituric acid under acidic conditions. To increase sensitivity, the MDA-TBA adduct formed is extracted into an organic solvent (butanol) and reading the absorbance of the MDA-TBA adduct at 532 nm.. SOD was assayed according to the procedure of CC. Winter-bourn et al [9].

This method is based on the method of Beauchamp and Fridovich and depends on the ability of the enzyme to inhibit the reduction of NBT by superoxide, which is generated by the reaction of photoreduced riboflavin and oxygen. Results are expressed as units of superoxide dismutase per gram of hemoglobin and 1 unit is defined for a particular system as that amount of enzyme causing half the maximum inhibition of NBT reduction.

GSH was estimated in the whole blood by method of Beutler, Duran and Kelly. The method is based upon the development of a relatively stable yellow colour when 5,5' –dithiobis-(2-nitrobenzoic acid) is added to sulfhydril compounds.

Vitamin E was estimated by using Emmerie-Engel reaction [10]. This method is based on the reduction by tocopherols of ferric to ferrous ions, which then form a red complex with α,α'–dipyridyl. ocopherols and carotenes are then extracted into xylene and the extinction read to 460nm to measure the carotenes. A correction is made for these after adding ferric chloride and reading at 520nm.

Vitamin C was estimated by [2],[6] dichlorophenol indophenol titration method [11], [2],[6]-Dichlorophenolindophenol is red in acid solution and on titration with a solution of ascorbic acid is reduced to the colourless leucobase, the ascorbic acid being oxidized to dehydroascorbic acid.

## STATISTICAL ANALYSIS

Statistical analysis was done using SalStat statistical software. t-test unpaired was used to compare means between cases and controls at 5% level of significance.

## RESULTS

Results of statistical analysis of oxidant and antioxidant parameters.

## DISCUSSIONS

The lipid peroxidation status was assessed in terms of MDA levels in the subjects. The MDA level was increased in cases of primary dysmenorrhea (mean ± SD = 4.31200 ± 0.48235) than controls (mean ± SD = 1.94880 ± 0.22775). The difference in the means is statistically highly significant as p = 0.0000001. In contrast to this, the antioxidant status was assessed by estimating MDA, SOD, GSH, vitamin E and vitamin C respectively. The SOD level was significantly decreased in cases (mean ± SD = 2278.84444 ± 182.67178) than control (mean ± SD = 3486.64000 ± 333.27665) as p = 0.0000001. Similarly the GSH was also significantly decreased in cases (mean ± SD = 52.55622 ± 5.71813) than controls (mean ± SD = 70.72000 ± 4.01580) as p = 0.0000001. Furthermore antioxidant vitamins were also significantly decreased in cases when compared to the controls. The vitamin E was significantly decreased in cases (mean ± SD = 0.78156 ± 0.07058) than controls (mean ± SD = 1.33800 ± 0.28746) as p = 0.0000001. Similarly the vitamin C was also significantly decreased in cases (mean ± SD = 0.68422 ± 0.08267) than control (mean ± SD = 1.13560 ± 0.19183) as p = 0.0000001 respectively as shown in the [Table/Fig-1].

Our study showed increased lipid per oxidation that is increased oxidative stress and decreased antioxidants levels in primary dysmenorrhea cases than controls. This study reveals increased oxidative stress and decreased antioxidants as one of the important contributing factor in the pathogenesis of primary dysmenorrhea. The decreased antioxidants level may be due to increased consumption of antioxidants to detoxify the increased oxidants or free radicals in primary dismenrrhoea. The most appropriate first-line choice of therapy in most women with primary dysmenorrhea is an non-steroidal anti-inflammatory drugs (NSAIDs). William's gyneclology says that, oral vitamins E and B (thiamine), magnesium, fish oil, low fat diet, and the herb Toki-Shakuyaku-San have all be shown to improve dysmenorrhea [12].The careful examination of these food items reveals that these food are very good sources of vitamin E, Vitamin A and other important nutrients. The vitamin E and vitamin A are very good antioxidants and administration of these along with other nutrients thus have improved the dymenorrhoea. This study reveals that along with NASID, the patients of primary dymenorrhea should be supplemented with natural antioxidants like vitamin E, vitamin c and β- carotenoids which would be extremely useful and may be helpful in combating the pain.

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| Parameters | MDA (nmol/ml) | SOD (units/gm Hb) | GSH (µmol/L) | Vitamin E (mg%) | Vitamin C (mg%) |
|---|---|---|---|---|---|
| Controls (n = 25) | 1.94880 ± 0.22775 | 3486.64000 ± 333.27665 | 70.72000 ± 4.01580 | 1.33800 ± 0.28746 | 1.13560 ± 0.19183 |
| Case (n = 45) | 4.31200 ± 0.48235 | 2278.84444 ± 182.87178 | 52.55622 ± 5.71813 | 0.78156 ± 0.07058 | 0.68422 ± 0.08267 |
| t(68) | –23.056 | 19.630 | 14.053 | 12.395 | 13.714 |
| p-value | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

**[Table/Fig-1]:** (Values are expressed in mean ± SD), MDA : malondialdehyde, SOD : superoxide dismutase, GSH : reduced glutathione

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