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Superoxide Dismutase and Malondialdehyde Levels in Adolescents with Primary Dysmenorrhea

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Abstract: Primary dysmenorrhea is painful menstruation without pelvic abnormalities and it usually occurs among adolescents and female adults. Primary dysmenorrhea processing the increasing of lipid peroxide (oxidative stress) and decreasing of antioxidant level. The aim of this study to identified the differences of Superokside Dismutase and Malondialdehyde levels among adolescents with primary dysmenorrhea. The research design used Case Control. The population of this study were all female students at Health Science Faculty of Unipdu Jombang who got menstruation, the sample of this study were 24 respondents who met the inclusion and exclusion criteria. Sampling method in this study used Purposive Sampling technique. NBT method was to measured SOD levels, Thiobarbituric Acid (TBA) test used to measured MDA levels, and the measurement in this study used Spektrofotometer. The data analyzed by using Independent Sample T-Test with $\alpha \leq 0.05$. Independent Sample T-Test results showed that there were significant differences between SOD and MDA levels in both of case group and control group ($p<0.05$). In primary dysmenorrea there is an increasing of Malondialdehyde (MDA) levels and a decrease of superoxide dismutase (SOD) levels, so the requiring efforts to balance the condition and reduce symptoms of primary dysmenorrea are needed.

1. Introduction

Dysmenorrhea describs a sensation of cramps, severe pain at the lower abdomen and half of it followed by systemic symptoms including sweating, headaches, nausea, vomiting, diarrhea, fatigue and irritability [1-2]. These symptoms occurrs at few hours before menstruation and continue until 48-72 hours of menstruation [3]. Primary dysmenorrhoea is painful menstruation without caused by pelvic abnormalities and commonly it occurs among adolescents and young adults [4-6]. Dysmenorrhoea has a negative impact to quality of life of women or teenagers such as the relationships with family and school friends, work performance and recreational activities and absence from their school [7-8].

The incident of primary dysmenorrhea ranged 34% - 95% occurred among women worldwide who experienced menstruation [8-9]. The incidence of dysmenorrhoea in Mexico was 48.4% - 64% [10-11], 85.4% was in Ethipia [12], 85.6% occurred among high-school students in Kuwait[13], 87.7% of Turkish University students [7], 88% of Australian Teenanger [14], 89.9% of students in Iran [15].

The pathogenesis of dysmenorrhoea associated with serious inflammatory and the release of large volume of free radical oxygen in typical tissue and endothelial injuries with dysmenorrhea [16-18]. Primary dysmenorrhea occurs an increasing of prostaglandin levels, so it also induces uterine contractions and reduce blood flow to the myometrium (ischemia) and it do an increase the sensitivity of peripheral nervus [19-22]. Ischemia in uterine muscle and endometrial cells are the condition of reperfusion ischemia so it produces free radicals of oxygen and takes antioxidant superoxide dismutase (SOD) [23]. The increasing of free radical activity can identify by using high levels of MDA in the body [24]. In primary dysmenorrhea occurs an increasing of lipid peroxidation (oxidative stress) and a decrease of antioxidant levels [23].

Pharmacological therapy to primary dysmenorrhea can use NSAIDs and contraceptives pills, but NSAIDs and contraceptives pills consumption have causes side effects such as discomfort of gastrointestinal, abnormal kidney and liver function [25-26], alternative interventions are needed, and the interventions does not have cause side effects. The interventions can be use β-carotene and Vitamin

E. β-carotene, because it can clean up free radicals and stop the process of lipid peroxidation [27-29]. Moreover, Vitamin E can inhibit arachidonic acid oxidation and prostaglandin production, so it can reduce the severity and duration of primary dysmenorrhoea [30-31]. Based on this description, the researchers are interest to conduct this research. The objective of this research was to found the differences of the Superoxides Dismutase and Malondialdehyde levels among adolescents who have primary dysmenorrhoea and without primary dysmenorrhoea.

2. Research Methodology

2.1 Design

The research design in this study used Case Control. Cases group in this research is the female student who got menstruation with dysmenorrhea and control group was female student who got menstruation without dysmenorrhea.

2.2 Participants

All female students in Faculty of Health Science of Unipdu Jombang who got menstruation were the population in this study, 24 respondents (case group n = 12 and control group n = 12) were taken as the sample by using inclusion and exclusion criteria. The inclusion criteria in this study are: 1) Case group: Students experienced primary dysmenorrhea; Has not received anti-pain therapy; Cooperative students; 2) Control group: Students who got menstruation without pain. Female students who experienced secondary dysmenohea was being exclusion criteria. Sampling method used Purposive Sampling technique.

2.3 Procedure of data collections

After getting permission from the head of the research institute and Ethical Clearance from the Ethics Commission of the Nursing Faculty, Airlangga University, Surabaya, the researchers approached the students who experienced menstrual pain (dysmenorrhoea) and the student who didn't have menstrual pain to get approval from them to being sample in this study. After that the researchers taken whole blood samples (3 mL) at peripheral vein. The blood sample of case group was taken at the first dysmenorrhea and blood sample was taken at the first menstruation for control group.

2.4 Measurement of SOD level and MDA levels

The measurement tool used a Spectrophotometer. NBT (Nitro Blue Tetrazolium) method used to measure SOD levels and the Thiobarbituric Acid (TBA) method used to measure MDA levels.

2.5 Statistical analysis

Statistical Package for Social Sciences (SPSS) version 16 used to analyze the data. Independent Sample T-Test with $\alpha \leq 0.05$ used to identify the differences of Superokside Dismutase and Malondialdehyde levels among adolescents with primary dysmenorrhea and without primary dysmenorrhea.

3. Results and Discussion

The age of respondents in both groups of this research were 20-25 years old, more than half participant 12-14 years old as their menarche age. The menstruation duration in case group was all respondent mentioned ≥ 7 days and in control group was more than ≥ 7 days. The characteristics of the research subjects were explained in detail in the following table 1.

Table 1. Characteristics of research subyect and homogeneity

Characteristics		Case group	Control group	P value
		N (%)		
1.	Age (year)			
	a. < 20	3 (25)	4 (33.3)	0.399
	b. 20 – 25	9 (75)	8 (66.7)	
2.	Age of Menarche (year)			
	a. 9 – 11	2 (16.7)	1 (8.3)	0.881
	b. 12 – 14	8 (66.6)	9 (75)	
	c. 15 – 17	2 (16.7)	2 (16.7)	
3.	Duration of menstruation (days)			
	a. 5 – 6	0 (0)	3 (25)	0.000
	b. ≥ 7	12 (100)	9 (75)	

Source: Primary data, 2019

To find the differences between SOD and MDA levels in case group and control group the researcher used the Independent Sample T-Test statistical test, as shown in table 2. below:

Table 2. The differences SOD and MDA level in case group and control group

Variable	Case group (Mean ± SD) (pg/ml)	Control group (Mean ± SD) (pg/ml)	Beda *Mean* (95% *CI*)	p
SOD level	1024.17 ± 68.57	1093.75 ± 20.72	-69.58 (-114.26 – -24.91)	0.005
MDA level	290.00 ± 31.21	76.83 ± 16.33	213.17 (192.08 – 234.26)	0.000

Independent Sample T-Test.

This research found that the mean of SOD levels in case group was 1024.17 pg/ml and in control group was 1093.75 pg/ml, this showed that the SOD level among case group was lower than control group. The mean of MDA levels in case group was 290 pg/ml and in control group was 76.83 pg / ml, this means that the mean of MDA level in case group was higher than control group. The result of Independent Sample T-Test statistic showed that there were statistical significant differences between SOD and MDA levels in both of case and control groups ($p<0.05$).

Primary dysmenorrhea is pelvic pain that occurs during menstruation without pathological abnormalities [32]. Dysmenorrhea occurs because of the increasing production of prostaglandins in uterine endometrium. Endometrial secretion contain arachidonic acid and it become prostaglandin F2α and prostaglandin E2 during menstruation. The release of prostaglandin during menstruation occurred along 48 hours (Koike et al, 1992 in Pramanik et al, 2015) [33]. The increasing of prostaglandins lead uterine contractions, decrease blood flow to the myometrium and the end of process is ischemic and increase peripheral nerve sensitivity [20-21]. Hypoxemia-ischemia occurs during uterine contractions by use activates phospholipase A2, hydrolyses acylgliserolipids and produces fatty acids, especially arachidonic acid. When the perfusion is maintained during myometrial relaxation and maximum of oxygen supply, arachidonic acid metabolized by three enzymes, it are cyclooxygenase, lipoxygenase, and cytochrome P450 which is they lead to formation process of eicosanoids and the release of Reactive Oxigen Species (ROS) [19] [22]. The release of Reactive Oxigen Species (ROS) are causes by lipid peroxidation and protein.

The results of this study showed that the average SOD level in group with primary dysmenorrhoea was lower than group without primary dysmenorrhoea and the average MDA level in group with primary dysmenorrhoea was higher than group without primary dysmenorrhoea. The results of examination of SOD and MDA levels in this study were taken on the first day of menstruation, where at 48 hours most of prostaglandins were released so this impacted on ischemia and pain. The decreasing of SOD levels in primary dysmenorrhoea group because of the increasing of free radical activity, this can be seen from the increasing of MDA level's respondents. SOD is one of the type of antioxidant and

has a function to protects cells against oxidant disorders (free radicals), which is it can cause several diseases by preventing the formation of new free radical or changing free radicals to be come less reactive molecules. SOD converts superoxide anions to be hydrogen peroxide and oxygen, and it called the primary defense against oxidative stress because superoxide is a strong initiator of chain reactions [34-35]. Free radicals in the body can cause lipid oxidation. Lipid peroxidation is an oxidative destruction of long-chain unsaturated fatty acids (Polyunsaturated Fatty Acid) that produce malondialdehyde (MDA) compounds. MDA is an index indicator to measure free radical activity. The increasing of free radical activity can be seen by the high levels of MDA in the body [24]. The increasing of oxidative stress and decreasing of antioxidants are important pathogenesis factors in primary dysmenorrhea [16] [36]. The decreasing of antioxidants caused by an increase consumption of antioxidants to detoxify the increasing of oxidants or free radicals in primary dysmenorrhoea [23]. Oxidative stress occurred when the balance of antioxidants and reactive oxygen species (ROS) were disrupted due to depletion of antioxidants or increasing of ROS formation [22] [37].

Some results from previous studies showed that MDA levels were higher among women with dysmenorrhoea compared to women without dysmenorrhoea [16] [18] [38-40]. This mean that dysmenorrhoea there is an increase in lipid peroxidation, an oxidative stress index and it characterized by an increasing of plasma malondialdehyde (MDA) levels.

The results of the study by Rao et al (2017) [23], found had a decreasing of antioxidant levels (SOD) among women with primary dysmenorrhoea compared with healthy women. The measure method to measure SOD in that study use the Beauchamp and Fridovich methods, while in this study using the NBT method. Although the methods used to measure SOD levels are different with this study but the results on both study were same. This mean that in primary dysmenorrhoea occurred the decrease of antioxidant levels (SOD), and this is needed to detoxify an increase of oxidants.

4. Conclusion

In primary dysmenorrea there is an increasing of Malondialdehyde (MDA) levels and a decrease of superoxide dismutase (SOD) levels, so the requiring efforts to balance the condition and reduce symptoms of primary dysmenorrea are needed.

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