

Comparison of Ovarial Malondialdehyde (MDA) Level between Endometriosis Rat Given with and without Curcumine Supplementation

Bernadetta Caroline Panjaitan¹, Ashon Sa'adi¹, Hendy Hendarto¹, Widjiati²

¹Department of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Dr. Soetomo Hospital, Surabaya

²Department of Embryology, Faculty of Veterinary Medicine, Airlangga University

ABSTRAK

Peradangan kronis pada endometriosis menyebabkan stres oksidatif pada sel granulosa yang menyebabkan ketidakseimbangan antara oksidan dan anti radikal bebas. Hal ini menyebabkan efek berantai terus menerus dan menyebabkan kerusakan membran sel. Efek akhirnya adalah ketidakstabilan dan fragmentasi produksi MDA. Peran kurkumin sebagai antioksidan yang dapat menurunkan MDA titer menyeluruh belum diteliti. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh kurkumin ke ovarium MDA di dalam tikus jenis endometriosis. Penelitian ini merupakan uji eksperimental dengan populasi 40 tikus (*Rattus novergicus strain Wistar*) betina yang memenuhi kriteria inklusi dilakukan randomisasi dengan 20 sampel dalam setiap kelompok. Untuk mendapatkan tikus endometriosis, kami menyuntikkan tikus dengan intra Estradiol cyclosporin A dan Ethynil muscular juga injeksi dalam jaringan endometrium dalam tumor ovarium jinak pada manusia ke dalam tikus intrapertitoneal. Kelompok perlakuan diberi suplementasi kurkumin 24 mg (240 g/ kg) setiap hari dalam 14 hari, kelompok lain diberi plasebo. Setelah semua tikus dioperasi untuk mendapatkan jaringan ovarium dan memeriksa titer MDA diperiksa dengan spektrofotometri. Didapatkan ada perbedaan dalam titer ovarium MDA di dalam tikus endometriosis yang diberikan dan tidak diberikan suplementasi kurkumin ($p < 0,0001$). Sebagai kesimpulan, titer MDA pada tikus endometriosis dengan suplementasi kurkumin lebih rendah dari plasebo. (MOG 2012;20:30-34)

Kata kunci: endometriosis, curcumin, MDA

ABSTRACT

Chronic inflammation at endometriosis causing oxidative stress at granulosa cell which cause unbalance between free radical and anti oxidant. This cause the chains effect continuously and cause the destruction of cell membrane and the last effect is unstable and the fragmentation producing MDA. The role of curcumin as antioxidant can decrease overall MDA titer has not been studied. The objective of this study was to investigate the effect of curcumin to ovarian MDA in endometriosis rat. This was eksperimental laboratory trial with population female 40 rat (*Rattus novergicus strain Wistar*) which met the inclusion criteria the rats were randomized and with 20 sample in each group. To become endometriotic the rats were, we injected with cyclosporin A and Ethynil Estradiol intra muscularly also injection in endometrial tissue of human benign ovarian tumor in human into intrapertitoneal rat. Treatment group was given curcumine supplementation 24 mg (240 g/kg) every day in 14 days, another group given placebo. All rats were operated to get the ovarian tissue and check the titer MDA was examined with spectrofotometry. There were difference in ovarian MDA titer at in endometriosis rat which given with dan without curcumine supplementation. ($p < 0,0001$). In conclusion, MDA titer at endometriosis rats with curcumin supplementation is lower than placebo. (MOG 2012;20:30-34)

Keyword : endometriosis, curcumin, MDA

Correspondence: Bernadetta Caroline Panjaitan, Department of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Dr. Soetomo Hospital, Surabaya, detta_p@yahoo.com

INTRODUCTION

Endometriosis is a gynecology abnormality which known as the growth of endometrial tissue outside the uterine cavum. This abnormality is often found in reproductive age. Most of them come with symptom of pain and infertility. Endometriosis therapies recently are medical therapy, surgery and combination of both. Medical therapy recently with hormonal therapy like GnRHagonis, progestin, danazol oral contraception. Hormonal therapy is the most effective in reducing pain at endometriosis. Commonly to get the optimal effect,

these drugs given in 3-6 months, with side effect the cease of ovulation progress and hipoestrogen. This can cause endometriosis patient cannot pregnant while in therapy

Chronic inflammation at endometriosis causes oxidative stress at granulosa cell which cause unbalance between free radical and anti oxidant. This leads to chains effect continuously and cause the destruction of cell membrane and the last effect is unstable and the fragmentation producing MDA. One of the alternative therapy recently is herbal therapy from plantation which is

Curcuma longa and has been used for many centuries in South East Asia. Many active ingredients in curcumin have different effect which are anti inflammation, anti oxidant, anti proliferative, anti angiogenesis. Phenolic and methoxy group in the structure of curcumin is the basic things which function is anti oxidant. Until know the role of curcumin as antioxidant that can decrease the overall MDA titer, has not been studied.

Ideal therapy is to suppress the target cell as effective, safety, cheap and with little side effect and without hypoestrogenic effect. Curcumin has not been used in human to cure endometriosis, which connected to infertility and in human experimental to know the effect of curcumin still constraint with ethic. So in this studying, we use endometriosis type rat. The aim is to prove the MDA titer in ovarian rat can decreasing with curcumin in endometriosis type rat compared with control group. With group fenolic in the structure of curcumin is the basic things of the effect as an antioxidant to clean the free radical directly and indirectly by activate the GSH reductase enzyme which it role to increasing the supply of GSH. In this experiment, the concept which shows that curcumin by oral can prevent peroxidation lipid membran which has been caused by oxidative stress. The damage of granulosa cell membrane causes interference in folliculogenesis. The effect of antioxidant curcumin can decrease the oxidative stress and lipid membrane peroxidation. In the future, curcumin can be the alternative therapy to cure endometriosis, particularly which related to infertility.

MATERIALS AND METHODS

Research was conducted in Embriology Laboratory, Faculty of Veterinary Medicine, Airlangga University, in March 2012. Sample was taken from two groups of rats: group A and B, injected by cyclosporin and got injected by human endometrial tissue intraperitoneally, then injected by estradiol at day 1 and 5, start from day 14, group A got curcumin by sondage for 14 days, as if group B got placebo per sondage for 14 days. The amount of sample got 19 with federer formula.

Inclusion criteria for this research are: female rat, (*Rattus novergicus* strains wistar), 3 months, weight 100-150 grams, virgin, health. Exclusion criteria: have been used by others research. All materials in this study was endometriosis type rat ovarian. Tissue was put in reaction tube. Ovarial tissue is separated and homogenizing in homogenizer with buffer contain 1,5% chloride potassium to get 1:10 (b/v) fully homogenized. Supernatan (MDA) was mixed with 1 ml TBA reagent, measure with spectrophotometry at 541nmol.

All experimental animals were taken from experimental animal unit in Faculty of Veterinary Medicine, Airlangga University. We use female rat (*Rattus novergicus*) with age less than three months and weight 100-150 grams, chosen by inclusion and exclusoin criteria. Rat will be in adaptation time for 1 weeks in clean nest, enough air, light, food and drink and homogenize. All rats were divided to 2 groups, with each group consisted 19 rats. Group A and B were endometriosis type rats. Group A received curcumin, group B only placebo. To make the endometriosis type rat is from endometrial biopsy which injected by cyclosporin and estrogen. The injection of cyclosporin in day 1 intramuscularly to group A and B, each 0.2 ml with disposable syringe 1 ml. Injection with endometrial biopsy at day 1 intraperitoneally to 2 groups A and B, each rat injected by 0.7 ml. Injection with disposable syringe 1 ml, with needle size 16 so the endometrial tissue can enter. Injection at day 1 and 5 intramuscularly for group A and B, each rat injected by 0,05 ml, injected with disposable syringe 1 ml, treatment with curcumin supplementation given in day 14 to group A. Group B was given with placebo. Treatment for two groups until day 27 (14 days). Sample was taken at day 28, and analyzed by spectrofotometry to measure the MDA titer in the ovary.

All data in this research were recorded in specific form. Data analysis was done with software SPSS (Software Package for Social Science). Conduct normality test with Kolmogorov Smirnov test. If the data were distributed normally, continued with t test 2 free samples. If data not normally distributed, continued with Mann-Whitney test. The confidence interval at this research is 0.05.

RESULTS AND DISCUSSION

After tested by normality test with distribution Kolmogorov-Smirnovone sample in variable which is rat weight placebo group, the result was $p = 0.431$ (before treatment) and $p = 0.381$ (after treatment) also with curcumin got $p = 0.141$ (before treatment) and $p = 0.411$ (after treatment). because $p > 0.05$ so the two groups are normally distributed (Table 1).

Because normally distributed, then tested with t test two sample and get $p < 0.05$ at two groups so can be concluded even we found increasing weight at curcumin groups more than placebo group, but as stastically with t test 2 sampe, got $p = 0.114$ ($p > 0.05$) show there are no difference at weight change in two groups and two group can be categorize as homogenize. Because the weight change is variable so weight is not put at next analysis (Table 2). In this research ovarian MDA titer

can be measure with spectrofotometry with kit OXItek TBARS produced by Zepto Metrix Corporation. The principle of this test was based on the measurement by spectrophotometry from pink, which produced by Thiobarbituric Acid (TBA) reaction with MDA, concentration of TBARS titer calculated by absorbancy calculation from MDA-TBA curve. The evaluation by spectrophotometry conduct in laboratory unit, consultation and training at Faculty of Veterinary Medicine, Airlangga University. Normality variable MDA ovarial titer was tested with Kolmogorof-Smirnov one sample, which $p = 0.972$ ($p > 0.05$) at placebo group and $p = 0.792$ ($p > 0.05$) at curcumin groups which means this two group were normally distributed (Table 3). Because of it normally distributed, stastically test which use is parametric test with t test two sample. T test two sample result was $p = <0.0001$ ($p < 0.05$) means there is differece between ovarial MDA average between curcumin group and placebo group. (Table 4)

On day 14 the treatment of curcumin and placebo started. We did not do confirmation examination about the incident of endometriosis histopatologically in our research design, but as a result we found that the variation coefficient was 12.7% for the placebo group and 22.2% for the curcumin group that shows MDA variable measurement.

Those narrow variation coefficient value shows that there might be other factors that can affect the result but it can be ignored. Success rate of incidence of endometriosis is based on previous studies conducted by Awwad (1999) 96%, Vika (2006) 87.5%, Kuswojo (2009) 89.5% and Sa'adi (2010) 80%. So we make a correction of the sample to anticipate the possibility of failure occurrence of endometriosis by adding the sample to remain unfulfilled.

Comparison of ovarian MDA levels in the placebo group and curcumin

Transcription factor NF- κ B known role in the pathogenesis of endometriosis, which stimulate the process of adhesion, invasion, angiogenesis, inflammation, cell proliferation and inhibits apoptosis endometriosis. The role of classical pathway activation of NF- κ B is a natural immune response that stimulates inflammation and maintain endometriosis lesions.¹⁰ Activation of transcription factor NF- κ B also associated with increased ROS.

Oxidative stress is believed to contribute negatively to the number of reproductive processes including folikulogenesis and endometriosis (Behrman, 2001). Suspected a link between oxidative stress and infertility. Women with endometriosis often show an increase in macrophage activity which may result in excessive ROS in the peritoneal environment.³ Increased oxidative stress and ROS concentration in peritoneal fluid and serum has also been shown in unexplained infertility, tubal factor infertility and endometriosis.^{8,3} Polak et al study the levels of MDA which is a marker of oxidative stress and also the enzymes catalase and SOD levels in endometrial tissue and blood on the woman's infertility, the results of these studies found that MDA levels were significantly higher and levels of the enzymes catalase and SOD were significantly lower in women with endometriosis and also in unexplained infertility compared with the levels of MDA in the control.⁸

Curcumin has potent antioxidant capabilities. Curcumin inhibits lipid peroxidation induced by iron.¹¹ the formation of ROS and oxidation of ferrous ions.¹¹ The ability of curcumin in removing free radicals especially with regard to O₂-and OH¹¹ may be due to the phenolic group of the donor H atom.^{2,6} Phenolic and methoxy groups on the phenyl ring and 1,3-diketon system seems to be an important structure that contributes to this effect. Another fact is that put forward in the antioxidant activity increases when the phenolic and methoxy groups are in the ortho position.

In our study, the mean levels of MDA in the rat model of endometriosis curcumin group was lower than the placebo group. Based on the free two-sample t test found significant differences between the two groups. Difference in results is likely due to the success of curcumin in inhibiting the process of apoptosis that occurs, while the mechanism may be through multiple pathways including inhibition of activation of the transcription factor NF- κ B with various results, suppression of inflammatory activity through the suppression of TNF- α directly or through its antioxidant effects. Singh and Aggarwal (1995) have shown that curcumin can inhibit the activation of the transcription factor NF- κ B with emphasis on the target before the I κ B- α phosphorylation. Research of Jobin (1999) have also proved the same. Curcumin was shown to reduce the negative impact of endometriosis patients with immune defects caused by anti-TNF- α , anti-NF- κ B, anti-oxidants, anti-JNK and anti-caspase activation pathway. Curcumin can suppress NF- κ B pathway and gene target of NF- κ B cytokines.

Table 1. Normality tes rat weight in curcumin dan placebo

Group		Mean	SD	P price	Information
Placebo	Before	131.0	13.3	0.431	Normal
	After	137.5	10.2	0.381	Normal
Curcumin	Before	135.5	16.7	0.141	Normal
	After	148.0	14.4	0.411	Normal

Note : $p > 0.05$ is normally distributed

Table 2. Homogenicity test result in rat weight fot group curcumin and placebo

Group	Before	After	delta	P
Placebo	131 $\pm$ 13.3	137.5 $\pm$ 10.2	6.5 $\pm$ 12.7	0.033
Curcumin	135.5 $\pm$ 16.7	148 $\pm$ 14.4	12.5 $\pm$ 10.7	<0.0001

Note: $p > 0.05$ is not meaningfull (homogen)

Table 3. Normality test result for MDA ovarial

Group	Mean	SD	p	Info
Placebo	0.63	0.08	0.972	Normal
Curcumin	0.18	0.04	0.792	Normal

Note: $p > 0.05$ is normally distributed

Table 4 T test two sample result from free MDA ovarial titer

	group		p	Info
	Placebo	curcumin		
MDA titer (nmol/ml)	0.63 $\pm$ 0.08	0.18 $\pm$ 0.04	< 0.0001	Difference Meaningfull

Note: $p < 0.05$ is meaningfull difference

With inhibition of apoptosis that occurs will cause the number of granulosa cells that survived became more so that proteins and hormones are secreted also higher. With more number of granulosa cells that survived the process of abnormal apoptosis, it is expected to increase fertility in patients with endometriosis.

CONCLUSION

Ovarian MDA level is lower in group receiving supplementation of curcumin.

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