Antioxidant status in women with uterine leiomyoma: relation with sex hormones

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ABSTRACT

Uterine leiomyomas are benign soft-tissues tumors that arise from uterine smooth muscle tissue. Etiopathogenesis of leiomyomas is not well understood. We aimed to examine whether antioxidant enzyme activities and lipid hydroperoxides level in patients with leiomyoma are influenced by changes in sex hormones and gonadotropins (estradiol (E2), progesterone, FSH, and LH) during menstrual cycle and in postmenopause. The material consisted of blood and uterine tissue specimens. Hormone concentrations were determined and assays for superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activities and lipid hydroperoxides concentration were performed. In blood of examined women, a significant difference in catalase, glutathione peroxidase and glutathione reductase activity was recorded among the phases. There was also a positive correlation between the estradiol/progesterone concentration and the catalase activity. Progesterone negatively correlated with lipid hydroperoxides level. In myoma tissue, we recorded a phase-related difference in lipid hydroperoxides level and activities of superoxide dismutase, glutathione peroxidase activities, and glutathione reductase. Negative correlation was observed between FSH and glutathione peroxidase. The results suggest that antioxidant status in patients with uterine leiomyoma is influenced by the changes in sex hormones during the menstrual cycle and in postmenopause, indicating a role of the observed relationship in the leiomyoma etiology.

Key words: antioxidant enzymes, lipid hydroperoxides, sex steroids, leiomyoma.

INTRODUCTION

Uterine leiomyomas are benign soft-tissues tumors that arise from uterine smooth muscle tissue (myometrium) and they are also called myomas or uterine fibroids. As non-cancerous growth arising from myometrium, leiomyomas are unrelated to the common forms of uterine cancer and uterine cervical cancer which typically

arise from the endometrium (the uterine lining) or cervical epithelium, respectively. Currently, the pathogenesis and etiology of leiomyomas are not well understood but several risk factors have been identified. These include early menarche (<10 years of age), nulliparity, age, hypertension, obesity, multiple uterine infections, tamoxifen use and ethnicity (Flake et al. 2003). Many studies are in agreement that African-American women present 2-3 times greater leiomyoma incidence

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than Caucasian, Asian or Hispanic women, as well as significantly higher serum levels of estrone (E1), estradiol (E2) and free E2 (Blake 2007). Some associations have been suggested with elevated levels of fibrotic growth factors and vascular abnormalities (Weintraub and Yemin 2011). Protective factors have also been identified, including smoking, multiparity, menopause, progestin-only injectable contraceptives, and oral contraceptive pills (Houston et al. 2001, Wallach and Vlahos 2004).

The growth of uterine leiomyomas is considered to be influenced by estrogen (E2), growth hormone, and progesterone. Such dependence is indicated by the fact that most of these tumors are diagnosed during the reproductive years when they exhibit maximum growth, and regress after menopause (Evans and Brunsell 2007). Many investigations also indicated differences between non-transformed and leiomyoma tissue concentrations of estrogens and progesterone and their respective receptors (ER-α, ER-β and PR-A, PR-B) (Ciavattini et al. 2013). Leiomyomas may occasionally increase in size during pregnancy, which can be caused by hormones, but also by increased blood flow and edema (Wallach and Vlahos 2004).

Reactive oxygen species (ROS) have many physiological regulatory functions and are also implicated in the development of a wide spectrum of diseases (Alfadda and Sallam 2012). The mammalian cell has an adequate antioxidant system to cope with excessive ROS production under normal physiological conditions. This system consists of antioxidant (AO) vitamins, thiol-containing compounds such as reduced glutathione (GSH), and antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). Variations in antioxidant capacity may influence individual susceptibility to some diseases associated with the deleterious effects of oxidative reactions (Shinde et al. 2012).

The relationship between sex hormones and antioxidant system was investigated during regular cyclic changes of endometrium, and in the reproductive system disorders. SOD plays an important role in maintaining luteal cell integrity and steroidogenic capacity (Sugino and Kato 1994) and its expression in endometrial stromal cells is associated with decidualization (Sugino et al. 2000). In amenorrheic women, estradiol but not progestagen replacement therapy stimulated erythrocyte GPx activity (Massafra et al. 2000). It is believed that estrogen decreases free radical production, and depressed estrogen synthesis in postmenopausal women is responsible for enhanced oxidative stress (Agarwal et al. 2005). In endometrial cells of women with endometriosis, Andrade et al. (2013) reported elevated levels of H_2O_2 and lower CAT activity, along with antiapoptotic effect of estradiol (E2), increased cell proliferation and increased mitogen-activated protein kinase (ERK) phosphorylation. These results support the findings of high endogenous oxidative stress in endometriotic cells due to alterations of ROS detoxification pathways and activation of ERK1/2, similar to tumor cells (Ngô et al. 2009).

We have previously shown that antioxidant status is altered in patients with endometrial hyperplasia or adenocarcinoma in comparison to women diagnosed with polyp or leiomyoma (Pejić et al. 2008, 2009). The aim of this study was to examine whether AO enzyme activities and lipid hydroperoxides (LOOH) level in patients with uterine leiomyoma are influenced by changes in sex hormones during the menstrual cycle and in postmenopause.

MATERIALS AND METHODS

SUBJECTS

The material used in this study consisted of 30 blood and tissue specimens of women admitted to the Department of Gynecology and Obstetrics for gynecological evaluation within routine checkups or for abnormal uterine bleeding (Metrorrhagia prolongata, Metrorrhagia recidivans, Metrorrhagia

postmenopausi). They were diagnosed with uterine leiomyoma, confirmed by histopathological findings. Study was conducted prospectively and it was approved by the Human Studies Ethics Committee of the Clinical Center. The protocol was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects). All specimens were taken after obtaining the informed consent and none of the subjects had undergone hormone therapy or any other medical treatment in the last 6 months. Patients were divided as follows: 11 in the follicular phase (F) (age, 39-49 yr; median 45 yr), 10 in the luteal phase (L) (age, 37-50 yr; median 44 yr), 9 in the postmenopause (PM) (age, 51-70 yr, median 56 yr).

SAMPLES

Venous blood samples were collected into heparinized tubes on the same day of uterine biopsy and aliquoted immediately. For SOD assay (OxisResearch™), blood was centrifuged at 2500 g for 5 min. Plasma was discarded and pellet was resuspended in 4 packed-cell volume of ice-cold demineralized ultrapure water (MilliQ reagent grade water system, Millipore Corp., Bedford, MA, USA). After addition of ethanol/chloroform extraction reagent (62.5/37.5 vol/vol) to remove hemoglobin interference, samples were centrifuged at 3000 g for 10 min (Eppendorf centrifuge 5417, Eppendorf AG, Hamburg, Germany). Upper aqueous layer was collected and kept at -70 ℃ until assay.

Myoma samples were washed in saline solution and homogenized in phosphate buffer containing $0.05 M KH_2PO_4$ and $1 mM EDTA$, pH 7.8 (1 g tissue per 2 ml buffer) in a Teflon/glass homogenizer (Spindler and Hoyer, Göttingen, Germany) and frozen at -70 ℃ for 20 h in order to disrupt cell membranes. For SOD assay (OxisResearch™), thawed homogenates were vortexed 1 min and centrifuged at 8600 g, for 20 min at 4 ℃ (Eppendorf centrifuge 5417, Eppendorf AG, Hamburg, Germany). According to manufacturer's

recommendation, after addition of ethanol/chloroform extraction reagent (62.5/37.5 vol/vol) to completely remove hemoglobin interference, samples were centrifuged at 6000 g for 20 min, at 4 ℃ (Beckman centrifuge J2-21, Beckman Instruments Inc., Palo Alto, CA, USA). Upper aqueous layer was collected and kept at -70 ℃ until assay. The enzyme activities and lipid hydroperoxide (LOOH) concentration were monitored spectrophotometrically (Perkin Elmer Spectrophotometer, Lambda 25, Perkin Elmer Instruments, Norwalk, CT, USA).

The specific enzyme activities were expressed as Units (U) or mU per milligram of total cell protein (U or mU/mg protein). LOOH concentration was expressed as nmol/mg protein. Determination of protein concentration was performed in tissues homogenates by the method of Lowry et al. (1951) and expressed as mg/ml. Plasma follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) and progesterone (P) levels were analyzed using standard radioimmunoassay (RIA) methods by the hormone analysis laboratory.

ASSAYS

ASSAY OF SOD ACTIVITY

Determination of total SOD activity was performed using Oxis Bioxytech® SOD-525™ Assay (Oxis International, Inc., Portland, OR, USA). The method is based on SOD-mediated increase of autoxidation of 5,6,6a11b-tetrahydro-3,9,10-tryhydroxybenzo[c]fluorene in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm. The SOD activity is determined from the ratio of the autoxidation rates in the presence (Vs) and in the absence (Vc) of SOD. One SOD-525 activity unit is defined as the activity that doubles the autoxidation rate of the control blank.

ASSAY OF CAT ACTIVITY

CAT activity was determined by the method of Beutler (1982). The reaction is based on the

rate of H_2O_2 degradation by catalase, contained in the examined samples. The reaction was performed in an incubation mixture containing 1M Tris-HCl, 5mM EDTA, pH 8.0, and monitored spectrophotometrically at 230 nm. One unit of CAT activity is defined as 1 µmol of H_2O_2 decomposed per minute under the assay conditions.

ASSAY OF GPX ACTIVITY

GPx activity was assessed using the Oxis Bioxytech® GPx-340™ Assay (Oxis International, Inc., Portland, OR, USA), based on the principle that oxidized glutathione (GSSG) produced upon reduction of an organic peroxide by GPx, is immediately recycled to its reduced form (GSH) with concomitant oxidation of NADPH to NADP+. The oxidation of NADPH was monitored spectrophotometrically as a decrease in absorbance at 340 nm. One GPx-340 unit is defined as 1 µmol of NADH oxidized per minute under the assay conditions.

ASSAY OF GR ACTIVITY

Activity of GR was measured using the Oxis Bioxytech® GR-340™ Assay (Oxis International, Inc., Portland, OR, USA). Assay is based on the oxidation of NADPH to NADP+ during the reduction of oxidized glutathione (GSSG), catalyzed by a limiting concentration of glutathione reductase. The oxidation of NADPH was monitored spectrophotometrically as a decrease in absorbance at 340 nm. One GR-340 unit is defined as 1 µmol of NADH oxidized per minute under the assay conditions.

LIPID HYDROPEROXIDES

Concentration of LOOH was measured by Oxis Bioxytech® LPO-560™ Assay (Oxis International, Inc., Portland, OR, USA), which is based on the ability of hydroperoxides to react with an excess of Fe^{2+} at a low pH when the xylenol orange (XO) dye is present. The amount of Fe^{3+} generated by this reaction is measured as the Fe-XO complex at 560 nm, as demonstrated by the following equations:

$$Fe^{2+} + ROOH \rightarrow Fe^{3+} + OH^- + RO^\bullet$$
$$Fe^{3+} + XO \rightarrow Fe\text{-}XO^\bullet \text{ (dye)}$$
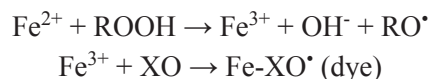
Since hydrogen peroxide content in many biological samples is much higher than that of other hydroperoxides, samples were pretreated with catalase to decompose the existing H_2O_2 and eliminate the interference.

STATISTICS

Statistical analysis was carried out with the use of the Kruskal-Wallis test and the Dunn's *post hoc test*, which considered the unequal and small sample sizes we used in this study. A linear regression model was used to evaluate associations between hormonal and antioxidant variables. Before plotting the data in the regression study, the normality test on the variables was performed and the values of estradiol and progesterone were log-transformed. The 95% confidence intervals (CIs) for the regression lines were calculated. Two-tailed *p* values are given throughout. All data were analyzed using GraphPad Prism software.
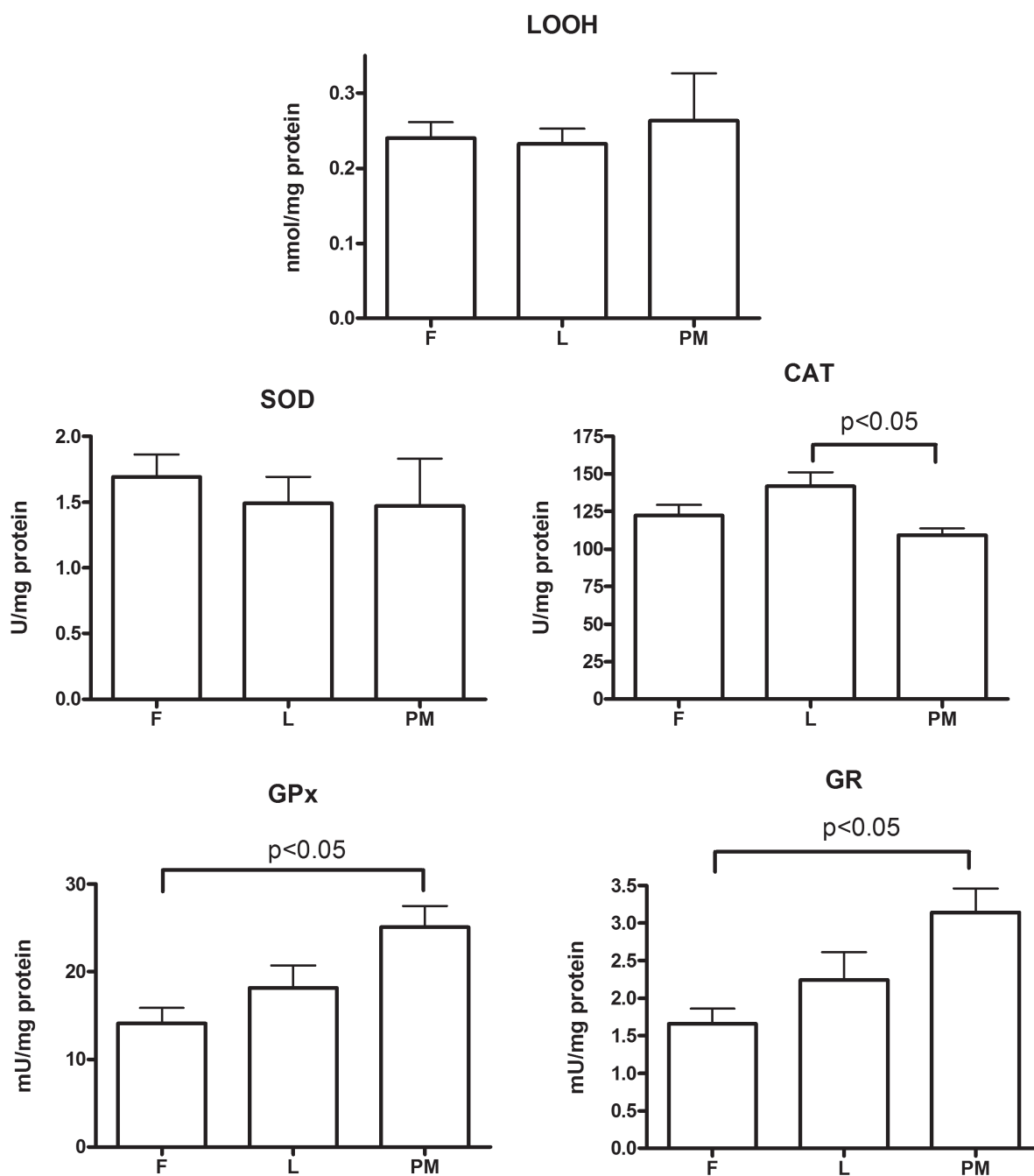
RESULTS

The phase related concentrations of gonadotropins and sex hormones are reported in Table I. Significant changes were observed in FSH (H=8.63, p<0.05, Kruskal-Wallis) and in estradiol concentrations (H=6.74, p<0.05, Kruskal-Wallis).

ANTIOXIDANT PARAMETERS AND CORRELATION STUDY WITH SEX HORMONES IN BLOOD

The phase related changes of LOOH concentrations and AO enzyme activities in the blood of examined patients are shown in the Figure 1. The LOOH concentration and SOD activity were not significantly different with respect to the phase. The activity of CAT varied significantly (H=6.17,

p<0.05, Kruskal-Wallis) and it was lower in postmenopause than in luteal phase (p<0.05, Dunn test). GPx and GR showed significant changes of enzyme activities from F phase toward postmenopause (H=6.36, H=6.83, respectively p<0.05, Kruskal-Wallis). The activities of both enzymes were higher in postmenopause than in F phase (p<0.05, Dunn test).

Figure 1 - Changes in LOOH concentrations and AO enzyme activities in follicular phase (F), luteal phase (L) and postmenopause (PM) in blood of patients with uterine leiomyoma. Data are shown as mean ± SEM. *P* values refer to the results of the Dunn test.

TABLE I
Changes in hormone levels during follicular phase, luteal phase and
in postmenopause (Data are expressed as mean ± SEM; * p<0.05).

	Follicular phase	Luteal phase	Postmenopause
FSH (U/L)* Median (Min/Max)	12.94±2.20 10.80 (7.80-20.10)	4.23±1.35 4.10 (1.90-6.80)	29.95±0.95 29.95 (29.00-30.90)
LH (U/L) Median (Min/Max)	4.62±1.57 3.10 (2.20-10.70)	2.00±1.24 0.95 (0.40-5.70)	5.75±2.25 5.75 (3.50-8.00)
Estradiol (pg/ml)* Median (Min/Max)	50.05±10.65 58.55 (18.50-64.60)	245.70±22.98 234.00 (213.00-290.00)	25.43±10.70 20.30 (10.00-46.00)
Progesterone (nmol/L) Median (Min/Max)	6.58±2.26 5.00 (1.60-14.70)	13.63±8.85 5.70 (3.00-40.10)	1.05±0.05 1.05 (1.00-1.10)

The linear regression analysis of individual hormonal variables against antioxidant parameters in blood (Fig. 2) showed significant positive correlation between the estradiol concentration and CAT activity (r=0.62, p<0.05), progesterone concentration and CAT activity (r=0.74, p<0.01), as well as a negative correlation between progesterone and LOOH concentrations (r=-0.65, p<0.05). No significant correlations were found between other hormones and antioxidant variables.

ANTIOXIDANT PARAMETERS AND CORRELATION STUDY WITH SEX HORMONES IN TISSUE

The phase related changes of LOOH concentrations and AO enzyme activities in uterine leiomyoma of examined patients are shown in Figure 3. Differently from the blood pattern, the LOOH concentration significantly differed with respect to the phase (H=6.68, p<0.05, Kruskal-Wallis). In follicular phase, it was significantly lower than in postmenopause (p<0.05, Dunn test) but not in comparison to the L phase.

The activity of SOD was significantly different (H=7.48, p<0.05, Kruskal-Wallis), and it was higher in luteal phase in comparison to both, follicular phase and postmenopause (p<0.05, Dunn test). The activity of CAT was stable, while the activities of GPx and GR varied significantly

(H=6.78; H=7.12, p<0.05, respectively, Kruskal-Wallis). Furthermore, the activities of both GPx and GR were significantly higher in luteal phase than in postmenopause (p<0.05, Dunn test).

The linear regression analysis of hormone levels and the examined AO parameters in uterine leiomyoma tissue (Fig. 4) showed negative correlation between FSH level and GPx activity (r= -0.64, p<0.01). No significant correlations were found between other hormones and anti-oxidant variables.

DISCUSSION

This study shows the changes of AO enzymes activities and LOOH level in blood and uterine leiomyoma tissue of cycling women, and women in postmenopause. In the blood of these patients, we found no difference in LOOH concentration and SOD activity. The CAT activity was lower in the postmenopause than in the luteal phase and GPx and GR activities were higher in the postmenopause than in F phase. As in healthy women (Burger et al. 2007), patients with fibroids examined in this study, also had a decline of estradiol level in the postmenopause compared to women with regular cycle. At higher concentrations estradiol has some antioxidant properties while its decline in postmenopause is associated with pro-oxidant effects. These effects
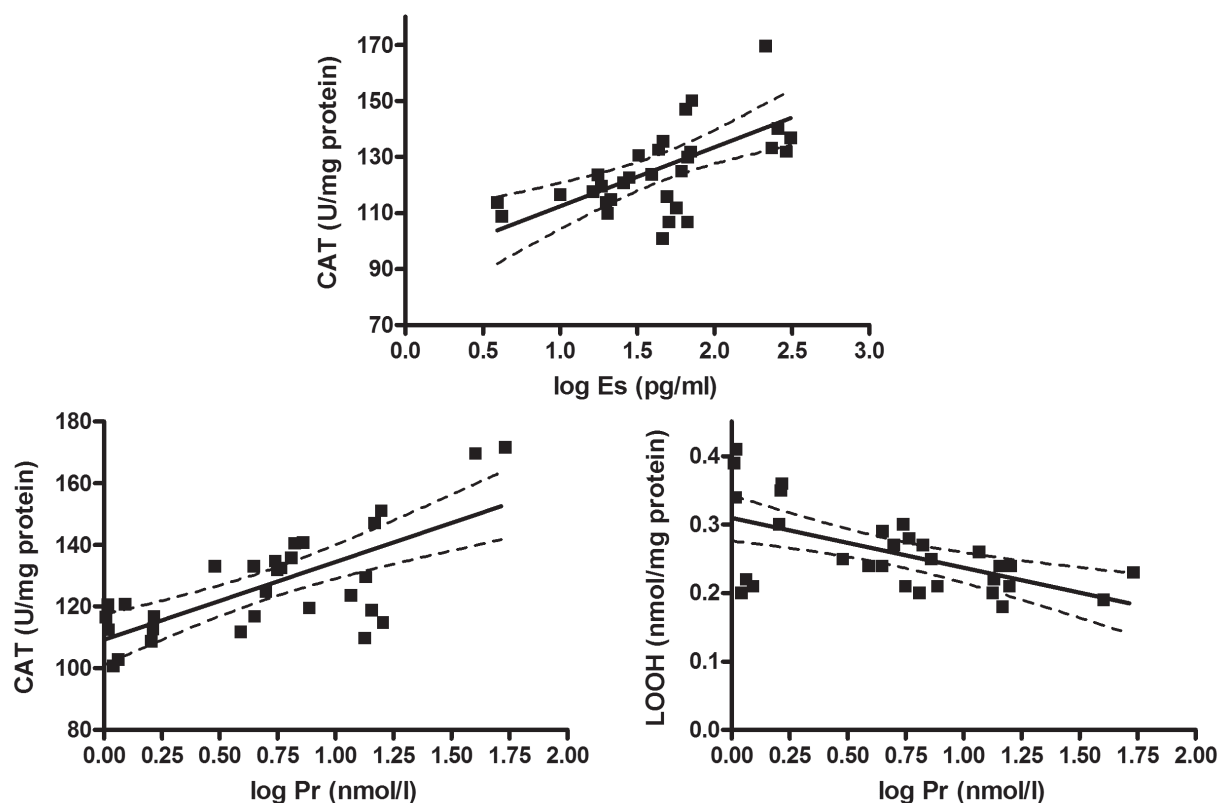
Figure 2 - Linear regression line and 95% CI to study the relationship between log Es/Pr and CAT activity, log Pr and LOOH concentration in the blood of patients with uterine leiomyoma.

include oxidation of bases and formation of DNA adducts (Doshi and Agarwal 2013).

Studies of healthy women and those with hormonal and ovarian disorders provided different results about the influence of sex hormones on AO enzymes activity during menstrual cycle. Bednarek-Tupikowska et al. (2001) found no significant differences of SOD activity in erythrocytes of healthy premenopausal and postmenopausal women. During the menstrual cycle, Massafra et al. (2000) observed no cycle-phase dependent changes in SOD and CAT activities as well as no correlation between activities of these enzymes and FSH, LH, estradiol or progesterone concentrations. Women with polycystic ovary syndrome were found to have higher SOD activity in blood than healthy women (Sabuncu et al. 2001). In women with ovulatory and anovulatory menstrual cycles it was

observed that non-ovulating women had lower plasma 17-beta-estradiol concentrations than the ovulating ones, in both follicular and luteal phase, while the progesterone concentration was lower only in the luteal phase (Lutosłavska et al. 2003). They also found higher SOD activity in non-ovulating women and no difference in CAT, GPx and GR activities in both groups of women with respect to the phase of the menstrual cycle. In ovulating women, inverse correlation was demonstrated between circulating estradiol and SOD activity (Lutosłavska et al. 2003).

In leiomyoma tissue, we observed similar pattern of SOD, GPx and GR activities, which were lower in the F phase and in the postmenopause than in the L phase. Furthermore, the elevated level of LOOH was recorded in postmenopause. Decreased activity of antioxidant enzymes, along with changes

LOOH

SOD

CAT

GPx

GR
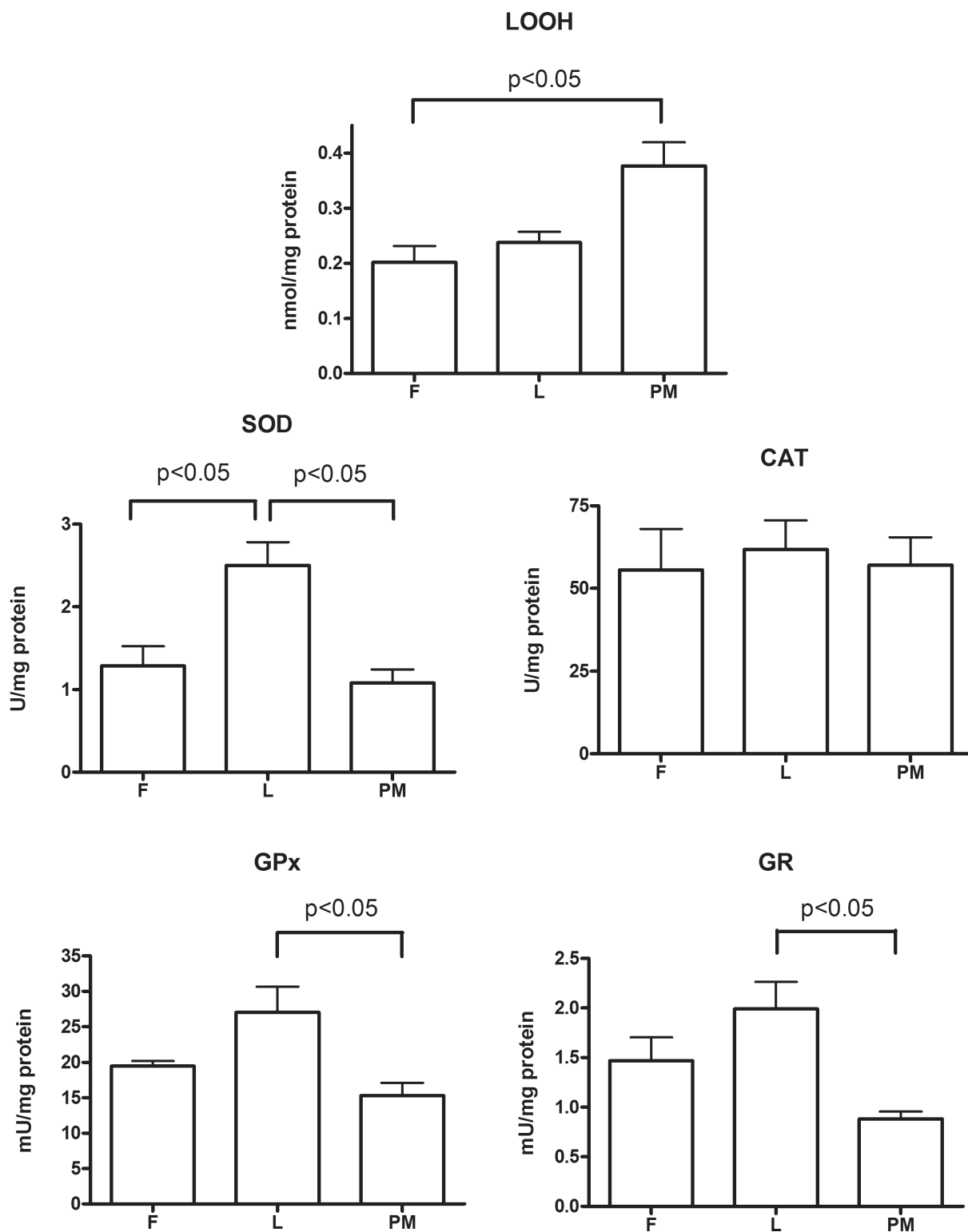
Figure 3 - Changes in LOOH concentrations and AO enzyme activities in follicular phase (F), luteal phase (L) and postmenopause (PM) in uterine leiomyoma tissue. Data are shown as mean ± SEM. *P* values refer to the results of the Dunn test.
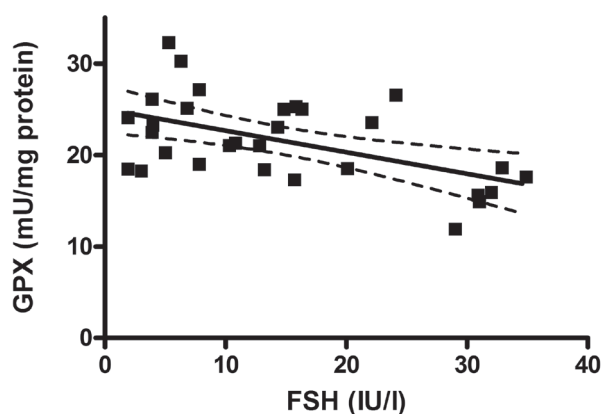
Figure 4 - Linear regression line and 95% CI to study the relationship between log FSH and GPx activity in uterine leiomyoma tissue.

of hormones and lipid peroxides level, indicate that postmenopausal women are particularly sensitive to peroxidative stress. Based on current data, GPx was shown to be influenced by hormones. In women with amenorrhea, Massafra et al. (1996, 1997) found that estradiol treatment, but not progestogen, stimulates GPx activity in erythrocytes of these patients. In addition, the increased activities of GPx and CAT in erythrocytes were observed during extended use of oral contraceptives (Massafra et al. 1993, 1996). Healthy women with a normal ovulatory cycle manifested changes in the activity of GPx depending on the phase of the cycle, so the activity of this enzyme was higher in the late stage of the F phase in comparison to the earlier L phase (Massafra et al. 1998, 2000). There was a positive correlation between serum estradiol and changes in GPx activity relative to the phase of the menstrual cycle (Massafra et al. 2000, Bednarek-Tupikowska et al. 2001). Similar results regarding GPx activity in healthy women with regular cycles were obtained by Serviddio et al. (2002). The activity of this enzyme increased in the follicular phase and decreased in the luteal phase. These authors also showed a positive correlation between the activity of GPx and LH concentrations in healthy women.

In this study, we found inverse correlation between GPx and FSH level in leiomyoma tissue. In the blood of the examined women, CAT activity was positively correlated with estradiol and progesterone, while LOOH concentration was inversely correlated to progesterone. Reports suggested a connection between CAT activity and estrogen exposure. Castanho et al. (2012) reported antagonizing effect of hormone therapy on CAT activity in menopausal women, while results of Vaishali et al. (2005) indicated higher CAT activity and lower GPx activity in blood of premenopausal women, compared with those in postmenopause. The relation we observed might indicate that leiomyoma patients have certain specificity in which although CAT activity is lower, GPx activity is still elevated to cope with a free radical production in blood, even in postmenopause. In addition to estrogen levels, cellular redox state may also influence CAT activity. It was observed that long-term oxidative stress reduces stability and half-life of catalase by stimulating ubiquitination and proteosomal degradation of the enzyme (Cao et al. 2003). However, in maintenance of cellular H_2O_2 levels, the harmonized CAT and GPX activity is most important since H_2O_2 plays an important role in many physiological processes like cell cycle and proliferation (Li et al. 2009), regulation of enzyme activity (Ferrer-Sueta et al. 2011), redox signalling (Sies 2014) and apoptosis (Andrade et al. 2013).

The role of progesterone in lipid peroxidation and AO body's defence is not clarified. Different results have been obtained, mainly as a consequence of progesterone preparations for hormone replacement therapy in postmenopausal women. Tranquilli et al. (1995) showed that treatment with medroxyprogesterone (MPA), whether used alone or in combination with estradiol, has antioxidant effects. On the other hand, Bednarek-Tupikowska et al. (2004) did not show the antioxidant effect of progesterone, because MPA in combination with estradiol, as part of hormone therapy, did not increase the observed antioxidant effect of estradiol. Negative correlation between the levels of progesterone and LOOH that we recorded

in patients with fibroids could also indicate the antioxidant properties of progesterone.

Some data suggest a greater impact of progesterone on the growth of uterine leiomyoma (Rein 2000), compared to estrogen, although others emphasize the role of estradiol (Barbarisi et al. 2001, Kovács et al. 2001) or of both steroids (Neuwirth and Moritz 2008). It is still unclear how estradiol and progesterone affect the growth and development of fibroids since during pregnancy there is a decrease in the size of fibroids, when circulating levels of these hormones are high (Strobelt et al. 1994). Recent studies suggest that progesterone exerts a dualistic, stimulatory and inhibitory, effect on cell survival and growth of fibroids, depending on local growth factors surrounding these tissues (Maruo et al. 2004). It is also believed that sex steroids promote development of leiomyomas by stimulating inappropriate expression of growth factors. Estrogen and progesterone act as physiologic regulators of gene expression by activating nuclear receptors that are, themselves, transcription factors. In this way estrogen and progesterone play a key role in regulating genes that direct cell growth (Neuwirth and Moritz 2008). Studies already supported a role of these hormones in leiomyoma growth through a feedback mechanisms and activation of MAP-kinase pathways (Yu et al. 2010).

It was also observed that the agonist-gonadotropin releasing hormone (GnRH) inhibits steroidogenesis and induces pharmacological menopause. Through this mechanism, GnRH can reduce fibroid volume by 50% within 3-6 months (Wang et al. 2002, Maruo et al. 2004). Clinical evidence of sex steroids involvement in leiomyoma growth can be seen with the shrinkage of leiomyoma during menopause and after GnRH administration in hypoestrogenic and hypoprogesterone states (Neuwirth and Moritz 2008).

The pathogenesis of uterine leiomyoma is still not clarified. Although all women of reproductive age experience significant exposure to estrogen, it is unclear what renders some individuals susceptible to leiomyoma formation. Our study suggests that in blood and uterine leiomyoma tissue, some AO enzymes are regulated by sex hormones. Whether the relation between endometrial AO status and changes of sex hormones level plays a role in its etiology, remains to be evaluated.

ACKNOWLEDGMENTS

RESUMO

Leiomiomas uterinos são tumores benignos dos tecidos moles que surgem da musculatura lisa uterina. A etiopatogênese dos leiomiomas não é bem compreendida. Nós objetivamos examinar se a atividade de enzimas antioxidantes e o nível de hidroperóxidos de lipídios em pacientes com leiomioma são influenciados por mudanças nos hormônios sexuais e nas gonadotropinas (estradiol (E2), progesterona, FSH e LH) durante o ciclo menstrual e na pós-menopausa. O material consistiu em sangue e espécimes de tecido uterino. As concentrações hormonais foram determinadas e foram realizados ensaios de atividade de superóxido dismutase, catalase, glutationa peroxidase e glutationa redutase e a concentração de hidroperóxidos de lipídios foi mensurada. No sangue das mulheres examinadas, foi verificada uma diferença significativa entre as fases nas atividades de catalase, glutationa peroxidase e glutationa redutase. Também houve uma correlação positiva entre a concentração de estradiol/progesterona e a atividade de catalase. A progesterona correlacionou negativamente com níveis de hidroperóxidos de lipídios. No tecido do mioma, foi verificada uma diferença relacionada à fase nos níveis de hidroperóxidos de lipídios e nas atividades de superóxido dismutase, glutationa peroxidase e glutationa redutase. Uma correlação negativa foi observada entre FSH e glutationa peroxidase. Os resultados sugerem que o estado antioxidante em pacientes com leiomioma uterino é influenciado por mudanças nos

hormônios sexuais durante o ciclo menstrual e na pós-menopausa, indicando um papel da relação observada na etiologia do leiomioma.

Palavras chave: enzimas antioxidantes, hidroperóxidos de lipídios, esteroides sexuais, leiomioma.

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