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Assessment of oxidant-antioxidant status alterations with tumor biomarkers and reproductive system hormones in uterine MYOMAS☆

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ABSTRACT

Objectives: Uterine myomas (UM) are responsible for significant morbidity and have adverse effects on quality of life in women. Reactive oxygen species (ROS) and antioxidant enzymes (AOE), as well as sex steroids play important roles in the reproductive physiology processes. Thus, we aimed to investigate the role of oxidant-antioxidant status in UM by measuring the AOE activities and lipid peroxidation (LPO) levels. This is the first study assessing these parameters together in UM based on also menopausal status and evaluating possible correlations between AOE activities, LPO markers, tumor biomarkers, female reproductive system hormone levels, comprehensively.

Study Design: The study group consisted of patients who have undergone surgical resection with confirmed pathology of uterine myoma (UM, n = 25) and divided into subgroups; premenopausal (UM_{pre}) and postmenopausal (UM_{post}). Erythrocyte copper-zinc superoxide dismutase (Cu,Zn-SOD), catalase (CAT), glutathione peroxidase (GPx1) activities were measured along with plasma malondialdehyde (MDA) and urinary 8-epi-prostaglandin F2α (8-epi-PGF2α) levels in patients with UM. The obtained data were compared to the data of healthy individuals (C, n = 25) and its subgroups; premenopausal (C_{pre}) and postmenopausal (C_{post}).

Results: All AOE activities were higher (~40% for Cu,Zn-SOD, p = 0.003; ~55% for CAT, p = 0.001; ~15% for GPx1, p = 0.169) and the LPO levels were lower (~60% for MDA, p = 0.011 and ~45% for 8-epi-PGF2α, p = 0.055) in patients with UM vs control. Approximately similar alterations were observed in UM_{pre} vs C_{pre} and in UM_{post} vs $C_{post.}$ A significant negative correlation between erythrocyte Cu,Zn-SOD activity and plasma MDA levels (r = -0.431, p = 0.005) was reported.

Conclusion: Decreased LPO levels might be the consequence of compensator high antioxidant enzyme activities against mild oxidative stress in the circulation of patients with UM. The marked negative correlation between erythrocyte Cu,Zn-SOD activity and plasma MDA levels also supported this finding.

Introduction

Uterine myomas (UM, uterine fibroids, leiomyomas) are benign, monoclonal tumors that arise from the myometrial smooth muscle cells and are classified according to their adjacent uterine layers (subserous, intramural, submucous). As it is most common with an estimated incidence of 20–40% in women of reproductive age and becomes symptomatic of women aged >35 years, the disease breaks out infrequently before menarche and goes into remission generally after menopause. It is associated with different clinical symptoms including abnormal uterine bleeding, pelvic pressure, mass or pain, urinary frequency or incontinence, constipation, infertility, spontaneous miscarriage, benign metastasis and malignancy [1,2].

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Various risk factors, including hormonal, environmental, dietary and hereditary factors trigger the development of uterine myomas. The disease is mainly associated with reproductive/hormonal factors such as nulliparity, low parity, early age at menarche, older age at menopause, delayed childbearing, short term breastfeeding and obesity [3]. Even though the underlying pathogenesis and etiology of myomas are not fully identified, there are two basic steps of myoma development; i) transformation of normal myocytes into abnormal cells by acquiring somatic genetic or epigenetic changes, ii) their growth into clinically apparent tumors due to their increased sensitivity to growth factors or hormones [1,4]. Genetic factors (up-regulation and/or down-regulation of sex-steroid associated genes: estrogen receptor α and β, progesterone receptor A and B, growth hormone receptor, prolactin receptor, extracellular matrix and collagen genes; genetic predisposition with about 40–50% chromosomal aberrations) have an important role in formation, cell growth, differentiation, proliferation, mitogenesis and angiogenesis [4,5]. In addition, overexpression of some growth factors trigger the smooth muscle cell proliferation, increase DNA synthesis, promote mitogenesis and angiogenesis in uterine myoma. Therefore, these types of tumors compose large amounts of extracellular matrix containing collagen, fibronectin and proteoglycan [1,3].

Besides these factors, biochemical, clinical and epidemiological evidences mostly suggest that both estrogen and progesterone are important hormones related to myoma development and maintenance with their modulating effects on growth and apoptosis related factors. Thus, factors triggering the overall lifetime exposure to estrogen increase the incidence of myoma and most medical treatments of uterine myoma are based on inhibition of sex steroids' production or action [3,6–8].

Oxidative stress (OS) is an imbalance between toxic reactive oxygen species (ROS) and antioxidant defense system. ROS are products of normal cellular metabolism, but if their production exceeds the normal levels they can easily react with the components of the cell and cause some metabolic and cellular damages (lipid peroxidation, oxidative DNA damage, cell membrane damage). Thus, they can alter the normal physiological functions in the body and play a critical role in the pathogenesis of various diseases, including cancer [9–11]. Moreover, ROS may have a regulatory role in oocyte maturation, folliculogenesis, ovarian steroidogenesis and luteolysis. They can act as cellular messengers in reproductive and menopausal periods of women and contribute to development of some conditions and diseases such as pregnancy, normal parturition, age-related infertility, premature labor, menopause, preeclampsia, endometriosis, uterine leiomyoma, and some gynecological cancers [12].

Antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] and molecules [glutathione, tocopherol, ascorbate]may prevent biological damage due to excessive ROS. Adequate antioxidants may be supplied through the antioxidant system in response to acute/mild OS. However, prolonged and severe OS due to elevated ROS levels induces consumption and/or decreases the activity of AOE which in turn further increases the risk of developing cancer. High LPO levels and low AOE activities were generally observed in gynecologic cancers compared to benign diseases of female reproductive system as reported in previous studies [13,14] including ours [15]. Moreover, a recent study pointed out to a close relationship between UM and increased endometrial cancer risk [16].Therefore, the balance between the severity of OS and AOE in UM becomes more crucial for developing cancer.

To understand the possible role of redox imbalance in the pathogenesis of UM, we aimed to find out alterations in the oxidant-antioxidant status of patients with UM. In addition, we tried to reveal all the possible correlations between antioxidant enzyme activities, LPO levels, tumor biomarkers and reproductive system hormones of UM patients, comprehensively.

Methods

Chemicals and reagents

Prolactin, free testosterone, progesterone, estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH) and total testosterone kits were purchased from Abbott (Abbott Laboratories, Abbott Park, IL, USA) and Siemens (Los Angeles, CA, US). CA125 and CA15-3 kits were purchased from Roche (Roche Diagnostics, Mannheim, Germany). All reagents and chemicals used to measure antioxidant enzyme activities and MDA levels were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich Co. Ltd. (St Louis, MO, USA). Commercially available competitive ELISA kit BIOXYTECH® 8-Epi-Prostaglandin-$F_{2\alpha}$ was obtained from (Oxis-Research, Portland, OR, USA).

Study groups

The women admitted to Hacettepe University Adult Hospital, Department of Obstetrics and Gynecology (Ankara, TURKEY) for routine gynecologic checkups and abnormal bleeding were recruited into this study. The study group consisted of patients undergone surgical resection with confirmed pathology of uterine myoma (UM, n = 25) and divided into subgroups; [premenopausal (UM_{pre}), n = 15 and postmenopausal (UM_{post}), n = 10] based on their menopausal status. Women who had a history of at least 12 months (>1 year) of amenorrhea without hormone/estrogen replacement therapy were accepted as postmenopausal patients. The control group consisted of healthy individuals whom were assessed by transvaginal ultrasonography for UM existence and had no benign or malign diseases (C, n = 25) and divided into subgroups [premenopausal (C_{pre}), n = 14 and postmenopausal (C_{post}), n = 11].

Written informed consent was obtained from all subjects and a comprehensive questionnaire was carried out to collect demographic and reproductive system data. The study was approved by the Local University Clinical Research Ethics Committee of the Hacettepe University. The protocol was consistent with the World Medical Association Declaration of Helsinki.

Preparation of blood and urine samples

10 ml venous blood samples were obtained in the morning after an overnight fasting. Serum tumor biomarkers and reproductive hormone levels were assessed by clinical chemistry laboratory of Hacettepe University Adult Hospital. Heparinized blood samples were centrifuged at 3000xg for 15 min to obtain plasma and erythrocyte samples for the determination of oxidant-antioxidant status parameters by our toxicology laboratory. 10 ml of spot urine samples were collected before the surgery for the measurement of 8-epi-PGF2α levels. All the samples were kept at −80 °C until the analyses.

Biochemical assays

Measurement of reproductive system hormone levels and tumor biomarkers

Serum prolactin, total testosterone, free testosterone, progesterone, estradiol, FSH, and LH levels were measured with chemiluminescent microparticle immunoassay (CMIA) while CA125, CA15-3 levels were measured with electrochemiluminescence immunoassay (ECLIA).

Determination of antioxidant enzyme activities and lipid peroxidation markers

Cu,Zn-SOD (EC 1.15.1.1) activity was determined with the pyrogallol autoxidation method in blood samples [17]. The inhibition of pyrogallol autoxidation by SOD was detected at 420 nm. One unite of Cu,Zn-SOD activity was defined as the amount of enzyme required to cause 50% inhibition of pyrogallol autoxidation under the assay conditions and expressed as units per mg of hemoglobin ($Umg^{-1}Hb$). Catalase (EC 1.11.1.6) activity was determined spectrophotometrically by following the enzymatic decomposition of H_2O_2 directly at 240 nm [18]. One unite of CAT activity was defined as the amount of enzyme required to decompose 1 μmol H_2O_2 per minute and expressed as units per mg of hemoglobin ($Umg^{-1}Hb$). GPx1 (EC 1.11.1.9) activity was measured in a coupled reaction with glutathione reductase [19]. One unite of GPx1 activity was defined as the amount of enzyme that oxidized 1 μmol NADPH to NADP per minute and expressed as units per g of hemoglobin ($Ug^{-1}Hb$).

The MDA levels were determined quantitatively by HPLC equipped with an autosampler (Hewlett Packard Agilent, 1100 series, Vienna, Austria) using a fluorescence detector (excitation wavelength of 515 nm and emission wavelength of 550 nm [20]. The analytical column was a reverse phase silica based C_{18} column (ACE®, Scotland) with length of 25 cm × 4.6 mm i.d., 5 μm particle size. The mobile phase was %65, 50 mM KH_2PO_4-KOH, pH 7.0, and % 35 MeOH. The flow rate was 0.6 ml/min, and the injection volume was 100 μl. The average retention time of the MDA-$(TBA)_2$ complex was 4.7 min. The levels were calculated directly by using calibration curves of peak area prepared for MDA standards and results were given as μM. The 8-epi-PGF2α levels of urine samples were determined using a competitive enzyme-linked immunoassay (ELISA) by performing manufacturer's instructions. A standard curve was obtained by fitting the standard absorbances at 450 nm to the concentration of 8-epi-PGF2α by the 4-parameter logistic curve fit method. Samples were run in duplicate to assure consistency, and intra-sample variability was less than 10%. Results were given as ng/ml.

Statistical analyses

Experimental data were analyzed with Shapiro-Wilk, test of normality followed by Student *t*-test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables using a Statistical Package for Social Sciences Programme (SPSS programme v23.0, SPSS Inc., Chicago, IL). Pearson's chi-square test with Yates' correction was used to determine relationships between categorical variables. The correlations were assessed using Pearson or Spearman correlation coefficients. The level of significance was defined as ($p < 0.05$). Values were given as mean ± standard deviation (SD) for normal variables and median (min-max) for non-normal variables.

Results

Demographic and reproductive system data

Demographic and reproductive system data of UM and C were illustrated in Table 1. The groups were matched in terms of their mean age, body mass index (BMI), dietary and smoking habits, existence of chronic diseases and total breastfeeding period. Significant differences were not observed in the distribution of UM and C in terms of smoking ($\chi^2 = 1.125$; $p = 0.29$) and having chronic diseases ($\chi^2 = 0.000$; $p = 1.00$). In addition, no statistically significant differences were found with respect to their dietary habits. Gravida and parity of UM were found to be high compared to C ($p = 0.028$ and $p = 0.007$, respectively).

Tumor biomarkers and reproductive system hormone levels

The serum CA125 levels of UM [14.98 (4.55–36.25) U/ml]were found approximately similar to that of C [15.79 (8.08–37.73) U/ml] ($p = 0.82$). Although there was no any cancer diagnosis (breast cancer, etc.) in patients with UM, the CA15-3 levels were obtained higher (34%) in UM [20.19 ± 6.33 U/ml] compared to C [15.04 ± 7.75 U/ml]($p = 0.027$). Prolactin, total testosterone, free testosterone, progesterone, estradiol, FSH and LH levels in the circulation of UM were not found to be different from the levels of C ($p > 0.05$). When we evaluated the results based on menopausal status, no significant differences were observed in the levels of tumor biomarkers and reproductive hormones except estradiol levels between UM_{pre} and UM_{post}. As expected, estradiol levels were found significantly higher (~7 times) in UM_{pre} vs UM_{post} ($p = 0.036$). A similar trend was also observed for estradiol levels in C_{pre} (~6 times higher) vs C_{post} ($p = 0.002$). In addition, when the menopausal status of UM and C were matched, estradiol levels were higher in UM_{pre} vs C_{pre} (~1.4 times, $p = 0.85$) and in UM_{post} vs C_{post} (1.3 times, $p = 0.53$) (Table 2).

Antioxidant enzyme activities and lipid peroxidation level

Erythrocyte antioxidant enzyme activities, plasma MDA levels and urinary 8-epi-PGF2α levels of UM and C were summarized in Table 3. Significant elevations of erythrocyte Cu,Zn-SOD and CAT activities (~40% for Cu,Zn-SOD, $p = 0.003$; ~55% for CAT, $p = 0.001$) and an insignificant elevation of GPx1 activity (~15%, $p = 0.17$) were observed in UM vs C. On the other hand, lower plasma MDA levels (~60%, $p = 0.011$ and urinary 8-epi-PGF2α levels (~45%, $p = 0.05$) were found in UM compared to C. A significant negative correlation was observed only between erythrocyte Cu,Zn-SOD activity and

Table 1
Demographic and reproductive data of patients with uterine myoma and controls.

Data/Study Groups	Uterine myoma (UM) (n = 25)	Control (C) (n = 25)	p values (UM vs C)[a,b]
Mean age (years)	48.86 ± 3.05	46.62 ± 4.45	0.06[a]
Range of age (years)	44–54	40–54	–
Body mass index (BMI)(kg/m^2)	29.82 (19.07–42.67)	26.22 (21.97–42.97)	0.08[b]
Breastfeeding period (month)	26.43 ± 17.66	22.19 ± 17.79	0.44[a]
Gravida (G)	4.00 (0.00–11.00)*	2.00 (0.00–7.00)	0.028[b]
Parity (P)	3.00 (0.00–5.00)*	2.00 (0.00–5.00)	0.007[b]
Abortus (A)	0.00 (0.00–2.00)	0.00 (0.00–3.00)	0.12[b]
Dilation&Cureattage (D&C)	1.00 (0.00–6.00)	0.00 (0.00–4.00)	0.27[b]

[a] Student's *t*-test ; data are given as mean ± standard deviation (SD).
[b] Mann Whitney U-test; data are given as median (minimum-maximum).
* UM vs C ($p < 0.05$).

Table 2
Estradiol levels, erythrocyte antioxidant enzyme activities and plasma-urine lipid peroxidation levels of patients with uterine myoma and controls based on menopausal status.

Variables/Study groups	Premenopause			Postmenopause		
	Uterine myoma (UM_{pre})(n = 15)	Control (C_{pre})(n = 14)	p values[a,b] (UM_{pre} vs C_{pre})	Uterine myoma (UM_{post})(n = 10)	Control (C_{post})(n = 11)	p values (UM_{post} vs C_{post})[a,b]
Estradiol (pg/ml)	106.0 (10.0–286.0)	76.0 (10.0–175,0)	0.85[b]	16.0 (10.0–92.0)	12.0 (10.0–22.0)	0.53[b]
Cu,Zn-SOD activity (U mg^{-1}Hb)	1.84 ± 0.48*	1.51 ± 0.63	0.028[a]	1.89 (1.63–2.12)*	0.92 (0.80–1.71)	0.002[b]
CAT activity (U mg^{-1}Hb)	317.38 ± 90.6*	192.20 ± 49.13	<0.001[a]	311.98 (245.85–380.51)*	260.21 (100.12–306.05)	0.050[b]
GPx1 activity (U g^{-1}Hb)	39.0 ± 13.16	33.17 ± 9.25	0.19[a]	37.88 ± 8.37	35.64 ± 9.99	0.67[a]
MDA (μM)	0.81 (0.48–2.56) *	1.01 (0.56–3.66)	0.019[b]	2.10 ± 1.82	2.78 ± 1.06	0.44[a]
8-epi-PGF2α (ng/mL)	0.90 (0.19–6.91)	1.87 (0.44–4.01)	0.08[b]	1.18 (0.26–3.73)	0.92 (0.40–3.86)	0.94[b]

Erythrocyte antioxidant enzyme activities were expressed as (U mg^{1}Hb) for copper,zinc-superoxide dismutase (Cu,Zn-SOD), catalase (CAT) and (U g^{-1}Hb) for glutathione peroxidase (GPx1). Plasma levels of malondialdehyde (MDA) were expressed as (μM); urinary 8-Epi-Prostaglandin-$F_{2\alpha}$ (8-epi-PGF2α) levels were expressed as (ng/ml).
[a] Student's *t*-test ; data are given as mean ± standard deviation (SD).
[b] Mann Whitney U-test; data are given as median (minimum-maximum).
* UM_{pre} vs C_{pre} ($p < 0.05$); UM_{post} vs C_{post} ($p < 0.05$).

Table 3
Erythrocyte antioxidant enzyme activities and plasma-urine lipid peroxidation levels of patients with uterine myoma and controls.

Variables/ Study Groups	Uterine myoma (UM) (n = 25)	Control (C) (n = 25)	p values (UM vs C) [a,b]
Cu,Zn-SOD activity (U mg^{-1}Hb)	1.86 ± 0.41*	1.36 ± 0.58	0.003[a]
CAT activity (U mg^{-1}Hb)	318.17 ± 80.18 *	208.66 ± 59.39	<0.001[a]
GPx1 activity (U g^{-1}Hb)	38.65 ± 11.63	33.99 ± 9.32	0.17[a]
MDA (μM)	0.82 (0.48–4.47)*	1.99 (0.56–3.87)	0.011[b]
8-epi-PGF2α (ng/mL)	1.02 (0.19–6.91)	1.78 (0.40–4.01)	0.05[b]

Erythrocyte antioxidant enzyme activities were expressed as (U mg^{-1}Hb) for copper,zinc-superoxide dismutase (Cu,Zn-SOD), catalase (CAT) and (U g^{-1}Hb) for glutathione peroxidase (GPx1). Plasma levels of malondialdehyde (MDA) were expressed as (μM); urinary 8-Epi-Prostaglandin-$F_{2\alpha}$ (8-epi-PGF2α) levels were expressed as (ng/ml).
[a] Student's *t*-test ; data are given as mean ± standard deviation (SD).
[b] Mann Whitney *U*-test; data are given as median (minimum-maximum).
* UM vs C ($p < 0.05$).

plasma MDA levels (r=-0.431, p = 0.005). However, no significant correlations were found between urinary 8-epi-PGF2α levels and all antioxidant enzyme activities.

When the AOE activities and LPO levels were evaluated based on menopausal status both in UM and C groups, approximately similar elevations of erythrocyte Cu,Zn-SOD, CAT and GPx1 activities and lower LPO levels were observed in UM_{pre} vs C_{pre} and in UM_{post} vs C_{post} (Table 2).

Discussion

This is the first study that evaluates the data of AOE together with LPO, tumor biomarkers, demographic and reproductive system data. The main results of the study were discussed below in detail: (i) erythrocyte Cu,Zn-SOD (∼40%) and CAT (∼55%) activities were increased, (ii) erythrocyte GPx1 activity (∼15%) was increased, (iii) plasma MDA levels (∼60%) and urinary 8-epi-PGF2α levels (∼45%) were decreased in UM vs C, (iv) approximately similar alterations were observed in AOE activities and LPO levels of UM_{pre} vs C_{pre} and UM_{post} vs C_{post}, (v) a marked negative correlation was observed between erythrocyte Cu,Zn-SOD activity and plasma MDA in UM.

Evaluation of demographic and reproductive system data

The mean age of UM patients was presented in Table 1. It was in compatible with the knowledge about age as a risk factor in myoma development. Moreover, despite a lack of definitive data, there are a number of large population-based studies concluded to a significant positive risk association between obesity and UM [21]. The alterations of endogenous sex steroid hormone synthesis and bioavailability in obese women might be effective in the etiology of UM. Obesity might be also a risk factor for UM due to its chronic inflammatory nature. Studies pointed out to high TNF-α and pro-fibrotic activin A expressions by macrophages in the adipose tissue and leiomyoma cells of the obese people [22]. In our study, although no statistically significant differences were found between groups according to their dietary habits, the mean BMI of UM was 29.82 kg/m^2 and most of them were overweight.

A large number of epidemiological studies predicted by several etiologic hypothesis remark an association between some reproductive risk factors and development of UM. Factors such as obesity, early age at menarche, older age at menopause, older age at first term pregnancy and nulliparity, trigger the continuous estrogen secretion/exposure and therefore, they can increase the UM development risk [3]. On the other hand, factors decreasing the estrogen exposure such as exercise and high parity can be protective against UM [3,4]. According to these knowledge, we evaluated all the risk and protective factors of UM and we found no protective role of higher gravida and parity in UM maybe due to limited population of study group.

Reproductive system hormones can play an etiologic role in the development of UM. Although there is no clear evidence that testosterone induce the UM onset or development, a longitudinal study demonstrates, for the first time, that women who undergo menopausal transition both with high testosterone and estrogen levels are more likely to develop UM [23]. Gonadotropins have both direct and indirect effects on malign transformed cells. They can activate the gonadotropin-sensitive genes directly or stimulate the estrogen production in ovaries by paracrine and autocrine mechanisms indirectly [24,25]. Baird et al. [26] hypothesized that if LH activates the same receptor with human chorionic gonadotropin (hCG) which has proliferative effects on uterine smooth muscle and leiomyoma tissue, premenopausal women

with high levels of LH would be at more risk with regard to develop UM. As hypothesized, a strong association was observed between high levels of LH and tumor onset in Uterine Fibroid Study. Moreover, Stewart et al. [27] demonstrated an increased production of prolactin after exposure to the pituitary and placental gonadotropins in UM and myometrium. The same laboratory group suggested prolactin as an autocrine and paracrine growth factor for both UM and myometrial cells with some differences in terms of their sensitivity to this hormone. They also observed a biphasic role for prolactin: it promotes cell growth at low concentrations, but inhibits the myoma cell proliferation at high concentrations [28]. Physiological regulations of estrogen and progesterone were also suggested as a contributing factor that promotes uterine myoma growth. The estradiol levels were found higher within myoma cells compared to normal myometrium due to high levels of aromatase enzyme that converts androgens to estrogen and low levels of enzymes that converts estradiol to estrone. Thus, accumulated estradiol levels might lead to estrogen receptor (ER) and progesterone receptor (PR) up-regulations and high PR expression was associated with overexpressed functional ER in myomas compared to adjacent normal myometrium [3,6,29,30]. The interaction of estradiol with estrogen receptor (ER) may cause transcriptional induction of genes involved in proliferation/extracellular matrix formation and induce myoma responsiveness to progesterone due to up-regulation of progesterone receptor (PR) expression. Ovarian progesterone interaction with PR regulates the transcription of key genes responsible for apoptosis, proliferation and extracellular matrix formation that stimulate the myoma growth and development [31].

In our study, prolactin, total testosterone, free testosterone, progesterone, estradiol, FSH and LH levels were not different in the circulation of patients with UM compared to C. At first, we believed that the overall circulating reproductive hormone levels of patients with UM may not reflect the elevated levels within myomas and their triggering effects on UM onset, growth and development. Afterwards, we decided to divide UM group into subgroups of menopausal status (UM_{pre} and UM_{post}) and found high estradiol levels in UM_{pre} vs C_{pre} and UM_{post} vs C_{post}. Therefore, as the estrogen and FSH are the two hormones related with menopause while, estrogen and progesterone are associated with UM development, we concluded that it is more reliable to evaluate the circulated hormones particularly estradiol levels according to the menopausal status in UM.

Evaluation of tumor biomarkers

Increased CA125 levels can be obtained in some type of cancers (ovarian, pancreatic, breast, bladder, liver, lung, etc.), benign gynecologic conditions (endometriosis, adenomyosis, pelvic inflammatory disease, benign ovarian cysts), some physiological conditions (pregnancy, menstruation) or non-gynecologic conditions (diverticulitis, liver/heart failure, etc.) [32,33] However, in our study, the serum CA125 levels of UM were found to be nearly similar to the levels of C. Although CA15-3 tests are positive generally for breast cancer, ovarian cancer, etc., we obtained significantly higher CA15-3 levels (34%) in UM compared to C.

Evaluation of antioxidant enzyme activities and lipid peroxidation level

Several studies support the steroidogenic effects of ovarian sex steroids on myoma growth regulation [34]. However, there is little known about their modulatory effects of on AOE in highly proliferated smooth muscle cells observed in UM. Strehlow et al. [35] observed a selective and specific effect of estrogens on SOD expression through ER activation, whereas CAT and GPx expressions were not affected. They also demonstrated different regulation patterns for SOD isoforms due to possible enhancing effect of estrogen on EC-SOD and Mn-SOD but not Cu,Zn-SOD promoter activities. Moreover, progesterone was found to increase Cu,Zn-SOD as well as Mn-SOD, whereas estrogen has only weak effects on human endometrium. Estrogens exert their radical scavenging effects through increased NO production, decreased angiotensin receptor-1 expression, and modulation of NADPH-oxidase enzyme activity. In our study, high estradiol levels and Cu,Zn-SOD activity were observed in UM_{pre} vs C_{pre} and UM_{post} vs C_{post}. Although estrogen might be ineffective on Cu,Zn-SOD expression as suggested by Strehlow et al. [35], high Cu,Zn-SOD activity observed in UM may have been caused by some post-translational modifications on this enzyme.

Mitochondria are the major source of ROS and, so, play a multifunctional role in malignant tumor progression. Mn-SOD has a critical importance in oxidative stress induced pathological conditions due to its location in mitochondrion. Mitochondrial function loss and increased number of size are common characteristics of cells undergoing neoplastic transformation [36]. Besides this, Tuncal et al. [37] observed ~1.5 fold increase in nuclear encoded electron transport chain genes and also suggested an increase in size, number and activity of mitochondria in UM tissues. Thus, these findings pointed out to a possible role for mitochondria in the transformation of smooth muscle cells in UM. Increased mitochondrial respiratory activity may result in oxidative stress and, therefore the increased activity of oxidative phosphorylation could be one of the mechanisms underlying the etiology of UM. Moreover, recent studies suggested that O_2 depletion stimulates mitochondria to further increase ROS, with subsequent activation of some signaling pathways that promote some adaptations such as cell survival, suppression of apoptosis and fibrotic growth [38]. Furthermore, Zhou et al. reported hypoxia as a risk factor in the pathogenesis of UM depending on severely hypoxic and oxygen-limited microenvironment of leiomyoma cells that protects the cells against apoptosis and maintains a proliferative state [39].

In the literature, lower SOD (87%, $p < 0.001$), CAT (50%, $p < 0.05$) activities and MDA levels (14%, $p > 0.05$) were observed in the microsomes of fibroid tumors ($n = 8$) compared to control [40]. On the contrary, higher GPx1 (23%, $p < 0.05$) [41] and CAT (52%, $p < 0.05$) [41] activities were observed in the leiomyoma tissues compared to normal myometrium in another study. Moreover, lower SOD (72%, $p < 0.05$) and CAT (43%, $p < 0.05$) activities due to elevated LPO products (105%) were observed in adenocarcinoma endometrii compared to UM [13]. Fletcher et al. [43] reported decreased SOD and CAT activities in UM cells compared to myometrial cells ($p < 0.05$), although under more hypoxic conditions they observed increased SOD activity only in myometrial cells and decreased CAT activity in UM cells ($p < 0.05$).

Different patterns were also observed in the studies that evaluate the circulating AOE and LPO markers of UM patients. Decreases in SOD_{Total} activities [14% ($p > 0.05$, $n = 25$); 14% ($p > 0.05$, $n = 12$); 20% ($p > 0.05$, $n =$ unknown)] were observed in patients with UM compared to control [44–46]. Besides this, insignificant and controversial alterations [37% decrease ($p < 0.001$), $n = 51$); 13% decrease ($p > 0.05$, $n = 25$); 9% increase ($p > 0.05$, $n = 12$)] for CAT activities [42,44,45] and for GPx1 activities [11% decrease ($p > 0.05$, $n = 25$); 7% decrease ($p > 0.05$, $n =$ unknown)] [44,46] were obtained in the circulation of UM patients compared to control. All parameters were studied in erythrocyte samples except CAT activity measured in serum samples by Vural et al [42].

As summarized above, contradictory and mostly insignificant data of AOE were obtained from the literature in the circulation of small number of UM patients. Moreover, menopause is one of the

triggering factors for elevated OS induced-LPO due to lower estradiol levels. It can also diminish the protective role of estrogen against endometrial ROS damage [12,47]. However, the measured parameters were not evaluated according to the patients' menopausal status in those limited studies above [42,44–46]. Therefore, we also evaluated our results based on menopausal status and pointed out to higher estradiol levels and lower LPO in UM_{pre} vs UM_{post}. The AOE activities were not found different between UM_{pre} and UM_{post} as Markowska et al. also observed the same trend in UM tissue samples [41]. In addition, we observed higher estradiol and AOE enzyme activities along with lower LPO levels in UM_{pre} vs C_{pre} as well as in UM_{post} vs C_{post}, although the alterations were more marked in premenopausal period.

Prolonged and severe oxidative stress elevates ROS production, induces consumption of antioxidants and makes more vulnerable normal cells to transform into malignant cells due to free radical damages. As mentioned above [13], higher LPO levels were observed in endometrial adenocarcinomas compared to UM at tissue site. However, less severe oxidative stress in UM compared to endometrial adenocarcinomas might be compensated by high antioxidant enzymatic activity as in our results. Besides this, the researchers found generally lower SOD and CAT activities in UM tissue/cells in their studies. It might be associated with severe oxidative stress due to mitochondrial or peroxisomal activities at tissue site. However, in erythrocytes, the results were found to be more confounding with regard to different cellular components and physiology of red blood cells from UM cells. Therefore, in our study, obtained low LPO levels might be the consequence of compensator high AOE activities. The marked negative correlation between erythrocyte Cu,Zn-SOD activity and plasma MDA levels also supported this hypothesis.

This is the first study pointed out to induced Cu,Zn-SOD and CAT enzyme activities which results in lower LPO levels independently from reproductive hormone levels and menopausal status in peripheral blood circulation of UM patients compared to control. Although we do not know precisely that redox imbalance has a causative and/or a consequential role in UM development, further clinical studies with a large number of study population are needed to clarify the possible involvement of OS and impaired antioxidant defense system in the pathophysiology of UM.

Disclosure

The authors declare no conflicts of interest.

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