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Oxidative stress markers in uterine fibroids tissue in pre- and postmenopausal women

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Summary

Uterine fibroids are common benign tumors of the reproductive organ and occur in approximately 50-80% of women of reproductive age. The pathogenesis of uterine fibroids is multifactorial and includes: sex hormones, genetic factors, cytokines, and oxidative stress. *Objective:* The aim of this study was to investigate the oxidative stress markers in tissue samples of women with uterine fibroids, with further analysis on size and menopausal status. *Materials and Methods:* Fifty-nine patients with the mean age 50.6 (35 premenopausal and 24 postmenopausal) who underwent standard gynecological procedures were recruited in the study. All women had histologically proven uterine leiomyoma. Samples were collected *ex vivo* immediately after resection. Glutathione peroxidase (GPX), catalase (CAT), and the ferric reducing ability of plasma (FRAP) were measured. *Results:* The activity of GPX was significantly higher in fibroid samples than in myometrium (0.070 +/- 0.042 *vs.* 0.057 +/- 0.027 U/mg of protein, $p < 0.05$), activity of CAT did not differ between samples (1.13 +/- 0.86 *vs.* 1.23 +/- 0.51 U/mg of protein, $p > 0.05$), and FRAP presented higher values in fibroid samples than in myometrium (4.58 +/- 6.29 *vs.* 3.04 +/- 3.81 mM Fe^{+2}/mg of protein), but the difference was not statistically significant ($p = 0.06$). In the subgroups analyses, there were no statistically significant differences when comparing the activity of GPX, CAT, and FRAP in fibroid samples from pre- and postmenopausal women, as well as when comparing fibroid samples of small size (< 50 mm) and large size (≥ 50 mm) tumors. *Conclusion:* Oxidative stress markers are changed in fibroid tissue samples showing that oxidative stress may play an important role in this tumor formation, although without influencing menopausal status nor tumor size.

Key words: Uterine fibroids; Oxidative stress; Menopause.

Introduction

Uterine fibroids are common benign tumors of the reproductive organ and occur in approximately 50-80% of women of reproductive age, mostly between 30 - 55 years of age. and two to three times more often in Black women [1-5]. Fibroids are monoclonal tumors that develop from myoblasts and vessel wall of the myometrium [6, 7]. Depending on the size and location, about 25% of fibroids present clinical signs: abundant, menstrual bleeding, signs of compression of the abdominal organs or discomfort. They cause reproductive failures - infertility or recurrent miscarriage and complications during childbirth [8-10]. The pathogenesis of uterine fibroids is multifactorial and includes: sex hormones (estrogens, estrogen receptors, progesterone) [2, 11-14], genetic factors (mutations, polymorphisms) [3, 7, 15-21], cytokines (insulin-like growth factor 1 - IGF1, transforming growth factor beta 1 - TGFβ1 and cathepsin D) [2, 22-25], and oxidative stress [26].

Free radicals are produced in the normal cell metabolism and are involved in physiological processes such as growth, proliferation, differentiation, and apoptosis of cells. Excessive production of free radicals, particularly reactive oxygen species (ROS) are injurious to the organism and is considered to be the cause of many diseases, including those of the female reproductive organs [26-29]. Antioxidant system is complex and includes a number of enzymes such as glutathione peroxidase (GPX) and catalase (CAT). GPX catalyzes, among others, the reaction between glutathione GSH and hydrogen peroxide, resulting in the elimination of excess hydrogen peroxide and blocking the formation of other free radicals, and protects the cell from the damaging effects of lipids [27, 30]. CAT is an enzyme that saves the cells against the toxic effects of hydrogen peroxide and its decreased activity is associated with inflammation. Small CAT activity was found in the connective tissue that forms part of the myoma [27, 30, 31]. Because of the difficulty in measuring each antioxidant component of plasma individually and of the interactions that take place among components, Benzie and Strain described a method to measure the total antioxidant capacity known as the ferric reducing ability of plasma (FRAP) [32].

The aim of this study was to investigate the oxidative stress markers in tissue samples of women with uterine fibroids, with further analysis on size and menopausal status.

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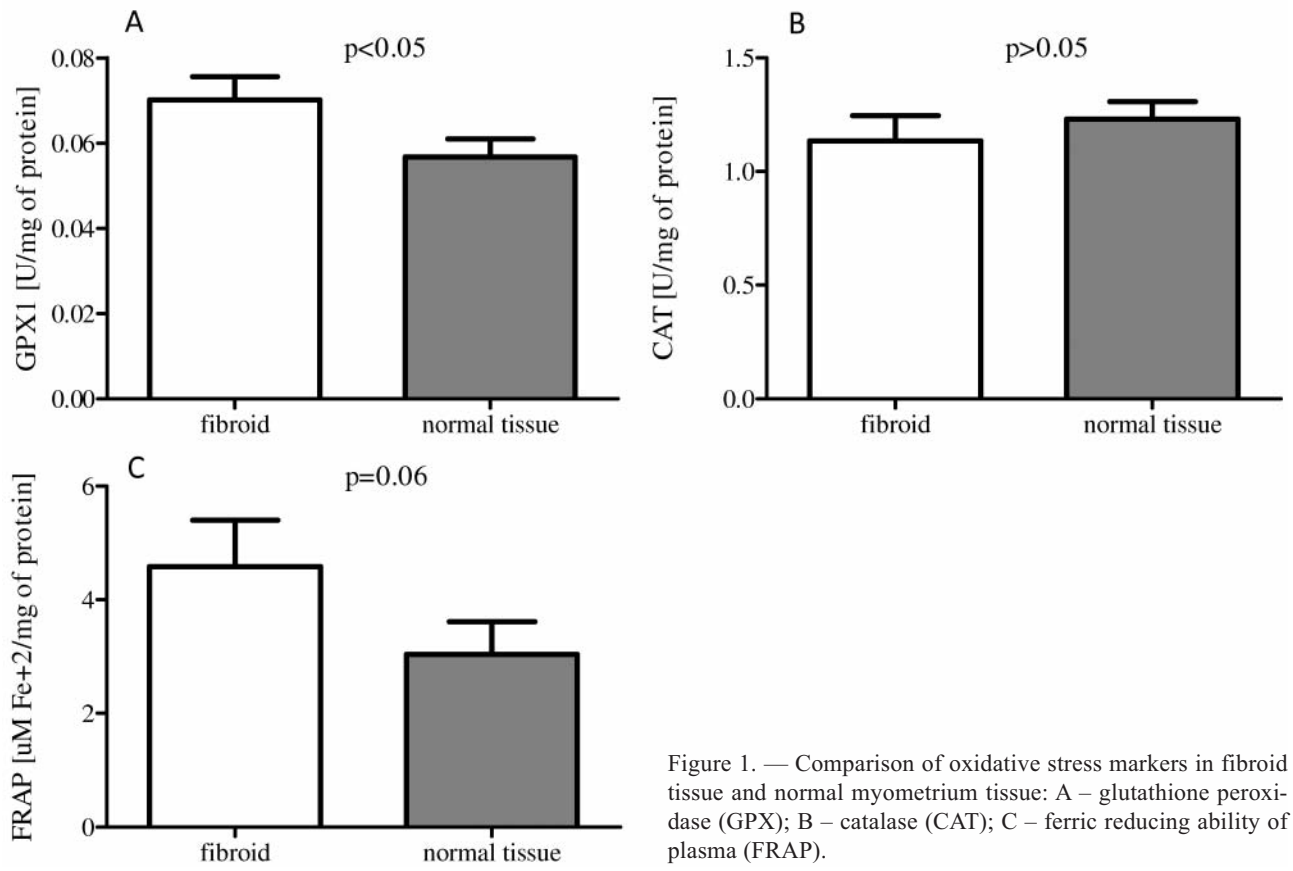

Figure 1. — Comparison of oxidative stress markers in fibroid tissue and normal myometrium tissue: A – glutathione peroxidase (GPX); B – catalase (CAT); C – ferric reducing ability of plasma (FRAP).

Materials and Methods

Participants

Fifty-nine patients with transvaginal ultrasound proven uterine fibroids who underwent standard gynecological procedures in the Department of Oncology at Poznan University of Medical Sciences were recruited in the study after informed consent. The mean age was 50.6 years (48.2-52.9, 95% CI). From 59 woman, 35 were described as premenopausal and 24 as postmenopausal. No woman in this study had a previous history of myomectomy, autoimmune or inflammatory disease. Women with virus C or virus B hepatitis or human immunodeficiency virus infection were not included in this study. None of included women was pregnant or had endometriosis. All women in the study group had histologically proven uterine leiomyoma

Samples collection

Samples were collected *ex vivo* immediately after resection in the size of approximately one cm^3. In woman who underwent hysterectomy, both fibroid sample as well as macroscopically normal myometrium sample were collected. In woman who underwent conservative surgery (tumor resection) only fibroid sample was collected. Samples were washed with distilled water and frozen (-76°C) until the analysis.

Evaluation of oxidative stress markers

The tissue samples were homogenized in phosphate buffer pH = 7.4. FRAP assay was performed according to Benzie and Strain [32] with some modifications [33]. The reducing ability of the sample was expressed in ferrous ion equivalents (mM Fe^{2+}/mg of protein) within the standard incubation period of 30.0 minutes after reagent addition. GPX activity was evaluated with hydrogen peroxide as the substrate, as described previously [34]. CAT activity was estimated according to Aebi [35]. Protein content was determined by Bradford method.

Statistical analysis

Statistical analysis was performed with the use of Statistica 10.0 Software. D'Agostino-Pearson omnibus test was used for normality of the data check. Data were evaluated using the Mann-Whitney U-test for comparison between groups, and the Wilcoxon signed-rank test for comparison within groups. The level of significance was set at the standard level of $p = 0.05$.

Results

Comparisons in oxidative stress markers between fibroid tissue and normal myometrium are presented in Figure 1. The activity of GPX shows to be significantly higher in fibroid samples than in myometrium (0.070 +/- 0.042 *vs.* 0.057 +/- 0.027 U/mg of protein, $p < 0.05$*),* activity of CAT did not differ between samples (1.13 +/- 0.86 *vs.* 1.23 +/- 0.51 U/mg of protein, $p > 0.05$) and FRAP presented higher values in fibroid samples than in myometrium (4.58 +/- 6.29 *vs.* 3.04 +/- 3.81 mM Fe^{+2}/mg of protein) but without statistical significance ($p = 0.06$). The subgroups analyses are presented in Figure 2. There were no statistically significant

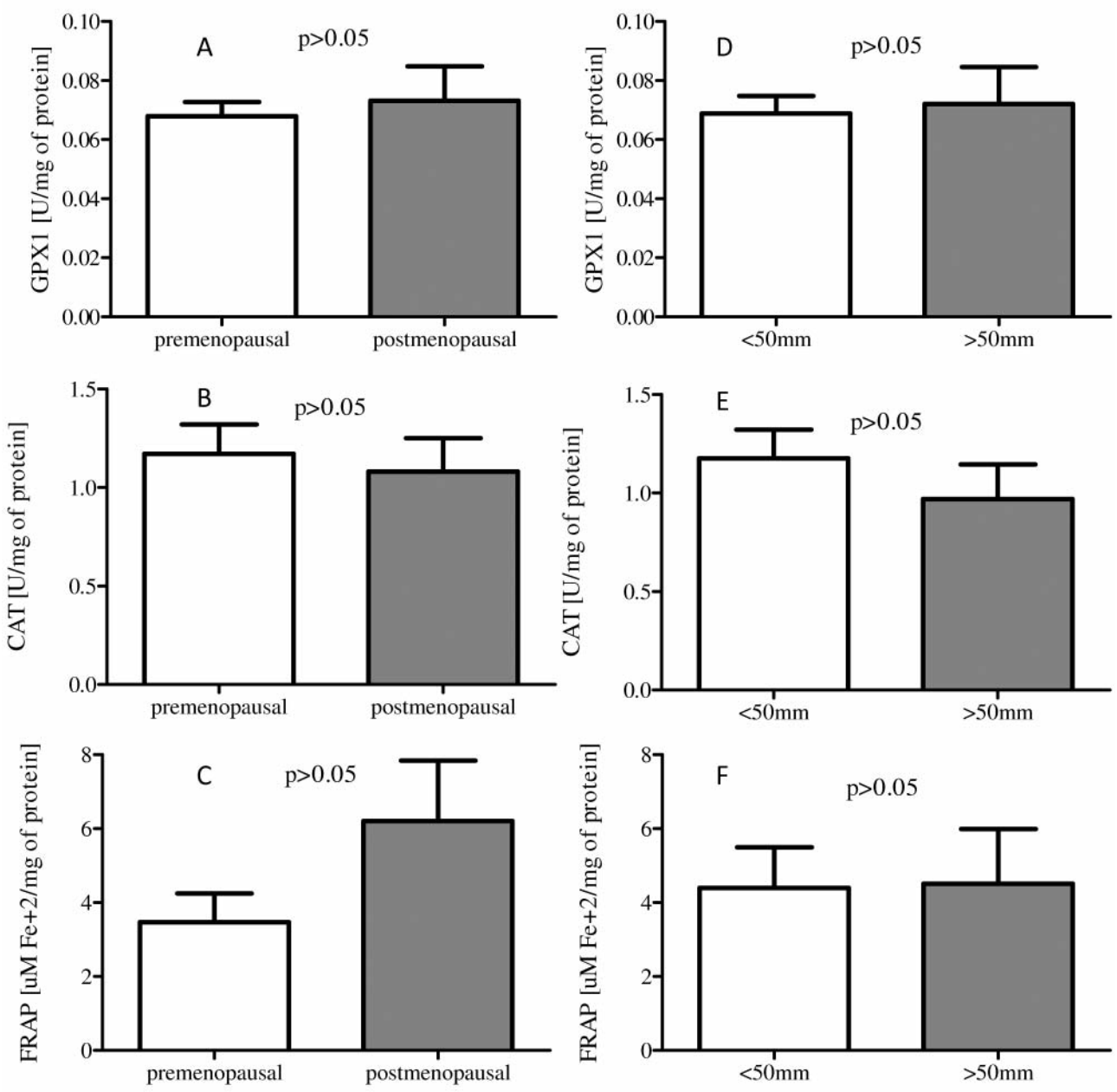

Figure 2. — Comparison of oxidative stress markers: glutathione peroxidase (GPX), catalase (CAT), and ferric reducing ability of plasma (FRAP) of fibroid tissue from pre- and postmenopausal women (A, B, C) and samples of small size (< 50 mm) and large size (≥ 50 mm) tumors (D, E, F).

differences when comparing the activity of GPX, CAT, and FRAP in fibroid samples from pre- and postmenopausal women, as well as when comparing fibroid samples of small size (< 50 mm) and large size (≥ 50 mm) tumors.

Discussion

To the present authors' knowledge, this is the first study which compares tissue oxidative stress markers between fibroid tissue and normal myometrium in the same patients and additionally compares tissue samples in pre- and postmenopausal woman. They found increased GPX activity in fibroid tissue ($p < 0.05$) as well as a nearly significant increased FRAP. Oxidative stress markers were independent from menopausal status and fibroid size.

Pejić *et al.* [26] compared endometrial tissue samples in patients with different gynecological disturbances. They found that CAT activity was not altered between polyps

and myoma, simple and complex hyperplasia, while in adenocarcinoma patients the activity was lower by 43% ($p < 0.05$). At the same time CAT activity compared with controls, was not altered in blood of subjects with polypus, myoma, hyperplasia simplex, and adenocarcinoma [29]. GPX activity was not different between polyps and myoma in endometrial tissue samples [26] as well as the serum activity was not changed in uterine myoma and controls [29]. Vural *et al.* [36] showed that ceruloplasmin, CAT, arylesterase, free sulfhydryl group, and prolidase activities were significantly higher in fibroid tissue than those in myometrial tissue. It was concluded that the study demonstrated increased antioxidative repair system in the fibroid tissue compared to the myometrium and serum of the same patients. Additionally, higher pathophysiological potential of the submucosal fibroids over intramural and subserosal fibroids were shown with the levels of oxidative stress markers and prolidase activity levels.

The results presented by Ohwada *et al.* [37] showed that endometrial GPX activity is regulated by sex hormones, being stimulated by estrogen and suppressed by progesterone, that is important especially in premenopausal woman. Foksinski *et al.* [38] showed a higher level of 8-hydroxy-2'-deoxyguanosine in uterine myoma tissues than in their respective tumor-free tissues what was more elevated in uterine tissues of premenopausal women when compared with postmenopausal ones. They also found the correlation between the size of the tumor and the amount of 8-OH-dG. In another study Chiou *et al.* [31] showed that plasma thiobarbituric acid-reactive substances (TBARS) were on significantly higher level ($p < 0.05$) in myoma patients than in controls when plasma and erythrocyte superoxide dismutase (SOD) activity was significantly lower than in controls. Erythrocyte GPX activity and CAT activity did not differ in myoma patients and in controls.

Conclusions

Oxidative stress markers are changed in fibroid tissue samples showing that oxidative stress may play an important role in this tumor formation, although without influence of menopausal status nor tumor size.

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