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Physical Chemistry

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and

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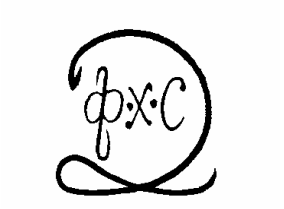

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ANTIOXIDANT ENZYMES IN BLOOD OF WOMEN WITH UTERINE HYPERPLASIA

A. Todorović, D. Drakulić, L. Gavrilović, V. Stojiljković, N. Popović, N. Đorđević, N. Todorović Vukotić, J. Miletić, S. Pejić, S. B. Pajović

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ABSTRACT

The literature emphasizes the involvement of oxidative stress in the etiopathogenesis of many uterine diseases. Antioxidant system (AOS) represents the protective mechanism used by cells to neutralize overproduced reactive oxygen species (ROS) and prevent oxidative stress. We have previously shown that in gynecological patients with various diagnoses, the reproductive and other factors may be associated with antioxidant capacity and the ability to defend against oxidative damage. In this study, we examined the changes in expression of antioxidant enzymes (AOE): superoxide dismutases (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) in the blood of women with endometrial hyperplasia. Our results indicate that hyperplasia induces perturbance in oxidative balance, particularly in glutathione redox cycle enzymes.

INTRODUCTION

According to the World Health Organization (WHO) classification system, simple endometrial hyperplasia (SH) refers to diffuse and variably sized glands with a normal ratio of glands to stroma. This type of hyperplasia is characterized by a spectrum of changes in the endometrium ranging from slight alterations seen in the late proliferative phase of the menstrual cycle to irregular, hyperchromatic lesions that are similar to endometrial adenocarcinoma [1].

There is growing literature evidence on the involvement of oxidative stress in the etiopathogenesis of various uterine diseases including endometriosis, pre-eclampsia and unexplained infertility. Antioxidant system (AOS) represents the protective mechanisms used by cells to neutralize reactive oxygen species (ROS) and prevent oxidative stress. The most important enzyme components in antioxidant system are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). Various studies demonstrated that compared to control, patients with benign and malignant changes in the genital tract have increased level of lipid peroxidation and altered antioxidant enzyme (AOE) activities in both peripheral blood and tissue [2].

We have previously shown that in gynecological patients with various diagnoses, the reproductive and other factors may be associated with antioxidant capacity and the ability to defend against oxidative damage [3]. In this study, we wanted to further examine the changes of antioxidant enzymes (SOD, CAT, GPx, and GR) expression, to better understand the changes in AOE activities found in patients with SH.

METHODS

This study utilized blood samples obtained from 30 subjects admitted to the Department of Gynecology and Obstetrics for gynecological evaluation within routine checkups or for abnormal uterine bleeding. The samples were taken after obtaining the informed consent. The study was conducted prospectively and it was approved by the Human Studies Ethics Committee of the Clinical Centre (No. 27/06-2006). The protocol was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects). Based on diagnosis and histological examination, subjects were divided into the following groups: healthy

control patients (C, n = 15, 49±3 yr) and patients with hyperplasia simplex endometrii (SH, n = 15, 48±1 yr). None of the subjects had undergone hormone therapy or any other medical treatment 6 months before sampling.

Venous blood samples were collected into heparinized tubes on the same day as endometrial biopsy. The protein levels of SOD, CAT, GPx, and GR were assayed by Western blot analysis as described previously [4].

Due to a non-Gaussian distribution of values, nonparametric Mann-Whitney U test was used to compare enzyme levels between SH patients and control subjects. A 2-tailed $p < 0.05$ was considered statistically significant. All data were analyzed using IBM SPSS Statistics version 23 and GraphPad Prism 4 software.

RESULTS AND DISCUSSION

Despite the incidence, treatment protocols for SH have not been standardized. The choice depends on lesion severity, patient age and medical history, but further progression of clinical diagnostic and treatment requires a better understanding of the molecular mechanisms that influence the disease. Oxidative stress seems to be important for disease development since it starts in the early stages of gynaecological pathologies [5].

Our study confirmed the existence of oxidative imbalance in women with SH. Results showed that among examined protein levels in the blood of SH patients, the GPx level was significantly increased (133.2%, $p < 0.05$), and the GR level was decreased (80.6%, $p < 0.01$), compared to healthy controls. The protein levels of CuZnSOD and CAT in SH patients were comparable between groups (**Figure 1.**).

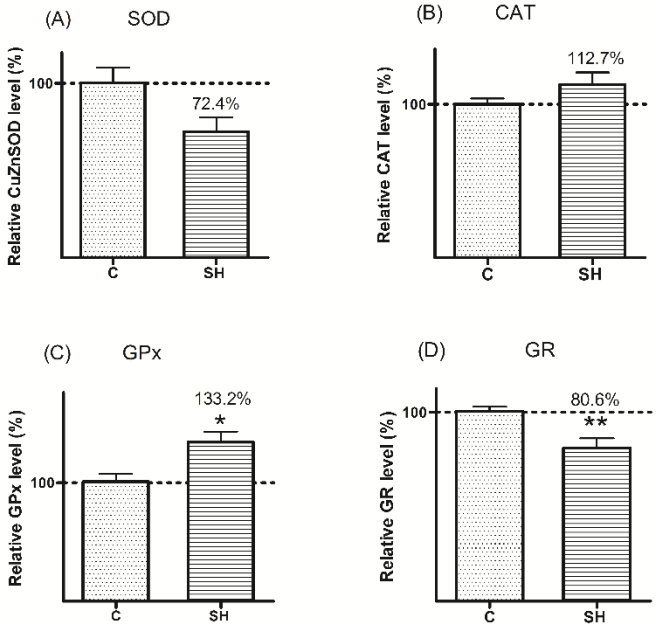

Figure 1. Relative protein levels of (A) CuZnSOD, (B) CAT, (C) GPx, and (D) GR in blood of healthy subjects (controls, C) and patients diagnosed with hyperplasia simplex (SH). Data are presented as the mean ± SEM, whereas the values of controls are set as 100%. *$p < 0.05$, **$p < 0.01$ vs. controls.

In our previous work, we reported significantly decreased activity of SOD in the blood of patients with SH [3]. Based on the unchanged SOD level detected in the current study, the results can be explained by inhibition of the enzyme activity that was not caused by the changes in its expression. Phosphorylation of proteins, for example, is one of the typical post-expressional mechanisms for the regulation of SOD activity. Transient phosphorylation of cytoplasmic SOD was firstly reported by Csar et al. [6] and it could explain the reduction of SOD activity in the blood of SH patients. Enzymatic activity of SOD could also be reduced by the peroxynitrite and myeloperoxidase system modification [7, 8], as well as by nitration, phosphorylation, glutathionylation, glycation, etc. The consequences of these modifications include altered structure and function of the protein, modulation of the catalytic activity, susceptibility to proteolysis, and disturbed signal transduction [9].

Similar to SOD, the CAT level in patients with SH was also unchanged. It seems that CAT functioning was unaffected by the existence of SH, although its adequate activity might be important for the disposal of free radicals and maintenance of the redox status of the cells. Unchanged expression and activity of CAT in SH patients could be elucidated by a decreased H_2O_2 production due to reduced SOD activity, but also by the role of CAT in the H_2O_2 removal. In the presence of high H_2O_2 concentration, CAT is the most effective for its metabolizing, but the glutathione system plays a critical role in the presence of low concentrations of H_2O_2 and other peroxides [10]. Ota et al. observed that CAT level was higher in patients with endometriosis than in controls. They hypothesized that GPx was not sufficient to dispose excessive free radicals in endometrium, so CAT was mobilized to support the neutralization [11].

GPx and GR are complementary pair of enzymes and important components of the glutathione redox system. GPx reduces toxic hydroperoxides, formed as a result of oxidative stress, while GR converts oxidized glutathione to the reduced form which GPx uses as an obligate co-substrate for normal enzyme activity. The significance of all components of the glutathione redox cycle is based on the great importance of glutathione molecules (GSH). They participate directly in free radicals neutralization and maintain vitamins C and E in their active forms. They are also involved in the regulation of the cell cycle, cell death, and many other important physiological processes [12]. Since GR is involved in sustaining the intracellular reducing environment by maintaining a high ratio of reduced to oxidized forms of GSH (GSH/GSSG), the inhibition of GR leads to the accumulation of GSSG, resulting in oxidative stress [13]. In our study, increased GPx and reduced GR levels observed in SH patients, might induce a reduction in GSH level, increasing the sensitivity to oxidative stress. This is particularly important for SH patients that are already in a state of oxidative stress since GSH deficiency enhances the cell's vulnerability to oxidative stress and the possibility of hyperplasia progression to adenocarcinoma [14].

CONCLUSION

Presented results provide evidence about AO disturbance in the blood of SH patients, with particular regard to glutathione redox cycle enzymes GPx and GR. Findings indicate the complex mechanisms that link the AO system with this endometrial disease and point to the need for further research to elucidate the mechanisms of observed AO shift.

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