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Changes in Catalase Activity Depending on Stages of Gynecological Cancers

(Submitted by corresponding member of NAS RA E. S. Gevorgyan 15/VII 2019)

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The female reproductive system is a complex multiorgan system which requires an optimal biological environment. Aerobic metabolism utilizing oxygen is essential for reproductive homeostasis and is associated with the generation of pro-oxidant molecules called ROS (reactive oxygen species) [1]. Many studies have investigated the presence of antioxidants and their transcripts in the female reproductive tract indicating that the balance between ROS and antioxidants greatly influences the reproductive activities, such as endometrial changes in different luteal phases, folliculogenesis, ovulation, fertilization, placental growth, embryogenesis, and implantation [2]. Thus, antioxidants are critical for maintaining the redox balance in the reproductive tract to support normal reproductive function [2].

Catalase (CAT, EC 1.11.1.6) plays a critical role in ROS metabolism. For this reason, it has been intensely studied in recent years [1-3]. Despite this, changes in CAT level during carcinogenesis, the mechanism of these changes, and their significance for course of the disease are still incompletely understood. CAT is frequently downregulated in human and rodent tumor tissues compared to normal tissues [4]. The low levels of CAT expression correlate with a high production of H_2O_2, which is involved in the activation of signaling pathways to induce proliferation, migration, and invasion in cancer cells [4]. However, increased catalase expression has been observed in tumors from patients with gastric carcinoma, skin cancer, and chronic myeloid leukemia and in human HL-60 cancer cells rendered resistant to chronic exposure to H_2O_2 [4]. In studies with ovarian carcinoma low levels of tumor CAT activities were observedin tumor tissue [5-6] and in blood samples [7-9], whereas Gorozhanskaia et al. [10] measured high CAT activities in ovarian cancer patients compared with control. Very recently, it was reported about decreased CAT level in tissue

samples of the patients with uterine adenocarcinoma [3]. Thus, additional studies required to understand the controversial data about CAT activity in cancer pathophysiology.

We hypothesized that such changes in CAT activity in pathophysiology of malignant tumors may be not only organ-specific, but also depend on the stage of the disease and the patient's age. Therefore, in present study we have considered advisable to investigate the changes in CAT activity in plasma and tumor tissue samples of the patients with primary ovarian and endometrial cancer depending on the stage of disease and the age of patients.

Material and methods. The blood and tissue samples frompostoperative material of untreated patients with the I (n=6), II (n=7) and III (n=5) stages of primary ovarian cancer and I (n=13), II (n=6) and III (n=5) stages of primary uterine adenocarcinoma were provided by the National Centre of Oncology (NCO MH RA). The plasma of healthy donors (n=6) and histologically checked healthy parts of remote tissue (n=8 for uterine and n=4 for ovaries) were used as a control. Histological study of the postoperative material was conducted by the Laboratory of Clinical Pathomorphology at the NCO MH RA. The most cases of ovarian cancer were diagnosed as a moderately and poorly differentiated adenocarcinoma. Age of patients ranged from 45-80, and the average age was 61 years. In case of uterine carcinoma, moderately differentiated endometrioid adenocarcinoma was diagnosed mostly. Age of patients ranged from 35-76, and the average age was 61 years as well.

Blood (1.5-2 ml) was collected into sodium citrate (3.2%)-coated vacutainer tubes and centrifugated at 1500 rpm for 10 min. Plasma was separated and stored at -32°C. Tissue samples were homogenized with 5 volumes of 50 mM Tris-HCI, pH 7.5 buffer, containing 0.05% Triton-X-100, 0.1 mM EDTA, 1 mM ditiothreitol (DTT), protease inhibitors, and centrifugated at 20000×g for 60 min at 4ºC. The supernatant was separated and stored at -32°C as well. The protein content in samples wasdetermined by Bradford assay [11].

The activity of catalase was studied using the spectrophotometric assay of hydrogen peroxide based on formation of its stable complex with ammonium molybdate [12].

Experiments were performed at least three times. The results were expressed as the means ± SEM. Statistical analysis was performed using Origin 6.1software. Statistical significance was defined at $P<0.05$ and was determined with one-way ANOVA.

Results and discussion. Data obtained demonstrates that catalase activity in both plasma (Fig. 1a) and tissue samples (Fig. 1b) of patients with ovarian cancer were changed in a parallel manner showing a modest increase (1.23 fold and 1.58 fold, respectively) in the I stage of disease compared with the control groups. In the II and III stages of disease CAT activity was shown to be increased 1.1 and 1.97 fold in plasma, and 1.36 and 2.24 fold in tumor tissue, respectively compared with the I stage of disease. It must be noted that the changes in CAT activity in plasma of patients with ovarian cancer depending on stage of disease were not statistically significant.

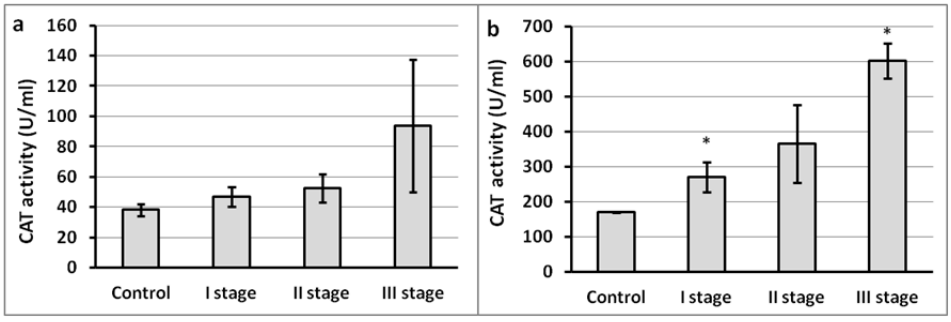

Fig. 1. Catalase activity in plasma (a) and tumor tissue (b) of the primary ovarian cancer patients with the I, II and III stages of disease. * p<0,05 for the I stage compared with control, and for the III stage compared with the I stage (using one-way ANOVA).

In plasma of the patients with uterine cancer CAT activity demonstrates 6 fold increase in the I stage of disease compared with control group, and 4.3 and 3.6 fold decrease in the II and III stages of disease, respectively, compared with the I stage (Fig. 2a). However, CAT activity in tumor tissue demonstrates 6.3 fold increase in the I stage of disease compared with control group, 1.7 fold increase in the II stage of disease compared with the I stage and dramatic 8.3 fold decrease in the III stage of disease compared with the I stage (Fig.2b).

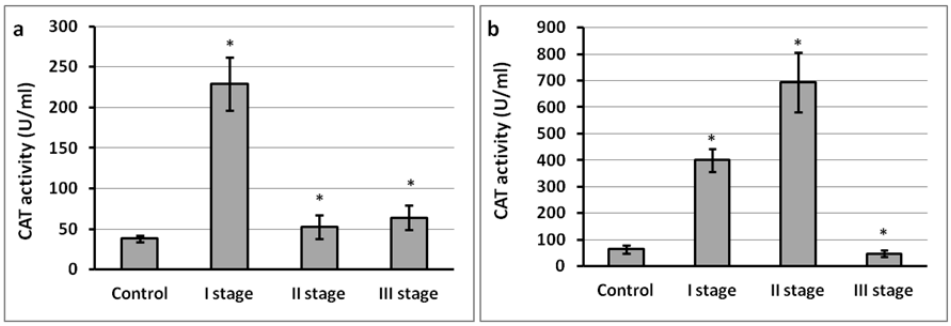

Fig. 2. Catalase activity in plasma (a) and tumor tissue (b) of the primary uterine cancer patients with the I, II and III stages of disease. * p<0,05 for the I stage compared with control, and for the II and III stages compared with the I stage (using one-way ANOVA).

Tumors undergo metabolic reprogramming to meet the increased energetic and anabolic demands [13]. Thus, most malignant tumor cells likely have high cell metabolic activity and, thereby may have high oxidant generation. As oxidative stress is a primary stimulus for the induction of antioxidative enzymes, their induction most commonly remains insufficient in response to enhanced oxidative stress and can reduce but not fully compensate the oxidative stress. The imbalance reveals a slightly pro-oxidant state and over a time creates a tumor

supportive oxidant environment in which tumor suppressor elements are downregulated, and tumor cells show enhanced proliferation and are more aggressive [14]. In this regard, gradually increased antioxidative enzyme levels, such as CAT activity in ovarian cancer, could be the reflection of cancer cells adaptation to exacerbating oxidative stress.

It was reported that activity of CAT in I stage uterine adenocarcinoma patients was significantly decreased compared to women with polyps or myoma [3]. The difference between that results and our data maybe because of different control groups. Furthermore, Punnonen et al. [15]found that Japanese women with endometrial cancer did not have significantly altered CAT activity. This means that perhaps changes in CAT activity in the same pathology may depend on nationality, that is genom, as well. Previously, the difference in the transcriptional regulation of catalase expression has been shown in normal versus cancer cells [4]. Although the precise mechanism of CAT regulation during the development of uterine malignancy remains unknown, the absence of a positive correlation between its activity and level [3] is suggesting that CAT activity in endometrial tissue is governed by a mechanism other than expression.

The low levels of CAT in the plasma of endometrial cancer patients with the II and III stages of disease may be due to their increased utilization to scavenge lipid peroxides as well as their sequestration by tumor cells and most probably would enhance the oxidative stress in patients by lowering the detoxification of H_2O_2[16]. It is already known that if the O^{2-} is not removed immediately, it may cause the inhibition of CAT activity [17]. Thus, the lower CAT activity we have found might be a consequence of the decreased SOD activity reported in patients with endometrial cancer [16]. It is possible that such relations contribute to a certain antiapoptotic milieu that is suitable for enhancing the mutations frequency, a condition which may lead to the cell transformation and cancer. Investigations already point to that mechanism [18].

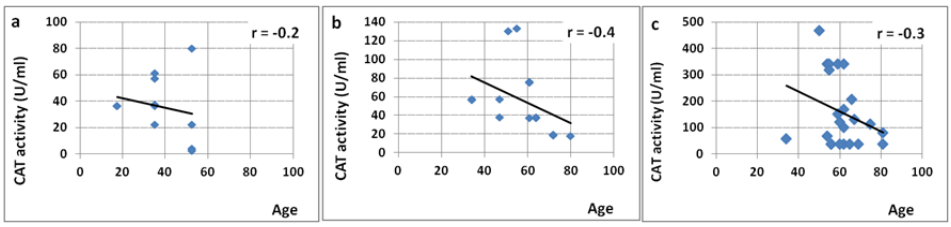

Fig. 3. Scatter plots of the plasma CAT activity against the age of the healthy donors (a; $p<0.05$), primary ovarian (b; $p<0.05$) and uterine (c; $p<0.05$) cancer patients.

We have also study the age dependent changes in CAT activity in plasma and tumor tissue of the patients with ovarian and endometrial cancer. Interestingly, we have found weak negative correlation between CAT activity and the age of patients only in plasma (r=-0.4 for ovarian and r=-0.3 for uterine cancer) (Fig. 3b, c), but not in tumor tissue (r=0.07 for ovarian and r=0.001 for uterine

cancer) samples. As we have demonstrated that CAT activity in plasma changes depending on the age of the healthy donors as well (r=-0.2; Fig. 3a), it means that it is a characteristic feature of catalase activity to be changed with the age. Thus, we have concluded that changes in CAT activity do not depend on the age of patients with gynecological cancers.

Since endometrial carcinoma is usually divided into Type 1 and Type 2 tumors, we considered advisable to study also the changes in CAT activity depending on this division. Data obtained demonstrated that there were no statistical significant changes in CAT activity in plasma of oncologic patients with Type 1 and Type 2 tumors (151.7±31.8 U/ml and 161.3±40.9 U/ml, respectively). However, in tumor tissue we have found statistically significant changes in CAT activity depending on the type of tumor. It was shown to decrease in Type 2 tumor tissues (36±4.4 U/ml) compared with the Type 1 tumor (315.1±51.6 U/ml). Thus, in tumor tissue we have found weak correlation (r = 0.4) between CAT activity and type of tumor, which means that tumor type in endometrial adenocarcinoma affects CAT activity only locally in tumor, but not in circulation.

In summary, we have demonstrated that changes in CAT activity in gynecological cancers are organ-specific; depend on the stage of disease, but not on the age of patients, as well as, on the tumor type in tumor tissue of the patients with endometrial adenocarcinoma.

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Changes in Catalase Activity Depending on Stages of Gynecological Cancers

The changes in catalase activity in plasma and tumor tissue samples of untreated patients with primary ovarian and endometrial cancers with the I, II and III stages have been studied. It was demonstrated for the first time that catalase activity in both ovarian and endometrial cancer changes depending on the stage of disease, but not on the age of patients. The changes in catalase activity in tumor tissue of the patients with endometrial cancer depend on the tumor type as well. Data obtained can be used for improving the main therapy strategies for the gynecological cancers treatment.

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Ն. Հ. Բարխուդարյան

Կատալազի ակտիվության փոփոխությունը գինեկոլոգիական քաղցկեղի փուլերից կախված

Ուսումնասիրվել են կատալազի ակտիվության փոփոխությունները բուժում չստացած և I, II, III փուլերում գտնվող ձվարանների ու էնդոմետրիումի առաջնային քաղցկեղով հիվանդների պլազմայում և ուռուցքային հյուսվածքում: Առաջին անգամ ցույց է տրվել, որ կատալազի ակտիվությունը ինչպես ձվարանների, այնպես էլ էնդոմետրիումի քաղցկեղի ժամանակ փոփոխվում է կախված հիվանդության փուլից, բայց ոչ հիվանդի տարիքից: Էնդոմետրիումի քաղցկեղով հիվանդների ուռուցքային հյուսվածքում կատալազի ակտիվությունը փոփոխվում է նաև ուռուցքի տեսակից կախված: Ստացված արդյունքները կարող են կիրառվել գինեկոլոգիական քաղցկեղի բուժման հիմնական թերապևտիկ մոտեցումների բարելավման նպատակով:

Г. А. Оганнисян, Ф. П. Саруханян, О. В. Унанян, Н. А. Бархударян

Изменения активности каталазы в зависимости от стадии гинекологического рака

Изучались изменения активности каталазы в плазме и опухолевой ткани ранее нелеченных пациентов с первичным раком яичников и эндометрия в I, II и III стадии. Впервые показано, что активность каталазы как при раке яичников, так и эндометрия меняется в зависимости от стадии заболевания, но не от возраста больного. Изменения активности каталазы в опухолевой ткани у пациентов с раком эндометрия зависят также от типа опухоли. Полученные результаты могут быть использованы для улучшения основных стратегий терапии при лечении гинекологических раковых заболеваний.

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