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ANTIOXIDATIVE BIOMARKERS AND CANCEROGENESIS

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Summary: Cancer development includes three major steps, initiation, promotion and progression, in which oxidative stress is involved. Oxidative stress is defined as an imbalance between the levels of prooxidants and antioxidants in favour of the former and resulting in irreversible cell damage. We examined the lipid peroxidation levels and antioxidant enzyme activities in women diagnosed with different forms of uterine cell transformations in order to evaluate the extent of oxidative stress in the blood of such patients. Blood samples of healthy subjects and gynecological patients were collected and subjected to assays for superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and lipid hydroperoxides. The results show that alterations of the measured parameters vary with the enzyme type and diagnosis. However, both reduction in antioxidants and elevation of lipid peroxidation were observed in general. In addition, lipid hydroperoxide levels were negatively correlated with superoxide dismutase and glutathione peroxidase activities, as well as positively correlated with catalase activity. The obtained results also show that perturbation of the antioxidant status is more pronounced in blood of patients with premalignant (hyperplastic) and malignant (adenocarcinoma) lesions, compared to those with benign uterine changes such as polypus and myoma.

Key words: antioxidant enzymes, biomarkers, cancer

Introduction

Carcinogenesis is a complex multisequential process leading a cell from a healthy to a precancerous state, and finally to an early cancerous stage. Cancer development includes three major steps, initiation, promotion and progression, in which oxidative stress is involved. Generally speaking, oxidative stress is defined as an imbalance between the level of prooxidants and the antioxidant defence system, in favour of the former, and resulting in irreversible cell damage (1–3).

When produced in excess, reactive oxygen species (ROS) can seriously alter the structure of biomolecules, such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA). Oxidative DNA damage may participate in ROS-induced carcinogenesis (4). A common form of damage is the formation of hydroxylated bases of DNA, which are considered an important event in chemical carcinogenesis (4). This adduct formation interferes with normal cell growth by causing genetic mutations and altering normal gene transcription. Several different pathways by which oxidative DNA damage leads to mutations have been proposed, including chemical modification of nucleotide moieties in DNA causing alteration in their hydrogen bonding, exacerbation of polymerase-specific hot spots, conformational change in the DNA templates, and the induction of a DNA polymerase conformation that is error prone (5). Formation of 8-hydroxy-2'-deoxyguanosine (8-OhdG), an oxidative modification of DNA produced by hydroxylation in the C-8 position of deoxyguanosine residues by the hydroxyl radical (6), has been used as a measurement of oxidative DNA damage.

Cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydroperoxides (7). Lipid peroxyl radicals can subsequently propagate into malondialdehyde (MDA). The formation of lipid damage may result in several possible sequelae, including protein oxidation (7). These lipid

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radicals can diffuse through membranes, thus modifying the structure and function of the membrane and resulting in a loss of cell homeostasis. In addition, lipid peroxides may bring about an interaction with cellular DNA and cause the formation of DNA-MDA adducts.

Proteins are also easily attacked by ROS directly or indirectly through lipid peroxidation. Protein radicals can be rapidly transferred to other sites within the protein infrastructure. This can result in further modification of enzyme activity, stimulation or inhibition (8, 9). In addition to enzymes, damage to the membrane transport proteins may produce cellular ionic homeostasis and lead to alterations in intracellular calcium and potassium that will trigger a series of changes in the cell (10). Changes to receptor proteins and gap junction proteins may also modify signal transfer in cells. In selective cases, alterations of protein structure may allow the target protein to be further attacked by proteinases (11). Thus protein oxidative damage can result in modifications in the structure, enzyme activity, and signaling pathways.

Signal transduction or cell signaling is a process enabling information to be transmitted from the outside of a cell to various functional elements inside the cell. Signals sent to the transcription machinery responsible for expression of certain genes are normally transmitted to the cell nucleus by a class of proteins called transcription factors. Many recent studies have shown that numerous oxidation-reduction reactions in the cell are involved in regulating several cell functions. According to their nature, quantity, source, and production kinetics in the cell, ROS affect cell regulation differently. The boundary between positive and negative ROS effects is hard to define according to the cell type studied (12). The same concentration may or may not trigger deregulation of signal transmission, with desirable or undesirable effects. For example, activation of factor $NF_\kappa B$ is positive when it triggers apoptosis, but negative when it causes expression of genes coding for proinflammatory agents (cytokines).

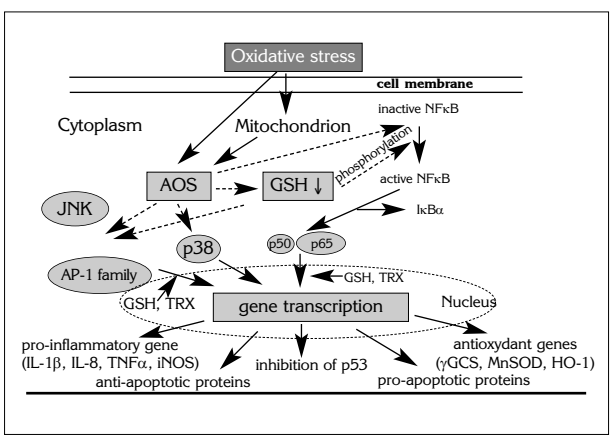

Figure 1 Control role of activated oxygen species (AOS) and glutathione (GSH) in the redox regulation of gene transcription

This duality depends on the cell type and also on the cell's antioxidant status. In this perspective, glutathione plays a prime role in maintaining a redox status that is optimal for the cell and in regulating transcription genes. In terms of cancer prevention, antioxidant strategies enabling the cell to maintain this optimal state as long as possible can be envisaged.

Antioxidant status in blood of patients with transformed endometrial cells

Deleterious effects of reactive oxygen species (ROS) and lipid peroxidation (LPO) products are counteracted by the antioxidative defense system (AOS), which consists of nonenzymatic antioxidant molecules such as tocopherol, carotenoids, ascorbate, glutathione (13) and antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione transferase (GST). SOD, the first line of defense against oxygen free radicals, catalyzes the dismutation of superoxide anion radical into hydrogen peroxide (H_2O_2), which can be transformed into water and oxygen by CAT or GPx. Besides hydrogen peroxide, GPx also reduces lipid or nonlipid hydroperoxides while oxidizing glutathione (GSH). The oxidized GSH is then reduced by GR (14). Antioxidants show different patterns during neoplastic transformation and tumor cells exhibit high variability of antioxidant enzymes (AOE) activities, when compared to their appropriate normal cell counterparts (15).

Endometrial carcinoma is the most frequently diagnosed malignancy of the female genital tract. It is often preceded by histopathologic lesions known as endometrial hyperplasia (16), which denotes a set of mixed epithelial and stromal proliferations (17), and is generally considered a precursor of endometrial cancer. According to the current World Health Organization (WHO) nomenclature, four categories of endometrial hyperplasia exist: simple (SH), complex (CH), simple atypical (SAH), and complex atypical (CAH). Recently, a new concept of classification (EIN nomenclature) has been proposed by Mutter et al (18), based on integrated morphological, genetic, molecular, cell biological, and morphometrical studies, according to which three disease categories are discerned: benign hyperplasia, endometrial intraepithelial neoplasia (EIN) and cancer (19).

Although endometrial hyperplasia is regarded as a preliminary stage of endometrioid carcinomas (20), there is a lack of data on the relationship between oxidative stress and antioxidant enzymes in such patients. Some investigations have so far revealed elevated levels of lipid peroxidation and perturbed

AOE activities in peripheral circulation and tissues of women with benign and malign diagnosis. Chiou and Hou (21) reported lowered plasma and erythrocytes SOD activity of both uterine cervicitis and myoma patients, while the activities of CAT and GPx were elevated in cervicitis patients and lowered in myoma patients. Similar observations on erythrocytes SOD, CAT and GPx activities of cervicitis patients were made by Manoharan et al (22), whereas activities of examined enzymes decreased in cervical cancer patients. Previous results of Kolanjiappan et al (23) and Manoharan et al (24) also demonstrated elevated levels of lipid peroxidation, lowered concentrations of GSH, vitamin E and CAT, disturbed antioxidant status as well as altered Na^+K^+-ATPase activity in erythrocytes of cervical cancer patients.

In the current study, we examined the level of lipid peroxidation in plasma and AOE activities in the blood cells of patients diagnosed with following diseases: uterine myoma (a hard benign growth that forms on the muscle layer), endometrial polypus (localized overgrowths of the endometrium that project into the uterine cavity), hyperplasia simplex, hyperplasia complex and adenocarcinoma in order to evaluate the extent of oxidative stress in the blood of such patients.

Results

Comparing data for the level of lipid hydroperoxides and antioxidant enzyme activities among six diagnosis groups, all parameters showed significant variations of the obtained values (Kruskal-Wallis, LOOH: $H=28.38$, $df=5$, $p<0.001$; SOD: $H=46.83$, $df=5$, $p<0.001$; CAT: $H=16.58$, $df=5$, $p<0.01$; GPx: $H=48.77$, $df=5$, $p<0.001$, GR: $H=37.92$, $df=5$, $p<0.001$).

Compared to controls, moderate elevation of lipid hydroperoxides concentration was observed in patients diagnosed with polypus endometrii or uterine myoma (4% and 27%, respectively, $p>0.05$), whereas it was significantly higher in both types of hyperplasia (SH: 41%, CH: 52%, $p<0.001$) and adenocarcinoma (57%, $p<0.01$). Also, patients with simple hyperplasia had more increased levels of LOOH than patients with polypus ($p<0.05$).

Superoxide dismutase activity was 26% ($p<0.05$) and 20% ($p>0.05$) lower in polypus and myoma patients respectively than in controls; and it was also significantly decreased in patients with hyperplasia simplex (42%, $p<0.001$), hyperplasia complex (45%, $p<0.001$) and adenocarcinoma (53%, $p<0.01$). Significant difference ($p<0.05$) between patients with hyperplasia or adenocarcinoma and the patients with myoma was also observed.

Compared with controls, it was found to be slightly elevated in the blood of subjects with polypus, myoma, hyperplasia simplex and adenocarcinoma (3%, 9%, 12% and 10%, respectively, $p>0.05$), and significantly elevated (24%, $p<0.01$) in subjects with hyperplasia complex. Among the groups, CAT activity differed between patients diagnosed with hyperplasia complex and polypus endometrii ($p<0.05$).

Patients with endometrial polyp had 26% higher GPx activity ($p<0.05$) than controls, while in other patient groups the activity was 7% lower ($p>0.05$) in uterine myoma; 27% and 35% ($p<0.001$) in hyperplasia simplex and complex respectively, and 37% ($p<0.01$) in adenocarcinoma subjects. Also, patients with either form of hyperplasia or adenocarcinoma had significantly decreased GPx activity ($p<0.05$) when compared to patients with polypus.

Compared to control values, significant reduction of GR activity was recorded in all examined groups: polypus endometrii: 65%, uterine myoma: 44%, hyperplasia simplex: 52%, hyperplasia complex: 51%, ($p<0.001$), and adenocarcinoma 51% ($p<0.01$). No significant difference ($p>0.05$) was found among patients with either form of diagnosis.

Plasma level of lipid hydroperoxides negatively correlated with SOD ($r=-0.33$, $p<0.001$) and GPx activity ($r=-0.42$, $p<0.001$), and positively correlated with CAT activity ($r=0.28$, $p<0.01$). Also, no correlation was found between lipid hydroperoxides and GR activity.

Discussion

In neoplastic transformation, antioxidant enzyme activities have shown different patterns and they are highly variable in tissues and blood of patients with different types of cancer (25–27).

This study indicates that antioxidant defense mechanisms are impaired in human uterine diseases, and it also points to elevated levels of lipid peroxidation products, as markers of oxidative stress, in the plasma of such patients. Namely, in all examined groups higher levels of LOOH were recorded than in controls, though the elevation was not significant only in patients with polypus endometrii. The observed changes are in accordance with other findings where elevated lipid peroxidation was reported for patients with uterine cervicitis or myoma (21) or cancer patients (28, 23). We also observed that circulating levels of lipid hydroperoxides are generally higher in subjects with either form of hyperplasia or adenocarcinoma than those found in polypus or myoma patients. Since oxygen radical production, which elevates lipid peroxidation, increases with the clinical progression of diseases (29, 30), this observation might indicate that patients with hyperplasia or adenocarcinoma potentially have a wider extent of cellular membrane degeneration (31) or DNA damage (32) than patients with myoma or polypus endometrii. The increase in LOOH may also be due to the

impaired antioxidant system as observed in the previous studies (26, 22).

Superoxide dismutase, a scavenger of superoxide anions, along with catalase and glutathione peroxidase, the preventive antioxidants, plays a very important role in protection against lipid peroxidation. In this study, SOD activities were lowered in blood of all examined patient groups than in healthy subjects. Besides, the decrease of SOD activity was much more pronounced in hyperplasia or adenocarcinoma patients than in subjects with polypus or myoma, thus making those individuals more vulnerable to oxidative stress. Decreased SOD activity in plasma of gynecological patients was also reported by Bhuvaraharamurthy et al (33), Chiou and Hou (21), Manoharan et al (22). Reduction of SOD activity may be due to an increased endogenous production of ROS as evidenced by increased lipid hydroperoxides. Also, it may be related to the consumption of activated enzymes during prolonged oxidative stress. In support of this observation, plasma LOOH were found to be negatively correlated with SOD activities in the examined patients.

Compared with controls, significant elevation of CAT activity was recorded only in patients with hyperplasia complex, while mild elevation was observed in the other groups. However, GPx activity was significantly increased only in patients with polypus endometrii. Other groups had lowered GPx activity, which was significant for the subjects diagnosed with either form of hyperplasia or adenocarcinoma. The observed changes also point to different antioxidant defense properties in various gynecological pathologies. Previous studies have reported elevated CAT and GPx activities in cervicitis patients (21, 22) and lowered activities of these enzymes in myoma patients (21). In cancer patients, both lowered (21, 22) and increased (34) CAT activity in blood was observed.

Catalase is considered to play a predominant role in protecting erythrocytes against oxidative stress in relation to GPx (35, 36), although their significance in H_2O_2 decomposition is still not clear (37). Also, it is well known that reactive oxygen metabolites such as hydrogen peroxide and superoxide anion increase in various pathological conditions, and superoxide anion radical inactivates CAT (38) and GPx (39). Decreased SOD activity, observed in this study, would be expected to further elevate superoxide anion levels. Also, it was proposed that superoxide anion channel allows the transport of superoxide and other free radicals into the red cell, where they are deactivated by the erythrocyte antioxidant system (40). According to our results, in the blood of examined gynecological patients, CAT activity seems to be unimpaired and GPx enzyme seems to be more sensitive to elevated levels of superoxide. In addition, lipid hydroperoxides were found to be positively correlated with CAT and negatively correlated with GPx activities in the examined patients.

Besides, decreased GPx activity recorded in all groups except in polypus patients might also be due to depletion of glutathione. Namely, oxidative stress was shown to induce the efflux of oxidized glutathione, consequently decreasing its content in erythrocytes and leading to their shortened life span (41–43). Similar observation was made by Manoharan et al (22) for cervical cancer patients and it was further supported with the finding of lowered glucose 6-phosphate dehydrogenase activity and NADPH production. Remarkably reduced glutathione reductase activity recorded in our study is in accordance with these observations. Also, reduction of erythrocyte GPx and GR activities, besides GSH depletion, is considered responsible for increased heme degradation as shown by Nagababu et al (37). Similar findings on GPx and GR activities in our study could also point to further deterioration of oxidative stress condition based on heme degradation in gynecological patients.

In summary, this study shows that patients with polypus or myoma, or either form of hyperplasia or adenocarcinoma, have enhanced lipid peroxidation and altered activities of antioxidant enzymes in peripheral blood circulation. Although alterations vary with the enzyme type and diagnosis, both reduction in antioxidants and elevation of lipid peroxidation were observed in general. The lowered activity of antioxidant enzymes in gynecological patients could be a result of disturbed redox status, while elevated lipid peroxidation seems to be a consequence of the disease rather than its cause.

It is known that, in response to acute oxidative stress, antioxidants may be consumed to prevent oxidative damage, and then may be supplied through the antioxidant network. However, in the cases of the observed gynecological pathologies, it seems that prolonged oxidative stress elevates free radical production and induces consumption of antioxidants, which in turn further aggravate the free radical damage and increase the chance of developing uterine cancer. Indeed, the results obtained in this study show that observed changes of AO status in peripheral circulation of gynecological patients are more pronounced in premalignant (hyperplastic) and malignant (ACE) lesions, compared with benign uterine changes (polypus and myoma). Further investigation should determine whether lipid hydroperoxide levels and AOE activities in blood of such patients might be used as additional parameters in the clinical evaluation of gynecological disorders.

Generally speaking, monitoring the levels of antioxidants as important markers in individuals may be useful in the diagnosis of disease and in researching therapies and disease processes.

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ANTIOKSIDATIVNI BIOMARKERI I KANCEROGENEZA

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Kratak sadržaj: Nastanak kancera odvija se u tri glavne faze (inicijacija, promocija i progresija) koje uključuju oksidativni stres. Oksidativni stres definiše se kao narušavanje ravnoteže između nivoa prooksidanata i antioksidanata, u pravcu povećanja nivoa prooksidanata, što za posledicu ima nastanak ireverzibilnih oštećenja ćelija. Da bismo procenili nivo oksidativnog stresa u krvi pacijentkinja sa dijagnozom različitih formi transformacije uterusa, ispitivali smo nivo lipidne peroksidacije i aktivnost antioksidativnih enzima. Prikupljeni su uzorci krvi zdravih žena i pacijentkinja i u njima je određivana aktivnost enzima: superoksid dismutaze, katalaze, glutation peroksidaze, glutation reduktaze, kao i nivo lipidnih hidroperoksida. Rezultati pokazuju da promene ispitivanih parametara variraju u zavisnosti od vrste enzima i dijagnoze. Međutim, može se uopšteno reći da je zapaženo sniženje antioksidativne aktivnosti i povećanje nivoa lipidne peroksidacije. Pored toga, nivo lipidnih hidroperoksida je negativno korelisan sa aktivnošću superoksid dismutaze i glutation peroksidaze i pozitivno korelisan sa aktivnošću katalaze. Dobijeni rezultati takođe pokazuju da je narušavanje antioksidativnog statusa u krvi izraženije kod pacijentkinja sa premalignim (hiperplazija) i malignim (adenokarcinom) lezijama, u poređenju sa pacijentkinjama kod kojih su benigne promene uterusa dijagnostikovane kao polip ili miom.

Ključne reči: antioksidativni enzimi, biomarkeri, kancer

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