Changes in lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with ovarian cancer

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Objective. This work was undertaken to assess oxidative stress and antioxidant status in patients with ovarian cancer. **Patients and Methods.** The study was conducted in thirty-eight patients with ovarian cancer, the control group being 38 healthy volunteers. Erythrocyte lipid peroxidation products (MDA), glutathione (GSH), ascorbic acid, plasma vitamin E and activities of antioxidant enzymes super oxide dismutase (SOD), glutathione peroxidase ($GP_{X)}$, catalase in erythrocytes and plasma glutathione – S – transferase (GST) were estimated in ovarian cancer patients. **Results.** In this study it was observed that there was a significant increase in erythrocyte MDA levels, SOD, GP_X and plasma GST activities and a significant decrease in erythrocyte GSH, ascorbic acid, plasma vitamin E levels and catalase activity in patients with ovarian cancer when compared to controls. **Conclusions.** The results of our study suggest higher oxygen free radical production, evidenced by increased MDA and decreased GSH, ascorbic acid, vitamin E and Catalase activity, as support to the oxidative stress in ovarian cancer. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress.

Key Words: Ovarian cancer, Malondialdehyde, Glutathione, Superoxide dismutase, Catalase, Glutathione peroxidase, Glutathione -S - transferase.

Introduction

Oxidative stress is the imbalance between the generation of reactive oxygen species and the activity of antioxidant defenses. Severe oxidative stress causes cell damage and cell death and it has been implicated in numerous human diseases including cancer (1). Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues (2). The uncontrolled production of free radicals is considered to be an important factor in the tissue damage induced by several pathophysiological processes (3). Moreover the body's defense

mechanisms would play a role in the form of antioxidants and try to minimize the damage, adapting themselves to the stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions (4) and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated (5). They exist in both the aqueous and membrane compartment of cells and can be enzymes or non-enzymes. Alteration in the oxidant -antioxidant reaction, relationship profile is known to occur in cancer (6).

Ovarian cancer remains one of the most lethal of all gynaecologic malignancies, accounting for more deaths than cervical and uterine cancers (7). More than 60% of patients with ovarian cancer do not present until they are at an advanced stage and the average 5 year survival rate is reported to be lower than 20% (8).

Patients and Methods

Blood samples were obtained from thirty-eight patients (mean age: 56 ± 10 years) with clinically and histopathologically proven ovarian cancer. Thirty-eight normal healthy age matched women volunteers served as controls. Written consents were also obtained from the patients prior to the study and the objectives of the study were fully explained. Ten of the participants were excluded from the study because they were not comfortable with the research protocol. An equal number of age matched healthy subjects were also investigated.

The complete clinical and personal history of the subjects was recorded. The subjects ranged in age between 46 – 65 years. All the patients in the study were clinically diagnosed as patients with ovarian cancer. None of these subjects were alcoholics or chronic smokers and did not suffer from any systematic diseases like hypertension or any diabetic complication. Subjects who had no other cancers and subjects with normal nutritional habits without supplementing any vitamins over 6 months were included. Subjects with a history of receiving anti-inflammatory drugs in the last 6 months and a history or present symptoms of any other stress induced disorder were excluded.

The controls and patients were divided into two groups.

- Group 1 (Controls): Thirty-eight healthy age matched women as controls.
- Group 2 (Study Subjects): Thirty-eight patients with clinically and histopathologically proven ovarian cancer.

Heparinised venous blood samples from these subjects "before meal intake" were used for the analysis. Plasma was separated by centrifugation at 1,000 g for 15 minutes. Separated plasma was used for the estimation of vitamin E and measurement of GST activity. The buffy coat was removed and the packed cells were washed three times with physiological saline. The erythrocyte suspension was prepared by the method of Dodge et al., (9) modified by Quist (10). The packed cells were used for the analysis of GSH, ascorbic acid, MDA, SOD, Catalse, GP_X. Erythrocyte GSH was estimated by the method of Beutler et al (11) using Di Thio Bis Nitro Benzoic acid (DTNB). Ascorbic acid levels were estimated by the method of Tietz (12). Plasma vitamin E levels were estimated by the method of Baker H et al (13). MDA was determined by the measure of TBARS (14). SOD (EC 1.15.1.1) activity was determined in the hemolysate by the method of Misra & Fridovich based on the inhibition of auto oxidation of epinephrine to adenochrome at Ph 10.2 (15). Catalase (EC 1.11.1.6) activity was measured by the method of Beers and Sizer (16). The activity of Glutathione Peroxidase (GPX, EC 1.11.1.9) was measured as described by Paglia and Valentine (17) in erythrocytes and

activity of GST (EC 2.5.1.18) was measured by using 1-Chloro-2, 4-Dinitro Benzene (CDNB) (18). All reagents used were of analytical reagent grade. DTNB, CDNB and Thio Barbituric Acid were obtained from sigma chemicals, St.Louis; MO.

Statistical analysis

Statistical analysis between the Control Group and the Ovarian Cancer Group was performed by the independent student – t test (parametric analysis) by using the SPSS statistical package for windows, Version 15. The data were expressed as mean ± SD. $P < 0.05$ was considered as significant.

Results

The mean ± SD of erythrocyte GSH, ascorbic acid, MDA, SOD, Catalase, GP_x, plasma vitamin E and plasma GST are indicated in Table 1. There was a statistically significant increase in the erythrocyte MDA levels in patients with ovarian cancer compared to controls. The activities of the erythrocyte antioxidant enzymes SOD, GP_X and plasma GST were significantly increased in group 2 (study subjects) compared to group 1 (controls). The levels of erythrocyte GSH, ascorbic acid, plasma vitamin E and catalase activity were significantly decreased in patients with ovarian cancer compared to controls.

Discussion

The lipid peroxidation product i.e. MDA levels, were significantly increased in the erythrocytes of the patients with ovarian cancer compared to controls in this study. A significant rise in MDA levels in our patients is indicative of elevated oxidative stress. The rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. Similar results were obtained in the work of Nayak SB et al (19) and Kumaraguruparan et al (20) who showed an increase in MDA levels in patients with ovarian cancer. In contrast to our results, Gerber et al (21) and Saintot et al (22) reported diminished MDA levels in patients with ovarian cancer and colorectal cancer (23) and they postulated that MDA is reported to be an unstable intermediate in the peroxidation sequence of unsaturated fatty acids, which may be metabolized further or be transported.

In the present study we observed a significant decrease in the levels of non enzymatic antioxidant parameters, i.e erythrocyte glu-

Table 1 Malondialdehyde (MDA), glutathione, ascorbic acid, vitamin E, super oxide dismutase (SOD), catalase, glutathione peroxidase (GP_X) and glutathione – S – transferase in controls and patients with ovarian cancer

Variables	Control group (n = 38)	Ovarian cancer group (n = 38)	P value
Glutathione (mg/gm of hemoglobin)	18.7 ± 2.7	11.7 ± 2.9	< 0.001
Ascorbic Acid (mg/dl)	4.5 ± 1.3	4.1 ± 1.2	< 0.001
Vitamin E(**µmoles/l**)	7.3 ± 1.4	6.9 ± 1.4	< 0.01
MDA (nmoles/gm of hemoglobin)	5.3 ± 0.3	5.9 ± 0.6	< 0.001
SOD (U/gm of hemoglobin)	645.1 ± 40.9	672.2 ± 57.1	< 0.05
Catalase(U/gm of hemoglobin)	7.2 ± 1.4	6.4 ± 1.3	< 0.01
GP_X (U/gm of hemoglobin)	48.7 ± 1.1	50.3 ± 1.2	< 0.001
GST(µmoles/dl of plasma)	9.2 ± 0.9	13.2 ± 0.6	< 0.001

tathione (GSH), ascorbic acid and plasma vitamin E, in patients with ovarian cancer when compared to controls. Reduced glutathione, a major endogenous antioxidant, plays an important role in antioxidant defense (24). Vitamin C, a major extra cellular non enzymatic antioxidant, has a crucial role in scavenging the ROS. Vitamin E is one of the most important free radical scavenging chain-breaking antioxidant with in the biomembrane (25). The decrease in levels of these non enzymatic antioxidant parameters may be due to increased turnover, to prevent oxidative damage in these patients, suggesting increased defense against oxidant damage in ovarian cancer. Various studies have reported the decreased GSH, Ascorbic acid and Vitamin E levels in patients with ovarian cancer (26). This indicates severe damage to the antioxidant system, which is unable to control the consequences of fighting the oxidative stress.

Enzymatic antioxidants (SOD, CAT and GP_x) form the first line of the antioxidant defense mechanism to protect the organism from ROS mediated oxidative damage (27). In ovarian cancer patients erythrocyte antioxidant enzymes, i.e. SOD & GP_x activities are significantly increased. SOD is an important antioxidant enzyme having an antitoxic effect against super oxide anion. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. GP_X, an oxidative stress inducible enzyme, plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes. The rise in the activity of GP_X could be due to its induction to counter the effect of increased oxidative stress. This is in accordance with the studies of Skrzydelwska et al (28). We observed a significant decrease in the activity of catalase in patients with ovarian cancer compared to controls. Catalase is the enzyme, which protects the cells from the accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase (29).

Glutathione – S – Transferase is a group of multifunctional proteins, which play a central role in detoxification of electrophilic chemicals and the hepatic removal of potentially harmful hydrophobic compounds from the blood (30). We observed a significant increase in the GST activity in patients with ovarian cancer compared to controls. The rise in the activity of GST could be due to its induction to counter the effect of increased oxidative stress. No significant difference in GST activity was observed in the human ovarian cancer tumor cell line and the adriamycin resistant cell line. This indicates that GST does not appear to play a role in drug resistance (31).

To conclude, this study confirmed the increased production of free radicals in ovarian cancer, which supports the existence of oxidative stress in this disease. The higher oxygen free radical production and decreased catalase activity support this finding. The increased activity of antioxidant enzymes is a compensatory activity as a response to increased oxidative stress. Our findings indicate the existence of an abnormal balance between the oxidative and protective mechanisms in these patients. The observations in the present study strongly suggest that treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent oxidative damage.

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