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Impact of therapeutic intervention on oxidants and antioxidants status in patients with ovarian malignancy

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Abstract

The present study was undertaken in patients of ovarian malignancy to evaluate the status of oxidative stress and antioxidant defence mechanisms undergoing chemotherapy treatment. Circulating plasma lipid peroxides as malondialdehyde [MDA] and activities of the defensive enzymes (Superoxide Dismutase [SOD] and Catalase [CAT]) were measured. Blood samples were collected before treatment and within 24 hours and six weeks after polychemotherapy consisting of doxorubicin, cisplatin and cyclophashamide. Material and methods: Newly diagnosed women with ovarian carcinoma (N=30), 30-60 years of age and age- matched clinically healthy women (N=20) were included in the present study. Circulating plasma lipid peroxides were quantified according to the method of Okhawa et al (1979) with modifications as described by Sanacka et al (1996) using thiolbarbuturic acid. Activities of the defensive enzymes SOD and CAT were evaluated by the method of McCord and Fridovin (1969) and Aebi and Suter (1974) respectively. Result: MDA level was found to be markedly elevated at 24 hours after therapy which decreased significantly ($p<0.001$) after six weeks of chemotherapy. CAT and SOD activities were significantly lower at 24 hours which significantly increased ($P<0.001$) after six weeks of polychemotherapy. Conclusion: Fall in MDA and rise in the activities of antioxidants SOD and CAT after six weeks of chemotherapy indicate long term protective and curative effect of above treatment modalities mediated through antioxidant defence processes.

Key word: Plasma Malondialdyhyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), Ovarian Carcinoma, Polychemotherapy.

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Introduction

Ovarian carcinoma often is called the "silent killer" because the disease is not detected until an advanced stage. In India, 15% of all gynaecological cancer is ovarian malignancy [1] and it represents the greatest clinical challenge. Risk factors for ovarian carcinoma includes inflammation excessive number of lifetime ovulations, increased in steroid hormone levels, heredity, infertility, oral contraceptive pills, age, asbestos exposure, and reproductive factors such as null parity [2,3]. Ovarian cancer in its early stage is asymptomatic, but later the main symptoms include abdominal swelling, bloating, pain and pressure symptoms [4]. Ovarian cancer is the leading cause of death from gynaecological cancer. The treatment of ovarian cancer is based on two cornerstones i.e. surgery and chemotherapy. Surgery is crucial in the treatment of ovarian cancer and should be performed by specialists. Optimal debunking surgery significantly increases the response rate to chemotherapy and the progression-free and overall survival [5]. In recent years, increasing experimental and clinical data has provided compelling evidence for involvement of oxidative stress in large number of pathological states including carcinogenesis [6-8]. Of the most active and widely used anticancer drugs, cisplatin, doxorubicin and bleomycin are known to generate free radicals [9]. Free radicals are highly reactive oxygen species (ROS) which can cause extensive tissue damage through reactions with all biological macromolecule, e.g., lipids, proteins and nucleic acids, leading to the formation of oxidized substances such as the membrane lipid peroxidation product-malondialdehyde [10]. Serious side-effects of chemotherapy such as cisplatin-induced nephrotoxicity, doxorubicin-related cardiomyopathy, and bleomycin induced pulmonary damage are, in part, the result of the formation of free radicals [11-13]. On the other hand, the anti-tumor effect of most cytostatic drugs is thought to be caused by

non-oxidative damage or functional impairment of DNA, leading to growth arrest and cell death (14). Under normal circumstances, there is a steady balance between the production of oxygen derived free radicals and their destruction by the cellular antioxidant defence system inside the human body. However, any imbalance between the levels of these oxidants and antioxidants might cause DNA damage and may lead to cancer development. Human body is equipped with certain antioxidants scavenging enzymes such as super dioxide dismutase (SOD) and catalase (CAT) which can counteract the deleterious actions of these ROS and protect against cellular and molecular damage. Disruption of this delicate balance between the free radicals and the antioxidants may cause cellular damage and trigger carcinogenesis. It is believed that oxidative stress plays a major role in the mechanism leading to the underlying side effects of cytotoxic chemotherapy. Patients who underwent several courses of polychemotherapy with free radical generating compounds are likely to have diminished anti-oxidative capacities. This may lead to manifestations of oxidative stress, depending on the drugs used and the different intrinsic biochemical conditions in human cells and the individual's defensive capacity. Present study evaluates the status of oxidative stress and antioxidant defence mechanism in patients of ovarian carcinoma undergoing polychemotherapy.

Materials and Methods

The present study was conducted in the Department of Biochemistry in collaboration with Obstetrics and Gynaecology and Radiotherapy Departments, Chhatrapati Shahuji Maharaj Medical University and Central Drug Research Institute, Lucknow. The study was divided into 2 groups comprising of 30 patients (age: 30-60 years) of ovarian carcinoma as a Study Group and Control Group consisting of 20 female volunteers of similar age group without any evidence of malignancy but having some gynaecological problems. Each patient was subjected to detailed history of present and past illness. All patients included in this study were histologically confirmed for epithelial ovarian malignancy. Both routine and specific investigations were done. Patients with a history of tobacco consumption were excluded from this study. Also women suffering from diabetes mellitus, chronic liver disease, rheumatoid arthritis and any other chronic disease like tuberculosis or concurrent second malignancy were excluded from the present study. Patients on prolonged medication of any kind which could have resulted in discrepancy during estimation of MDA, CAT and SOD were not included in this study. Ethical clearance for this study was obtained from the Institutional Ethics Committee and was in accordance with the Declaration of Helsinki. Blood samples were taken just before the surgery and within 24 hours of the polychemotherapy and also after 6 weeks of the completion of last chemotherapy cycles for biochemical estimations.

Patient were given chemotherapy in the form of doxorubin, cisplatin and cyclophosphamide combination and oxidant and antioxidant status was evaluated after six weeks of complete course of chemotherapeutic medication. Correlations were done between the response to the therapy and status of the response to the therapy to know the status of the oxidant and antioxidants for better therapeutic management in such situations.

Method of free radical and antioxidant measurement

Five ml of venous blood was collected from the control as well study group by disposable plastic syringes previously rinsed with heparin. Samples were transported in ice packed flasks to the Department of Biochemistry for biochemical analysis.

Plasma circulating lipid peroxides in terms of MDA was estimated by the spectrophotometric procedure as described by Okhawa et al. (1978) and modified by Sanacka et al. (1996). Standard absorbance of MDA (2.5 nmol) was used to calculate the amount of lipid peroxides in the samples and results were expressed as nmol/ml.

SOD activity was measured by the method of McCord and Fridovin (1969) and the unit of enzyme activity was defined as the amount of enzyme required to inhibit the optical density at 560 nm of Nitro Blue Tetrazolium (NBT) reduction by 50% in one minute under the assay conditions and results were expressed as units/ml.

CAT activity was determined by the method of Aebi and Suter (1974) and results were expressed as U/ml. One unit of CAT decomposes 1.0 mM of hydrogen peroxide per minute under specified conditions.

Result

Mean MDA levels were found to be significantly elevated before and after 24 hours of Polychemotherapy in ovarian cancer patients as compared to levels seen in controls from the baseline value(control group) and significantly reduced ($p<0.001$) after six week of polychemotherapy Mean CAT and mean SOD activities were found to be significantly lower ($p<0.001$) before and after 24 hours of polychemotherapy in ovarian cancer patients whereas it was markedly elevated ($p <0.001$) after six weeks of Polychemotherapy versus controls (Table 1).

Statistical analysis

The data analysis was carried out by using SPSS (Ver.15.0). The statistical significance of difference between the various groups was determined by using the student 't' test. Results were expressed as Mean $\pm$ SEM. The statistical significance of observed differences is the parameters between the various groups was determined

by the student 't' test, P > 0.05 = Not significant; P < 0.05 Significant; P between 0.05 to 0.001 = Moderately significant and P < 0.001 = Highly significant.

Table 1. *Pre and post chemotherapy MDA levels and CAT, SOD activities in patients (n=30) and controls (n=20).*

	Controls	**Prechemotherapy of patients**	**Within 24 of therapy**	**After 6 week of chemotheray**
MDA	2.12±0.61	5.63±0.26	15.44±2.06	3.42±0.04
CAT	16.02 ±0.35	9.52±0.22	6.17±0.55	12.53±0.84
SOD	10.66±0.14	7.11±0.62	5.97±0.35	8.61±0.49

MDA=nmole/ml CAT=units/mlRBC SOD=units/mlRBC

Table 2. *Comparison of changes in plasma MDA, CAT and SOD activity in carcinoma ovary patients undergoing chemotherapy*

	MDA		**CAT**		**SOD**	
	'*t*'	**'p'**	**'t'**	**'p'**	**'t'**	**'p'**
Pre Vs. within 24 hours	16.36	<0.001	19.59	<0.001	11.03	<0.001
PreVs-6 weeks	13.84	<0.001	12.06	<0-001	10.53	<0.001
Within24 Hours Vs 6 weeks	19.65	<0.001	21.97	<0.001	5.15	<0.001

Discussion

Lipid peroxidation is a one of the most frequently used parameters for assessing the involvement of free radicals in cell damage. Lipid peroxidation is a free radical mediated phenomenon occurring in biological tissue where polyunsaturated fatty acids are generally abundant. Accurate measurement of lipid peroxide products is quite difficult due to their rapid degradation in vitro. The enzymes CAT and SOD catalyze cell defence reactions against the potentially harmful effects of superoxide anion generated by a wide variety of biological process. The disturbance of the pro-/anti-oxidant balance, resulting from increased free radical production, antioxidant enzyme inactivation, and excessive antioxidant consumption, is the causative factor in oxidative damage (15-17). The increase in pre-treatment levels of circulating lipid peroxides of cervical cancer patients in the present study correlates with the decline in SOD and CAT activity, as reported earlier in patients with advanced cancer cervix undergoing neoadjuvant chemoradiation [18]. Significantly increased levels of lipid peroxides with concomitant decrease in antioxidant levels in cancer cervix patients was earlier observed by Manoharan et al. (19) and Mila-Kierzenkowska et al. (20). Several authors have observed similar alterations in pro-/anti-oxidant levels in other cancers as well (21-22). After therapy, the levels of antioxidants were normalized when compared to untreated cervical carcinoma. The present study describes an effect of Polychemotherapy on oxidative status in patients with ovarian carcinoma. It shows how antioxidant defence mechanisms are impaired in ovarian carcinoma. Compared to the control group, the activity of MDA was found to be remarkably higher while the activities of CAT and SOD were significantly declined in patients before therapy and also within 24 hours of therapy. Extensive work has been carried out on the relationship between free radical activities, antioxidants scavenging of free radicals and their relation with chemotherapy in patients of the ovarian carcinoma. We find a significant relationship between chemotherapy and change in the status of oxidant enzymes and lipid peroxide in the blood of ovarian carcinoma patients (Table2). The enzymes CAT and SOD catalyze cell defence reactions against the potentially harmful effects of superoxide anion generated by a wide variety of biological processes. We have found that SOD and CAT were lower in all cancer patients as compared to controls. This showed increased oxidative stress because of raised free radical injury to the tissues. We observed a significant relationship between chemotherapy and changes in the status of oxidant enzymes and lipid peroxides in patients with ovarian carcinoma. There was elevation in levels of MDA within 24 hours of chemotherapy as campared to the controls and pre therapy levels of MDA and low levels of antioxidants within 24 hours of chemotherapy could be responsible for undesirable side effects of chemotherapy which could probably in part be due to generation of reactive oxygen species. There was significant fall in the levels of MDA from the base line to those 6 weeks after chemotherapy. Significant rise from the pre-treatment levels was observed in the activity of antioxidant enzymes after 6 weeks of chemotherapy. Normalization of MDA

and antioxidants enzymes after 6 weeks of chemotherapy indicates efficacy and curative effect of chemotherapy on circulating antioxidants system in human ovarian carcinoma.

The present observations confirm the role of free radicals in ovarian carcinoma and showed that there were marked variations in the status of free radicals and their scavengers following chemotherapy, however, further studies are required to know whether these oxidants and antioxidants depending upon their levels can be used as markers for predicting long-term prognosis in such patients after chemotherapy.

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