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Status of Oxidant and Antioxidant in Patients with Gynaecological Malignancy Undergoing Therapy

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Abstract: *The present study was undertaken in patients of gynaecological malignancy including carcinoma cervix, endometrium and ovarian to evaluate the status of oxidative stress and antioxidant defence mechanisms after radiotherapy, surgery and chemotherapy. 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 surgery, chemotherapy and radiotherapy. Newly diagnosed women with gynaecological carcinoma [N=111], 30-65 years of age and age- matched clinically healthy women [N=50] were included in the present study. Circulating plasma lipid peroxides and activities of the defensive enzymes SOD and CAT were evaluated. Result: MDA level was found to be markedly elevated at 24 hours after therapy which decreased significantly [p<0.001] after six weeks of therapy. CAT and SOD activities were significantly lower at 24 hours which significantly increased [P<0.001] after six weeks of chemotherapy, surgery and radiotherapy. 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*

Keywords*: chemotherapy, radiotherapy, cervical, ovarian, cancer*

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I. Introduction

Gynaecological cancers include malignancies affecting female reproductive system, uterus, cervix, ovary etc. If gynaecologic cancers are discovered at an early stage, surgery can be curative, and other treatments, such as radiotherapy or chemotherapy may be given post-operatively to consolidate treatment. Malignancy or neoplasm literally means "new growth", and neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change. Endometrial carcinoma is the third most common cause of gynecologic cancer death behind ovarian and cervical cancer. 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 [1-3]. Of the most active and widely used anticancer drugs, cisplatin, doxorubicin and bleomycin are known to generate free radicals [4]. 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 [5]. 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 [6-8]. 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 [9]. 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 chemotherapy, surgery and radiotherapy 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 was aimed to develop a baseline data on the status of free radicals and its scavenging enzymes and oxidative stress produced by imbalance between the two in etiological female genital malignancy.

II. Material and Methods

The present study was conducted in the Department of Biochemistry in collaboration with Obstetrics and Gynaecology and Radiotherapy Departments, King George's Medical University .The present study was concluded to assess the free radical induced oxidation stress in females with genital malignancy. The study group (n=112) consisted of 90 cases of cancer cervix, 10 cases of cancer endometrium, 12 cases of cancer ovary. Control group consisting of 50 female volunteers of similar age group without any evidence of malignancy. 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.

Mode of Treatment

Patients with carcinoma cervix stage IA, IB and IIA underwent surgery followed by radiotherapy. Patients with IIB, IIIA and IIIB were given telecoblat therapy (Co-60). The dose given was 5000 cGy in 25 sittings (fractions) as 5 Fraction/week by AP-PA fields to whole pelvis, each fraction being the order of 200 cGy. Patients with endometrial carcinoma underwent surgery. Total abdominal hysterectomy followed by bilateral salpingo-oophrectomy. Patients with ovarian cancer underwent surgery followed by polychemotherapy (Doxorubicin cisplatin and cyclophosphamide (CCF Regime).

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. as described by Sanocka et al. [10]. 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 Fridovich [11] 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 [12] and results were expressed as U/ml. One unit of CAT decomposes 1.0 mM of hydrogen peroxide per minute under specified conditions.

III. 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 + 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 between0.05 to 0.001 = moderately significant and $P < 0.001$ = highly significant.

IV. Results.

Present study was conducted to assess the status of MDA, SOD and CAT in patients with carcinoma cervical, ovarian and endometrial undergoing therapy. We found in **(Tab No. 1**) that MDA levels within 24 hours of chemo-radiotherapy was significantly high ($p<0.001$) compared from the pre treatment levels in patients with cervical cancer .There was significant fall ($p<0.001$) in the levels of antioxidant enzymes CAT and SOD from the pre-treatment levels levels and after six week of therapy there was significant fall in MDA and increases in SOD and CAT .In ovarian cancer (**Table. No.2)** significant rise in MDA levels was observed with 24 hours of chemotherapy. There was significant fall in the levels of antioxidant enzymes SOD and CAT. Significant fall in MDA level was observed after 6 weeks of Polychemotherapy. Similarly there was significant rise in levels of antioxidant enzymes after 6 weeks of chemotherapy. In patients with endometrial cancer **(Table No. 3)** there was Significant rise ($p<0.001$) in MDA level was observed within 24 hours of surgery from the baseline values in cancer endometrial. Similarly significant fall was observed in the mean CAT and SOD activity from the baseline or pre-treatment levels Significant fall in the mean MDA level was observed after 6

weeks of therapy and there was significant rise in the levels of antioxidant enzymes units/ml after 6 weeks of surgery. High levels of MDA within 24 hours of radiotherapy, chemotherapy and surgery indicates increased oxidative stress due to free radical generation which may be responsible for undesirable side effects of radiotherapy surgery and anticancer drugs. Fall in MDA levels and rise in levels of antioxidant enzymes after 2-6 weeks of treatment (surgery, radiotherapy, and chemotherapy) indicates long term protective and curative effects of above treatment modalities. This study gives a convincing support in favour of etiological role of free radical injury in genital cancer. The imbalance between oxidants (Malandialdehyde) and antioxidants (catalase and superoxide dismutase) leads to oxidative stress which may be responsible for number of side effects of radio chemotherapy and surgery

Table No .1. Status of MDA, SOD and CAT in patients with cervical cancer undergoing chemo-radiotherapy

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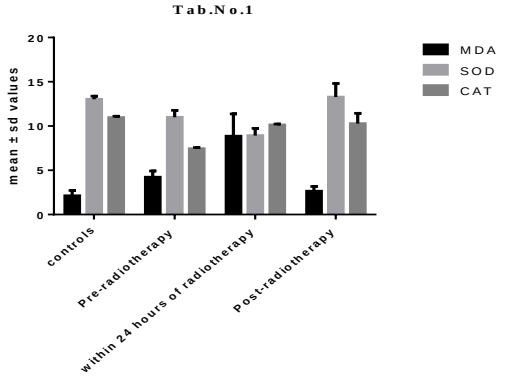
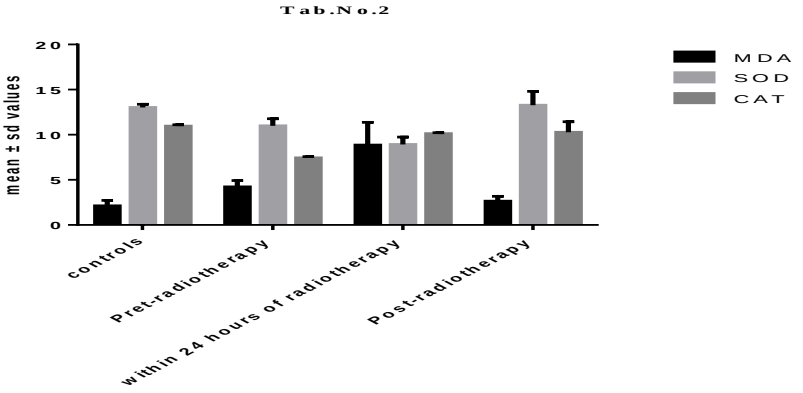


Status of MDA,SOD and CAT in cancer cervix pateints and control

Table no 2 Status of MDA, CAT and SOD levels in patients with ovarian cancer undergoing chemotherapy



Status of MDA,SOD and CAT in ovarian cancer pateints and controls

Table No 3. Status of MDA, CAT and SOD in patients with endometrial cancer after sugary

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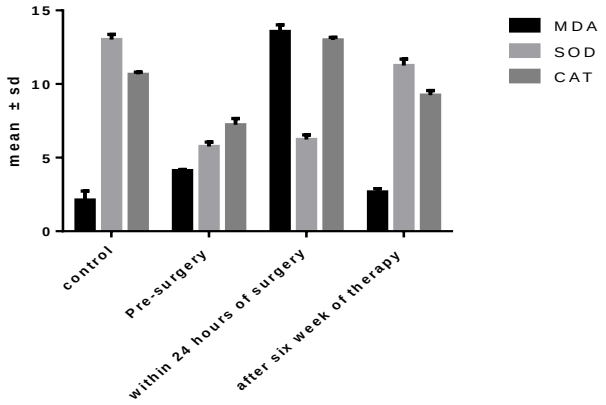
Tab.No.3



Status of MDA , SOD and CAT in Endometrium cancer pateints and control

V. 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 [13-15]. 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 [16]. Significantly increased levels of lipid peroxides with concomitant decrease in antioxidant levels in cancer cervix patients were earlier observed **[**17, 18]. Several authors have observed similar alterations in pro-/anti-oxidant levels in other cancers as well [19-23]. 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 therapy and change in the status of oxidant enzymes and lipid peroxide in the blood of cervix, ovarian and endometrium carcinoma patients 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 therapy 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 therapy as compared to the controls and pre therapy levels of MDA and low levels of antioxidants within 24 hours of therapy could be responsible for undesirable side effects of therapy 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 therapy. Normalization of MDA and antioxidants enzymes after 6 weeks of therapy 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 and radiotherapy.

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