Implication of Therapeutic Intervention on Putative Oxidative stress markers in Cervical Cancer

Suman Gautam[1], M.L.B Bhatt [3,] Seema Mehrotra[2], A.A Mahdi[1], Ranjana Singh[1], komal S Pasi[3] , B Anjnum [1]*

[1]Department of Biochemistry, King George's Medical University, Lucknow, India.
[2]Department of Obstetrics & Gynaecology, King George's Medical University, Lucknow, India.
[3]Department of Radiotherapy, King George's Medical University, Lucknow India
*Corresponding author: *Suman Gautam*

Abstract

Background: *The present study was undertaken in patients of carcinoma cervix to evaluate the Implication of chemo radiotherapy on status of oxidative stress biomarker such as protein carbonyl, lipid hydro peroxide and antioxidants defence mechanism of melatonin and total thiol levels.* ***Methodology***: *Patients were delivered radiotherapy by external beam radiotherapy (EBRT) followed by brachytherapy. All patients were given chemotherapy in the form of injection cisplatin. Blood samples were collected from patients as well as control before treatment and within 24 hours and six weeks after chemo radiotherapy. Newly diagnosed women with cervical cancer [N=192], 30-65 years of age and age- matched clinically healthy women [N=192] were included in this study.*
Result &conclusion: *The mean LOOH and PC levels in all three groups of cases were comparatively higher than controls. Further, the mean LOOH and PC levels increased in cases after the chemo-radiotherapy as compared to pre chemo-radiotherapy., P<0.001) .Study also found that the mean Melatonin level and total thiol level in all three groups of cases lowered comparatively than controls. Further, the mean Melatonin and total thiol in cases decreased after the chemo-radiotherapy as compared to pre chemo-radiotherapy. We suggest that plasma PC, LOOH, PC, Melatonin and T-SH may serve as biomarkers for oxidative stress in patients with gynecological malignancy. A highly structured study with a larger sample size is required to establish the precise role of oxidative stress in pathobiology of cancer. Such oxidative biomarker can be used for diagnosis and prognosis of diseases in future.*
Keywords: *chemotherapy, oxidative stress, radiotherapy, cervical cancer*

I. Introduction

Cancer is the leading cause of death worldwide. Despite the significant research effort, underlying mechanisms of carcinogenic processes are still poorly understood. In recent decades, a group of extremely reactive oxygen metabolites, reactive oxygen species (ROS), have been linked closely to carcinogenesis. Levels of ROS are constantly controlled by antioxidants to ensure stable redox balance in our cells. An aberrant cellular redox balance is thought to be connected to carcinogenesis by inflicting damage to cellular macromolecules and disturbing normal cellular signalling. Reactive oxygen species (ROS) are a group of highly reactive oxygen metabolites that are constantly produced in our cells to meet the needs of several crucial physiological processes such as cellular signalling, immune responses, hypoxia adaptation, aging and wound healing [1]. Endogenous sources such as the mitochondrial electron transport chain and specific ROS-producing enzymes are the main origin of cellular ROS, although some exogenous sources such as ionizing radiation and various xenobiotics can contribute significantly to the total cellular ROS levels [1-2]. Modern research has linked the aberrant cellular balance between oxidizing and reducing agents to the pathogenesis of several diseases such as Alzheimer's disease, Parkinson's disease, cardiovascular diseases and cancer [3, 1]. Interest in ROS has been increasing significantly, especially in the fields of cancer research, since it would seem that ROS-mediated cellular damage and abnormal signalling contribute to the initiation, promotion and progression of the carcinogenic process [4]. Gynaecological cancers represent a great clinical challenge in oncology. Since most cases are asymptomatic until the disease has metastasized, two-third is diagnosed with advanced stage. Hence, most of gynaecological cancers have the highest fatality-to-case ratio of all women malignancies [5]. Cervical cancer represent the third most commonly diagnosed cancer and rank the first leading cause of cancer death in female in the developing countries [6]. Squamous cell carcinoma and adenocarcinoma are most epidemic and account for 70% and 20% of cervical cancer cases respectively [7]. Cervical cancer may occur at any age, but increasing incidence is seen in young women and often is associated with serious consequences. Present study has been designed to study the changes in the status of oxidants and antioxidant in patients with cervical cancer undergoing chemo-

radiotherapy. Six month following the completion of complete treatment patient will be again evaluated for the status of oxidant and antioxidants. Correlations will be done between the response to the therapy and status of the oxidant and antioxidant level of the patient and variation with the same following therapy. We consider that the developing and improved therapies targeted towards the redox balance and it's identify extensive studies on the consequences of enhanced oxidative stress at biochemical levels to identify the corresponding particularities of broad panel of gynaecological cancers. Oxidative stress associated biomarkers in health and disease, early indication of disease onset/progression and of the response to a particular therapy. This study may reinforce the toxicological and pharmaceutical armentarium for assessing the toxicity of xenobiotics, of convention and innovative therapeutics.

II. Material and Method

Sample collection:
The present study was conducted in the Department of Biochemistry in collaboration with Departments of Obstetrics and Gynecology and Radiotherapy, King George's Medical University and Central Drug Research Institute (CDRI) Lucknow .The present study was conducted to assess the free radical induced oxidation stress in females with cervical cancer undergoing chemradiotherapy .The study group consisted of total 192 cases of cervical cancer and control group also consisting of 192 female volunteers of similar age group (30-75) without any evidence of malignancy. 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 protein carbonyl (PC), total lipid hydrperoxide (LOOH) and total thiol (T-SH) and Melatonin 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 from patients and control following an overnight fasting into EDTA vials to avoid the probable influence of nutritional factors on the ROS level. The plasma was separated by centrifugation at 3000 rpm for 15 minutes.

Protein Carbonyl Estimation:
Plasma protein carbonyl (PC) content was measured by spectrophotometric detection of protein hydrazone formed with 2, 4-dinitropa phenyl hydrazine (DNPH) [8]. The results are expressed as n moles of PC per milligram of protein by using molar extinction coefficient $\varepsilon_{370}=22\,000$ M-1cm-1 at370 nm. The protein content was determined by the Lowry et al.using bovine serum albumin as a standard [9].

Lipid hydroperoxide estimation:
Total hydroperoxide (LOOH) concentration was determined using the FOX-2 method with minor modifications. The FOX-2 test system is based on the oxidation of ferrous iron to ferric iron by the various types of peroxides present in the plasma samples, in the presence of xylenol orange which produces a coloured ferric-xylenol orange complex whose absorbance can be measured at 560 nm wavelength [10]. Using a solution of H_2O_2 as standard. The coefficient of variation for individual plasma samples was less than 5%.

Total Thiol Estimation:
Total thiols in plasma were measured by the method of Hu [11]. To 0.05 ml plasma, 0.6 ml of 0.25 M tris buffer containing 0.02 M EDTA, pH 8.2 was added followed by addition of 0.04 ml of 0.01 M 2,2-dithiobisnitrobenzoic acid (DTNB) in absolute methanol and 3.31 ml of absolute methanol. The tubes were capped and color was developed for 15 min at room temperature. The tubes were centrifuged at 3,000 x g for 20 min. Supernatant was collected and absorbance measured at 412 nm. Total thiol groups were calculated using molar extinction coefficient of 13,600 at 412 nm.

Urinary Melatonin :
Urinary melatonin was assayed as melatonin sulfate (6-Sulfatoxymelatonin) according to manufacturer's procedure.Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle.

Statistical analysis
Data were summarized as Mean ± SD. Groups were compared by one way analysis of variance (ANOVA) and the significance of mean difference between the groups was done by Tukey's post hoc test. Groups were also compared by independent Student's t test. A two-sided (α=2) P value less than 0.05 (P<0.05) was considered statistically significant. Analyses were performed on SPSS software (PSAW, Windows version 18.0).

III. Results

Level of protein carbonyl (PC) in patients with cervical cancer undergoing chemo-radiotherapy:

The mean ± SD of PC level in controls, cases at pre chemo-radiotherapy, within 24 hour of chemo-radiotherapy and post chemo-radiotherapy were 0.80 ± 0.39 nmole/mg, 1.47 ± 0.54 nmole/mg, 1.84 ± 0.38 nmole/mg and 1.63 ± 0.48 nmole/mg.(Fig.No.1) The mean PC level in all three groups of cases (pre chemo-radiotherapy, within 24 hour of chemo-radiotherapy and post chemo-radiotherapy) was comparatively higher than controls. Further, the mean PC level in cases increased after the chemo-radiotherapy as compared to pre chemo-radiotherapy. Comparing the PC level of four groups (controls, pre chemo-radiotherapy, within 24 hour of chemo-radiotherapy and post chemo-radiotherapy), ANOVA revealed significantly different PC level among the groups (F=189.90, p<0.001) (Table 1).
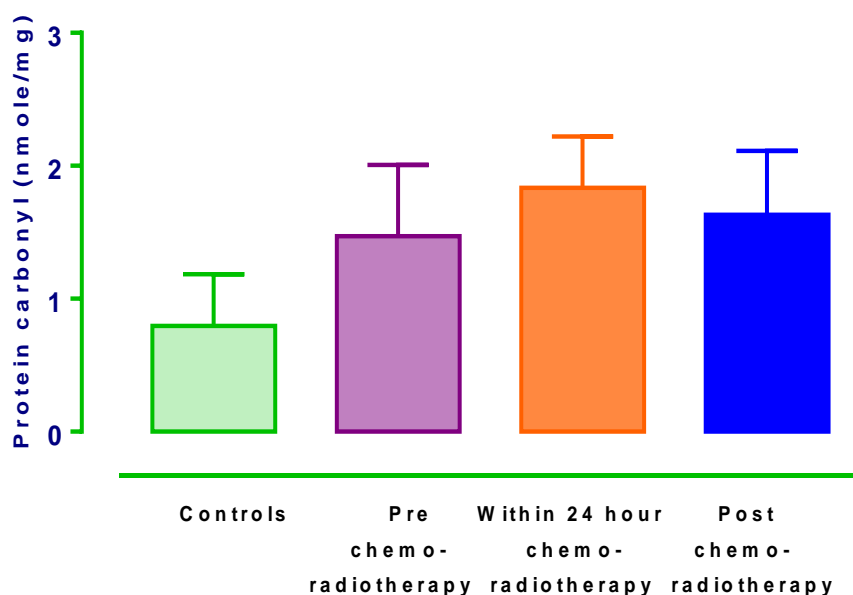
Figure.No.1

Table 1: Comparison of mean difference in Protein carbonyl level between the groups by Tukey post hoc test

Comparisons of PC between the groups	Mean Diff.	Q	P value	95% CI of diff.
Control vs. Pre chemo-radiotherapy	0.67	20.59	P<0.001	-0.793 to -0.553
Control vs. Within 24 hour of chemo-radiotherapy	1.04	31.80	P<0.001	-1.159 to -0.919
Control vs. Post chemo-radiotherapy	0.84	25.59	P<0.001	-0.956 to -0.716
Pre chemo-radiotherapy vs. Within 24 hour of chemo-radiotherapy	0.37	11.21	P<0.001	-0.486 to -0.246
Pre chemo-radiotherapy vs. Post chemo-radiotherapy	0.16	5.01	P < 0.01	-0.283 to -0.043
Within 24 hour of chemo-radiotherapy vs. Post chemo-radiotherapy	0.20	6.21	P<0.001	0.083 to 0.323

Level of LOOH in patients with cervical cancer undergoing chemo-radiotherapy:

The Lipid hydroperoxide (LOOH) level of controls and pre and post cervical cancer patients are summarized in Table 5. The mean (± SD) LOOH level of controls, cases at pre chemo-radiotherapy, within 24 hour of chemo-radiotherapy and post chemo-radiotherapy were 0.36 ± 0.17 mmol/l, 0.84 ± 0.33 mmol/l, 1.04 ± 0.48 mmol/l and 0.88 ± 0.37 mmol/l.(Fig.No.2) The mean LOOH level in all three groups of cases was comparatively higher than controls.

Further, the mean LOOH level in cases increased after the chemo-radiotherapy as compared to pre chemo-radiotherapy. Comparing the LOOH level of four groups, ANOVA revealed significantly different LOOH level among the groups (F=129.50, p<0.001) (Table 5). Further, comparing the mean LOOH level between the groups (Table 6), Tukey test revealed significantly different and higher LOOH level in all three groups of cases as compared to controls.
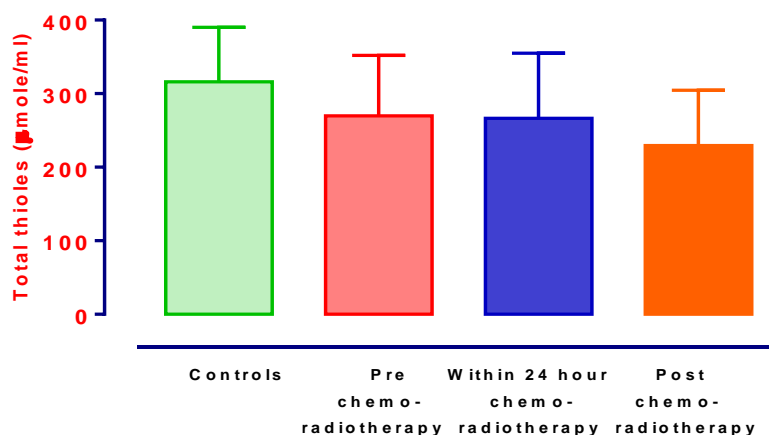
Figure.No.2:
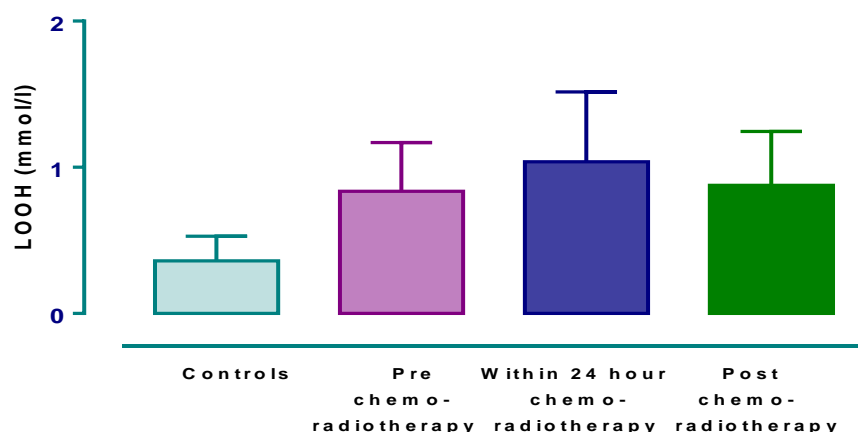
Comparisons	Mean Diff.	Q	P value	95% CI of diff.
Control vs. Pre chemoradiotherapy	0.47	18.54	P < 0.001	-0.569 to -0.381
Control vs. Within 24 hour of chemoradiotherapy	0.68	26.46	P < 0.001	-0.772 to -0.583
Control vs. Post chemoradiotherapy	0.52	20.13	P < 0.001	-0.610 to -0.421
Pre chemoradiotherapy vs. Within 24 hour of chemoradiotherapy	0.20	7.92	P < 0.001	-0.297 to -0.109
Pre chemoradiotherapy vs. Post chemoradiotherapy	0.04	1.59	P > 0.05	-0.135 to 0.0534
Within 24 hour of chemoradiotherapy vs. Post chemoradiotherapy	0.16	6.33	P < 0.001	0.068 to 0.256

Table 2: Comparison of mean difference in Lipid hydroperoxide level between the groups by Tukey post hoc test

Level of T-SH in patients with cervical cancer undergoing chemo-radiotherapy

The Total thioles (T-SH) level of controls and pre and post cervical cancer patients are summarized in Table 7. The mean (± SD) T-SH level of controls, cases at pre chemo-radiotherapy, within 24 hour of chemo-radiotherapy and post chemo-radiotherapy were 316.18 ± 74.09 µmole/ml, 269.66 ± 82.48 µmole/ml, 266.62 ± 88.32 µmole/ml and 229.31 ± 75.22 µmole/ml(Fig.No.3). The mean T-SH level in all three groups of cases lowered comparatively than controls. Further, the mean T-SH level in cases also decreased after the chemo-radiotherapy as compared to pre chemo-radiotherapy. Comparing the T-SH level of four groups, ANOVA revealed significantly different T-SH level among the groups (F=37.77, p<0.001) (Table 7). Further, comparing the mean T-SH level between the groups (Table 8), Tukey test revealed significantly different and lower T-SH level in all three groups of cases as compared to controls.
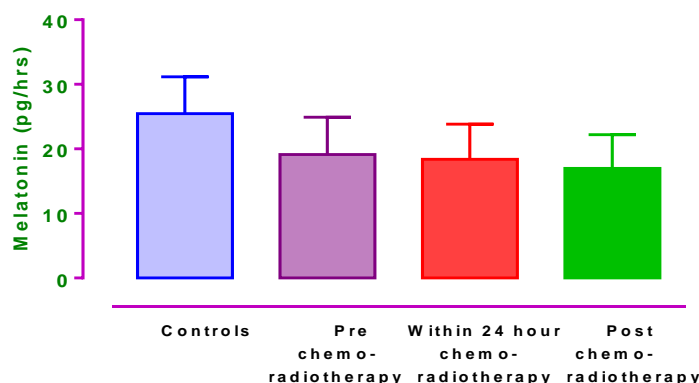
Figure.No: 3.

Comparisons	Mean Diff.	Q	P value	95% CI of diff.
Control vs. Pre chemoradiotherapy	46.52	8.03	P < 0.001	25.25 to 67.79
Control vs. Within 24 hour of chemoradiotherapy	49.56	8.56	P < 0.001	28.29 to 70.83
Control vs. Post chemoradiotherapy	86.87	15.00	P < 0.001	65.60 to 108.1
Pre chemoradiotherapy vs. Within 24 hour of chemoradiotherapy	3.04	0.52	P > 0.05	-18.23 to 24.31
Pre chemoradiotherapy vs. Post chemoradiotherapy	40.35	6.97	P < 0.001	19.08 to 61.62
Within 24 hour of chemoradiotherapy vs. Post chemoradiotherapy	37.32	6.44	P < 0.001	16.04 to 58.59

Table 3: Comparison of mean difference in Total thioles level between the groups by Tukey post hoc test

Level of melatonin in patients with cervical cancer undergoing chemo-radiotherapy

The Melatonin level of controls and pre and post cervical cancer patients are summarized in Table 9. The mean (± SD) Melatonin level of controls, cases at pre chemo-radiotherapy, within 24 hour of chemo-radiotherapy and post chemo-radiotherapy were 25.47 ± 5.70 µg/hrs, 19.12 ± 5.78 µg/hrs, 18.42 ± 5.43 µg/hrs and 16.99 ± 5.20 µg/hrs.(Fig.No.4) The mean Melatonin level in all three groups of cases lowered comparatively than controls. Further, the mean Melatonin level in cases decreased after the chemo-radiotherapy as compared to pre chemo-radiotherapy. Comparing the Melatonin level of four groups, ANOVA revealed significantly different TH level among the groups (F=88.44, p<0.001) (Table 9). Further, comparing the mean Melatonin level between the groups (Table 10), Tukey test revealed significantly different and lowered Melatonin level in all three groups of cases as compared to controls.

Figure No.4:

Comparisons	Mean Diff.	Q	P value	95% CI of diff.
Control vs. Pre chemoradiotherapy	6.35	15.89	P < 0.001	4.881 to 7.817
Control vs. Within 24 hour of chemoradiotherapy	7.09	17.74	P < 0.001	5.621 to 8.557
Control vs. Post chemoradiotherapy	8.48	21.22	P < 0.001	7.014 to 9.950
Pre chemoradiotherapy vs. Within 24 hour of chemoradiotherapy	0.74	1.85	P > 0.05	-0.729 to 2.208
Pre chemoradiotherapy vs. Post chemoradiotherapy	2.13	5.34	P < 0.01	0.664 to 3.601
Within 24 hour of chemoradiotherapy vs. Post chemoradiotherapy	1.39	3.49	P > 0.05	-0.075 to 2.861

Table 4: Comparison of mean difference in Melatonin level between the groups by Tukey post hoc test

IV. Discussion

Data from this investigation revealed that a significant relationship between the treatment response and the changes in antioxidants level and level of oxidants markers in patient with cervical cancer .There was significant changes found in the level of Protein carbonyl in cervical cancer patient undergoing chemo radiotherapy. Protein carbonyl is a product of irreversible non-enzymatic oxidation or carbonylation of proteins and indicators of free radical generation in cell [12].Carbonylation of proteins often leads to a loss of protein

function, which is considered a widespread marker of severe oxidative stress damage and disease-derived protein dysfunction [8].The usage of protein carbonyl groups as biomarker of oxidative stress has some advantages in comparison with measurement of other products because of relatively early formation and the relatively stability of carbonylated protein. When we study the level of protein carbonyl under chemo radiotherapy we found that the mean PC level in all three groups of cases (pre chemo-radiotherapy, within 24 hour of chemo-radiotherapy and post chemo-radiotherapy) was comparatively higher than controls. Further, the mean PC level in cases increased after the chemo-radiotherapy as compared to pre chemo-radiotherapy. Comparing the PC level of four groups (controls, pre chemo-radiotherapy, within 24 hour of chemo-radiotherapy and post chemo-radiotherapy) we found significantly different PC level among the groups. Moreover, the mean PC level in cases decrease significantly (P<0.001) at post chemo-radiotherapy as compared to at within 24 hour of chemo-radiotherapy. Results of our study also showed that plasma level of LOOH level was significantly high in treatment groups as compared to the healthy volunteers. At the time of diagnosis, the mean plasma value was significantly higher in patients Evidences from previous studies showed that there was a remarkable high lipid peroxidation occurs in various pathologies including cancers [13-14]. The level of LOOH was high in breast cancer, gynecological malignancies and oral cavity cancer [15-16]. Previous studies on breast cancer have shown increased level of LOOH in plasma as compared to controls [13].

Lipid peroxidation has been linked to a variety of disorders, including atherogenesis, ischemia–reperfusion injury, and carcinogenesis [17].Lipid hydroperoxide (LOOH) level of controls and pre and post cervical cancer patients. The mean LOOH level in all three groups of cases was comparatively higher than controls. Further, the mean LOOH level in cases increased after the chemo-radiotherapy as compared to pre chemo-radiotherapy. Comparing the LOOH level of four groups found significantly different LOOH level among the groups. Further, comparing the mean LOOH level between the groups and found significantly different and higher LOOH level in all three groups of cases as compared to controls .Furthermore, the mean LOOH level in cases increased significantly at within 24 hour of chemo-radiotherapy as compared to pre chemo-radiotherapy. However, in cases, the mean LOOH level not differed between pre chemo-radiotherapy and post chemo-radiotherapy i.e. found to be statistically the same. Moreover, the mean LOOH level in cases decreases significantly at post chemo-radiotherapy as compared to within 24 hour of chemo-radiotherapy. The presented result may indicate a visible effect on oxidants and antioxidants level changes in patients with cervical cancer treated with chemo radiotherapy. It is possible that in our study, an enormous production of free radical after radiation occur in blood plasma of patients Total T-SH play a prominent role in antioxidant defense system, and also in reactions of catalysis, regulation, electron transport and those preserving the correct structure of proteins [18-19] .It was reported that T-SH level can be used to evaluate excess free radicals generation in both physiological and pathological conditions [20, 19]. In our study we found that the mean T-SH level in all three groups of cases lowered comparatively than controls. Further, the mean TH level in cases also decreased after the chemo-radiotherapy as compared to pre chemo-radiotherapy. Comparing the T-SH level of four groups founds significantly different T-SH level among the groups. Further, comparing the mean T-SH level between the groups revealed significantly different and lower T-SH level in all three groups of cases as compared to controls. Furthermore, the mean T-SH level in cases decreased significantly at post chemo-radiotherapy as compared to pre chemo-radiotherapy. However, in cases, the mean T-SH level not differed between pre chemo-radiotherapy and within 24 hour chemo-radiotherapy i.e. found to be statistically the same. Moreover, the mean T-SH activities in cases decrease significantly at post chemo-radiotherapy as compared to within 24 hour of chemo-radiotherapy.

We also found that urinary melatonin level in patients with cervical cancer undergoing chemo-radiotherapy changes significantly. There is some evidence that any external or internal insult that initiates free radical generation in an organism may lead to a reduction in circulating melatonin levels [21-22]. The ability of melatonin to scavenge free radicals is undoubtedly an important property in its protection against oxidative stress. In our study we found that the mean Melatonin level in all three groups of cases lowered comparatively than controls. Further, the mean Melatonin level in cases decreased after the chemo-radiotherapy as compared to pre chemo-radiotherapy. Comparing the Melatonin level of four groups, revealed significantly different level among the groups. Further, comparing the mean Melatonin level between the groups revealed significantly different and lowered Melatonin level in all three groups of cases as compared to controls. Furthermore, the mean Melatonin level in cases decreased significantly at post chemo-radiotherapy as compared to pre chemo-radiotherapy. However, in cases, the mean Melatonin level not differed between pre chemo-radiotherapy and within 24 hour chemo radiotherapy, and within 24 hour chemo-radiotherapy and post chemo-radiotherapy i.e. found to be statistically the same.

V. Conclusions

Oxidative stress is closely related to all aspects of cancer, from carcinogenesis to the tumor-bearing state, from treatment to prevention. Oxidative stress is also involved in the problem of resistance to these

treatments. The efficacy of antioxidants in the prevention of carcinogenesis is currently under investigation. Issues to be addressed in the future include the establishment of easy, accurate methods of measurement and evaluation of the extent of oxidative stress in the body as well as the clinical application of experimentally obtained knowledge to the prevention and treatment of cancer. To date, data concerning the induction of the stress response by chemotherapeutic agents have not been successful in providing conclusions on clinical value, mainly because of the reports derived from inconsistent experimental protocols. Until now, there has been a decided lack of evidence not only with regard to the clinical efficacy of antioxidants, but also the diminishing effect against anticancer therapy. In particular, very little information is currently available regarding oxidative stress and the efficacy of antioxidant intervention from a radiotherapy perspective. Therefore, further investigations into the clinical significance of oxidative stress will be necessary to clearly assess the feasibility of the clinical use of antioxidative intervention. Based on these results, further investigations containing interventional and longitudinal studies will be required to assess the effects of the changes in oxidative stress markers on radiotherapy and chemotherapy -related adverse events. Also, the clinical application of these findings has to be considered to minimize radiotherapy-related adverse events and to improve Oxidative stress to (quality of life) QoL in cancer patients.

References

[1]. Brieger K,Schiavone S,Miller FJ Jr & Krause KH Reactive oxygen species:frm health to disease. Swis Med Wkl .2012; 142:13659.
[2]. Nathan C & Cunningham-Bussel A.Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nat Rev Immunol.2013; 13:359-361.
[3]. Hekimi S,Lapointe J&Wen Y Taking a "good " look at free radicals in the aging process. Trends cell Biol. 2011; 21:569-576
[4]. Fiaschi T&Chiarugi P Oxidative stress.tumor microenvironment and metabolic reprogramming: a diabolic liaison.Int J Cell Biol, 2012;762-825.
[5]. Holschneider CH,Berek JS ovarian cancer :epidemiology, biology and prognostic factors. Semin Surg Oncol. 2002;19:3-10.
[6]. Jemal A ,Bray F,Center MM,Ferlay J,Ward E & Forman D Global cancer statistics.CA cancer J Clin . 2011;61:69-90.
[7]. Silverberg SG & Ioffe OD.Pathology of cervical cancer,J 2003;9(5),335-47.
[8]. Levine RL, Garland D, Oliver CN, Amici A, Climment I, Lenz AG, Ahn BW, Shaltiel S,Stadtman ER . Determination of carbonyl content of oxidatively modified proteins.Methods Enzymol. 1990;186:464-47
[9]. Lowry, OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagents. J Biol Chem. 1951; 193:265-275.
[10]. Wolf SP. Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxide. Methods enzymmol. 1994; 233:182-189.
[11]. Hu ML. Measurement of protein thiol group and glutathione in plasma. Methods Enzymol 1994; 233:381-5.
[12]. Kharb S,Singh V, Ghalaut PS,Sharma A,Singh GP.Glutathione levels in health and sickness. Ind J Med Sci.2002;54:52-4.
[13]. Kumaragurupan R,Subapriya R,Kabalimoorthy J et al.Antioxidant profile in circulation of patients with fibroadenoma and adenocarcinoma of breast .Clin Biochem. 2002;35:275-279.
[14]. Giacomo CD,Acquaviva R,Lanteri R,et al.Nonproteic antioxidant status in plasma of subjects with colon cancer.Exp Biol Med.2003;228:525-528.
[15]. Beevi SS,Rasheed AM,Geetha A.Evalution of oxidative stress and nitric oxide levels in patients with oral cavity cancer.Jpn J Clin Oncol.2004;347;379-385.
[16]. Sener DE ,Genonc A,Akmer M & Torun M.Lipid Peroxidation and total antioxidant status in patients with breast cancer.Cell Biochem.Funct.2007,25:377-382.
[17]. Wulf Droge Free radicals in the physiological control of cell function.Physiol Rev.,2002 82,pp 47-95
[18]. Khoory R, Stemme D.Plasma Melatonin levels in plasma patients suffering from colectal carcinoma.J Pineal Res 1998:251-258.
[19]. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine, 2nd ed. Oxford, Oxford University Press, 1989.
[20]. Pang SF,Allen AE.Extra – pineal melatonin in the retina :Its regulation and Physiological Function.In :Reiter RJ,ED.Pineal Research Review.New York:Alan R.Lies, 1986; Vol 4:pp55-95,.
[21]. Wolf SP. Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxide. Methods enzymmol. 1994; 233:182-189.
[22]. Vaughan GM, Taylor TJ. Pruitt BAJ,Mason ADJ.Pineal function in burns:Melatonin is nat a marker for general sympathetic activity.J Pineal Res. 1985; 2:1-12.