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

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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-

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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 hydroperoxide (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-dinitrophenyl hydrazine (DNPH) [8]. The results are expressed as n moles of PC per milligram of protein by using molar extinction coefficient ε370=22 000 M⁻¹cm⁻¹ at 370 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 xylene orange which produces a coloured ferric xylene orange complex whose absorbance can be measured at 560 nm wavelength [10]. Using a solution of H2O2 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).
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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](image)

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

<table>
<thead>
<tr>
<th>Comparisons of PC between the groups</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>P value</th>
<th>95% CI of diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs. Pre chemo-radiotherapy</td>
<td>0.67</td>
<td>0.20</td>
<td>P&lt;0.001</td>
<td>-0.793 to -0.553</td>
</tr>
<tr>
<td>Control vs. Within 24 hour of chemo-radiotherapy</td>
<td>1.04</td>
<td>3.18</td>
<td>P&lt;0.001</td>
<td>-1.159 to -0.919</td>
</tr>
<tr>
<td>Control vs. Post chemo-radiotherapy</td>
<td>0.84</td>
<td>2.55</td>
<td>P&lt;0.001</td>
<td>-0.956 to -0.716</td>
</tr>
<tr>
<td>Pre chemo-radiotherapy vs. Within 24 hour of chemo-radiotherapy</td>
<td>0.37</td>
<td>1.12</td>
<td>P&lt;0.001</td>
<td>-0.486 to -0.246</td>
</tr>
<tr>
<td>Pre chemo-radiotherapy vs. Post chemo-radiotherapy</td>
<td>0.16</td>
<td>0.51</td>
<td>P&lt;0.01</td>
<td>-0.283 to -0.043</td>
</tr>
<tr>
<td>Within 24 hour of chemo-radiotherapy vs. Post chemo-radiotherapy</td>
<td>0.20</td>
<td>0.62</td>
<td>P&lt;0.001</td>
<td>0.083 to 0.323</td>
</tr>
</tbody>
</table>

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.

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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 0.0334 |
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.
**Table 3:** Comparison of mean difference in Total thioles level between the groups by Tukey post hoc test

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

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

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

**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 found 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 in cancer.Cell, ile in circulation of patients with fibroadenoma and -ineal function in burns:Melatonin is nat a marker for general sympathetic ticular, 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.

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