Glutathione level and its relation to radiation therapy in patients with cancer of uterine cervix

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Glutathione functions as an important antioxidant in the destruction of hydrogen peroxide and lipid peroxides by providing substrate for the glutathione peroxidase and also promotes the ascorbic acid. Glutathione plays a vital role in detoxification of xenobiotics, carcinogens, free radicals and maintenance of immune functions. The study was aimed to determine plasma glutathione as well as erythrocyte glutathione and glutathione peroxidase in patients with invasive cervical carcinoma (n=30) before initiation and after completion of radiotherapy and subsequently, at the time of first three monthly follow-up visit. The levels of plasma glutathione, erythrocyte glutathione and glutathione peroxidase activity were found to be lower in all cervical cancer patients as compared to age matched normal control women. The study indicates a change in antioxidant status in relation with the glutathione system among patients with invasive carcinoma of the uterine cervix. This study also demonstrates the effect of radiation therapy on this antioxidant system.

Cancer of the uterine cervix is the leading malignancy affecting women in developing countries and is the commonest malignancy among Indian women. Although a number of risk factors have been identified like early sexual activity (before 18 years of age), multiple sexual partners, cigarette smoking and low socio-economic status, the aetiology of cervical cancer is not yet clearly understood. Numerous clinicopathological studies have implicated venereally transmitted agents, viz. HSV-2 and, more recently, HPV (Human papilloma virus, mainly types 16 and 18) in the genesis of cervical neoplasm.

Cancer of the uterine cervix does not arise de novo, but are preceded by a spectrum of abnormal epithelial changes known as dysplasia which is the important precancerous lesion. Several studies have also suggested the role of antioxidant elements in diet in prevention of cervical cancer. Diet deficient in vitamin A, vitamin C (ascorbic acid) and folic acid appears to play a role in the aetiology of this disease. Peroxidation of lipids exposed to oxygen is responsible for damage to tissues in vivo and has been implicated as a probable cause of cancer. To control and reduce lipid peroxidation, nature invokes the use of antioxidants. Glutathione (GSH) functions as an important antioxidant in the destruction of hydrogen peroxide and lipid peroxides by providing substrate for the glutathione peroxidases (GPX). Glutathione also functions as an antioxidant by promoting formation of the reduced forms of other antioxidants such as ascorbic acid and detoxification of xenobiotics, carcinogens and free radicals and maintenance of immune functions.

Radiation therapy is the most commonly used therapeutic modality for inoperable cervical cancers. The effects of radiation therapy are mediated by the production of free radicals. Since glutathione is the most important cellular antioxidant and a quencher of free radicals, it can be postulated that those cell systems containing a high ratio of glutathione, as reflected by the plasma and erythrocyte glutathione levels, may be found to be relatively radioresistant. Studies on blood GSH in several malignancies have reported an altered GSH redox system. Bhuvarahamurthy et al. studied the effect of radiotherapy and chemoradiotherapy on circulating antioxidant system
of human uterine cervical cancer and observed that reduced levels of GSH and GPX become normalized after treatment.

The present study was aimed to determine plasma GSH, erythrocyte GSH and GPX activity as well as their variations in patients with invasive cervical carcinoma before and after radiotherapy.

Materials and Methods

Patients—30 patients of histopathologically proven carcinoma of the uterine cervix (Stages Ib-Illb) were randomly selected from patients attending the Radiotherapy Department of Maulana Azad Medical College and associated L.N. Hospital, New Delhi.

Controls—30 age-matched females also were selected from the Obstetrics and Gynaecology Department of Maulana Azad Medical College, New Delhi. The controls had no history or evidence of malignancy, pelvic inflammatory disorders, or any systemic illness and had 2 consecutive PAP smears negative for cervical abnormality.

A detailed clinical history of the subjects was collected by personal interview and clinical examinations were performed. The cervical tumour grading was done as per FIGO guideline10. Blood samples at three time points were collected from all patients included in the study and only one sample was drawn from the control women. For patients, first blood sample was drawn at the time of presentation in the Department of Radiotherapy prior to initiation of therapy. Second blood sample was collected at the completion of treatment. Subsequently, the third sample was collected on the first three monthly follow-up visit. All patients were treated as per treatment protocol being followed at the Radiotherapy Department of Maulana Azad Medical College by external radiation therapy with Theratron 780 machine and/or intracavitary irradiation by Selectron LDR/MDR brachytherapy unit.

Biochemical analysis—5 ml of peripheral venous blood was collected in heparinised tubes. The plasma was separated by centrifugation and used for GSH assay. Total plasma GSH was estimated according to the method of Tietze11. Briefly, an aliquot of plasma was mixed with precipitating solution (1.67% glacial metaphosphoric acid, 0.20% EDTA, 30% NaCl) and the mixture was kept on ice for 10 min. After centrifugation at 800 g for 10 min, the supernatant was separated out and used for the assay. The assay was carried out in 3 ml of reaction mixture. The reaction mixture contained 1.0 ml of 1M Tris (pH 7.2); 100µl of 12 mM NADPH; 60 µl of DTNB (40 mg/ml) and 100 µl of supernatant. The reaction was initiated by adding 0.3 U/ml of glutathione reductase enzyme. The linear changes in absorbance were monitored at 412 nm.

After separation of plasma from the whole blood, erythrocytes were washed twice in cold normal saline (0.9% NaCl solution). Washed erythrocytes were lysed in distilled water, and were centrifuged at 800 g for 15 min at 4°C. Supernatant was used to determine enzyme activities of the selenium dependent GPX according to the method of Paglia and Valentine12. Reduced GSH content in erythrocytes was measured spectrophotometrically using DTNB as colouring reagent as per the method described by Beutler et al.13. Haemoglobin was determined by cyanmethemoglobin method.

Statistical analysis—The data of the study were subjected to univariate and bivariate analysis. In order to test the significance of observed relationships between various variables, the Student's t test was employed.

Results

The mean age of the patients included in the study was 48.8 years (SD ± 11.4), while control group of women had a mean age of 49.0 years (±13.4). Amongst the cases, the mean age at menarche was observed to be 14.56 ± 1.22 years, whereas the control women had a mean age of 14.06 ± 0.72 at menarche. The mean age at marriage for patients was 16.1 ±1.04 years and 15.88 ± 1.77 years for controls. Average parity of the patient group was 5 ± 2, whereas the control group had only 3 ± 1. Out of total 30 patients, 17 patients belonged to postmenopausal and 13 patients were premenopausal.

Out of the total 30 patients studied, 83% cases (n=25) had a histopathological diagnosis of squamous cell carcinoma of the cervix and the rest were adenocarcinoma. Of the patients with squamous cell carcinoma, 9 patients had well differentiated tumour, 13 had moderately differentiated and the rest 3 cases belonged to poorly differentiated squamous cell carcinoma. With regard to clinical staging of the patients, 3 patients were of stage I, while 10 and 17 patients were of stage II and stage III respectively.

The levels of plasma glutathione (GSH), erythrocyte glutathione and glutathione peroxidase (GPX)
activity were found to be reduced in all patients with invasive carcinoma of the uterine cervix as compared to age matched control group. These differences in the levels of GSH and GPX were found to be statistically significant (P<0.001) (Table 1). Further, the mean value of plasma GSH in patients after radiotherapy was found to be lower than that of normal controls. Similarly, the mean value of erythrocyte GSH and GPX activity in post-radiotherapy cases were lower as compared to matched normal controls. Similar decreased levels of plasma GSH, erythrocyte GSH and GPX values were also observed in the patients on follow-up (3 months after radiotherapy). However, no remarkable alterations in the levels of plasma GSH, erythrocyte GSH and GPX were found at the two different time points after treatment (Table 1).

Clinical stage-wise analysis of the levels of GSH showed an increase in the mean values of plasma GSH levels in all patients who had completed the treatment, except amongst the follow-up patients with invasive cervical carcinoma stage III wherein mean plasma GSH level was lower than the corresponding post-radiotherapy state (Table 2). The histological type of cervical carcinoma also revealed an interesting observation (Table 3). Significant difference (P<0.05) was observed in the plasma GSH values among patients with well differentiated squamous cell carcinoma and moderately differentiated squamous cell carcinoma before treatment. The difference became non-significant after treatment.

Out of the total 30 patients studied 11 patients (stage I: 3 patients, stage II: 4 patients and stage III: 4 patients) showed complete response (>75% regression) whereas 6 patients (Stage II: 3 patients, stage III: 3 patients) revealed partial response (50% regression) and the equal number of patients (stage II: 1 patient, stage III: 5 patients) showed no response (<50% regression). 7 patients left without completing their treatment. Among the patients who showed no response (n=6), 5 patients left after completing the treatment and did not attend the follow-up study. The changes of the levels of plasma GSH, RBC GSH and GPX activity of the patients with cervical carcinoma

<table>
<thead>
<tr>
<th>Mode of treatment</th>
<th>No. of patients</th>
<th>Plasma GSH (µg/ml)</th>
<th>RBC GSH (µg/g Hb)</th>
<th>GPX (IU/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>32</td>
<td>209.1 ± 55.15</td>
<td>389.8 ± 113.3</td>
<td>35.5 ± 8.01</td>
</tr>
<tr>
<td>Pret RT</td>
<td>30</td>
<td>117.8 ± 44.54*</td>
<td>267.4 ± 86.18*</td>
<td>28.23 ± 7.55*</td>
</tr>
<tr>
<td>Post RT</td>
<td>23</td>
<td>125 ± 47.58*</td>
<td>252.3 ± 85.07*</td>
<td>26.9 ± 5.53*</td>
</tr>
<tr>
<td>Follow up</td>
<td>18</td>
<td>131 ± 58.49*</td>
<td>243.4 ± 84.05*</td>
<td>27.72 ± 6.78*</td>
</tr>
</tbody>
</table>

* P < 0.001

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>No. of cases</th>
<th>Plasma GSH</th>
<th>RBC GSH</th>
<th>GPX</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>83.20</td>
<td>378.8</td>
<td>20.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± ± ±</td>
<td>± ± ±</td>
<td>± ±</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>128.1</td>
<td>259.9</td>
<td>28.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± ± ±</td>
<td>± ± ±</td>
<td>± ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56.14</td>
<td>70.54</td>
<td>5.63</td>
</tr>
<tr>
<td>III</td>
<td>17</td>
<td>117.9</td>
<td>251.2</td>
<td>29.56</td>
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<td></td>
<td></td>
<td>± ± ±</td>
<td>± ± ±</td>
<td>± ±</td>
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<td></td>
<td></td>
<td>37.92</td>
<td>82.28</td>
<td>7.57</td>
</tr>
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</table>
Discussion

The study has highlighted the alteration of GSH and GPX in patients with invasive cervical carcinoma before, during and after radiotherapy treatment. This is in conformation with earlier reports on cervical carcinogenesis in relation with plasma GSH\(^{14}\). Utilization of GSH may be mediated by the glutathione S-transférase (GST) or the glutathione peroxidase system\(^{15}\). Red cells are found to have very low oxidised glutathione content since oxidised glutathione is known to be continually transported outward from the red cells into the plasma\(^{16}\). In humans, the level of glutathione is too low to make a distinction impracti-
cal between the reduced and oxidised forms. Therefore, only total GSH (oxidised + reduced) was estimated. Several reports have shown altered GSH content in a number of cancers of human origin as well as of animal origin. Increase in red cell GSH has been also reported in leukaemia and gastrointestinal adenocarcinoma. The present study has revealed lower levels of plasma GSH in patients of invasive cervical carcinoma before and after radiotherapy as well as during follow-up in comparison to controls. Interestingly, differences between GSH levels in relation to histological differentiation were also observed. Contrary to the findings of Bhuvarahamurthy et al., levels of GSH and GPX of our study did not change to normal values (normal controls' values) even after treatment.

Erythrocyte glutathione peroxidase acts as an antioxidant enzyme. However, it has been observed in the present study that the erythrocyte glutathione peroxidase activity among patients of cervical cancer was significantly lower in comparison to normal healthy controls which is in conformity of the findings on CIN and invasive cervical carcinoma, as reported by Kumar et al. and Bhuvarahamurthy et al. respectively. Balasubramaniam et al. reported that the activities of glutathione peroxidase were significantly lower in stage III and IV patients of carcinoma of human uterine cervix than that of normal controls. Similar findings were also observed in CIN III as well as in invasive cervical cancer by Kumar et al. It has also been reported that the reversion of erythrocyte GPX activity to normal level occurred following radiotherapy in patients with uterine cervical carcinoma. These findings reflect that GSH detoxification system, one of the defense systems against exogenous as well as endogenous factors (i.e., free radicals, carcinogens and other peroxides, etc.) is greatly influenced during the process of cervical carcinogenesis. As such, lower level of GPX together with the lower GSH contents may favor an overproduction of free radicals and lipid peroxides which in turn may induce the damage to cell membrane and cellular molecules (DNA, RNA) leading to neoplastic changes. It is possible that GSH scavenger system may be impaired as a consequence of reduced synthesis of the enzymes due to the carcinogenic process.

Ionizing radiation is toxic to organisms because it induces deleterious structural changes in essential macromolecules. These changes can be the result of direct interaction with radiation. However, it is generally considered that, because of the abundance of water in living organisms, a more important mechanism is the interaction with free radicals formed by photolysis of water. GSH plays a key role in protecting cells against electrophiles and free radicals. This is due to the nucleophilicity of the SH group and to the high reaction rate of thiols with free radicals. Radiation resistance of many cells is associated with high intracellular levels of GSH. Administration of radioprotective agents like WR-2721 has shown an elevation of the blood GSH levels. Cells containing low levels of GSH were found to be much more sensitive to the effect of irradiation than controls, although Backer et al. reported that loss of GSH does not affect radiosensitization. However, in normal cells, GSH or non-protein cellular thiols are present at levels far in excess of that required for the repair of radiation-induced damage. Further Cholon et al. observed that ionizing radiation decreased the levels of total GST activity.

Numerous studies have shown a correlation between the erythrocyte glutathione peroxidase activity and the radiation response. Glutathione peroxidase is an important 'scavenger' enzyme which has an incorporated selenium molecule to which it owes its activity. Bewick et al. observed the deficiency of this enzyme in erythrocytes of lymphoma patients. Groupkowski et al. observed an inverse correlation between the activity of this enzyme and the extent of the disease in patients with colon cancer.

In a study conducted by Jadhav et al., a good correlation was detected between the degree of GSH depletion and the tumour response. However, the present study did not reveal any significant relationship between the treatment response and the changes of the levels of either glutathione in blood plasma and RBC or glutathione peroxidase in RBC of cervical cancer patients.

The data of the present study clearly suggest the changes in antioxidant status with special reference to the glutathione system among patients with invasive carcinoma of the uterine cervix in comparison with the normal controls. This study also demonstrates the effect of radiation therapy on this antioxidant system. However, study on larger sample size, with a longer follow-up period and measuring other related antioxidant levels also, may yield more meaningful data on the role of antioxidant system in the clinical course of cervical tumours.
References
