Original Article

Does fall in serum glutathione predict the long-term outcome to concurrent chemoradiation for cervical cancer patients?

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ABSTRACT

Background: Wide variations are seen in clinical practice with respect to response to concurrent chemo radiation among cervical cancer patients. Fall in serum glutathione (GSH) level directly correlates with early response to treatment. Whether this early response translates to a better long term outcome is the subject of this prospective study.

Materials and Methods: Thirty eight women with cervical cancer were treated with concurrent chemo radiation followed by brachytherapy. Serum GSH was measured before and after two fractions of radiation and first chemotherapy. Patients were followed for a median follow up of four years. Fall in GSH was correlated with response at six weeks and disease status at four years.

Results: Median fall in serum GSH was 171.16 µg per ml. Fall in GSH was 170.42, 103.54 and 37.25 µg per ml (*P* value of <0.0001, 0.05 and 0.18) in patients showing complete response, partial response and no response respectively. Among 26 patients who had no disease at six weeks, 22 women remained disease free at four years ($P < 0.0001$), two recurred ($P < 0.05$) and two died of other causes respectively. Non bulky tumours and patients more than 50 years of age showed a fall of 190.69, 265.17 µg per ml respectively.

Conclusion: Greater fall in serum GSH predicts better early response as well as long term disease control.

KEY WORDS: Cervical cancer, glutathione, radiotherapy

INTRODUCTION

Cervical cancer is the second most leading cancer in the less-developed world[1] and most patients are diagnosed in advanced stages. In our department, it constitutes 18.39% of all cancers.[2] Concurrent chemoradiation followed by brachytherapy is the standard treatment.[3] There are many factors that predict the response of cervical cancer to concurrent chemoradiation; some of them are age of the patient, stage of the disease, size of the tumor, etc. Within a particular stage, varying results are observed in clinical practice, and hence, there is a need to extend our understanding to the cellular level to differentiate responders from nonresponders. Several studies have been conducted about the role of antioxidant status, glutathione (GSH) content, survival fraction, potential doubling time, and micronuclei for predicting tumor radioresponse in cancer of the cervix.[4] GSH activity is one of the most promising predictive factors. GSH functions as free radical scavenger and plays an important role in protection against cellular oxidative and free radical damage. Tumors are associated with higher GSH which helps them to survive in adverse conditions.[5] Depletion of GSH in these tumor cells makes them more vulnerable to the effects of radiation as well as chemotherapy (CT).[6] GSH is present in the tumor as well as in the blood. Following exposure to radiation, GSH in the tumor gets used up and is replenished by its content in the blood. If more GSH is generated, its level remains high and response to radiotherapy (RT) and CT will be sub-optimal; on the other hand, if less GSH is produced by body, its level falls and tumor cells are rendered more susceptible to the effect of radiation. Thus, fall in GSH values can predict the ultimate response of the tumor to RT and CT.

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This has been observed by Vidyasagar *et al*. in 98 patients of cervical cancer, in which GSH values were measured before and after two fractions of radiation and first cycle of cisplatin CT.[7] There was a remarkable fall in GSH values in complete responders (CRs) and partial responders (PRs) compared to no-response (NR) group.

The early response is studied by different authors; however, long-term disease status has not been correlated with GSH levels. Hence, this prospective study was conducted to correlate the fall in serum GSH level with early response as well as long-term control.

MATERIALS AND METHODS

Thirty-eight patients of carcinoma cervix treated between October 2010 and March 2012 were the subjects of this study. Postoperative patients were excluded from the study. The Institutional Ethical Committee has approved the study. Informed consent was taken from all the patients. Each patient was clinically assessed by two clinicians and staged according to FIGO staging system for cervical cancer. At the same time, tumor size was also assessed and recorded. Complete blood count, renal function tests, and chest X-ray were done to know the general condition of the patient. Ultrasound abdomen was done to rule out liver metastases and to assess renal status. External beam RT (EBRT) to a dose of 46 Gy in 23 fractions with 200 cGy per fraction and 5 fractions per week was delivered either on telecobalt or linear accelerator along with weekly CT with injection cisplatin 40 mg/m^2 body surface area once a week for 4–5 cycles. Patients also underwent intracavitary brachytherapy, 10–15 days after the completion of EBRT to a dose of 30 Gy to Point A with manual afterloading system using caesium source.

Two samples of blood were taken for serum GSH estimation. The first sample (pre-RT) was taken before the commencement of radiation and the second sample (post-RT) was taken after the second fraction of radiation and first cycle of cisplatin CT. Both samples were collected between 11 am and 1 pm to overcome diurnal variation. GSH levels were estimated using Beutler's method.[8] This test is simple and accurate for estimation of GSH. The serum sample is made to react with 2-nitrobenzoic acid in neutral pH and a yellow color is produced. This is transferred to a cuvette and optical density (OD) was recorded. The standard OD on a spectrophotometer was set in the range of 412–422 nm. The difference in the OD was recorded and the amount of GSH was calculated.

Six weeks later, the patient was examined by two physicians and the response was classified as per the WHO criteria into CR, PR, and NR. These patients were followed up till June 2015 and were assessed for the disease status. The fall in the GSH level was documented and correlated with early response at 6 weeks as well as disease status at a mean follow-up of 4 years.

Furthermore, an effort was made to correlate age, bulky disease status, and stage of the disease with the response and GSH fall.

Statistical analysis

Descriptive method of statistical analysis was performed. Nonparametric tests were also used. Data were collected and tabulated using Microsoft Excel worksheets. SPSS version 16.0 18.0, (IBM, Chicago, US) was used for analysis of the data.

RESULTS

A total of thirty-eight patients were the subjects of the study. The patient characteristics are shown in Table 1.

Median serum GSH pre-RT was 511.5 µg/ml (158.304–921.888) whereas post-RT was 365.7 µg/ml (69.322–695.296) after two fractions of radiation and first cycle of CT. The median fall was 171.6 (−99.328–664.256). GSH levels with respect to response groups are as shown in Table 2.

Twenty-six (68.4%) patients showed CR, 10 (26.3%) showed PR, and 2 (5.3%) showed NR. The fall was highly significant ($P < 0.0001$) in CR compared to PR and was negligible in NR as shown in Table 3.

Table 1: Patient characteristics

Characteristics	*N* (%)
Age	
</=50 years	27 (71.06)
>50 years	11 (28. 94)
Staging	
1B2	4 (10.53)
IIA	1 (2.63)
IIB	15 (39.47)
IIIA	2 (5.27)
IIIB	15 (39.47)
IV A	1 (2.63)
Differentiation	
Well differentiated SCC	14 (37)
Moderately differentiated SCC	22 (57.8)
Poorly differentiated SCC	1 (2.6)
Adeno squamous cell carcinoma	1 (2.6)
Bulky (>4cm)	
Yes	29(60)
No	09(40)

Table 2: Correlation of glutathione levels (in µg per ml) with response at 6 weeks

	PRE RT GSH	POST RT GSH	FALL IN GSH	*P* value
CR	527.9	357.49	170.42	>0.0001
PR	464.11	360.57	103.54	0.05
NR	535.44	498.19	37.25	0.18

Table 3: Correlation of response at 6 weeks with the outcome at 4 years

Response at 6 weeks	Disease status at 4 years			Lost to F/U	*P* Value
	NED	REC	DIED		
CR (26)	22	2	0	2	<0.0001
PR (10)	5	2	1	2	<0.05
NR (2)	1	0	1	0	0.42

At a median follow-up of 4 years (39–52 months), four patients (10.5%) were lost to follow-up, two died (5.3%), four had recurrence (10.5%), and 28 patients (73.7%) had no evidence of disease (NED). Patients who were NED at 4 years also had a significant fall in GSH with $P < 0.0001$.

Early response was correlated with the outcome at 4 years and is shown in Table 3. Of 26 patients who had CR, 22 patients were NED at 4 years. Among the two patients who died, the cause was unrelated to the disease as shown in Figure 1.

The fall in GSH was higher among patients who were CR at 6 weeks and NED at 4 years compared to those patients who were CR at 6 weeks but recurred at 4 years ($P < 0.0001$). The fall was higher in patients who were PR at 6 weeks and NED at 4 years compared to patients who were PR at 6 weeks and recurred at 4 years [$P < 0.05$, Figure 2].

The other factors correlated were age of the patient, bulky status, and grade of the tumor. There was a higher fall in GSH among patients with nonbulky tumors compared to bulky disease with $P = 0.0001$ as shown in Figure 3. Furthermore, patients older than 50 years had a greater fall in GSH compared to <50 years ($P < 0.05$) as shown in Figure 4.

The CR, PR among well-differentiated tumors were seen in 10 (71.4%) and 3 (21.4%) patients, respectively. The CR, PR in moderately differentiated tumors were seen in 16 (72.7%) and 6 (27.3%) patients, respectively. GSH values too showed similar trends in well and moderately differentiated tumors with no statistically significant difference between them.

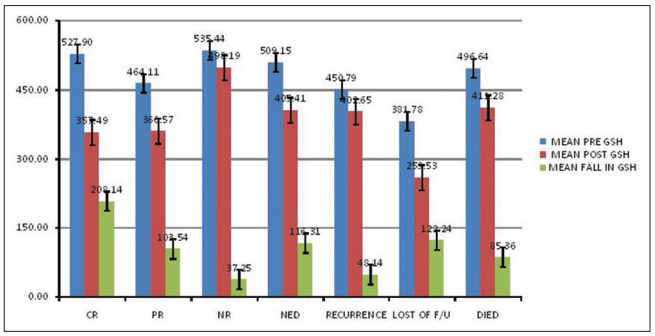

Figure 1: The median fall in glutathione with respect to early response as well as disease status at 4 years

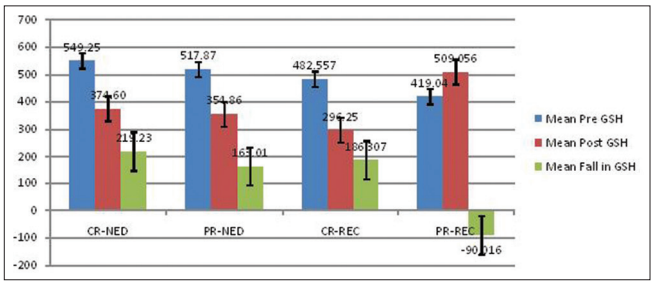

Figure 2: Correlation of outcome with glutathione levels

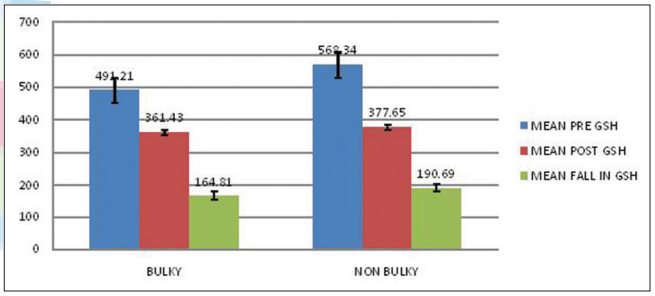

Figure 3: Glutathione levels and bulk of disease

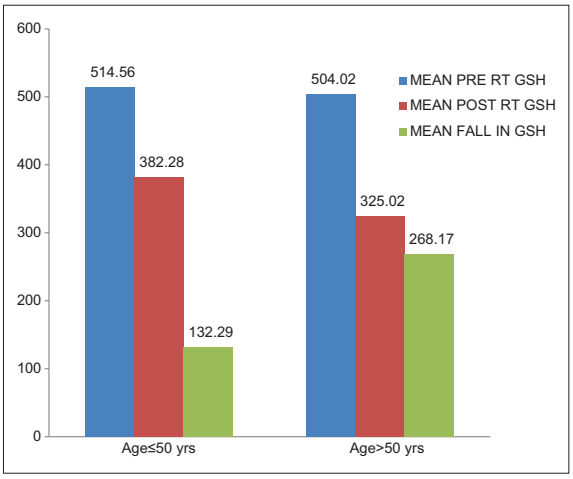

Figure 4: Graph correlating age with glutathione levels

DISCUSSION

The present study was conducted to determine the value of serum GSH level assessment in predicting response to chemo-RT in cervical cancer patients. In our study, 68% of patients achieved CR, 26% achieved PR, and NR was seen in 6% of study population. Similar results were obtained by Vidyasagar *et al*. with 72% of patients achieving CR in their study.[7] In a study by Jadhav *et al*., the response rates were less than the present study with only 33% of patients achieving CR and significantly a larger number of patients showing NR to the treatment. The probable reason for this could be that no concurrent CT was given and a lesser dose of EBRT, 35 Gy in 16 fractions, was delivered to Stage IIB patients.[9]

In the present study, there was significant fall in post-RT GSH values compared to baseline values in both CR and PR groups. The fall in GSH values was larger in CR group as compared to PR group. There was a meager fall in GSH values in the group that showed NR and this fall was not statistically significant.

Similar results were observed by Vidyasagar *et al*.; baseline GSH values were compared with GSH values post two fractions of RT and one cycle of cisplatin CT similar to the present study. There was a statistically significant fall in GSH values in

groups showing CR and PR but an insignificant fall in patients showing NR. Results in this study comply with the results of the present study.[7]

Results of an earlier study by Jadhav *et al*. too show a similar trend. They studied 45 patients in whom serum and tumor GSH were checked before and after one fraction of radiation. All patients who had CR showed more than 70% fall, who had PR showed between 50% and 70% and those who had NR showed <50% fall. This was seen both in tumor and blood GSH levels.[9]

Demirci *et al*. have published a similar study of 35 women. In this study, post-RT GSH values were measured on the day of completion of RT. They also compared with healthy women and observed that GSH levels were significantly lower compared to controls. They found that there was no difference between pre- and post-RT GSH values. This is contrary to our results; the possible reason could be that significant tumor response to treatment generally occurs during the 3rd or 4th week of RT. Thus, one can hypothesize that alterations of the antioxidant system may return to the pre-RT levels at the end of the treatment since there is only a part or no tumor left. Patients who did not respond had a higher GSH levels compared to those who responded ($P \leq 0.01$).[10]

We have correlated with the immediate response at 6 weeks after the treatment as well as at 4-year follow-up. When we compared the fall in GSH before and after two fractions of radiation and first cycle of cisplatin with response, there was a significant correlation. Those who had no disease at a mean follow-up of 4 years had a greater fall compared to those who recurred.

In a retrospective study of cancer of cervix Stage IIIB cases by Saibishkumar *et al*., it was found that response to EBRT was a predictor of long-term survival.[11] Those who had no residual tumor had a better pelvic control, Disease-free survival (DFS) and overall survival (OS) (75.6% vs. 54.6%, 60.6% vs. 31.9%, 62.2% vs. 33.7%, respectively: All $P < 0.0001$) compared to those who had gross residual tumor. Hence, immediate response to RT is a strong prognostic factor for DFS and OS.

When disease status at a mean follow-up of 4 years was compared with the initial response, we observed that 84.61% of patients in CR remained disease free which was statistically significant. We cannot comment on the PR and NR groups as there were very few patients. Two of the CR group died; however, the cause of death was not related to the disease.

Subgroup analysis based on FIGO stage of the cervical cancer was done in the present study. There were no significant differences in response rates between early Stage (I, II) and locally advanced Stage (III and IV) groups and both groups showed statistically significant fall in GSH values post two fractions of RT. Hence, the stage of the disease influenced neither the response rates nor the fall in GSH values.

Demirci *et al*. also have shown similar results. There was no statistically significant difference between Stages I, II, and III with respect to pre, post, or fall in GSH values.[10] Moreover, the present study, as well as this one quoted here, are underpowered in terms of sample size to find such a difference.

Various trials such as Saibishkumar *et al*. have reported age to be a significant predictor of the outcome, but this and many other such studies have not correlated with GSH levels. In the present study, there is a difference in response rates between ≤50 and >50 years age groups. Sixty-three percent of younger age group showed CR and 7.2% were NR whereas 81% of older than 50 years group showed CR with no-NR in this group. Response rates were paralleled by corresponding trends in GSH values.[11] Both pre- and post-RT GSH values were more pronounced in older age group. However, none of these differences were statistically significant.

Dattoli *et al*.[12] and Delaloye *et al*.[13] also showed similar trends of poor outcome in younger patients. A similar analysis done by Demirci *et al*., with 60 years as cutoff, also showed similar results. Though >60 years age group showed as consistently smaller pre- and post-GSH values versus younger age group, the difference was not statistically significant.[10]

Histology and degree of differentiation were analyzed for their influence on response to treatment and on GSH levels. There were no significant differences between well-differentiated and moderately differentiated tumors. There was only one poorly differentiated tumor; hence, no statistical test could be applied to analyze it. There was one adenosquamous carcinoma which ended up being an NR. Correspondingly, this patient showed a persistently high pre- and post-RT GSH with a small insignificant fall after starting radiation.

We have correlated with the immediate response at 6 weeks after the treatment as well as at 4 years follow-up. When we compared the GSH before and after two fractions of radiation and first cycle of cisplatin with response, there was a significant correlation. Those who had no disease at a mean follow-up of 4 years had a greater fall compared to those who recurred. Similar observations are also done by many other authors. Hence, we can say that GSH level can predict the outcome in cervical cancer, also studied other factors such as age <50 years, stage of the disease, and bulky growth.

To the best of our knowledge, this is the first study in literature to correlate fall in GSH with not only early response but also long-term control at 4 years. The limitation is that there are only few patients, thereby difficult to come to a sound conclusion.

CONCLUSION

In this prospective study, higher fall in blood GSH levels after two fractions of radiation and first CT not only correlated with immediate response but also sustained even at longer follow-up at 4 years. Elderly patients and nonbulky disease also had a greater fall and did better. Further studies with a bigger sample size are necessary before it can be taken as the standard of care. Patients who show lesser fall probably require more aggressive treatment such as addition of surgery or adjuvant CT.

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Conflicts of interest

There are no conflicts of interest.

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