Study of Glutathione-*s*-transferase and Reduced Glutathione Receiving Chemotherapy

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Authors' contributions

This work was carried out in collaboration among all authors. Author RSA Conceptualization of research paper, literature search, writing manuscript, Data collection and analysis of the results, Writing and editing of manuscript. Authors SN, RKJ and MK Literature search, Writing manuscript, Data collection and analysis of the results. All authors read and approved the final manuscript.

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ABSTRACT

Cervical cancer is the third most prevalent cancer in women worldwide, and the fourth leading cause of death from cancer in women. Recent advances, such as the availability of broad scale genome data, articulated gene tag (EST) data bases, innovative sequence alignment techniques, and X-ray crystallography determination of three-dimensional structures, have significantly expanded our understanding of structure–function relationships in this important enzyme superfamily. Total 36 histologically confirmed patients, locally advanced FIGO stage IIB–IIIB cervical cancer were enrolled. Based on the findings of our research, it can be concluded that improvements in GSH concentration during the treatment of locally advanced cervical cancer can have a major impact on the treatment response. In comparison to the lack of

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concentration changes in the blood serum of patients who have had no reaction to medication or who have had a reported relapse following treatment, GSH tends to be an effective indicator.

1. INTRODUCTION

It is now well understood that glutathione-*s*-transferase (GST) play an important role in step II of enzymatic detoxification. Recent advances, such as the availability of broad scale genome data, articulated gene tag (EST) data bases, innovative sequence alignment techniques, and X-ray crystallography determination of three-dimensional structures, have significantly expanded our understanding of structure–function relationships in this important enzyme superfamily. Although mammalian GSTs have been thoroughly studied and categorized using widely accepted standards, a variety of novel GST groups have been discovered in non-mammalian sources and only recently recognized in mammals. Furthermore, non-mammalian GSTs have a disproportionately high number of crystal structures. These advances have helped us to recognise novel roles that were not historically identified with GSTs. In cells, these enzymes perform a host of roles, including the elimination of reactive oxygen species and the regeneration of S-thiolated proteins, catalysis of endogenous ligand conjugations, as well as catalysis of reactions in metabolic processes not related to detoxification. The classification method developed for mammalian GSTs has proven to be stable enough to be generalized to non-mammalian enzymes; on the other hand, new groups identified in non-mammalian sources have later been discovered in mammals. The classification of non-mammalian GSTs is the subject of this study, which focuses on their importance in expanding our understanding of structure–function relationships in these enzymes as well as the implications for the evolution of this dynamic multifunctional superfamily [1].

Glutathione-*s*-transferase are a family of enzymes present in both eukaryotes and bacteria, with at least 18 GSTs expressed in humans. GSTs catalyse a number of reactions involving the addition of glutathione (GSH) to substrate compounds, but their most well-known role is in xenobiotic detoxification. GST pi has been shown to control JNK signalling, and GST mu from mice forms inhibitory complexes with ASK1, a MAP kinase. Members of the alpha and sigma groups are implicated in the biosynthesis of sex steroids and prostaglandins, respectively, and mutations or a lack of particular GSTs has been linked to a variety of human disorders, including Parkinson's and Alzheimer's disease, as well as an elevated risk of cardiovascular disease. GSTs are believed to be responsible for chemotherapeutic drug resistance as well as carcinogenic drug resistance [2].

GSTs are classified into classes denoted by mu, omega, and sigma based on historical conventions. Different classes have different general substrate profiles, whereas members of the same class have more nuanced variations in substrate identification. There is a Specific GSTs appear to be promiscuous in converting a series of similar compounds, and there are usually several GSTs transcribed within the same organism. In terms of structure, cytosolic GSTs function as dimers, with each monomer consisting of a conserved thioredoxin domain containing the glutathione (GSH) binding site followed by a more variable -helical domain (the GST C domain) containing the GSH acceptor substrate binding site. The activation of GSH for transition to a substrate by stabilization of the GSH thiolate is the central theme in GST catalysis [3].

Cervical cancer is the third most prevalent cancer in women worldwide, and the fourth leading cause of death from cancer in women [4]. It is the fourth most prevalent female cancer and ranks seventh in terms of female cancer mortality rates in Lithuania, although it is the most common cause of cancer death among women aged 15 to 44 [5].

Cisplatin-based chemoradiation is the recommended procedure for locally advanced cervical cancer [6]. The treatment's benefits, though, are insufficient; the prognosis for a more severe condition remains low, with elevated rates of local and/or remote relapses. There is a desire for modern and more efficient care modalities. New chemotherapy regimens, chemotherapy and

new formulations of target drugs, and changes of chemotherapy doses or schedules are also being investigated in clinical trials. New cytostatic combinations without cisplatin, as well as new cisplatin-based combinations, are used in these studies as neoadjuvant or adjuvant therapies of radiation or chemoradiation. New prognostic and predictive variables are being sought in order to personalize care. In the case of cervical cancer, a number of well-known clinical, morphological, and molecular variables are used to determine definite predictive significance: the point, tumor size, regional lymph node metastases, histological form, grade, lymph node and blood vessel invasion, and blood hemoglobin amount. And with similar data, however, patient reactions to medication and recovery rates typically differ greatly [6].

Which allows scientists to do further experiments in the fields of molecular biology and genetics. Changes in oxidative-reductive systems, as calculated by the rate of lipid peroxidation and the activation of antioxidative system enzymes, play an important role in cancerogenesis [7].

Glutathione (GSH) is one of the most important detoxifying substances. It is well understood that the amount of GSH in a cell affects its susceptibility to anticancer therapy as well as its toxicity (a decrease in GSH increases drug toxicity). As a result, determining the amount of GSH is critical in predicting if cancerous cells would be vulnerable to the drug's impact or whether the drug's effect on normal cells would be harmless [8].

Patients of cervical cancer have a slightly smaller level of GSH in the blood and tumor while they have a full reaction to medication, as opposed to a partial response. GSH changes can be caused by metabolism enzymes that use tripeptide as a substrate. In cancer chemotherapy, adequate activity of the enzyme glutathione S-transferase (GST) is important, because as the enzyme catalyzed conjugation interacts with GSH, the solubility of medications and other harmful materials in the water increases, and they are removed more effectively; hence, the effect on the organism is decreased, which can result in a worse reaction to treatment and a shorter survival [9].

2. MATERIALS AND METHODS

In a research performed in the Dept. of Biochemistry in collaboration with Dept. of Medicine. Total 36 histologically confirmed patients, locally advanced FIGO stage IIB–IIIB cervical cancer were enrolled.

2.1 Exclusion Criteria

1. pregnant or breastfeeding,
2. previous diagnosis of cancer,
3. active cardiac disease.

2.2 Neoadjuvant Chemotherapy

Weekly neoadjuvant chemotherapy (NACT) with a combination of cisplatin (30 mg/m2) and gemcitabine (125 mg/m2) was given for four weeks. Normal instructions for hydration, infusion length, and antiemetic medication were followed. A thorough physical examination, including a pelvic examination, normal and perfusion computer tomography (CT) scan, was conducted after a 14-day post-NACT duration to determine the response to the procedure. External beam radiation (EBRT) and brachytherapy were planned using CT images and immobilization methods. A three-dimensional (3-D) conformal EBRT dosimetry plan was devised.

2.3 Chemoradiation

Chemoradiation began in week six. Beginning on the first day of radiation, a mixture of cisplatin (40 mg/m2) and gemcitabine (125 mg/m2) was provided weekly for 5 weeks during EBRT. Prior to radiation, cytostatics were given as an infusion. Over the course of 5 weeks, EBRT was administered to the whole pelvis using a linear energy accelerator (15 MeV energy) at a cumulative dosage of 50–50.4 Gy in 25–28 fractions. Following the completion of EBRT, intracavitary brachytherapy was administered. Patients were given 4 fractions (7 Gy each) at a high dose rate (HDR) of 1–2 per week to point A. A total of 89 Gy was shipped to point A. Two months after the procedure was done, the reaction to the treatment was measured. Following that, patients were monitored according to a normal monitoring protocol for cervical cancer patients.

2.4 The Treatment's Efficacy and Safety was Tested

A pelvic inspection, normal and perfusion CT scan, and complex assessment of the size and function of the cervix, parametrical invasion, and regional lymph nodes were used to determine

the response to NACT. A full response (CR) to therapy means that the tumor symptoms have vanished, while a partial response (PR) means that the tumor signs have decreased. When the tumor symptoms stayed constant, it was diagnosed as stable disease (SD). The diagnosis of progressive disease was granted if the tumor size grew by more than 20% or if a new lesion appeared (PD). A pelvic examination, cytological or histological examination (a biopsy may be performed if a residual tumor is suspected), and CT scan were used to evaluate the reaction to full chemoradiation. Monitor radiological tests of the chest, liver, and pelvis were done to rule out distant metastases. If no clinical or cytological tumor symptoms were seen, CR was diagnosed.

2.5 Collection and Examination of Blood Samples

GSH and GST blood samples were taken before the start of therapy, 2 weeks after NACT (before the start of chemoradiation), and 2 months after chemoradiation ended. GSH and GST samples were held at 70°C before analysis. GSH and GST levels were determined using an enzyme-linked immunosorbent assay (ELISA) (CUSABIO, BIOTECH, China), as instructed by the manufacturer. A microtiter reader (Shenzhen Mindray Bio-Medical Electronics Co, China) was used to test the absorbance of each well at 450 nm.

A statistically meaningful increase in GSH concentration levels after NACT was discovered after a study of the GSH and GST concentrations in patients with stage IIB–IIIB cervical cancer. Patients' GSH levels did not improve after further chemoradiation. There were no statistically relevant improvements in GST during the procedure (Table 1).

3. RESULTS

Patients were split into two groups to evaluate improvements in GSH and GST concentrations in response to NACT therapy based on a mixture of cisplatin and gemcitabine: one group (n = 26) had a supportive (all patients partial) response to the treatment, while the other group (n = 10) had no response (stable disease). GSH concentrations displayed a statistically significant rise after NACT and a statistically significant decrease after chemoradiation, in comparison to their amount after NACT, considering the positive reaction to the procedure. During the care of patients who did not respond to NACT, there were no major differences in GST and GSH concentrations (Table 2).

Calculations showed that two patients did not come to the evaluation of the treatment's results, two patients were diagnosed with disease development, and 32 patients had a full reaction after completing the protocol's complete chemoradiation. GST and GSH levels were calculated during the treatment period, and the findings indicated that GSH levels in patients with CR increased statistically after NACT and decreased statistically after chemoradiation (Table 3).

Table 1. Changes in GST and GSH levels as a result of therapy

Factor	Before treatment	After NACT	After chemoradiation
GST, ng/mL	0.62 ± 0.99@	0.84 ± 1.36@	0.71 ± 0.93
GSH, µg/mL	21.73 ± 13.64*	34.98 ± 22.38*	31.87 ± 24.47

P -value@-0.4353, 0.0034*

Table 2. GST and GSH levels fluctuate through therapy depending on how the body responds to NACT

Factor	Before treatment	After NACT	After chemoradiation
Positive response to NACT			
GST, ng/mL	26 0.52 ± 0.71	26 0.83 ± 1.38*	26 0.64 ± 1.02
GSH, µg/mL	26 24.68 ± 12.72	26 36.93 ± 22.67@	26 31.42 ± 28.89π
No response to NACT			
GST, ng/mL	10 0.91 ± 1.63	10 0.99 ± 2.02	10 0.84 ± 0.87
GSH, µg/mL	10 18.87 ± 10.95	10 26.68 ± 26.13	10 22.73 ± 20.74

* P – 0.313, @ P-0.923, π P-0.001

Table 3. GST and GSH levels fluctuate through therapy, suggesting a full chemoradiation reaction

Factor	Before treatment	After NACT	After chemoradiation
GST, ng/mL	0.78 ± 1.31	0.65 ± 0.97	0.59 ± 0.86
GSH, µg/mL	22.84 ± 15.73*	34.81±26.92*	30.07 ± 26.22$^{@}$

*@ - p-0.0337, * P-0.653*

4. DISCUSSION

On the one hand, free radicals are necessary for the control of most cell processes; on the other hand, high levels of free radicals, especially active oxygen types, are associated with oxidative stress. The antioxidative mechanism defends the body from oxidative stress. Various tumors, including breast cancer, have been studied extensively for changes in oxidative stress. However, there is a scarcity of evidence that represents the significance of oxidative stress in terms of prognostic and predictive factors.

Mukundan et al. [10] looked at the levels of GSH and glutathione-peroxidase (GSH-Px) in cervical cancer patients before and after radiation, as well as a stable control group. The levels of GSH and GSH-Px in patients with cervical cancer were slightly lower than those in the control group, according to the findings. In comparison to our findings, this research revealed that radiation therapy has an impact on the antioxidant system, but no correlation was identified between GSH, GSH-Px shifts, and treatment reaction. Unlike Bhuvarahamurty et al. [11] 's findings, GSH and GST levels after treatment did not exceed those of the control group.

Sharma et al. [12] compared the levels of blood lipid peroxide, GSH, GSH-Px, GST, catalase (CAT), and superoxide dismutase (SOD) in 60 cervical cancer patients before neoadjuvant chemotherapy, 2 weeks after chemotherapy, and 2 weeks after radiation to evidence from a comparison group of 60 healthy people. The lipid peroxide level was slightly higher in the blood of cervical cancer patients before surgery, although GSH, GSH-Px, GST, SOD, and CAT were significantly lower, in comparison to the lipid peroxide levels and enzyme activity in the blood of the control group. The amount of lipid peroxide dropped significantly after chemotherapy. GSH, GSH-Px, GST, SOD, and CAT levels all increased marginally. The mentioned amounts returned to normal after radiation (P 0.01). According to the researchers, normalizing lipid peroxide levels and antioxidative system enzymes can aid in reaction evaluation. Following the discovery of NACT in our study, there was a statistically significant increase in GSH concentration levels. Despite further chemoradiation, the patients' GSH levels did not increase. During the procedure, there were no statistically significant changes in GST concentration levels. Jadhav et al. looked at the predictive role of GSH in evaluating the response to radiotherapy. Blood and tissue samples were collected from 45 cervical cancer patients before and after the first fraction of radiation. After the first fraction, GSH levels in blood and tissue saw a substantial decrease; additionally, an association between treatment reaction and GSH levels was discovered. GSH concentrations in the blood and tumors were found to be down by more than 70% in all patients who got CR. Patients with tumor reductions of less than 50% (stable disease) had GSH concentration reductions of less than 50%. Prior to surgery, those who had been diagnosed with PR had GSH concentrations in their blood serum and tumor tissue fell to about 50%–70%. Patients who responded to NACT and chemoradiation had a statistically significant decrease in GSH in their blood serum after chemoradiation, according to our findings. There were no statistically important differences in GSH concentration in patients whose reaction was not detected [13].

GST plays a significant role in the development of tumor cell resistance to chemotherapy, according to the majority of the authors' in vitro and in vivo research. Increased GST expression in tumor cells has been related to resistance to cisplatin and other platinum compounds [14].

A reduction in serum GSH may be a predictive factor in the clinical response for cervical cancer patients treated with radiation, according to the findings of Vidyasagar et al. [15]. The data indicates that GSH levels drop significantly after chemoradiation, particularly in patients who achieve complete remission (CR), as opposed to those who do not respond at all. The same findings were used in our research.

Before and after radiation, Prabhu et al. [16] assessed serum GST levels. Changes in serum GST levels can help predict radiation response, according to the findings of the report. Our findings indicate that there were no statistically important improvements in GST concentrations during therapy. However, as patients were compared based on when their condition worsened following chemoradiation, a statistically important discrepancy was found [17-20].

5. CONCLUSION

Based on the findings of our research, it can be concluded that improvements in GSH concentration during the treatment of locally advanced cervical cancer can have a major impact on the treatment response. Statistically important increases in GSH concentration levels during the recovery period in the patient population with a promising outcome and little evidence of disease development after 2 years, In comparison to the lack of concentration changes in the blood serum of patients who have had no reaction to medication or who have had a reported relapse following treatment, GSH tends to be an effective indicator. During the procedure, no statistically meaningful differences in GST concentration levels were found.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline patients consent and ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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