GLUTATHIONE S-TRANSFERASES AS A PROGNOSTIC BIOMARKER FOR LEUKEMIA

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ABSTRACT

The present study has been undertaken to investigate the prognostic role of plasma Glutathione-s-transferases (GSTs) as a biomarker for leukemia. GSTs comprise a family of water-soluble enzymes involved in the detoxification of foreign compounds. The increase in GSTs is a major contributing factor to drug resistance. The present study revealed that the mean GST activity in the plasma of leukemia patients was significantly higher as compared to the control group. There was no significant difference ($p<0.05$) between plasma GST activity of different classes of leukemia. There was a significant increase ($p<0.01$) in plasma GST activity of leukemia patients in each class of leukemia after chemotherapy. The patients who achieved complete remission demonstrated significantly low plasma GST activity, but patients who achieved partial remission and the non-responsive patients demonstrated significantly high plasma GST activity.

Keywords: Biomarkers, chemotherapy, GSTs, Leukemia, Prognostic

INTRODUCTION

The term biomarker refers to a measurable variable that is associated with disease outcome (Ballman, 2015). A prognostic biomarker is one that indicates an increased (or decreased) likelihood of a future clinical event, disease recurrence or progression in patients. Prognostic biomarkers are useful in the selection of patients who required more intensive surveillance or adjuvant therapy. Prognostic markers are also important to place patients into different risk categories for guiding decisions on clinical management, to treat or not to treat (Lee et al. 2020). Keeping this in view the present study was undertaken to determine the activity of Glutathione s-transferases as prognostic biomarker in pre-and post-stage of chemotherapy for different sub-classes of leukemia. Glutathione-s-transferases (GSTs) is a family of enzymes involved in detoxification of foreign compounds. They participate in antioxidant defences through several mechanisms including reactive oxygen species (Ambad and Nagtilak. 2015).

GSTs play roles in both normal cellular metabolism as well as in the detoxification of a wide variety of xenobiotic compounds (Marrs et al. 1996). GSTs are referred to as phase II enzymes. They are actively involved in second phase of xenobiotic metabolism. The family of mammalian GSTs consists of eight classes of cytosolic isoenzymes namely, Alpha, Mu, Pi, Sigma, Theta, Zeta, Kappa and Omega. The mechanism of detoxification involves neutralization of the electrophilic, reactive sites of toxic compounds and attaching them to the tripeptide glutathione (GSH). GSTs studies are of great importance since they have been implicated in the development of drug

resistance in tumoral cells (Dourado et al. 2008). In many normal and malignant cells, increased GSH level is associated with a proliferative response and is essential for cell cycle progression (Messina et al. 1989, Lu et al. 1992). The increase in GSH is a major contributing factor to drug resistance by binding to or reacting with, drugs, interacting with ROS, preventing damage to proteins or DNA, or by participating in DNA repair processes. In melanoma cells, GSH depletion and GGT inhibition significantly increased cytotoxicity via oxidative stress (Nicola et al. 2013). In view of this, the present study was carried out with the aim to know the role of GST activity and its predictive value in leukemia progression and chemoresistance.

MATERIALS AND METHODS

The study was conducted at the Animal Physiology Laboratory, Department of Zoology RTM Nagpur University, Nagpur, and all samples were collected from the Ganju Hematology Clinic & Hospital Nagpur and Kane Hematology and Oncology Laboratory Nagpur. In this study total numbers of 26 subjects were selected in which 6 were normal healthy individuals and 20 were leukemic patients who were divided into four subgroups: 6 were diagnosed as CML (Chronic Lymphoid Leukaemia), 6 as AML (Acute Myeloid Leukaemia), 6 as ALL (Acute Lymphoid Leukaemia) and 2 were as Chronic Lymphoid Leukaemia (CLL). All the cases were newly diagnosed. The case selection was based on clinical features. Informed consent was taken from all the patients and the study was approved by Research Recognition Committee (RRC). Blood samples were collected three times from each patient, before chemotherapy, at the end of first induction chemotherapy and after the end of last chemotherapy.

Plasma Glutathione-s-transferase activity was measured by, using 1-chloro-2,4-dinitrobeneze (CDNB) as substate purchased from Himedia (EZAssayTM GST Activity Estimation Kit, Product Code: CCK028). IBM SPSS version 18 was used for statistical analysis. Data were expressed as mean and standard deviation. Mean values were assessed for significance by paired and unpaired t-Test. Probability value $p<0.05$ were considered statistically significant.

OBSERVATIONS AND RESULTS

The mean GST activity of control group was 5.32±0.68 and mean GST activity of leukemia patient was 8.23±1.07. The Mean GST activity in the plasma of leukemia patients was significantly higher as compared to control group. There was no significant difference ($p<0.05$) between GST activity of different classes of leukemia. GST activity of different classes of leukemia was shown in the Figure 1. The Mean GST activity of patients after chemotherapy was 12.31±1.11. There is significant increased ($p<0.01$) in GST activity of leukemia patients in each class of leukemia after chemotherapy (Figure 2). The patients who achieved complete remission demonstrated significantly low GST activity, but patients who achieved partial remission and the non-responsive patients demonstrated significantly high GST activity.

DISCUSSION

Glutathione-s-transferases are the family of detoxifying enzymes. They catalyzed the binding of large variety of electrophiles to the sulfhydryl group of Glutathione yielding less harmful and more water-soluble molecules which can be easily excreted. Hance, GSTs takes considerable importance as a mechanism for detoxification of carcinogen as well as chemotherapeutic drugs (Habig et al. 1974 and Nagao et al. 1978). The capability of GSTs to provide

resistance against chemotherapeutic drugs makes this enzyme family a potent prognostic marker for leukemia and chemoprotective activity (Bruin et al. 2009). The Present study was carried out to determine the role of Glutathione s-transferases activity as a prognostic biomarker in pre- and post-stage chemotherapy for different sub-classes of leukemia.

In the present study plasma GST was significantly higher ($p<0.01$) in all leukemia patients in each class of leukemia after chemotherapy. Similar findings reported by Ambad and Nagtilak, in-stomach cancer. Increase activity of GSTs in blood plasma can be due to overexpression of GSTs gene in response to chemotherapeutic drugs (Mukanganyama et al. 2010) GSTs have been implicated in the development of resistance toward chemotherapy agents. It is plausible that GSTs serve two distinct roles in the development of drug resistance via direct detoxification as well as acting as an inhibitor of the MAP kinase pathway (Townsend et al. 2003). In addition to their well-established GSH-conjugating enzymatic activity, GSTs of the α, π, and μ classes have been shown to modulate signaling pathways that control cell proliferation, cell differentiation, and cell death by interacting with important signaling proteins in a non-enzymatic way (Laborde et al. 2010, Tew et al. 2011). In the present study, there was no significant difference ($p<0.05$) between the GST activity of different classes of leukemia. Similar results were reported by Koberda and Hellmann in different leukemia types according to the French-American-British (FAB) classification (Koberda and Hellmann, 1991). In the present study, the patients who achieved complete remission demonstrated significantly low GST activity, but patients who achieved partial remission and the non-responsive patients demonstrated significantly high GST activity. Similar findings were reported by Chen et al. (2002). The lowest values of GST activity and GST mRNA expression were observed in those patients who achieved complete remission. The highest values of GST activity and GST mRNA expression were observed in those patients with no response to treatment (Chen et al. 2002). Thus, a progressive increase in the activity of GSTs was associated with progression in leukemia. It has been also associated with poor prognosis.

CONCLUSION

It is concluded from this study that enhanced plasma GST activity was associated with leukemia. Hoverer, there is no significant difference in the GST activity of different classes of leukemia but it may be a very useful prognostic biomarker for leukemia. GST enzyme-linked system may be a determining factor for the sensitivity of some tumors to various chemotherapeutic agents. It might be helpful to predict the response of chemotherapy. The involvement of GST in the carcinogenesis and in the drug resistance of leukemic cells is clear, but further studies, aimed at understanding the GST-GSH driven molecular pathways, might be crucial to design new therapeutic strategies to fight cancer progression and overcome chemoresistance.

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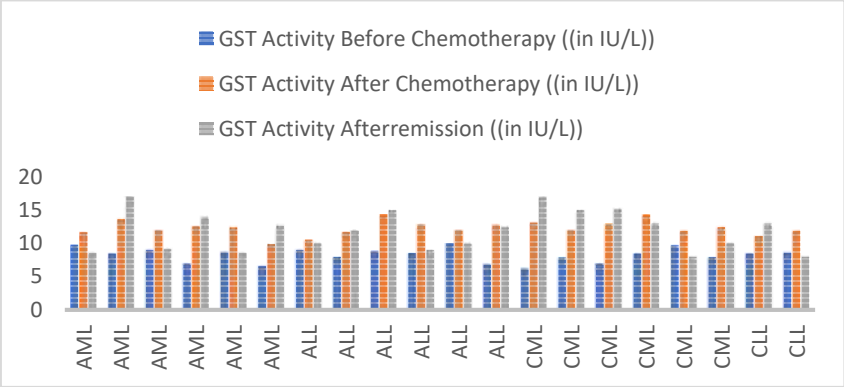

Figure 1: Plasma GST Activity of All Classes Leukemia Patients

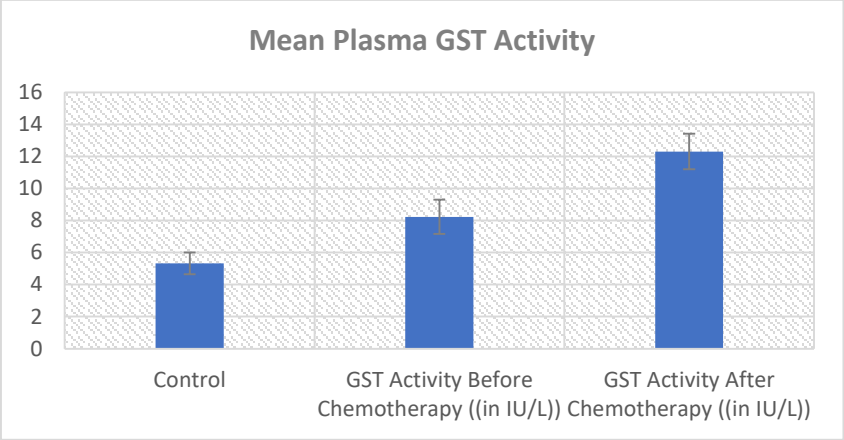

Figure 2: Mean Plasma GST Activity of leukemia patients before and after chemotherapy

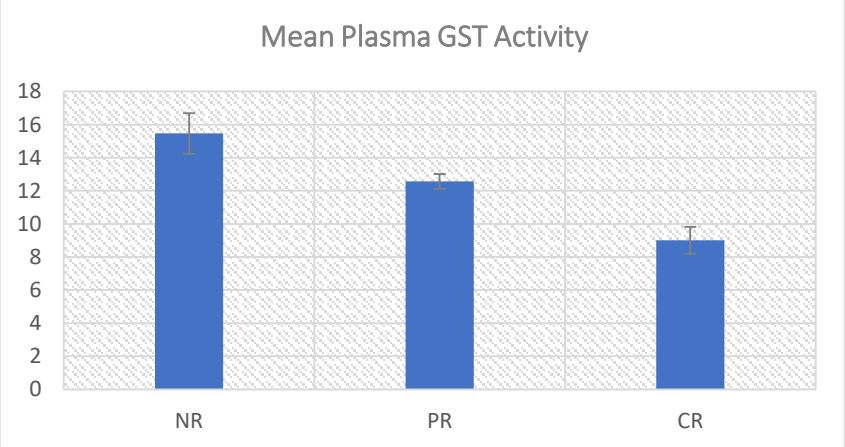

Figure 3: Mean Plasma GST Activity of leukemia patients in three different groups after chemotherapy

NR= Non-Responsive group, PR= Partial Responsive group, CR= Complete Responsive group

S.N.	Subjects	GST activity (in IU/L)
1	Sub-1	5.76
2	Sub-2	4.98
3	Sub-3	5.33
4	Sub-4	6.45
5	Sub-5	4.68
6	Sub-6	4.73

Table 1: Plasma GST activity of 6 healthy individuals (control).

S.N.	Leukemia Type	GST Activity Before Chemotherapy ((in IU/L))	GST Activity After Chemotherapy ((in IU/L))
1	AML	9.78	11.67
2	AML	8.45	13.65
3	AML	8.98	12.02
4	AML	6.98	12.55
5	AML	8.67	12.40
6	AML	6.56	9.87
7	ALL	8.97	10.54
8	ALL	7.98	11.73
9	ALL	8.87	14.34
10	ALL	8.56	12.87
11	ALL	9.98	12.05
12	ALL	6.86	12.80
13	CML	6.22	13.09
14	CML	7.87	12.06
15	CML	6.99	12.98
16	CML	8.45	14.32
17	CML	9.67	11.87
18	CML	7.89	12.45
19	CLL	8.43	11.09
20	CLL	8.63	11.89

Table 2: Plasma GST activity of 20 Leukemia patient before, and after chemotherapy

Non-Responsive group (NR)	Partial Responsive group (PR)	Complete Responsive group (CR)
16.98	12.63	7.98
13.87	12.98	8.56
14.98	11.9	9.98
14.98	12.4	9.09
16.89	12.98	8.98
15.09		9.93
		8.56
		7.98
		9.98

Table 3: Plasma GST activity (in IU/L) of 20 Leukemia patient after completion of chemotherapy. Patient were classified according to the response of chemotherapy