



Down Regulation in Glutathione S-Transferase Stimulate Oxidative Stress and Genetic Susceptibility of Human Breast Cancer

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Glutathione S-Transferase is an enzyme that catalyzed the formation of thio-ether conjugates between the endogenous tripeptide glutathione and xenobiotic compounds. Imbalance of glutathione level is observed in a variety of disease including breast cancer. In the present study we observed the alteration in the glutathione level and variation in glutathione S-transferase enzyme supergene family to influence susceptibility to breast cancer disease. Our study include 50 breast cancer tissues sample along with 25 ANCT control. The experiments were performed through high performance liquid chromatography to determine the endogenous glutathione level and multiplex PCR was used to determine GSTM1 & GSTT1 genotypes. The relationship between breast cancer and disease progression and histological grade were estimated by one way ANOVA and descriptive statistics followed by spearman correlation {S.D±SEM, 95% CI (P<0.05)}. The study show decreased level of glutathione (GSht P<0.05, GSH, GSSG P>0.05) in breast cancer tissue sample when compared to control, while no significance difference was occurred in GST (P>0.05) concentration when compared to control. In addition significance difference was observed

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when correlated histological grades {(grade II $P < 0.05$) grade I & III $P > 0.05$ } with control subjects. We therefore, also investigated the role of GST genotypes to associate the role of this genotype in breast cancer. Genotyping study of GST show that the GSTM1 ($P > 0.05$) null genotype was significantly associated with breast cancer and we found no association with breast cancer and GSTT1 ($P < 0.05$) null genotype. In conclusion our study suggest that the dysregulation of glutathione level were associated with breast cancer and GSTM1 null genotype could be a useful marker for breast cancer prognosis.

Keywords: Oxidative stress; breast cancer; glutathione level; GSTs genotypes.

1. INTRODUCTION

Glutathione S-Transferases (GSTs) is a superfamily of phase II detoxification enzymes, involved in the neutralization of xenobiotics, regulate oxidative stress providing cellular protection against oxidative stress. GSTs enzymes catalyze the formation of thio-ether conjugates among endogenous tripeptide glutathione and xenobiotic compounds [1]. Initially Phase I metabolizing enzymes (cytochrome P450) metabolize pro-carcinogens into carcinogenic facilitating GSTs to inactivate and detoxify these toxic substances through biotransformation and to excrete from the body [2]. Eight classes of mammalian cytosolic GSTs are currently recognized, designated as alpha (A), mu (M), kappa (K), omega (O), pi (P), sigma (S), theta (T), and zeta (Z) [3]. Among them, Alpha (GSTA), Mu (GSTM), Pi (GSTP) and Theta (GSTT) are actively involved in neutralization of toxic compounds and prevent tissues from oxidative damage. GSTM1 and GSTT1 isoforms are the most studied genes that revealed genetic variations in human population [4].

Variations in the nucleotide sequence of xenobiotic metabolizing gene give rise to aberrant enzyme activity which differ the capabilities of xenobiotics metabolism [5]. Null genotypes of GSTM1 and GSTT1 have been widely explored in relation to several types of cancer [6] including mouth, lung, bladder, hepato cellular carcinoma and breast cancer [7]. Glutathione (GSH), a tripeptide thiol along with antioxidant enzymes glutathione peroxidase (GPx), catalase and glutathione S-transferase neutralizing free radicals and lipid hydroperoxides, restore damage molecules by hydrogen donation [8]. High intracellular glutathione level is associated with resistance of many cells against oxidative stress [9]. GSTs can regulates GSH by catalyzing the conjugation of glutathione to several electrophilic compounds

including polyaromatic hydrocarbons, preventing various lipophilic compounds from being stored in adipose tissues including breast tissues [10, 11]. Aromatic adducts were found to be higher in women with breast cancer than in healthy control individuals [12].

Epidemiological studies revealed that genetic variations of genes in individual are associated with diseased susceptibility to cancer including breast cancer. Estrogens level is regulated by GSTM1 & GSTT1 that is crucial for breast cancer tumorigenesis [13]. It has been reported that normal or high GSTM1 enzyme activity protects predisposed tissues from somatic DNA mutations by facilitating the detoxification of carcinogens [14].

Oxidative stress is associated with the initiation progression and metastasis in breast cancer development [15]. Imbalance among antioxidants and ROS as well as and RNS have potential threaten to biomolecules damage by these species [16]. Various reactive oxidative species such as hydrogen peroxides, alkoxyl (ROU), peroxy radicals (ROOU), nitric oxide (NO) and malondialdehyde (MDA) have currently been recognized to cause cellular toxicity to damage [17]. During highly oxidative stress, tumor stroma releases high energy nutrients that fuel cancer cells and facilitate their growth and survival [18]. Various studies revealed that oxidative stress and production of reactive oxygen species are critical to damage cell membranes, mitochondria as well as proteins and DNA to promote carcinogenesis. The aim of the current study was to explore the role of GSTs enzyme and the critical burden of oxidative stress that has been proposed to have crucial role in breast cancer pathogenesis. Our study will be helpful to understand the biochemical & molecular mechanism in cancer pathogenesis for the early prognosis of breast cancer. The study will also open new therapeutic fronts for disease management.

2. MATERIALS AND METHODS

2.1 Recruitment of Patients and Samples Collection

Patient's recruitment and sampling collected were performed after a prior approval from departmental ethical committee of Kohat University of Science & Technology, (KUST) Kohat and the collaborating hospitals. This study included 120 breast cancer patients (diagnosed by oncologist) along with controls. Tumors tissues were performed from patients having histologically confirmed breast carcinoma and adjacent normal controls (ANCTT) were taken (by oncologist) as 2 cm away from tumor site of same patient. All tissues samples were collected in RNA Later solution stored at -80°C for further analysis. Written approvals were taken from the patients before samples collection. Patients were divided into two studied cohorts. One was equal or less than 45 years and the second cohort contained the patients with age greater than 45 years. Demographic and clinical characteristics of the study show that the average age of patients with breast cancer was 46.36 years, ranging from 28 to 66. In the control group, the average age was 44.74 years, ranging from 23 to 68 years (Table 1).

2.2 Biochemical Analysis

2.2.1 Samples preparation and tissues glutathione analysis by HPLC

Glutathion (GSTt, GST reduced and GST oxidized) were determined using HPLC (PerkinElmer Life and Analytical Sciences 710 Bridgeport Avenue Shelton, CT 06484-4794 USA). Briefly tissue sample (100 mg) was homogenized in 3 ml buffer solution (10 mM EDTA, 50 mM NACLO4 and 0.1% H3PO4) and total protein was precipitated by adding 0.5ml metaphosphoric acid (5% w/v). The samples were vortexed for 20 min and centrifuged at 8000×g for 10 minutes at 4 °C. The supernatant was transferred to auto-sampler vials of HPLC for analysis. Standard curve was generated at different concentration of glutathione (sigma Aldrich). The column used in HPLC was MZ Inertsil ODS, 5 µm with dimension was 125mm x 4mm. The injection volume was 20 µL and the flow rate was adjusted as 1.0 mL/min. Detection was implemented at 385 nm and emission 515 nm by fluorescence detection excitation, while column temperature was set at 30°C.

2.3 Tissue Glutathione S Transferase (GST) Enzyme Assay

Tissue glutathione S transferase enzyme assay of breast cancer patients was performed using commercially available kit (sigma Aldrich). GST catalysis the conjugation of 1-Chloro-2,4-dinitrobenzene (CDNB) with L-glutathione through their thiol group. The complex (glutathione-DNB) formed as a result of this reaction absorbs at 340 nm. The absorbance was directly proportional to GST activity in sample.

2.4 Molecular Analysis of Glutathione S Transferase (GSTs) Genotypes

2.4.1 DNA extraction and quantification

Genomic DNA was extracted from all recruited samples (tumors and controls) for the amplification of GSTs isoforms (GSTM1 & GSTT1) gene, by phenol-chloroform DNA extraction protocol. Extracted DNA were substantiated by horizontal electrophoresis on 1% agarose gel, stained with ethidium bromide under UV eliminator and quantification was performed by nanodrop based on spectrophotometric principle.

2.5 PCR Amplification of GSTs (GSTM1 & GSTT1) Genotypes

Identification of GSTs genotype was performed by multiplex PCR as described by Farmanullah (2013) using gene specific primers along with β-globulin (internal control). Genomic DNA (100 ng) from tumors and control was amplified with 1 U taq polymerases (solis biodyne) in total volume of 25 µL. briefly the PCR reaction contained 1x reaction buffer, 200 pm of each primers (GSTT1-forward GAACTCCCTGAAAAGCTAAAGC, GSTT1-reverse GTTGGGCTCAAATATACGGTGG, GSTM1-forward TTCCTTACTGGTCCTCACATCTC, GSTM1-reverse TCACCGGATCATGGCCAGCA, β-Globuline-forward GAAGAGCCAAGGACAGGTAC, β-Globuline-reverse CAACTTCATCCACGTTCCACC), 2.3 mM MgCl₂, 200 µM dNTPs and 1U polymerases. Thermocycler condition were: 95°C for 5 minutes (initial denaturation), followed by 35 cycle of 95°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute and finally one cycle at 72°C of 5 minute. The PCR product were verified in 2% agarose under

UV eliminator. DNA size marker (100 bp) was run to evaluate PCR products on agarose gel. The determination of GSTT1 was based on the presence of a 480 bp band (wild GSTT1), while

its absence is GSTT1 (-). Similarly the GSTM1 band appear on 512 bp (wild GSTM1) and its absence is GSTM1 (-). The β -globuline used as an internal control was present at 268 bp (Fig. 1).

Table 1. Demographic and Clinico-pathological coherence of Breast Cancer patients

Study Variable		N (%)	Frequency of Study Cohort I (< 45 Years)	Frequency of Study Cohort II (\geq 45 Years)
Menopausal Status	Pre-menopausal	33(66%)	11	02
	Post-menopausal	17 (34%)	00	12
Histological Types	IDC	15(60%)	03	12
	ILC	05(20%)	02	03
	DCI	05(20%)	03	02
Disease Stages	Stages I	6 (12%)	10	06
	Stage II	39 (78%)	0	06
	Stage III	05 (10%)	0	03
Disease TNM Grading	Well Differentiated (G1)	03 (12%)	0	03
	Moderate Differentiated (G2)	19 (76%)	08	11
	Poor Differentiated (G3)	03 (12%)	0	03

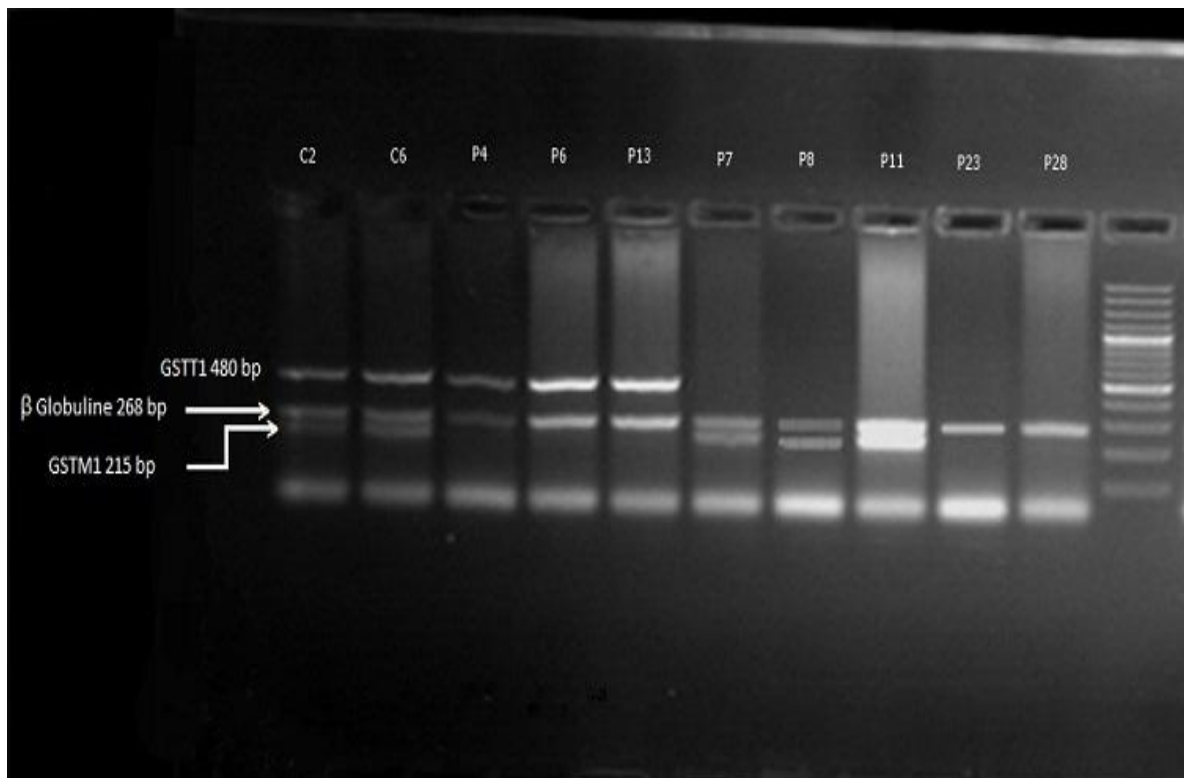


Fig. 1. Gel electrophoresis of amplified product of GSTM1 & GSTT1 genotypes

2.6 Statistics Analysis

OriginPro 2015 (OriginLab, Northampton, MA) statistic software was used for data analysis. The results were expressed as S.D and SEM to determined descriptive statistics. One way ANOVA, pearson's correlation, Kruskal wallis and Mann-Whitney's test were used at 95% confidence interval to determine the influence of age, age at menarche, age at menopause and area of cancer in breast cancer. To evaluate possible effects of glutathione level. P-value lower than 0.05 was considered as significant.

3. RESULTS

3.1 Evaluation of Serum Glutathione Level and GST Concentration in Breast Tumors

Evaluation of tissue glutathione level (GSht, GSH, GSSG and redox) and GST concentration in breast cancer patients and control show that the glutathione level (GSht $p < 0.001$, GSSG $p = 0.0865$) were significantly lower in tumor than control tissues. While the GSH (< 0.001) level were significantly higher in tumor tissues than control tissues which increased the redox ratio (GSH/GSSG $p < 0.05$). The tumor cells are under highly oxidative stress which increased its own GSH level and this elevated level of GSH show resistance to chemotherapy. The GST

($p = 0.90972$) antioxidant exhibited no change in tumor and control tissues.

3.2 Downregulation of GSTs Activities promote Oxidative Stress in Breast Cancer Patients

Serum glutathione level and GST concentration was analyzed among two studied cohort (≤ 45 years and > 45 years) in both tumor tissues and adjacent normal control tissue in breast cancer patients. In the 1st cohort the glutathione level (GSht $P < 0.001$, GSSG $P < 0.001$) and GST concentration ($p < 0.001$) were significantly decreased in tumor tissues than control tissues and the GSH level ($p = 0.201$) were non significantly decreased in tumor tissues than control tissues. In the 2nd cohort the glutathione level (GSht $p < 0.001$, GSSG $p < 0.001$) were lower in tumor tissues as compare to control tissues where the GSH level ($p = 0.201$) and redox ratio ($p = 0.001$) was increased in tumor tissues as compared to control. The concentration of GST show no significance difference in tumor and control tissues. Moreover the glutathione level were also observed in pre and post-menopausal women which is increased in premenopausal women (GSH $p = 0.608$, GSSG $p < 0.001$, redox $p = 0.006$) and post-menopausal women with decreased glutathione level (GSH $p = 0.608$, GSSG $p = 0.001$). The GST concentration ($p < 0.001$) increased in pre-menopausal women.

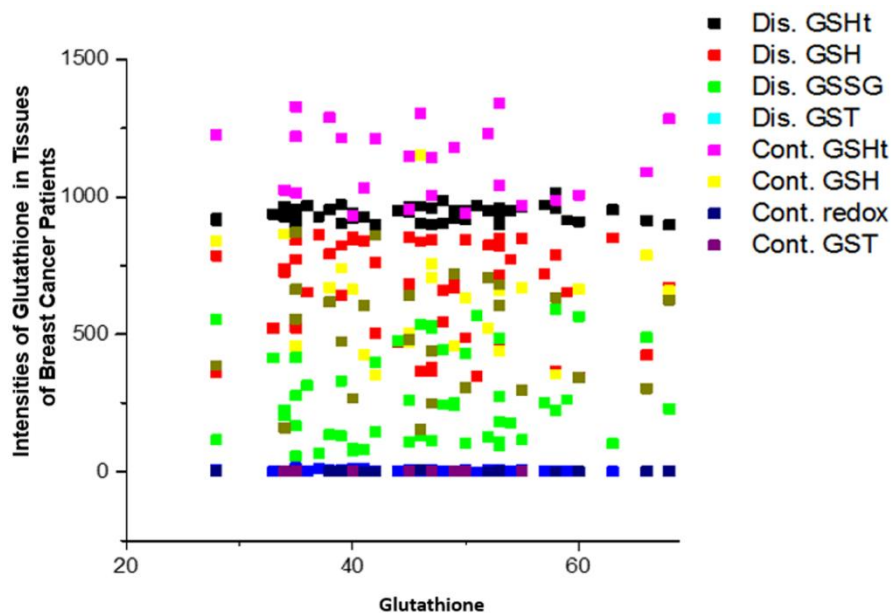


Fig. 2. Association of GSH level with age

Table 2. Analysis of endogenous GSH levels in breast cancer patients and its correlation with above and below 45 years age of patients

Variables	Analysis	Samples	N	Mean	SD	SEM	95% CI of Mean		Skewness	P value
							Lower	Upper		
Overall	GSH total	Control	25	1124.31	134.32	26.86	1068.87	1179.76	0.09976	<0.001
		Diseased	50	937.25	27.021	3.821	929.578	944.937	0.2849	
	GSH red	Control	25	618.511	183.75	36.751	542.658	694.363	0.86314	<0.001
		Diseased	50	670.941	174.901	24.734	621.234	720.647	-0.66699	
	GSH oxi	Control	25	505.808	206.55	41.31	420.54	591.071	-0.0907	0.08654
		Diseased	50	268.512	166.332	23.522	221.241	315.783	0.57845	
Redox	Control	25	1.740	1.636	0.327	1.065	2.416	2.407	0.00119	
	Diseased	50	4.076	3.558	0.503	3.0656	5.088	1.2238		
GST	Control	10	2.156	0.751	0.237	1.6191	2.6933	0.34659	0.90972	
	Diseased	10	2.156	0.751	0.237	1.6191	2.6933	0.34659		
>45	GSH total	Control	13	1117.07	138.59	38.438	1033.32	1200.82	0.361	<0.001
		Diseased	28	938.516	30.468	5.758	926.702	950.331	0.3502	
	GSH red	Control	13	651.044	197.787	54.856	531.522	770.565	1.06552	0.201
		Diseased	28	637.066	188.255	35.577	564.068	710.064	-0.410293	
	GSH oxi	Control	13	466.025	200.623	55.643	344.79	587.26	-0.0543	<0.001
		Diseased	28	299.61	175.386	33.145	231.602	3687.618	0.43953	
Redox	Control	13	1.979	1.855	0.514	0.858	3.101	2.485	0.001	
	Diseased	28	3.066	2.589	0.489	2.062	4.071	0.97201		
GST	Control	4	6.054	1.377	0.688	3.863	8.245	-0.0933	<0.001	
	Diseased	4	2.262	0.471	0.235	1.512	3.012	-0.328		
≥ 45	GSH total	Control	12	1132.17	135.206	39.031	1046.27	1218.079	-0.183	<0.001
		Diseased	22	935.656	22.478	4.792	925.689	945.623	-0.12711	
	GSH red	Control	12	583.267	168.501	48.64	476.21	690.327	0.497	0.201
		Diseased	22	714.055	149.448	31.862	647.794	780.317	-0.968	
	GSH oxi	Control	12	548.906	212.801	61.43	413.699	690.327	-0.227	<0.001
		Diseased	22	228.934	148.578	31.677	163.057	294.809	0.7003	
Redox	Control	12	1.481	1.394	0.402	0.595	2.366	2.3594	0.001	
	Diseased	22	5.362	4.221	0.9001	3.491	7.234	0.87517		
GST	Control	6	2.0857	0.9309	0.38	1.108	3.0627	0.673	<0.001	
	Diseased	6	2.0857	0.9309	0.38	1.108	3.0627	0.673		

Table 3. Association of tissue glutathione level in relation to their hormonal effect

Variables	Analysis	Samples	N	Mean	SD	SEM	95% CI of Mean		Skewness	P value
							Lower	Upper		
Pre-menopaus	GSH total	Control	17	1127.03	133.479	32.373	1058.41	1195.66	-0.10926	<0.001
		Diseased	33	934.553	25.052	4.361	925.67	943.435	0.11986	
	GSH red	Control	17	629.777	203.184	49.279	525.309	734.245	0.9783	0.608
		Diseased	33	679.382	169.258	29.464	619.366	739.399	-0.66374	
	GSH oxi	Control	17	497.258	223.658	54.245	382.263	612.252	0.04134	<0.001
		Diseased	33	260.058	161.008	28.027	202.9678	317.149	0.48514	
	Redox	Control	17	1.9214	1.90688	0.46249	0.94097	2.90182	2.09283	0.006
		Diseased	33	4.63	3.9133	0.6812	3.24242	6.0176	1.0409	
GST	Control	9	5.645	1.125	0.375	4.780	6.510	0.12429	<0.001	
	Diseased	9	2.115	0.784	0.261	1.512	2.718	0.5372		
Post-menopaus	GSH total	Control	8	1118.55	145.183	51.330	997.172	1239.925	0.5751	<0.001
		Diseased	17	942.508	30.602	7.422	926.774	958.243	1.0136	
	GSH red	Control	8	594.571	142.995	50.556	475.023	714.117	-0.6228	0.608

Table 4. Glutathione status in breast cancer patients at various stages

Variables	Analysis	Samples	N	Mean	SD	SEM	95% CI of Mean		Skewness	P value
							Lower	Upper		
Well differentiated Grade I	GSH Total	Control	25	1124.3	134.320	26.86	1068.87	1179.76	0.100	0.00
		Diseased	29	933.12	24.763	4.598	923.701	942.539	-0.086	
	GSH red	Control	25	618.51	183.759	36.75	542.658	694.363	0.863	0.056
		Diseased	29	689.37	173.260	32.17	623.470	755.280	-0.856	
	GSH oxi	Control	25	505.80	206.558	41.31	420.545	591.071	-0.091	0.004
		Diseased	17	249.30	163.908	30.43	186.960	311.655	0.671	
	Redox	Control	25	1.697	1.391	0.278	1.122	2.271	2.719	0.007
		Diseased	29	4.906	3.974	0.738	3.394	6.418	0.957	
GST	Control	10	5.793	1.160	0.366	4.963	6.624	-0.094	0.048	
	Diseased	7	2.083	0.849	0.321	1.297	2.870	0.697		
Moderately differentiated Grade II	GSH Total	Control	25	1124.3	134.320	26.86	1068.87	1179.76	0.100	0.00
		Diseased	18	946.10	28.907	6.813	931.724	960.475	0.497	
	GSH red	Control	25	618.51	183.759	36.75	542.658	694.363	0.863	0.053
		Diseased	18	644.99	178.840	42.15	556.061	733.932	-0.540	

	GSH oxi	Control	25	505.80	206.558	41.31	420.545	591.071	-0.091	0.00
		Diseased	18	298.24	171.708	40.47	212.851	383.628	0.522	
	Redox	Control	25	1.697	1.391	0.278	1.122	2.271	2.719	0.765
		Diseased	18	3.145	2.679	0.631	1.813	4.478	1.113	
	GST	Control	10	5.793	1.160	0.366	4.963	6.624	-0.094	
		Diseased	3	1.972	0.834	0.481	-0.101	4.046	-0.535	
Poorly differentiated Grade III	GSH Total	Control	25	1124.3	134.320	26.86	1068.87	1179.76	0.100	0.087
		Diseased	3	924.20	31.511	18.19	845.924	1002.48	1.307	
	GSH red	Control	25	6.18.51	183.759	36.75	542.658	694.363	0.863	0.067
		Diseased	3	648.41	213.525	12.32	117.985	1178.83	-0.453	
	GSH Oxi	Control	25	505.80	206.558	41.31	420.545	591.071	-0.091	0.001
		Diseased	3	275.79	194.189	11.21	-206.59	758.184	1.023	
	Redox	Control	25	1.697	1.391	0.278	1.122	2.271	2.719	0.028
		Diseased	3	1.641	1.121	0.647	-1.143	4.426	1.628	

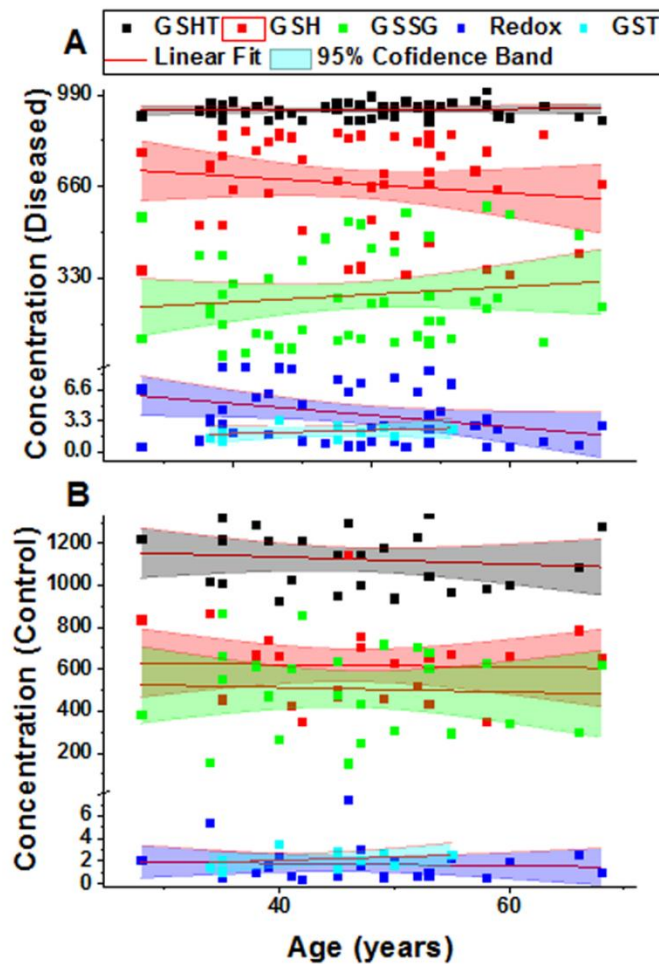


Fig. 3. Correlation of endogenous GSH level with different age group

Curiously the glutathione level (GSht $p < 0.001$, GSH $p = 0.360$, and GSSG $p < 0.001$) decreased in stage I & III than control while the GSH level was increased in stage II than control. Redox index increased in all grades ($p = 0.024$). The GST concentration decrease with advance grades. When compared the diseased status in all the stages it revealed that the GSH level increased from stage I to stage II and then dropped in stage III. According to the TNM grading system the GSH level ($p = 0.549$) decreased from grade I to III. The GST antioxidant exhibited variation of demotion. This reduce concentration of GST indicates its prime role in the inhibition of development of cancer.

3.3 Evaluation and Association between GSTM1 & GSTT1 null Genotypes and Breast Cancer Risk

The risk of breast cancer associated with GSTM1 & GSTT1 genotypes was assessed in 50 cases

and 25 control. Demographical characteristics and genotyping distribution in normal controls and breast cancer patients are shown in the Table 5. The overall frequencies of null genotype GSTM1 in controls and in patients with breast cancer were 36.0% and 72.0%, respectively, and the frequency of GSTT1 null is 20% in control and 38% in patients. Whereas GSTM1 & T1 null genotype is 10% in patients which is not appear in control show oxidative stress.

3.4 Evaluation of GSTM1 & GSTT1 Null Genotypes among Different Age Group and Diseased Status in Breast Cancer Patients

By separating the total patients and control into two groups on the basis of their respective ages, such as less than forty-five years ≤ 45 and greater than forty-five years ≥ 45 . The population range is between ages of 23 to 68 years. Almost 44% patients and 68% control

were under the age of ≤ 45 and 56% patients and 32% control was above the age of 45. The genotype age wise distribution of ≤ 45 age patients is GSTM1 polymorphism 41.66%, GSTT1 is 47.368% and GST1 & M1 null polymorphism is 20%. The genotype greater than the age of 45 (> 45) GSTM1 was 58.33%, GSTT1 was 52.631% and null GSTT1 & GSTM1 was 80%. The genotype distribution of control in age ≤ 45 years GSTM1 null is 3(33.33%) and GSTT1 null is 3(60%) and in age >45 years of GSTM1 null is 6(66.66%) and GSTT1 null is 2 (40%).

GST genotypes in well differentiated patients show that GSTM1 null was 65.517%, GSTT1 was 41.379% and GSTM1 & GSTT1 was 6.896%. Similarly in moderately differentiated patients the GSTM1 null was 77.777%, GSTT1 was 38.888% and GSTM1 & GSTT1 null polymorphism was

16.666%. But in all 50 breast cancer patients, the poorly differentiated patients was only 3 and they all have GSTM1 null.

3.5 Significant Correlation between Tissues Glutathione S-transferase Level and Glutathione Level in Breast Cancer Patients

There is significant correlation between tissue glutathione level and breast cancer. Present study further evaluate the expression of various biomarkers, including tumor, proliferation, and invasion and metastasis in cancer tissue and ANCT samples of breast cancer patients The dysregulation of glutathione level were associated with breast cancer and GSTM1 null genotype could be a useful marker for breast cancer prognosis.

Table 5. Genotyping frequency of patients and control

Genotypes	Frequency		p-value
	Patients(n=50)	Control(n=25)	
GST M1 Polymorphism			
M1 (null)	36(72%)	9(36%)	0.264
M1 (wild)	14(28%)	16(64%)	
GSTT1 Polymorphism			
GSTT1 (null)	19(38%)	20(80%)	0.025
GSTT1 (wild)	31(62%)		
GSTT1 & M1 null Polymorphism	5(10%)	0	

Table 6. Age wise genotype distribution of patients

Variables	GSTM1 null genotype		GSTT1 null genotype		GSTM1 & T1 null genotype Patients
	Control	Patients	Control	Patients	
≤ 45 years	3 (33.33%)	15 (41.66%)	3 (60%)	9 (47.368%)	1 (20%)
>45 years	6 (66.66%)	21 (58.33%)	2 (40%)	10 (52.631%)	4 (80%)

Table 7. Correlation between GSH level and breast cancer

Study Cohorts	Sample types	DF	Pearson's r	Adj. R-Square	F Value	Prob>F
GSH total	Control	23	-0.12743	-0.02653	0.37966	0.54384
	Diseased	48	0.04862	-0.01842	0.11375	0.73739
GSH red	Control	23	-0.03264	-0.04237	0.02453	0.87691
	Diseased	48	-0.1386	-0.00122	0.94016	0.3371
GSH oxi	Control	23	-0.05383	-0.04045	0.06684	0.7983
	Diseased	48	0.1306	-0.00342	0.83287	0.366
Redox	Control	23	-0.07048	-0.03829	0.11483	0.73778
	Diseased	48	-0.28135	0.05997	4.12605	0.04778
GST	Control	23	0.29332	-0.02821	0.75308	0.41078
	Diseased	48	0.29332	-0.02821	0.75308	0.41078

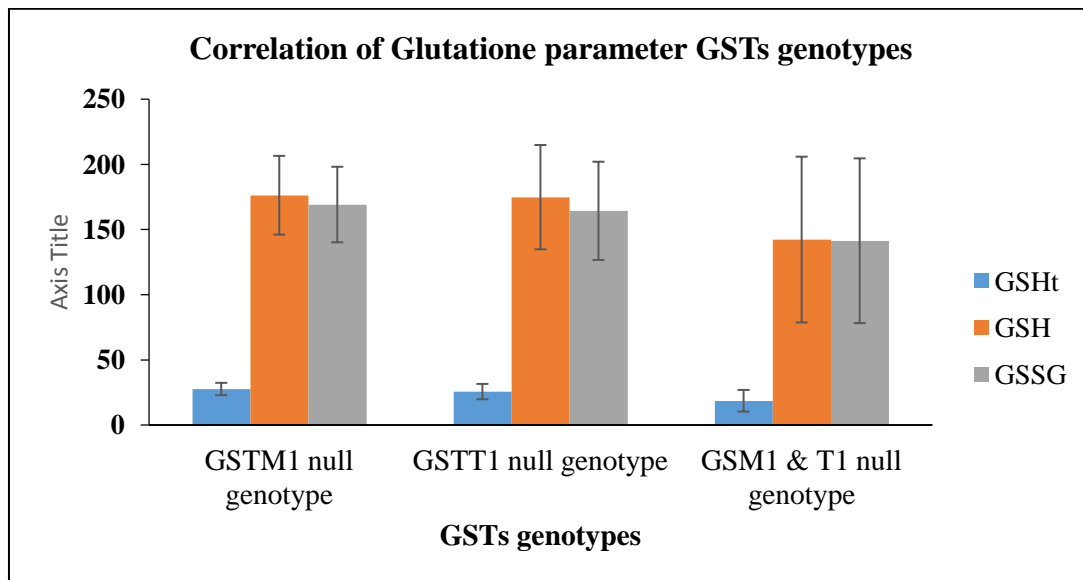


Fig. 4. Correlation of Glutathione parameter GSTs genotypes

4. DISCUSSION

Lipid peroxidation and increased oxidative stress are concerned in carcinogenic processes. The aggregate of this damage depends not only on ROS levels but also on the body's defense mechanisms against them intermediated by various cellular antioxidants [19,20]. Humans have well defined defense mechanism to prevent body against stress and ROS for the protection of integrity of genome, in which the most important one is glutathione which directly decreases reactive oxygen species and acts as first line of defense against oxidative stress [21, 22]. In present study we evaluated the glutathione level in different parameters, glutathione total (GSht), glutathione reduced (GSH), glutathione oxidized (GSSG), redox index (GSH/GSSG) and GST concentration in breast cancer patients compared with ANCT control. Comparing different age group of breast cancer patients with their respective control. We observed that there was a decrease in GSht, GSH and GSSG activities in all age group of patients except >45 years and >50 years of post-menopausal women where the level of GSH and GSSG is higher in patients than control. Several studies reported lower level of GSht, GSH and GSSG in breast cancer patients which supported our results [23,24]. More than a few study shows a decrease in blood GSH level in circulation has been described in numerous diseases comprising malignancies. In breast cancer patients the lower GSH levels support the hypothesis that the glutathione status is inversely

related to malignant transformation. Numerous studies have reported lower levels of GSH in the blood of the patients with breast cancer compared to those of the control subjects. They considered the decrease in GSH concentration can be explained by decreased GSH synthesis and/or increased GSH consumption in the removal of peroxides and xenobiotics [25]. The GSH level is also reported in decreased concentration in head and neck cancer which show that the decreases level caused cancer (Komatsu et al., 2008; Trachootham et al., 2009). The decrease level of glutathione may be due to increase consumption of glutathione can act as an indicator of increase oxidative stress. Moreover (Ballatorri et al) demonstrated that the antioxidant capacity of neoplastic cells increased due to high level of GSH which making the cell more resistant to chemotherapy. So these high expression of GSH can be considered as a sign of low response to chemotherapy which may have further contributed to the development of metastasis [26]. The increased levels of glutathione (GSH, GSSG) in breast cancer tissues may be result of stress and excess need of glutathione in turn increase tumor load and results in un-controlled cell growth and division resulting in cancer pathogenesis.

Cell and tissues can use GSH for their defense system against oxidative stress which is the most efficient substance and is demonstrative of redox environment of the cell (Cacciatore et al., 2010) [27]. Our study also indicated that the concentration of antioxidant enzyme GST show

not significant difference in all age group, however the concentration of GST is slightly lower in the age of >45 years and \leq 45 years of pre-menopausal women. This indicates that GST not play a significant role in breast cancer, however the reduce activity of GST may be due to depletion of GSH. My results was related to the (Sharmila et al. 2004) which showed similar GST levels in tumor tissue of colorectal cancer when compared to the normal colorectal tissue of subjects. The excessive oxidative stress in breast cancer has been correlated to the severity of the disease e.g. Stage III ($P>0.05$) patients have been shown to have higher levels of oxidative stress compared to II, who have been shown to have higher levels of ROS stress compared to Stage I ($P<0.05$) patients. Our finding is in accordance with the previous study reports [28]. GSTs play an important role in the detoxification process and are involved in the biotransformation of exogenous substances, containing mutagens, carcinogens, and other toxic chemicals, in this manner defending cells from these compounds [29]. In the second part of the study we found significant association between polymorphism in breast cancer susceptibility gene and clinicopathological features of breast cancer and disease development. This finding providing useful prognostic information. We evaluate that age was significantly related with the vulnerability to development of breast cancer disease. In our study we observed the relationship of GSTM1 and GSTT1 genotypes and the risk of breast cancer. Polymorphic GST alleles to be associated with effect on various diseases or different risk of breast cancer have been studied. The GST variations modify the catalytic function of enzymes. Therefore, those individuals may be at a higher risk of chronic effects of disease who produce less specific detoxification enzymes. Sohail et al. [30] shown a study on 100 patients diagnosed with breast cancer and Lu et al. [31] lead a meta-analysis of 34,658 patients; both studies found an increased susceptibility of heterozygote genotypes for breast cancer,. The frequency also suggests that this gene might be involved in chemical detoxification. According to our results of association studies, it can be clearly understood that GSTM1 can be the major cause for cancer association. However, our result show that the combine lack of GSTM1 and GSTT1 polymorphism increase cancer progression. Therefore, it is important to test the predictive value of the GSTM1 and GSTT1 null variant in a population study.

We also investigated that the risk of breast cancer is higher in post-menopausal women related to GSTM1 null genotype. In women with prolonged exposure to sex hormones, e.g., early menarche and late menopause epidemiological studies designated an increased breast cancer risk [32,33]. There is no visible influence of age factor on GSTT1 null genotype. However the post-menopausal women or women above 45 years show greater GSTT1 oxidative stress and breast cancer risk. In addition, both GSTM1 and GSTT1 null genotypes are also associated with the occurrence of cancer or disease, between GSTM1 and GSTT1 null genotypes an association has been reported in various cancer, e.g. lung, bladder and colon cancer. Consequently, patients might be prone to develop hepatic liver disease with HCV infection who are genetically predisposed to produce less specific enzyme activity. [34,35] have also observed an increased risk of breast cancer associated with combined effects of GST genotypes, but the genotype combinations were not the same for both papers.

5. CONCLUSION

In our study we concluded that there is significant correlation between tissue glutathione level and breast cancer. Our study suggest that the dysregulation of glutathione level were associated with breast cancer and GSTM1 null genotype could be a useful marker for breast cancer prognosis.

CONSENT

All the specimens were collected after getting signed and informed consent from the patients for this study.

ETHICAL STANDARD

The study was conducted after prior approval from departmental ethical review committee of Kohat University of Science Technology, Kohat (KUST), Pakistan and the collaborating hospital (LRH, Peshawar, Pakistan). Members of this committee include Dean ORIC (Office of Research Innovation and Commercialization) Pro. Dr. Fida Younas, Pro. Dr. Shafiq ur Rehman, Prof. Dr. Murad Ali, and Prof. Dr. Syed Tasleem Hussain (Head of the Department). The ethical review board approved the project entitled "Down Regulation in Glutathione S Transferase

Stimulate Oxidative Stress and Genetic Susceptibility of Human Breast Cancer Patients”.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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