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Interaction of glutathione S- transferase M1 and T1 gene polymorphism and oxidative stress associated biomarkers (GSH and GST) in pesticide exposed workers in Himachal Pradesh

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ABSTRACT

Humans get oxidative stress as a result of using several pesticides and herbicides in excess. Occupational and environmental exposures harm the public's health even if their effectiveness has led to their widespread use. The current study's goal was to assess the activity of the antioxidants glutathione reductase and glutathione S-transferase in a group of HP, India residents who had been exposed to pesticides. Pesticide exposed individuals had their glutathione transferase (GSTM1 and GSTT1) genetic polymorphisms investigated to check for any connections to oxidative stress and toxicity. For the genotyping of GSTM1, GSTT1, and antioxidant enzymatic assays such as GSH and GST, 5 ml of blood was drawn from the population under study. Antioxidant enzymatic activity of GSH (205.63 ± 42.42) and GST (1.65 ± 0.39) were decreased in pesticide exposed group as compared to unexposed group GSH (273.76 ± 56.70) and GST (2.40 ± 0.81). We observed non-significant ($p>0.05$) results for the association of GSTM1 with GSH and GST enzyme activity as well as similar result have been observed for the association of GSTT1 with GSH and GST activity in the studied population. According to the study, prolonged pesticide exposure results in oxidative stress, which is connected to the necessary genetic variance. The findings may pave the way for further investigation into the toxicogenetics and health impacts of pesticides.

Key words: *Pesticide, GSTM1, GSTT1, GSH and Oxidative stress.*

Introduction

The term "pesticide" refers to a broad and diverse class of compounds designed to prevent and eliminate weeds or other pests, has various biological effects, targets, and chemical structures. The public health is threatened by occupational and environmental exposure, despite the fact that their effectiveness has led to their widespread usage (Martenies and Perry, 2013). Low quantities of pesticides are still present in the environment, but some workers, like those who work in greenhouses might be ex-

(*Associate Professor)

posed to excessive doses, which might have negative health effects. Although the phrase "pesticide exposure" would seem to be quite general, epidemiological studies are frequently biased by an inadequate evaluation of exposure and cannot take individual pesticide exposure into account, because they are virtually always together (Sultana Shaik *et al.,* 2016).

Acute exposure causes symptoms that are simple to recognise, but persistent exposure can lead to the onset of chronic pesticide-related disease (Gangemi *et al.,* 2016, Mamane *et al.,* 2015; Parrón *et al.,* 2014; Yu *et al.,* 2013; Fenga *et al.,* 2017; Costa *et al.,* 2017; Gangemi *et al.,* 2016; Suratman *et al.,* 2015; Falzone *et al.,* 2016; Polo *et al.,* 2017). Chronic pesticide exposure has been linked to genetic and epigenetic changes underpinning the onset of several illnesses (Collotta *et al.,* 2013). Certain types of pesticides have been identified as the root cause of gene alterations and polymorphisms that disrupt crucial regulators of hazardous chemicals and xenobiotic metabolisms. (Koutros *et al.,* 2011). Additionally, it has been shown that pesticides can alter the expression levels of micro RNAs (Weldon *et al.,* 2016, Yuan *et al.,* 2018), which are known to have a role in the emergence of several chronic degenerative disorders (Falzone*et al.,* 2019; Falzone*et al.,* 2019; Candido *et al.,* 2019).

Additionally, pesticides can operate as endocrine disruptors, especially if used in combinations, and recent research have linked oxidative stress to the harmful health consequences of pesticides (Petrakis *et al.,* 2017). The physiological equilibrium between the creation and the removal of oxidant chemical species by antioxidant enzymes is altered during oxidative stress, which is caused by the cell's inability to neutralise an excess of oxidative species. The generation of free radicals has the potential to damage all cellular constituents, including proteins, lipids, and DNA (Surajudeen *et al.,* 2014; Wafa *et al.,* 2013). Additionally, oxidative stress magnifies the inflammatory response, which affects the pathophysiology of many diseases (Aouey *et al.,* 2017; Fenga *et al.,* 2017). Numerous studies show that oxidative stress, which is brought on by an excess of reactive free radicals and a reduced ability to neutralise them, is a major factor in the development of cancer, its progression, and the treatment of cancer patients with cytostatic medications. Special enzymatic components of the antioxidative system, such as glutathione S-transferase and glutathione-dependent enzymes, as well as non-enzymatic components (vitamin E, vitamin C, glutathione, and flavonoids), regulate the levels of free radicals. The cellular defence against oxidative stress involves reduced glutathione (GSH) and GSH-dependent enzymes such as glutathione S-transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR).

The detoxification of xenobiotic or endogenous substances is carried out by enzymes known as glutathione S-transferases (GSTs), which are engaged in phase II metabolization. The interaction of glutathione (GSH) and the substrate is significantly influenced by GSTs. Polymorphic alleles are responsible for the genetic variations in these enzymes' expression and activity. These polymorphisms affect GST activity, which in turn changes how susceptible we are to hazardous chemicals.

Materials and Methods

The study was carried out among 123 pesticides exposed individuals and 112 unexposed healthy individuals of Himachal Pradesh. The studied population have 185 males and 50 females with ages, ranging from 20 to 62 years. The study's goals were well explained to all the participants and were asked to fill out a questionnaire to collect personal details as well as information related to their socioeconomic status. The research was duly approved by Institutional Ethical Committee, Maharaja Agrasen University, Baddi (HP) India.

Blood sampling from control and exposed population

For DNA extraction and enzymatic assay, 5 ml blood sample was obtained from each individual of studied population in a vacutainer tube containing K_2EDTA. All blood samples were transported to the laboratory in an insulated ice bucket.

Glutathion S-transferase (GST)

Glutathione S-transferase (GST) activity in serum was determined using the substrate 1-chloro-2,4-dinitrobenzene (CDNB), spectrophotometrically in accordance with the method of Habig *et al.*, (1974) with minor modifications. 100 µl blood serum, 2.7 ml 100 mM phosphate buffer (pH 6.5), 100 µL 75 mM GSH and 100 µl 30 mM 1-chloro-2,4-dinitrobenzene (CDNB) made up the test mixture. The net increase in absorbance at 340 nm in comparison to the

blank was used to track the development of CDNB-2,4-dinitrophenyl glutathione. The extinction coefficient of 9.6 mmol/cm will be used to compute the enzyme activity, which will then be reported as units of product generated per milligram of protein per minute.

Reduced glutathione (GSH)

The method described by Tietze, (1969) was used to measure the reduced glutathione (GSH) level of whole blood. 500 µl blood was mixed with 125 µl of 25% TCA and cooled on ice for 5 min followed by further dilution of the mixture with 600 µl of 5% of TCA and centrifugation at 3000 rpm for 5 min to settle down the precipitate. 150 µl of the supernatant was mixed with 350 µl of sodium phosphate buffer (0.2M, pH 8) and 1 ml of DTNB (0.6 mM in 0.2M, pH 8 phosphate buffer). The yellow colour observed at 412 nm against a blank which contained 5% TCA in the place of sample. A standard curve graph was prepared using different concentrations (10.50 nM) of GSH.

Genotyping of *GSTM1* and *GSTT1*

Genomic DNA was extracted from 200 µl of whole blood using ReliaPrep™ Blood gDNA Miniprep kit (Promega, USA). The presence or absence of the *GSTM1* and *GSTT1* genes were determined by multiplex-PCR. As an internal control, a portion of exon 7 of the housekeeping gene such as *CYP1A1* was also co-amplified. The primer sequences (Abdel-Rahman *et al.*, 2007 and Kumar *et al.*, 2012) and product size of the *GSTM1* and *GSTT1* genes are listed in Table 1. Both genes were genotyped using a 25-µl reaction mixture containing 1 µl of genomic DNA template (100 ng/µl), 1µl of each primer (20 pmol/ml), 0.5 µl of dNTPs (200 µM), 2.5 µl of PCR buffer with 15 mM/l $MgCl_2$, and 0.5 µl of Taq polymerase (3 U/ µl) and 19.5 µl sterile nuclease free water. In total 30 thermal cycles were performed. PCR reaction cycle included, initial denaturation (at 94 °C for 10 minutes); denaturation (at 94 °C for 60 seconds); annealing (at 59 °C for 45 seconds); and extension (at 72 °C for 60 seconds). The final extension was carried out at 72 °C 10 minutes and the amplification results were examined on 2% agarose gel.

Statistical analysis

The student t test was used for comparison of age, GST and GSH enzyme activity between studied groups. ÷2 test was applied for difference in gender, consumption habit (smoking) and exposure history among studied population. The influence of *GSTM1* and *GSTT1* polymorphism on studied antioxidant assay among multiple sub-groups was done by post hoc analysis using multivariate ANOVA test. The interaction of different confounding factors such as age, gender, consumption habit (smoking), exposure and *GSTM1* and *GSTT1* genotypes with GST and GSH enzyme activity was studied using linear regression model. All of the statistical analyses were performed with SPSS.20. The level of significance was set at 0.05.

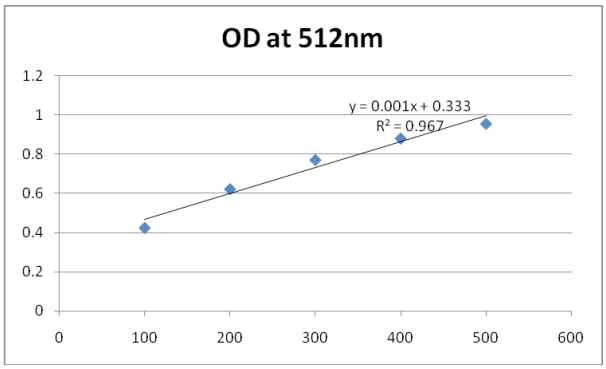
Results and Discussion

Epidemiological data of studied population

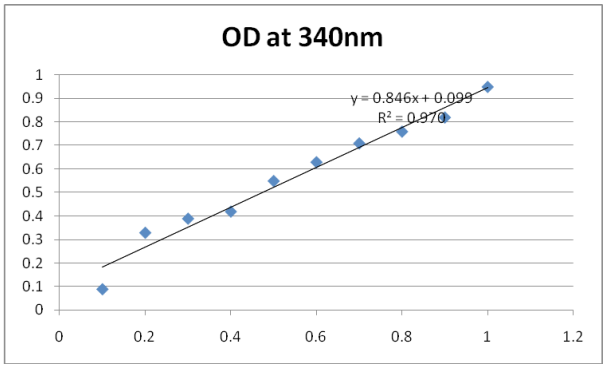
In our study, we have included 123 pesticide exposed individuals, who had been exposed to several pesticides and 112 control population. The information regarding age, gender, exposure, alcoholic status, smoking status and tobacco status have been shown in Table 1.

GSH and GST activity in studied Population

In our current study, we found significant ($p<0.05$)



Standard Curve for GSH



Standard Curve for GST

Table 1. Demographical Characteristics of the control and exposed groups

Variables	Control	Exposed	*P* value
All *N* (%)	112 (100)	123 (100)	
Age in years (Mean ± SD)	37.65±10.14	40.56±11.98	0.046
Age			0.367
20-30	30 (26.7)	26 (21.2)	
31-45	58 (51.7)	67 (54.4)	
46-60	24 (21.6)	30 (24.4)	
Gender			
Male	77 (68.7)	108 (87.8)	0.000
Female	35 (31.3)	15 (12.2)	
Smoking			
Current Smoker	34 (30.3)	48 (39.0)	
Never Smoker	78 (69.7)	75 (61.0)	0.396
Alcohol users			
Current Alcohol User	50 (44.6)	59 (48.0)	0.013
Never Alcohol User	62 (55.4)	64 (52.0)	
Tobacco Chewers			
Current Tobacco Chewer	37 (33.0)	49 (39.8)	0.265
Never Tobacco Chewer	75 (67.0)	74 (61.2)	
Exposure duration			
1-15	-	63 (51.3)	-
15-30	-	46 (37.3)	-
30-45	-	14 (11.4)	-

*The mean age of the control and exposed groups were compared using the Student's t test. The Chi-square test was used to look for variations in the research population's age, gender, Exposure duration and consumption patterns.

Table 2. Enzymatic Activities of GSH and GST in exposed and control Population in Himachal Pradesh

Antioxidant Enzyme	Control (N=112) Mean ± SD	Exposed (N=123) Mean ± SD	*P* value
GSH (Units/mg protein)	273.76 ± 56.70	205.63 ± 42.42	<0.05
GST (Units/mg protein)	2.40 ± 0.81	1.65 ± 0.39	<0.05

The mean values of the control and exposed groups were compared by using the Student's t test.

difference between GSH activity among exposed (205.63 ± 42.42), and control population (273.76 ± 56.70). GST activity among exposed (1.65 ± 0.39) and control (2.40 ± 0.81) were also significantly different (Table 2). The primary oxidant in the cell is GSH, which directly neutralizes free radicals and defends biomolecules from their damage. A significant drop in GSH levels was seen in this study. This drop may be due to the suppression of GSH synthesis and increased use of GSH for the detoxification of free radicals caused by the pesticides. The conversion of GSSG to GSH has likely been adversely affected if the GSH: GSSG ratio has decreased (Singh *et al*., 2001; Das *et al*., 2009). GST is a phase II enzyme that helps to detoxify the end products of phase I processes. Increased expression of GST-pi and total GST activity, as seen in the current study, may constitute a compensatory response to counteract the harmful effects of pesticide exposure (Patel *et al*., 2006). The fact that GST has a strong affinity for GSH level compounds and reduces the ROS burden may serve as additional support (Tavazzi *et al*., 2000; Hubatsch *et al*., 1998; Sharma *et al*., 2013).

Influence of confounding factors on GSH and GST activity

In the current study, we found non-significant ($p>0.05$) association between different confounding factors such as age, gender and consumption habits (smoking, alcohol intake and tobacco chewing) with GSH and GST enzymatic activities in the control population. GSH and GST activity shows significant ($p<0.05$) results for smokers and tobacco chewers in the exposed group. We have also observed non-sig-

nificant (p>0.05) values for age, gender and alcoholic factors for GSH and GST activity in exposed group (Table 3).

Influence *of GSTM1* and *GSTT1* genotype on GSH and GST activity

We found non-significant (p>0.05) results for the association of *GSTM1* with GSH and GST enzyme activity as well as similar result have been observed for the association of *GSTT1* with GSH and GST enzyme activity (Table 4). One of the many biological functions of GSTs, a class of phase II metabolizing enzymes, is to protect cells against harmful and oxidative stress compounds. These enzymes have the capacity to associate GSH to hydrophobic and electrophilic compounds like as medicines, oxidative metabolic products, and carcinogens, which makes them less hazardous and allows additional modification and/or removal (Allocati *et al.*, 2018). The *GSTM1* and *GSTT1* genes are deleted in the null genotype, which results in the lack of the enzymes. Because of the decreased clearance of carcinogens caused by the homozygous deletions, carrier patients are more vulnerable to oxidative damage (Kumar *et al*., 2012). In our study we found non-significant (p>0.05) association of *GSTM1* and *GSTT1* with oxidative stress and contradicting our findings (Ercegovac *et al*., 2015). According to several researches, deletion of the *GSTT1* and *GSTM1* genes is linked to an increase in lipid peroxidation indicators such MDA (Datta *et al*., 2010; Kumar *et al*., 2012; Ghelli *et al*., 2021).

Conclusion

The results of the present investigation seem to indicate a mild augment in oxidative stress associated

Table 4. Influence of age, gender, confounding factors and exposure duration on SOD, catalase and lipid peroxidation

Variables	Control (112)			Exposed (123)		
	N	GSH activity (Mean±SD)	GST Activity (Mean±SD)	*N*	GSH activity (Mean±SD)	GST Activity (Mean±SD)
Age Group20-30	30	259.67 ± 60.49	2.63 ± 0.93	26	205.36 ± 44.4	1.59 ± 0.29
31-45	58	281.95 ± 55.55	2.23 ± 0.70	67	206.77 ± 38.95	1.66 ± 0.39
46-60	24	271.57 ± 53.55	2.53 ± 0.87	30	203.33 ± 49.03	1.70 ± 0.47
Gender MaleFemale	7735	268.79 ± 49.04	2.40 ± 0.77	108	207.26 ± 42.53	1.63 ± 0.36
		284.68 ± 70.51	2.39 ± 0.91	15	193.39 ± 48.30	1.81 ± 0.56
Current Smoker	34	275.66 ± 42.01	2.42 ± 0.82	48	206.99 ± 39.32	1.54 ± 0.25*
Never Smoker	78	272.39 ± 62.37	2.39 ± 0.82	75	204.77 ± 44.53	1.72 ± 0.45*
Current Alcohol User	50	275.32 ± 50.31	2.30 ± 0.75	59	213.14 ± 39.67	1.60 ± 0.33
Never Alcohol User	62	272.49 ± 61.89	2.49 ± 0.86	64	198.71 ± 43.99	1.70 ± 0.44
Current Tobacco Chewer	37	271.66 ± 48.37	2.37 ± 0.83	49	209.75 ± 37.69	1.56 ± 0.30*
Never Tobacco Chewer	75	274.79 ± 60.79	2.42 ± 0.81	74	202.91 ± 45.33	1.72 ± 0.44*
Exposure duration (in years)1-15	-	-	-	63	206.36 ± 46.12	1.62 ± 0.37
15-30	-	-	-	46	202.19 ± 38.11	1.70 ± 0.43
30-45	-	-	-	14	213.68 ± 40.05	1.65 ± 0.41

* Significant at P<0.05, the comparison of SOD activity, catalase activity, and lipid peroxidation assay in numerous subgroups between the exposed and control groups individually was done using a multivariate ANOVA test with post hoc analysis.

Table 5. Influence *of GSTM1* and *GSTT1* genotype on GSH and GST activity

Genotypes	Control Group (112)			Exposed Group (123)		
	N	GSH activity (Mean±SD)	GST Activity (Mean±SD)	*N*	GSH activity (Mean±SD)	GST Activity (Mean±SD)
*GSTM1*Positive	65	274.41 ± 56.99	2.39 ± 0.84	50	203.95 ± 41.63	1.57 ± 0.30
*GSTM1*Null	47	275.61 ± 57.06	2.42 ± 0.79	73	206.79 ± 43.21	1.71 ± 0.44
*GSTT1*Positive	61	278.90 ± 64.72	2.33 ± 0.86	56	208.80 ± 36.31	1.65 ± 0.34
*GSTT1*Null	51	267.60 ± 45.40	2.48 ± 0.75	67	202.99 ± 47.03	1.66 ± 0.44

*NON-Significant at *P* >0.05. Multivariate ANOVA was used for comparison of value among control and exposed population

with pesticide exposure, followed by an adaptive response to increase the antioxidant defences. This phenomenon could be essential for preventing the adverse effects of sustained oxidative stress. In order to advance biomarkers of exposure in workers exposed to pesticides and assess early harm, the evaluation of oxidative stress may be used.

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Conflict of Interest

The authors declare no potential conflict of interest.

Authors Contributions

Shiv Kumar Giri conceived, designed the study and review the original draft. Hemlata collected blood and urine samples, conducted experiments writing original draft. Monika Rani helped to conduct analysis of data. Anil Kumar, Gulab Singh, Anita Saini, and Anuradha Bhardwaj provided critical inputs during the data analysis and manuscript preparation.

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