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Glutathione-S-transferase (GSTM1 and GSTT1) polymorphism and lead toxicity in individuals environmentally exposed to lead

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Abstract

Genetic variation in enzymes involved in metal detoxification could have influence on susceptibility to lead-induced oxidative stress. The present study aimed at evaluating the effects of genetic variation of Glutathione-S- transferase gene on oxidative stress markers (GSH, SOD, CAT and MDA) among individuals environmentally exposed to lead. GSTM1 and GSTT1 null homozygous genotypes were determined by polymerase chain reaction. Blood lead levels were determined using Microwave Plasma Atomic Emission Spectroscopy (MP- AES-Agilent 4210 USA). Activities of superoxide dismutase, catalase and glutathione levels were assayed using Cayman's commercial kits. The study found mean blood lead levels of the study subjects of 78.16± 5.79µg/dl with range from 26.6 to 191.0 µg/dl. The frequencies of GSTM1 and GSTT1 null genotypes were 23% and 50.8% respectively. The frequency was high in individuals with blood lead levels greater than 70µg/dl. Significant decreased activity of antioxidant enzymes (SOD and CAT), glutathione levels and concomitant increased of MDA concentrations were observed in individuals with GSTM1 and GSTT1 null genotypes. Therefore, findings from this study were enough to infer a causal relationship between environmental exposure to lead and oxidative stress. Individuals who are homologous null at GSTM1 and GSTT1 loci predispose to increased risk of multi-organs toxicity from environmental exposure to lead.

Keywords: Lead; Glutathione S-transferase; Polymorphisms; Superoxide Dismutase; Catalase

1 Introduction

Humans tent to accumulate heavy metals primarily due to modern lifestyle and persistent environmental contamination due to mining activities and ever growing industrialization. Detoxification of such metals is an essential process in all living organisms. Glutathione S-transferases (GSTs) are most crucial of phase II enzymes that involved in the detoxification of xenobiotics including the toxic metals. The enzymes initiate the catalytic nucleophilic conjugation of reduced glutathione into various hydrophobic and electrophilic substrates making them more soluble and easier for excretion, thus, protecting the cell from oxidative damage [1, 2]. Therefore, GSTs constitute the major cellular defensive antioxidant mechanism against oxidative onslaught by quenching the reactive free radicals and simultaneous conjugation of GSH to different hydrophobic and electrophilic substrates. GSTs are a multigene family of isozymes identified and broadly classified into eight different classes as alpha, mu, kappa, omega, pi, sigma, theta and zeta that are encoded by GSTA, GSTM, GSTK, GSTO, GSTP, GSTS, GSTT and GSTZ genes, respectively [2, 3].

Molecular epidemiological studies on heavy metal toxicity are now receiving serious attention due to growing problem of metal toxicity. Reported evidences had indicated that individual susceptibility to metal toxicity is strongly mediated by genetic variation. Variations in GST genes both in deletion and single nucleotide polymorphisms produce significant

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alterations in GST activity and hence, ability to protect cell against oxidative stress become compromised [4]. The GSTT1 is identified on chromosome 22q11, encodes by GST theta while GSTM1 is found on chromosome 1p13.3, encodes by GST mu. The two enzymes are identified to have functional polymorphisms (deletion) resulting in impaired catalytic activity, which is associated with greater sensitivity to toxic metals. This type of polymorphism had been reported in the general population [4, 5, 6]. The homozygous deleted GSTM1 and GSTT1 genotypes are believed to be associated with increase susceptibility to metal toxicity.

Gundaker *et al.* [7] have documented the effect of the GSTs (GSTT1 and GSTM1) deletion polymorphisms as potential risk factor for increased mercury toxicity among students in Austria. Deletion in GSTM1 gene and high blood lead concentration were also documented in individuals exposed to lead [4]. Lamichhane *et al*. [8] have reported evidenced reduced birth weight and head circumference in male babies born to the mothers with high blood lead concentration and deleted GSTM1 gene. A number of scientific research findings support the hypothesis that either GSTT1 or GSTM1 null genotype may increase susceptibility of an individual to the risk of heavy metal toxicity. However, Nabgha-e-Amin *et al*. [9] have indicated non-significant relationship between environmental exposure to heavy metal and GSTs deletion (GSTT1 and GSTM1) among children with ASD in Pakistani population.

2 Material and methods

2.1 Study subject

The study comprised sixty five (65) artisanal gold miners from five different mining sites of Zamfara State that have been linked to lead poisoning due to artisanal mining of gold-rich ore. The study was approved by Zamfara State Health Research Ethics Committee (ZSHREC/03/06/2021). Consent form and questionnaire were given to participant to fill and sign, authorizing their participation in the research. Moreover, the researchers explained the informed consent and questionnaire in local (Hausa) language to those that could not read and understand English language.

About ten milliliters (10 mL) of whole blood sample were collected by venipuncture under standard condition; five milliliters (5 mL) were taken into heparinized tubes from each individual and quickly centrifuged at 5000 g for five minutes. The remaining five milliliters of blood and serum obtained from after centrifugation were stored at – 20^{o}C until genotyping and biochemical analyses were conducted.

2.2 Lead Determination

The blood lead concentrations of each individual of the study were assayed using Microwave Plasma Atomic Emission Spectroscopy (MP- AES-Agilent 4210 USA), after applying standard wet digestion method [10].

2.3 Determination of Superoxide Dismutase

The serum activity of the enzyme was analyzed from each participant as described by Sun *et al*. [11]. About two hundred microliter (200 µL) of the diluted radical detector was put into standard wells (tubes A-G) and sample wells as described in the manual instructions. Ten microliter (10 µL) of standard was then taken into designated wells (standard) and ten microliter (10 µL) of sample into the sample wells. Reaction was initiated by immediate addition of 20 µL of diluted xanthine oxidase to all the wells, mixed for ten seconds and covered with plate cover. The reaction plate was then incubated on a shaker for 30 minutes at 25^{o}C. The absorbance was taken at 550 nm using the Rayto (RT100C) plate reader.

2.4 Determination of Catalase Activity

The serum activity of catalase (CAT) enzyme was assayed as described in the method of Johansson and Borg [12]. The enzyme reacts with substrate (methanol) in the presence of excess concentration of hydrogen peroxide. The reaction product produced (formaldehyde) was then quantitatively determined using spectrophotometer with chromogen (4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole) at 450nm wavelength.

2.5 Determination of Glutathione by the DTNB Method

Whole blood (0.1 mL) was added to distilled water (1.9 mL) together with 3ml of precipitating solution (1.67 g glacial metaphosphoric acid, 0.2g disodium ethylenediaminetetraacetic acid (EDTA) and 30 g sodium chloride). Then, the filtrate (0.5 mL) obtained was put into 2 mL of phosphate buffer (pH 6.4). Hence, 0.25 mL of one millimolar (1 Mm DTNB) was then added mixed thoroughly. The product of reaction measured using spectrophotometer at absorbance of 412 nm wavelength [13].

2.6 Determination of MDA in Serum

Concentration of MDA in serum reflects the status of lipid peroxidation. MDA was determined by a thiobarbituric acid reactive substance method. The assay was done using a commercial kit and according to the instruction by the manufacturer. In principle, serum MDA reacts instantly with TBARS producing a red color compound at maximal absorbance at 532 nm wavelength. The concentrations of MDA was calculated from a standard curve and expressed in µmol/mL [14].

2.7 DNA extraction and Genotyping

The genomic DNA was extracted from the whole blood of individuals in the study area using the commercial kit (Thermo Scientific GeneJET Genomic DNA Purification kit #K0722). Genomic DNA (100–150 ng) was amplified together with 0.5µl of each pair of the following primers: GSTM1 gene, DNA samples were amplified with the primers: 5´GAACTCCCTGAAAAGCTAAAGC' 3' and 5'GTTGGGCTC AAATATA CG GTGG3' and GSTT1 primers: 5'GTTGGGCTCAAATATACGGTGG3' and 5'GCATCAGCTTCTGCTTTATGG3' using polymerase chain reaction (PCR). HBB (408 bp) with the primers 5'GAAGA GCCAAG GA CAGGTAC3' and 5'GGAA AATAGAC CAATA GGCAG3' was used as an internal control. The PCR was conducted in a final volume of 25µl for a single reaction mixture consisting of 3.0 µl genomic DNA, 0.5µl dNTPs, and 2.5µl PCR buffer of 3.0µl MgCl2, 14.5µl PCR water and 0.5 units of Taq polymerase. Reaction mixtures were heated at 94^{o}C for 3min for initial denaturation followed by 35 cycles of amplification at denaturing step of 94 ^{o}C for 30 s, an annealing step at 58.3^{o}C for 45 s, extension step at 72^{o}C for 30 s and final extension at 72^{o}C for 10 min. The PCR reaction products were observed using electrophoresis on ethidium bromide-stained 2% agarose gel. The presence of a band at 408 bp (corresponding to HBB) indicated a successful amplification. The presence or absence of a band at 219 and 433 bp indicated wild or deletion genotypes of GSTM1 and GSTT1 respectively.

2.8 Statistical analysis

Analysis of data was performed using statistical software GraphPad Prism (version 5.0). Data were expressed as mean ± SEM. Associations between Lead and GST genotypes were evaluated by tertiles (T1, < 40 µg/dl, T2 45-69 µg/dl and T3, > 70 µg/dl). The comparison between variables was determined by chi-square. Odds ratios (ORs) and their 95% confidence intervals were obtained using logistic regression analysis. Differences were considered significant when $p < 0.05$.

3 Results and discussion

3.1 Demographics, Blood lead Levels and markers of Oxidative Stress

Table 1 depicted some characteristics of study subjects; the mean age of the study subjects was 22.02 ± 7.21 with blood lead concentration range of 26.6 to 191.0 µg/dl and mean of 78.16 ± 5.79µg/dl. Smoking status showed significant ($p < 0.05$) increases of blood lead concentrations in some subjects (smokers, 91.21±1.83 vs nonsmokers 72.90±1.79). The gel electrophoretogram showing the separation of GSTT1 and GSTM1 gene amplicons on agarose gel are presented in Figure 1, 2 and 3.

Table 1 Blood Lead Levels and Oxidative Stress Markers in the Study Population

Variable	BLLS(µg/dl)	SOD(U/ml)	CAT(nmol/ min/ml)	GSH(µg/dl)	MDA(µM)
Age					
10 – 20 yrs.	75.26±8.16	3.05±0.26	35.52±3.89	14.77±0.64	3.32±0.25
21 – 40 yrs.	81.67±8.28	3.32±0.16	24.58±2.99[a]	13.58±0.35	4.24±0.39[a]
Smoking status					
Smokers	91.21±11.83	2.72±0.15	24.78±1.87	12.85±0.23	5.76±0.54
Non-smokers	72.90±9.71	3.13±0.19	33.65±4.35[a]	14.72±0.45a	4.17±0.32[a]

10-20yrs, n = 35, 21-45yrs, n= 30 while n = 38 in smokers and n = 27 in non-smokers. Values are mean ± SEM. Values bearing superscripts on the same column differ significantly ($p< 0.05$) when compared by unpaired test using Graph Pad Prism (version 5.0). GSH: glutathione, GPx: glutathione peroxidase, SOD: superoxide dismutase, CAT: catalase, MDA: malondialdehyde

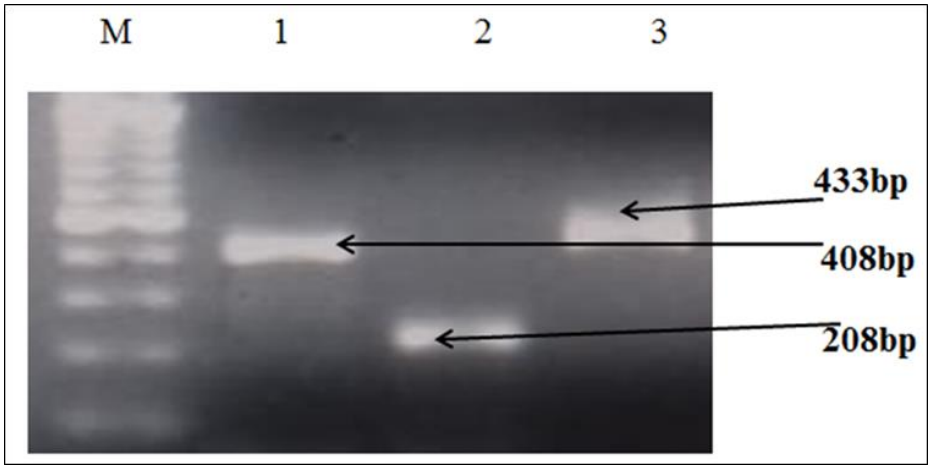

Figure 1 Gel electrophoretogram of PCR product of GSTM1 and GSTT1 and β- globin. M,1,2 and 3 are ladder GBB, GSTM1 and GSTT1 respectively

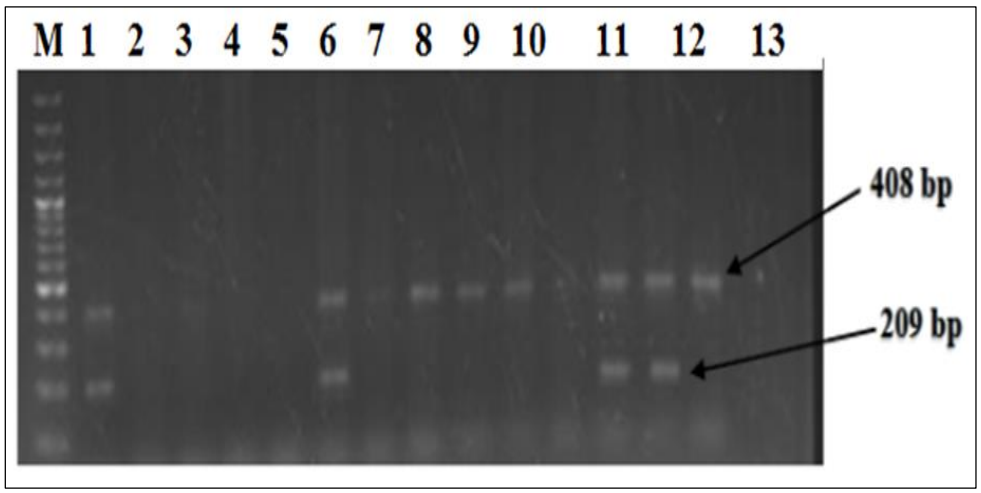

Figure 2 Identification of GSTM1 genes with β- globin.(control) on 3% ethidium bromide – stained agarose gel M and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 are ladder and samples respectively

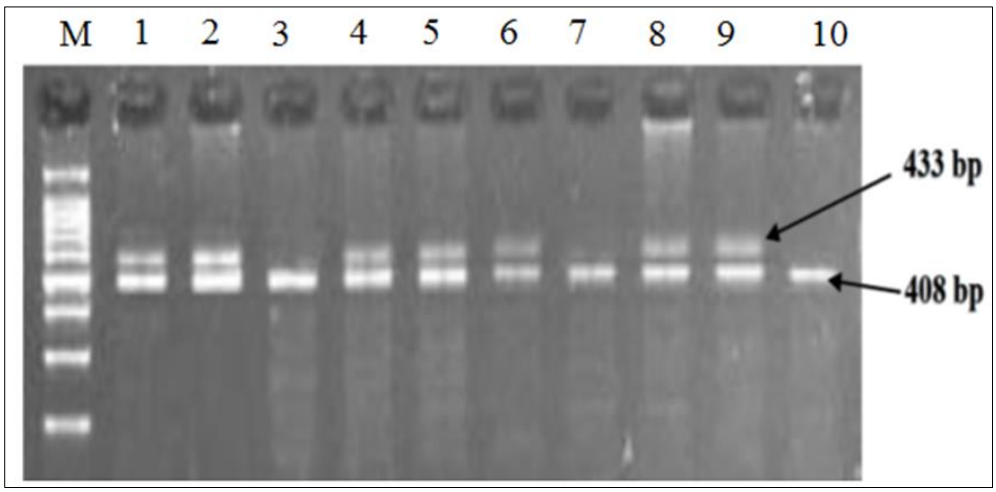

Figure 3 Identification of GSTT1 genes with β- globin.(control) on 3% ethidium bromide – stained agarose gel M and 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 are ladder and samples respectively

The progressive accumulation of heavy metals due to modern life style, industrialization, agricultural practices and of course poor monitoring and evaluation by relevant environmental agencies had continued to exposed human, animal and environmental quality into devastating health conditions. Numerous health implications that affect kidney, liver, brain and reproductive organs had been documented in literature [15, 16]. Therefore, detoxification of these toxic metals is highly essential process in all living organisms. The present study indicates mean blood Pb concentration of 78.16±5.79µg/dl and is influenced by cigarette smoking. Galazyn-Sidorczuk *et al*. [17] had reported that lead in cigarette is recovered and distributed in the process of burning in the smoke, butt and ash which consequently is inhaled by smokers. This could be one of the reasons for increase blood lead concentrations observed among smokers in this study. Similarly, the study observed that the individuals could also be exposed to this toxic metal via ingestion and inhalation of the atmospheric dust generated due to manual gold ore processing methods, rudimentary tools used and poor environmental practices.

Increased ROS production was evidently observed in many experimental and epidemiological studies involving lead toxicity [18, 19]. Lead has been implicated to interfere with pro-oxidant/antioxidant balance which leads to over generation of ROS and reduced cell's antioxidant defense mechanism which eventually leads oxidative stress. Individual's susceptibility and degree of toxicity are strongly mediated by genetic variation in gene that involves detoxification and maintaining antioxidant/oxidant balance that eventually produce oxidative stress. GSTs play significant roles in the mitigation of ROS generated by toxic metal lead, thus protecting the integrity of the cells from oxidative onslaught. Therefore, polymorphisms in GST gene would no doubt, produce an important change in the activity of these crucial detoxifying enzymes [20].

3.2 Prevalence of GSTM1 and GSTT1 Genes Deletion among Study Population

The genotypic frequencies of GSTM1 and GSTT1 are shown in Table 2. The deletion frequencies of the GSTM1 and GSTT1 recorded among the studied population were found to be 23.1% and 50.8% respectively. This indicated that about 10.8% of the individuals in the population were found to have null genotypic deletion of both GSTM1 and GSTT1 genes.

Table 2 Genotype Frequencies for GSTM1 and GSTT1

Gene	Variation	Genotype	Frequency	
			Number	Percentage (%)
GSTM1		Null	15	23.1
	Deletion	Present	50	76.9
GSTT1	Deletion	Null	33	50.8
	Deletion	Present	32	49.2
GSTM1(+/+)&GSTT1(+/+)		Both Present	24	36.9
GSTM1(+/+)&GSTT1(-/-)		Either Null	26	40.0
GSTM1(-/-)&GSTT1(+/+)		Either Null	8	12.3
GSTM1(-/-)&GSTT1(-/-)		Both Null	7	10.8

n= 65, GSTM1: Glutathione-S- Transferase (mu)1: GSTT1: Glutathione-S-Transferase (theta)1 Present: no gene deletion and Null: gene deletion

A number of studies had documented that the prevalence of GSTs (GSTM1 and GSTT1) null genotypes appeared differently among ethnic groups and across geographical locations. Saitor and Ishida [21], had previously shown that the prevalence of GSTM1 null genotype of 10-63%, 42-54% and 42-60% for Caucasians, Asians and Africans respectively, However, the percentage of the GSTT1 null genotype was found as low as 13-26% in Caucasian population but high in Asian population (35-52%). About 27% and 26% prevalence for GSTM1 and GSTT1 null genotypes respectively were recorded in eastern and southern African population [22]. Moreover, considerably high prevalence for GSTM1 and GSTT1 (80% and 58% respectively) was documented in Chinese population [23]. Ebeshi *et al*. [24] have indicated homozygous null GSTM1 genotypic frequency of 37%, 23% and 31% in the three major Nigerian ethnic groups of Hausa, Ibo and Yoruba respectively, while GSTT1 null genotype was as high as 42%, 36% and 35% in the Hausa, Ibo and Yoruba population respectively.

In this study, we found genotype frequencies of GSTM1 and GSTT1 deletion in lead exposed individuals were 23% and 50.8% respectively (Table 2). Khansakom *et al*.[4] reported similar genotype frequencies of GSTT1 and GSTM1 deletion

(32.7% and 57.6% respectively) in Thai population environmentally exposed to lead. Moreover, the observed prevalence of GSTM1 and GSTT1 null genotype in this study was high compared to values documented by Oshodi *et al*. [25] in Austistic subjects from Lagos Nigeria. The study observed that individuals with null genotype of either GSTM1 or GSTT1 had high concentrations of lead in their blood. This indicates that GST polymorphism may invariably influence lead bioaccumulation and toxicity. Therefore, increased blood lead concentrations observed in the individuals with high percentage of GSTM1 and GSTT1 null genotype could be due to complete absence of activity of these genes. As such, increased risk of lead bioaccumulation and of course toxicity was noticed.

3.3 Effect of GSTs Polymorphism on Different Blood Lead Levels

Data generated from blood lead concentrations were divided into three tertiles and analyzed to evaluate possible effects of GSTs polymorphism in the population (Table 3). The GSTs gene variation showed influence on blood Pb in tertile 2 and 3. In the tertile 2, blood Pb concentrations in subjects with non-deleted GSTM1 were significantly (P< 0.05) lower compared to those with deleted genotypes (52.80±3.1 vs. 66.30±3.2 µg/dl). Similarly, subjects with non-deleted GSTM1 had significantly (p< 0.05) lower blood Pb concentrations compared to those with deleted genotypes in tertile 3 (125.90±6.5 vs. 195.80±18.1µg/dl). However, in tertile 2, subjects with non-deleted GSTT1 demonstrated significantly (p< 0.05) higher blood Pb concentrations compared to those with deleted genotypes. Non-significant (P>0.05) increase of blood Pb concentrations in individuals with deleted genotypes was observed in tertile 3.

Table 3 Blood lead level in Tertiles for Different Genotypes

Genotype	Tertile1	Tertile 2	Tertile 3
	Blood lead (< 40 µg/dl)	Blood lead (45-69 µg/dl)	Blood lead (> 70 µg/dl)
GSTM1			
Present	24.4 ±1.9	52.8±3.1	125.9±6.5
Null	33.1±3.4	66.3±3.2[a]	194.8±18.1[a]
GSTT1			
Present	24.1±1.6	62.5±2.4	123.9±8.2
Null	27.9±5.9	50.5±3.4[a]	152.4±12.1

n= 65, Glutathione-S- Transferase (mu)1: GSTT1: Glutathione-S-Transferase (theta)1 Present: no gene deletion and Null: gene deletion. Values are mean ± SEM. Values bearing superscripts on the same column differ significantly (p< 0.05) when compared by unpaired test using Graph Pad Prism (version 5.0).

Table 4 Odd Ratio for Blood Lead Levels according to GST Genotypes

Genotype	BLLs (< 40µg/dl)	BLLs (> 70µg/dl)	OR	P- value
GSTM1				
Present	19	20		
Null	3	10	3.17	0.1938
GSTT1				
Present	17	9		
Null	5	21	7.933[a]	0.0017
GSTM1 & GSTT1				
Both Present	15	7		
Either Null	9	17	4.048[a]	0.0415
Both Null	0	7	8.143	0.1493

n= 65, Glutathione-S- Transferase (mu)1: GSTT1: Glutathione-S-Transferase (theta)1 Present: no gene deletion and Null: gene deletion, BLLs: blood lead levels, OR: odd ratio, Significant level [a] $p < 0.05$

The study also demonstrated that the prevalence of GSTM1 and GSTT1 null genotype were considerably high among individuals with blood lead concentrations greater than 70µg/dl and there was significant (P<0.05) association between frequency of GSTs null genotype and blood lead concentration. The most likely explanation could be viewed from the angle of possible partial or complete loss of detoxification activity of the GSTs enzymes. The Mu and theta class of GSTs have been reported to play a significant role in the detoxification of product of oxidative degradation like lipid peroxide.

Moreover, analyses were carried out to investigate the genetic influence on the blood Pb concentrations (particularly in < 40µg/dl and >70µg/dl respectively) as depicted in Table 4. The degree of frequencies of the null genotypes of both GSTM1 and GSTT1 were found high in individuals with high blood Pb concentrations (70µg/dl) compared to those having lower blood Pb concentrations (40µg/dl). The putative high risk null genotype (GSTT1) was calculated and 7.9 fold increases in the risk of having more accumulated blood Pb concentrations compared to other gene.

3.4 Impact of GSTs (GSTM1 and GSTT1) Polymorphism on Markers of Oxidative Stress

The effect of GSTs (GSTM1 and GSTT1) polymorphism on potential markers of oxidative stress is depicted in Figure 4 and 5 respectively. The oxidative stress markers like SOD and CAT enzymes, reduced glutathione molecule and lipid peroxidation product (MDA) were affected by both GSTM1 and GSTT1 null genotypes. Moreover, in all the GSTs genotypes, antioxidant enzymes activity and concentration of glutathione molecule were significantly ($p < 0.05$) decrease in the null genotypic individuals under study. However, the concentrations lipid peroxidation molecules (MDA) were found statistically high in both GSTM1 and GSTT1 null genotypic individuals.

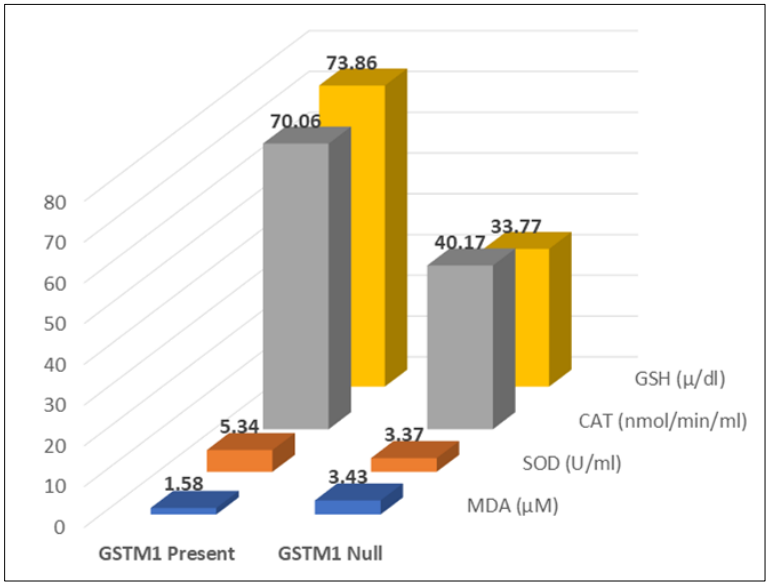

Figure 4 Impact of GSTM1 genotypes on Oxidative Stress Biomarkers. Values are mean ± SEM. GSH: glutathione, SOD: superoxide dismutase, CAT: catalase, MDA: malondialdehyde

Evidences from epidemiological and animals studies have suggested that lead-induced toxicity via oxidative stress by generation of reactive oxygen species, depletion of endogenous antioxidant biomolecules and essential minerals [26, 27]. The study has demonstrated that the activity of CAT and SOD enzymes was significant decreased in individuals with GSTM1 and GSTT1 null genotypes. The decrease activity was also associated with increased blood lead concentrations which could be due to lead's competitiveness with the cofactors of the enzymes or excessive ROS production by lead exposure. Similarly, the concentration of GSH was significantly reduced. Therefore, the reduction of blood GSH concentration could be attributed to direct reaction of GSH with lead for detoxification purpose or with ROS generated by lead due to prolong exposure. However, concentration of blood MDA, which is a marker of lipid peroxidation increased significantly in individuals with GSTM1 and GSTT1 null genotypes and was associated with increase blood lead concentrations. The decreased activity of the antioxidant enzymes, reduction of blood GSH concentration and increased MDA concentration could be further reasoned by GSTM1 and GSTT1 deletions which leads to impaired GSTs activity and consequent over production of ROS evidently seen during chronic lead exposure. Lots of GSH would definitely be exhausted just to reduce the destructive effects of lead exposure, a typical adaptive mechanism obtained in the human body.

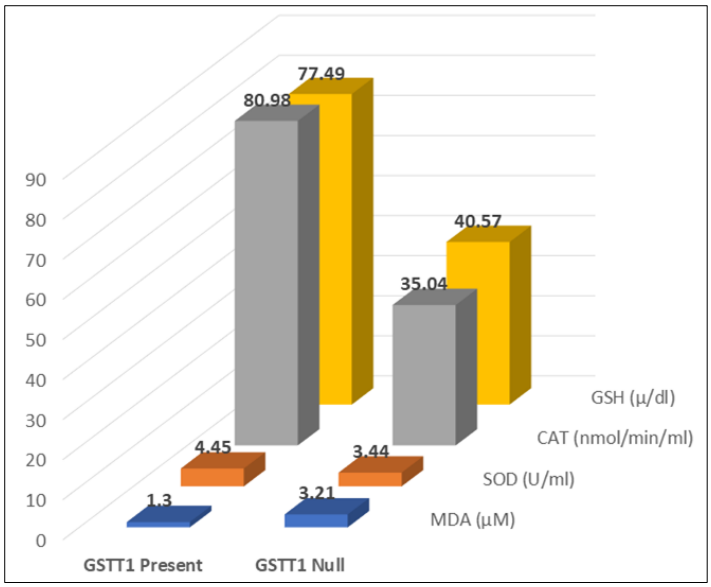

Figure 5 Impact of GSTT1 Genotypes on Oxidative Stress Biomarkers. Values are mean ± SEM. GSH: glutathione, SOD: superoxide dismutase, CAT: catalase, MDA: malondialdehyde

4 Conclusion

The present study indicated that GST genes polymorphism play a crucial role in individual's susceptibility to lead toxicity from environmental exposure. The activity of antioxidant enzymes (SOD and CAT) and GSH level were inversely proportional to blood lead concentrations and GSTs null genotypes. Therefore, considering the important role play by GSTs in metals detoxification, it is highly possible to suggest that individuals who are homologous null at GSTM1 and GSTT1 loci predispose to increased risk of multi-organs toxicity from exposure to lead or any other environmental pollutant detoxify by GST enzyme.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declared no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

Statement of ethical approval

Ethical permission was granted by Zamfara State Ministry of Health Research Ethic Committee on Human before the commencement of the research work.

Statement of informed consent

Consent form and questionnaire were given to participant to fill and sign, authorizing their participation in the research. Moreover, the researchers explained the informed consent and questionnaire in local (Hausa) language to those that could not read and understand English language.

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