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*Full Length Research Paper*

# Impact of GSTM1, GSTT1, GSTP1 polymorphism and environmental lead exposure on oxidative stress biomarkers

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**Oxidative stress and genetic related to antioxidants could have influence on susceptibility to lead (Pb) toxicity. In this study, we aim to examine the effects of genetic variations of glutathione S-transferase (GST) gene on oxidative stress alterations (by the measurements of malondialdehyde; MDA and glutathione; GSH) among general population. Real-time-PCR with Taqman probes was performed to analyze GSTM1, GSTT1 and GSTP1 Ile105Val. Blood lead and GSH levels were determined by spectrophotometer. MDA level was measured by HPLC with fluorescence detector. Mean blood Pb level in this study group was 4.85 µg/dl (ranged 2.00 and 18.50 µg/dl). Gender, cigarette smoking and alcohol consumption affected significant blood Pb levels. To further investigation, blood Pb levels were calculated into 3 tertiles and statistical results found only in tertile 3. Individuals with the Val/Val allele for GSTP1-105 had higher blood Pb, and MDA levels but lower GSH level as compared to individuals with Ile/Ile genotype ($p < 0.05$). Similar results were found in GSTM1 deletion, except for the GSH levels. In contrast, no effects of GSTT1 on three parameters were observed. Our findings support consideration of genetic variations of GSH-related genes as the important risk factor for lead toxic effects in the general population with environmental exposure.**

**Key words:** Lead, glutathione S-transferase, polymorphisms, glutathione, malondialdehyde.

## INTRODUCTION

Lead, an environmental toxicant, caused various health effects in different target organs. Major routes of exposure in humans are inhalation and ingestion. More than 75% of lead-exposure in the non-occupational exposed population comes from ingestion (Patrick, 2006).

For adults, the gastrointestinal absorption rate is

approximately 5 to 10%, mostly via contaminated food and beverages. After absorption, lead deposits accumulates in some soft tissues and ultimately in bones. Absorbed lead is eliminated primarily in the urine and bile. Blood lead (Pb) level has been considered as a good biomarker for lead toxicity.

Over production of reactive oxygen species (ROS) are implicated in lead-induced oxidative stress. This oxidative process resulted in the damaging of critical biomolecules such as DNA, proteins and lipid. Several studies reported the toxic effects of lead on cell membrane alterations, such as lipid component, membrane integrity, perme-ability and function, thereby increasing suscep-tibility to lipid peroxidation (Bolin, 2006). Generally, lipid peroxidation is monitored by the level of malondialdehyde (MDA). Kasperczyk et al. (2009) observed MDA concentration was significantly higher in lead-normotensive group. Moreover, the increase in lipid peroxidation due to lead toxicity may affect the antioxidant defense system, including enzymes (glutathione peroxidase; GPx, superoxide dismutase; SOD, catalase; CAT and glutathione S-transferases; GSTs) and nonenzymatic molecule like glutathione (GSH). GSH plays a major role in protecting cell against free radical attacks and its functional group (-SH) also directly binds to toxic metal with high affinity. Study in environmental exposure to lead found that there was statistically significant correlation of blood Pb levels with MDA ($r = 0.46$, $p = 0.00018$) and GSH ($r = -0.62$, $p = 0.0001$) (Ahamed et al., 2005).

An individual genetic make-up related to lead metabolism or oxidative stress is the current trend in medicine concerned with growing problems of toxic heavy metals. GSTs play the important function as GSH-utilizing enzymes in ROS and lead-detoxifications. Variations in GST genes both in deletion and polymorphism produce significant alterations in GST activity. Impacts of GSTM1 and GSTT1 deletion as well as GSTP1 Ile105Val (amino acid change from isoleucine at codon 105 to valine) exhibit the decreased conjugation of GSH to various electrophilic compounds, including lead and other free radicals (Hayes, 2000). *In vivo* and *in vitro* experimental studies also addressed alteration of GSH level and up-regulation of GSH-related enzymes (that is, glutathione reductase, GR; and GPx) in lead exposure condition (Bokara, 2009). Moreover, there was an increase in MDA level with a concomitant decrease of GSH in subjects with blood lead level 11.39 ± 1.39 µg/dl compared with those of subjects with blood lead level 7.11 ± 1.25 µg/dl and 3.93 ± 0.61 µg/dl ($p = 0.0001$ and 0.0002, respectively) (Ahamed et al., 2005). To the best of our knowledge, this is the first study that aims to further investigate the effects of genetic variations of all three GSTs, as GSTM1, GSTT1 and GSTP1 Ile105Val on oxidative stress alterations in environmental lead exposed population.

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## MATERIALS AND METHODS

### Study population

The Electric Generating Authority of Thailand (EGAT) study was the first cohort study of chronic disease in Thailand, originally designed in 1985 (known as EGAT 1), and mainly covered multidisciplinary researches related to cardiovascular disease (CVD) risk factors such as nutrition and toxicology. The 370 subjects in this study were participants in the third survey of EGAT 2 in 2009 (the first survey started in 1998 and second survey in 2003). This study was approved by the Committee on Human Rights related to Researches involving human subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand. All participants that completed a self-administered questionnaire, underwent a physical examination, and performed laboratory analysis, including tests for diabetes, liver and kidney diseases, etc. Toxicological profile of heavy metals was investigated in both first and third surveys of EGAT 2 and further genetic analysis started in 2009. Ten milliliters of blood were collected by venipuncture into EDTA and heparinized tubes from each subject and immediately centrifuged at 2000g. Buffy coat, erythrocytes and plasma were separated and stored at -20°C until genotyping analysis and biochemical measurements were performed.

### Determination of Pb in blood

Blood lead concentrations were measured by graphite furnace atomic absorption spectrometry (GFAAS) with Zeeman background correction. The analytical procedure was based on the method described by Subramanian (1983). The measurements were calculated as micrograms per deciliter (µg/dl) and expressed by means of total Pb in blood.

### Determination of glutathione by the DTNB method (Beutler et al., 1963)

Whole blood (0.1 ml) was added to distilled water (1.9 ml) together with 3 ml of precipitating solution (1.67 g glacial meta-phosphoric acid, 0.2 g disodium ethylenediaminetetraacetic acid; EDTA and 30 g sodium chloride). Then, the filtrate (0.5 mL) was added to 0.3 M phosphate buffer, pH 6.4 (2 ml). Finally, 1 mM DTNB (0.25 ml) was added, mixed well, and the absorbance was read at 412 nm within 4 min.

### Determination of MDA

MDA was determined using an HPLC method with fluorescence detection, as described by Khoschsorur et al. (2000). The CV was 4% within runs and 3% between days. Detection limit was 0.25 µmol/L and this method exhibited a linear response of MDA in a range of concentration from 1.50 to 15.0 µmol/L and calibration curve presented high correlation coefficient ($r^2 > 0.90$, $p = 0.001$; n=10).

### Genotype analysis

The genomic DNA was extracted from the lymphocytes by a

**Table 1.** Profile of the study population and blood Pb, MDA and GSH levels.

| Variable | Blood Pb (µg/dL) | Blood MDA (µmol/L) | Blood GSH (mg/dL) |
|---|---|---|---|
| All (n = 370) | 4.85 ± 2.71 | 7.40 ± 5.73 | 31.08 ± 7.04 |
| Male (n = 267) | 5.24 ± 2.82 | 7.26 ± 5.41 | 31.09 ± 7.14 |
| Female (n = 103) | 3.84 ± 2.10[a] | 7.75 ± 6.50 | 31.04 ± 6.81 |
| Age | | | |
| 45-55 yrs (n = 144) | 4.99 ± 2.79 | 8.08 ± 6.24 | 31.25 ± 6.90 |
| >55 yrs (n = 226) | 4.76 ± 2.66 | 6.96 ± 5.35 | 30.97 ±7.14 |
| Smoking status | | | |
| Smokers (n = 76) | 6.08 ± 3.09 | 8.04 ± 5.67 | 31.02 ± 6.97 |
| Nonsmokers (n = 294) | 4.54 ± 2.51[b] | 7.23 ± 5.74 | 31.28 ±7.34 |
| Cigarettes smoked per day | | | |
| 1-9 (n = 20) | 5.78 ± 2.01 | 7.54 ± 3.89 | 30.18 ± 5.67 |
| 10-19 (n = 32) | 6.03 ± 2.95 | 8.02 ± 3.57 | 29.88 ± 7.72 |
| ≥ 20 (n = 24) | 6.89 ± 3.07 | 7.96 ± 2.99 | 31.25 ± 6.58 |
| Alcohol consumption | | | |
| Yes (n = 192) | 5.34 ± 2.84 | 7.33 ± 5.50 | 31.13 ±6.81 |
| No (n = 178) | 4.32 ± 2.46[c] | 7.47 ± 5.98 | 31.02 ± 7.26 |
| Frequency of alcohol consumption (drinks/week) | | | |
| 1-3 (n = 64) | 4.94 ± 2.74 | 7.11 ± 4.64 | 31.78 ± 5.67 |
| 3-6 (n = 86) | 5.58 ± 3.99 | 7.84 ± 3.97 | 32.26 ± 4.87 |
| ≥ 7 (n = 42) | 5.99 ± 4.37 | 7.68 ± 5.24 | 31.96 ± 6.84 |

[a, b, c] p<0.05 compared to male, smokers and drinkers, respectively.

modified salting out procedure (Miller, 1988) and frozen at -20°C until analysis. The TaqMan Assay included the forward target-specific polymerase chain reaction (PCR) primer, the reverse primer, and the TaqMan MGB probes labeled with 2 special dyes: FAM and VIC. The reaction consist of 10 ng of DNA added into the PCR reaction, consisting of TaqMan_Universal Master Mix (1x), and TaqMan_MGB probes for *GSTM1*, *GSTT1* and *GSTP1Ile 105Val* (1x) in a total volume of 10 µl. The real-time PCR reaction protocol was 10 min at 95°C, 40 cycles of 15 s at 92°C, and 1 min at 60°C using 7500 Real-Time PCR System (Applied Biosystems, USA). Information of specific probe and primers are available on the National Cancer Institute's SNP500 database web page at http://snp500cancer.nci.nih.gov/ (Packer et al., 2004).

### Statistical analysis

Statistical analysis was carried out using the SPSS 16.0 for window software (SPSS, Inc., Chicaco, IL). The overall values were expressed as means ± SD. Associations between blood Pb and other parameters were analyzed by tertiles (T1, < 2.99 µg/dl; T2, 3.00 to 6.00 µg/dl, and T3 > 6.00 µg/dl) The comparisons between variables were examined by the Student's t-test and analysis of variance (ANOVA). Hardy-Weinberg equilibrium (HWE) testing of GSTP1 Ile105Val (rs 1695) was evaluated by chi-square test. Odds ratios (ORs) and their 95% confidence interval (CI) were obtained by using logistic regression analysis. Statistical significance was defined as p < 0.05.

## RESULTS

The subject characteristics are presented in Table 1. The blood Pb concentrations in this study group ranged between 2.00 and 18.50 µg/dl, and the mean was 4.85 µg/dl. Sex, smoking status and alcohol consumption showed some significant differences with respect to blood Pb levels. Female subjects have lower blood Pb level than males (3.84 ± 2.10 vs. 5.24 ± 2.82 µg/dl, p < 0.050). Smoking cigarette and alcohol consumption also affected the blood Pb levels (Smokers, 6.08 ± 3.09 µg/dL vs. Nonsmokers, 4.54 ± 2.51 µg/dl, p < 0.050; Drinkers, 5.34 ± 2.84 µg/dl vs. Nondrinkers, 4.32 ± 2.46 µg/dl, p<0.050). Although, slight increases in the blood Pb concentration with number of cigarette smoked per day could be observed, these differences were not statistically significant. With respect to alcohol consumption, frequency of alcohol drinking per week did not influence on the blood Pb level.

**Table 2.** Genotype frequencies for GSTM1, GSTT1 and GSTP1 (N=370).

| Gene | Variation | Genotype | Frequency | |
|---|---|---|---|---|
| | | | Number | Percentage (%) |
| GSTT1 | Deletion | Null | 121 | 32.7 |
| | | Present | 249 | 67.3 |
| GSTM1 | Deletion | Null | 213 | 57.6 |
| | | Present | 157 | 42.4 |
| GSTP1-105 (rs1695) | Ile105Val | Ile/Ile | 212 | 57.3 |
| | | Ile/Val | 139 | 37.6 |
| | | Val/Val | 19 | 5.1 |

**Table 3.** Blood lead level for different genotypes.

| Genotype | Tertile 1 | | Tertile 2 | | Tertile 3 | |
|---|---|---|---|---|---|---|
| | Blood lead (< 2.99 μg/dl) | No. | Blood lead (3.00-6.00 μg/dl) | No. | Blood lead (> 6.00 μg/dl) | No. |
| GSTT1 | | | | | | |
| Null | 2.79 ± 0.40 | 49 | 4.47 ± 0.50 | 55 | 8.09 ± 2.14 | 32 |
| Present | 2.63 ± 0.48 | 80 | 4.34 ± 0.47 | 91 | 8.69 ± 3.13 | 63 |
| GSTM1 | | | | | | |
| Null | 2.68 ± 0.46 | 75 | 4.44 ± 0.50 | 85 | 8.73 ± 2.58 | 53 |
| Present | 2.72 ± 0.45 | 54 | 4.31 ± 0.46 | 61 | 6.84 ± 2.10 [a] | 42 |
| GSTP1-105 | | | | | | |
| Ile/Ile | 2.70 ± 0.48 | 70 | 4.42 ± 0.49 | 80 | 6.20 ±0.44 | 62 |
| Ile/Val | 2.75 ± 0.43 | 49 | 4.35 ± 0.48 | 62 | 7.42 ±1.77 | 28 |
| Val/Val | 2.70 ± 0.48 | 10 | 4.25 ± 0.50 | 4 | 9.15 ±3.10 [b] | 5 |

[a, b] Significantly different from GSTM1 null genotype and GSTP1 Ile/Ile, p<0.05, respectively.

The means of MDA and GSH concentrations among the study population were 7.40 ± 5.73 μmol/L and 31.08 ±7.04 mg/dl, respectively. No significant differences were found between males and females in MDA and GSH levels. Both biomarkers of oxidative stress were not significantly influenced by age, cigarette smoking and alcohol consumption (Table 1).

Table 2 represents the genotype frequencies of GSTM1, GSTT1, and GSTP1 Ile105Val. The frequencies of GSTM1 and GSTT1 deletion among this population were 57.6% and 32.7%, respectively. For GSTP1 gene, 19 (5.1%) were homozygous for the 105 Val /105Val, 139 (37.6%) were heterozygous (105Ile/105Val), and 212 (57.3%) were homozygous for the 105Ile/105Ile GSTP1 genotype. The genotype distributions of GSTP1 Ile105Val were in Hardy-Weinberg equilibrium, with non-significant chi square values (p > 0.05, data did not show).

Data of blood Pb were divided into 3 tertiles and further analyzed to elucidate effects of GSTs polymorphisms (Table 3). All three GSTs gene variations had no influence on blood Pb in tertile 1 and 2. In the highest tertile, blood Pb concentrations in individuals with non-deleted GSTM1 were significantly lower than those with deleted genotypes (6.84 ± 2.10 vs. 8.73 ± 2.58 μg/dl, p < 0.050). Moreover, after analysis in the same fashion we found a significant function of GSTP1 on blood Pb level. This effect was observed only in individuals with the GSTP1 Val/Val and Ile/Ile genotype (9.15 ± 3.10 vs. 6.20 ± 0.44 μg/dl, p < 0.050). However, no effect of GSTT1genotype on blood Pb levels was found in this study group.

Table 4 illustrates the effect of smoking and GSTs genotypes on blood Pb (< 6 μg/dL vs. > 6 μg/dl). Smoking status did not increase the risk of high blood Pb level among individuals with and without the GSTT1 and GSTM1 null genotypes. For GSTP1 polymorphism, we combined subjects with the Ile/Val and Val/Val alleles for further analysis with appropriate sample size. In this statistical model, the odds of blood Pb > 6 μg/dl in the nonsmokers and smokers with heterozygous and homozygous variant were 1.7 (95% CI: 1.1 to 2.6) and 1.8 (95% CI: 1.1 to 3.1) times higher than those with homozygous wild type allele.

We further analyzed the genetic impact on the blood Pb

**Table 4.** Odds ratio for blood lead level according to smoking status and GST genotypes[a].

| Genotype | Variable | Blood Pb <6 µg/dl vs. > 6 µg/dl | | | |
|---|---|---|---|---|---|
| | | Nonsmokers | | Smokers | |
| | | OR | 95% CI | OR | 95% CI |
| GSTT1 | Present | 1.0 | Reference | 0.9 | 0.6-1.4 |
| | Null | 0.6 | 0.4-1.0 | 1.1 | 0.6-1.7 |
| GSTM1 | Present | 1.0 | Reference | 1.2 | 0.7-2.0 |
| | Null | 1.4 | 0.9-2.4 | 1.5 | 1.0-2.2 |
| GSTP1-105 | Ile/Ile | 1.0 | Reference | 1.1 | 0.7-1.8 |
| (rs1695) | Ile/Val and Val/Val | 1.7 | 1.1-2.6 | 1.8 | 1.1-3.1 |

[a] Adjusted for data of age, gender and alcohol consumption.

(only in the tertile 3), MDA and GSH levels (Figure 1). These biomarkers were affected by GSTM1 and GSTP1 genotypes where individuals with the Val/Val allele for GSTP1-105 had higher blood Pb, and MDA but lower GSH level compared with individuals with Ile/Ile genotype (Pb, 9.15 vs. 6.20 µg/dl; MDA, 7.65 vs. 6.18 µmol/L, and GSH, 25.02 vs. 28.21 mg/dl, $p < 0.05$). In addition, the GSTM1 null genotype exhibited the statistically higher blood Pb and MDA levels than those with GSTM1 genotype (Pb, 8.73 vs. 6.84 µg/dl; MDA, 9.63 vs. 6.99 µmol/L, $p<0.05$), but no difference in GSH concentration. However, no effects of GSTT1 on three parameters were observed.

## DISCUSSION

In our study, blood Pb levels are influenced by sex, cigarette smoking and alcohol consumption. As expected, male subjects have higher blood Pb level than female subjects probably due to higher lead exposure and partially differences in hormonal metabolisms (Batariova et al., 2006). Generally, tobacco plants very easily take up lead from soil and concentrate it in leaves, leading to relatively high amount of lead in tobacco and cigarettes. It has been suggested that lead, presented in a cigarette, is recovered and distributed in the process of burning in the smoke, butt and ash which ultimately is inhaled by smokers (Galazyn-Sidorczuk et al., 2008). The average lead content in Thai cigarettes was reported to be 8.07 µg per cigarette (Poovapanit, 1982). This information could support the increase of blood Pb level in smokers in the present study. In addition, a possible source of lead contamination in alcoholic beverage is considered for elevated blood Pb level in drinkers. The data of lead content in various brands of currently produced cigarettes and alcohol beverage, especially in Thailand are incomplete due to no obligation to control these products for contamination with heavy toxic metals. However, high cigarette smoking and alcohol consumption were not strongly related to elevated blood lead concentration in this study population (Table 1).

Based on the phase-out of Pb in gasoline and restriction of Pb-based paint, the biological monitoring of blood Pb levels declined among population in various countries. An overall decline in national rates of elevated BLLs among US adult from 14.0 in 1994 to 7.8 in 2007 has been observed (CDC, 2009). In addition, a study conducted in 1995 among French men demonstrated that geometric mean of blood Pb was 4.45 µg/dl and decreased to 3.67 µg/dl in 2006 (Falq et al., 2011). In Thailand, mean of blood Pb level (4.85 µg/dL) reported in the present study was comparable to our previous report in 2002 (3.25 µg/dl) (Sirivarasai et al. 2002). Adverse health effects by occupational lead exposure have been recognized for a long time. Meanwhile environmental exposure with detected low blood Pb causing threats for cardiovascular risks was also investigated. Menke et al. (2006) found that an increased risk of cardiovascular, myocardial infarction, and stroke mortality was evident at blood Pb levels > 2.0 µg/dl. Additional epidemiological studies observed associations between blood Pb with increased blood pressure and altered renal function markers at blood Pb levels < 10 µg/dl (Kuo et al. 2006; Scinicariello et al., 2011). Plausible mechanisms of these adverse effects may be a disruption of lead-oxidation-reduction system, resulting in production of ROS and depletion of GSH or other antioxidant enzymes.

Virulence of health risks induced by lead exposure principally depends on factors related to degree of exposure (that is, dose, route, duration and formulation) and its biotransformation pathway. Furthermore, one of the most important factors to take into account is genetic variation in lead-metabolizing enzymes. GST genes play

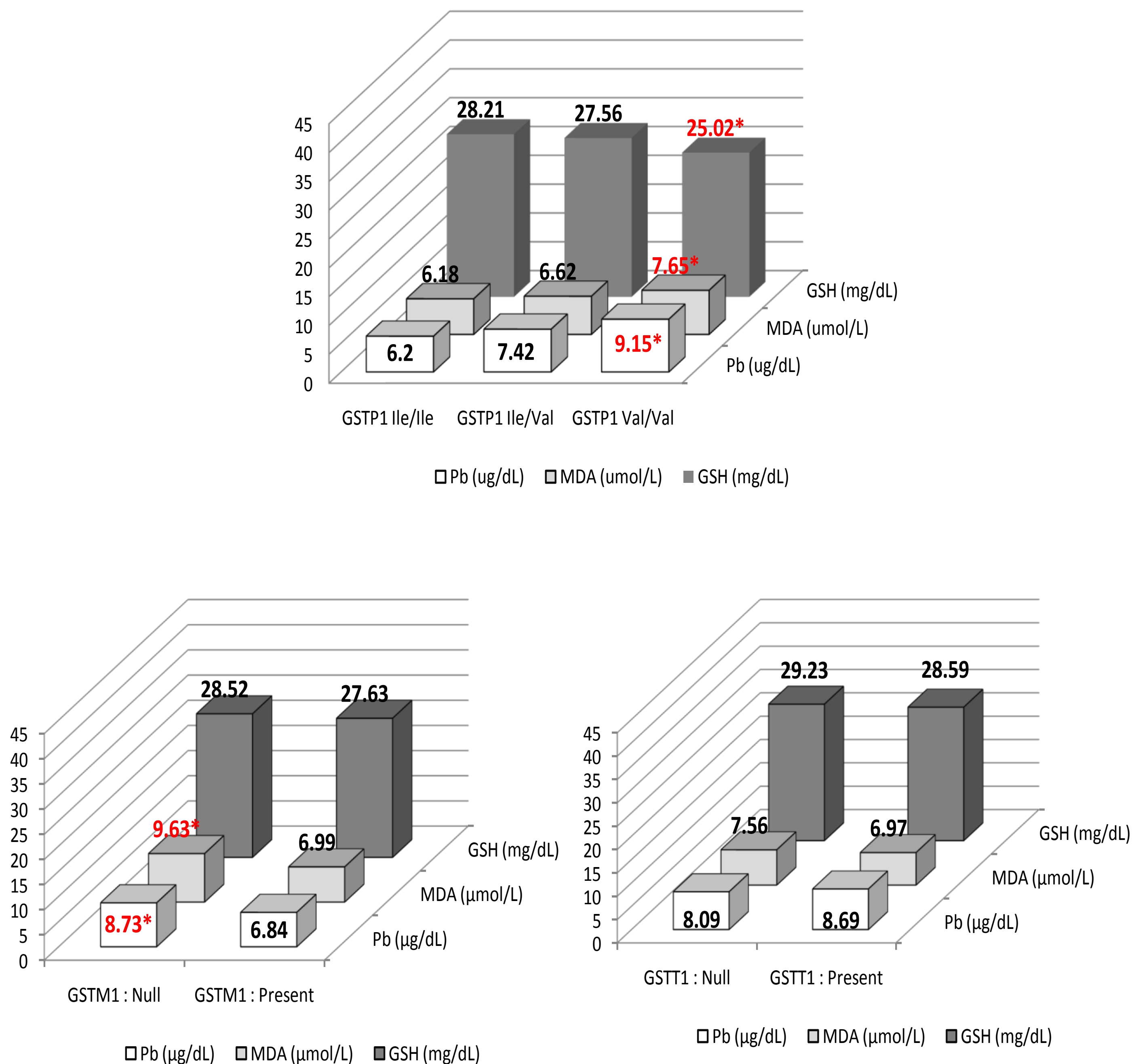


**Figure 1.** Genetic impact on the blood Pb, MDA and GSH levels.

a prominent role to reduce lead-induced oxidative stress. Generally, previous researches presented the associations between lead exposure and only one genetic variant of GSTM1, GSTT1 or GSTP1 (Lee et al., 2007; Kim et al., 2007). This is the first study, to our knowledge, to examine the impact of GSTM1, GSTT1 and GSTP1 Ile105Val on biomarkers related to oxidative stress among general population exposed to environmental lead.

The prevalence of GSTM1 and GSTT1 null genotypes varies among ethnic groups. We found that the genotype frequencies of GSTT1 and GSTM1 deletions in this Thai population were 32.7 and 57.6%, respectively. The results were also comparable to previous studies. The prevalence of GSTT1 and GSTM1 null in Chinese were 53.3 and 58.7%, respectively (Mak et al., 2007) and in Austrian were 17.45 and 55.4% (Gundacker et al., 2009). This study confirmed other observations that the frequency of homozygous GSTP1 Val/Val genotype was uncommon in Thai (5.1%), Chinese (3.5%) (Mak et al.,

2007), and Korean (5.20%) (Kwon et al., 2011), but considerably lower than that reported in Austrian (10.5%) (Gundacker et al., 2009).

Significant impacts of genetic factors on lead levels were found in our study, particularly for the GSTM1 and GSTP1 Ile105Val genotypes (Table 3). In tertile 3 of blood Pb level (> 6.00 µg/dl), the carriers of the GSTM1 null and GSTP1 variant allele showed significantly higher blood Pb levels than those with GSTM1 present and GSTP1 wild type genotypes. To clarify the simultaneous influence of cigarette smoking and GST genetic variations on blood Pb level, we further analyzed these data with ORs (Table 4). Only the GSTP1 polymorphism showed significant outcomes, but no statistical differences in risks of elevated blood Pb level between nonsmokers and smokers with wild type and variant alleles. These results could be an additional data in supporting the potential role of GSTs gene on lead level. Moreover, some evidences suggested that oxidative stress play a role in lead-induced toxicity by induction of ROS production and depletion of various antioxidant proteins (Bolin et al., 2006). In this study MDA level, a representative indicator of ROS production, showed a significant increase of blood lead level in tertile 3. In contrast, a significant decline of GSH level with blood PB level was observed. Overall significant changes found only in individuals with GSTM1 null and GSTP1 Val/Val genotypes (Figure 1). The reduction of blood GSH was associated with an increase in blood lead level could be partially explained by the reaction of GSH with lead or with the free radicals promoted by lead exposure. The other possible explanation may be GSTM1 deletion and amino acid change from Ile to Val in GSTP1 affected GST activity, leading to impaired ability to detoxify lead-induced ROS. More specifically in high level of lead exposure, all GSH would be consumed in order to balance more MDA level, as an adaptive mechanism in the human body.

## Conclusions

Our data suggested the putative role of GST gene polymorphisms in susceptibility to toxic response from lead exposure. The more lead entered the body, the higher glutathione was consumed. Due to potential role of GST in detoxifying toxic metals, it is plausible that individuals with GSTM1 null genotype and GSTP1 105 Val alleles may be at increased risks from exposure to lead/ or other toxicants detoxified by the this enzyme. Information related to oxidative stress, caused by environmental lead exposure, and genetic factors take an important step towards elucidating the biochemical mechanisms underlying these processes. Valuable knowledge provided by these data may be useful in designing treatment or clinical practices to enhance antioxidants in order to mitigate lead-induced toxicity both in the occupational and non-occupational population.

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