JCOMS

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Journal of

Current Oncology and Medical Sciences

Vol. 2, No.3

Original Free Access

MT1A single Nucleotide Polymorphism and Blood Mercury Levels

Maryam Salacheh [1], Amir Jalali [2]*, Javad Babaei [1], Hamid Galehdari [3], Amal Saki [4]

[1] Department of Toxicology, School of Pharmacy and Toxicology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2] Department of Applied Cellular Sciences and Tissue Engineering, Langroud School of Allied Medical Sciences and Medical Biotechnology Center, Guilan University of Medical Sciences, Rasht, Iran

[3] Department of Genetics, School of Sciences, Chamran University, Ahvaz, Iran

[4] Department of Statistics, School of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Abstract

Introduction: The risk factors for the metallothionein (MT) polymorphism in concentrations of heavy metals, especially mercury, in the blood are subject to several confounding factors, including differences in the ethnicity of the population analyzed, the sample size, and the type of the studied environment heavy metals to which population is exposed. This study aimed to investigate the effects of the MT1A(A>G) and MT1A(C>G) single nucleotide polymorphisms (SNPs) on blood mercury levels in the city of Ahvaz (located in southwest of Iran).

Materials and Methods: 300 unexposed (control group) and 150 exposed (case group) were included. DNA extraction, PCR-RFLP and DNA sequencing were performed, and blood mercury levels were determined by AAS method with DMA-80.

Results: Blood mercury levels in the case group were higher than those in the control group (p-value <0>G), with MT1A (C>G) polymorphism and P-values of blood mercury levels of 0.69, 0.44, and 0.59. 0.56 for the case and control groups, respectively. Results showed that these two SNPs were not associated with mercury-induced toxicity in the case group despite high blood mercury levels and exposure.

Conclusion: In conclusion, this takes look shows that MT1A (A>G) and MT1A (C>G) polymorphisms aren't related to susceptibility to excessive blood mercury attention in individuals.

Keywords: Mercury, Metallothionein, MT1A (A>G), MT1A(C>G), Iranian population

*Corresponding Author: Amir Jalali

✉ Email: amjalali@hotmail.com

Received: 2022.8.9, Accepted: 2022.9.16

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Introduction

There are three forms of mercury in the environment such as elemental, inorganic, and organic mercury compounds (1). However, organic mercury (mostly methyl mercury) exposure is via dietary fish consumption (2). Mercury is widely found in the environment and foods and so are life-threatening organisms (1). Therefore, mercury exposure may lead to toxicity. Genetic polymorphisms and individual differences have a leading role in heavy metals toxic effects (3-4). The different studies on the genetic susceptibility genes of heavy metals levels have become of interest.

Metallothioneins (MT) are cysteine-rich low molecular weight proteins. These proteins bind to physiological and xenobiotic heavy metals (1). The intracellular binding prevents the toxic effects and cellular damage of heavy metals.

MT1, MT2, MT3 and MT4 are the main isoforms expressed in humans. The Liver and kidney are the main prominent for synthesis that needs dietary minerals (Zn, Cu, and Se) and amino acids (His and Cys) (2). Our study focused on two SNPs of metallothionein in a regulatory region, including MT1A missense (A>G) (rs8052394) and MT1A 5′ near gene (C>G) (rs9922957).

There are studies on MTs and their relation to heavy metal levels, such as cadmium, lead, zinc and copper in blood samples (3, 4) and mercury levels in human urine and hair samples (5). Also, there is a relation to diseases such as the risk of ductal breast cancer (6), type2 diabetes mellitus (11), intestinal, and gastric cancer (12), lung cancer (13) and inflammatory bowel disease (14). Furthermore, it was demonstrated that MT gene polymorphism is related to metal levels in the placenta (15) and kidney tissues (16).

MT plays a crucial role in the detoxification of mercury blood concentration, and altered gene coding was suggested as a potential role to explain high mercury blood levels. The modification of neurobehavioral effects of mercury by genetic polymorphisms of MT and the relationship between MT1A (A>G) and MT1A (C>G) to hair and urine mercury level was studied (17). Individual differences as a result of genetic polymorphisms lead to different adverse effects of environmental factors (18). This genetic diversity can cause changes in whole blood mercury levels. SNP in MTs may influence mercury biomarker levels in the human body (19). Individual difference studies, for example, can help doctors pinpoint an illness, suggest further tests and prescribe appropriate drugs. Like MT genes, association studies of polymorphisms to heavy blood levels should further investigate. This study attempted to investigate the two MT polymorphisms as a dependent risk marker for mercury blood levels. This is the first research on the relationship between SNPs of MT and heavy metal levels in the Iranian population.

Materials and Methods

Study population

This study was performed following a protocol approved by the commission of Bioethics at the Jundishapur University of Medical Sciences, Ahvaz, Iran. This case-control study was performed in the Toxicology Research Center (TRC). Whole blood samples that included 300 normal, healthy volunteers (150 males and 150 females) with 25-70 years old (mean age 44.00±19.23) as the control group and 150 exposure people (75 males and 75 females) with 29-45 years old (mean age 37.30±8.19) as case group collected from accredited medical diagnostic laboratories in Ahvaz city (southwest of Iran) and factory workers exposed to mercury in Mahshahr city (southwest of Iran), respectively, in the period from October 2014 to December 2014. The control group was healthy blood donors having no evidence of any personal or family history of high blood mercury concentration. Five milliliters of venous blood were drawn into a sterile tube containing EDTA and stored at -20 ºC until the isolation of genomic DNA. The subjects signed informed written consent to take part in the study. Individuals filled out the brief questionnaire. All the molecular analysis was performed in the Toxicology Research Center of Jundishapur University of Medical Sciences.

Genotype analysis

Genotyping was performed on DNA extracted from whole blood samples that were considered for mercury assessments. Polymerase chain reaction based on the restriction fragment length polymorphism (PCR-RFLP) was used for genotyping (Figures 1 and 2).

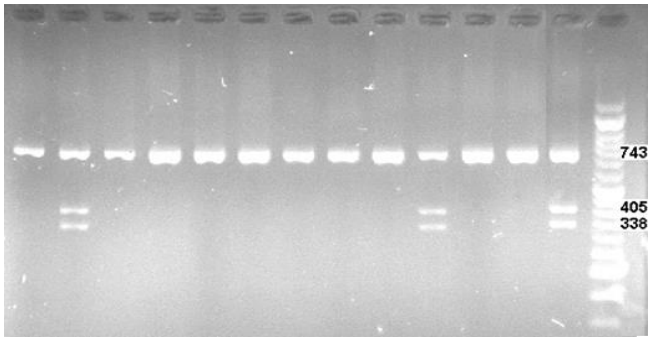

Figure1. PCR-RFLP of MT1A missense (A>G) polymorphism on agarose gel 2% (paya pajoohesh, Iran). In some cases, in addition to 743bp bond, it's visible 405bp and 338bp bonds, which represent the heterozygous genotype (AG).

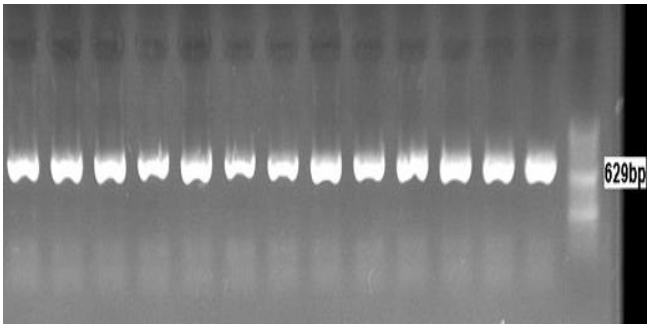

Figure2. Electrophoresis PCR products of MT1A 5' near gene (C>G) polymorphism on agarose gel 1.5%.

Information about the MT1A (A>G) and MT1A (C>G) and PCR conditions are listed in Table 1.

Table1. SNP name, Primer Sequence, PCR Conditions, RFLF and DNA sequencing for the MT1A (A>G) and MT1A (C>G) (20).

SNP name	Primer sequence	PCR condition	Time
MT1A ((A>G Missense rs8052394	F:CAAACTGAGGCCAAGAGTGCACCA R:TGACCTGAGGCAGGTGCCTGATTT	94c°-4min (initial (denaturation {94c°-30sec,↓ 62c°-30sec, 72c°-45sec} for 30 cycle↓ 72-5min (extension)	743 RFLP 405, 338
MT1A ((C>G 5´ near rs9922957	F:ACATCGGTGGCRGTTGCTCTGCAC R:CTAGCATCCCTTACCAGTGGCGCA	94c°-4min (initial (denaturation {94c°-30sec,↓ 62c°-30sec, 72c°-45sec} for 30 cycle↓ 72-5min (extension)	629 ABI Sequencing

The PCR products were digested with the restriction enzymes *PstI* (Vivantis, Malysia). Automated Sanger sequencing ABI (Applied Biosystem, USA, 2012) was used for DNA sequencing analysis (Figure 3). Digestion conditions were performed according to the manufactures instructions and products were separated at the appropriate concentration on a low-melting point agarose gel and stained with ethidium bromide. All PCR reactions were carried out in an independent, blinded duplicate manner and for each polymorphism, some samples were confirmed by sequencing the PCR products.

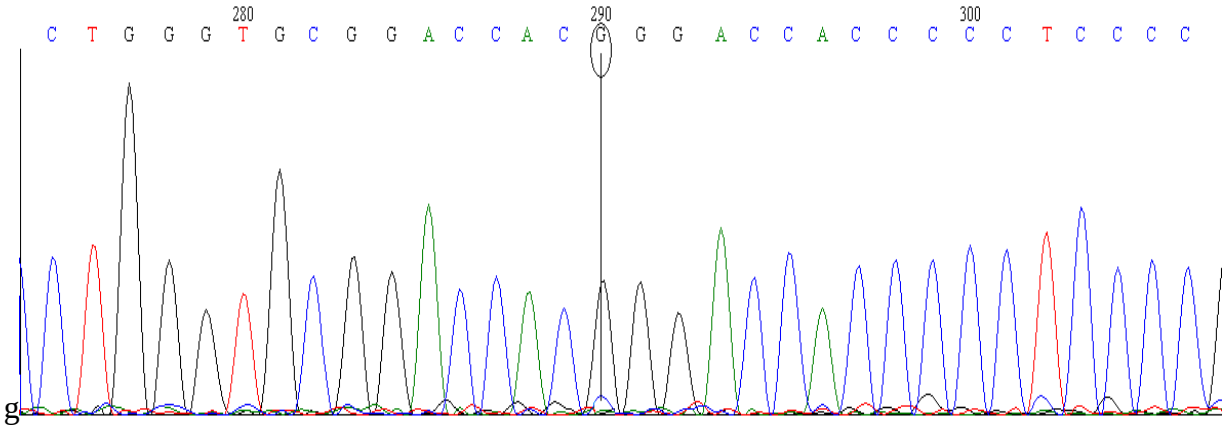

Figure 3. DNA sequencing of MT1A (C>G) polymorphism and single nucleotide mutation that cytosine mutated to guanine. This case is a heterozygote (Reverse primer was used).

Determination of mercury levels

Whole blood mercury was measured based on Atomic Absorption Spectrometry (AAS) technique by Direct Mercury Analyzer (DMA-80, Italy) instrument. We entered 100µl of individual whole blood to DMA, and then the sample temperature rises in the curve segment named Catalyst; the heated sample arrives at Amalgamator segment that contains gold pieces for the release of mercury; eventually, the atomized mercury absorbs light in Cuvette segment and determines the amount of mercury according to light absorption and calibration curves (www.milestonesrl.com).

The ethical approval of this study is IR.ajums.REC.1393.142. This code is addressed at the following: Behsan.ajums.ac.ir/webdocument/load.action/webdocument_code=1000&masterCode=33005332.

Statistical analysis

Deviations from Hardy–Weinberg equilibrium was tested using the Chi-square ($\chi 2$) test. Data analysis was performed by Statistical Package for Social Sciences (SPSS) version 22 software. Values of $P < 0.05$ were considered statistically significant.

Results

The average whole blood mercury levels of exposure (the case group) and non-exposure (control group) people were measured. It was found that the amounts of mercury were 58.79±51 ppb and 6.65±3.5 ppb in the case and control groups, respectively. The blood mercury levels in the case group are approximately nine times higher than those in the control group. These results show that there are significant differences (p value<0.001) between the two groups of mercury; therefore, exposure to mercury in the case group has been effective in increasing blood mercury levels.

Individuals in the case and control groups were genotyped by DNA PCR-RFLP and DNA sequencing techniques; genotype and allele frequencies were determined (20). All genotype distribution did not diverge significantly from Hardy-Weinberg equilibrium for both control and case groups separately. In the control group, the genotype frequencies of MT1A (A>G) were 77%, 23% and 0.0% for wild-type, heterozygous and homozygous; and allele frequencies were 88.5% and 11.5% for A and G alleles, respectively. In case group for MT1A (A>G), the genotype and allele frequencies were obtained 74% (AA), 26% (AG), 0.0% (GG) and 87% allele A, 13% allele G. About MT1A (C>G) polymorphism the genotype and allele frequencies were as follows: 92% wild-type, 8% heterozygous and 0.0% homozygous, 96% allele C and 4% allele G in the control group and 80% wild-type, 20% heterozygous and 0.0% homozygous, 90% allele C and 10% allele G in case groups. The genotype and allele frequencies P values were obtained by using the Chi-Squire test. P values for MT1A (A>G) and MT1A (C>G) were 0.69 and 0.03, respectively.

Statistical evaluation of gender (p=0.76) and age (p=0.60) significances in these two polymorphisms was performed and no significant association was found.

In the main part of the study, the results show that MT1A (A>G) and MT1A (C>G) polymorphisms have no significant effects on mercury blood levels in the case and control groups (Fig 4, 5). The results indicated that these two SNPs of the MT gene were not associated with susceptibility to mercury blood levels in the Ahvaz population of Southwest Iran. Mercury level was measured by an AAS technique in male and female blood samples in exposure and non-exposure groups and there was no significant difference between blood mercury levels in female and male groups (p=0.73). The average blood mercury levels in people with MT1A (A>G) and MT1A (C>G) polymorphisms that have wild-type, heterozygous and homozygous genotypes demonstrate that SNP changes were not considerable, however, about MT1A (C>G) SNP changes lead to an increase in blood mercury level, however it is not significant (Table 2). A comparison of blood mercury based on genotypes between case and control groups is shown on the graph in figures 4 and 5.

Table2. MT1A (A>G) and MT1A (C>G) polymorphisms and mercury concentrations of blood samples in case (exposure) and control (non-exposure) groups.

polymorphisms	**MT1A (A>G)**				**MT1A (C>G)**			
Groups	Case		Control		Case		Control	
N & Hg amount	N	Hg(ppb)	N	Hg(ppb)	N	Hg(ppb)	N	Hg(ppb)
Wild type	111	58.44	231	6.47	120	58.34	276	6.62
Heterozygote	39	59.80	69	2.27	30	60.59	24	7.07
Homozygote	0	---	0	---	0	---	0	---
P value	---	0.69	---	0.44	---	0.59	---	0.56

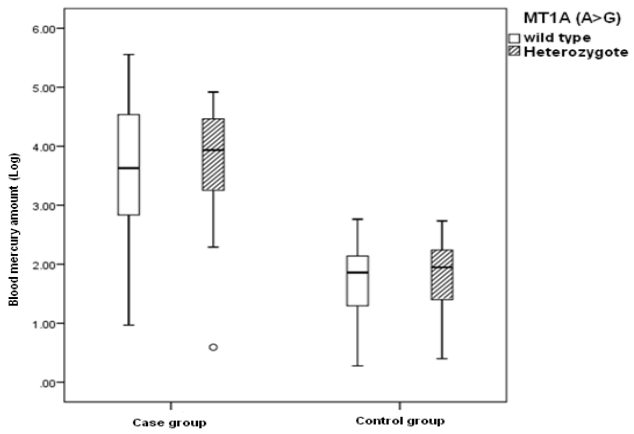

Figure 4. The bar chart of the blood mercury concentrations of MT1A (A>G) in case (n=150; p=0.69) and control (n=300; p=0.44) groups.

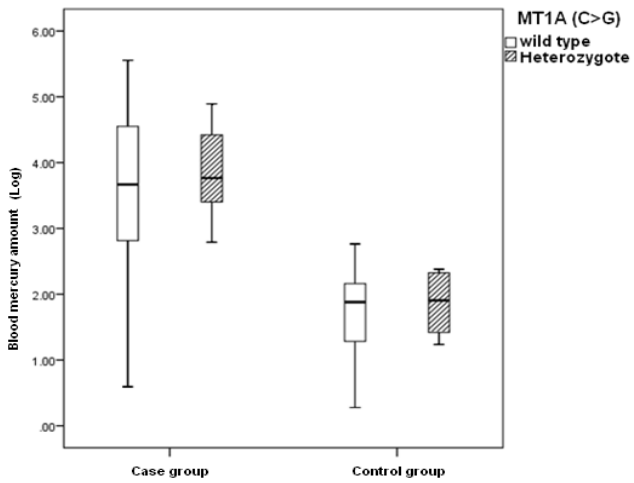

Figure 5. The bar chart of the blood mercury concentrations of MT1A (C>G) in case (n=150; p=0.59) and control (n=300; p=0.56) groups.

Discussion

This report aimed to use a case-control study to establish a database of the effect of MT1A (A>G) and MT1A (C>G) SNPs in the Ahvaz population from

Southwest Iran and to evaluate these SNPs as an indicator of blood mercury level susceptibility.

There is an excellent mechanistic ground for finding an interaction between the putative high-inducibility-associated MT genotypes and heavy metal blood levels. For further comprehension of the role played by MT1A (A>G) and MT1A (C>G) SNPs of MT gene in mercury blood levels, the two gene polymorphisms were associated with mercury blood levels. In Iran country, the industry is growing in particular the oil, and petrochemical industry and the accumulation of toxic metals in the human body is increasing; thus, it is necessary to use time and energy to investigate the effect and damage of toxic metals in the body and identify the prevention methods (21). Expression of MTs proteins increases via oxidative stress and heavy metal effect in the regulatory area of the gene. MTs expression various in different tissues, so there is a significant correlation between the metal level and MTs expression. Induction of this protein is a highlight biomarker of heavy metal exposure (22-24). MTs expressed polymorphically and these changes in polymorphism affect on proteins that bind to heavy metals for to homeostasis and detoxification. MT1A (A>G) and MT1A (C>G) are two studied MT polymorphism that is located in the regulatory region of chromosome 16 (25). A mutation in one nucleotide, such as changing in nucleotide A and turning it to nucleotide G in MT1A (A>G) and changing nucleotide C to nucleotide G in MT1A (C>G) lead to SNP and may influence the toxicity of mercury. In this study, the effect of these changes on blood mercury levels was investigated in the Iranian population. There are several studies in the field of this polymorphism such as polymorphism in MT1A (A>G) gene and the risk of type 2 diabetes mellitus in Chinese and Nepalese people. The results showed that the incidence of type 2 diabetes was significantly related to G allele in SNP rs8052394 (11, 26). Another study in the Italian central female population proved that polymorphism in MT1A (A>G) gene coding region is associated with longevity. Also, in Greece, it was found that the AG and GG genotype significantly increased the risk of cardiovascular disease (27-28). It was demonstrated that the variations of MT1A SNPs may influence urine uric acid and N-acetyl-beta-D-glucosaminidase excretion in chronic lead-exposed workers (29). The other reports were not found about the effects of MT1A polymorphisms on mercury metabolism or toxicity. These reports are unlike our results and SNP in MT1A (A>G) was effective. There is just one study about MT1A (C>G) polymorphism in the USA population and there was no significant relationship between MT1A (C>G) allele frequency and hair and urine mercury levels in accordance with our results (9,19). These results may display and explain that MT1A polymorphisms had no strong modifying effects on mercury metabolism and toxicity. Much research has been done in the field of metallothionein polymorphisms and heavy metal levels in the human body, which the most typical are: A) The effect of metallothionein 2A-5A/G single nucleotide polymorphism on blood Cd, Zn, Pb and Cu levels in the Turkish population that highly statistically association were detected between MT2A and these heavy metals except Cu (8). B) The association between 13 polymorphisms of metallothionein and urine and hair mercury level were examined in the USA population, and the results showed that there is no significant difference between hair and urine mercury level and all of the polymorphisms except MT1M (T>C) rs9936741. In this polymorphism individuals with TC genotype significantly have high hair mercury levels than wild type genotype (19).

Our study is the first research about MT1A (A>G) rs8052394 and MT1A (C>G) rs9922957 and their association with blood mercury levels. As shown in Table 2, the mean amount of mercury blood levels in the MT1A (C>G) SNP control group is more than MT1A (A>G) SNP control group. This study identifies that MT1A (C>G) SNP changes may influence mercury blood concentration and toxicity. Therefore, individuals with MT1A (C>G) SNP may be more sensitive to mercury toxicity than MT1A (A>G) SNP (Figure 4,5). In general, according to the results and other studies all over the world, it can be stated that in most cases, the SNP is effective on heavy metals concentration in the human body and it can say that should take more care of because these people are susceptible to heavy metals. Thus, we can identify more susceptible individuals. With the genetic database of people, safety advice was earnest and they banned their exposure to heavy metals and other toxins. It is hoped that in the future only by performing genetic

testing of people working in the industry and determining their genetic susceptibility to heavy metals. There are several conceivable factors for the inconsistent outcomes in the previous studies. First, the difference in the study design (sample size, ethnicity, and selection of subjects) may have contributed. Second, it is not easy to elucidate the relationship between genotype and phenotype. The phenotype is often influenced by environmental factors in addition to genotype. The perfect model would be to obtain cell lines that have MT1A (A>G) and MT1A (C>G) SNPs of MT genes and their sensitivity towards mercury studied.

Conclusions

In summary, our findings show that MT1A (A>G) and MT1A (C>G) SNPs of MT gene don't influence the Iranian population's susceptibility to mercury blood levels. This result should be confirmed in more studies on various ethnic groups

Author contribution

MS, **JB**, **HG** and **AS** collected the data and compiled this article. **AJ** wrote and edited the manuscript comprehensively. All authors confirmed final version.

Acknowledgments

This study was supported by the Deputy research of Jundishapur University of Medical Sciences. We acknowledge Mr Miah for his aid to provide Blood samples. Also, the authors would like to thank all participants who willingly contributed to the study.

Conflict of interest

The authors have declared that no competing interest exists.

Funding

This project was financially supported by the vice chancellor of Research affairs of Ahvaz Jundishapur University of Medical Sciences (Toxicology Research Center).

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