# DNA Repair and Detoxifying Gene Polymorphisms in Patients with Multiple Sclerosis and Dental Amalgam Fillings

Multipl Sklerozlu ve Amalgam Dolguları Olan Hastalarda DNA Onarım ve Detoksifikasyon Gen Polimorfizmleri

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**ABSTRACT**

**Objective:** The present study was designed to evaluate the influence of Metallothionein (*MT*), detoxifying (*GSTs*) and repair (*OGG1, XRCC1*) gene polymorphisms on the risk of Multiple Sclerosis (MS) related to metal exposure.
**Materials and Methods:** One heavy (Mercury; Hg) and two essential metals (Copper; Cu and Zinc; Zn) from dental amalgam fillings were selected. Our study subjects were divided into 4 groups, namely, MS patients not carrying dental amalgam fillings (n=20); patients carrying dental amalgam fillings (n=20); control subjects not carrying dental amalgam fillings (n=20) and control subjects carrying dental amalgam fillings (n=20). The levels of urinary metals detected with Atomic Absorption Spectrometry (AAS) and gene polymorphisms were analyzed with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).
**Results:** Subjects with polymorphic *OGG1* genotype had 3 times higher MS risk and subjects with dental amalgam fillings had significantly more urinary Hg levels compared to subjects without dental amalgam fillings.
**Conclusion:** Considering the information on urinary metal levels, *OGG1 Ser326Cys* gene polymorphisms may modify the repairing of oxidative products. However, no significant association between *OGG1* gene polymorphism and urinary Hg levels was observed.

**Key Words:** Metallothionein; genetic polymorphisms; *OGG1*, PCR-RFLP; AAS; metals

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**ÖZET**

**Amaç:** Bu çalışma, Metallothionein (MT), detoksifikasyon (*GST*) ve onarım (*OGG1, XRCC1*) gen polimorfizmlerinin metal maruziyetiyle ilişkili Multipl Skleroz (MS) riski üzerindeki etkisini değerlendirmek için tasarlanmıştır.
**Yöntem:** Amalgam dolgularındaki bir ağır (Cıva; Hg) ve iki temel metal (Bakır; Cu ve Çinko; Zn) analiz için seçildi. Çalışma deneklerimiz 4 gruba ayrıldı: Amalgam dolgusu taşımayan MS hastaları (n = 20); amalgam dolgusu taşıyan hastalar (n = 20); amalgam dolgusu taşımayan kontrol bireyleri (n = 20) ve amalgam dolguları taşıyan kontrol bireyleri (n = 20). İdrar metallerinin seviyeleri Atomik Absorpsiyon Spektrometresi (AAS) ile tespit edilirken gen polimorfizmleri Polimeraz Zincir Reaksiyonu-Restriksiyon Fragment Uzunluğu Polimorfizmi (PCR-RFLP) ile analiz edildi.
**Bulgular:** Polimorfik *OGG1* genotipine sahip bireyler, 3 kat daha yüksek MS riskine sahipti ve amalgam dolgusu olan bireyler, amalgam dolgusu olmayanlara kıyasla önemli ölçüde daha fazla idrar Hg seviyelerine sahipti.
**Sonuç:** İdrar metal seviyeleri hakkındaki bilgiler dikkate alındığında, *OGG1 Ser326Cys* gen polimorfizmleri oksidatif ürünlerin onarımını değiştirebilir. Ancak *OGG1* gen polimorfizmi ile idrar Hg seviyeleri arasında anlamlı bir ilişki gözlenmedi.

**Anahtar Sözcükler:** Metallotiyonein; genetik polimorfizm; *OGG1*; PCR-RFLP; AAS; metaller

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## INTRODUCTION

Multiple sclerosis (MS) is a chronic, progressive, and degenerative disease of the central nervous system (CNS) and its etiology has not been fully elucidated. Environmental and genetic factors play roles in MS risk (1). Many studies have proposed the association of MS with toxic metals (aluminum; Al, lead; Pb and mercury; Hg) and essential metals (Zinc; Zn, Copper; Cu) (2; 3). At present, 2.3 million people in the world suffer from MS; thus, researchers investigate the factors which have roles in MS pathogenesis. Recently, considerable attention has been paid to the risk of dental amalgam fillings on the development of MS due to their potential neurotoxic effects. The presence of dental amalgam fillings is suggested to be associated with a variety of neurological disorders. Therefore, studies on associations with neurological diseases and metal exposures take into account dental amalgam fillings for accurate evaluation.

Mercury is a naturally occurring element released from a variety of sources such as natural sources and the burning of fossil fuels. 3-4 % of total Hg sources are dental amalgam fillings consisting of 50 % metallic Hg mixed with an alloy (silver, tin and Cu) (4). Animal studies showed that Hg released from dental amalgam fillings is absorbed and accumulated in various organs such as kidney, brain, liver and lungs (5). There are comparative studies on MS patients with or without dental amalgam fillings. Significant correlations were shown between urinary Hg levels and the number of amalgam fillings. Removal of amalgam fillings results in a significant reduction in body burden of Hg (6). There have been controversial studies on removing amalgam fillings or chelation therapy on MS patients. Chelation therapy relieves general symptoms due to decrease burden of metals. When amalgam fillings are removed, small amounts of Hg are slowly released and accumulate in various tissues especially in brain tissue (7).

The first report between amalgam fillings and MS was suggested by Craelius (1978) and reported a strong correlation. Certain health problems may occur due to amalgam fillings (8). In a case-control study (9), elevated MS risk was found for subjects with a large number of amalgam fillings, however no statistically significant difference was found between patients and controls. MS patients with amalgam fillings had significantly lower levels of haemoglobin and haematocrit, higher Hg levels in their hair and poorer mental health status (10; 11). On the other hand, there were case-control studies showing no association of amalgam fillings with MS risk (12; 13). Despite these contradictory studies, the potential risk of amalgam fillings still remains a concern for MS.

Oxidative stress (OS) is a common pathological feature in neurological diseases (14). CNS is particularly sensitive to OS due to its high oxygen consumption (15) Disruption of redox-active metal homeostasis and accumulation of redox-active metals (Hg) lead to an uncontrolled formation of ROS toxicity which may cause oxidative damage to DNA and OS occurs (16; 17). Both Metallothionein's (*MT*s) and Glutathione S- transferases (GSTs) are important detoxification proteins. Their detoxification mechanisms are different from each other but both proteins have roles the detoxification of metals. *MT*s contain a high proportion of cysteine groups to which certain metals, including mercury, preferably bind. For *GST*, the intracellular binding reaction with GSH is catalyzed by the *GST*s and leads to stable GSH-metal conjugates being transported out of the cell probably by *MRP2* (Multidrug Resistance associated protein 2) and excreted via faces and urine. (18; 19). DNA damage due to metal exposure in neuronal cells is repaired by detoxifying and DNA repair enzymes (20). Most of these enzymes are polymorphic, this may alter the efficiency of their activities and can contribute to genetic susceptibility of DNA damage and toxicities. XRCC1 and OGG1 are major Base Excision Repair (BER) enzymes. They provide protection and repair the DNA damage from metals.

In order to identify genetic factors underlying the inter-individual variance in detoxification and repair capacity for the metal exposure, it was investigated the impact of genetic background on metal exposure in the development of MS and evaluate metals exposure from amalgam fillings. For this reason, we investigated whether DNA repair (*OGG1, XRCC1*) and detoxifying (*GST*s and *MT2A*) gene polymorphisms have impact on MS patients related to the levels of metals (Hg, Cu and Zn).

## MATERIALS and METHODS

40 controls (25 female, 15 male, 18-58 years of age) and 40 MS patients (28 female, 12 male, 24-52 years of age) were participated in our study, which was approved by Ankara Numune Education and Research Hospital Ethics Committee. As shown in Table 1, 20 controls and 20 MS patients had more than 3 amalgam fillings for more than 10 years. All patients were diagnosed according to the criteria as revised by McDonald et al. (21). Disability of patients was graded as mild (EDSS; Expanded Disability Status Scale 0–4). Gender and age-matched hospital-based controls who did not have any neurological disorder or problems were selected. The dietary and occupational exposure to metals could be negligible with respect to the questionnaire. Hence, we assumed that the source of metal exposure was based on the presence of dental amalgam filling.

DNA was isolated from peripheral blood samples (22) and genotyping was made by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Table 1 demonstrates the descriptive and clinical characteristics of study groups

**Table 1.** General and clinical characteristics of the Multiple Sclerosis (MS) patients and controls

| | Controls (n=40) | MS patients (n=40) | *p* |
|---|---|---|---|
| **Age, years (mean±SD),** | 32.9 ± 10.9 | 37.2 ± 7.3 | |
| **min-max** | 18-58 | 24-52 | 0.047* |
| **Gender** | 40 | 40 | |
| **Female** | 25 | 28 | |
| **Male** | 15 | 12 | 0.47* |
| **Smoking status** | | | |
| **Smokers** | 20 | 19 | |
| **Nonsmokers** | 20 | 21 | |
| **With Amalgam filling** | 20 | 20 | |
| **Without amalgam filling** | 20 | 20 | |
| **The mean of no. of amalgam** | 4.3 | 4.9 | |
| **Clinical types*** | | | |
| **RRMS** | | 39 | |
| **PRMS** | | 1 | |
| **SPMS, PPMS** | | - | |
| **Duration of disease (year)** | | | |
| **min-max** | | 1-14 | |
| **Mean** | | 6.24 | |
| **The number of relapses** | | | |
| **min-max, Median** | | 1-4.2, 2 | |

**P<0.05 is* considered statistically significant. **Clinical types of Multiple Sclerosis*

*RRMS: Relapsing-Remitting Multiple Sclerosis*
*PRMS: Progressive-Relapsing Multiple Sclerosis*
*SPMS: Secondary Progressive Multiple Sclerosis*
*PPMS: Primary Progressive Multiple Sclerosis*

*Genotyping Analysis*
*OGG1* Ser326Cys (rs1052133), *XRCC1* Arg399Gln (rs25487) and *MT2A* -5A/G (rs28366003) gene polymorphisms were made as previously described (23; 24) and *GSTP1* (rs1695), *GSTT1* (rs4630) and *GSTM1* (rs366631) genotyping were performed as previously described (25).

*ROS Activity Measurement*
The levels of serum ROS were detected by Enzyme Linked Immune Sorbent Assay (ELISA) according to the manufacturer's instructions (EastBiopharm).

*Determination of Hg, Zn and Cu in Urine*
*Reagents*
All chemicals used were of analytical reagent grade. Each Hg, Cu and Zn standard solutions were prepared from 1000 mg $L^{-1}$ stock solution. Deionized (DI) water, from Milli-Q Water Purification System was used throughout the experiments. Nitrogen gas was used as carrier gas. Recovery studies were carried out in order to validate the methods for determination of Hg, Zn and Cu.

*Instrumentation*
*Cold Vapor Atomic Absorption Spectrometry (CVAAS), Flame Atomic Absorption Spectrometry (FAAS)*
All measurements (Hg, Zn and Cu) were performed using Unicam 939 AAS with a deuterium background correction. The hollow cathode lamp of each element was operated at respective wavelengths according to the instrumental parameters recommended by the manufacturer. Cold Vapor Atomic Absorption Spectrometry (CVAAS), Flame atomic absorption spectroscopy (FAAS) and slotted tube atom trap flame atomic absorption spectrometry (STAT-FAAS) techniques were used for the determination of Hg, Zn and Cu respectively. Air-acetylene flame was used during Cu and Zn measurements and peak height was measured throughout the experiments.

*Sample preparation*
The collected urine samples were stored at -80 °C freezer until analysis. The digestion of urine samples was performed according to the method used by Burguera, Burguera and Gallignani (26) with slight modifications.

*Sampling procedure*
For Hg determinations, three replicate measurements were performed using CVAAS method for each parallel and external calibration was used.

For determination of Zn, three replicate measurements were performed for each sample and external calibration was used for determination of Zn and Cu. Recovery studies was performed for each analyte by spiking known amount of Hg, Zn and Cu to urine samples before the sample digestion step. The obtained recoveries for Hg, Zn and Cu were 96±3 %, 101±4 % and 97±4 % respectively and the analytical parameters for each analyte were given in Table 2.

**Table 2.** LOD, Linear range and calibration equation parameters for metals (Hg, Zn and Cu)

| Parameter | Urinary Hg * | Urinary Zn * | Urinary Cu * |
|---|---|---|---|
| **LOD (Limit of Detection)** | 0.2 | 13 | 20 |
| **Linear range** | 0.5 – 12.5 | 30 - 1000 | 30 – 500 |
| **Calibration equation** | 0.0188x-0.0007 | 0.0003x+0.0074 | 0.00031x + 0.0008 |

**concentration in ng/ml.*

Our detection limits are similar or lower value than other studies such as 8-5 ng $L^{-1}$ for Zn and 20.9 ng $mL^{-1}$ for Cu in Li et al.'s paper (27) and 4.5 ng $mL^{-1}$ for Zn and 24.5 for Cu in Eyeson et al.'s study (4).

*Statistical Analysis*
Statistical analyses were performed using the SPSS software version 22.0. The univariate analyses to identify variables associated with disease outcome were investigated using Chi-square and Mann-Whitney U tests, where appropriate. For the multivariate analysis, the possible factors identified with univariate analyses were further entered into the logistic regression analysis to determine independent predictors of disease outcome. p-values less than 0.05 is considered statistically significant.

## RESULTS

*The Levels of Urinary Metals*
The levels of urinary metals were measured between control and patients and also comparisons were made among subgroups (with and without amalgam fillings). Urinary Hg levels in MS patients with dental amalgam fillings were statistically higher than controls and patients without amalgam fillings. The mean level of urinary Hg in patients with amalgam fillings was almost 2 times higher compared to controls without amalgam fillings (0.78±0.52 and 0.38±0.20, Table 3). On the other hand, statistically significant difference was found between with and without amalgam fillings) in MS subjects (0.78±0.52 and 0.56±0.45 ng/ml Hg, Table 3, Mann-Whitney U test). It is well-known that Zn is not a major source from amalgam filling; nevertheless, urinary Zn levels were also compared among groups (patient versus control, amalgam filling versus no amalgam fillings). We found a statistically significant difference in Zn levels between MS patients with and without amalgam fillings ($p$=0.046) (Table 3).
Cu levels in urine samples were under the detection limit (Table 2 shows Limit of Detection of metals) therefore any comparisons could not made.

**Table 3.** The levels of urinary Hg and Zn in controls and in MS patients

| | N | Mean±SD (µg/g creatinine) Hg | Zn | Median Hg | Zn | Min-Max Hg Min-Max | Zn Min-Max | p value |
|---|---|---|---|---|---|---|---|---|
| **CONTROLS** | | | | | | | | |
| **Total** | 40 | 0.51±0.27 | 219.66±168.42 | 0.47 | 196.52 | 0-1.22 | 0-788.29 | |
| **Subjects with Amalgam** | 20 | 0.64±0.28 | 208.79±140.95 | 0.65 | 196.52 | 0.30-1.22 | 0-481.65 | |
| **Subjects without Amalgam[1]** | 20 | 0.38±0.20 | 230.54±195.21 | 0.35 | 181.69 | 0-0.78 | 13.97-788.29 | 0.002 |
| **PATIENTS** | | | | | | | | |
| **Total** | 40 | 0.67±0.49 | 210.80±190.24 | 0.54 | 171.57 | 0-2.31 | 0-964.88 | |
| **Subjects with Amalgam[1,2]** | 20 | 0.78±0.52 | 185.13±219.30 | 0.85 | 108.96 | 0-2.31 | 0-964.88 | 0.049 |
| **Subjects without Amalgam[2]** | 20 | 0.56±0.45 | 236.46±157.52 | 0.42 | 197.69 | 0-1.98 | 53.09-652.58 | 0.046 |

*p<0.05 is considered statistically significant; Hg, Mercury; Zn, Zinc; Mann-Whitney U test; [1]p = 0.002 MS patients with dental amalgam compared with controls without dental amalgam in terms of Hg; [2]p = 0.049 MS patients with dental amalgam compared with patients without dental amalgam in terms of Hg; [2]p = 0.046 MS patients with dental amalgam compared with patients without dental amalgam in terms of Zn.

*The Levels of Serum ROS*

The levels of ROS were detected by ELISA. Contrary to our expectation, controls with amalgam fillings had almost 2 times higher ROS activity compared to patients with amalgam fillings (Table 4). Lower ROS activity in patients than controls may be related with more use of antioxidant supplements among MS patients.

**Table 4.** The levels of serum ROS (U/L) in controls and in MS patients

| | N | ROS activity (U/L) Mean±SD |
|---|---|---|
| **Healthy controls** | | |
| **With Dental Amalgam*** | 19 | 1401.5±1051.8 |
| **Without Dental Amalgam** | 20 | 828.9±697.4 |
| **MS patients** | | |
| **With Dental Amalgam*** | 20 | 809.2± 882 |
| **Without Dental Amalgam** | 20 | 939±981.2 |

Mann-Whitney U test; *p<0.05 is considered statistically significant; *p<0.05 MS patients with dental amalgam compared with healthy controls with dental amalgam.

*Gene Polymorphisms and the Risk of MS*

The allele frequencies of *GSTs*, *OGG1*, *XRCC1* and *MT2A* gene polymorphisms in Turkish population were within the range described for Caucasians (28; 29; 30). Multivariate analysis was made for comparisons among gene polymorphisms, metal exposure and the risk of MS. The possible risk factors were identified with univariate analyses and then entered into the logistic regression analysis to determine independent predictors of disease outcome. When the impacts of gene polymorphisms on MS risk were evaluated, it was found that subjects with *OGG1 Ser/Cys+ Cys/Cys* genotype had 3 times higher MS risk (AOR=2.952 CI%95=1.118-7.794, *p*=0.029) (Table 5). No association was found between other gene polymorphisms (*GSTs, MT2A* and *XRCC1*) and the risk of MS in regard to metal exposure (data not shown). The mean levels of urinary Hg and Zn in controls were 0.51±0.27 and 219.66±168.42 and 0.67±0.49 and 210.80±190.24 µg $g^{-1}$ creatinine in patients (Table 3). Although, there was an increase the levels of urinary Hg and Zn in subjects with *OGG1 Ser/Ser* genotypes, no significant associations were found between other gene polymorphisms and the levels of urinary Hg, Zn. (*p*>0.05, not shown).

**Table 5.** Associations between the allele/genotype frequencies of *OGG1*, *GSTP1*, *XRRC1*, *MT2A*, *GSTM1* and *GSTT1* gene polymorphisms and the risk of MS

| Allele/Genotype frequencies | Controls N (%) | MS patients N (%) | COR (95% CI) | *p* | AOR (95% CI)* | *p*** |
|---|---|---|---|---|---|---|
| ***OGG1* Locus-326** | | | | | | |
| ***Ser/Ser*** | 22 (57.9%) | 13 (32.5%) | 1 | | 1 | |
| ***Ser/Cys*** | 13 (34.2%) | 20 (50%) | 2.604 (0.979-6.927) | 0.055 | 2.603 (0.925-7.327) | 0.07 |
| ***Cys/Cys*** | 3 (7.9%) | 7 (17.5%) | 3.949 (0.867-17.989) | 0.076 | 4.496 (0.906-22.316) | 0.066 |
| ***Ser/Cys+Cys/Cys*** | 16 | 27 | **2.856 (1.134-7.19)** | **0.026** | **2.952 (1.118-7.794)** | **0.029** |
| ***Ser*** | 28 (73.7%) | 23 (57.5%) | | | | |
| ***Cys*** | 10 (26.3%) | 17 (42.5%) | | | | |
| ***GSTP1* Locus-105** | | | | | | |
| ***Ile/Ile*** | 15 (39.5%) | 18 (45%) | 1 | | 1 | |
| ***Ile/Val*** | 23 (60.5%) | 18 (45%) | 0.652 (0.259-1.64) | 0.363 | 0.721 (0.271-1.919) | 0.513 |
| ***Val/Val*** | 0 | 4 (10%) | 1 | | 1 | 1 |
| ***Ile/Val+Val/Val*** | 23 | 22 | 0.797 (0.324-1.962) | 0.622 | 0.914 (0.354-2.357) | 0.852 |
| ***Ile*** | 26 (68.4%) | 27 (67.5%) | | | | |
| ***Val*** | 12 (31.6%) | 13 (32.5%) | | | | |
| ***XRCC1* Locus-399** | | | | | | |
| ***Arg/Arg*** | 14 (38.9%) | 15 (40.5%) | 1 | | 1 | |
| ***Arg/Gln*** | 19 (52.8%) | 17 (46%) | 0.835 (0.314-2.223) | 0.718 | 0.985 (0.349-2.783) | 0.978 |
| ***Gln/Gln*** | 3 (8.3%) | 5 (13.5%) | 1.556 (0.312-7.751) | 0.59 | 1.754 (0.33-9.329) | 0.51 |
| ***Arg/Gln+Gln/Gln*** | 22 | 22 | 0.933 (0.365-2.384) | 0.885 | 1.098 (0.407-2.964) | 0.853 |
| ***Arg*** | 23 (63.9%) | 23 (62.2%) | | | | |
| ***Gln*** | 13 (36.1%) | 14 (37.8%) | | | | |
| ***MT2A*** | | | | | | |
| ***AA*** | 33 (84.6%) | 34 (85%) | 1 | | 1 | |
| ***AG*** | 6 (15.4%) | 6 (15%) | 0.971 (0.284-3.317) | 0.962 | 0.755 (0.205-2.775) | 0.672 |
| ***A*** | 36 (92.3%) | 37 (92.5%) | | | | |
| ***G*** | 3 (7.7%) | 3 (7.5%) | | | | |
| ***GSTM1*** | | | | | | |
| ***Null*** | 22 (56.4%) | 18 (45%) | 1.757 (0.72-4.286) | 0.215 | 1.902 (0.744-4.864) | 0.179 |
| ***Positive*** | 17 (43.6%) | 22 (55%) | | | | |
| ***GSTT1*** | | | | | | |
| ***Null*** | 5 (12.8%) | 9 (22.5%) | 1.974 (0.579-6.533) | 0.265 | 1.873 (0.534-6.567) | 0.327 |
| ***Positive*** | 34 (87.2%) | 31 (77.5%) | | | | |

*Adjusted to age and gender; ***p<0.05 is* considered statistically significant.

## DISCUSSION

The effects of genetic polymorphism related to Hg kinetics may influence the development of MS besides the dietary intake and occupational exposure. Several animal studies and epidemiological studies suggested that genetic factors might affect urinary Hg levels and Hg accumulation in the body (31). Based on these data, we focused on the impact of polymorphisms of repair and detoxifying genes on MS development related to metal exposure. Polymorphisms of *MT2A, GSTM1, GSTT1* and *GSTP1, OGG1* and *XRCC1* genes which have protective roles in MS as defense systems, were analyzed. These polymorphisms may alter enzyme activities thus there may be association between these gene polymorphisms and the risk of MS (32). Previous studies found a strong correlation between metal exposure and levels of OS products in urine such as 8-OHdG. 8-OHdG is repaired by 8-oxoguanine DNA glycosylase (OGG1) enzyme (33; 34). According to PubMed database, so far, there has been a lack of information about the possible associations between genetic polymorphisms and metal exposure from dental amalgam fillings in the development of MS.

Lee et al. (32) investigated the association of gene polymorphisms on *GST*s with the risk of MS in a meta-analysis. They observed significant association between *GSTT1* null genotype, and the risk of MS however, there was no significant association observed with others (*GSTM1* or *GSTP1)*. Stavropoulou et al. (35) found higher frequency of *GSTM1* null genotype in female MS patients and suggested that there might be a possible role of *GSTM1* detoxification pathway in a gender-dependent manner. Alexoudi et al. (36) and Živković et al. (37) found significant associations between *GSTT1* gene polymorphism and MS risk. Barcelos et al. (38) found higher Hg levels in blood and hair samples of subjects with null *GSTM1* genotype.

Due to rich thiol groups of *MT2A*, they bind to trace metal ions. They regulate distribution and extraction for heavy metal toxicity (39). MT2A protein is polymorphic and A/G gene polymorphism is most commonly studied polymorphism. Due to polymorphism, MT2A may have less or lack of activity therefore elimination of metal toxicity may be change. Several studies investigated the associations between *MT2A* (A/G) gene polymorphism and the levels of toxic metals. Kayaaltı, Aliyev and Söylemezoğlu (25) showed that subjects with *GG* genotype had significantly higher Cd and Pb levels and lower Zn levels compared to *AA* and *AG* genotypes. In our study, same comparisons were made however no statistically significant difference was found in regard to *MT2A*. We found 3 times higher MS risk in subjects with *OGG1* genotypes (*Ser/Cys* and *Cys/Cys*) (Table 5). Based on this finding, we investigated the possible influence of polymorphic *OGG1* genotype on the levels of urinary Hg, however we did not observe a significant effect (not shown).

Gundacker et al. (18) evaluated the relationship between *GSTM1* and *GSTT1* polymorphisms and Hg levels in blood, urine, and hair samples. They showed that the epistatic effect of *GSTT1* and *GSTM1* null genotypes were risk factors for Hg exposure and found an increased susceptibility to Hg. We also evaluated the epistatic effect of *GSTM1* and *GSTT1* null, *GSTP1* heterozygote and mutant, *OGG1* and *XRCC1* mutant genotypes, but we did not find any significant difference (data not shown).

There is no study the associations between Hg (non-occupationally exposure), Zn, Cu, other metals and *OGG1 Ser326Cys* gene polymorphism in regard to the development of MS. There were two studies showing the associations of *OGG1* and *XRCC1* gene polymorphisms with occupationally exposure to Pb and arsenic (As). In these studies, the authors concluded that *OGG1* and *XRCC1* gene polymorphisms had a significant impact on these metal exposures (40; 41). These two-study encouraged us to investigate the associations between *OGG1* and *XRCC1* gene polymorphisms with metal exposure in MS disease.

We found statistically significant higher MS risk in subjects with *OGG1 Cys/Cys* genotypes than subjects with *OGG1 Ser/Ser* genotypes. However, we did not find any impact of *XRCC1 Arg399Gln* gene polymorphism and of the levels of Hg, Zn and Cu in the risk of MS. Quite recently, considerable attention has been paid to dental amalgam fillings and their potential neurological toxic effects in terms of their metal content especially, Hg. As reported by "Food and Drug Administration (FDA) White Paper" (42) the weight of evidence from human clinical and epidemiological studies did not support the hypothesis that Hg exposure from amalgam fillings causes adverse health effects. According to FDA (42), the range of urinary Hg levels in subjects without any occupational exposure is up to 20 µg $L^{-1}$. Urinary Hg levels were known to increase by 6 % for each amalgam surface, but they were not associated with fish consumption. For this reason, except for occupational exposure, it was suggested that amalgam fillings constitute the main source of Hg exposure (31).

Factor-Litvak et al. (43) found that Hg exposure was not associated with detectable deficits both in cognitive or motor functions. Normal background urinary Hg levels in the general population without amalgam fillings are considered to be approximately 0.7 ug $L^{-1}$ or 0.5 ug $g^{-1}$ creatinine and 3.1 ug $L^{-1}$ or 2.4 ug $g^{-1}$ creatinine with amalgam fillings. Previous studies found a positive correlation between urinary Hg levels and the number of amalgam fillings. They confirmed that in general population, not occupationally exposed to Hg, average urinary Hg values were in the range of 1.3-3.8 µg $L^{-1}$ (42). Others have reported background levels for urinary Hg in an unexposed population as 5 ug $g^{-1}$ creatinine (44). In another study, it is suggested that there was no indication of Hg-associated adverse effects at levels below 5 µg Hg $g^{-1}$ creatinine or 7 µg Hg $L^{-1}$ in urine (45). Attar et al. showed that serum Hg levels in MS patients were statistically higher than controls. Their data revealed that high Hg levels might contribute to MS development in susceptible individuals (46).

Our study groups were not occupationally exposed to Hg and intake of Hg with diet can be negligible (according to the questionnaire information). Therefore, it was assumed that their exposure to Hg was from dental amalgam fillings. Hg levels in patients were statistically higher than control subjects. This significance was remarkable for Hg accumulation derived from amalgam fillings. MS patients also had higher levels of urinary Hg compared to patients without amalgam fillings and controls with or without amalgam fillings. It was concluded that this additional Hg burden needed to be taken into consideration. Excess levels of Zn suppress Cu and Fe absorption thereby promoting ROS production and disruption of metabolic enzymes. They lead to apoptosis, consequently neurodegeneration. However, in our study, urinary Zn levels were found to be lower in MS patients with amalgam fillings than those without amalgam fillings.

The key limitation of our study is that having relatively small sample size. Based on previous published studies, we expected that there might be an association between Hg exposure from amalgam fillings and *OGG1* gene polymorphism. However, we could not find any associations between urinary Hg levels and OGG1 gene polymorphism since the number of samples in subgroup comparisons is even smaller.

Hg is one of the reasons for the development of MS, however, there is no sufficient evidence on the association between Hg derived from dental amalgam fillings and the risk of MS. Inadequate detoxification or repair due to genetic polymorphisms might cause metal accumulation in body. It was investigated the impact of detoxifying and repair gene polymorphisms on the risk of MS related to Hg exposure from amalgam fillings. As a result, significantly higher levels of urinary Hg in subjects with amalgam fillings caused an additional Hg burden. Based on our study and previous findings, polymorphic *OGG1* genotype is a risk factor for the development of MS. Furthermore, it can be said that excess Hg exposure may be a risk factor in subjects having reduced or lack of activity of OGG1 enzyme due to genetic polymorphism. In future, conducting similar studies with larger sample sizes will help to make a robust judgment.

**Conflict of interest**

No conflict of interest was declared by the authors.

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