



## INFLUENCE OF COPROPORPHYRINOGEN OXIDASE 4 POLYMORPHISM ON URINARY BIOMARKERS OF DENTAL MERCURY EXPOSURE IN PATIENTS WITH AMALGAM FILLINGS

AL-FAWAEIR SAAD<sup>1</sup> | KURT I<sup>1</sup> | OKTAY E.A.<sup>2</sup> | KUBAR A<sup>3</sup> | SERDAR M.A<sup>1</sup> | AYKUT O<sup>4</sup>

<sup>1</sup> DEPARTMENT OF MEDICAL BIOCHEMISTRY, GULHANA MEDICAL SCHOOL, ANKARA, TURKEY.

<sup>2</sup> DEPARTMENT OF DENTISTRY, GULHANA MEDICAL SCHOOL, ANKARA, TURKEY.

<sup>3</sup> DEPARTMENT OF VIROLOGY, GULHANA MEDICAL SCHOOL, ANKARA, TURKEY.

<sup>4</sup> REFIK SAYDAM HYGIENE CENTER PRESIDENCY, ANKARA, TURKEY.

### ABSTRACT

**Background:** Dental amalgam filling contains 50% Hg in its gradients and has been accepted as a part of dental treatment for more than 170 years ago, the side effects of dental amalgam is still controversial subject and in many European countries it was prohibited or restricted.

**Aim:** in this study we aimed to study the effects of coproporphyrinogen oxidase 4 (CPOX-4) polymorphism on dental mercury exposure urinary biomarkers in patients with amalgam fillings.

**Subjects and Methods:** The study group consisted of 102 patients have amalgam fillings from different periods of time and 32 healthy subjects as control group. Urine and blood samples were collected from these subjects. Mercury levels in urine were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), urinary total porphyrins levels were measured by High Performance Liquid (HPLC), for genomic assays the following methods were used, DNA extraction, real time-PCR and Amplification Refractory Mutation System (ARMS).

**Results:** The total urinary porphyrins concentrations in patients group were statistically significant higher than in control group (60.2 ±41.8 nmol/L and 41.2 ±19.5 nmol/L respectively) (p<0.001). After adjusting to creatinine total urinary porphyrins concentrations in patients group were significantly higher than that in control group (5.6± 3.20 vs. 3.7±0.18 nmol/mmol creatinine). The concentrations of urinary Hg was statistically significant higher in patients group (6.4 ± 3.8 µg/L) than in healthy group (2.5± 1.2µg/L) (p<0.001). The frequencies in patients group of the homozygous common allele (A/A), heterozygote genotype (A/C) and homozygous genotype (C/C) were 85%, 13% and 2% respectively. Where in healthy groups the frequencies were 97%, 3% and 0 %. In all subjects (n=134) the frequencies were 88%, 10% and 2 %.

**Conclusion:** collective results states that (CPOX-4) polymorphism affects urinary biomarkers of dental mercury exposures in patients with amalgam fillings showing a significant increase in urinary mercury levels and urinary porphyrins concentration in patients with homozygous genotype of (CPOX-4).

**Keywords:** Mercury, Dental Amalgam Fillings, Porphyrins, Coproporphyrinogen Oxidase 4, Polymorphism.

### Introduction

Mercury is well known as a toxic metal in the human body and at high levels, it causes liver and kidney damage as well as neurological disorders. This toxic metal not only inhibit various enzymatic reactions and metabolic processes, but also enhances lipid peroxidation, progression of atherosclerosis and the risk of myocardial infarction and stroke, leading to death (1,2).

Mercury is a highly hazardous pollutant with an estimated global natural mercury emission of 1800-5800 tons per annum (3) and global anthropogenic mercury emission to the atmosphere was estimated to be 2190 tons in 2010 (4). Since mercury is ubiquitous in the environment, it is nearly impossible for most human and animals to avoid exposure to some forms of mercury, be it elementary, organic or inorganic. Mercury, through biotransformation and bioaccumulation, has found its way through the food chain to humans. Anthropogenic activities and

industrialization are also sources of mercury pollution that had resulted in several catastrophe of mercury poisoning in many countries in world (5). Moreover, mercury pollution and poisoning have imposed a huge economic cost on environmental remediation and public health (6).

Dental amalgam filling contains 50% Hg in its gradients and has been accepted as a part of dental treatment for more than 170 years ago, the side effects of dental amalgam is still controversial subject and in many European countries it was prohibited or restricted (7).

Recently, an increasing interest in the health hazards associated with mercury exposure has been raised, and the mercury release from dental amalgam has been blamed for a variety of health complaints .In particular, prenatal exposure to mercury may affect the blood pressure and ability to respond to sensory stimuli in the exposed fetus and infants later in life. Thus dental amalgam fillings and restoration have become increasingly recognized as a

potentially significant source of inadvertent chronic exposure to mercury, particularly in populations with no dietary dependency on foods of marine origins. Over the past decade, in the Western countries with little mercury intake from fish consumption, mercury release from dental amalgam fillings has been blamed for a variety of health complaints (7, 8).

In this work we aimed to study effects of coproporphyrinogen oxidase 4 on urinary biomarkers of dental mercury in patients with amalgam fillings to investigate any possible effects.

## Methods and Materials

### Study populations

During the period between 15 March 2012 and 30 May 2013 samples were collected from patients attending to the Department of Dentistry in Gulhane Medical Military Academy in Ankara-Turkey, 102 patients with amalgam filling were selected with different number of amalgam fillings and different duration of these fillings, on the other hand 32 subject without amalgam filling were selected as healthy control group.

After written informed consent was obtained, a 2-3 min face to face interview was conducted about (a) the number of amalgam fillings (b) the duration of the amalgam fillings (c) the age (d) smoking status (who smokes, daily average of cigarettes consuming). The average of amalgam filling number were classified into five categories (1-5, 6-10, 11-15, 16-20 and > 20) the same categories were used to classify duration of amalgam fillings.

From each patient the following samples were collected: 25 ml urine sample, 4 ml whole blood in EDTA tube. Urine and blood samples were stored at refrigerator at  $-20\text{ }^{\circ}\text{C}$  until day of analysis.

### Exclusion criteria

The Exclusion criteria were: patients with bridges, crowns, gold inlays or dentures, and those with relevant organic or metal disorders. Females were excluded because they usually using dyes for their hair this may increase heavy metals concentrations.

### Determination of urinary mercury by ICP-MS

#### Standards preparations

A solution of 1000 mg/L concentration was prepared from stock standard of mercury. Then a 10 ml of the intermediate standard of 1000 mg/L were taken and 90 ml of deionized water were added for 100mg/L standard preparation. A 10 ml of 100 mg/L intermediate standard were taken and 90 ml of deionized water were added to prepare 10 mg/L standard. Then a 10 ml of 10 mg/L intermediate standard were taken and 90 ml of deionized water were added to prepare 1 mg/L standard.

For linearity calculation the following serial standards were prepared before start working 1,2,5,10,15 and 20  $\mu\text{g/L}$ .

### Sample preparation:

The urine sample was homogenized by well shaking, 5 ml  $\text{HNO}_3$  were added to about 1 ml urine, then 0.5 ml HCl and 5ml deionized water were added, then the solution was placed in microwave, after the heat processes finished, 25 ml deionized water were added to solution, after cooling for 30 minutes the sample was injected to ICP-MS.

### Determination of urinary porphyrins by HPLC

#### Sample preparation

500  $\mu\text{l}$  were taken from urine sample in eppendorf tube and 50 $\mu\text{l}$  of concentrated HCl (37%) were added then vortexed for 1 minute and centrifuging for 5 minutes at 1000g, 200 $\mu\text{l}$  of supernatant were placed into a vial and then injected to HPLC.

#### Standards preparations

Stock standard was prepared by adding 2ml of 3M HCl to porphyrin acid chromatographic marker; intermediate standard was prepared by adding 950 $\mu\text{l}$  of 0.1 M HCl to 50 $\mu\text{l}$  from stock solution.

#### Preparation of Mobile Phase 1

Mobile phase was prepared by dissolving 77.08 g of ammonium acetate in 500 ml distilled water, on the other hand 27 ml of 100% glacial acetic acid to 473 ml of distilled water, after will mixing of the two solutions 100 ml was removed and 100 ml of acetonitrile were added, the solution was filtered using filtration pump.

#### Preparation of Mobile Phase 2

Mobile phase 2 was prepared by adding 450 ml of methanol to 50 ml of acetonitrile.

### Determination of urinary creatinine levels

The levels of urinary creatinine were measured using Olympus AU 2700(Olympus Optical Co.Ltd.Shizuoka-ken, Japan). (Kinetic Jaffe method)

### Genomic assay

#### DNA extraction

DNA samples were extracted by the inorganic method, 12.5 $\mu\text{l}$  of pronase were added to 500 $\mu\text{l}$  of K buffer, and then 50 $\mu\text{l}$  of whole blood were added, the solution was incubated at  $47\text{ }^{\circ}\text{C}$  for 1 hour.

500 $\mu\text{l}$  of phenol were added to the solution followed by centrifugation at 1300 rpm for 6 minutes. Then the upper colorless layer was taken and putted in a clean eppendorf tube, then 500  $\mu\text{l}$  of isopropyl alcohol were added then centrifugation for 6 minutes at 1300 rpm.

500 $\mu\text{l}$  of ethyl alcohol (75%) were added to pellets, after that it was centrifuged, and then the upper layer of pellets was poured. Then it was diluted by adding with deionized distilled water (300-500 $\mu\text{l}$ ) followed by drying in oven for 20 minutes at ( $37\text{-}65\text{ }^{\circ}\text{C}$ ).

### Real time PCR reaction

CPOX exon 4 gene (GTAGGCa / cACAAGC) single-nucleotide polymorphism (SNP) was determined by real time PCR analysis using PCR analyzer (7500 Applied Biosystems, USA). The standard conditions of real-time PCR were performed. Real Time PCR cycles during operations were performed at the end of each line fluorescence reading at 60 °C. TaqMan-based measurements were used for detailed analysis of SNPs.

In this study COPX exon 4 gene polymorphisms were determined using a primers and specific probes. SNP alleles were analyzed by fluorescence-based 5'-nuclease. ARMS primers were designed in this study using the OLIGOYAP5.0 program (10). The nucleotide sequences of the primers prepared as follows:

- CPOXEX4NP2 CACCAAACCACCACTGCTTGAT
- CPOXEX4MP2 ACCAAACCACCACTGCTTGAG
- CPOXEX4OP1 ATTAAGCTGCTAAATTAAGTGTCTTA
- CPOXEX4PR FAM-CCAGTAATGCTGAATCTCAAAAGTCCACA-BHQ

PCR amplification of the initial denaturation begin at 95 °C for 5 minutes with the continuation of the process, with 40 cycles of denaturation at 95 °C for 15 seconds followed by 60 °C, 60 seconds to connect to the continued and at the end of cycles performed during the fluorescence reading.

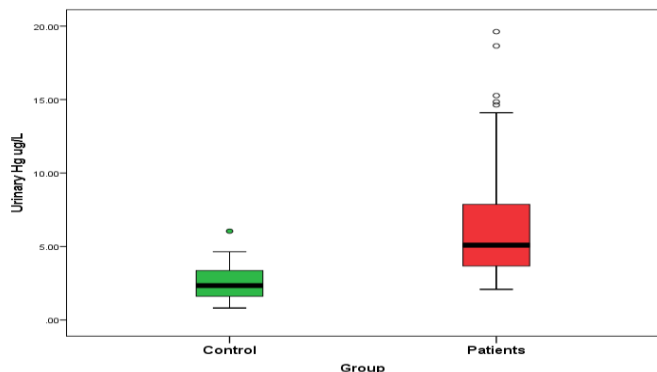
**Statistical analysis**

All statistical analysis were performed using SPSS for windows 15.0 (SPSS Inc. Headquarters, Chicago, Ill., USA) software program and Microsoft Excel 2007 program. The data were expressed as mean ± standard deviation (SD). In all of our statistical analysis a two-tailed P-value of <0.05 was considered statistically significant.

**RESULTS**

**Urinary mercury**

Analysis of urinary mercury was performed among the two groups using ICP-MS. The mean ± SD of urinary Hg was significantly higher in patients group (6.5 ± 3.9 µg/L) than in healthy group (2.5681± 1.271µg/L) (p<0.001) Figure 1. We found that there is a strong correlation between the duration of amalgam fillings and the level of mercury (Pearson rank 0.727). Also a strong correlation between the number of amalgams and urinary mercury level (Pearson rank 0.875).

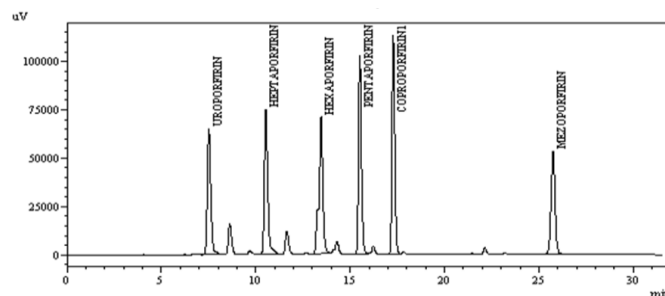


**Figure 1** The distribution of urinary mercury levels among groups (Box-plot graphic).

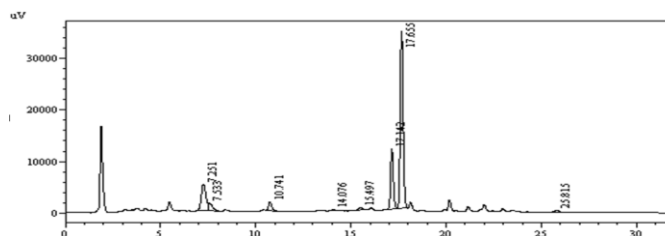
**Urinary total porphyrins**

Porphyrins are formed as intermediates in the biosynthesis of heme. In human and other mammals, porphyrins with eight, seven, six, five, and four carboxyl group are excreted in the urine in a well-established pattern. Mercury selectively alters porphyrin metabolism in kidney proximal tubule cells, leading to an altered urinary porphyrin excretion pattern. Dental amalgams are a commonly used as a dental restorative material, and amalgams contain about 50% mercury. In our study, urinary total porphyrins were examined to in 102 patients with different numbers and duration of amalgam fillings.

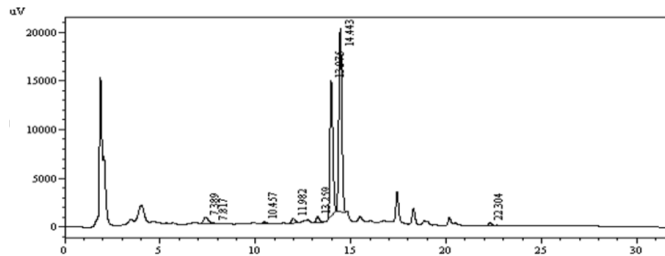
In our study HPLC was used to measure the total porphyrins in patients group and control group (Figure 2, 3, 4).



**Figure 2:** Chromatogram of HPLC porphyrin standard profiles.



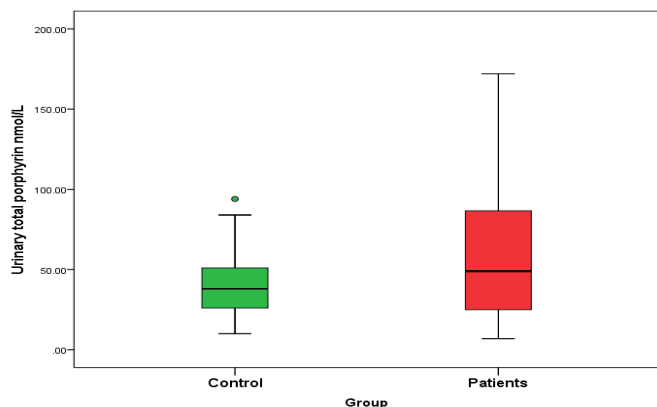
**Figure 3:** Chromatogram of HPLC porphyrin profile of unexposed healthy subject.



**Figure 3:** Chromatogram of HPLC porphyrin profile of dental mercury- exposed subject

In our study we found that the mean ± SD of total porphyrins concentrations in patients were significantly higher than in control subjects (60.2±41.8 nmol/L and 41.2±19.5 nmol/L respectively) (p<0.001) (Figure4.). The mean of total porphyrins in overall subjects was (50.28 nmol/L), strong correlation between total porphyrins and

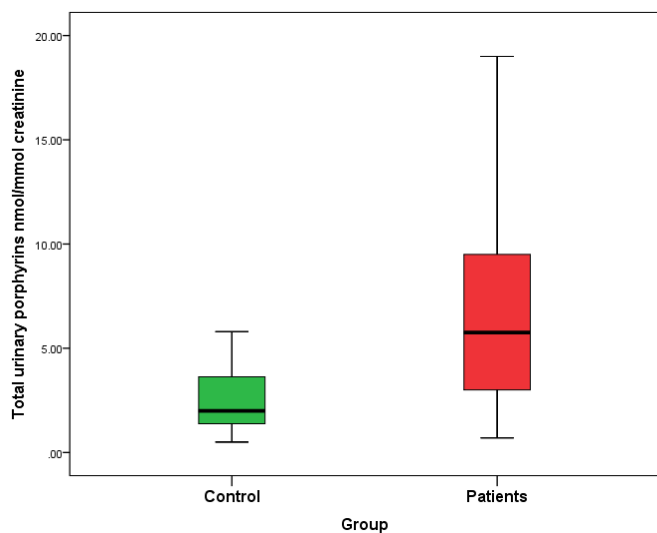
duration of amalgam fillings was found (Pearson Rank 0,192) , also a strong correlation was found between both Coproporphyrins and Uroporphyrins and duration( Pearson Rank 0.182,0.198 respectively ). There is no correlation between urinary total porphyrin and age of subject (-0.234).



**Figure 4:** The distribution of urinary total porphyrins among the groups (Box-plot graphic).

The mean ± SD of coproporphyrin was significantly higher in patient in comparison with healthy subjects (42.2 ± 13.8 vs. 27.98 ± 17.87 nmol/L).

After adjusting to creatinine total urinary porphyrins concentrations in patients group were statistically significant higher than in control group (5.60± 3.20 vs 3.70±0.18 nmol/mmol creatinine).



**Figure 5:** The distribution of urinary total porphyrins among the groups after adjusting to creatinine (Box-plot graphic).

The mean ± SD of heptaporphyrin, hexaporphyrin and pentaporphyrin in patients is higher than that of healthy subjects (Table1).

**Table1:** Summary of porphyrins fraction levels (nmol/L) among groups.

	Patients group (n=102)		Control group (n=32)		P
	Mean +SD	geometric mean	Mean +SD	geometric mean	
UrinaryHg (µg/mmol creatinine)	0.98±.54	0.83	0.18±0.16	0.12	<0.001
Hg (µg/L)	6.5±3.8	5.5	2.5±1.3	2.3	<0.001
Total Porphyrin (nmol/L)	60.0±43.6	47.8	41.2±19.5	38.8	<0.001
Uro (nmol/l)	12.1±6.7	8.9	8.3 ± 2.8	6.8	0.002
Hepta (nmol/L)	2.8± 1.8	2.6	2.0 ± 0.8	1.9	0.456
Hexa (nmol/L)	2.5 ±2.5	2.5	1.8 ± 0.6	1.6	0.014
Penta (nmol/L)	3.0 ± 2.4	2.34	1.8 ±0.8	1.6	0.962
Copro-total (nmol/L)	42.0± 13.8	39.2	27.8 ±17.8	25.4	<0.001
Copro-I (nmol/L)	15.2±12.6	9.3	8.7±6.2	6.7	<0.001
Copro-III (nmol/L)	28.3± 16.3	25.4	18.8 ±12.3	15.6	<0.001
(Penta+Copro) (nmol/L)	33.6 ± 21.8	30.5	30.8± 18.8	25.8	0.008

**Polymorphism of CPOX**

Automated genomic sequencing-based assays were performed on DNA acquired from 28 atypical high porphyrin excretors to investigate the presence of polymorphisms in genes encoding CPOX. All Subjects of this study were included in genomic assay; a single nucleotide polymorphism (SNP) encoding was identified in the CPOX gene at exon 4.

The frequencies of the homozygous common allele (A/A), heterozygote genotype (A/C) and homozygous genotype (C/C) were 85%, 13% and 2% respectively. Where in healthy groups the frequencies 97%, 3% and 0 were %.( Table 2,).

**Table 2:** CPOX polymorphism investigated with fluorescent 5' nuclease assays

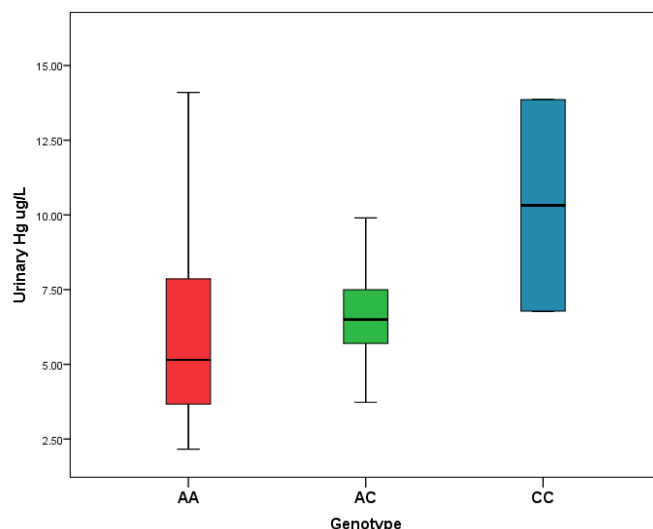
Group	Region	Sequence	Frequency
Patient s	CPOX exon 4	GTAGGCa/cACAAG C	WT(A/A)= 0.85 Het(A/C)=0.13 Mut(C/C)=0.02
Control	CPOX exon 4	GTAGGCa/cACAAG C	WT(A/A)= 0.97 Het(A/C)=0.03 Mut(C/C)=0.00

<b>Total</b>	CPOX exon 4	GTAGGGCa/cACAAG C	WT(A/A)= 0.88 Het(A/C)=0.10 Mut(C/C)=0.0 2
--------------	----------------	----------------------	--

The total urinary porphyrins and Hg levels in urine was higher in patients with homozygous polymorphic(C/C) in comparison with patients with homozygous common (A/A) and heterozygous (A/C) in patients group (Table 3, Figure 6).

**Table 3:** Hg levels in urine and total urinary porphyrins in comparison with genomic assay results.

Test	Genotype (A/A) n=90	Genotype (A/C) n=10	Genotype (C/C) n=2
Urinary Hg (µg/L)	6,5±3,9	5,4±2,9	10,30±4.70
Urinary Hg (µg/g kreatinin)	0.65±0.24	0.78±.34	0.98±0.45
Hair Hg (µg/g saç)	0,55±0,14	0,63±0,28	0,76±0.19
Urinary total porphyrin (nmol/L)	54±38	56±44	58±43
Urinary total porphyrin, (nmol/mmol kreatinin)	4.5±1.2	6.2 ±2.3	6.5±1.4
Uro (nmol/l)	7.22.8	7.3±3.7	8.5±3.6
Hepta (nmol/l)	2.5± 1.4	2.6±1.8	2.9±1.2
Hexa (nmol/l)	2.3±1.1	2.2±1.3	2.6±1.5
Penta (nmol/l)	1.4±0.77	1.5±0.8	1.7±0.9
Copro I (nmol/l)	11.0±8.7	13.0±9.8	14.0±12.2
Copro I (nmol/l)	14.0±12.5	15.0±13.2	17.0±15.6
( Copro + Penta) (nmol/l)	25.0±19.0	30.0±11.0	33.0±13.0



**Figure 6:** The distribution of urinary mercury in different genotype in patients group (Box-plot graphic)

**DISCUSSION**

**Urinary mercury**

To date, the issue of safety in the use of amalgams is still being debated, with conflicting researches findings. In 2009, the FDA concluded that dental amalgam is a safe and effective restorative treatment; however, after receiving several petitions raising concerns on specific issues, the FDA reviewed the use of amalgams in late 2010. In our study, urinary Hg levels were measured by ICP-MS from 102 subjects with amalgam fillings and 32 subjects without amalgam fillings from Turkish population.

The results of our study suggest that extent of excretion of Hg in the urine is related to the exposure from dental amalgam in a number and duration -dependent fashion. The findings of our results are consistent with previous studies examining Hg exposure from dental amalgams. In USA, investigators examined US subjects over a 5-year period and found that the number of amalgam restorations had a significant dose-response relationship with Hg urine levels (15). Similarly, other investigators found that the number of amalgam was related to the emission rate of Hg into the oral cavity and to the excretion rate of Hg by urine. Finally, a positive correlation was found between urine Hg concentration and duration of amalgam restoration (11).

A further study found that, in adults, Hg excretion correlated with the number of amalgam fillings (12). They also found that immediately post removal (up to 6 days after removal), there was a mean increase of 30% and that within 12 months after removal of all amalgam fillings, the participants showed substantially lower urinary Hg levels. Other investigators found, in adult males, a significant correlation between amalgam exposure and both urinary and blood Hg amalgam concentration. They estimated from the data that, on average, each 10-fold increase with an increase of 1µg Hg/L in urine concentration. This observation was found in numerous studies.

In our study it was observed that the urinary Hg concentrations were highly correlated with the number of amalgam fillings when comparing subjects in the dental amalgam group to the healthy group this finding agree with that found by Woods et al 2006.

It should be pointed out that urinary Hg excretion is a minor excretory pathway and that about 90% of excretion of inhaled Hg vapor is eliminated in feces (13). Also, it is well known that Hg is a retained toxicant, with high central nervous system half-life estimated to be many years (14).

### Urinary total porphyrins

Previous studies noted specific changes in urinary porphyrin excretion patterns associated with exposure to mercury in animals and humans. In our study urinary porphyrin concentrations were examined in patients with amalgam fillings from different periods of time and compared these concentrations with healthy group without amalgam fillings, in order to evaluate the potential health consequences of prolonged exposure to Hg from dental amalgam fillings.

Our study found that the characteristics pattern of porphyrinuria associated with Hg -burden, specifically, elevated pentacarboxylporphyrins, coproporphyrins and total porphyrin concentrations in patients with amalgam filling when compared with that without amalgam fillings, and these elevated concentrations correlated with the number of amalgam fillings and the duration of amalgam.

The findings from the present study are consistent with previous studies examining Hg exposure from dental amalgam and other biomarkers of Hg exposure. For example, Dunn et al. (2008) examined US children over 5-year period and found that the number of amalgam restoration had a significant dose-response with Hg urine levels (15). Likewise, Wood et al. (2007), in a longitudinal study in children, found urinary Hg concentrations were highly correlated with the number of amalgam fillings (16). Post-mortem studies also show this same dose-dependent central theme. Guzzi et al, for example, found that at autopsy, total Hg levels in all types of tissue were significantly higher in subjects with a greater number of amalgam surfaces (> 12) compared with those fewer amalgams (0-3)(17). These authors also reported that the greater the number of amalgams, the greater the likelihood that Hg was found in the brain.

Several studies found this same dose-dependent occurrence when determining the rate of Hg absorption from amalgams. For example, Abraham et al. looked at Hg levels in blood and in mouth air before chewing in 47 persons with and 14 persons without dental amalgam restorations and found that blood Hg concentration were positively correlated with the number and surface area of amalgam restorations (18). Olstad et al, also found a positive correlation between urine Hg concentration and extent of amalgam restoration (19). Vimy and Lorscheider found that subjects with 12 or more occlusal amalgam surfaces were estimated to receive a daily Hg dose 29 µg, as compared to subjects with four or fewer occlusal

amalgam surfaces, for whom the dose was 8 µg (20).

The steps in the heme pathway most vulnerable to heavy metal inhibition are those in which uroporphyrin decarboxylase (UROD) and coproporphyrinogen oxidase (CPOX) are involved (21,22). The presence of Hg inhibits specific enzymes that are necessary for the heme synthesis pathway to progress.

Consistent with previous observations, the results of our study suggest that urinary porphyrin testing was able to detect the low-dose continuous exposure to Hg among individuals with dental amalgams. The relative 5-10% increases in the Hg -associated porphyrin observed over a long period of presence of amalgam fillings, suggest that dental amalgams for the average individual do not cause an acute high-dose exposure to Hg, but instead reflects a significant life-long continuous chronic low-dose exposure to Hg.

As a result, our data suggests that the chronic low-dose exposure to Hg from dental amalgams continuously makes a significant contribution to Hg-associated urinary porphyrin levels, and hence to contributes to an ever-increasing body-burden of Hg. This type is particularly evident when comparing the relative 5-10% increase in the Hg-associated porphyrins observed in some studies with other studies linking elevated Hg-related neurological disorders. Several recent studies revealed twofold to threefold significantly higher Hg-associated porphyrin among subjects diagnosed with neuro-developmental disorders in comparison to neurotypical subjects (23, 24).

Numerous studies have proposed the potential utility of monitoring urinary porphyrin excretion patterns as biomarker of chemical exposure and effects in target tissues (25, 26). The present study defines the characteristics of porphyrins profile changes elicited by dental mercury during, low-level exposure in patients with amalgam fillings.

In the present study we observed that mercury exposure elicits specific changes in the porphyrin excretion pattern. These patterns characterized by significantly increased urinary concentration of coproporphyrin, pentaporphyrin and total porphyrins, these results agree with pervious results.

### CPOX polymorphism

Urinary mercury concentration have long been used for quantifying exposure to elemental mercury, representing recent exposure (over period of 2-3 months), but may not adequately represent risks associated with longer periods of exposure. In contrast, an increase in the urinary concentration of specific porphyrins has been described as a biomarker of prolonged exposure to all forms of Hg ([Bowers et al,1992] and [Wood et al, 1991]) (27), based upon selective interference with the fifth (UROD) and sixth (CPOX) enzymes of the heme biosynthesis pathway in kidney cells, a principal target of the Hg. Whereas urinary porphyrin concentrations may also be influenced by other

environmental exposures (especially other heavy metals and halogenated hydrocarbons, medications and disease conditions (28), Hg induces a specific change in the urinary porphyrin excretion pattern characterized by increased concentration of pentacarboxyporphyrin (5-CP) and coproporphyrin (4-CP), along with the appearance of an atypical porphyrin identified empirically as keto-isocoproporphyrin (KICP)(29).

This pattern of the dose-and time -related porphyrinogenic response to Hg has been observed in both animals and humans and has been proposed as a potential biomarker of prolonged Hg exposure. However, while this response has been shown to be predictable among most human subjects occupationally exposed to Hg (30), approximately 15% of subjects from several population studies have been found to display an atypical response to Hg exposure, characterized by excretion of substantially higher concentrations of all three porphyrins, especially KICP, independent of Hg dose. This atypical porphyrinogenic response to Hg has been attributed to a specific SNP exon 4 of the CPOX gene (CPOX4 polymorphism) encoding a N272H substitution in the CPOX4 gene product (31). These findings represent the first report of a polymorphism in a human gene that modifies the effect of Hg on a biological process and suggest that the atypical porphyrinogenic response, which occurs as a consequence of Hg exposure in subjects with the CPOX4 polymorphism, might serve as a biomarker of susceptibility to Hg toxicity. The porphyrinogenic response to Hg, characterized by an increase in the urinary concentration of 4-CP and 5-CP, is attributable to the preferential inhibition by Hg of the UROD and CPOX enzymes. It is further characterized by the appearance and increased concentration of an atypical porphyrin, KICP, which is postulated to be formed as a consequence of excess 5-CP accumulation in kidney cells, and the preferential use of 5-CP as a substrate of CPOX4.

It was previously observed that the 5- to 4-decarboxylation step is exquisitely sensitive to Hg<sup>+2</sup> both in vivo and in vitro, resulting in the selective accumulation of 5-CP in kidney epithelial cells. Notably, no polymorphism in UROD that modified the effect of Hg on porphyrin excretion was observed previously (32). This finding was somewhat unexpected in light of numerous reports of UROD polymorphisms that render the gene product potentially more susceptible to inhibition by chemical agents (32), our study suggest, instead, that the CPOX4 polymorphism may encode an enzyme that preferentially utilizes 5-CP, as opposed to 4-CP, as substrate and that Hg exacerbates this effect by inhibiting the decarboxylation of 5-CP, resulting in 5-CP accumulation and its conversion to KICP by CPOX4.

The frequencies of the homozygous common (A/A), heterozygous (A/C), and homozygous polymorphic (C/C) genotypes of CPOX were 85%, 13% and 2% in patients group and 97%, 3% and 0% in control group in our result. These findings in comparison with a study in American population (21) the concentration of Hg in urine and hair

are significantly higher in patients with homozygous polymorphic(C/C) in comparison with those have homozygous common (A/A), heterozygous (A/C). These findings from our study are consistent with previous studies heterozygous (A/C) in Turkish population is less (%25).

## REFERENCES

1. Radcliffe HE, Swanson GM, Fisher LJ. Human exposure to mercury: a critical assessment of the evidence of adverse health effects. *J. Toxicology Environ Health*, 1996; 49:221.
2. Watanabe C, Satoh H, Evolution of our understanding of methyl mercury as a health threat. *Environ Health Perspect* 1996; 104(Supp 2):367.
3. Li P, Feng XB, Qiu GL, Shang LH, Li ZG, Mercury pollution in Asia : A review of the contaminated sites *J Hazard Mater* 2009. 03.03119345013.
4. Pacyna EG, Pacyna JM, Steenhuisen F, Wilson S, Global anthropogenic mercury emission inventory for 2010 *Atmos Environ* Year:j atomsenv. 2010.0.041.
5. Kudo A, Fujikawa Y, Miyahara S, Zheng J, Takigami H, Sugahara M, Muramatsu T, Lessons from minamata mercury pollution. *Japan-After a continuous 22 years of observation Water Sci Technol* Year:1998 :38:1871-93.
6. Trasande L, Landrigan PJ, Schechter C, Public health and economic consequences of methyl mercury toxicity to the developing brain, *Environ Health Persp* 2005.
7. Mutter J; is dental amalgam safe for human? The opinion of the scientific committee of the European Commission. *J Occup Med Toxicol*. 2011 Jan 13; 6(1):2.
8. Lorscheider, F.L., Vimy, M.J., and Summers, A.O. Mercury exposure from "silver" tooth fillings: emerging evidence questions a traditional dental paradigm. *FASEB Journal* 9:504-508, 1995.
9. Leong, C.C.W., Syed, NLL., and Lorscheider, F.L. Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following in vitro exposure to mercury. *NeuroReport* 12: 733-737, 2001.
10. Mehmet Y. Hakan A. Alladdin P, Cakir G, Ismail Y.A, Kenan Sener, Ahmet C.B, Mohammad A. Ayhan K. Rapid and quantitative detection of Crimean-Congo Hemorrhagic Fever Virus by one- step Real - time Reverse transcriptase-PCR. *JPN.J. Nefect Dis*:58,358-362, 2005.
11. Kingman A, Albertini T, and Brown LJ. Mercury concentrations in urine and whole blood associated

with amalgam exposure in a US military population. *J DentRes* 1998; 77: 461-467.

12. Woods JS, Martin MD, Leroux BG, DeRouen TA, Leitaõ JG, Bernardo MF. The contribution of dental amalgam to urinary mercury excretion in children. *Environ Health Perspect* 2007; 115: 1527-1531.

13. Geier DA, Carmody T, Kern JK, King PG, and Geier MR. A significant relationship between mercury exposure from dental amalgams and urinary porphyrins: a further assessment of the Casa Pia children's dental amalgam trial. *Biometals* 2011.

14. Berglund A, Molin M: Mercury vapor release from dental amalgam in patients with symptoms allegedly caused by amalgam fillings. *Eur J Oral Sci* 104; 56-63: 1996.

15. Dunn JE, Trachtenberg FL, Barregard L, Bellinger D, McKinlay S (2008). Scalp hair and urine mercury content of children in the Northeast United States: the New England Children's Amalgam Trial. *Environ Res* 107(1):79-88.

16. Wood JS, Martin MD, Leroux BG, DeRouen TA, Leitao JG, Bernardo MF, Luis HS, Simmonds PL, Kushleika JV, Huang Y (2007). The contribution of dental amalgam to urinary mercury excretion in children. *Environ Health Perspect* 115(10):1527-1531.

17. Guzzi G, Grandi M, Cattaneo C, Calza S, Minoia C, Ronchi A, Gatti A, Severi G (2006). Dental amalgam and mercury levels in autopsy tissues: food for thought. *Am J Forensic Med Pathol* 27(1):42-45.

18. Abraham JE, Svare CW, Frank CW (1984) The effect of dental amalgam restoration on blood mercury levels. *J Dent Res* 63(1):71-73.

19. Olstad ML, Holland Ri, Wandel N, Pettersen AH (1987) Correlation between amalgam restoration and mercury concentration in urine. *J Dent Res* 66(6):1179-1182.

20. Vimy MJ, Lorscheider FL (1985) Serial measurements of intra oral air mercury: estimation of daily dose from dental amalgam. *J Dent Res* 64(8):1072-1075.

21. Wood JS, Kardish RM (1983) Developmental aspects of hepatic heme biosynthesis capability and hemotoxicity -II. Studies on uroporphyrinogen decarboxylase. *Biochem Pharmacol* 32(10):73-78.

22. Woods SJ, Echaverria, Heyer NJ, Simmonds PL, Wilkerson J, Farin FM (2005). The association between genetic polymorphism of coproporphyrinogen oxidase and an atypical porphyrinogenic response in humans. *Toxicol Applied Pharmacol* 206(2):113-246.

23. Gerier AD, Gerier MR (2006) A prospective assessment of porphyrins in autistic spectrum disorders. *J Toxicol Environ Health A* 70(20):1723-1730.

24. Buchet JP, Lauwerys R, Roels H, Bernard A (1980). Relationship between exposure to heavy metals and prevalence of renal dysfunction. *Arch Toxicol Suppl* 4:215-218.

25. Woods JS, Fowler BA. Altered regulation of mammalian hepatites heme biosynthesis and urinary porphyrin excretion during prolonged exposure to sodium arsenate. *Toxicol Appl Pharmacol* 1978. 43(2):361-371.

26. Bellinger DC, Daniel D, Trachtenberg F, Tavares M, McKinlay S. 2007. Dental amalgam restorations and children's neuropsychological function: the New England Children's Amalgam Trial. *Environ Health Perspect* 2007; 115: 440-446.

27. Woods JS, Bowers MA, Davis HA: urinary porphyrin profile as biomarker of trace metal exposure toxicity: studies on urinary porphyrin excretion patterns in rats during prolonged exposure to methyl mercury. *Toxicol Appl Pharmacol* 1991, 110(3):464-476.

28. Daniell W.E, Ellefson R.D, Moore M.R, Pierach C.A, Schreiber W.E, Tefferi A and Franklin G.M. Environmental chemical exposures and disturbances of heme synthesis. *Environ. Health Perspect.* 105 Suppl.1(1997), pp. 33-53.

29. Wood JS, Martin MD, Naleway CA, Echeverria D (1993). Urinary porphyrin profiles as biomarkers of mercury exposure: studies on dentists with occupational exposure to mercury vapor. *J Toxicol Environ Health* 40(2-3):235-246.

30. Gonzalez -Ramirez D, Maiorino RM, Zuniga-Charles M, Xu Z, Diaz Gama JH, Echeverria D, Wood JS, Aposhian H (1995) Sodium 2,3-dimercaptopropane -1-sulfonate challenge test for mercury in humans II: Urinary mercury, porphyrins and neurobehavioral changes of dental workers in Monterrey. *J Pharmacol Exp Ther* 272(1):246-274.

31. Luglie PF, Campus G, Chessa G, et al: Effect of amalgam fillings on the mercury concentration in human amniotic fluid. *Arch Gynecol Obstet* 271; 138-142: 2005.

32. Elder GH. 1974. The metabolism of porphyrins of the isocoproporphyrin series. *Enzyme* 17,61-68.