Urinary Mercury Level, Neurobehavioral Performance And Some Biochemical Markers In Children with Amalgam Restorations

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Abstract: At present, there is a lack of scientific evidence on toxicity from low-level mercury exposure in children. Despite the debate over the safety of dental amalgam fillings, amalgam is still widely used to restore posterior teeth in pediatric dentistry. Although, children could be at greater risk to harm from low-level exposure due to their developing nervous systems. Hence, this research was carried out to define some potential health effects from dental amalgam on children's health. Children were selected from those attending the Pedodontic clinic, Faculty of Dentistry, Tanta University. They were subjected to clinical examination, neurobehavioral and intelligence quotient (IQ) assessment, urinary mercury level, serum; malondialdehyde (MDA), reduced glutathione (GSH), zinc (Zn), and gamma amino butyric acid (GABA) measurement. The present study revealed that the mean urinary mercury level was significantly higher in the amalgam group (8.15+0.99 µg/L) than in the control group (3.53+0.94 µg/L). The urinary mercury level in children who had more than two amalgams or had duration 2 years or more was higher than children who had less than 2 amalgams or had duration less than 2 years. There was no difference in IQ between children with and without amalgam fillings. The children who had amalgam restoration were estimated to be more withdrawn, more anxious/depressed, and to have more social problems than the control group. Furthermore, greater attention problems and delinquent/rule-breaking problems were recorded in the children with amalgam filling than in the control children. Also there was significant increase of serum MDA level and significant decrease of serum levels of GSH, Zn, and GABA levels in amalgam group than the control. These changes were more evident in children who had more than 2 amalgams or had duration 2 years or more.
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1.Introduction

Dental amalgam is the most widely used dental restorative material since the early nineteenth century to repair cavities in teeth throughout the world **(Timothy *et al.*, 2002 and Bates, 2011).** Amalgam fillings currently comprise about 50% mercury, with the remainder principally silver, plus small amounts of copper, tin, or zinc **(Fredin, 1994).** In many countries amalgam is still the most commonly used filling material especially in posterior teeth **(Clarkson, 2002).**

Dental caries may affect more than 90% of children **(Al Dosari *et al.*, 2004 and Al-Malik & Rehbini, 2006)** which offers a good argument for amalgam persistent use. However, their use has been controversial particularly in children, as they continually release small amounts of mercury **(Bates, 2011).** Amalgam is still a valuable material in pediatric dentistry because of its superior physical properties, ease of manipulation, and low cost (**Levy *et al.*, 2004).**

Amalgam was thought to be relatively inert once it hardened. However, the elemental mercury it contains readily vaporizes under pressure. It is postulated that stress on the amalgam surface, such as that produced by chewing, grinding of teeth, or tooth brushing, causes the breakdown of a surface barrier and the release of mercury vapor into the mouth. (**Al-Saleh and Al-Sedairi, 2011)**. When mercury vapors are inhaled, 80% is readily absorbed in the blood through the lungs and distributed in various organs, mainly in the kidneys where it may become incorporated before being excreted **(Gerhardsson and Lundh, 2010)**. Other organs (brain, lungs, liver, gastrointestinal tract, endocrine glands) show varying degrees of elevated concentrations of mercury although, the brain is the site of greatest sensitivity**.** Metallic mercury, being lipophilic, can readily cross the blood–brain and placental barriers where it is

oxidized to inorganic mercury. In this state, mercury is not lipophilic and has a limited ability to cross these biological membranes. Thus, mercury can be retained in the brain and fetal tissues **(Levy *et al.*, 2004 and Barregard *et al.*, 2010)**. The amount of mercury from amalgam passing through the gastrointestinal tract may be large but is poorly absorbed Other routes of exposure through the oral mucosa appear to be of less importance than inhaled vapor **(Levy *et al.*, 2004)**.

Mercury can cause biochemical damage to tissues and genes through diverse mechanisms, such as interrupting intracellular calcium homeostasis, disrupting membrane potential, altering protein synthesis, interrupting excitatory amino acid pathways in the central nervous system, mitochondrial damage, lipid peroxidation, microtubule destruction and alteration of antioxidant defense mechanisms **(Brownawell *et al.*, 2005).**

Human cerebral cortical activity may be under the influence of a large number of neuroactive substances which are important for the normal integrity and function of the central nervous system (CNS) which controls a variety of physiological, behavioral, and endocrinal function **(Greengard, 2001).** Gamma-aminobuttyric acid (GABA) is an important amino acid-based signaling molecule in basic neuronal pathways and acts as the major inhibitory neurotransmitter in CNS. **(Kleppner & Tobin, 2001).**

A recent Food and Drug Administration (FDA) staff draft white paper stated that no scientific studies have demonstrated harm from dental amalgam**.** This conclusion, however, was questioned by a scientific advisory panel, which recommended a more extensive review, including data from other countries **(Food and Drug Administration, 2006).** Based on the ongoing controversy over the safety of dental amalgam, this study was carried out to investigate the effect of dental amalgam restorations on urinary mercury level, intelligence, neurobehavioral function and some biochemical markers among children who had dental amalgam fillings.

2.Patients and Methods

Study design

Patients were selected from outpatient clinic of pedodontic department, faculty of dentistry, Tanta University. Seventy nine children aged 6-14 years old were selected, 59 of them had 1 or more amalgam restoration in their mouths and 20 were control. Informed consent was obtained from the accompanying parents or guardian explaining the nature and purpose of the study.

Exclusion criteria

Children who had systemic disorders, mental retardation, and those who previously had psychiatric disorders before amalgam filling were excluded from the study. Children who had amalgam filling placement or replacement for a minimum of 1 month (as the release of mercury from amalgam restoration is at its peak just subsequent to placement in the cavity, declining to steady level by 10 to 15 days **(Derand and Johansson, 1983)** were also excluded from the study.

The selected children of the present study were divided into two main groups; **amalgam group and control group.**

Amalgam group was divided into:

Group I: included children who had two amalgam fillings or less and was divided into two subgroups;

Group I a: include the children who had two amalgam fillings or less since less than two years and

Group I b: include the children who had two amalgam fillings or less since two years or more

Group II: included children who had more than two amalgam fillings and was divided into two subgroups;

Group II a: include the children who had more than two amalgam fillings since less than two years and

Group II b: include the children who had more than two amalgam fillings since two years or more

All groups were subjected to:

1- **Clinical examination:** to detect the oral and systemic condition of the child including number of amalgam filling and duration of first amalgam filling.

2- **Questionnaire collection:** it included age, sex, and frequency of fish eating.

3- **The Child Behavior Checklist (CBCL) (Achenbach *et al.*, 1991)** was used to evaluate psychosocial competence and behavioral problems in the recruited children. An Arabic-translated and validated version of the CBCL **(El – Defrawi, 1997)** was completed by a parent and scored using a computerized scoring software system (Assessment Data Manager-version 9.1). CBCL yields four global T-scores: Competence, Internalizing Behavior Problems, Externalizing Behavior Problems, and Total Problem Behaviors. Three subscales contribute to the Competence score: Activities, Social Adaptation, and School. Eight subscales contribute to the Behavior scores: Withdrawn, Somatic Complaints, Anxious/Depressed, Social Problems, Thought Problems, Attention Problems, Delinquent Behaviors, and Aggression**.**

4- **Intelligence test (IQ)**: The Arabic Version of the Revised Wechsler Intelligence Scale for Children (WISC-R) **(Wechsler, 1977 and**

Kamel *et al.*, 1997). This is the most widely used test for intellectual assessment and covers an age range of 6-16 years. The test is scored according to a manual from which verbal and performance scores and intelligent quotient are obtained.

5- **Estimation of urinary mercury level:** Urine sample was collected from the children in the Morning. Urine samples were immediately frozen and sent for analysis. Inorganic urine mercury was determined by inductively coupled plasma mass spectrometry (ICP-MS)**(Qin** *et al.,* **2009).**

6- **Spectrophotometric determination of serum malonaldehyde (MDA) level:** This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured at 532 nm **(Ohkawa** *et al.,* **1979).**

7- **Spectrophotometric determination of reduced glutathione (GSH) level:** The method is based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm by using a commercial kit (Biodiagnostic, Egypt) **(Sedlak and Lindsay, 1968).**

8- **Estimation of serum levels of zinc (Zn):** Zn level was measured by an atomic absorption spectrophotometery (mode l2380; Perkin Elmer). The monochromatic slit was adjusted to 0.7 and the wave length was set to the zinc resonance line at 213.9 nm. **(Pekarek *et al.*, 1972).**

9- **Flurophotometric determination of serum GABA level:** Serum gamma amino butyric acid (GABA content was estimated according to the method of **Lowe *et al.*, 1958.**

Statistical analysis

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, Analysis of variance [ANOVA] tests, Linear Correlation Coefficient and chi-square test by SPSS V. 16. *P* value was considered insignificant if more than 0.05, significant if ≤ 0.05 and highly significant if ≤ 0.001.

3.Results

The demographic characteristics of children with and without amalgam fillings were similar as shown in **table 1**. Children in the amalgam group had on average two amalgam fillings (range=1–7). The duration of amalgam exposure (time since first amalgam treatment) ranged from 1 to 50 month. Other exposure indices as sex, type of food and fish consumption did not show statistically significant difference between control and amalgam group regarding the urinary mercury level as shown in **tables 2 and 5.**

The mean level of urinary mercury was significantly higher in the amalgam group (8.15+0.99 µg/L) than in the control group (3.53+0.94 µg/L). The urinary mercury level in children who had more than two amalgams (8.3±1.1 µg/L) was significantly higher than children who had less than 2 amalgams (7.97±0.83 µg/L). Additionally, insignificant higher mercury level was observed in children who had treatment for 2 years or more (8.99±0.35 µg/L) than that in children who had amalgam for less than 2 years (7.35±0.60 µg/L). Moreover, all amalgam groups by different number or different duration revealed significant difference in comparison with control as shown in **table 3**.

No significant differences in intelligence was detected between the two main groups; Children with and without amalgam fillings (**Tables 4 and 5)**.

Regarding neurobehavioral functioning, Children with amalgam fillings had significantly less total competence scores on the CBCL than the control group (**Table-6**). This difference was mainly due to significantly less competence in general activities and scholastic achievement in children of the amalgam group **(Table-7**). When compared to the control group, the amalgam group also scored significantly higher on the total internalizing and externalizing behavioral problems **(Table-6**). On further analysis of these results, differences between the two groups were significant in some but not all behavioral parameters measured by the checklist (**Table-8**). The children with amalgam were estimated to be more withdrawn, more anxious/depressed, and have more social problems than the control group. Furthermore, greater attention problems and delinquent/rule-breaking problems were recorded in children with amalgam filling than in control children (**Table-8**).

As regards the biochemical changes in the present study, **table 9** revealed significant increase of serum MDA level (4.75+1.71 nmol/ml) in amalgam group than the control (3.53±0.96 nmol/ml) group, with significant higher levels in children who had more than 2 amalgams or duration 2 years or more when compared to those who had less than 2 amalgams or has duration less than 2 years**.**

There was significant decrease of serum levels of GSH (1.76+0.27 mg/dl), Zn (84.99+12.94 µg/dL), and GABA (49.64+16.38 µg/L) levels in amalgam group than the control (2.50±0 .11mg/dl, 109.9+6.95

µg/dL and 108.11+13.16µg/L respectively). Additionally, more changes were observed in the children who had more than 2 amalgams or had duration more than 2 years when compared to children who had 2 amalgams or less or has duration less than 2 years concerning GSH and GABA but, not in zinc. Although, GSH, Zn and GABA levels in all amalgam groups revealed significant decrease compared to control (**Tables 10-12)**.

The present study showed significant positive correlation between urinary mercury level and serum MDA level in amalgam group. However, significant negative correlation was found between urinary mercury level and serum GSH and GABA levels and not zinc (**Table -13).**

Table 1: Demographic data of control and amalgam groups.

	Control group (*N*=20)	**Amalgam group** (*N*=59)
Age (year) Mean±SD	10+1.85	9.80+2.27
Gender (%)		
Male	40	49.15
Female	60	50.85
Hot food consumption habit (%)		
Yes	60	84.75
No	40	15.25
Fish consumption (%)		
no	40	5
1\month	20	35.59
1\2week	20	28.81
1\ week	20	25.42
2\ week	-	5.08
Median (range) of amalgam fillings		2 (1–7)

Percentages were calculated based on the numbers of subjects with data on this item.

Hot food consumption habit: answered "usually" to the question "How often do you eat foods, soups, and drinks when they are still hot?"

Table 2: Relation of mercury level (µg/L) to demographic data.

	Mercury level	
	Control (Mean+SD)	**Amalgam** (Mean+SD)
Sex		
Male	3.98+1.2	8.31+0.75
Female	3.23+1.14	8.01+1.18
T	0.365	0.753
P	0.477	0.615
Hot food consumption habit (%)		
Yes	3.22+1.13	8.12+1.02
No	3.99+0.05	8.36+0.89
T	1.632	0.626
P	0.099	0.502
Fish consumption		
no	3.99±0.01	8.14±2.72
1\month	3.99±0.99	7.79+0.78
1\2week	3.98±0.36	8.41+0.91
1\ week	3.71±0.04	8.15+0.80
2\ week	-	9.27+0.51
F	1.491	1.253
P	0.582	0.663

Table 3: Urinary mercury levels (µg/L) among control group and different amalgam group.

Group	***N***	**Mean ± SD**	**T test**	***P* value**
control	20	3.53±0.94	**16.185**	**0.001***
Amalgam (total)	59	8.15±0.99		
By total number of amalgams at the time of participation (*N*)				
Group I	26	7.97±0.83	9.65	0.003*
Group II	33	8.3±1.1		
By time since first amalgam filling at the time of participation				
Group Ia and IIa	30	7.35±0.60	0.117	0.733
Group Ib and IIb	29	8.99±0.35		
By number and time of amalgam filling with control				
Group Ia	12	7.31+0.79	*All are significant* ***F**= 205.44* ***P**= 0.000**	
Group Ib	14	8.55+0.18		
Group IIa	18	7.37+0.45		
Group IIb	15	9.07+0.85		

Table 4: Intelligence of children with and without fillings.

Parameter	Control	Amalgam
NO.	15	23
IQ	108.07±16.24	102.05±16.84
T	0.115	
P value	0.736	

Table 5: Correlation between mercury level and other variables.

	Urinary mercury	
	r.	*p*. value
Fish eating	0.253	0.528
Age	0.241	0.352
Sex	0.159	0.425
Food	0.259	0.741
IQ	0.529	0.698

Table 6: The four global t-scores of the Child Behavior Checklist (CBCL) as compared in the amalgam group versus control group.

Score	Control Group (n=20)	Amalgam Group (n=26)	T test	*P* value
	Mean ± SD	Mean ± SD		
Total Competence score	42.8±10.27	32 ± 6.57	3.9	0.001*
Internalizing Behavior Problems	58.9 ± 10.1	67.9 ± 7.91	3.4	0.002*
Externalizing Behavior Problems	55.5 ± 9.05	63.7 ± 9.58	2.9	0.005*
Total Problem Behaviors	58.0 ± 9.17	66.3 ± 7.96	3.3	0.002*

Table 7: The three competence t-scores of the Child Behavior Checklist (CBCL) as compared in the amalgam group versus control group.

Score	Control Group (n=20)	Amalgam Group (n=26)	T test	*P* value
	Mean ± SD	Mean ± SD		
Activities score	42.4 ± 7.8	31.1 ± 7.7	4.7	0.001*
Social adaptation score	42.6 ± 8.8	40.9 ± 8.8	0.62	0.5
Scholastic achievement	52 ± 7	44.4 ± 7.4	3.5	0.001*

Table 8: The t-scores of eight behavioral subscales in the Child Behavior Checklist (CBCL) as compared in the amalgam group versus control group.

Score	Control Group (n=20)	Amalgam Group (n=26)	T test	*P* value
	Mean ± SD	Mean ± SD		
Withdrawn	59.5 ± 9.5	65.7 ± 10.5	2.1	0.04*
Somatic Complaints	57.8 ± 7.6	60.7 ± 7.1	1.3	0.19
Anxious/Depressed	59.1 ± 7.4	68.5 ± 9.7	3.6	0.001*
Social Problems	57.3 ± 7.2	64.4 ± 7.5	3.2	0.002*
Thought Problems	60.2 ± 6.7	58.4 ± 8.1	0.81	0.4
Attention Problems	55.4 ± 4.5	60.5 ± 7.7	2.8	.007*
Delinquent Behaviors	52.4 ± 3.4	63 ± 8.1	6.01	0.001*
Aggression	59.1 ± 9.3	64.6± 11.5	1.7	0.08

Table 9: Serum MDA level (nmol/ml) among control group and different amalgam group.

Group	*N*	Mean ± SD	T test	*P* value
control	10	3.53±0.96	2.165	0.03*
Amalgam (total)	40	4.75+1.71		
By total number of amalgams at the time of participation (*N*)				
Group I	20	3.86±1.02	45.449	0.000*
Group II	20	5.65±1.81		
By time since first amalgam filling at the time of participation				
Group Ia and IIa	20	4.41±0.5	149.33	0.000*
Group Ib and IIb	20	5. 1±2.3		
By number and time of amalgam filling with control				
Group Ia	10	4.82±0.96	All are significant except control andGroup Ib & IIa ***F***= 74.95 ***P***= 0.000*	
Group Ib	10	2.90±0.31		
Group IIa	10	3.99±0.29		
Group IIb	10	7.29±o.87		

Table 10: Serum GSH (mg/dl) levels among control group and different amalgam group.

Group	*N*	Mean ± SD	T test	*P* value
control	10	2.50±0 .11	3.165	0.04*
Amalgam (total)	40	1.76+0.27		
By total number of amalgams at the time of participation (*N*)				
Group I	20	1.56±0.21	11.34	0.002*
Group II	20	1.97±0.14		
By time since first amalgam filling at the time of participation				
Group Ia and IIa	20	1.62±0.27	12.38	0.001*
Group Ib and IIb	20	1.91±0.19		
By number and time of amalgam filling with control				
Group Ia	10	2.06±0.14	All are significant ***F***= *158.09* ***P***= 0.000*	
Group Ib	10	1.88±0.03		
Group IIa	10	1.75±0.09		
Group IIb	10	1.37±0.06		

Table 11: Serum Zinc (µg/dL) level among control group and different amalgam group

Group	*N*	Mean ± SD	T test	*P* value
control	10	109.9+6.95	5.639	0.008*
Amalgam (total)	40	84.99+12.94		
By total number of amalgams at the time of participation (*N*)				
Group I	20	94.33±8.61	0.31	0.580
Group II	20	75.67±9.31		
By time since first amalgam filling at the time of participation				
Group Ia and IIa	20	92.64±9.55	1.17	0.287
Group Ib and IIb	20	77.96±12.19		
By number and time of amalgam filling with control				
Group Ia	10	100.47±3.07	All are significant ***F***= 79.75 ***P***= 0.000*	
Group Ib	10	88.18±7.94		
Group IIa	10	83.60±5.01		
Group IIb	10	67.74±4.24		

Table 12: Serum GABA (µg/L) level among control group and different amalgam group

Group	*N*	Mean ± SD	T test	*P* value
control	10	108.11+13.16	15.325	0.001*
Amalgam (total)	40	49.64+16.38		
By total number of amalgams at the time of participation (*N*)				
Group I	20	59.01±17.49	109	0.000*
Group II	20	40.27±7.79		
By time since first amalgam filling at the time of participation				
Group Ia and IIa	20	61.61±14.84	110.14	0.000*
Group Ib and IIb	20	37.68±5.44		
By number and time of amalgam filling with control				
Group Ia	10	75.77±3.71	All are significant ***F***= 224.59 ***P***= 0.000*	
Group Ib	10	42.26±2.87		
Group IIa	10	47.45±2.45		
Group IIb	10	33.09±2.73		

Table 13: Correlation between mercury level and biochemical variables.

	Urinary mercury	
	r.	***P*. value**
Serum MDA	0.642	0.001*
Serum GSH	-0.556	0.001*
Serum Zinc	0.247	0.084
Serum GABA	-0.559	0.001*

4.Discussion

Mercury (Hg) is a naturally occurring metal that exists in three chemical forms: organic, inorganic and elemental. Each form has its own profile of toxicity and source of exposure. While, diet, especially fish and other seafoods are the main sources of exposure to organic Hg, dental amalgam is an important source of elemental Hg vapor **(Clarkson and Magos, 2006).**

The present study has clearly demonstrated the association between dental amalgam fillings and the levels of Hg in children urine samples. The urinary mercury level (UHg) showed significant increase in the amalgam group (8.15+0.99) compared to control (3.53+0.94). Previous studies comparable to the present study showed widely varying results of mean mercury level in children with and without amalgam respectively 0.4 and 0.2 **Wilhelm *et al.*, (2006)** 1.5 and 1.4 **Woods *et al.*, 2007** 0.92 and 0. 21 **Link *et al.*,2007** 0.1 and < 0.1 ***Schulz et al.*, 2009** 3.749 and 2.853 µg/L **Al-Saleh and Al-Sedairi, 2011**. Although, **Ye *et al.*, 2009** found that urinary mercury concentrations for children with and without amalgam filling were not different.

Urine samples provide the best marker of body burden of mercury from low-level long-term exposure to elemental and inorganic mercury. As mercury release from amalgam is absorbed and then oxidized to inorganic divalent mercury (Hg^{2+}) *in vivo* then excreted via the urine **(International Program on Chemical Safety, 2003).**

The level of urinary mercury of control children in the present study was higher than many studies in other countries. However, the level of the present study is in the range of general background levels of unexposed children who should have urinary Hg levels < 5 µg/L **(Ozuah *et al.,* 2003 and Bose-O'Reilly *et al.,* 2010**). Comparing results of this study to the defined reference value for UHg by the German Commission of Human Biomonitoring for UHg in children (3–14 year olds), 100% of children's urinary Hg concentrations without fillings were above the reference value of 0.4 µg/L **(Schulz *et al.,* 2009).** Additionally, **Schulz *et al.,* 2009** reported that UHg for children with more than two dental amalgams was 3.1 µg/L. In this study, children with dental amalgam had a considerably higher level of mercury (8.15+0.99 µg/L). this is in accordance with a study done in Saudi Arabia which reported that children with dental amalgam had a considerably high mercury level of 8.538 µg/L, with a range of 3.129 to 15.575 µg/L (**Al-Saleh and Al-Sedairi, 2011).** This may be attributed to that mercury hygiene is not strictly adhered.

The present study revealed absence of correlation between fish consumption and urinary mercury level; this is in agreement of **Ye *et al.,* 2009** who stated that there is no effect of fish consumption on the urinary mercury level. Furthermore, **Leistevuo *et al.,* 2001** found a three-fold increase of mercury levels in saliva of individuals with dental amalgam compared to individuals without amalgam, although frequency and kind of fish consumption were identical in both groups. These findings are contradicted by another study which found that fish intake significantly influenced the UHg levels as children who reported higher levels of fish consumption excrete significantly elevated amounts of Hg **(Apostoli *et al.,* 2002 and Levy *et al.,* 2004**). The outcome of the present study on fish is not somewhat surprising since Hg in fish is mainly methyl-Hg, which is not excreted through the kidney **(Clarkson *et al.,* 1988 and WHO, 1996).** The null results on fish consumption may be due to the difference in fish species consumed across populations. Mercury levels in fish vary also in different areas **(International Program on Chemical Safety, 1990).** Additionally, food consumption in the children of the present study was very low compared to the frequency of fish eating in other countries.

The present study revealed significant increase in urinary mercury level in children with more than two amalgams than those with 2 amalgams or less. Previous studies in children have shown that urinary mercury concentrations were correlated with the number of amalgam fillings **(Woods *et al.,* 2007 and Dunn *et al.,* 2008).** This association was not found in the studies of **Khordi-Mood *et al.,* 2001 and Ye *et al.,* 2009**.

The present research did not find positive relation of urinary mercury levels and time since first amalgam filling, this finding is inconsistent with that of the two recent clinical trials **(Woods *et al.,* 2007 and Ye *et al.,* 2009**). The mechanism of this association is unclear and may be related to cumulative deposition of inorganic mercury in the kidney and its excretion in urine. However, as a matter of fact, after two years of mercury exposure the route of kidney excretion of mercury appears to be less effective as increased mercury exposure inhibits its own excretion. Additionally, over 90% of mercury leaves the body through the biliary transport system of the liver and excreted in the feces, not in the urine **(Lorscheider *et al.,* 1995). Mutter *et al.,* 2004** reported that possible adverse effects of mercury may need more than five years of mercury exposure to develop. If mercury is involved in the pathogenesis of Alzheimer's disease, the disease may need up to 50 years to be clinically diagnosed.

Absence of correlation between amalgam filling and intelligence in the present study were consistent with two clinical trials. The first study was conducted in Lisbon, Portugal, 507 children were randomly assigned to receive either amalgam (*n*=254) or composite (*n*=253) and were followed for 7 years **(1997–2005).** No statistically significant differences in neurobehavioral assessment (memory, attention, motor development, nerve conduction velocities) or intelligence were found between the two groups **(DeRouen *et al.,* 2006)**. The second study was conducted in two US cities followed 534 children (267 for amalgam and 267 for resin composite) for 5 years. Likewise, there were no statistically significant differences in full-scale IQ scores, memory, or visuomotor ability between children with and without amalgam **(Bellinger *et al.,* 2006, 2007 and 2008).** The result of the present study on IQ could be explained by the fact that heritability is a major factor in general cognitive ability **(Plomin *et al.,* 1994)**.

The neurobehavioral outcome predictors of the current study were totally different from those of the main trials done in other countries. While our results indicate clear differences between the amalgam group and the control group regarding several competence

and behavioral parameters, other trials **(Bellinger *et al.*, 2008 and Ye *et al.*, 2009)** showed no significant differences between the two groups regarding those parameters or any other behavioral parameters. The second study even showed better competence and behavioral performance in children with amalgam when compared to the control group. This disparity of the results comes in spite of using the same psychometric tool, namely the Child Behavior Checklist, validated for different cultures (the Chinese culture in the first study and the Arabic culture in ours).

These differences between our study and previous studies come in accordance with the disparity in the urinary mercury levels reported to be much higher in our study than in the cited studies. The differences between the amalgam types or manipulations used in Egypt versus those used in other countries, might explain some of the adverse competence and behavioral outcomes reported in our children. Other possible explanations may include the decreased levels of the inhibitory neurotransmitter, GABA, in our study. This decreased level might lead to dysfunctioning of the inhibitory control circuits in the brains of children with amalgam fillings leading to less attention, more delinquent behavior and hence lower scholastic achievement despite their average intelligence. Alteration of GABA plasma levels was previously associated with neurobehavioral and mood disorders in children **(Prosser and Hughes, 1997)**.

As regards the biochemical changes in the present study, there was significant increase of serum MDA level accompanied by significant decrease in the serum GSH, zinc and GABA levels in amalgam group as compared to the control group, more changes were documented in the children who had more than two amalgams and those who had treatment for 2 years or more than other groups.

Malondialdeyde (MDA) is one of the termination end products of lipid peroxidation generated during the oxidative breakdown of lipids, and it is a marker of oxidative stress **(Eraslan et al., 2004).** Both *in vivo* and in vitro models showed that Hg exposure can cause oxidative stress in biological systems with generation of reactive oxygen species (ROS), glutathione (GSH) depletion, and decrease of sulphydryl groups (–SH) of proteins, which can lead to pathological processes **(Shenker *et al.*, 2002, Crespo-López *et al.*, 2007 Augusti *et al.*, 2008 and Grotto *et al.*, 2009).**

GSH is the main antioxidant in mammalian cells, constituting nearly 90% of the intracellular non-protein thiol. It is important for maintaining the intracellular redox status of protein thiols, for protection against endogenous and exogenous sources of oxidative stress, and for the conjugation and excretion of toxic molecules **(Rico *et al.*, 2006).** In the present study, we observed decreased level of GSH. Only **Pizzichini *et al.*, 2002 and 2003** has demonstrated a negative correlation between total antioxidant power and salivary and plasma Hg in amalgam treated patients . Similarly, (**Grotto *et al.*, 2010)** observed negative correlation between mercury exposure and GSH level in Amazonian communities. He attributed this to the interaction of Hg with sulphydryl groups of GSH, resulting in diminished GSH concentration which, is considered as the most important mechanism for Hg-induced oxidative damage.

The increased serum MDA and concomitant decrease of GSH levels in amalgam group in our study can be considered as a good indicator for the effect of mercury in aggravation of oxidative stress. it has been revealed that exposure to mercury (organic or inorganic) can enhance the induction of oxidative stress and generation of free radicals as result of the depletion of the GSH **(Flora *et al.*, 2008).** A growing amount of data provides evidence that mercury capable of interacting with nuclear proteins and DNA and increasing the production of reactive radicals such as superoxide, hydrogen peroxide and hydroxyl radicals which cause oxidative deterioration of biological macromolecules, resulting in cellular damage like depletion of enzyme activities, damage to lipid bilayer membrane as well as DNA fragmentation, which can result in the disruption of nerve cell function and integrity **(Nur Özdabaka *et al.*, 2008).**

Zinc (Zn) is an essential trace element for all forms of life. It contributes to a number of important biological processes include gene expression, DNA synthesis, enzymatic catalysis, hormonal storage and release, memory process as well as neurotransmitter **(Vallee & Auld, 1993).** There are several potential mechanisms for the decreased zinc level in the present study. A possible explanation might be that Hg causes Zn displacement and execrtion **(Grotto et al., 2010)**. A second explanation may be damage to stomach and intestinal lining by mercury which along with its ability to bind to SH in cell membranes can alter permeability and adversely alters bacterial populations in the intestine causing leaky gut syndrome and enzyme blockages with poor nutrient absorption **(Bensefa-Colas *et al.*, 2011 and Suzuki *et al.*, 2011).**

Given that GABA is the main inhibitory neurotransmitter in the mammalian nervous system, prolonged disruptions of its function may underlie the sub-clinical impacts of Hg on health **(Basu *et al.*, 2010).** The decreased GABA level in the present study may be due to the inhibitory effect of mercury on neurotransmitters production by inhibiting:

calcium-dependent neurotransmitter release **(Gassó *et al.*, 2001)** or blocking neurotransmitter amino acids synthesis **(Belletti and Gatti, 2002).** Furthermore, the neurotransmitter GABA is biosynthesized from glutamate catalyzed by the enzyme glutamic acid decarboxylase (GAD). *Mercury* inhibited GAD activity at low micromolar concentrations in the cortical tissues. This inhibition was likely due to the interaction of Hg with essential sulfhydryl groups on the GAD protein **(Basu *et al.*, 2010).**

Many calls to continue, reduce, or ban mercury use have been issued, while some suggest that patients should be informed of the recognized benefits and risks **(Spencer, 2000, Mitchell *et al.*, 2005 and Martin & Woods, 2006).** Few restrictions limit the use of amalgam worldwide. Sweden may become the first country to entirely eliminate the use of amalgam **(Gelband, 1998).** Germany has recommended the restriction of its use in young children, pregnant women, and patients with severe kidney problems **(Harhammer, 2001).** Likewise, its use has seen a decreasing trend in the USA, Australia, Scandinavia, and to a lesser extent in the UK **(Burke, 2004).**

Recommendations:

1-It is clear from this study that research and public enquiry on this issue should continue to solve the subject of debate.

2-Improvements in the alternative restorative materials should be encouraged. The use of precapsulated alloy should be used to eliminate the sources of mercury vapor from spilling large quantities of mercury and subsequent squeezing of the amalgam mass to express excess mercury before packing the amalgam into cavity.

3-The possible adverse effects associated with mercury toxicity can be minimized with proper mercury hygiene. Mercury rich particles during condensation of amalgam should be strictly dispersed otherwise it can be inhaled by the patients and dental personnel.4-

4-Contaminated disposable materials should be placed in polyethylene bags and sealed before disposal by environmental agencies.

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