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"CHRONIC MERCURY EXPOSURE FROM AMALGAM" IS IT ASSOCIATED WITH RECURRENT MISCARRIAGE?

By

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ABSTRACT

Mercury is a hazardous metal that has become an important issue of environmental contamination. The main sources of exposure of pregnant women is dental amalgam fillings and canned fish consumption. Mercury levels in blood and abortuses of 40 women with repeated miscarriage were determined using Perkin-Elmer 2380 Atomic Absorption Spectrophotometer and compared with 24 pregnant women (control). Highly significant elevation of blood mercury levels was observed in: a) all aborted women ($20.82 \pm 4.55 \mu\text{g/dL}$) compared to all controls ($11.11 \pm 4.8 \mu\text{g/dL}$); b) aborted women with dental amalgams ($23.16 \pm 2.85 \mu\text{g/dL}$) when compared to control women with amalgams ($14.62 \pm 2.9 \mu\text{g/dL}$); c) aborted women without dental amalgams ($15.36 \pm 2.52 \mu\text{g/dL}$) when compared to control women without amalgams ($7.59 \pm 0.67 \mu\text{g/dL}$). Mercury levels in abortuses of the studied cases were found to be $11.06 \pm 1.98 \mu\text{g/gm}$. It was 11.94 ± 1.7 in cases with amalgams and $9.01 \pm 0.47 \mu\text{g/gm}$ in cases without amalgams. Aborted women with one amalgam had lower mercury levels in both blood and abortuses than women with 2, 3, or 4 amalgams. Also, blood mercury level was significantly elevated in aborted women with 1, 2, 3, and 4 when compared to those of control women. Increase duration of amalgam fillings was associated with more mercury levels in both blood and abortuses in aborted women and in blood of controls. Dental filling during pregnancy was associated with high mercury levels in both blood ($23.96 \pm 4.07 \mu\text{g/dL}$) and abortuses ($12.53 \pm 2.44 \mu\text{g/gm}$) of the aborted women. Canned fish consumption was associated with highest mercury levels in blood of aborted women ($24.74 \pm 2.15 \mu\text{g/dL}$) than those of controls ($16.86 \pm 1.14 \mu\text{g/dL}$) and also in abortuses ($12.78 \pm 1.5 \mu\text{g/gm}$). There were significant positive correlations between blood mercury level and age of the studied women ($r = 0.8$) and mercury concentration of abortuses ($r = 0.9$).

In conclusion, Mercury was found to be associated with cases of recurrent miscarriages especially in women with dental amalgam fillings and women who consumed canned fish. So, development of preventive strategies and governmental actions against the problem of mercury contamination should be done. Dental professionals should understand the impact of various levels and types of mercury on the environment and human health. Production, uses and disposal of dental amalgams should be decreased. Dental amalgam fillings in girls and women of reproductive age should be used with caution, to avoid increased prenatal mercury exposure. Consumption of canned fish should be restricted to only one / month. Every woman with repeated miscarriages should be investigated for mercury level in her blood. If it is elevated, removal of amalgams if present should be done six months before pregnancy. If still high, chelation therapy should be started cautiously in a hospital in order to avoid its complications. Antioxidants especially selenium may have a role in protection against oxidative stress induced by mercury.

INTRODUCTION

Elemental (metallic) mercury (Hg^0) is a highly toxic metal that can cause serious adverse health effects. It is found in thermometers, fluorescent light bulbs, barometers, switches in children's shoes that light up, and in dental amalgams (IPCS, 1991). It has been used in both medicine and dentistry for centuries. It is also contained in many over-the-counter drugs and cosmetics; e.g., mascara, contact lens solution, hemorrhoid preparations and beauty cream. Recent media attention regarding the increased levels of mercury in dietary fish, high levels of mercury in air emissions, and conjecture that certain diseases may be caused by mercury exposure has increased public awareness of the potential adverse effects of mercury (Kao, et al., 2004). Because Hg^0 has a high vapor pressure, the primary route of human exposure is by inhalation of Hg^0 vapor released from dental amalgams (IPCS, 1991). Levels commonly found in oral tissues of those with amalgam fillings were 100 to 1200 times the FDA/ EPA action level for health warnings in food, which is 1 ppm.

Dental amalgam contains approximately 50% of metallic mercury (Palkovicova, et al., 2007). Recent evidence that small amounts of mercury are continuously released from filling (Bates, 2006). Individuals with amalgam have 2-12 times more mercury in their body tissues compared to individual without amalgam. Mercury vapor, is known to be the most toxic nonradioactive element, and is toxic even in very low doses (Mutter et al., 2007). There is no safe level of mercury. If a person, have 4 amalgam fillings present in their mouth, the average person's saliva exceeds the EPA maximum legal municipal discharge standard for mercury. The upper level of mercury exposure recommended by the German Commission on Human Biomonitoring is 10 $\mu\text{g/L}$ in blood (Mottet et al., 1985) but adverse effects such as increases in blood pressure and cognitive effects have been documented as low as 1 $\mu\text{g/L}$ thus according to the European and U.S. National Academy of Sciences, mercury limit was lowered to 5 $\mu\text{g/L}$ (Grandjean, 1999).

According to animal experiments, case reports and epidemiological studies, exposition to a high concentration of metallic mercury vapor may cause an increase in reproductive problems (Schuurs, 1998). Because Hg^0 is highly lipid soluble, it readily penetrates the placental barrier (Lutz et al., 1996) and is taken up by fetal tissues, then oxidized to Hg^{2+} which is much less likely to re- cross the placental barrier. This oxidation serves to trap Hg^0 in tissues leading to more accumulation in the fetus than in the mother (Goering et al., 1992) with potential developmental toxicity, spontaneous abortion and stillbirths (Shuurs, 1999).

The average amalgam filling contains 50 % mercury (Palkovica et al., 2007). A single large amalgam filling contained 1 gm of mercury lost a significantly toxic 10 $\mu\text{g/day}$; there would be enough mercury for 100,000 days or about 274 years of exposure. A small tenth of gram mercury filling would last 27 years. So, enough mercury is within amalgam fillings to provide a consistent chronic toxic exposure and causes spontaneous abortions and birth defects (Allan, 2001, Choy et al., 2002).

AIM OF THE WORK

The objective of this study is to evaluate the chronic toxicity of exposure to mercury in cases of recurrent miscarriages by evaluating mercury levels in blood and abortuses of aborted women and correlating them to amalgam fillings in comparison to pregnant control women.

SUBJECTS AND METHODS

The present study was conducted on 64 women divided into two groups. The first group included 40 women with at least two miscarriages in the first trimester (first 14 weeks gestation), aged from 20 to 39 years, who attended Obstetric & Gynecology Department of Mansoura University Hospital over one year period. They were admitted complaining of inevitable miscarriage and surgical evacuation or manual vacuum aspiration was required. All women were subjected to ultrasound investigation to ensure diagnosis and exclude major anomalies of the uterus. The second group consisted of 24 females with no history of miscarriage and they were pregnant (controls) without problems. Their age ranged from 22 to 38 years. They were aged, weight, and height matched with cases. None of the aborted women or controls was receiving medication or suffering from any infection during the study. Informed consent was taken from all women to share in this study. Through history was taken from each woman with special emphasis on:

- 1- Age, residence and occupation.
- 2- Dental history: number of dental amalgam fillings, duration and dental fillings during pregnancy.
- 3- Consumption of canned food.
- 4- History of diabetes, hypertension, hepatic and renal diseases.
- 5- History of drug or alcohol intake.

For each participant, five ml. venous blood were withdrawn from anticubital vein under complete aseptic condition, added to EDTA, into polypropylene tubes, shaken gently. One gram from abortuses (product of conception) was put in a clean flask. Both blood and abortuses kept at 4 °C until assay.

Digestion of blood and aborted samples were done by wet method of digestion (nitric – perchloric acid) according to the method of Vanloon (1985). Analysis of total mercury was done by the method of Stockwell and Corns (1993) by using Perkin –

Elmer 2380 Atomic Absorption Spectrophotometer (AAS cold steam technique in combination with flow injection system). Preparation of standards and samples was carried out under clean conditions using deionized water. All chemicals and reagents used were of ultra pure reagent grade. All glassware and plasticware were washed three times with deionized water, then soaked in 20% nitric acid overnight. After soaking the glassware was rinsed three times with deionized water and dried. Quality assurance was achieved by measuring blank test solutions. All metal contents were measured in duplicate (S.D. <10%) by the working curve method.

Data were compared by using student's t-test (to compare two groups), Chi square (χ^2) was used for qualitative data (frequency and proportion). Correlation co-efficiency

was used to test association between variables. These data were run on an IBM compatible personal computer by using Statistical Package for Social Scientists (SPSS) for windows 11 (SPSS Inc., Chicago, IL , USA).

RESULTS

There was insignificant variations between age & residence of aborted women and control women as shown in table (1). Eighteen of aborted women (45%) and eight (33.3%) control had consumed canned fish. Dental amalgams were found in 70 % of aborted women and 50 % of controls. Eight aborted women have done amalgams filling during pregnancy.

Table (2) reveals highly significant elevation of blood mercury levels in: a) all aborted women ($20.82 \pm 4.55 \mu\text{g/dL}$) compared to all controls ($11.11 \pm 4.8 \mu\text{g/dL}$); b) aborted women with dental amalgams ($23.16 \pm 2.85 \mu\text{g/dL}$) when compared to control women with amalgams ($14.62 \pm 2.9 \mu\text{g/dL}$); c) aborted women without dental amalgams ($15.36 \pm 2.52 \mu\text{g/dL}$) when compared to control women without amalgams ($7.59 \pm 0.67 \mu\text{g/dL}$). Mercury levels in abortuses of the studied cases were found to be $11.06 \pm 1.98 \mu\text{g/g}$. It was 11.94 ± 1.7 in cases with amalgams and $9.01 \pm 0.47 \mu\text{g/gm}$ in cases without amalgams.

Aborted women with one amalgam had lower mercury levels in both blood and abortuses than women with 2, 3, 4 amalgams as shown in table (3). Also blood mercury level was significantly elevated in aborted women with 1, 2, 3, and 4 amalgams when compared to those of control women. Increased duration of amalgam fillings was associated with more mercury levels in both blood and abortuses in aborted women and in blood of controls. Dental filling during pregnancy was associated with high mercury levels in both blood ($23.96 \pm 4.07 \mu\text{g/dL}$) and abortuses ($12.53 \pm 2.44 \mu\text{g/gm}$) of the aborted women. Canned fish consumption was associated with highest mercury levels in blood of aborted women ($24.74 \pm 2.15 \mu\text{g/dL}$) than those of controls ($16.86 \pm 1.14 \mu\text{g/dL}$) and also in abortuses ($12.78 \pm 1.5 \mu\text{g/gm}$) as shown in table (4). There were significant positive correlations between blood mercury level and age of the studied women ($r = 0.8$) and mercury concentration of abortuses ($r = 0.9$) as shown in table (5).

Table (1): Characteristics of the studied cases.

Parameter	Cases of abortion n = 40	Controls n = 24	Statistical data P	
Age(years): Mean \pm S.D	30.32 \pm 5.43	32.33 \pm 4.13	t = 1.99	0.52
Residence : Urban n (%)	26 (65.0)	14 (58.3)	X ² =0.51	0.18
Rural	14 (35)	10 (41.7)		
Canned fish consumption n (%)	18 (45)	8 (33.3)	X ² =0.88	0.32
Dental amalgams: n (%)	28 (70)	12 (50)	X ² =0.95	0.36
Number : One	6 (21.4)	2 (16.7)	X ² =1.01	0.46
2, 3	14 (50)	6 (50.0)	X ² =0.72	0.29
4	8 (28.6)	4 (33.3)	X ² =0.53	0.16
Duration : 2- 6 years	8 (28.6)	4 (33.3)	X ² =0.53	0.16

8 – 20 years	20 (71.4)	8 (66.7)	$X^2=0.71$	0.28
Dental filling during pregnancy	8 (28.6)	-		

Insignificant at $p > 0.05$.

Table (2): Mercury levels among the studied cases.

Parameters	Cases of abortion n = 40	Controls n = 24	Statistical data P
1) Blood mercury ($\mu\text{g/dL}$) conc. for all: Mean \pm S.D	20.82 \pm 4.55	11.11 \pm 4.80	t = 6.15 < 0.0001
2) Bl. Hg$^\circ$ in women with amalgams: Mean \pm S.D	n = 28 (70 %) 32.16 \pm 2.85	n = 12 (50%) 14.62 \pm 2.90	t = 6.07 < 0.0001
3) Bl. Hg$^\circ$ in women without amalgams: Mean \pm S.D	n = 12 (30 %) 15.36 \pm 2.52	n = 12 (50 %) 7.59 \pm 0.86	t = 6.86 < 0.0001
4) Abortus mercury ($\mu\text{g/gm}$) for all: Mean \pm S.D	11.06 \pm 1.98		
5) Abortus Hg$^\circ$ in women with amalgams: Mean \pm S.D	11.94 \pm 1.70		
6) Abortus Hg$^\circ$ in women without amalgam: Mean \pm S.D	9.01 \pm 0.47		
Statistical comparison between 1,4			t=9.8 < 0.0001
Statistical comparison between 2,5			t=10.89 < 0.0001
Statistical comparison between 3,6			t = 5.61 < 0.0001

Highly significant at $p < 0.001$.

Table (3): Association of blood and abortus mercury concentrations with dental amalgams.

Parameters	Cases of abortion n = 40	Controls n = 24	Statistical data P
(A) Number of amalgams:			
i) One tooth : n (%)	6 (21.4)	2 (16.70)	
a- Bl. Hg $^\circ$ ($\mu\text{g/dL}$) Mean \pm S.D	19.99 \pm 0.50	10.05 \pm 0.01	t = 48.73 < 0.0001
b- Abortus Hg $^\circ$ ($\mu\text{g/g}$) Mean \pm S.D	10.47 \pm 0.40		
Statistical comparison between a and b :			t = 43.27 < 0.0001
ii) Two, three amalgams n (%)	14 (50)	6 (40.00)	
a- Bl. Hg $^\circ$ ($\mu\text{g/dL}$) Mean \pm S.D	22.92 \pm 2.33	14.42 \pm 0.90	t = 11.76 < 0.0001
b- Abortus Hg $^\circ$ Mean \pm S.D	11.64 \pm 1.56		
Statistical comparison between a and b			t = 15.07 < 0.0001

iii) Four amalgams	n (%)	8 (28)	4 (33.3)		
a- Bl. Hg°(µg/dL)	Mean ± S.D	25.98 ± 1.45	17.62 ± 0.04	16.29	< 0.0001
b- Abortus Hg°	Mean ± S.D	13.55 ± 1.15			
Statistical comparison between a and b				19.01	< 0.0001
Statistical comparison bet. iii) a and i) a				10.89	< 0.0001
Statistical comparison bet. iii) b and i) b				7.03	< 0.0001
(B) Duration of dental fillings:					
i) From 2- 6 years	n (%)	8 (28.6)	4 (33.3)		
a- Bl. Hg°(µg/dL)	Mean ± S.D	22.88 ± 3.75	13.85 ± 3.81	4.44	< 0.001
b- Abortus Hg°	Mean ± S.D	11.74 ± 2.02			
Statistical comparison between a and b :				7.38	< 0.0001
ii) From 8 – 20 years	n (%)	20 (71.4)	8(66.7)		
a- Bl. Hg°(µg/dL)	Mean ± S.D	23.28 ± 2.49	15.00 ± 1.68	8.67	< 0.0001
b- Abortus Hg°	Mean ± S.D	12.02 ± 1.56			
Statistical comparison between a and b :				17.06	< 0.0001
(C) Dental filling during pregnancy	n (%)	8 (28.6)	-		
a- Bl. Hg°(µg/dL)	Mean ± S.D	23.96 ± 4.07			
b- Abortus Hg°	Mean ± S.D	12.53 ± 2.44			
Statistical comparison between a and b :				6.81	

Highly significant at p<0.001.

Table (4): Mercury concentrations of the studied cases as regard canned fish consumption.

Parameters	Cases of abortion n = 40	Controls n = 24	Statistical data P
i) Positive consumption:	18 (45)	8 (33.3)	
a) Bl. Hg°(µg/dL)	24.74 ± 2.15	16.86 ± 1.14	11.45 < 0.0001
b) Abortus Hg°:	12.78 ± 1.50		
ii) No consumption:	22 (55)	18 (75)	
a) Bl. Hg°:	17.62 ± 3.20	9.30 ± 2.60	6.39 < 0.0001
3) Abortus Hg°:	9.65 ± 0.82		
Statistical comparison between i) a, ii) a			t = 7.82 < 0.0001

Highly significant at p<0.001.

Table (5): Correlation between blood mercury level and different studied parameters

Parameters	Correlation coefficient (r)	Significance
a) Age of the studied cases	+ 0.8	0.01
b) Abortus Hg°:	+ 0.9	0.01

DISCUSSION

Toxicity caused by mercury exposure is now becoming recognized as a widespread environmental problem and is continuing to attract a great deal of public attention. In the present study, mercury was found to be elevated in aborted women compared to controls. This was double the level recorded by Belson et al., (2005) who mentioned that level of mercury should be less than 10 ($\mu\text{g/dL}$) and 10 times the level recorded by Mckelvey et al., (2007) for New York City population. This level was found to be positively correlated with the number and duration of amalgam fillings.

Many authors recorded similar results. A large cohort study of exposed women found an increased risk of spontaneous abortion and other pregnancy complications (Sikorsky, 1987). Women with hormonal problems seeking help at a gynecological clinic in Germany; women with idiopathic menstrual problems had higher mercury levels than controls (Gerhard, et al., 1998). Mercury levels in placenta increased with an increasing number of maternal dental amalgam fillings (Ask et al., 2002). In a study by Björnberg et al. (2005) concentration of mercury in cord blood were correlated with its concentration in mother and dental amalgams. A significant association between the numbers of dental amalgam fillings and the mercury concentration in blood was recorded by Lindberg et al., (2004). In the period from 1999 to 2002 a report data showed that all women of childbearing age had levels 58 $\mu\text{g/dL}$, a concentration associated with neurological effects in fetus (Rice, 2004). A slightly increased risk of miscarriage was found by Lindbohm (2006) for exposure to mercury amalgams.

In the present study, relation of mercury concentrations to canned fish consumptions was consistent with that of Leistevuo (2001). Canned fish mostly contained big sized tuna fishes with high levels of mercury inside. The Environmental Working Group recommends restricting consumption of canned tuna to only one a month and to avoid high mercury seafood.

In contrast to the present results, Luglie et al. (2005) found insignificant dependence of mercury concentration on the number of amalgam fillings and fish consumption. Low level of mercury levels was detected in amniotic fluid and no adverse outcomes were observed through pregnancies and in newborns.

It has been well documented that maternal amalgam fillings are a major source of exposure for the fetus (Leistevuo, 2001). The average amalgam filling contains 50 % mercury (Palkovica et al., 2007). It emits mercury vapor 24- hours a day. It is known to have a low vapor pressure and to be continuously vaporized and absorbed by the body. Amalgam has been shown to act like a battery, setting up galvanic currents in the mouth, resulting in high levels of poisonous mercury being deposited through this action in the oral tissues and mucosa, from which it also spreads to other parts of the body (Nogi, 1989).

Based on animal studies as well as human studies, mercury from amalgam in blood of pregnant women crosses the placenta and appears in amniotic fluid and fetal blood within two days of placement (Björnberg et al., 2005). Fetal mercury content after maternal exposure to vapor was found to be over 20 times that for mother (Warfving, et

al., 1994). Mercury is often stored in fetus at much higher levels than that in mother (Vimy et al., 1997).

Many recent studies have found reproductive effects including infertility, miscarriage and developmental effects in fetus at much lower levels than those having significant effects in adults. The fetus have been found to be much more susceptible to the effects of low levels of mercury exposure due to low body weight with high absorption rate, less effective renal excretion and a less effective blood brain barrier (Kostial et al., 1991).

One of the major cellular mechanisms of mercury toxicity is the oxidative stress which may lead to membrane peroxidation and generation of reactive oxygen species (Pinhero et al., 2007). It acts as a free radical which is highly reactive, charged particles that damage body tissues. Free radicals prevent nutrient from entering the cells and wastes from leaving and block enzymes necessary for the body's detoxification processes. Mercury can bind to cell membranes, distorting them and interfering with normal cell functions. The immune system no longer recognizes the target as part of the body and will attack it. Once mercury reaches its destination tissue, it has many ways in which it may express its toxicity. It is a multipotent cytotoxin that intervenes in the primary processes of the cell by bonding strongly with sulfhydryl and selenohydryl groups on albumin molecules in cell membranes, receptors and intracellular signal links, and by modifying the tertiary structure. The structure of albumin molecules is genetically determined, and this leaves ample scope for genetic polymorphism to manifest itself in varying sensitivity and types of reaction to mercury exposure (Palkovicova et al., 2007).

Mercury has been documented to be a reproductive and developmental toxin in humans. Some of its documented hormonal effects at very low level of exposure include effects on the reproductive system resulting in menstrual disturbances, infertility, spontaneous abortions and birth defects (Yang et al., 2002). The uterus is a collection center for mercury. It is reported that more than 90% of the imbalances, created by sex hormone disturbances were corrected within a few weeks of amalgam removal. The patients noted differences in fertility, less pain during periods and a trend toward optimization of the days of menstrual flow. Mercury seems to have a negative impact on ovarian as well as on pituitary function. It has inverse associations with progesterone, prolactin and thyroid stimulating hormone and direct association with oestradiol. It may induce immunological changes, such as an increase of natural killer cells and changes of T suppressor and T helper cell counts, may interfere with the physiological adaptation of the immune system to the state of pregnancy with the result of miscarriage. The observed hormonal and immunological changes may be important factors in the pathogenesis of repeated miscarriage (Gerhard et al., 1998).

Studies found that very low levels of exposure to mercury cause genetic /DNA damage (Ben-Ozer et al., 2000; Leung et al., 2001; Prati et al., 2002, Sheiner et al., 2003) and inhibits DNA & RNA synthesis (Gazette, 2001); reduces blood's ability to transport oxygen to fetus, and transport of essential amino acids, nutrients including magnesium, zinc and vitamin B12 (Urbach et al., 1992); decrease enzyme function and isocitric dehydrogenase in fetus (Ng and Liu, 1999); causes reduced iodine uptake,

inhibited ATP activity, and causes spontaneous abortions and birth defects (Choy et al., 2002). It also, inhibits release of follicle stimulating hormones from the pituitary by damaging membranes of cells in the anterior pituitary.

Since mercury vapor is known to rapidly cross cellular membranes and to bioaccumulate over time with chronic exposure, these relationships get stronger with age, as evidenced in the positive correlation between the age of the studied women and the blood mercury levels, with the most serious health effects occurring more commonly in middle – aged women.

In conclusion, Mercury was found to be associated with cases of recurrent miscarriages especially in women with dental amalgam fillings and women who consumed canned fish. So, the following recommendations are very important:

- Development of preventive strategies and governmental actions against the problem of mercury contamination and continued international efforts should be done to reduce anthropogenic sources of mercury.
- Dental professionals should understand the impact of various levels and types of mercury on the environment and human health. The global production and consumption of mercury should be decreased as well as the production, uses and disposal of dental amalgams.
- Dental amalgam fillings in girls and women of reproductive age should be used with caution, to avoid increased prenatal mercury exposure. Consumption of canned fish should be restricted to only one / month.
- Every women with repeated miscarriages should be investigated for mercury level in here blood. If it is elevated, removal of amalgams if present should be done six months before pregnancy. If still high, chelation therapy should be started cautiously in a hospital in order to avoid its complications.
- Antioxidants especially selenium may have a role in protection against oxidative stress induced by mercury.

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