Estimation of Serum Mercury Levels in Users of Dental Fillings

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تقدير مستويات الزئبق في مصل دم مستخدمين لحشوات الاسنان

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Abstract:

Dental amalgam is a combination of mercury with other metals used by dentists to adapt tooth cavity. However, serious concerns present because of its toxicological accumulation in human body. For these reasons, our present study aimed to determine the internal mercury concentration of two groups differing in their attitude towards possible health hazards by mercury from amalgam fillings. It was to be examined if the two groups differ with regard to the mercury concentration and to compare the results with current reference values.

Specific analytical method for determination of mercury was illustrated by using Cold Vapor Atomic Absorption Spectrometry technique which is with higher sensitivity that can be reach to the low detection limits (in ppb units). Blood samples were analyzed from 36 men where 14 men of total samples were concerned as control samples.

Results showed that the Median (range) for mercury levels in blood were 6.393 (2.025 – 10.772) µg/L, while normal whole blood mercury was usually about <10 ng/mL.

Keywords: Dental Amalgam, Mercury, Cold Vapor-AAS.

المستخلص

الملغم هو مزيج من الزئبق مع معادن أخرى يستخدم من قبل اطباء الاسنان لعلاج تجاويف الاسنان. وبالرغم من ذلك، فقد كانت هناك مخاوف بسبب تراكمه السمي في جسم الانسان. ولهذه الاسباب تهدف دراستنا الحالية الى تقدير التعرض الداخلي للزئبق لمجموعتين تختلف في احتمالية التسمم بالزئبق من حشوات الملغم. لقد تم فحص الزئبق لمجموعتين تختلفان في محتوى الزئبق ومقارنته بالقيم المرجعية الحالية. لقد استخدمت طريقة تحليلية دقيقة لتقدير الزئبق وهي تقنية قياس الامتصاص الذري بالبخار البارد ، ذات الحساسية العالية يمكن الوصول بها إلى حدود كشف منخفضة جدا وبحدود الجزء بالبليون (ppb). حللت عينات الدم من 36 رجلاً ، 14 رجال من عدد النماذج الكلي اعتبر كنماذج سيطرة. لقد اوضحت النتائج أن متوسط (المدى) لمستويات الزئبق في الدم كان 6.393 (2.025 – 10.772) ميكروغرام / لتر ، بينما عادة ما يكون مستوى زئبق الدم الطبيعي العادي أقل من 10 نانوغرام لكل مللتر.

الكلمات المفتاحية: ملغم الاسنان، الزئبق، تقنية قياس الامتصاص الذري بالبخار البارد.

Introduction

Mercury is a heavy metal that can cause very serious damage to human health when individuals are chronically exposed to it. Furthermore, mercury has been used because it has certain therapeutic properties, being used in medicines such as laxatives, antihistamines and antiseptics, and in silver - mercury amalgam in dental flings. The conventional dental amalgam is an alloy composed of 65% silver (Ag), 28-29% tin (Sn), 6% copper (Cu), and 1% zinc (Zn). Mercury was added to the alloy because of its ability to agglutinate fine particles, forming a metal alloy at room temperature. In some cases, mercury can reach 50% of the mix (Richardson *et al*., 2011).

Dental amalgam alloy is very strong, durable, soft enough to adapt to the size and shape of the tooth cavity resulted from its damage, and hardens fast sufficiently to make it practical. There were many scientific notices about possible harmful effects of the mercury present in dental amalgams. It has been argued that the release of mercury from amalgam fillings is of toxicological relevance (Ahlqwist *et al*., 1988; Echeverria *et al*., 1995) .

Members of the public will occasionally become concerned about exposure to mercury from dental amalgams. Restorative dentistry has used a mercury-silver amalgam as a filling material. A small amount of mercury (2-20) mcg/day is released from a dental amalgam when it was mechanically manipulated, such as by chewing. The habit of gum chewing can cause release of mercury from dental amalgams greatly above normal. The normal

bacterial flora present in the mouth converts a fraction of this to Hg^{2+} and $Hg(CH_3)_2$, which was shown to be incorporated into body tissues. The World Health Organization safety standard for daily exposure to mercury is 45 mcg/day. Thus, if one had no other source of exposure, the amount of mercury released from dental amalgams is not significant. Many foods contain mercury. For example, commercial fish considered safe for consumption because it contained < 0.3 mcg/g of mercury, but some game fish contain > 2.0 mcg/g and, if consumed on a regular basis, contribute to significant body burdens (WHO and UNEP, 2008; WHO, 2008)

Millions of dentists around the world routinely use dental amalgam as a filling material in decayed teeth. Often referred to as "silver fillings," all dental amalgams actually consist of 45-55% metallic mercury. Mercury is a known neurotoxin that can cause harm to humans, especially children, pregnant women, and fetuses. Furthermore, the use of dental amalgam results in substantial quantities of toxic mercury released annually into the environment. Once in the environment, mercury pollution damages animals, plants, and the entire ecosystem, while creating "hotspots that last for centuries (WHO, 2008).

Our present study aimed to determine the internal mercury concentration of two groups differing in their attitude towards possible health hazards by mercury from amalgam fillings.

Materials and Methods

Two groups of sera sample of 36 men were taken. The first group (14 men) was as control samples while the second group (22 men) were with amalgam filling. The age was ranged (21 - 27) years old.

A blood sample in the range of several milliliters was collected from a vein into an injection tube already containing an anticoagulant (heparin) and

transferred into a sealed container to the laboratory for analysis. The sample is then centrifuged at 3,000 rpm for 10 minutes to separate the red blood cells from plasma then to be stored for a long period of time should be frozen condition.

The present method involving reduction and cold vapor atomic absorption spectrometry (CVAAS) is, in principle, similar to the conventional circulation system in that the method includes the following: reduction of Hg^{2+} ions in the sample test solution with stannous chloride to generate elemental mercury vapor (Hg^{2+}); and the introduction of mercury vapor into the photo-absorption cell for the measurement of absorbance at 253.7 nm.

However, unlike the conventional closed system in which the elemental mercury vapor generated is continuously circulated with a diaphragm pump through a reaction vessel, a U-shaped tube packed with a drying agent, and the photo absorption cell, the present method used a circulation-open air flow system. The apparatus constitutes a closed system and comprises a

diaphragm pump, reaction vessel, acid gas trap, moisture trap (ice bath), and a 4- way cock.

Cold Vapor Atomic Absorption Spectrometry is a much more sensitive method as compared with conventional flame atomic absorption spectrometry. Other advantages include its ability to measure mercury in the samples with simple mercury lamp. It is roughly classified into the reduction/aeration procedure and the sample combustion procedure according to the generation mode for mercury in the elemental form.

The former involves wet digestion with a mixture of strong acids followed by the addition of a reducing agent to generate elemental mercury vapor Hg^{2+}. In the latter, elemental mercury vapor Hg^{2+} is generated through direct combustion of the sample to be analyzed. Currently, the most common method is based on the form technique.

During its operation, the elemental vapor generated by the addition of stannous chloride is circulated via the 4-way cock at a flow rate of (1-1.5) L/min. for 30 seconds to homogenize the concentration in the gas phase. The 4-way cock is then rotated by 90°C to introduce the gas phase into the photo-absorption cell all at once. The measurement is completed within one minute per sample with this apparatus, which can measure even 0.1 ng of mercury with high accuracy. Additionally, in the method for preparing the sample test solution for the present method, the conventional wet digestion method is improved by the use of a 50-ml flask with a long neck (at least 10

cm), such as a thick-walled volumetric flask with a ground glass stopper, as well as a mixed acid system with an increased rate of sulfuric acid, HNO_3 - $HClO_4$ - H_2SO_4 (1:1:5), that already contains Perchloric acid, for the sample digestion.

This is innovative in that sample digestion can be completed in a relatively short time without loss of mercury. It is a simple method where the sample is subjected to wet digestion on a hot plate at (200-230)°C for 30 minutes and cooled followed by topping up to a fixed volume with water. This method can be applied directly to the digestion of biological samples including hair, blood, and fish as well as various solid samples such as sediment and soil. A reflux condenser is not required during heating (Chen *et al*., 1998).

Here in description, among these highly sensitive analytical methods- a method involving wet digestion, reduction and cold vapor atomic absorption spectrometry (AASCV) (the circulation-open air flow system), which offers substantial improvements over the conventional method(El-Awady *et al*., 1976).

Results and Discussion

The claimed association between dental amalgam fillings and a range of diseases or symptoms of unknown etiology has been the starting point for much concern over mercury absorption from dental amalgam (Pleva, 1992). A long list of possible adverse symptoms has been incorporated into

questionnaires for practitioners to use. Certainly patients with dental amalgams do report symptoms such as irritability, depression, numbness and tingling in the extremities, frequent night urination, chronic fatigue, cold hands and feet, bloating, memory loss, anger and constipation. However, this does not establish causation the problem with observing symptoms of unknown etiology in patients with dental amalgam restorations is confusing commonality with causality. Both the symptoms and dental amalgam fillings are commonplace, thus one being observed with the other is not unexpected This is what one researcher called the menace of daily life (Feinstein, 1988).

A small step forward is made in cross-sectional comparisons of a sub-sample of the population with and without dental amalgam fillings. This was the approach of Ahlqwist *et al.* compared the prevalence of 30 specified symptoms and complaints in dentate women with equal to or greater than 20 and (0- 4) tooth surfaces with dental amalgam fillings (Ahlqwist *et al*., 1988). They found no symptom or complaint to be more common in those with higher numbers of dental amalgam fillings. Instead, chest pain, over-exertion, abdominal pain, poor appetite and loss of weight were significantly less common in those with more tooth surfaces filled with dental amalgam**.**

A similar study by Saxe *et al*. among US nuns found no association between dental amalgam surface area and eight different tests of cognitive function (Saxe *et al*., 1995). Studies like those by Ahlqwist *et al*. can be further improved to support or refute causality (Ahlqwist *et al*., 1988)..

Their cross sectional design leaves the issue of time precedence unaddressed.

The next higher level of evidence on the link between dental amalgam fillings and adverse health effects would be cohort studies, where individuals exposed and not exposed to dental amalgam fillings are measured and followed over time for a range of adverse health effects. Even higher level evidence comes from randomized controlled trials. Here, the use of dental amalgam for fillings is on a random basis. This reduces the risk of bias in who receives and does not receive dental amalgam fillings.

However, the issue of participants knowing whether they receive dental amalgam fillings or not remains problematic. Alternatively, a randomized controlled trial could be conducted on the removal of dental amalgam fillings from patients with claimed mercurialism.

Health effects of mercury from dental amalgam fillings are formal toxicological risk assessment. Risk assessment initially focuses well away from the direct clinical or epidemiological study of individuals with dental amalgam fillings to the area of occupational exposures. Risk assessment involves four processes: hazard identification, dose-response assessment, exposure assessment and risk characterization (Reinhardt, 1998).

Mercury has a long history as an identified health hazard. There is no doubt that mercury at high levels of absorption causes severe adverse health

effects. Attention then shifts to the dose-response assessment, essentially to answer the question of the effects of low levels of mercury absorption.

A level of mercury absorbed from dental amalgam fillings which exceeds the TDI (Tolerable Daily Intake) would raise concerns but does not imply that an adverse effect will occur. Public policy might have to weigh those concerns against any possible benefits from the exposure, for example, the restoring of tooth function and avoidance of pain and suffering through the use of the material when alternatives are not either available or as cost-effective.

Exposure to low levels of mercury in air can be an occupational hazard in a number of industries. While traditionally these have included industrial sites such as chloralkali factories (Fawer *et al*., 1983), there is now an increasing trend to assess risk of exposure to mercury in dental office staff (Echeverria *et al*., 1995).

Concentration of mercury (Hg) in two groups (21-23, 24-27) and the results are illustrated in Tables (1,2). The Hg content in blood in the two groups was found in this study ranged from (2.025 – 10.772) μg/L. The higher Hg content was found in group B (10.772) μg/L in the man with age 25 year. A minority of the public has expressed concern about the use of dental amalgam in dentistry. While dental amalgam use has decreased markedly overall and in age groups up to late middle-aged adults, a vast number of dental amalgam fillings exist in the population.

However, there is no evidence that mercury absorbed from dental amalgam fillings is associated with signs or symptoms of adverse health effects or that the removal of dental amalgam fillings is associated with better health outcomes. Despite this lack of evidence, it is a principle in toxicology to accept there are more sensitive groups in the community, especially foetuses and young children, and as a result to recommend minimization of exposure among pregnant women and young children. The trend in dental amalgam use is toward minimization, but there is still wide-spread opinion that dental amalgam is the material of choice in certain clinical situations.

However, wider environmental issues with dental amalgam use and disposal and increasing concern over methyl mercury exposure will maintain community concerns. Dentists and their patients will need to be well informed and patient autonomy and choice respected.

Table (1) Concentrations of Mercury in blood A age (21-23)

Samples	Age	Hg Conc. (µg/L)	Dental amalgam
1	21	3.112	-
2		3.826	-
3		2.967	-
4		5.697	+
5		7.064	+
6		8.440	+
7	22	4.031	-
8		4.067	-
9		6.364	+
10		6.890	+
11		7.332	+
12	23	3.568	-
13		5.980	+
14		7.469	+
15		7.552	+
16		9.662	+

Table (2) Concentrations of Mercury in blood group B age (24 - 27)

Samples	Age	Hg Conc. (µg/L)	Dental amalgam
1	24	3.670	-
2		4.012	-
3		6.025	+
4		6.368	+
5		8.147	+
6	25	2.025	-
7		3.921	-
8		8.117	+
9		9.276	+
10		9.885	+
11		10.772	+
12	26	8.364	+
13		7.524	+
14		4.071	-
15		2.971	-
16		10.751	+
17	27	10.652	+
18		8.349	+
19		5.710	-
20		5.528	-

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