

Relation Between Serum Copper and Iron with Mercury Pollution in Dentists Used Amalgam Fillings

Assistant Lecturer. Soud Turkey Alazzawi
AL-Mam'on University College, Department of Medical Laboratory
Techniques

Dr. Kawther Alzubaide

Dr. Salah Alnuaimi

Abstract:

The aim of this study is the investigation of copper and iron levels in blood serum of dentists exposed to mercury which was undertaken from mercury dental amalgam preparation .The study includes (70) dentists divide into three groups according to the period of exposure (Group B exposed for (3-10) years), (Group C exposed for (10-15) years), (and Group D exposed for (15-30) years). Twenty healthy controls with no chance of exposure to mercury were chosen as control group (A). Mercury, copper and iron concentration were measured.

The result revealed that copper level increased with duration of exposure compared to the control group. A decrease in iron level was noticed in all dentists group compared with the control. It's concluded that mercury vapour from the dental amalgam could be a serious pollution poisoning source of dentists inhaling mercury vapor which inhibits some metabolic enzymes through their sulfhydryl (-SH) group. The alteration of essential and trace elements in the body could be due to damage the occur to the biological membranes and the imbalance of the ion transports across these membranes.

Key Words: Mercury Toxicity, Amalgam Restoration ,Mercury and Trace Element ,Pollution from Mercury Amalgam Restoration.

العلاقة بين العناصر الأساسية الحديد والنحاس والتلوث بالزئبق من خلال تعرض أطباء الاسنان للحشوات الزئبقية

م.م. سعاد تركي علي
كلية المأمون / قسم تقنيات التحليلات المرضية
د. كوثر عبدالرزاق الزبيدي
د. صلاح الدين النعيمي

المستخلص:

إن الهدف من هذه الدراسة هو قياس مستوى النحاس والحديد في مصل دم اطباء الاسنان الذين يستخدمون الزئبق في حشوة الاسنان الدائمة وذلك من خلال مزجه مع مسحوق السبيكة لتكوين مادة بلاستيكية تحشى في الحفرة المحضرة. كان عدد المتطوعين من اطباء الاسنان سبعين طبيبياً وقد قسموا الى ثلاث مجاميع:
(مجموعة (B) فترة التعرض (٣ - ١٠) سنة)، (مجموعة (C) فترة التعرض (10 - 15) سنة)، (مجموعة (D) فترة التعرض (15 - 30) سنة). كما اخذت عينات من دم متطوعين اصحاء من غير العاملين في هذا المجال واعتبروا كمجموعة سيطرة (المجموعة A) وعددهم (٢٠) متطوعاً. اظهرت النتائج زيادة في تركيز النحاس وقلّة في تركيز الحديد لدى العاملين بزيادة فترة التعرض مقارنة مع مجموعة السيطرة، مما يعطي دلالة على ان التعرض للزئبق من خلال تحضير الحشوة قد يؤدي الى التسمم بالزئبق نتيجة استنشاق الابخرة وقد يعزى ذلك الى ان ايون الزئبق يعمل على تثبيط بعض الانزيمات الايضية والحاوية على مجموعة الثايول (-SH) في الجسم كما ويعمل على عدم حصول موازنة في العناصر الأساسية والضرورية نتيجة تضرر اغشية الخلايا الحية وعدم السيطرة على عمليات النقل خلال المضخات المختصة بعمليات نقل الايونات.

Introduction:

Total and inorganic blood mercury levels which appear to be elevated in dentists organomercurial is not similarly elevated. It's found that the daily intake of mercury from removal of about (14) amalgams surface was estimated to be about 1.3µg,

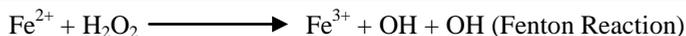
whereas toxic signs of mercury intoxication begin at blood levels of about 30µg/ml.

Levels of mercury in the blood and urine correlate with the number of amalgam fillings⁽³⁾.

Every tissue of the body contains iron. Iron is an essential element; its major function in the animal, body is the formation of hemoglobin. Enzymes also contain iron: these include cytochrome and xanthine oxidase⁽⁴⁾. Under normal condition the body burden is about 4gm. Hemoglobin is the major iron compound in the body is highly concentrated in the erythrocyte. Sixty seven percent of total iron is contained in the hemoglobin. 72% is stored as ferritin mainly in the liver, or as hemosiderin in case of excess intake⁽⁵⁾

Iron plays key role in oxygen transport and electron transport reaction of all living cells. Humans are unable to eliminate excess iron and regulate body iron stores by limiting absorption⁽⁶⁾.

Divalent iron (Fe^{2+}) is taken by intestinal mucosa and converted to the trivalent form (Fe^{3+}). The trivalent form is bound to transferring glycoprotein with two iron-binding sites^(7,8). Iron is transported as transferrin to the liver or spleen, where it is stored as ferritin which has a high iron storage capacity and prevents iron from participating in the ferritin which has a high iron storage capacity and prevents iron from participating in the Fenton reaction^(6,9).



Of the typical 4-gram body iron stores found in adult, 66% is bound as hemoglobin, 10% as the protein myoglobin, with a

minute amount in iron containing enzymes and the rest as intracellular storage proteins⁽¹⁰⁾.

Iron is absorbed through the mucosal barrier in the ferrous (Fe^{2+}) state where it oxidizes to the ferric (Fe^{3+}) state and attach to the storage protein.

Control of iron depends on variation in absorption rather than excretion.

Iron is released from the ferritin to the globulin transferring in the plasma and then transported to the blood-forming sites⁽¹¹⁾

Adult lose up to 2mg of iron daily. The primary therapeutic use of iron salts today is in the treatment of iron deficiency anemia⁽⁴⁾.

Major toxicity occurs when serum iron level exceeds the iron-binding capacity of transferrin free circulating iron damages systematic blood vessels⁽¹¹⁾.

Free iron is an oxygen - reactive substance, highly toxic to cells and will enhance the formation of free radicals and peroxidation at membrane lipids^(10,11,12,13,14).

Copper is an essential trace element that catalyzes processes in heme synthesis and iron absorption⁽¹¹⁾. It is an essential part of the antioxidant enzymes systems⁽¹⁵⁾.

The copper ions undergo unique chemistry due to their ability to adopt distinct redox states, either oxidized $[\text{Cu}(\text{II})]$ or in the reduced state $[\text{Cu}(\text{I})]$. Because of its highly reactive nature, it would, therefore, be extremely harmful for $[\text{Cu}(\text{I})]$ to exist as free ions in cells where it can participate in reactions which products ultimately damage cell membranes proteins and nucleic acids⁽¹⁶⁾. Copper is incorporated into several enzymes involved in hemoglobin formation, carbohydrate metabolism, catecholamine biosynthesis, and cross-linking of collagen, elastin, and hair keratin^(10,17).

The enzyme includes cytochrome oxidase, dopamine β -hydroxylase, ascorbic acid oxidase, and superoxide dismutase, as well as interaction with ceruloplasmin and metallothionein. Copper deficiency causes anemia, neutropenia, and impaired growth, particularly in children ⁽¹⁸⁾ Copper overload is normally further controlled by binding to metallothionein. Copper is either active or in transit, with little or no excess copper being normally stored ⁽¹⁹⁾.

Following absorption, copper is bound to albumin and transferrin, and is mainly deposited in liver hepatocytes with lesser amounts in the kidney. Biliary excretion is the major route with small amounts secreted in the urine ⁽¹⁰⁾. The normal serum level of copper is 120 to 145 $\mu\text{g/L}$ The liver and bone marrow are the storage organs for excess copper. While copper is an essential element in most organisms, the range between deficiency and toxicity is low in those without effective barrier to controlled absorption ⁽⁴⁾.

Aim of the study is to determine the concentrations of mercury in blood serum of dentists to prove the inhalation of mercury vapor released from mercury dental amalgam is a risk. And to measure the levels of copper and iron in dentists blood serum, and study the effect of mercury on it.

MATERIALS AND METHODS

Selection of Subjects

The study is conducted according to the following:

- Volunteers dentists from.
- Ministry of Health.
- The college of dentistry.
- Two central health of dentistry.

During the period from October 30, 2000 to May 30, 2001. (70) dentists females and males were examined in this study.

They have been classified into three groups according to the period of exposure to Mercury vapor aged between (28-66) years.

1. Exposure period from (3-10) years (group B).
2. Exposure period from (10-15) years (group C).
3. Exposure period from (15-30) years (group D).
4. Non-exposed (group A) females & males aged between (38-46) years (control n-20).

Blood Sampling:

(5 ml) of blood samples are collected from all subjects by vein puncture, (1ml) of blood sample was transferred into an anticoagulant tube with EDTA to use it for determination of hemoglobin (Hb).

(4ml) of blood samples were transferred into plane tubes and left for 45 min at room temperature (25°C) for clotting then centrifuged at (2000 rpm for 10 min) to get the serum which is stored at (-20°C). Unless used in work directly.

Determination of Mercury in Blood Serum:

An analytical method has been established to determine the concentration of mercury in blood, such as by using cold atomization and sodium borohydride reaction, and certain wavelength of 253.7nm. Comparing these levels with the health or controls.

Atomic Absorption Method for Determination of Trace and Essential Elements in Serum:

Principle:

Concentration of (Fe) and (Cu) in serum samples are measured by flame atomization atomic spectroscopy (AAS). This technique is based upon absorption of electromagnetic radiation by atoms at certain wave lengths of that element. The absorption in density is directly proportional to the

concentration of that element, by using Atomic absorption spectroscopy (AAS) for direct measurement of (Fe and Cu) concentrations of wave length (248.3 nm) and (324.8 nm) respectively using the hallow cathode (He) lamp of each element. The flame is air - C₂H₂ mixture.

Serum is diluted 1:10 then atomized by AAS. The absorbance (A) is recorded. The concentration of the sample calculated by the following equation.

$$\text{Concentration (mg/L)} = \frac{A_{\text{sample}}}{A_{\text{Standard}}} \times \text{standard ... concentration}$$

Determination of Hemoglobin (Hb).

Principle:

Hemoglobin is the main constituent of the red blood cells and carries out the important function of transportation of oxygen from lungs to various parts of the body. The (Hb) was measured by cyanomethemoglobin of (WHO 1990) in the medical center of the Iraqi Atomic Energy Commission.

Statistical Analysis Data:

1. Comparison of data was made by evaluation of significance between mean values, utilizing students t-test ≤ 0.05 were considered significant for all data shown in the results.
2. Drawings of Histograms, which show the significant differences between the patients or dentists and the controls according to the period of exposure to mercury vapor.

RESULTS AND DISCUSSION:

The Aim of the study is to determine the concentrations of mercury in blood serum of dentists since those workers expose to mercury from mercury dental amalgam fillings, polishing dental amalgam restorations, the removal of amalgam fillings and the storage of waste amalgam, and its toxicity.

Concerning mercury concentration and the period of exposure to mercury from mercury dental amalgam. Table No. (1) and Fig. No. (1) show the tendency of (Hg). **Concentrations** is significantly increasing ($P \leq 0.05$) as the period of exposure increases in dentists or patients groups comparing with the control group, and this agreed with previous studies⁽²¹⁻²⁴⁾ but it does not agree with other studies^(25,26).

Table (1) Mean of Mercury Concentration in Dentists Or Patient's Groups and Control or Normal Group

Groups	Number of Samples	Period Exposure (year)	Age (year) Mean \pm S.D	Conc. Of Hg $\mu\text{g}/100 \text{ ml}$ Mean \pm S.D	P value
A	20	0	40 \pm 7.52	0.292 \pm 0.123	< 0.05
B	13	3 - 10	31 \pm 3.17	0.810 \pm 0.043	<0.05
C	14	10 - 15	40 \pm 2.87	1.343 \pm 0.183	<0.05
D	43	15 - 30	53 \pm 7.56	5.130 \pm 3.402	<0.05

S.D = Standard Deviation.

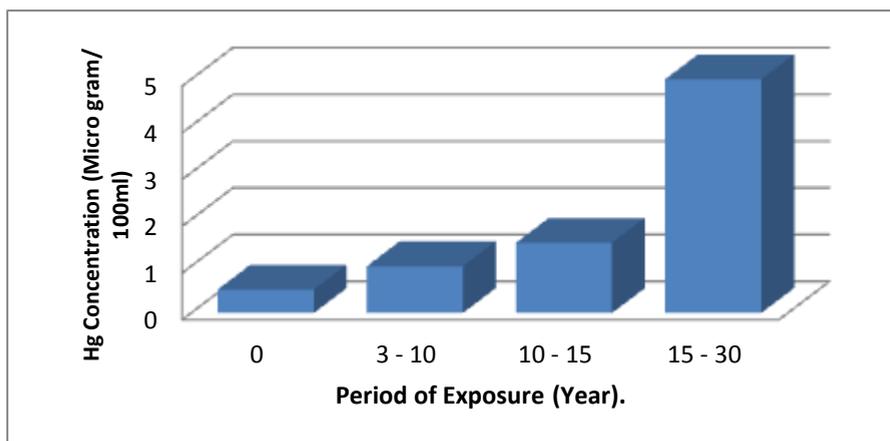
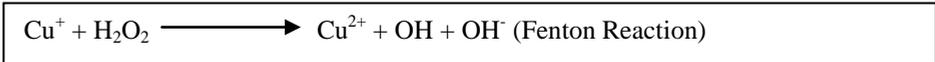


Fig.(1) The increasing in (Hg) Concentration in Dentists or Patients Groups and Control Groups with The Period of Exposure.

Copper concentration (Cu):

Table No.(2). shows the increasing of copper concentration in dentists or patients groups (B,C,D) with increasing period of exposure comparing with the control groups (A) and it was a significant increasing ($P \leq 0.05$). According to the results of the study it is concluded that the increasing in copper explains the increasing in lipid peroxidation (i.e. increasing in MDA). It is known that serum free copper ions (I), if presents, reacts with hydrogen peroxides (H_2O_2) to produce hydroxyl radicals (OH) in fast reaction in vivo.



Consequently, this (OH) radical attacks the biological cells and macromolecules at once leading to peroxidation of (PUFA) of cell membrane lipids. Accordingly, oxidative stress takes place (27)

The above explanations support results of this study which pointed to the existence of a positive correlation between increasing copper concentrations (malondialdehyde) levels in dentists or patients groups (B,C,D).

Table (2) mean of Copper concentration in dentists or patient’s groups and control or normal group

Groups	Number of Samples	Period Exposure (year)	Conc. Of Hg $\mu\text{g}/100 \text{ ml}$ Mean \pm S.D	P value
A	20	0	86.400 \pm 9.405	≤ 0.05
B	13	3 - 10	124.231 \pm 6.193	$\square 0.05$
C	14	10 - 15	144.929 \pm 4.665	$\square 0.05$
D	43	15 - 30	184 \pm 62.788	$\square 0.05$

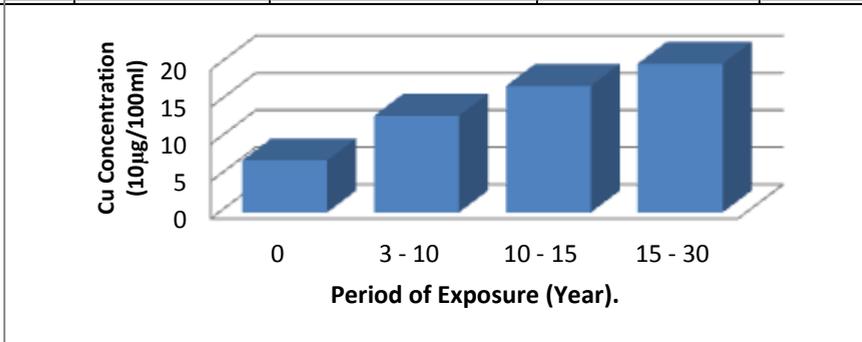


Fig.(2) The Increasing in (Cu) Concentration in Dentists or Patients Groups and Control Groups With The Period of Exposure.

Iron Concentration (Fe):

In this study, table (3) and Fig (3) show a decrease in serum iron concentrations in dentists or patients groups (B,C,D) with increasing of the period of exposure to mercury vapor. As comparing with control groups (A), There was a significant decrease ($P \leq 0.05$).

This result indicates that the hemoglobin levels in all dentists or patients groups tend to be normal or slightly increase more than control groups, and these (Hb) levels may explain the decrease in iron since iron is one of the components of Hemoglobin.

Results agree with other study by measuring serum iron concentration⁽²⁸⁾.

Table (3) Mean of Iron Concentration in Dentists or Patient's Groups And Control or Normal Group

Groups	Number of Samples	Period Exposure (year)	Conc. Of Fe $\mu\text{g}/100\text{ ml}$ Mean \pm S.D	P value
A	20	0	228.450 \pm 1.669	$\square 0.05$
B	13	3 - 10	127.077 \pm 2.216	$\square 0.05$
C	14	10 - 15	121.714 \pm 1.139	$\square 0.05$
D	43	15 - 30	112.023 \pm 4.940	$\square 0.05$

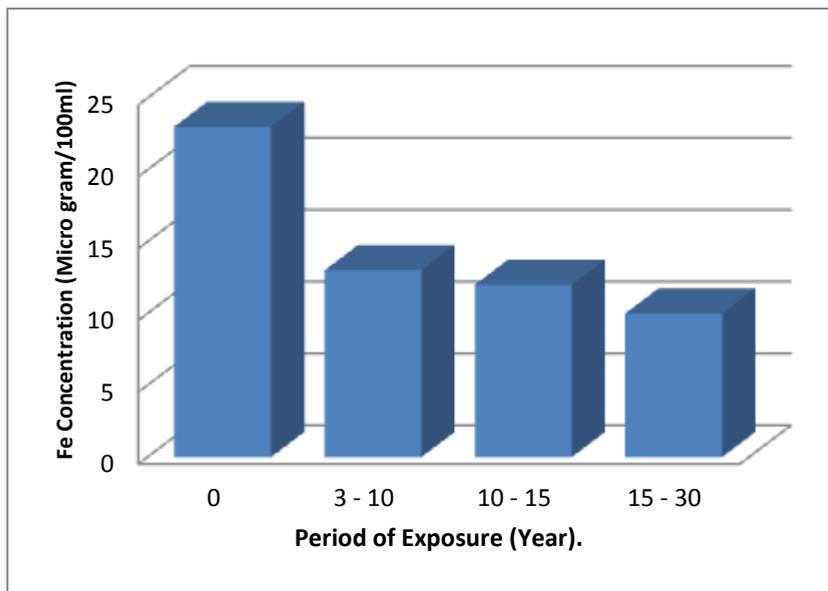


Fig.(3) The increasing in (Fe) Concentration in Dentists or Patients Groups and Control Groups With The Period Of Exposure.

Conclusion:

The presented data in this study enable us to conclude the following:

1. The increase in the serum levels of copper could explain the increase in lipid peroxidation and tissue damage.
2. The decrease in the serum levels of iron could explain the imbalance state due to increase of lipid peroxidation.
3. The normal levels of Hemoglobin in dentists or patients and the increasing in copper indicates the role of copper in increasing the lipid peroxidation.

References:

1. Jaueg, C., Sanfilippo. D.J., Rowens, B., Szeide, J and I esse, .Jl., Clin Toxicol 30:63-67.(1992)
2. Bergman, M., Systemic and environmental Int Dental J : 40:4-10. (1990)
3. Eli, S. Weisman, R.S., and Hoffman, R.S., vet Hum toxicol: 34:354. (1992)
4. Casarent, L.J., and Doulls, J., "Toxicology the Basic Science of Poisons" 2 nd ed. Machmillan publishing Co. Inc. New York. P691. (1980)
5. "Summary of scientific facts "DAMS Inc. U.S.A. April the dental amalgam issue. (Abstract). (2001).
6. Bast, A., Hanen, G.R., and Doelman C.J. Am. J. Med. 91 (Suppl.3c): 2-13. (1991)
7. Belies, R.P. "The metals in pattys industrial Hygien and Toxicolg" John Wiley and Sons, New York. PP. 1879-2352. (1994).
8. MacGillivary, R.T., Mendex, E., Sinha, Sinha, S.K., and et al PROC. Natl. Acad. Sci, 79:2504-2508. (1982).
9. Medeiros.D.M., Wildman, R., and Liebes, R. "Metal metabolism and toxicilies "Handbook of human toxicology. CRC.Press, New York. PP. 149-188. (1997).
10. Wallace Hayes, A., "Principle and Methods of Toxicology" 4th ed. Taylor and Francis England. PP. 669-670. (2001).
11. Ellenhorns, M.T.. "Medical Toxicology Diagnosis and Treatment of human Poisoning" 2nd ed. Williamas &

- Wilkins publishing company, Inc, U.S.A. PP. 1588-1602. (1997).
12. Bacon, B.R. and Britton, R.S., *Hepatology*: 11:127-137. (1990).
13. Bacon, B.R., Tavill, A.S., Brittenham, G.M., and et al *J.Clin. Invest.* 71:429-439 (1983).
14. Ryan, T.P., and Aust. S.D., *CRC. Crit. Rev. Toxicol*: 22:119-141. (1992).
15. Albala, C., Calazar. G., Vio. F., Arya, F, I., Feuerhacke. W., Olivores. S., and Alvarez, G. *Rev. Med. Chil*: 155:887. (1997).
16. Pena, M.M., Lee. L., and Thiele. D.J. *J. Nutr*; 129:125-126. (1999).
17. Agency for toxic substances and disease registry (ATSDR): *Toxicological profile for copper Atlants.* G.A. (1990).
18. N., R., C., *Recommended Dietary Allownees.* 10th ed. Natonder Academy press. U.S.A. (1989).
19. LINDER, M.C.. and Hazegh., M., *Am. J. Clin. Nutr*: 63:7975-8115. (1996).
20. Galic, N., Prpic-Mehicici. G., Prester, L., Blanusa, M., Krnic., and Ferencic, *Biometals Sep*: 12(3):227-231. (1999).
21. Wasylko, L., Matsui. D., Dykxhoorn, S.M. Rieder, M.J., and Wwinberg, S., *J-Can-Dent-Assoc.* June: 64(6):434-439. (1988).

22. Bjorkman, L., Sandborgh-England, G., and Ekslrand, J., Toxicol-Appl-Pharmacol. May: 144(1): 156-162. (1997).
23. Lang worth, S., and Syrombverg, R., Eur-J-Oral-Sci Jun, 104(3): 320-321. (1996).
24. Kostyniak, P.J., NOY-State-Dent-J-Apr, 64(4):40-43. (1998).
25. Muller-Miny, H., Erber, D., Moller, H., Muller-Muny, B., and Bong artz, G., j-Magn-Reson-Imaging Jan-Feb: 6(1):258-260. (1996).
26. Schiele, R., Kohoul, J., and Senft. V., Cas-Lek-Cesk: Oct. 7:136(19): 58-90. (1997).
27. Aruoma, O.J., Halliwell, F., Gajewsk. E., and Dizdarglu, M., Biochem, J. 273-601. (1991).
28. Zabinski, Z., Dabrowski, Z., Moszczunki, P., and Rutowski., J., Toxicol-IndHealth. Feb: 16(2):58-64. (2000).