The Effect of Mercuric Exposure on Oxidative Stress and Enzymatic Antioxidant Defense System

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Abstract

Throughout the centuries, several incidents of mercury toxicity have been reported. Mercury is found in many industries such as battery, thermometer and barometer manufacturing, in the agricultural industry is used in fungicides and in medicine, mercury is used in dental amalgams. An important mechanism involved in cellular injury is induced by exposure to different forms of mercury involves in the induction of oxidative stress.

This study was conducted on non-smoker, male working in a chloroalkali plant for different periods, all workers were not suffering from chronic disease. Healthy non-smoker males that are not exposed, matched age were used as controls(C), workers aged (22-61) years, they were divided into three groups:

G_1: workers with exposed period less than 10 years, $G_1 < 10$ years.

G_2: workers with exposed period (10-19) years .

G_3: included workers with exposed period more than 19 years, $G_3 > 19$ years.

The result we had through examining the different parameters led us to add another group which included individuals with high mercury levels regardless the occupation period, in this group we found high significant changes in the defense system parameters that we measured.

This study showed an elevation in MDA levels in all workers group, specially those with high level mercury , which were 9.31, 12.78, 12.99, 14.73, and 18.11nmol/dl for C, G1, G2 , G3 ,and high level mercury workers respectively .

No alteration was found in SOD activity in erythrocyte (0.67, 0.73, 0.72, and 0.77 U/g.Hb) for C, G_1, G_2 and G_3 respectively.

There were highly significant decrease in catalase activity ($P < 0.001$) in erythrocyte of all worker groups compared to normal control. The values were (1.5, 0.8, 0.88, 0.815 and 0.45 U/gm/Hb) for C, G_1, G_2, G_3 and G_4 respectively. While there were high elevation in glutathione S-transferase activity in worker groups compared to control ($P < 0.001$) and values were (1.85, 2.589, 2.441, 2.776 and 3.2 U/gm.Hb) for C, G_1, G_2 and G_3 respectively.

Introduction

Free radical induced oxidative damage has been implicated in the toxic action of many chemicals and environmental agents and in the pathogenesis of a number of diseases [1].

Mercury may occur in the elemental form or as inorganic and organic compounds [2]. From biochemical point of view, no other metal better illustrates the diversity of effects caused by different chemical species than does mercury [3,4] . Each of the three forms of mercury has characteristic toxicokinetics and health effects [5] .

In as much as mercury is ubiquitous in the environment, it is nearly impossible for most humans to avoid exposure to some form or forms of mercury on a regular basis[6]. All forms of mercury cause toxic effects in a number of tissues and organs, depending on the chemical form of mercury, the level of exposure and the duration of exposure[4,7,8]

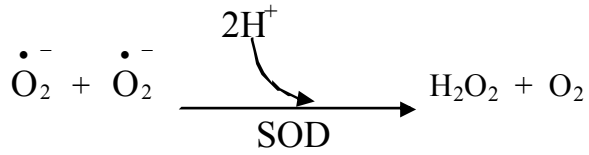
This work is apart of a PhD. Thesis of the first co-author

Normally the body burden of mercury in humans is predominantly caused by the diet(3,4) and the dental amalgam [9] Other sources such as air or drinking water contribute, substantially to the total burden only in the case of local mercury contamination .
The high vapour pressure of metallic mercury is the main reason for the occupational burden where ever mercury is mined, melted, refined, treated or recycled [4]

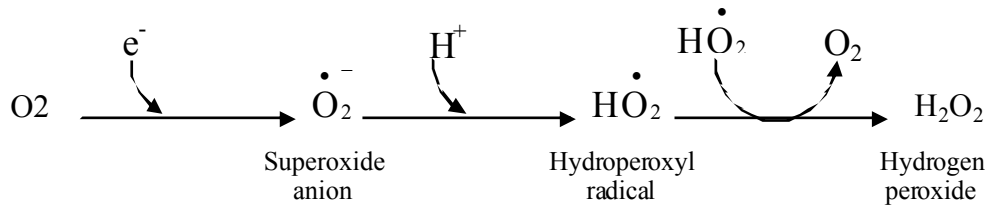
Free radical induced lipid per-oxidation may be involved in MeHg-induced cell damage[10,11]. This hypothesis is supported by findings in which MeHg exposure in vivo elevates ROS in brain regions sensitive to MeHg [12,13].

Living organisms have devised several antioxidant defense mechanisms. These mechanisms consist of a variety of metallo-enzymes and several types of antioxidant molecules [14] .

Superoxide dismutase SOD is a metalloprotein that is present in all aerobic organisms[15]. It catalyzes the conversion of two superoxide anions into hydrogen-peroxide and molecular oxygen [16] .



The hydrogen peroxide formed by superoxide dismutase, and by the uncatalyzed reaction of hydroperoxy radicals



Area scavenged by catalase [17] which is an ubiquitous heme protein that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen [18].

Glutathione – S – transferases (GSTs) constitute a family of enzymes that catalyze the conjugation of glutathione to electrophilic compounds including metabolites of several mutagens and carcinogens, and major class of phase – II enzymes involved in xenobiotic biotransformation and detoxification.

Methods

Sampling was conducted in a chloroalkali plant, that used mercury electrodes to produce chlorine and soda, during the period Feb. 2001 – May 2002. Forty one non-smoker males working in the plant for different periods were included in the study. All workers were not suffering from chronic diseases.Sixteen healthy non-smoker males, who are not occupationally exposed, matched age, were used as a control group(C) .

Workers aged 22-61 years, they are divided into three groups:

G_1: included workers with exposed period less than 10 years, $G_1 < 10$ years.

G_2: included workers with exposed period 10-19 years, G_2 10-19 years.

G_3: included workers with exposed period more than 19 years, $G_3 >$ 19 years .

About 10 mls of venous blood were drawn by using disposable needles and syringes. Samples were collected between 9.00-12.00 a.m. with no regard to meals.

Plasma samples were used to measure MDA content .The anticoagulant was added to the blood sample, centrifuged for 15 minutes at 3000 g then plasma was separated from red cells. Red cells samples were used to determine catalase, SOD and GST activities.

Mercury was analyzed by atomic absorption spectrometric technique uses sodium borohydride as a reducing agent, which is capable of reducing all the mercury in the sample to elemental mercury vapour [19] .

Lipid peroxidation was determined by using the thiobarbituric acid method [20]. In this method malondialdehyde (MDA), formed from the breakdown of polyunsaturated fatty acids was identified as the product of lipid peroxidation that reacts with thiobarbituric acid (TBA) to give a red chromophore absorbing at 532 nm. Malondialdehyde concentrations were calculated using molar absorptivity coefficient of 1.56×10^5 $L.Mol^{-1}.cm^{-1}$.

Superoxide dismutase (SOD) was assayed according to Winterbourn [21].This assay depends on the enzyme ability to inhibit the reduction of nitro blue tetrazolium (NBT) by ($\dot{O}_2^-$) anions that were generated subsequent to the reduction of riboflavin by illumination in the presence of methionin which is an oxidizable compound.

Catalase activity was determined by using assay method that depends on it's ability to decompose H_2O_2 to give H_2O and O_2. This assay was based on the reduction in the absorbance of hydrogen peroxide H_2O_2 at 240 nm. The difference in absorption (ΔA_{240}) per unit time is a measure of catalase activity [22].

Glutathione-S-transferase GST catalyzes the conjugation of GSH with 1-Chloro-2,4-dinitrobenzene (CDNB), as co-substrate, to form 2,4-dinitrophenyl glutathione which absorbs light at 340 nm, and the determination of the rate of it's formation reflects enzyme activity in the system.

Result and Discussion

Fig. (1) shows the concentrations of mercury in the study groups, male workers, in chloroalkali plant and occupationally unexposed control group. The concentrations of mercury in serum were significantly higher in the Hg-groups compared to control group .There were no significant differences between the Hg-groups them-selves ($p > 0.05$). Though there were some individual cases within the Hg-groups which showed values more than the acceptable level (3 μg/dl) [23]. Four cases from G_3, three from G_2 and four cases from G_1 showed values of Hg concentration in serum above(6 μg/dl) Mercury in blood of chloroalkali-workers correlated significantly with current type work, but not with length of exposure[24,25].

Lipid peroxidation products measured as malondialdehyde (MDA) content were detectable in a significantly higher concentration in all mercury exposed groups compared to the normal control group ($p < 0.001$) as shown in Fig. (II) and Table (1). Mean MDA levels were increased 37%, 39% and 58% over the mean control level for G_1,G_2 and G_3 respectively. Fig.(III) correlates MDA production in workers and control with the increase of Hg-concentration, as we can see that levels of serum Hg-concentration when became higher than 3 μg/dl the MDA levels increased rapidly ($r = 0.911$). Workers from different occupation periods having mercury levels less than (3 μg/dl) have serum MDA concentration within normal range of that for control. MDA production of high mercury level workers (Hg > 3 μg/dl) was increased (172%) over normal control. A number of studies have proposed that elevated blood level of lipid peroxidation might be involved in cellular dysfunction [26,27].Moreover, it is well known that mercury increases the level of oxygen reactive intermediates in tissue cells [28] .The data in table (1) indicates that a state of oxidative stress and increase in process of lipid peroxidation in workers is compatible with the observation of Queiroz et al., 1998 [29], these authors reported that the average of lipid peroxidation in serum is higher in workers exposed to mercury than in normal control.The highly significant

increase of MDA level in the third group of workers (58%) over control level could be due to age as it is known that oxidative stress is age related [30].

Table (2) shows activity of SOD in mercury exposed workers and control as it is noticed there are no significant differences between the three groups of workers and normal control ($p > 0.05$). Our results are in agreement with reported values for SOD where the authors found no significant differences in mercury exposed workers in SOD systems. SOD plays a significant role in erythrocyte defense against oxidation so the balance between antioxidant and oxidative damage maintains equilibrium between oxidative injury and defense system (29). On the other hand some authors reported that SOD activity in erythrocytes of workers occupationally exposed to mercury was significantly lower than control group as it can be explained that workers in elemental mercury leads to increase lipid peroxidation in erythrocytes, and they postulated that this exposure leads to decrease activity of SOD in erythrocytes (1). In mercury exposed groups SOD activity was incompatible and no alteration was found by Barregared, 1990 [31], who described a normal concentration of SOD in plasma and blood of mercury exposed workers. On the other hand, Perrin-Nadif., 1996 [32]observed increased SOD activity in workers with higher urinary mercury concentrations

Our results showed a highly significant decrease ($p < 0.001$) in catalase activity in mercury exposed workers compared to normal control group. 50% decrease was found in the enzyme activity in each worker group relative to the normal control group, while no significant differences ($p > 0.05$) were noticed within the worker groups themselves as shown in Fig. (IV) .Fig. (V) showed the levels of catalase activity in mercury exposed workers as a function of Hg-concentration, a highly negative correlation between the two parameters ($p < 0.001$) is shown $r = -0.8517$. In this figure only levels of mercury over 3 μg/dl are represented for the workers. Catalase enzymatically removes hydrogen peroxides formed from enzymatic removal of superoxide. Changes in catalase activity occuring after chronic exposure to high levels of mercury were investigated in the red cell system and an increase in catalse activities in worker groups was reported, they reported also that no alteration is detected in red cell antioxidant system [29].In a recent review of the influence of antioxidant systems in the erythrocyte survival Kurata[33] reported in all mammals, a positive correlation between the red cell life-span and the intracellular levels of SOD and GSH. However, no correlation was observed for catalase. The authors suggested that the relative strengths of the effectiveness of the cellular antioxidant systems and oxygen radical formation are potential candidates in governing the ageing process and in the determining red cell life span in mammalian species. Catalase activity was reported to be unaltered in oxidant injury and oxidant response induced by mercury [34,25]. Catalase activity was reduced in the renal cortex of rats exposed to mercury[35].

GST activities in all exposed groups were significantly higher than normal control levels as shown in Fig. (VI), there is 39%, 31% and 50% increase in GSTs activities in groups G_1, G_2 and G_3 respectively. Fig. (VII) showed a highly positive relation ($r = 0.76$, $p < 0.001$) between mercury and GST activity in high mercury levels workers (Hg > 3 μg/dl) regardless the occupation period. Fig. (VIII) shows the positive relationship between MDA levels for workers (Hg > 3 μg/dl) and GST activity ($r = 0.54$, $p < 0.001$) compared to normal control.
GST activity in high Hg-level workers (average 3.18 U/gm.Hb) was increased by 172% over control levels.The elevation in activity of the enzyme for mercury exposed subjects in our study is due to the detoxification effect of the enzyme against lipid peroxidation and xenobiotics [36]. Our result is compatible with researches performed on laboratories animals exposed to inorganic mercury which confirmed the protection effect of GST enzyme as a general mechanism against a wide variety of pulluent including heavy metals and carcinogenic aromatic compounds [37].

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Table (1): MDA product levels in sera of the four studied groups

Groups	No.	MDA level µg/dl Mean ± S.D.	T-test
C	16	9.31 ± 0.79	–
G_1	15	12.78 ± 0.53	P < 0.005
G_2	14	12.99 ± 3.94	P < 0.001
G_3	13	14.74 ± 5.5	P < 0.001

Table (2): Shows the mean values and the standard deviation of SOD activities for the four studied groups.

Groups	No.	U / g.Hb mean ± S.D.	T-test
C	16	0.674 ± 0.109	–
G_1	14	0.736 ± 0.187	P = 0.26
G_2	12	0.72 ± 0.149	P = 0.3
G_3	13	0.77 ± 0.289	P = 0.22

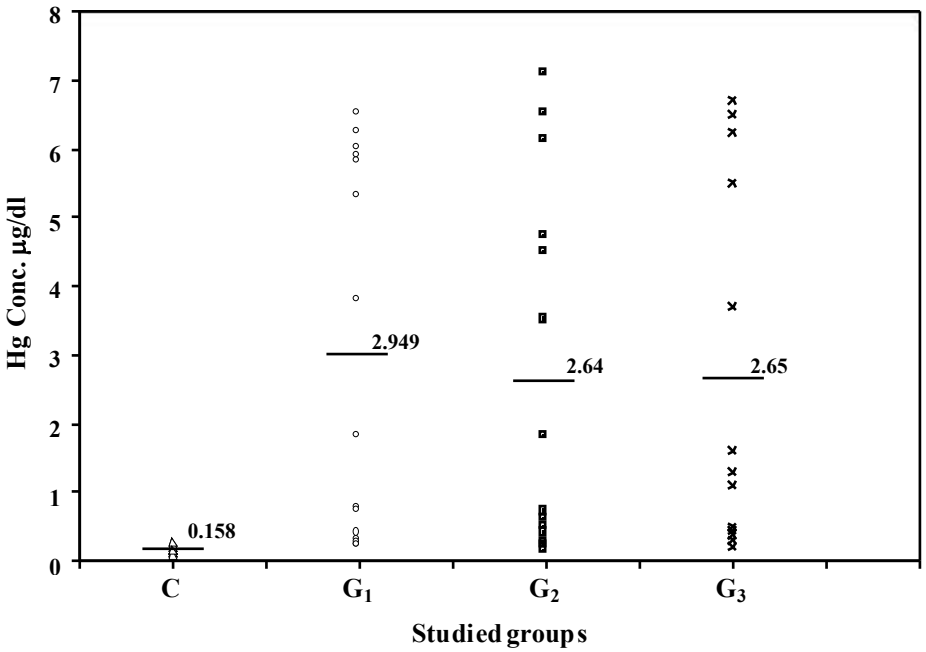

Fig. (1): Concentrations of mercury in the study groups (workers and normal control

Where △ represents C group (normal control group)

○ represents G_1 group (workers with exposed period less than 10 years)

■ represents G_2 group (workers with exposed period 10-19 years)

× represents G_3 group (workers with exposed period more than 20 years)

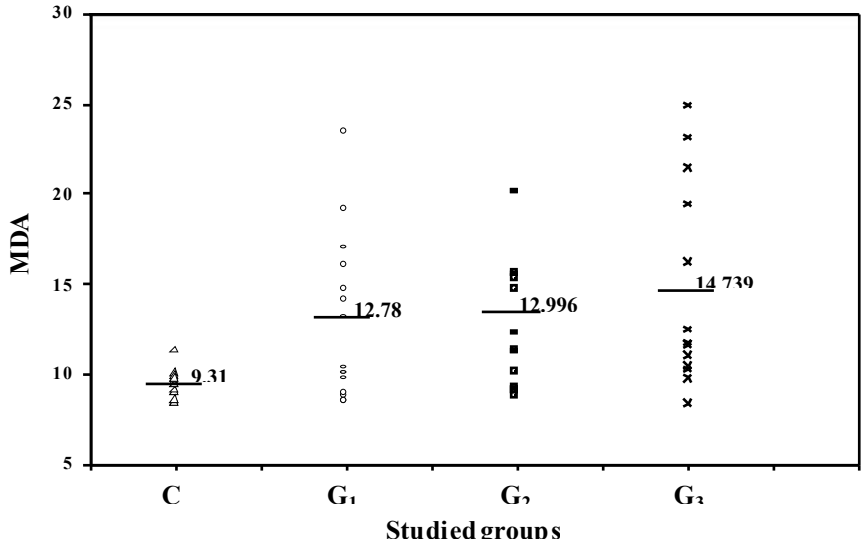

Fig. (2): Levels of MDA products in control and worker groups.

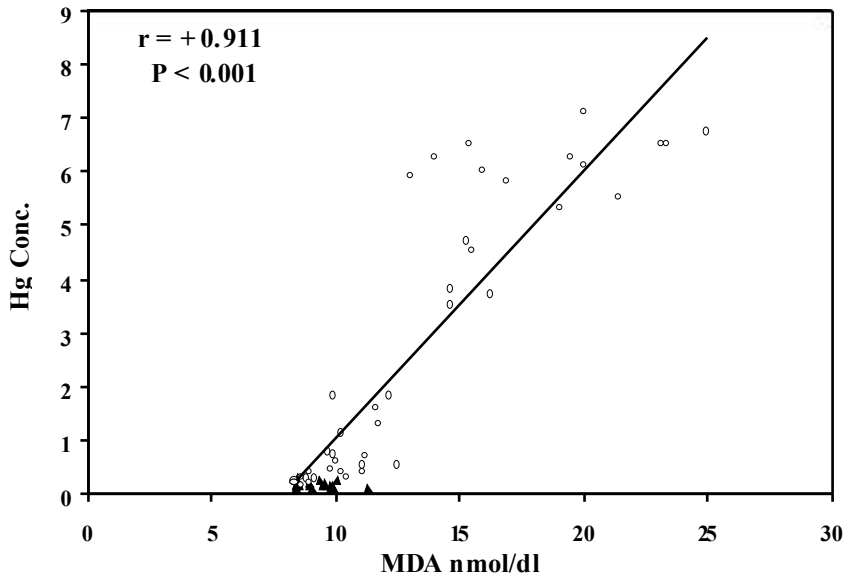

Fig. (3): Relationship between MDA production and Hg concentration in workers and control.

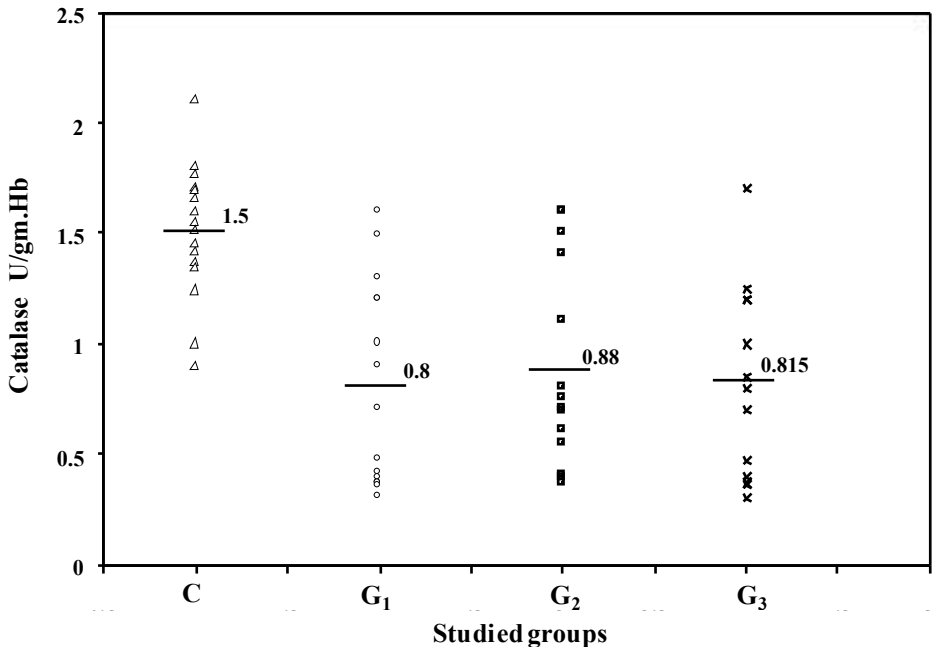

Fig. (4) :Erythrocyte catalase activity for the studied groups

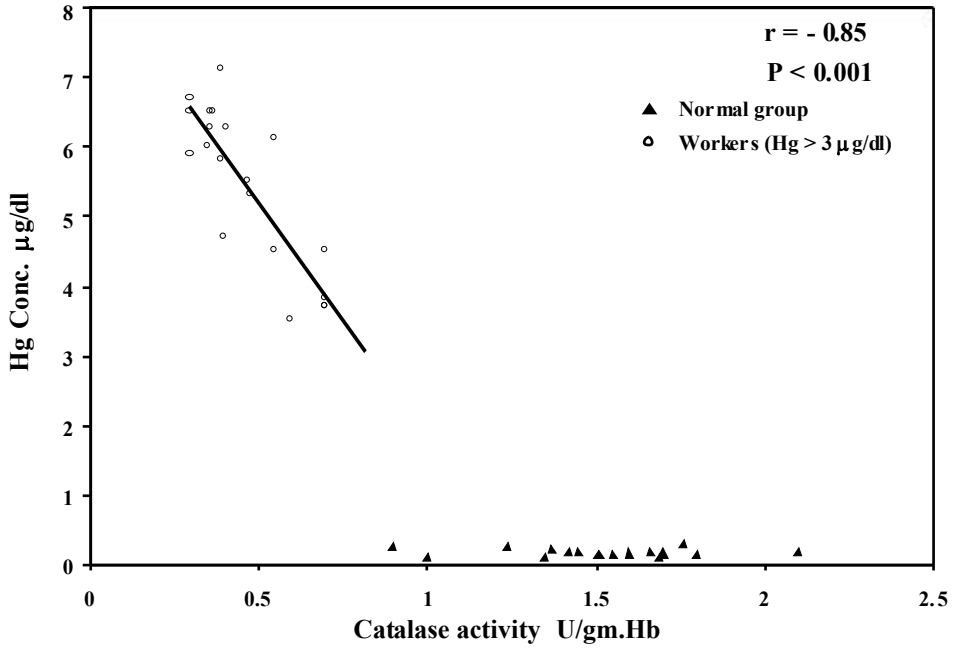

Fig. (5): Relation of catalase activity against workers (Hg > 3 µg/dl) and control

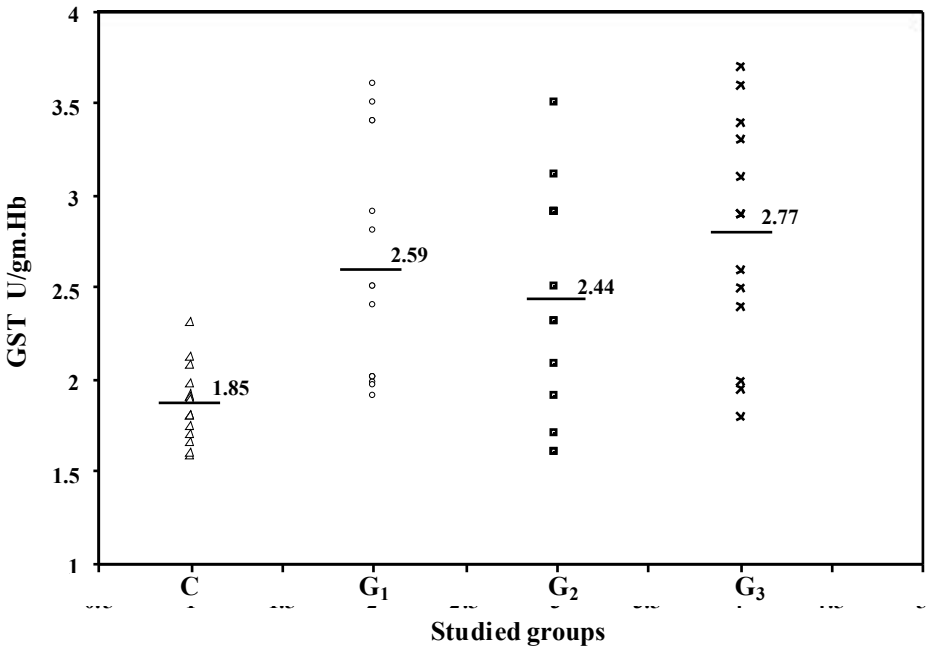

Fig. (6): GSTs activities in exposed groups and normal control

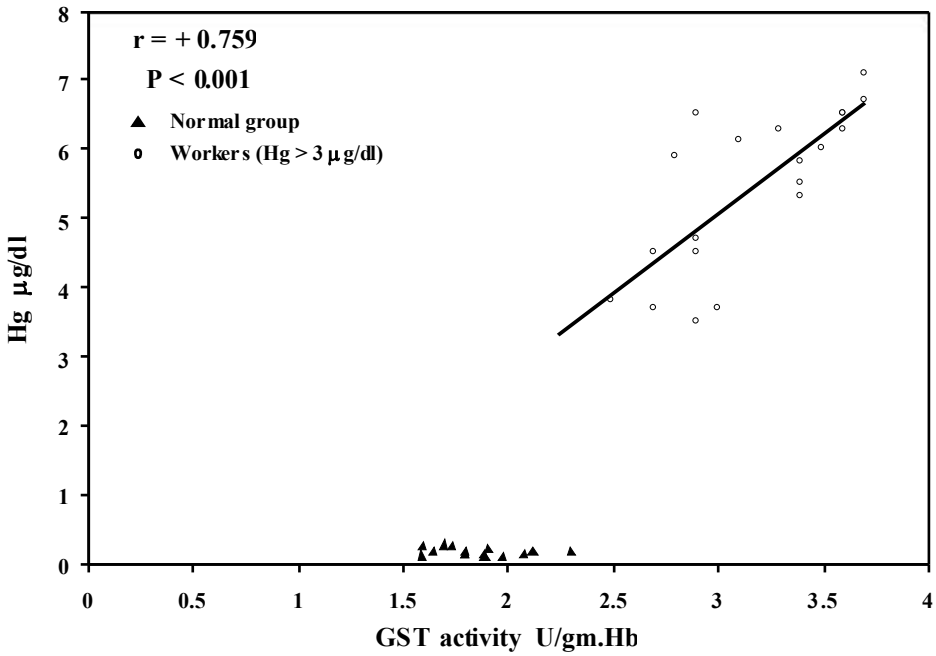

Fig. (7): Relation of serum mercury for workers (Hg > 3 µg/dl) and control versus GST activity.

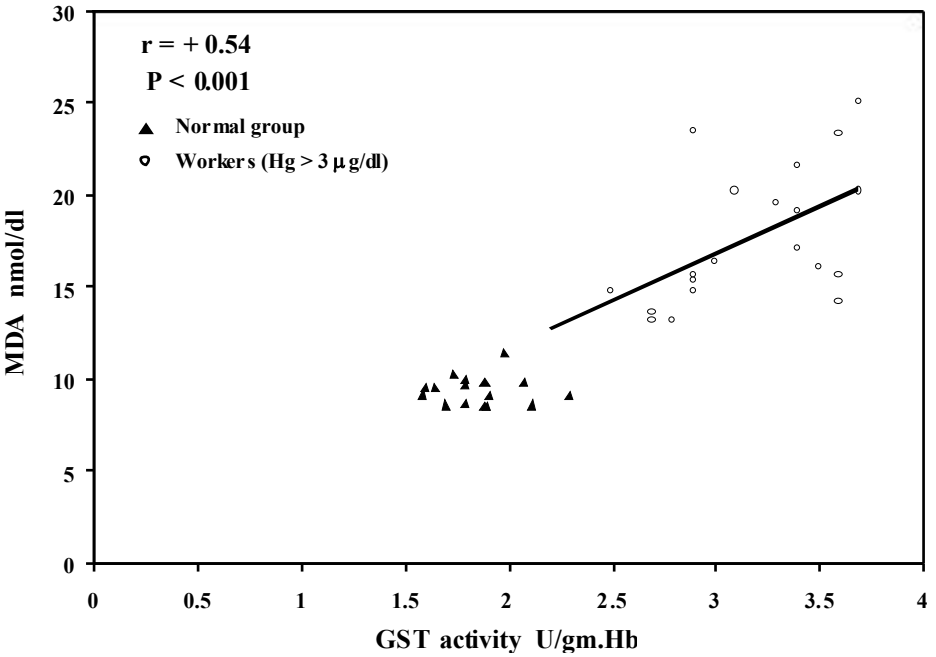

Fig. (8): Relationship of MDA for workers (Hg > 3 µg/dl) and normal control against GST activity.

تأثير التعرض للزئبق في الشد التأكسدي وفي نظام الدفاع الانزيمي ضد الأكسدة

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الخلاصة

خلال القرون السابقة وثقت حوادث عديدة للتسمم بالزئبق، إذ انه يدخل في العديد من الصناعات، مثل: صناعة البطاريات، والمحارير، والباروميترات. وفي الزراعة يستعمل الزئبق في تعفير الحبوب أما في الطب فيدخل في سبيكة حشوة الأسنان.

هناك ميكانيكية مهمة تؤدي إلى تصدع الخلية وتحدث نتيجة للتسمم بالأنواع المختلفة للزئبق، إنها ميكانيكية حث الشد التأكسدي.

هذه الدراسة أجريت على مجاميع من العمال (الذكور) العاملين مددا مختلفة في شركة الفرات من غير المدخنين وممن لا يشكون من أمراض مزمنة.أما مجاميع السيطرة (C) فهم من الذكور الأصحاء وغير المتعرضين للزئبق، وغير المدخنين ومن الأعمار نفسها عند المقارنة بمجاميع العاملين.

أعمار العاملين تتراوح بين (22–61) سنة وقد قسمت على ثلاث مجاميع :

G1 = تتضمن العاملين مدة تعرض أقل من (10) سنوات.

G2 = تتضمن العاملين مدة تعرض بين (10–19) سنة.

G3 = تتضمن العاملين مدة تعرض أكثر من (19) سنة.

إن النتائج التي حصلنا عليها من خلال فحص المعايير المختلفة قادتنا إلى إضافة مجموعة أخرى تتضمن نماذج من العاملين أعطت فحوصاتها تراكيز عالية من الزئبق بصرف النظر عن مدة التعرض. في هذه المجموعة لاحظنا تغييرات معنوية عالية في معايير المنظومة الدفاعية التي فحصت.

إن نتائج هذه الدراسة أظهرت ارتفاعا واضحا في مستويات المالون داي ألديهايد (المؤشر على مقدار الأكسدة الفوقية للدهون) في كل مجاميع العاملين ولاسيما الذين وجدت لهم تراكيز عالية للزئبق في مصل الدم، التي كانت: 9.31، 12.78، 12.99، 14.73، 18.11 نانومول/100 مل لكل من المجاميع C، G1، G2، G3 وأخيراً مجموعة التراكيز العالية للزئبق G4 على التوالي.

لم نجد هناك تغيرا في فعالية أنزيم السوبراوكساید دايسميوتيز في كريات الدم الحمراء، اد كانت النتائج(0.67،0.73،0.72،0.77 وحدة/غم هيموغلوبين) لكل من(G3,G2,G1,C) على التوالي.

كما أظهرت النتائج انخفاضاً معنوياً كبيراً ($p < 0.001$) لفعالية إنزيم الكتليز في كريات الدم الحمراء لكل مجاميع العاملين مقارنة بمجموعة الأصحاء. والقيم كانت كماياتي: 1.5، 0.8، 0.88، 0.815، 0.45 وحدة/غم من الهيموغلوبين للمجاميع C، G1، G2، G3، G4 على التوالي.

بينما كان هناك ارتفاع كبير في فعالية إنزيم الكلوتاثايون – س – ترانسفريز في مجاميع العاملين مقارنة بمجاميع الأصحاء ($p<0.001$)، كما موضح في القيم 1.85، 2.589، 2.441، 2.776، 3.2 وحدة/غم من الهيموغلوبين للمجاميع C، G1، G2، G3، G4 على التوالي.