The Role of Oxidative Stress In Lead Poisoning

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ABSTRACT

To investigate the relationship between increased lipid peroxidation, and the lowering of both plasma total proteins and albumin in lead-exposed workers, and the effectiveness of antioxidants (vit. C and E) in modulating oxidative stress in those workers.

Thirty male and females workers employed in the Iraqi storage battery (age range 20-40 years) were participating in this study. Additionally, 11 healthy subjects were served as healthy controls, with the same age range compared to workers group, to avoid the effects of age variations on the studied parameters. Blood lead levels, erythrocytes and plasma MDA, erythrocytes and plasma GSH, total protein and albumin levels in healthy controls and lead-exposed workers pre- and post-treatments with antioxidant were measured.

Comparison with healthy control groups reveal 360% increase in blood lead levels, 150% increase in erythrocyte MDA, 117% increase in plasma MDA, 28% decrease in erythrocyte GSH, 56% decrease in plasma GSH, 13% decrease in total plasma protein and 23% decrease in albumin levels in lead-exposed workers. Treatment with a combination of antioxidant vitamins (1000 mg/day vit. C and 200 mg/day vit. E) for one month produced significant reduction 12% in lead levels, 54% in erythrocyte MDA, 53% in plasma MDA; significant increase 41% in erythrocyte GSH, 120% in plasma GSH and 11% in plasma albumin levels in comparison with pre-treatment levels.

In conclusion, there is a beneficial effect of antioxidants on the oxidative stress parameters that not only related to their ability to remove lead from target cells, but also associated with antioxidant potential for bolstering thiol antioxidant capacity, and this makes these vitamins a good candidate for therapeutic intervention in lead poisoning.

الخلاصة

يعتبر التسمم بالرصاص من المشاكل التي تمت دراستها بصورة مكثفة عبر السنين السابقة، وبالرغم من الكم الهائل من المعلومات المتراكمة حول هذا الموضوع، فان ميكانيكية التسمم المعروفة غير قادرة على تفسير الكثير من الظواهر السمية لهذه المادة.إحدى النظريات المتداولة والتي تبحث في كيفية التأثير السمي للرصاص، تقترح أن الإجهاد التأكسدي المحدث بواسطة الرصاص، من المحتمل أن يكون له دور مهم في نشوء العديد من الأعراض الناتجة عن هذا التسمم.لذا تمت دراسة المالوندای ألديهايد (MDA)، الذي يعتبر مؤشر لزناخة الدهون، والكلوتاثايون (GSH)، والألبومين، اللذان يعتبران من موانع الأكسدة الطبيعية، قد تم فحصها لدى العمال المتعرضين للرصاص والعاملين في المنشأة العامة لصناعة البطاريات في العراق، قبل وبعد إعطاء موانع الأكسدة والتي تتضمن حامض الأسكوربيك (Vit. C) والألفاتوكوفيرول (Vit. E) كما تم اختبار مدى قابلية كريات الدم الحمراء والبلازما على مقاومة الإجهاد التأكسدي المحدث ببيروكسيد الهيدروجين ومن ثم قياس مدى تأثير موانع الأكسدة عليه.أظهرت نتائج الدراسة وجود زيادة في مستوى MDA في كل من كريات الدم الحمراء والبلازما لدى العمال المعرضين للرصاص، مع انخفاض ملحوظ في مستويات الكلوتاثايون والألبومين. كذلك لوحظ زيادة في حساسية الكريات والبلازما للإجهاد التأكسدي المحدث ببيروكسيد الهيدروجين مترافقة مع ارتفاع في مستوى الرصاص في الدم لدى العمال المعرضين له. إن موانع الأكسدة التي تم استخدامها أدت إلى تحسن في جميع المعايير التي تمت دراستها مما يعزز من إمكانية تأثيرها من الناحية السريرية في توفير الحماية للجسم ضد أضرار الإجهاد التأكسدي الناتج عن التعرض للرصاص.

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INTRODUCTION

Lead, similar to many heavy metals, is a complex toxin, exerting numerous pathophysiologic effects in many organ systems [1]. At the molecular levels, lead interacts with biomolecules and functions in different ways, like binding to numerous structural and enzymatic proteins [2], interference with metabolic pathways of mitochondria [3], and exhibiting mutagenic and carcinogenic effects in mammalian cells [4].

Oxidative stress which refers to a cellular situation characterized by elevation of the study state concentrations of reactive oxygen species (ROS), and this could be a possible contributor to the pathogenesis of lead poisoning[5]. Some in-vitro and in-vivo studies showed an elevated production of ROS due to lead treatment [6,7,8], and increased lipid peroxidation associated with altered antioxidant defense systems [9].

The effects of lead on the oxidative stress parameters like glutathione (GSH), and malondialdehyde (MDA), suggests ROS as a possible contributor to cell damage due to lead exposure [10]. The increase in lipid peroxidation during lead poisoning were found to be accompanied by alterations in the antioxidant defense system, including decreased GSH levels in all body compartments [11].

This study was conducted to investigate the extent to which ROS-related processes are involved in lead poisoning and the possibility of therapeutic intervention with antioxidant vitamins in this case.

SUBJACT and METHOD :-

This study was carried out on workers employed in the Iraqi storage battery plant, and selection was made on the basis that they must be directly involved with lead exposure, and have been employed for at least 8 months, before the investigation were carried out.

Thirty, male and females volunteers (age range 20-40 years) from the workers of the battery plant, participate in this study. Eleven healthy subjects served as healthy controls, with the same age range compared to workers group, to avoid the effects of age variations on the studied parameters.

The average working time (hrs/day) for each worker is 6 hrs, with a period of exposure to lead ranging from 8 months to 28 years.

Individual symptom survey was performed by clinical and physical evaluation of the workers involved in the study, concerning the presence of lead-associated signs and symptoms for the purpose of proper selection.

Blood samples (10 ml) were drawn by vein puncture from each subject prior to starting treatment with antioxidants (as baseline sample). After that, all subjects receive a combination of antioxidant vitamins (ascorbic acid 1000 mg/day and α-tocopherol 200 mg/day) orally for a period of 4 weeks, then second blood sample was drawn for evaluation of the effect of treatment on the studied parameters.

Blood samples were placed into heparinized tubes and refrigerated until separation of erythrocytes and analysis.

Blood lead levels were measured by graphite France atomic absorption spectrophotometer according to the method of Parson et al [12].

Erythrocytes and plasma malondialdehyde (MDA) level as indicator of lipid peroxidation were assessed utilizing thiobarbituric acid assay method of Stock and Dormandy [13], and the susceptibility of plasma and erythrocytes to in-vitro hydrogen peroxide-induced oxidative stress was measured according to the method of Gilbert et al [14], and the results were expressed as nmole (MDA)/gm Hb, based on the molar extinction coefficient of MDA ($1.56x10^5$ M^{-1} cm^{-1}). Glutathione levels were determined in erythrocytes and plasma according to the method of Godin et al [15]. Plasma albumin levels were determined utilizing a ready-made kit for this purpose (Randox company, England) according to the method of Doumas et al [16]. Total plasma protein was measured according to the Biuret method [17]. Erythrocyte hemoglobin concentration was measured using Drapkin's reagent method [18].

Statistical analysis of the data was done using Student's t-test, and P-values of less than 0.05 were considered significant.

Table (1) showed a significant elevation in lead levels in blood of exposed workers (360%) compared to controls, produced 12% decrease in lead levels, which is a significant value compared with pre-treatment levels.

The results of the study indicates that the base line erythrocytes and plasma MDA levels were elevated by 150% and 117% respectively compared to that of controls (Table 1). MDA levels in both compartment decrease after treatment with a combination of antioxidant vitamins (1000 mg/day ascorbic acid and 200 mg/day (α-tocopherol) for one month, which was significant, compared to the pretreatment values (Table 1). The response of erythrocytes and plasma to in-vitro hydrogen peroxide challenge showed that, MDA production in both compartments of the exposed worker's blood were significantly higher, compared to that of controls. Treatment with antioxidants as indicated before, significantly increase the

resistance of erythrocytes and plasma of lead-exposed subjects to the hydrogen peroxide-induced lipid peroxidation, reflected by a significant decrease in the MDA production after antioxidant treatment, compared to pre-treatment levels (Figures I and II).

Table (1): Blood lead levels, erythrocytes and plasma MDA levels in lead-exposed workers pre- and post-treatments with vit. C and vit. E

Parameters	Control n =11	Lead exposure workers	
		Before treatment n=30	After treatment n=30
Blood lead µg/dl	10 ± 0.64	46.4 ±2.07*	41.03 ± 1.96* ¥
Erythrocyte MDA nmole/gm Hb	7.7 ± 0.29	19.81 ± 20*	9.07 ± 0.58* ¥
Plasma MDA nmole/L	0.97 ± 0.08	2.11 ±0.08*	1.00 ± 0.06¥

Each value represents mean ± SE
*** Significantly different from control (P<0.05).**
¥ Significantly different with respect to pre-treatment (P<0.05).

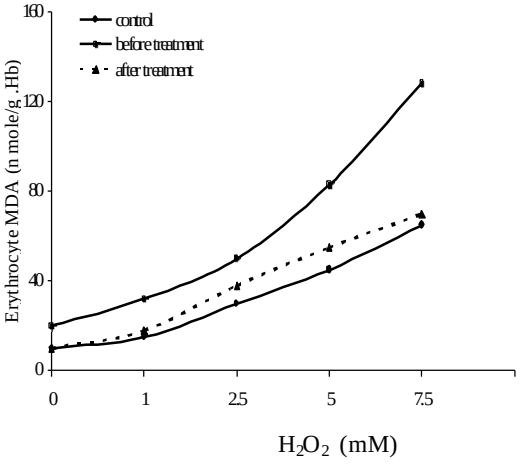

Fig.1Erythrocyte MDA of Control Subjects and lead – exposed Workers Befor and After Treatment with Vitamins (C&E)in response to in vitro Challenge with Various H_2O_2 Concentration

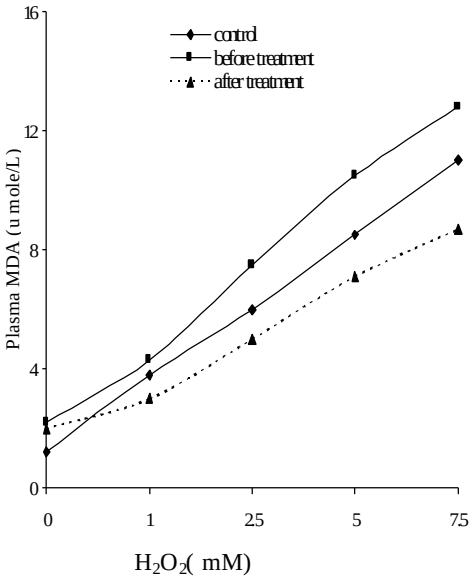

Fig.2 Plasma MDA of Control Subjects and lead – exposed Workers Befor and After Treatment with Vitamins (C&E)in response to in vitro Challenge with Various H_2O_2 Concentration

In lead-exposed workers, there was 28% and 56% depletion in erythrocytes and plasma glutathione (GSH) levels respectively, observed before antioxidant treatment, and compared to controls (Table 2). After treatment with antioxidants for one month, there were a significant increase in GSH levels in both compartments (41% and 120% respectively) compared to pretreatment levels (Table 1). Total plasma protein and albumin, the general antioxidants in the body, were found to be affected due to lead exposure, and their levels in lead workers were significantly decreased (13% and 23% respectively) compared to controls. Antioxidant treatment produced a significant elevation on plasma albumin only after one-month duration of treatment (Table 3).

Table (2): Erythrocytes and plasma glutathione (GSH) levels in lead-exposed workers pre- and post-treatment with vit. C and vit. E.

Parameters	Control n =11	Lead exposure workers	
		Before treat - ment n=30	After treat - ment n=30
Erythrocyte GSH µmole/gm Hb	11.92 ± 0.36	8.62 ± 0.4*	12.17 ± 0.37* ¥
Plasma GSH µmole/L	0.88 ± 0.15	0.39 ± 0.05*	0.86 ± 0.12¥

Each value represents mean ± SE
*** Significantly different from control (P<0.05).**
¥ Significantly different with respect to pre-treatment (P<0.05).

Table (3): Plasma total protein and albumin levels in lead-exposed workers pre- and post-treatment with vit. C and vit. E.

Parameters	Control n =11	Lead exposure workers	
		Before treat-ment	After treat-ment
Total plasma protein gm/dl	7.51 ± 0.11	6.56 ± 0.13*	6.6 ± 0.12*
Plasma albumin gm/dl	5.06 ± 0.1	3.88 ± 0.05*	4.29 ± 0.11¥ *

Each value represents mean ± SE
*** Significantly different from control (P<0.05).**
¥ Significantly different with respect to pre-treatment (P<0.05).

DISCUSSION

Free radicals activity has been implicated in the pathogenesis of a variety of human diseases and the analysis of our data showed that, oxidative stress was quite clear in lead exposed workers (Table 1, Figure I and II), as noticed by increased erythrocytes and plasma MDA levels, which is in agreement with other studies [19, 20].

The mechanisms by which lead causes it's deleterious effects has yet to be elucidated, however, part of lead's effect may be due to the accumulation of delta-aminolevulinic acid dehydratase (ALAD), an enzyme in the heme synthesis pathway, which catalyzes the condensation of two molecules of δ-ALA to porphobilinogen[21] .At a pH range of 7.0-8.0 δ-ALA enolizes, and the resulted enol undergo autooxidation resulting in the formation of superoxide and hydroxyl radicals. ALA has also been shown to undergoes iron-catalyzed oxidation with ROS generation, and to induce Ca^{+2} release from mitochondria through oxidative damage to inner membrane[22].

The effect of antioxidant vitamins (ascorbic acid and tocopherol) on lipid peroxidation parameter, MDA, as shown in table (1) and figure I and II, suggested that they did produce a decrease in the basal MDA levels and the susceptibility of both, erythrocytes and plasma to the oxidative stress induced in-vitro by H2O2. The antioxidant treatment lead to increase in erythrocytes and plasma GSH levels (Table 2), which may be due to a direct scavenging activities of the generated ROS, and decreasing utilization and damage of GSH, or indirect through the improvement of the oxidant/antioxidant balance in the cells after treatment [23].

In normal conditions, as well as during oxidative stress (lead exposure) a daily dose of ascorbic acid and α-tocopherol, appear to be protect the oxido-reductive state of red blood cells, by modulating the extent of lipid peroxidation, as well as the activities of the antioxidant enzymes [24].

In this study, daily supplementation with a combination of ascorbic acid and α-tocopherol, resulted in a significant decrease in lead levels in the blood after one month (Table 1), and this may provide an economic and convenient method of reducing blood lead levels, possibly by decreasing intestinal absorption of lead [25], or it may increase the renal excretion of this metal.

Albumin is known to act as an effective antioxidant, due to its ability to bind the catalytic copper ions [26], free fatty acid, and hypochlorus acid (HOCl), and also showed a significant capability to destroy H2O2 in the presence of reduced glutathione [27].

The present study clearly demonstrated the relationship between increased lipid peroxidation, and the lowering of both plasma total proteins and albumin in lead-exposed workers (Table 3), which may be attributed to the structural modification, which may lead eventually to impair the antioxidant properties of albumin, and even may act to induce oxidative stress, through it's action as a prooxidant in presence of catalytic ions [28].

Antioxidants treatment resulted in significant elevation in albumin levels (Table 3), which may be due to their direct scavenging activity, or protection of albumin against ROS-induced damage.

In conclusion, results of this study suggested that the beneficial effects of antioxidants on the oxidative stress parameters are not only related to their ability to remove lead from target cells, but also associated with antioxidant potential for bolstering thiol antioxidant capacity, and this makes these vitamins a good candidate for therapeutic intervention in lead poisoning.

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