Association of Blood Lead level with elevated Blood Pressure in Hypertensive Patients

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Abstract:

Background: Lead is a metal with many important industrial uses. The relationship between lead exposure and the rise of blood pressure has received a great deal of attention as it was implicated that the mortality from cardiovascular diseases might be reduced by lowering lead levels in the environment**.**

Objectives: The study was to investigate the correlation between the blood lead (B-Pb) levels and the values of blood pressure in hypertensive patients. Moreover, the plasma activities of angiotensin converting enzyme (ACE), plasma levels of nitric oxide (NO), total antioxidants (TAOX) and malondialdehyde (MDA) were estimated to investigate the correlations between the measured parameters and B-Pb levels in hypertensive patients.

Methods: Fifty-five hypertensive patients were compared with fifty-three age and sex matched control group. The B-Pb levels were detected by flame atomic absorption spectrometry. The plasma levels of ACE activities, NO, TAOX and MDA were measured by colorimetric methods.

Results: In the hypertensive patients, B-Pb levels were significantly higher than controls. Concomitantly, the plasma levels of ACE activities and MDA were significantly increased while the plasma levels of NO and TAOX were significantly reduced in the hypertensive patients in comparison with controls. There were significant positive correlations between B-Pb and each of MDA, and systolic as well as diastolic blood pressure. Conversely, a significant negative correlation was found between B-Pb and NO.

Conclusions: Our study indicated that a positive relationship exists between blood pressure and B-Pb levels. The increased B-Pb levels were associated with oxidative stress. Moreover, The B-Pb level was negatively correlated with NO and this may clarify the implication of Pb as leading risk factor for the cardiovascular diseases and hypertension. These findings provide support for continued efforts to reduce lead concentration in the population at Qassim region.

Key words: Hypertension, B-Pb, NO, ACE, TAOX, MDA.

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Introduction

Lead is a common environmental and industrial pollutant with no beneficial biological role. It has been detected in all phases of environment and biological system. The persistence of lead in the blood of animals and humans and the associated health risk is a topic of current debate and concern. [1-2] Moreover, rapid industrialization and the continued use of leaded gasoline appear to be increasing lead exposure throughout the developing world. [3]

Considerable attention has been paid to the possibility that low level lead exposure among adults in the general population can elevate blood pressure and increase the risk for cardiovascular diseases' morbidity and mortality. [4-6] Evidence for this association from the epidemiological literatures is compelling [7] but the exact causal nature of the relationship remains controversial.

The notion that lead exposure may influence blood pressure in humans is biologically plausible. Hu et al. [8] reported significantly higher levels of lead in skeletal and blood compartments among men with hypertension. Previously, some investigators reported that lead induces hypertension in rats. [9-10] Other animal data suggested that lead acts at multiple sites within the cardiovascular system, including direct effects on the excitability and contractility of the heart, alteration of the compliance of the vascular smooth muscle tissue, and direct action on parts of the central nervous system responsible for blood pressure regulation. [4] Evidence in animals also suggested that lead may affect blood pressure through the renin-angiotensin system. [9]

Many studies have provided new insights into the mechanisms by which metals can influence vascular function. Basal vascular tone impairment has been suggested as a possible mechanism of lead-induced hypertension. Thus, studies described by Khalil- Manesh et al. [11] suggested that low-concentration of lead exposure produced an imbalance in endothelial derived vasoconstrictor and vasodilator factors. Moreover, the increased vasoconstriction was also accompanied by a rise in plasma levels of endothelin-3 and a decrease in plasma and urinary cyclic guanosine monophosphate. [11] Moreover, Sanchez-Mendoza et al., [12] have demonstrated in hypertensive rats that, decreased vasodilator tone is associated with increased vasoconstrictor response and that decreased NO production was responsible for this decreased vascular tone.

Oxidative stress is a condition of oxidant / antioxidant disequilibrium, in which increased reactive oxygen species (ROS) generate overwhelms antioxidant defence mechanisms that lead to oxidative damage of cellular molecules. Of these, lipids are the most sensitive in that polyunsaturated fatty acids in cell membrane react with free radicals to form peroxidation products. Malondialdehyde (MDA) is the end product and best-known indicator of lipid peroxidation. To deal with harmful effects of ROS, a network of antioxidant defence mechanisms exist. Insufficient antioxidant protection or excess production of ROS can result in oxidative stress. [13]

Farmand et al., [14] mentioned that lead-induced hypertension is related to an over-production of ROS. ROS may increase blood pressure directly or indirectly by increasing the concentration of calcium in endothelial cells or inactivating NO. The release of ROS increases the arterial blood pressure and induces atherosclerotic changes in blood vessels. In consequence, it may lead to arterial hypertension and coronary heart disease. [15-16]

Although the evidence in support of a role of lead-induced hypertension is strong, it is not conclusive, nor is the mechanisms by which lead may act on the vascular system clear. Lead exposure may be more of a risk factor for certain ''susceptible populations'', and thus may not be apparent in the general population. Inasmuch as hypertension remains a significant risk factor for other forms of cardiovascular disease, the contribution of potential environmental lead exposure to this risk is an important public health concern. [2]

According to this background, the current study was conducted to determine the blood lead (B-Pb) levels in hypertensive patients and to investigate the correlation between B-Pb levels and the values of blood pressure in hypertensive patients. Moreover, the plasma activities of angiotensin converting enzyme (ACE), plasma levels of nitric oxide (NO), total antioxidants (TAOX) and malondialdehyde (MDA) were measured to investigate the correlations between these parameters and B-Pb levels.

Patients and Methods

Patients and Controls. In the present study, 55 consecutive hypertensive patients were recruited, during their routine check-up in the OPD clinic, Qassim University, College of Medicine, during January – March, 2010. After approval by the ethics committee of the College of Medicine, Qassim University, Saudia Arabia, an informed consent was obtained from each subject enrolled in the study. The patients were males and their ages ranged from 24 - 59 years. Fifty-three apparently healthy volunteer normotensive controls were matched hypertensive patients in age, sex and body mass index (BMI) were used as controls.

Each patient was subjected to full complete medical history including duration of the disease and details of therapy. Before inclusion, all participants underwent careful physical examination and detailed laboratory investigations to exclude any conditions that may interfere with the disease. Full clinical examination including fundus examination and routine investigations (ECG, ultrasonography, chest X-ray, and complete urine analysis) were done. Also, the BMI (weight, Kg/(height)^2, m^2) was calculated. All patients were examined for any complications. Exclusion criteria included smoking, history of recent acute illness, clinical evidence suggestive of any liver or kidney diseases were kept in consideration.

Blood Pressure and Hypertension. We used the mean of 3 systolic and diastolic blood pressure measurements, all of which were taken by a physician in the Clinical Unit of Qassim University. Patients were categorized as hypertensive if any of the following criteria were met: current user of blood pressure medication (self-report), a systolic blood pressure of 140 mm Hg or higher, or a diastolic blood pressure of 90 mm Hg or higher. [17] The patients did not receive ACE inhibitors within the week preceding the study.

Biochemical analysis

The following biochemical indices were measured for all patients and controls: B-Pb levels and plasma levels of NO, MDA, TAOX and ACE activities. Blood sample (10 mL) was collected in two heparinized tubes from each participant. The first tube was centrifuged at 5000 rpm for 10 min for plasma separation. The plasma sample was divided into aliquots and kept at -70^{o}C until biochemical analyses. The second tube contained whole blood that was used for B-Pb determination.

Lead analysis in the whole blood. The lead levels in the blood were determined by employing flame atomic absorption spectrometry according to previously reported method. [18] Calibration curve was constructed by adding known amounts of lead standard (E. Merck). Analysis of diluted samples of blood was injected into the atomic absorption spectrophotometer (Perkin-Elmer Model 400, Shelton, CT, USA). Hollow cathode lamp of Pb was used at wavelength of 283.3 nm. The results of B-Pb levels were expressed as μg/dl.

Determination of plasma total antioxidant. The plasma levels of TAOX capacity were determined according to Koracevic et al., [19] using commercial kit.

Determination of plasma MDA. The plasma level of *MDA* was determined using 1,1,3,3-tetraehoxypropane as malondialdehyde standard and mixing equal volumes of the coloring reagent (0.375% thiobarbituric acid, w/v, in 15 % trichloroacetic acid + 0.25% N HCl) and samples or standards and 10 μL of butylated hydroxyltoluene (0.8, w/v, in hexane), incubated at 100 ^{o}C for 15 min, cooled and centrifuged at 3000 rpm for 10 min. MDA and thiobarbituric acid react to form a pink color. Absorbance at 535 nm of the supernatant was recorded against reagent blank and concentration in μM was calculated from standard curve (0.25 - 20 μM). [20]

Determination of plasma nitric oxide analysis. The *plasma* levels of NO was determined as total nitrite after deproteinization with $ZnSO_4$ (30%), and the color developed by the reaction with Griess reagent (1% sulfanilamide/ 0.1% naphthyelthylene diamine diHCL, w/v in 2.5% H_3PO_4) was recorded at 550nm against reagent blank using sodium nitrite 10-100 μM as standard. [21]

Determination of plasma ACE activities. The ACE activities were determined by using furylacrylyl phenylalanyl glycylglycine (FAPGG) as substrate as described previously. [22]

Statistical analysis

The statistical analysis was performed using Prism Statistical Package version 5.0 (Graphpad, San Diego, CA, USA). Data comparisons were performed by using student *t*-test and ANOVA with Bonferroni's post

multiple comparisons test and the correlations among the clinical and biochemical parameters were performed using Spearman's rank correlation coefficient. The levels of significance were accepted with $P < 0.05$ and the results were presented in Tables as mean ± SEM.

Results

The clinical criteria of patients and controls are summarized in Table (1). Age and BMI did not show significant differences among the comparable groups.

The B-Pb levels and the plasma levels of bioindices of hypertensive patients comparing with controls are shown in Table (2) and Figs. (1-2). The B-Pb levels were significantly higher in hypertensive patients than controls. The plasma ACE activities and the plasma levels of MDA were significantly increased in the hypertensive patients in comparison with controls. Whereas, the plasma levels of NO and TAOX were significantly lower in hypertensive patients than control group.

The correlations between the B-Pb levels and clinical and biochemical indices in hypertensive patients are shown in Table (3). There were significant positive correlations between B-Pb and each of MDA, systolic and diastolic blood pressure. Conversely, a significant negative correlation was found between B-Pb and NO. No significant correlations were found between the B-Pb levels and age, TAOX, ACE and BMI in hypertensive patients.

Discussion

There is considerable public health interest in the possible toxic effects of environmental lead exposure on the cardiovascular system, with additional concern regarding the role of chronic low-level lead exposures in the pathogenesis of hypertension, as a leading risk factor for cardiovascular morbidity and mortality. [7; 2]

Vupputuri et al., [23] suggested that increased levels of B-Pb remain an important environmental risk factor for elevated blood pressure. Many studies [24-25] but not all [26] have indicated that environmental exposure to lead is associated with an increased risk of hypertension. Harlan [24] reported that both environmental and occupational exposure to lead has long been associated with hypertension. Although the cardiovascular system is not typically viewed as a primary target of lead toxicity, high concentrations of lead, such as might occur during occupational exposure, are toxic to both the heart and vascular smooth muscles. [2] Some reports suggested that, even transient exposure to lead during childhood can have a long-term and delayed hypertensive effect. [27] Studies in experimental animals also supported a correlation between low-level exposure to lead and development of hypertension. [11; 28] Martin et al., [29] suggested that, lead has an acute effect on blood pressure and a chronic effect on hypertension risk due to cumulative doses.

In the present study, the B-Pb levels were significantly higher in hypertensive patients than controls and positively correlated with the increase of systolic and diastolic blood pressure level. In this regard, Dursun et al., [30] found a significant elevation of the mean systolic and diastolic blood pressures in the lead-treated rats when compared to healthy controls. Robles et al., [31] confirmed the hypertensive effect of lead exposure in rats. Vupputuri et al. [23] also reported significant associations between blood lead level and high systolic and diastolic blood pressures in black men. Nash et al., [17] found that, B-Pb level is positively associated with both systolic and diastolic blood pressure in postmenopausal women. Recently, Kasperczyk et al., [32] found that, B-Pb levels were positively correlated with high arterial blood pressure (both systolic and diastolic). Moreover, Afridi et al., [33] reported higher levels of B-Pb as well as a lower level of Zn, correlated well with the consequences of hypertension. Conversely, Staessen et al., [34] did not find a significant relation between B-Pb and hypertension.

Age and BMI are very strong factors in the genesis of hypertension but they seem to be independent of lead effect. Kasperczyk et al.,[32] did not find a correlation between B-Pb and BMI or age. Our study showed similar finding. Harlan, [24] found that, BMI has an inverse relation to B-Pb in the US population. deQueiroz et al.,[35] found that, BMI is significantly correlated with the increase in blood pressure levels.

Lead is reported to cause enhanced sympathetic nerve activity with increases in circulating epinephrine and norepinephrine levels in conjunction with decreased density of

vasodilating beta 2 adrenergic receptors. [36] Chronic low-level exposure to lead results in increased activity of angiotensin converting enzyme and increase in plasma renin, angiotensin II and aldosterone levels. [37] Plasma kininase I and II levels are higher during Pb exposure. This can lead to decreases in plasma bradykinin levels resulting in a reduction in endothelial NO production. [38]

There is very strong evidence that lead decreases the functional availability of the potent vasodilator NO, most likely through direct or indirect mechanisms involving oxidative stress. [37] In the current study, the plasma levels of NO were significantly lower in hypertensive patients than controls. The B-Pb levels were negatively correlated with NO levels in hypertensive patients. In this regard, Dursun et al., [30] showed that, exposure to low lead results in a marked decrease of plasma NO levels. Robles et al., [31] demonstrated that, lead treatment was associated with increased ROS production and inactivation of NO in the lead-induced hypertensive animals. Thus, high ROS levels after lead-exposure may increase the presence of superoxide anion, raising the probabilities of an interaction between NO and ROS to produce a peroxynitrite, highly deleterious molecule, which has been implicated in tissue injury. It is known to initiate lipid peroxidation and might lead to irreversible tissue damage, and impaired vascular function. [39] Taken together, the data suggest that upon lead exposure, a higher production of ROS is generated, inactivating NO.

Many reports have suggested that lead-induced hypertension is related to an over production of free radicals and/or a decrease of the antioxidative mechanisms in the body. [28;14] The release of ROS increases the arterial blood pressure and induces atherosclerotic changes in blood vessels. In consequence, it may lead to arterial hypertension and coronary heart disease. [15-16]

In the current study, plasma levels of TAOX were significantly lower but plasma levels of MDA were significantly higher in hypertensive patients than controls. The B-Pb concentrations were positively correlated with MDA levels in hypertensive patients. In this regard, many studies [40-41] have proven that the exposure of human to lead compounds results in increased peroxidation of lipids in blood, due to changes in the activity of antioxidant enzymes. Moreover, Kasperczyk et al., [32] found a positive correlation between B-Pb and MDA among human exposed to lead. Conversely, Dursun et al., [30] showed that, lead administration results in hypertension and a nonsignificant rise in plasma concentration of lipid peroxides.

Previous reports have shown that lead-induced hypertension could be related to an increase in ROS. [15-16] In addition, it is widely accepted that, oxidative stress may contribute to the generation of hypertension and endothelial dysfunction via the inactivation of NO. [28] Robles et al., [31] demonstrated that rats exposed to lead had a higher production of ROS, such that coadministration of lead with antioxidant treatment was able to prevent the oxidative stress and the hypertensive effect of lead treatment. As demonstrated by experiments in which the treatment with antioxidants, such Vitamins E [28] and C [42] led to a reversal of hypertension.

Renin-angiotensin system is a critical pathway for blood pressure control and kidney function. Angiotensin II (Ang II), considered as the main effector molecule of the renin-angiotensin system, is a potent vasoconstrictor produced by the ACE. [43]

In the current study, plasma activities of ACE were significantly higher in hypertensive patients than controls. Lead exposure has been reported to increase ACE activity in the plasma in the early phase of lead exposure. [44] This suggests that, Ang II may be an important mechanism of lead-induced hypertension. Indeed, previous studies have suggested that, acute or chronic lead exposure results in elevation of plasma renin activity, [45] probably, by interfering with a calcium dependent mechanism of renin secretion. [46] Thus, Robles et al., [31] suggested that, increased blood pressure in lead treated animals may be associated with an increased vasoconstriction tone, due to increased synthesis or sensitivity to vasoconstrictor agonists as Ang II, and/or to decreased vasodilatory effect due to either decreased synthesis or increased catabolism of NO. This process is associated with increased ROS.

Conclusion

The blood lead level is significantly and positively correlated with blood pressure

among hypertensive patients. Lead exposure may increase blood pressure through NO-dependent and independent mechanisms that increasing vascular sensitivity to vasoconstrictors thereby inducing hypertension and accelerating the development of hypertensive complications. Further biochemical and epidemiological studies are needed, especially in the field of metal exposure-induced hypertension, to determine the causative relationship, pathological changes, their clinical significance and the underlying mechanisms.

Table (1): Clinical characteristics in hypertensive patients compared with controls.

Parameters	**Controls (n=53) Mean ± SEM**	**Hypertensive Patients (n=55) Mean ± SEM**	**P-Value**
Age (years)	39.92 ± 1.209	43.16 ± 1.292	P<0.07 NS
BMI (kg/m²)	28.53 ± 0.541	29.87 ± 0.509	P<0.07 NS
Blood pressure Systolic (mmHg) Diastolic (mmHg)	 114.30 ± 0.831 80.26 ± 0.505	 137.60 ± 2.045 90.29 ± 1.063	 P<0.0001 P<0.0001

BMI, Body mass index

Table (2): Blood lead and plasma levels of bio-indices in hypertensive patients compared with controls.

Parameters	**Controls (n=53) Mean ± SEM**	**Hypertensive Patients (n=55) Mean ± SEM**	**P-Value**
B-Pb (μg/dL)	1.85 ± 0.132	2.21 ± 0.125	P<0.05
NO (nmol/ml)	27.87 ± 2.443	18.67 ± 0.923	P<0.001
ACE (U/L)	47.82 ± 3.588	71.05 ± 3.258	P<0.001
TAOX (mmol/ L)	1.189 ± 0.017	1.073 ± 0.036	P<0.01
MDA (μmol/L)	1.74 ± 0.036	2.16 ± 0.049	P<0.001

B-Pb, blood lead; NO, nitric oxide; ACE, angiotensin converting enzyme; TAOX, total antioxidant capacity; MDA, malondialdehyde.

Table (3): Correlations of B-Pb levels with age, BMI, systolic BP, diastolic BP, NO, ACE, TAOX and MDA in hypertensive patient.

Variables	Correlation Coefficient (r)	P-value
B-Pb vs		
ACE	+0.23	NS
NO	-0.46	P<0.01 *
TAOX	-0.30	NS
MDA	+0.51	P<0.05*
Age	+0.11	NS
BMI	+0.14	NS
Systolic BP	+0.41	P<0.01 *
Diastolic BP	+0.39	P<0.0 5*

*, significant; B-Pb, blood lead; BP, blood pressure; NO, nitric oxide; ACE, angiotensin converting enzyme; TAOX, total antioxidant capacity; MDA, malondialdehyde; BMI, body mass index.

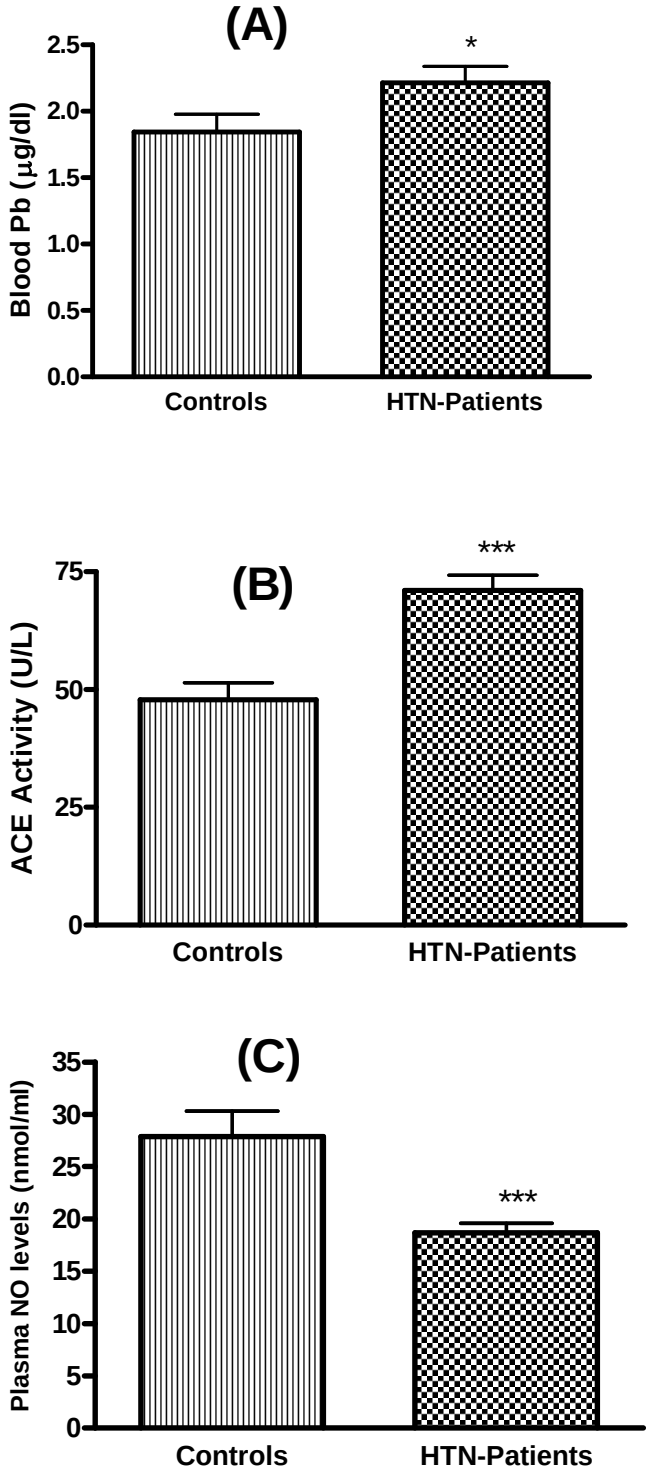

Fig. (1): The blood lead (A), angiotensin converting enzyme activity (B) and plasma level of nitric oxide (C) in hypertensive (HTN) subjects in comparison with controls. Data are mean ± SEM. ***, P<0.001; and *,P<0.05.

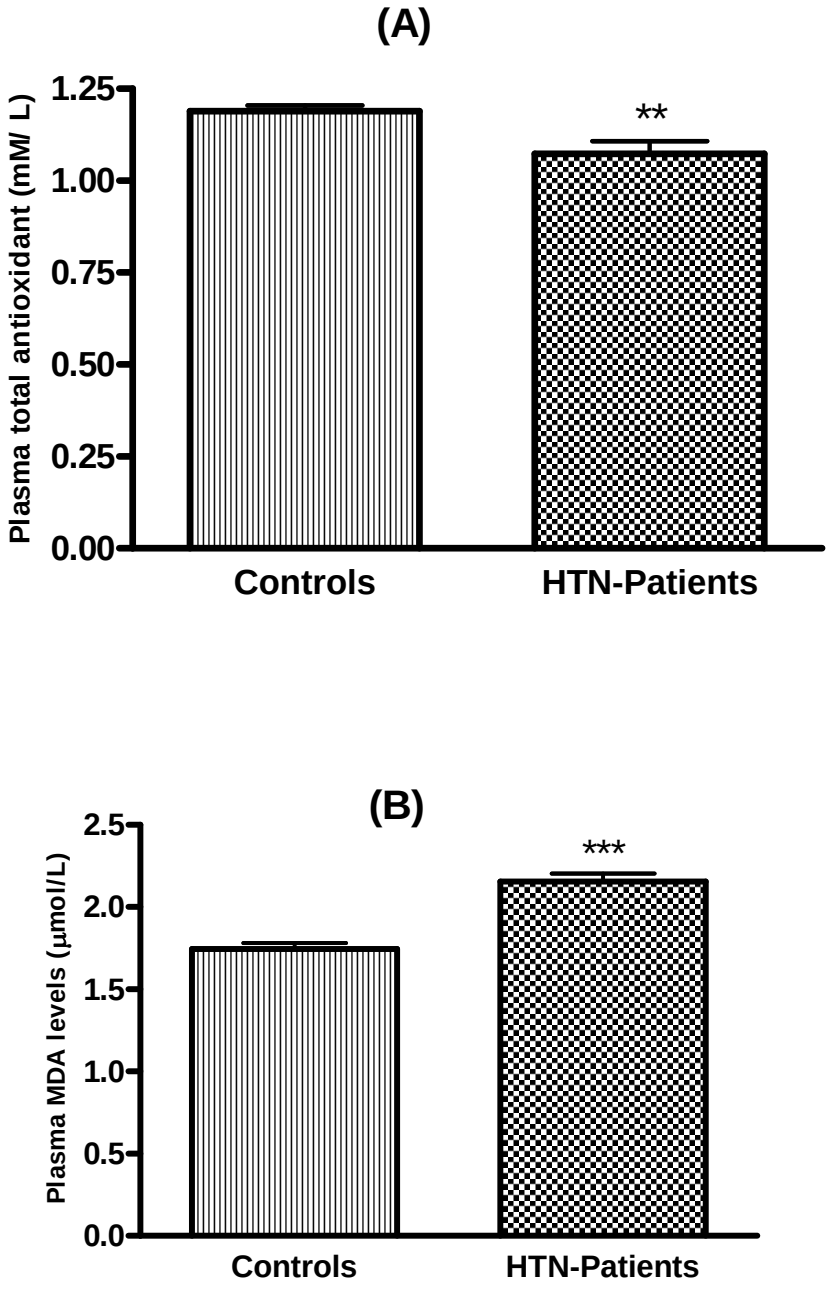

Fig. (2): The plasma levels of total antioxidant (A) and MDA (B) in hypertensive (HTN) subjects in comparison with controls. Data are mean ± SEM. ***, P<0.001; and **,P<0.01.

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