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Serum Heavy Metals, Oxidized Low-Density Lipoproteins and Antioxidant Vitamins Levels as Risk Factors for Coronary Artery Diseases in Hypertensive Patients

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Abstract: ***Background: Hypertension (HTN) is a significant risk factor for various cardiovascular diseases (CVDs), including heart failure, stroke, end-organ damage, and atherosclerosis. Oxidized-low-density lipoprotein (ox-LDL) absorbed by artery macrophages is a significant factor in the development of atherosclerosis. This study aims to evaluate the levels of serum lipid profile (cholesterol, triacylglycerol, LDL, and high-density lipoprotein (HDL), ox-LDL, heavy metals, and malondialdehyde (MDA) as a crucial technique for the diagnosis of coronary artery diseases (CAD). Methods: The study involved 90 participants categorized into three groups (n= 30) as follows: negative control (NTC), hypertensive patients (HTN), and cardiac patients (HTN and CAD). Blood samples were taken to perform the previous assessments. Results: The serum levels of HDL in groups two and three were significantly decreased (p<0.05), while total triacylglycerol, cholesterol, and LDL were significantly elevated (p<0.05) compared with the control. Also, the results were significant increased (p<0.05) for the serum cobalt, chromium, copper, lead, and the ox-LDL levels in HTN and HTN with CAD, compared to the control. Besides, the MDA level showed significant elevation in the HTN group. The positive correlation between oxidative stress (OS) and atherosclerosis and the association between heavy metals contamination and CVD were (r=0.6) and (r=0.055). Conclusions: This study focuses on the heavy metal-induced toxicity and OS in hypertensive and cardiac patients; however, further studies are essential for a deeper understanding of the underlying molecular mechanism.***

Keywords: Heavy metals, Hypertension, Oxidative stress

Abbreviations

CAD, coronary artery diseases; CVDs, cardiovascular diseases; eNOS, Endothelial NOS; HDL, high-density lipoprotein; MDA, malondialdehyde; HDL-c, high-density lipoprotein cholesterol; HHD, hypertensive heart disease; HTN, Hypertension; HTN, hypertensive patients; LDL-c, low-density lipoprotein cholesterol; NO, Nitric oxide; NTC, negative control; OS, oxidative stress; ox-LDL, Oxidized-low-density lipoprotein; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substance

1. Introduction

Arteries deliver blood from the heart to all body regions, and blood pressure is the force exerted by the blood against the artery walls (1). Blood pressure is divided into two types: systolic and diastolic. HTN is a prevalent health issue where blood pressure increases above normal (2). In 2011, the World Health Organization (WHO) estimated that HTN might cause 7.5 million (12.8%) deaths. HTN represents a substantial risk factor for cardiovascular diseases and stroke, besides being a significant cause of mortality and morbidity. Moreover, heart issues occurring due to HTN are defined as hypertensive heart disease (HHD). These issues include CAD, Heart failure, and cardiac hypertrophy (3).

Blood pressure, serum cholesterol, and smoking are the common risk factors that account for more than 50% of the atherosclerosis death rate (4). Furthermore, oxidative free radicals have a role in the development of atherosclerosis (5). Oxidative damage increases the peroxidation of LDL, thus increasing its engulfment by the phagocytic cell, leading to the formation of foam cells, and increasing CAD, though other mechanisms may exist. Different water or lipid-soluble antioxidants have diverse mechanisms of action. Several lipid-soluble compounds, including beta-carotene, lipophilic molecules, and vitamin E, are integrated into LDL particles, preventing LDL peroxidation and CAD (6).

Heavy metal toxicity is a less recognized factor among the known causes of high blood pressure and CAD. The

poisoning of water resources with heavy metals is a severe issue to the living system, particularly in the urban areas (7). Toxic heavy metals include cadmium, zinc, uranium, nickel, mercury, lead, chromium, silver, arsenic, selenium, gold (8). Under certain environmental conditions, some crucial elements like lead, mercury, arsenic, cadmium, and chromium can accumulate to toxic levels causing ecological damage (9). Moreover, other metals such as nickel, zinc, copper, and cobalt are not as toxic; however, their increased level may cause less serious issues (10).

OS is a condition with excessive production of cells' reactive oxygen species (ROS) and an impaired antioxidant function, resulting in a deleterious imbalance and cell damage (11). Vascular OS precedes HTN progression, contributing to increased vascular resistance and endothelial dysfunction by uncoupling Endothelial NOS (eNOS) and increasing Nitric oxide (NO) scavenging (12).

The toxicity of heavy metals results in creating free radicals contributing to HTN and CAD. This study has evaluated the lipid profile, serum heavy metals, and ox-LDL level and their significance for early diagnosis of CAD in hypertensive patients (13).

2. Materials and Methods

Participants

The study was performed on Saudi participants, and written informed consent was obtained from all participants. The study protocol has been approved by the Ethics Committee of the King Abdulaziz University (Ref. No.356-14). Questionnaires covering previous and current disease status, lifestyle factors, diet, age, sex, and medication were completed by the participants at the time of sample collection. An age-and sex-matched strategy were not considered in the selection criteria. The study included 90 participants with ages ranging from 20-70 years, who were categorized into three groups (n= 30) as follows: the negative control (NTC), the hypertensive patients (HTN), and the cardiac patients (HTN + CVD). HTN is diagnosed when blood pressures >140/90 mmHg, according to The World Health Organization. After fasting for 12 hours, venous blood samples (5ml) were collected from all participants. The serum was obtained by centrifuging the blood samples at 3000 rpm for 5 min.

Biochemical analysis

Determination of Lipid Profile

Serum triglyceride, low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) were evaluated by Medica'sEasyRA®, a fully automated clinical chemistry analyzer using three kits (Cholesterol, Triglycerides, HDL-cholesterol) from Medica Corporation, Bedford, MA 01730, USA. The serum level of LDL-C was determined by the protocol of (14) utilizing the following equation:

LDL-c = TC-TG/5 + HDL-c

where, TC is total cholesterol, and TG is triglyceride. The value was demonstrated in mg/dl.

Measuring serum Heavy Metals

The analysis of blood samples was conducted by ICP-AES (ICPE-9000, Shimadzu, Japan) equipped with ASC-6100 autosampler. A multi-element AccutraceTM reference standard for ICP (1000 mg/L, Accu Standard, USA) was utilized to determine cadmium, cobalt, chromium, copper, and lead levels.

Determination of serum ox-LDL concentration

An OxiSelect™ Human ox-LDL ELISA kit (MDA-LDL Quantitation) by Cell Biolabs, Inc. was utilized to measure ox-LDL concentrations. The optical density (OD) was determined at OD 450 nm via a microplate reader.

Determination of serum MDA using a thiobarbituric acid reactive substance (TBARS)

The lipid peroxides were assayed through a thiobarbituric acid reactive substance (TBARS) per the procedure of (13). In the TBARS assay, one molecule of MDA interacts with two molecules of thiobarbituric acid, resulting in the production of pink pigment with a maximum absorbance at 532 nm under acidic conditions.

Statistical Analysis

Data were demonstrated as Means of outcome measurements and Standard Error (SE) of the means in the bars of each group. ANOVA test was used to determine the statistically significant differences between variables ($P < 0.05$) using version 15 for the Windows software package.

3. Results

Blood lipid profile test

Compared to the negative control, significantly ($\boldsymbol{p} < 0.001$) high cholesterol, triglycerides, and LDL levels were observed in the hypertensive and cardiac patients, as shown in Figure 1. However, no significant difference ($p = 0.056$) in the triglycerides, cholesterol, and LDL level was noticed between hypertensive and cardiac patients. Unlikely, the HDL level was significantly ($\boldsymbol{p} < 0.001$) higher in the negative control, as demonstrated in Figure 1. Nevertheless, an insignificant difference ($\boldsymbol{p} = 0.080$) in the HDL level was observed between hypertensive and cardiac patients, as displayed in Figure1. The value of different lipids is presented in Table I.

Heavy Metals analysis using Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES)

Heavy metals including cobalt, chromium, copper, lead, and cadmium were studied in the different groups of subjects. The levels of cobalt, chromium, and lead were significantly higher in hypertensive patients and cardiac patients, as demonstrated in Figure 2. intriguingly, the

cadmium level was almost identical in all the studied groups, as shown in Figure 2. ICP analysis of heavy metals (Cobalt, Lead, Chromium, Cadmium, and Copper) was demonstrated in Table II.

Serum ox-LDL and MDA Concentration

The ox-LDL and MDA levels were demonstrated in Tables III and IV. The ox-LDL level was significant in the cardiac and hypertensive patients, compared to the control group, as demonstrated in Figure 3. Moreover, a significant elevation in the MDA level in hypertensive patients was noticed, but no difference was observed between the cardiac patients and the control group, as demonstrated in Figure 4. However, an insignificant difference in the ox-LDL and MDA levels was observed between the hypertensive and cardiac patients, as shown in Figure 3, 4.

4. Discussion

CAD is a complex disease with numerous risk factors, including dietary, genetic, behavioral, and environmental factors. Moreover, it can be caused by HTN, dyslipidemia, diabetes, obesity, Mellitus, high-fat, physical inactivity, a high-cholesterol diet, and cigarette smoking (14).

Recently, HTN represented the leading cause of mortality globally, particularly in developed countries (15). HTN is a prevalent modifiable risk factor for cardiovascular disease (18). It is strongly associated with an increased risk of cardiovascular diseases (stroke, kidney illness, and myocardial infarction) and mortality. It is diagnosed when the average of two or more diastolic or systolic blood pressure measurements taken over at least two visits are 90 mmHg or 140 mmHg. Essential HTN is characterized by elevated blood pressure without secondary reasons such as renal failure, renovascular disease, aldosteronism, and pheochromocytoma (19). It represents 95% of HTN. Factors like aging and obesity have been shown to cause HTN (18).

Our results reported elevated TG, cholesterol, and LDL levels, but low HDL was observed in hypertensive and cardiac patients. These results were in line with many previous findings that reported significantly high cholesterol, triglyceride, and LDL levels but low HDL levels in hypertensive and cardiac patients (15, 19).

The potential correlation between cardiovascular disease and chronic heavy metal exposure has not been determined well. Although the mechanism by which heavy metals increase cardiovascular risk factors is uncertain, decreased antioxidant metabolism and high OS might have a significant role (22). In this study, heavy metals like copper, cadmium, cobalt, chromium, and lead were tested from hypertensive and cardiac patients' serum. Compared with the control group, no significant alteration in serum cadmium concentration of hypertensive patients and cardiac patients was observed. It was found that cadmium depletes glutathione and impairs sulfhydryl homeostasis, promoting lipid peroxidation, OS, kidney proteins inactivation, and elevating the blood pressure (23). Unlikely, a previous study did not find any associations between blood cadmium and HTN; The observed associations varied by smoking status and gender (22).

Dietary copper is crucial for the normal functioning of the cardiovascular system (25). In this study, copper concentration showed a significant elevation in hypertensive and cardiac patients relative to the control. The increased copper level in the hypertensive and cardiac patients was consistent with numerous studies reported about the increase in the levels of serum copper in those with HTN or atherosclerosis (26). The serum copper concentrations were associated positively with the atherosclerosis severity in atherosclerotic patients (25, 26). Similarly, the femoral atherosclerosis patients demonstrated increased serum copper concentrations, positively associated with total LDL and cholesterol (27). HTN can occur in copper-deficient rats due to reduced vasoactive activities in the lower resistant blood vessels, implying that copper plays a role in microvascular regulatory mechanisms (15).

The association between the serum lead and cobalt concentration with HTN has been reported in various (28, 29). A significantly high concentration of lead in hypertensive and cardiac patients was observed relative to the control. Previous findings have suggested the mechanism whereby lead exposure promotes HTN through impairing calcium metabolism (30). Acute lead exposure impairs cardiac function, whereas chronic lead exposure impairs the mechanical and electrical activity of the heart and the function of vascular smooth muscle (22). Lead may contribute to the generation of reactive nitrogen and oxygen species and trigger inflammation in the target tissue and CVD progression, causing endothelial dysfunction and vascular smooth muscle cell proliferation and transformation (20, 29, 30). It can also impair the NO homeostasis essential for vasodilatation (30-33).

Similarly, cobalt concentration demonstrated a significant elevation in hypertensive and cardiac patients compared to the control. A previous study reported that the individuals exposed to cobalt during work experienced more suffering from reversible electrocardiographic (ECG) changes and HTN (27). These findings agree with previous studies reporting the consequences of environmental lead exposure on HTN and the pathogenesis of CVD (36, 37). Besides, the metal-induced toxicity influenced the reactive nitrogen and oxygen species in biological systems (5).

LDLs represent the leading carriers of cholesterol (36), and the LDL elevation causes accumulating cholesterol, thus forming atherosclerotic lesions in the vessels (17). Various studies involving macrophage foam cell formation have concentrated on the oxidized form of LDL-cholesterol (37-40).

LDL can be oxidized through the major cell types within the wall of arteries (39). The ox-LDL was reported to be more significant in the progression and genesis of atherosclerosis relative to native unmodified LDL-c (43). It was impossible to demonstrate that native LDL causes

foam cell development because the cellular receptor for LDL is scarcely expressed on differentiated macrophages and impaired by total cholesterol buildup (38, 40). The significant elevation of ox-LDL level in hypertensive and cardiac patients was observed compared to the control. Our results are consistent with other studies implicating that cardiovascular disease sometimes starts with inflammatory fluctuations in the endothelium (35). Native LDL in the circulation becomes oxidized by free radicals into ox-LDL. Ox-LDL accumulation in the subendothelial space begins to express the adhesion molecule. Macrophages generated from monocytes absorb the ox-LDL and transfer it into the subendothelial region. Failure of removal of subendothelial ox-LDL molecules leads to their accumulation. In early atherosclerosis, these lipid-laden macrophages generate foam cells appearing as fatty streaks. Matrix metalloproteinase deposition, subsequent local inflammation, fibrosis, and neointimal proliferation result in the development of mature atherosclerotic plaque (33).

MDA is an excellent indicator for OS and lipid peroxidation (11). We observed that the hypertensive patients showed a significant elevation of MDA when compared with the control. The finding was consistent with a prior study demonstrating the significance of OS in HTN patients, with hypertensive patients having much higher serum MDA levels than the control group (11, 44-46). In addition to this, high levels of MDA were also found to play a role in atherogenesis (42).

It was determined that the toxicity of heavy metals has resulted in the creation of free radicals, which have produced vascular OS, thus contributing to CAD and HTN. By uncoupling eNOS and increasing NO scavenging, an early vascular OS may increase endothelial dysfunction and vascular resistance. Further studies are required to clarify the role of heavy metals in cardiovascular toxicity, which will provide significant implications for disease prevention and health policy.

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Tables

Table I: Lipid Profile Tests (TC, TG, HDL-c, and LDL-c) in all Studied Groups

Outcome Measure (mg/dL)	Experimental Groups						*p*-value (HTN vs. NTC)	*p*-value (HTN+CVD vs. NTC)	*p*-value (HTN+CVD vs. HTN)
	NTC		HTN		HTN+CVD				
	Mean	SE	Mean	SE	Mean	SE			
TC	159.5	5.27	210.23	7.75	190.3	6.64	<0.001***	< 0.001***	0.056
TG	124.7	5.05	185.93	14.88	153.07	7.81	<0.001***	0.003**	0.055
HDL-c	42.87	0.92	37.1	1.16	39.87	1.04	<0.001***	0.035**	0.080
LDL-c	91.69	5.59	135.95	7.45	119.82	7.13	<0.001***	0.003**	0.123

All values were demonstrated as Mean ±Standard Error of the mean (SE) of each group. *P*-value is significant when it is < 0.05*, or highly significant when < 0.01**, and very highly significant when < 0.001***. *P*-value is determined by t-test for continuous variables.

Table II: ICP analysis of heavy metals (Cadmium, Chromium, Copper, Cobalt, and Lead) in all Studied Groups

Outcome Measure (mg/L)	Experimental Groups						*p*-value (HTN vs. NTC)	*p*-value (HTN+CVD vs. NTC)	*p*-value (HTN+CVD vs. HTN)
	NTC		HTN		HTN+CVD				
	Mean	SE	Mean	SE	Mean	SE			
Cd	0.01	0.0004	0.01	0.0006	0.01	2.96855E-05	0.4237	0.5810	0.553
Co	0.03	0.0003	0.03	0.0008	0.03	8.47896E-05	0.009**	< 0.001***	0.703
Cr	0.03	0.0003	0.03	0.0008	0.03	6.97011E-05	0.008**	< 0.001***	0.701
Cu	0.05	0.0007	0.06	0.0012	0.06	0.000228726	0.014*	< 0.001***	0.549
Pb	0.15	0.0007	0.16	0.0029	0.16	0.000467528	< 0.001***	< 0.001***	0.394

Table III: ox-LDL Test in all Studied Groups

Outcome Measure	Experimental Groups						*p*-value (HTN vs. NTC)	*p*-value (HTN+CVD vs. NTC)	*p*-value (HTN+CVD vs. HTN)
	NTC		HTN		HTN+CVD				
	Mean	SE	Mean	SE	Mean	SE			
Ox-LDL (mg/dL)	1.008	0.19	1.661	0.07	1.671	0.09	0.001***	0.003**	0.652

Table IV: Serum MDA Levels in all Studied Groups

Outcome Measure	Experimental Groups						***p*-value** (HTN vs. NTC)	***p*-value** (HTN+CVD vs. NTC)	***p*-value** (HTN+CVD vs. HTN)
	NTC		**HTN**		**HTN+CVD**				
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**			
MDA (µmol/L)	0.582	0.027	0.88	0.08	0.653	0.141	0.001***	0.622	0.17

Figures

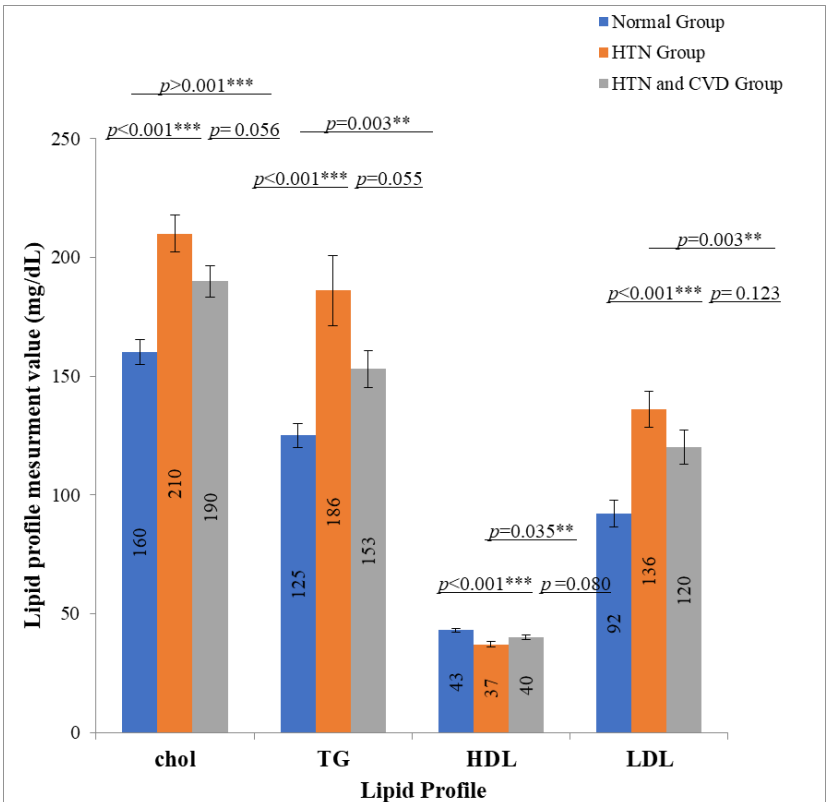

Figure 1: Lipid Profile (Cholesterol, TG, HDL, and LDL) results in all Groups

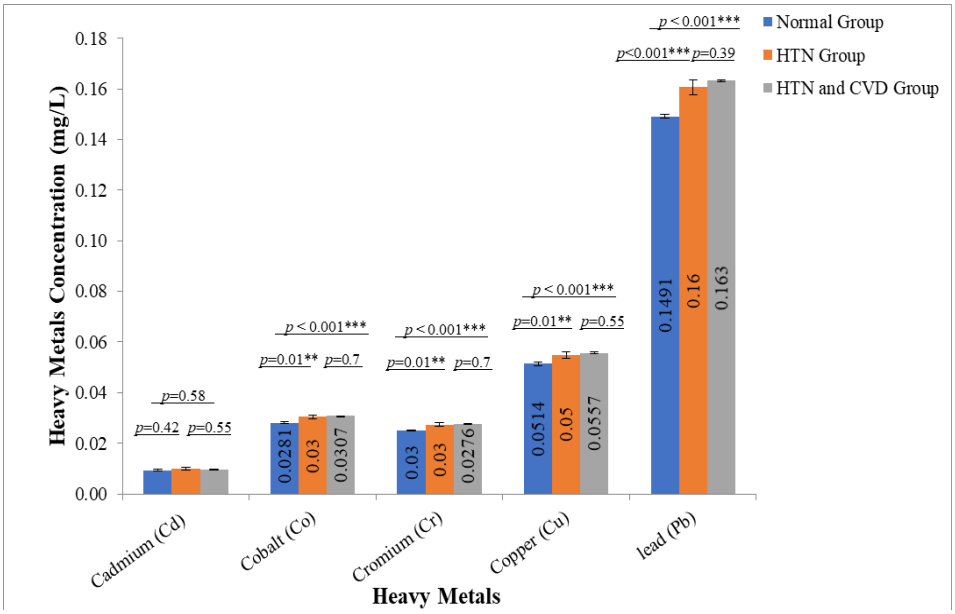

Figure 2: Values of Heavy Metals (Cadmium, Cobalt, Chromium, Copper, and Lead) in all groups

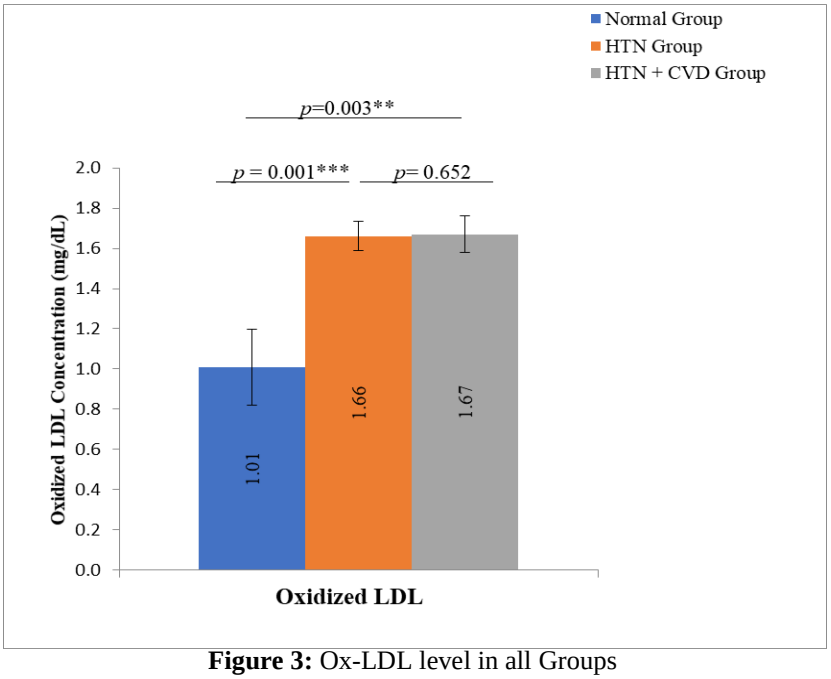

Figure 3: Ox-LDL level in all Groups

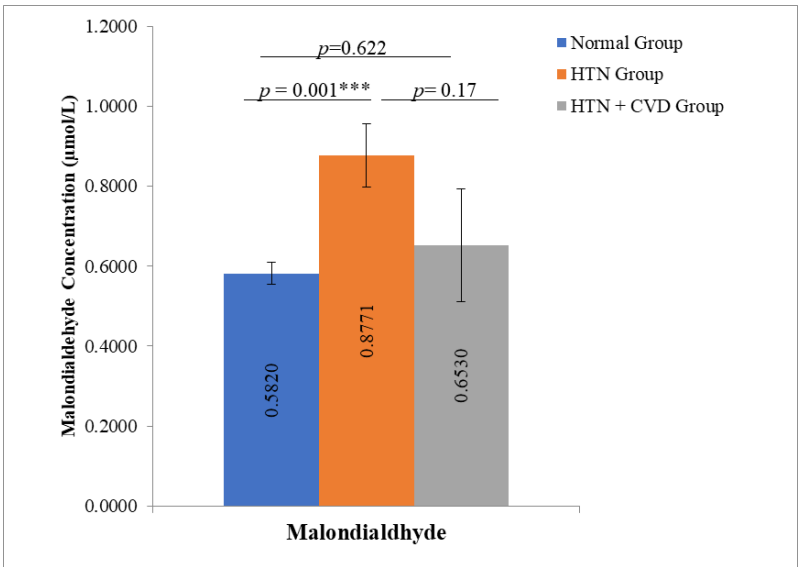

Figure 4: Serum MDA Levels (µmol/L) in all Studied Groups