Pak. J. Chem. 1(3): 120-125, 2011 Full Paper
ISSN (Print): 2220-2625
ISSN (Online): 2222-307X DOI: 10.15228/2011.v01.i03.p03

Serum Malondialdehyde and Lipid Profile Levels of Young Patients "Haven't a Family History of Hypertension": A New Study for Cases in the Civic Society

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ABSTRACT

This study was designed to evaluate the effect of the oxidative stress in sera of young patients without a family history of hypertension, then find the relation of it to lipid profile. The study involved 56 young healthy (30.5 ± 4.7 years) and 23 healthy elderly volunteers (66.3 ± 3.5 years), these individuals were compared with 67 patients (28.6 ± 5.0 years), attending the Gastro Intestinal and Liver Centre at Al Sader Medical City and several specialized clinics in Najaf government. Malondialdehyde level was measured by TBARS assay as reflection to the oxidative stress effect. Using standard enzymatic assays, TG, TC, HDL-C, VLDL- -C, and LDL-C values were measured for patients and controls on the same day of sample obtainment. Levels of serum MDA, TG, VLDL-C, and LDL-C were significantly raised ($p<0.005$) in hypertensive patients group when compared with young and elderly controls, while non significant variations were obtained when the control groups were compared together. Daytime SBP and DBP were both strong positive correlated ($r=0.82$, $p<0.005$ for SBP, and $r=0.95$, $p<0.005$ for DBP) with the MDA in hypertensive patients, but in elderly controls only the correlation between SBP and MDA levels was statistically significant ($r=0.61$, $p<0.005$). Sera TG, VLDL-C, and LDL-C levels showed the same of MDA results when they were correlated to SBP and DBP, while correlations between blood pressure with sera TC were moderate positively in the study groups. In spite of; HDL-C levels in hypertensive patients were within the levels of those of two control groups, HDL-C levels showed negatively correlation with both SBP and DBP. Notably, there was a positive correlation ($r=0.60$, $p<0.005$) between serum LDL-C levels and SBP of elderly controls, no such correlations were observed when the relation was between Daytime SBP and DBP and VLDL-C or LDL-C of young controls the highly significant positive correlation ($r = 0.94$ at $p<0.005$) of MDA and TG of the hypertensive patients. Significant positive correlations were also observed for MDA with VLDL-C ($r = 0.74$ at $p<0.005$), and with LDL-C ($r = 0.71$ at $p<0.005$). It is well known that the endogenous female sex hormones have significant effect on lipid levels, according to that, testing the gender effect was occurred. MDA level in the study subgroups revealed a significant increase ($p<0.005$) in male patients when compared to females, while, student's *t-test* failed to exhibit significant changes among male and female subgroups in the control groups. Except for the significantly variation ($p<0.005$) of TG in the patient subgroups, all the other lipid profile parameters showed no significant differences between male and female subgroups. Concerning the controls' subgroups, TG, TC, HDL-C, VLDL-C, and LDL-C were comparabltween male and female subgroups. Finally, treatment for hypertension is similar for all demographic groups, but socioeconomic factors and lifestyle may be barriers to BP control in some patients, therefore; dealing with transitory hardships and arrangement of food style may be consider as a prim factors in the hypertension treatment.

Keywords: oxidative stress, lipid profile, hypertension

1. INTRODUCTION

Recent few years have witnessed an increasing the number of young hypertensive patients (persistent and sustained high blood pressure) in the contrapuntal centres and clinics, regardless their gender, profession type, and dietary habits, but they have one subscriber peculiarity "**they haven't a family history of hypertension**". For the aforesaid reason, the present study was designed, by measurement of malondialdehyde "MDA" level to assess lipid peroxidation in addition to lipid profiles levels.

The pressure of blood flowing through the blood vessels against the vessel walls, it depend on the blood pumped from the heart and elasticity of the blood vessels. And varies with the age and human activity. Hypertension (a silent killer) is define as the blood pressure >140/90mmHg[1]**.** hypertension is now a global epidemic affecting 1.5 billion people world wide and claiming about 7 million lives every year, and the number will increase to reach 1.56 billion in 2025[2, 3]**.** In Iraq, like many other countries in Arab Gulf region, where adoption of western lifestyles and the stress of urbanization both of which are expected to increase the morbidity associated with unhealthy and environmental factors are reported to play a key role in hypertension that major risk factor for cardiovascular events, such as myocardial infarction and stroke, as well as for microvascular complications, such as retinopathy and nephropathy[4]**.** Hypertension problems become more dangerous in the young aged, where it may be decrease the medial people age. Hypertension is classified into two groups–primary or essential hypertension and secondary hypertension. Primary hypertension is defined as a 'rise of blood pressure of unknown cause'. Secondary hypertension is the 'increase in blood pressure caused by diseases of kidney, endocrines, or some other organs'. It is further graded into 3 stages based on the elevated blood pressures. Less than 5% of hypertensive patients develop malignant hypertension[5].

O_2 is both essential to human life and toxic [6]. Cellular, through a number of enzymatic and nonenzymatic processes, O_2 accepts single electrons, it is transformed into highly reactive oxygen species (ROS), that damage cellular lipids, proteins, and DNA, that contributes to cellular death and degeneration in a wide range of diseases[6-8]. ROSs can attack polyunsaturated fatty acids in cell membrane phospholipids, resulting in the formation of lipid peroxides which can

*Corresponding Author Received June 2nd 2011, Accepted June 29th 2011

then fragment to numerous small compounds, like MDA, [9, 10], 8-isoprostane[11], 8-isoprostaglandins-F2α (8-iso-PGF)[12], and 8-hydroxy-2O-deoxyguanosine (8-OHdG)[13]**.** Oxidative stress occurs when there is an imbalance between the generation of ROS and the antioxidant defence systems so that the latter become overwhelmed[11].

The main aims of the present study are: evaluation of MDA levels as a reflection to the oxidative stress effect in sera of patients and healthy controls, then compared patient and healthy groups together, firstly, investigation whether serum MDA and lipid profile are harmoniously changed in young patients, age-matched and elderly, secondly; healthy individuals, and finally, sifting about the cause of the hypertension.

2. EXPERIMENTAL

2.1 *Study Individuals*

Blood samples (approximately 5-10ml) were collected in appropriate sterile vials by venous arm after overnight fasting, through the present study that extended between July 2009 and September 2010. The study involved 56 young healthy (30 male and 26 female, mean age ± SD 30.5 ± 4.7 years) and 23 healthy elderly volunteers (11 male and 12 female; mean age ± SD 66.3 ± 3.5). These individuals were compared with 67 patients (35 male and 32 female, 28.6 ± 5.0 years), attending Gastro Intestinal and Liver Centre at Al Sader Medical City and several specialized clinics in Najaf government. The majority of patients studied had evidence uncontrolled hypertension (the definition of hypertension was based on questionnaire information and blood pressure measurements; inclusion criteria included the patients with systolic blood pressure (SBP) ≥140 mm Hg and diastolic blood pressure (DBP) ≥90 mm Hg), pin-head, annoyance, and recur vomiting, but none had been hospitalized in the previous 6 months. In addition, the majority of patients were also receiving antihypertensive patients and antinational agents. Health was defined as an absence of major medical or surgical illness in the previous5 years, no hospital admissions, no current medication, and a subjective perception of good health as determined by health questionnaire. None of the patients and control subjects had concomitant diseases such as diabetes mellitus, liver disease and rheumatoid arthritis.

2.2 *Measurement of Serum Malondialdehyde Level*

Malondialdehyde level is measured by the thiobarbituric acid-reacting substances (TBARS) assay[14]. Abridgment,150 µl of the serum sample was mixed with 1 ml of trichloro acetic acid (17.5 %) and 1ml of thiobarbituric acid (0.6 %). Using the vortex, the final mixture was mix, the reaction mixture was then heated at 100 °C for 15 minutes in a water bath. After the mixture was cooled with tap water, it was extracted with 1 ml TCA (70 %), the mixture was stand for 20 minutes at 25 °C, and centrifuged at 3000 xg for 15 minutes. The organic phase was measured by use of a spectrophotometer with a wavelength of 534 nm.

2.3 *Measurement of Serum Lipid Profile Levels*

Using standard enzymatic assays, triglyceride (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C), VLDL-Cholesterol (VLDL-C), and LDL-Cholesterol (LDL-C) values were measured for patients and controls on the same day of sample obtainment.

2.4 *Statistical Analysis*

The findings were expressed as the mean ± standard deviation (S.D.).The data were analyzed with **Student's independent *t* test**. All statistical analyses were performed with the program Statistical Package for the Social Science (SPSS for Windows, Version 10.0).Pearson's correlation was applied to determined the relations among the blood pressure and the clinical and laboratory parameters of the present study, significance was determined regression. A *p*-value of <0.005 was accepted as statistically significant.

3. RESULTS AND DISCUSSION

In the present study, the mean SBP of the three study groups was165±25, 110±10, and 120±15 for patients, young, and elderly individuals, respectively, while the mean DBP was 95±25, 70±10, and 85±5 for the contributor groups. Complete results of clinical and laboratory parameters are given in table 1. Serum MDA levels in hypertensive patients were significantly higher than young ($p<0.005$), and elderly ($p<0.005$) controls, but MDA levels were comparable between two control groups ($p<0.01$). Levels of TG, VLDL-C, and LDL-C were significantly raised ($p<0.005$) in hypertensive patients group when compared with young and elderly controls, while non significant variations were obtained when the control groups were compared together. On the other hand, non significant variations were observed when the mean TC as well as HDL-C values of patients group was compared to those of young and elderly healthy individuals, with same manor; non statistical variation was noted when the two control groups where compared together.

Table-1: Levels (µM) of MAD and (mg/dL) of lipid profiles in patient and control subjects (mean ± S.D.)

Individual	MAD	TG	TC	HDL-C	VLDL-C	LDL-C
P (67)	20.53±7.24	230±50	200±30	42.5±2	46.0±10	111.5±22
Y (56)	9.69±2.63	120±30	170±20	47.0±8	24.0±6	99.1±13
E (23)	13.52±3.33	140±35	175±35	43.2±6	34.0±11	97.8±14
p-value	<0.005 for P vs Y <0.005 for P vs E <0.01 for Y vs E	<0.005 for P vs Y <0.005 for P vs E <0.1 for Y vs E	<0.01 for P vs Y <0.05 for P vs E <0.5 for Y vs E	<0.5 for P vs Y <0.3 for P vs E <0.3 for Y vs E	<0.005 for P vs Y <0.005 for P vs E <0.01 for Y vs E	<0.005 for P vs Y <0.005 for P vs E <0.01 for Y vs E

P: Patients, Y: Young Controls, E: Elderly Controls

Daytime SBP and DBP were both strong positive correlated (r=0.82, p<0.005 for SBP, and r=0.95, p<0.005 for DBP) with the MDA in hypertensive patients, but in elderly controls only the correlation between SBP and MDA levels was statistically significant (r=0.61, p<0.005). Both SBP and DBP didn't show statistical relation with sera MDA levels of young healthy individuals. Sera TG levels showed the same of MDA results when they were correlated to SBP and DBP, particularly, the results were so clear (r=0.91, p<0.005) when TG was correlated to the DBP in patients with the hypertension. Correlation between blood pressure (SBP and DBP) with sera TC were moderate positively in the study groups. In spite of; HDL-C levels in hypertensive patients were within the levels of those of two control groups, HDL-C levels showed negatively correlation with both SBP and DBP, no such correlations were observed in the control groups. Daytime SBP and DBP were both positive correlated (r=0.67, p<0.005 for SBP, and r=0.78, p<0.005 for DBP, respectively) with VLDL-C levels, and (r=0.79, p<0.005 for SBP, and r=0.88, p<0.005 for DBP, respectively) with LDL-C levels in patients with hypertension. Notably, there was a positive correlation (r=0.60, p<0.005) between serum LDL-C levels and SBP of elderly controls, no such correlations were observed when the relation was between Daytime SBP and DBP and VLDL-C or LDL-C of young controls. The complete results are illustrated in Table-2.

Table-2: Correlation of various study parameters to the dating blood pressure (SBP and DBP) in patients and controls

Parameters Attachment to BP	P	*p*	Y	*p*	E	*p*
SBP and MDA	0.82	<0.005	0.28	>0.5	0.61	<0.005
SBP and TG	0.77	<0.005	0.09	>0.5	0.39	<0.01
SBP and TC	0.69	<0.005	0.59	<0.005	0.62	<0.005
SBP and HDL-C	-0.75	<0.005	-0.09	>0.5	0.22	>0.5
SBP and VLDL-C	0.67	<0.005	0.25	<0.5	0.31	<0.5
SBP and LDL-C	0.79	<0.005	0.30	<0.01	0.60	<0.005
DBP and MDA	0.95	<0.005	0.12	<0.05	0.35	<0.01
DBP and TG	0.91	<0.005	0.11	>0.5	0.21	<0.5
DBP and TC	0.73	<0.005	0.61	<0.005	0.63	<0.005
DBP and HDL-C	-0.68	<0.005	-0.12	<0.5	-0.32	<0.5
DBP and VLDL-C	0.78	<0.005	0.23	<0.5	0.30	<0.5
DBP and LDL-C	0.88	<0.005	0.31	<0.05	0.40	<0.01

P: Patients, Y: Young Controls, E: Elderly Controls

According to the study outresults, highlight on the relation between MDA and the lipid profiles that appeared significantly variation than healthy controls was done, for tracking the possible relationship. Thus, the correlations were happened with TG, VLDL-C, and LDL-C, respectively. Figure 1. A illustrates the highly significant positive correlation (r = 0.94 at p<0.005) of MDA and TG of the hypertensive patients. Significant positive correlations were

also observed for MDA with VLDL-C (r = 0.74 at p<0.005), and with LDL-C (r = 0.71 at p<0.005), (figure 1. B and C).

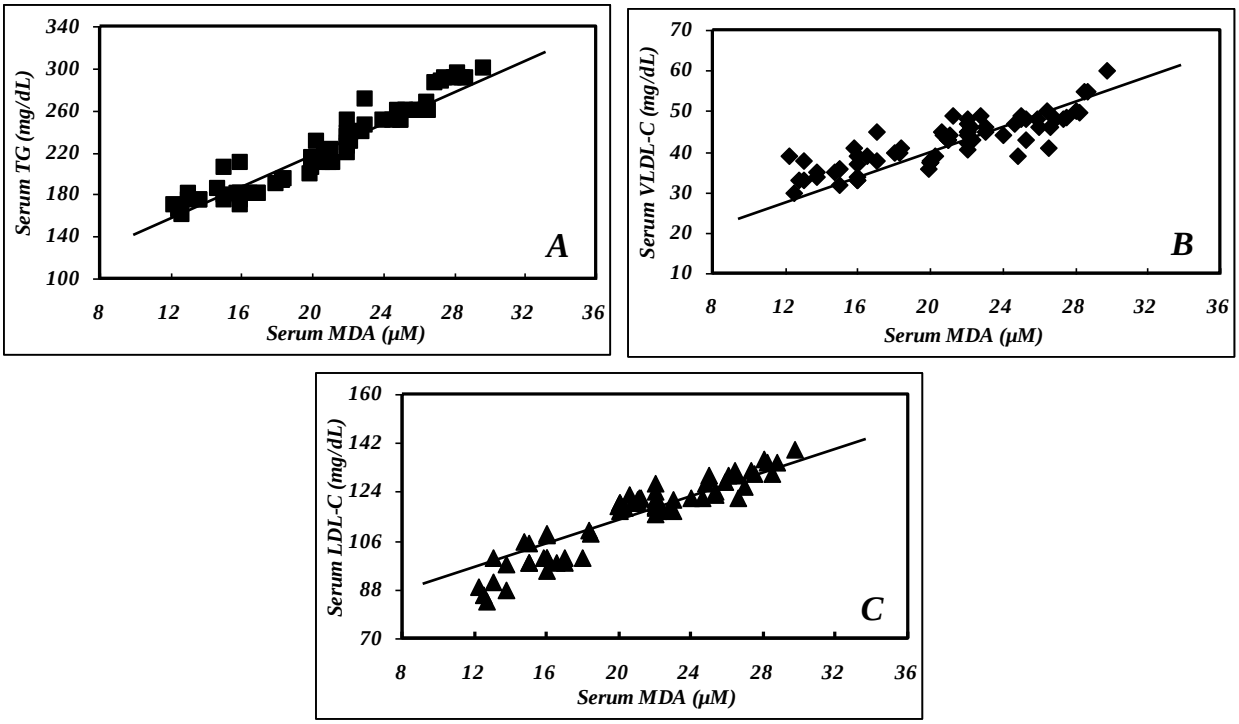

Fig-1: *Correlation of Serum Malondialdehyde and (A): TG, (B):VLDL-C, and (C): LDL-C.*

It is well known that the endogenous female sex hormones have significant effect on lipid levels[15], thus it may effect on the present study parameters, according to that, testing the gender effect became so important. The evaluation of the MDA level in the study subgroups revealed a significant increase (p<0.005) in male patients when compared to females, while, student's *t-test* failed to exhibit significant changes among male and female subgroups in the control groups, as shown in table 3. Except for the significantly variation (p<0.005) of TG in the patient subgroups, all the other lipid profile parameters showed no significant differences between male and female subgroups. Concerning the controls' subgroups, TG, TC, HDL-C, VLDL-C, and LDL-C were comparable between male and female subgroups, data shown in table 3.

Table-3: Levels (μM) of MAD and (mg/dL) of lipid profiles in male and female patient and control subjects (mean ± S.D.)

Individual	MAD	TG	TC	HDL-C	VLDL-C	LDL-C
P						
Male (35)	22.93±5.22	240±60	190±29	43.8±9	48.0±12	101.2±8
Female (32)	17.42±8.55	190±35	205±35	42.0±2	38.0±7	125±26
***p*-value**	<0.005	<0.005	<0.01	<0.5	<0.05	<0.05
Y						
Male (30)	9.88±1.2	122±20	167±10	35.1±3	24.4±4	87.5±3
Female (26)	8.79±2.3	115±35	155±25	44.2±4	23.0±7	67.8±14
***p*-value**	<0.5	<0.5	<0.1	<0.05	<0.5	<0.05
E						
Male (11)	14.00±2.12	150±40	190±17	38.5±2	35.0±1	113±14
Female (12)	13.08±5.87	135±32	170±30	39.9±1	33.2±2	91.9±27
***p*-value**	<0.1	<0.05	<0.01	<0.5	<0.05	<0.01

P: Patients, Y: Young Controls, E: Elderly Controls

Hypertension is may due to the genetic predisposition, secondary life styles, fatty food consumption, saturated fat, cholesterol in the food increase the blood cholesterol and saturated fat is the main culprit, smoking and increased alcohol intake[16, 17]. Generally; more than 80% of people with hypertension have additional comorbidities, such as

obesity, glucose intolerance, hyperinsulinemia, etc. More than 50% of people with hypertension have two or more comorbidities[5]. The contributor patients in the present study were non smoking young individuals (<35 years), most of them were within normal body mass index or less, city population, with multifarious style of aliment, without a family history of hypertension. The present study is done to study the pattern of lipid peroxidation (MDA) and lipid profile in young hypertensive patients compared to the controls (young and elderly).

Current work would specifically like to mention about serum MDA, it showed a progressive rise with age. The relationship between ages versus serum MDA was ascertained by correlation coefficient and was found out to be 0.68 ($p<0.005$) which is highly significant. These observations were highly interesting.

The SBP is increased as the age progresses in the different study cases and the highest levels was recorded in the hypertensive patients, while the DBP in hypertensive patients is also higher than the controls. The DBP is not increased as the age increased, it is stabilized. These observations suggest that SBP is the best predictor in elderly than DBP.

ROSs form as a natural by product of the normal metabolism of oxygen and have important roles in a number of biological processes, such as the killing of bacteria[18]. During times of environmental stress ROS levels can increase dramatically which can result in significant damage to cell structures, especially in absence of anti-oxidant defences, such as the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase or antioxidant vitamins A, C and E and polyphenol antioxidants[11]. Cardiovascular system regulation is underlying several molecules, two of the most important compounds are angiotensin II (Ang II) and nitric oxide (NO), the central role of these molecules in the development of hypertension and hypertension-induced organ damage have been firmly established [19]. When the genetic effect excluded the hypertension may explain through the effect of oxidative stress. Oxidative stress may contribute to the generation and/or maintenance of hypertension via a number of possible mechanisms, included: (1) Quenching of the vasodilator NO by ROS such as superoxide. (2) Generation of vasoconstrictor lipid peroxidation products. (3) Depletion of tetra hydro biopterin (BH4), an important NO synthase cofactor.(4) Structural and functional alterations within the vasculature[20]. These vascular changes may be mediated in several ways[7, 21], thus, oxidative stress promotes vascular smooth muscle cell proliferation and hypertrophy and collagen deposition, leading to thickening of the vascular media and narrowing of the vascular lumen. In addition, increased oxidative stress may damage the endothelium and impair endothelium-dependent vascular relaxation and increases vascular contractile activity. All these effects on the vasculature may explain how increased oxidative stress can cause hypertension[20, 22]. The role of oxidative stress in the complex mechanisms involved in blood pressure control has been extensively analyzed[16, 23]. Several clinical studies are pointing to the fact that therapies reduce blood pressure (manufactured or natural), possibly exhibit their effect via stimulation of the antioxidant systems[24, 25].

In the present study, It has been estimated that the risk of hypercholesterolemia is higher in 40% of healthy menopausal females, while no such observation was recorded in healthy young females or elderly males; on the other hand, it has been observed that the risk of hypertriglyceridemia is raised in 17% of young healthy males, and in 37% of elderly healthy males. These observations agreed with several studies[16, 17, 26]. Non significant variation were observed when TC and HDL-C levels of hypertensive patients were compared to those of nontensive groups, these findings disagree with most of hypertension related lipid profile studies[5,27], thus; these results prompted to determination of the TC/HDL-C ratio for the study groups. According to the recommendations of Joint British Societies TC/HDL-C ratio should be below 4.5[28], in the present study this ration was within the normal level for both control groups (3.6 in young and 4.1 for elderly) , while patients group recorded high TC/HDL-C ratio (5.2), as table 1 illustrated.

Different serum lipids vary significantly in various population groups due to difference in geographical, cultural, economical, social conditions, dietary habits and genetic makeup[2]. Age and gender differences also affect serum lipids considerably[29,30]. Elevated levels of triglyceride, cholesterol and LDLC are documented as risk factors for atherogenesis[2]**.** LDL-C in its oxidized or acetylated form has been identified as a major atherogenic particle; as it not only load macrophages with cholesterol for the formation of foam cells but also because it is chemotactic for circulating monocytes, iscytotoxic and can adversely alter coagulation pathways[31-33]. The blood level of HDL-C in contrast bears an inverse relationship of the risk of atherosclerosis and coronary heart disease that is higher the level, smaller the risk[2]. The association of alteration in serum lipid profile in hypertension is well documented in many various studies[8, 17].

The reason behind the abnormal lipid metabolism in hypertension may be the genetic locus associated with dyslipidemia accompanying hypertension[8].

According to the information in the Gunderson guides[23] in persons aged 40–70 years, each increment of 20 mmHg in SBP or 10 mmHg in DBP doubles the risk of CVD across the entire BP range from 115/75 to 185/115 mmHg, these pitfalls may increase as the person infected with the malignant hypertension in the young age, as the present study patients. Therefore, the current research indicates that the maintaining of the oxidative balance and monitoring of the lipid level in hypertensive patients would be helpful in preventing the CVD and other diseases associated with hypertension. Finally, treatment for hypertension is similar for all demographic groups, but

socioeconomic factors and lifestyle may be barriers to BP control in some patients[16, 34], therefore; dealing with transitory hardships and arrangement of food style may be consider as a prim factors in the hypertension treatment.

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