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Some oxidant Parameters in Cardiovascular Iraqi females

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Abstract: Cardiovascular diseases (CVDs) are heart and blood vessels related groups of disorders which supply myocardium with oxygen and nutrients. Reactive oxygen species (ROS) is a group term that is used for a group of oxidants, which are either free radicals or molecular species being able to generate free radicals. This study was conducted on a group of 28 cardio diseased females' patients from Ibn Al-Nafese in Baghdad, Iraq. and 28 apparently healthy individuals as control group. Oxidation parameters were determined such as malondialdyhide by Walker method, 8-Hydroxydeoxyguanosine by CUSABIO/China and albumin by biosystem company/Spain. The deletion of Glutathione S-transferase mu gene was detected and the deletion of Glutathion S transferase mu (GSTM1) gene was studied using polymerase chain reaction PCR using primers pair from Promega/USA. Results of this study showed there was a high significant decrease ($p \leq 0.01$) in albumin concentration of patient related to control, and there was a high significant increase ($p \leq 0.01$) in each MDA and 8-OHdG for patients group in comparison with controls, and the electrophoresis study for GSTM1 gene shows a significant difference ($p < 0.01$) in deletion of this gene.

Keywords: Cardiovascular, Malondialdehyde, 8-Hydroxydeoxyguanosine, Glutathione S-transferase

INTRODUCTION

Cardiovascular diseases are the result of the accumulation of atheromatous plaques within the walls of the arteries that supply myocardium with oxygen and nutrients (Parikh N. I et al., 2008). After decades of progression, some atheromatous plaques may rupture and may thus severely restrict the flow of oxygen carrying blood to the myocardium. Consequently, a heart attack can occur (WHO, 2017).

Reactive Oxygen species (ROS) are raised in response to vessel stimulation by mechanical stretch or angiotensin II (AII). Reaction of ROS with endothelium released NO inhibits vasodilatory or antisclerotic effects of NO and thus can exacerbate the disease (Steven D., 2014; Rodrigo R. et al., 2011). Among the many biological targets of oxidative stress, membrane lipids are the most commonly involved class of biomolecules. Lipid peroxidation yields a number of secondary products able to boost oxidative damage (Vogiatzi G. et al., 2009; Biomarkers Definitions Working Group, 2001). In addition to their cytotoxic properties, lipid peroxides are increasingly recognized as being important in signal transduction for a number of events in the inflammatory response (Bartoli M. L. et al., 2011). A byproduct of polyunsaturated fatty acid peroxidation caused by ROS, malondialdehyde (MDA) is regarded as a typical biomarker of oxidative stress. Since it has high reactivity,

MDA is toxic, potentially mutagenic, and atherogenic due to its reactions with biomolecules such as proteins and nucleic acids (Chen J et al., 2015; Yang T. et al., 2014). Alteration of MDA level in the living organism often reflects pathological changes (Siegert E. et al., 2016; Jiangang L. et al., 2008; Seemma L.J. et al., 2010) which have been verified in various types of illnesses. Therefore, it is of great significance to detect MDA to monitor the progression of the diseases and elucidate the underlying pathology mechanisms in living subjects (Rio D.D. et al., 2005).

8-Hydroxydeoxyguanosine (8-OHdG) is a most common stable product of oxidative DNA damage following enzymatic cleavage after ROS induced 8-hydroxylation of guanine based on mitochondrial and nuclear DNA (Arunima P.R. et al., 2014). It can be recognized at an increase level in all bodily fluids and tissues, where an inflammatory process exists (Caraiane A. et al., 2014) and is considered a measure of DNA oxidation in response to free radicals (Ibrahim A. et al., 2014).

MATERIALS AND METHODS

This study was conducted on group of 28 cardio diseased females' patients and 28 apparently healthy individuals as control group. These patients were submitted from Ibn Al-Nafesein Baghdad, Iraq. Samples collected were divided into two types: whole blood for DNA isolation and serum for biochemical parameters. DNA isolated using ReliaPrep Blood gDNA Miniprep System (Hook B. et al., 2011). The thiobarbituric acid reactive substance method (TBAR test) has been frequently used to assess MDA concentrations (Cipierre C. et al., 2013). Serum MDA was measured by spectrophotometric methods supplied by Shah and Walker by reaction with thiobarbituric acid (TBA) that involves reaction one molecule of MDA with 2 molecules of Thiobarbituric acid (Shah S.V. et al., 1988). The 8-OHdG measured according to the instruction of manufacture [Test Kit No.CSB-E10140h] produced by CUSABIO/China assay employs the competitive inhibition enzyme immunoassay technique. The serum albumin was measured by kit supplied by Biosystem Company (Doumas B.T. et al., 1971).

Statistical Analysis

All the statistical analyses in the study were performed using SPSS version 15.0 for Windows [Statistical Package for Social Science, Inc., Chicago, IL, USA] (Cary N.C., 2012). Descriptive analysis was used to show the mean and standard deviation of variables. Student T-Test. estimated the significance of difference between mean values. The probability $P< 0.05$ is significant, and $P> 0.05$ is non-significant. Correlation analysis was used to test the linear relationship between parameters. ANOVA test was used to show the differences between variables of differentiated groups.

RESULTS AND DISCUSSION

Samples from controls and patients were used for DNA isolation procedure and at the same day blood sample were taken. Approximately, all the samples yielded intact genomic DNA, as shown in figure (1).

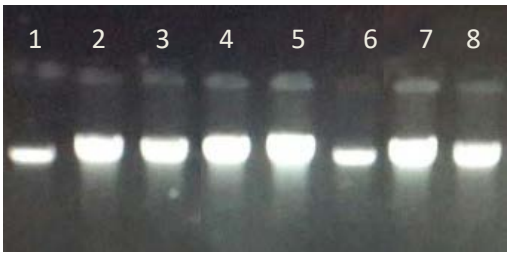

Figure 1: Genomic DNA Profile from Blood Sample of Subjects, Agarose gel (1%), 5 V/cm for 1 hr., Stained with Ethidium Bromide. Lane 1-4 control's DNA, lane 5-8 CVDs' group DNA

The concentration and purity of DNA was determined by Nano drop spectrophotometry and results are shown in table (1).
The 260/280 purity ratio of DNA purity with values for "pure" nucleic acid are commonly in the range of (1.8-2.2) (Desjardins Ph. et al., 2010).

Table 1: Concentrations and purity of DNA of Study Groups (Mean± SD):

Group	Cardiovascular patients n=28	Control n=28	*p*-value
DNA conc. ng/µL	88.05 ± 10.86	81.76 ±4.45	0.210
DNA purity	1.80 ± 0.02	1.83 ± 0.02	0.24

GSTM1 (glutathione S-transferase mu 1) is a protein-coding gene. GSTM1 locus was amplified at exons 4 and 5 by polymerase chain reaction (PCR) technique as previously described to differentiate between the null polymorphism and the presence of one or more copies of the gene.

The microdeletion of GSTM1 gene was detected by using the primers pairs and the results were observed in several levels of deletion.

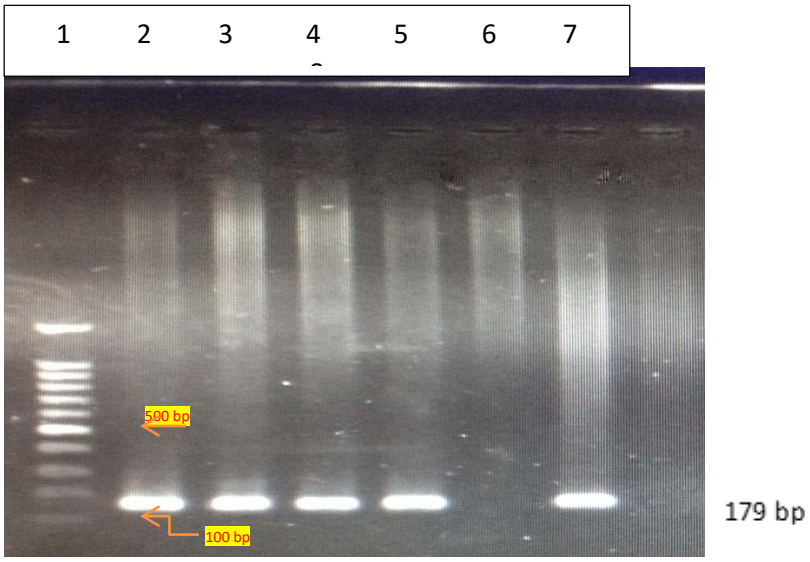

Figure 2: Gel electrophoresis for GSTM1 genotype. Ladder PCR products resolved by (2%) agarose gel electrophoresis (1h/70v). Lane1 DNA molecular weight marker, lane 2-4 controls' DNA, lane 6-8 samples DNA for patients

This study was designed to investigate the role of genetic polymorphisms of GSTM1 and biomarkers of oxidative stress in cardiovascular and healthy controls as shown in table (2).

The distribution of GST genotype and alleles of the study groups were given; the GSTM1 positive genotype occurs at higher frequencies in healthy controls (46.42%) and lower for Cardiovascular (33.33%). Ramprasath *et al.* (Ramprasath T. et al., 2011) found out that the GSTT1 positive genotype occurs at higher frequencies in controls than in Type 2 Diabetes Mellitus (T2DM) with cardiovascular and T2DM without cardiovascular patients. As for the GSTM1 positive genotype occurs at higher frequencies in controls than in T2DM with CAD patients and T2DM without CAD patient. (Dadbinpour et al., 2013) observed that the absence or deletion of detoxification pathway of GSTT1 has no significant effect on the side effects of T2DM, but GSTM1–null had significant relationship with diabetes retinopathy, indicating the role of detoxification of this genes in this regards.

(Kolla K. et al., 2011) found that the frequency of both positive and null of GSTT1 and GSTM1 did not differ significantly between control and patients.

The mean levels of serum 8-OHdG, is the main DNA modification formed by (ROS), which showed a significant increase in patients group (104.67±24.94 ng/ml), when compared to control group (82.62±19.13 ng/ml) as revealed by figure (3).

The concentration of MDA of patients group increased significantly in comparison to control group ($p<0.01$). In these patients, the oxidation of lipids increases and produces more MDA. Our findings in cardiovascular patient are also found by (Jawalekar *et al.*) and (Chopra et al., 2015). Also the significantly elevated ($p<0.01$) in diagnosed patient and this agrees with (Dhananjay V. et al., 2013). In addition, it has been demonstrated that increased intracellular generation of ROS plays an important role in chronic inflammatory responses to arterial diseases, so this causes damage to the membrane polyunsaturated fatty acids leading to the generation of MDA cause elevation in MDA in these patients (Hasan R. et al., 2013).

The mean level of albumin showed a significant decrease in patients group when compared to control group ($p<0.01$) and this is found also by Liu *et al.* and (Oda E) in (Liu M. et al., 2012; Oda E., 2012). The atherosclerosis has been considered as an inflammatory and immunizing disease. It is widely accepted that inflammation represents a risk factor for atrial fibrillation and for prothrombotic conditions. Different molecules behave differently during an inflammatory phase; albumin synthesis decreases, while other inflammatory globulins rise (Zecca B. et al.,2014). In hypertension which is not inflammatory disease in basic, the decrease in albumin levels may be due to antioxidant activity more than its behavior as acute phase protein.

Results show a significant difference between cardiovascular patient and control group in 8-OHdG ($p<0.01$). These results agreed with (Kaya Y. et al., 2012) who found a significant difference in 8-OHdG levels in hypertensive patient. Also (Fructaci A. et al., 2015) found an elevation in 8-OHdG in cocaine related cardiomyopathy cases.

DNA damage has been related to the development of cardiovascular pathologies in the general population, which is supported for the monoclonal origin of cells from human atherosclerotic plaques (Kaya Y. et al., 2012).

8-OHdG significantly increased in patients' subjects as compared to the control group. This may be due to the increased generation of ROS in certain type of white blood cells, which contributes in reduction bioavailability of nitric oxide and thus to the endothelial dysfunction, as some of the hypertension-induced organ damage, which occur due to hyperactivity of mechanisms that increase ROS production.

The results have shown that absence of GSTM1 gene has significant influence on MDA concentration for all the cases and controls. Furthermore, it has association with albumin concentration just for control group while for patient group, it has influence on 8OHdG. Partial deletion has unique effect on cardiovascular patient.

Table 2: The biochemical parameters of different studied groups (Mean± SD)

Characteristic	Cardiovascular patients n=28	Control group n=28	p–value
Age (year)	52.3±9.3	43.4±9.3	>0.01
BMI (Kg/ m^2)	32.59 ± 1.24	27.27 ±0.97	<0.05
Family history of disease no.(%)	14(50%)	12(32.14%)	>0.05
Menopausal no. (%)	17 (60.72%) *	10(35.71%)	<0.01
Menstruating no. (%)	11(39.28%)*	18(64.29%)	<0.01
Presence of GSTM1	9 (33.33%)	13 (46.42%)	<0.01

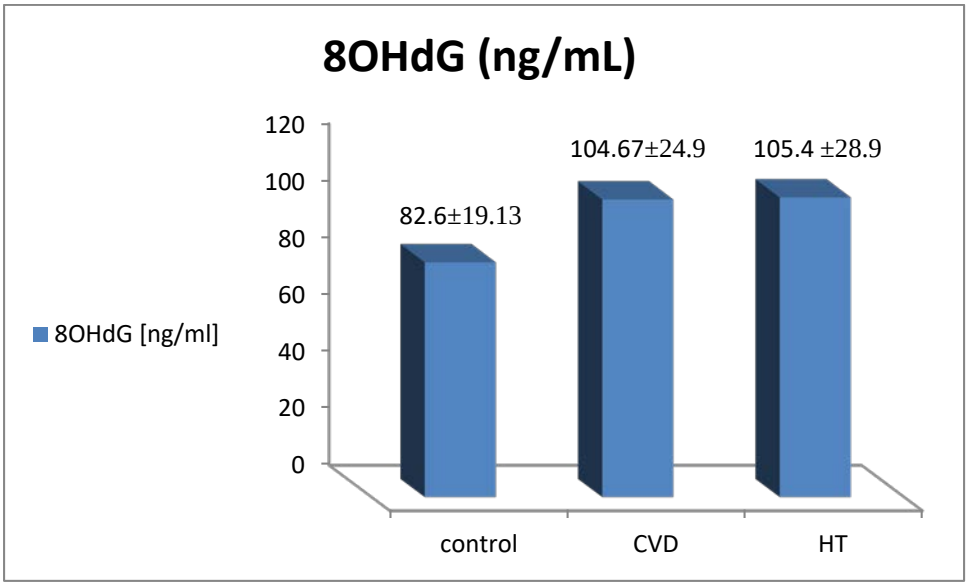

Figure 3: OHdG Levels in Patients and Control Group

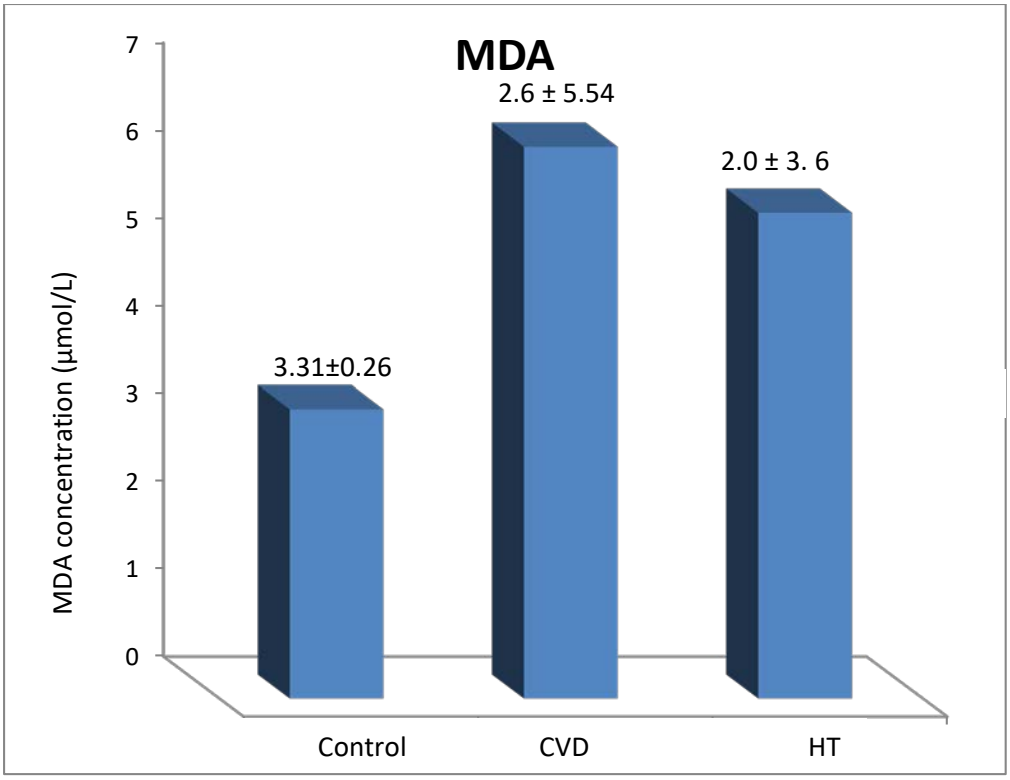

Figure 4: Mean Conc. (µ mol/L) of Oxidative Marker MDA for Studied Groups.

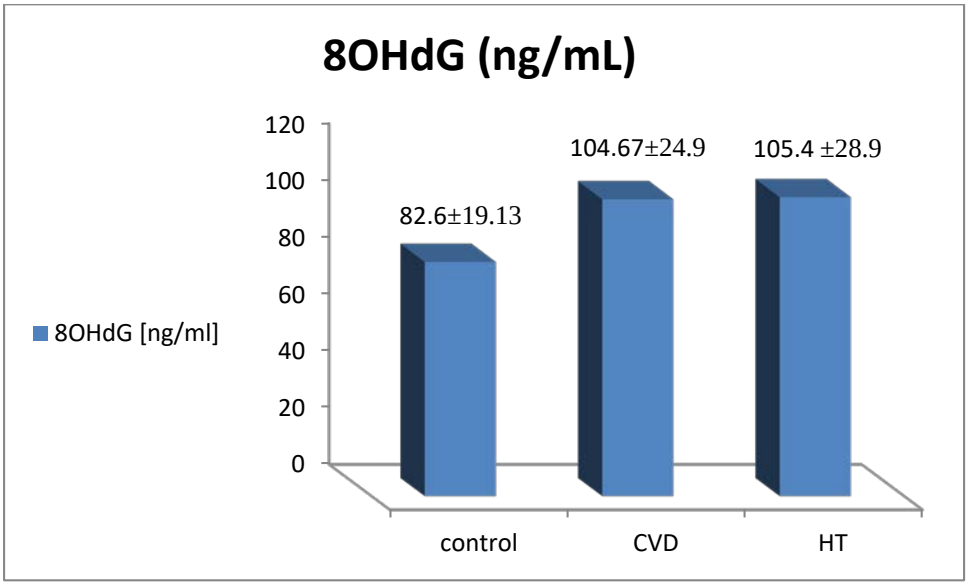

Figure 5: 8-OHdG Levels in Patients and Control Group

Table (3-14): Effect of Gene in Study Parameters in Cardiovascular groups (Mean ± SD)

Characteristic	Null genotype	Presence of primers of gene	P value
MDA µmol/L	6.20 ±1.71	4.00 ± 0.45	0.025*
Albumin g/L	32.39 ±1.3	38.77 ±3.1	0.051
8OHdG ng/mL	106.3±33.3	97.5±17.3	0.042*

* significant difference with $p<0.05$.

CONCLUSION

Oxidative stress is the main chemical manifestation of cardiovascular diseases in the patients of the underlying study. The results revealed a high significant difference between serum malondialdehyde, 8-OHdG and albumin in patients with cardiovascular diseases. The association between deletion of Glutathione-S- transferase gene (in genome) with increasing serum malondialdehyde leads to believe that oxidation damage has adverse effect on defense system in addition to biomolecules.

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