Study the Relation between Homocystein and Some Physiological and Oxidative Stress Parameters in Iraqi Diabetics Patients Type2

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Abstract: The aim of this study is to explain the effect of type2 DM on the concentration of blood glucose, HbA1c, serum uric acid. Lipid profile concentration, Homocysteine (Hcy) concentration, High sensitive CRP (hsCRP) concentration. Oxidative stress parameters that include Lipid peroxidation Malondialdehyde (MDA). Antioxidant parameters that include Glutathione (GSH), water soluble vitamin (vitamin C) and Lipid soluble vitamin (vitamin E). Sixty diabetic patients was used in this study with the range of age from 50 to 69 years from Specialized Center For Endocrine diseases and Diabetesof Baghdad Health Department/Al-Rusafa .This study was included 30 man and 30 woman suffers from diabetes mellitus type 2 , and this samples was divided in to three groups according to the type of treatment they use, group take oral hypoglycemic tab (group A) , group take Insulin (group B) and group take both oral hypoglycemic tab and Insulin (group C).And we take 30 sample it was use as a control group to compare with patients sample. Results explained significantly increases ($p < 0.05$) in F.B.S, HbA_{1c}, Hcy and hsCRP concentration as compared with control group, Results showed significant differences in the

concentration of uric acid as compared with control group in Male and Female patients, While the result of Lipid profile concentration showed significant differences as compared with control group. Oxidative stress parameter include MDA showedsignificantly increases ($p < 0.05$) as compared with control group. While Antioxidant parameters include GSH, vitamin C and vitamin E showed significantly decreases ($p > 0.05$) as compared with control group.

Keywords: Diabetes mellitus type2,Homocysteine,high sensitive CRP(hsCRP), Malondialdehyde (MDA), Glutathione (GSH).

1. Introduction

Diabetes mellitus is a metabolic disease that affects almost 300 million people worldwide, and this number is expected to approach 450 million by 2030[1,2]. Type 2 diabetes is the commonest form of diabetes that associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species (ROS) and oxidative stress[3] .The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, that include

retinopathy, nephropathy, angiopathy and atherosclerosis, that impose a tremendous burden on individual with diabetes and on the health care system[4]. Patients with type 2 diabetes have a high incidence of atherosclerosis, which leads to increased morbidity and mortality from coronary artery disease (CAD), cerebrovascular disease, and peripheral vascular disease (PVD) [5]

Oxidative stress is associated with diabetes, because of excessive production of reactive oxygen species (ROS) and an impaired antioxidant defense mechanism [6,7]. The occurrence of free radical induced lipid peroxidation causes considerable changes in the cell membrane[8]. LipidPeroxidation of the lipid membrane hasbeen related to the pathogenesis of many degenerative diseases, such asatherosclerosis, aging, carcinogenesis and diabetes mellitus[9]. Lipoprotein abnormalities in patient with diabetes are likely to play important role in development of atherogenesis[10]this abnormalities in

lipoproteins are recognized as predictors for coronary heart disease, including elevated total cholesterol (TC) and Very Low Density Lipoprotein –Cholesterol (VLDL) and a predominance of low density Lipoprotein –Cholesterol (LDL)[11] , while reduced plasma levels of high density Lipoprotein –cholesterol (HDL-C) concentration and elevated plasma levels of Triacylglycerol (TAG)[12'13].

Serum uric acid is the final product of purines metabolism, has recently been associated with metabolic syndrome[14'15]Moreover many studies have assessed the association of serum uric acid levels with the incidence of impaired fasting glucose in type 2 diabetesmellitus (T2DM)[16'17], which suggest hyperuricemia to be an early importance indicator of impaired glucose control[18'19].

Glycatedhaemoglobin (HbA_{1c}) is recognized as the best index for long-term glucose control in diabetic patients [20] also, it is regarded as a useful screening tool for detecting diabetes in

general population[21'22]. HbA_{1c} has been found to be associated with atherosclerosis[23'24]incidence of diabetes, cardiovascular disease (CVD)[25'26]

Homocysteine (hcy) is a nonessential sulphur-containing amino acid and an intermediary metabolic product derived from the demethylated essential amino acid methionine [27]. Circulating homocys-teine derives from the interplay of genetic and environmental factors involved in the homocysteine/methionine metabolic cycle. Ageing and gender, renal function, the status of nutritional coenzymes of vitamin B12, B6 and folate, together with lifestyle factors such as smoking, are known deter-minants of plasma homocysteine concentration[28]. Hyper homocysteinemia (Hhcy) has been associated with pathological and stressful conditions and is a risk factor for Cardiovascular disease (CVD)[29] The C-reactive protein being as an acute phase reactant protein that serves as a novel biomarker for inflammation and atherosclerosis.

Generally, it is a risk factor for cardiovascular disease and may specifically predict the development of Myocardial infarction (MI)and stroke in human[30]. CRP may cause atherogenesis by the production of oxygen free radicals (ORs) and expression of adhesion molecules. The oxidative theory of atherosclerosis is based upon the pathophysiological generation of ORs[33].

The human body is equipped with a variety of antioxidants that seem to counterbalance the effect of the reactive oxygen species (ROs) these can be divided into two categories enzymatic and nonenzymetic antioxidant, the non–enzymatic antioxidant include Vitamin C , E , uric acid and GSH triple peptide [32]. Antioxidants can be defined as substances whose presence in relatively high concentration significantly inhibits the rate of oxidation of lipids, proteins, carbohydrates and DNA. Antioxidants such as uric acid (UA and glutathione GSH) act as potent electron donors; they donate hydrogen

atoms to pair up with unpaired electrons on free radicals. Thus, they convert reactive free radicals into inactive substances[33].

2. Subject and method

Subjects: This study was carried out atSpecialized Center For Endocrine diseases and Diabetesof Baghdad Health Department/Al-Rusafa, from September 2016 to March, 2017. That included 60 diabetic patient (30are male and 30 female)and this samples was divided in to three groups according to the type of treatment they use, group A take Oral tape , group B take Insulin and group C take both Oral hypoglycemic drug and Insulin. And 30 normal subject as control, all of them in the range of age 50-69 years.

Blood sample: Ten milliliter (10 ml) of venous blood sample was taken, using plastic disposable syringes . Tow milliliter (2 ml) were added to an ethylene diamine tetra acetic acid (EDTA) tube for Hemoglobin A_{1C} measuring, The remaining 8 ml of the blood were transferred to disposable plain tube. The

serum was separated by centrifugation at 3000 rpm for 5 minutes, and collected in plain tube and kept frozen at (-20ºC) until assayed. Each serum samplewas analyzed for Glucose, Lipid profile, uric acid and Homocysteine, High sensitive CRP(hsCRP), Malondialdehyde (MDA), Glutathione (GSH), Vitamin C&E .

Methods: Fasting Blood Sugar was measured according to Thomas[34]. Uric acid was measured by colorimetric method Thomas[34] . Measurement of glycosylated hemoglobin A1c (HbA1c) according toBaynes, et al.[35].

Measurement of Lipid profile, Serum total cholesterol (TC), Triacylglycerol (TAG) determined according to enzymatic colorimetric method Thomas,[34]High Density Lipoprotein (HDL –C) concentration measured according to Tietz,[36], Estimation of (LDL –C)by the following equation accordimg to Friedewald, et al.[37]. LDL –C = TC – (HDL –C + VLDL –C), and Very Low Density Lipoprotein (VLDL –C) concentration that was

estimated by the following equation according to Friedewald, *et al.,*[37] VLDL –C = TAG / 5. Measurement of serum Homocysteine waspreformed using sandwich enzyme immune assay technique kit was measured according to Upchurch et al.[38].

Measurement of High sensitive CRP protein (hsCRP) by using ichroma[1m]hsCRP by using sandwich immunofluorescence assay, reader analyzes and reads the florescence intensity the CRP concentration I sample Oh SW et al.[39].

Measurement of Malondialdehyde (MDA)according toBurtis&Ashwood[40]. Serum Glutathione (GSH) measured according to Ellman[41].

Serum vitamin C and vitamin E measured by using sandwich enzyme immune assay technique according to lin[42], Bieri, et al.[43].

3. Result

The results of Fasting blood sugar and HbA1C for type 2 diabetesmellitus showed higher significant difference(p<0.05) in Male and Female diabetics groups when compared to the healthy controls.

Table (1): Explain the effect of Diabetes mellitus type2 on Fasting blood sugar (FBS) and Glycosylated hemoglobin A1c (HbA_{1c}) in Male and Female.

FBS . HbA_{1c}		Mean ± SE				LSD value
		Control	group A Oral tab	group B Insulin	group C tab +Insulin	
Male	FBS (mmol/L)	5.62 ± 0.62 b	13.43 ± 3.13 a	13.13 ± 2.42 a	11.85 ± 4.41 a	2.564*
Female		7.64 ± 3.53 c	10.48 ± 2.94 b	10.20 ± 1.56 a	13.13 ± 2.55 a	2.715*
Male	HbA_{1c} (mg/dL)	4.89 ± 0.56 b	11.50 ± 1.47 a	11.63 ± 2.33 a	9.13 ± 2.12 a	3.073*
Female		4.53 ± 0.31 b	9.23 ± 1.65 a	9.45 ± 1.52 a	11.67 ± 2.05 a	2.866*
*(p< 0.05).						

FSB = Fasting Blood Sugar, Mean ± SE = Mean ± Slandered error.

LSD = Less significant differences,HbA_{1c} = Hemoglobin A_{1c}.

serum uric acid concentration showedsignificant differences (p<0.05) in Male and Female diabetics groups when compared with healthy control group.

Table (2): Explain the effect of Diabetes mellitus type2 on serum Uric acid in Male and Female.

Uric acid		Mean ± SE				LSD value
		Control	group A Oral tab	group B Insulin	group C tab +Insulin	
Male	Uric acid (µmol/L)	338.57 ± 31.53 a	282.4 ± 51.79 b	342.54 ± 62.84 a	363.00 ± 13.22a	39.67*
Female		275.60 ± 33.60 a	260.4 ± 50.36 a	266.18 ± 72.92 a	192.00 ± 11.63b	45.09*
*(p< 0.05).						

Mean ± SE = Mean ± Slandered error.

LSD = Less significant differences.

Serum total Cholesterol (TC) result showed non-significant differences (p>0.05) between diabetic groupsin Male and Female with healthy control group. Serum Triacylglycerol (TAG) results explained significant increases (p<0.05)

between diabetics groups among Male and Female compared with healthy control group.High Density Lipoprotein Cholesterol (HDL-C) results showednon-significant differences (p>0.05) inMale groups as compared with control group. While the results of High Density Lipoprotein Cholesterol (HDL-C) in Female groups showed significant differences (p<0.05) as compared with control group.Low Density Lipoprotein (LDL-C) results showed significant differences (p<0.05) in Male groups as compared with control group. While the results of Low Density Lipoprotein in Female groups showed non-significant differences (p>0.05) as compared with control group.

Table (3): Explain the effect of Diabetes mellitus type2 on Lipid profile in both Male and Female.

Lipid Profile	Mean ± SE				LSD value
	Control	group A Oral tab	group B Insulin	group C tab +Insulin	

Male	**cholesterol (mmol/L)**	4.59 ± 0.63 **a**	4.42 ± 0.75 **a**	4.05 ± 0.62 **a**	4.10 ± 0.75 **a**	0.772 **NS**
Female		5.19 ± 0.52 **a**	4.51 ± 0.78 **a**	4.82 ± 0.49 **a**	4.93 ± 0.73 **a**	0.630 **NS**
Male	**Triglyceride (mmol/L)**	2.285 ± 0.44 **c**	3.02 ± 0.92 **b**	2.41 ± 0.45 **c**	3.55 ± 2.28 **a**	0.334*
Female		1.86 ± 0.63 **b**	1.77 ± 0.23 **b**	2.53 ± 0.60 **a**	2.90 ± 1.09 **a**	0.405*
Male	**HDL (mmol/L)**	1.014 ± 0.08 **a**	0.806 ± 0.12 **a**	0.911 ± 0.23 **a**	0.925 ± 0.15 **a**	0.338 **NS**
Female		1.846 ± 0.79 **a**	1.05 ± 0.18 **b**	1.036 ± 0.22 **b**	1.10 ± 0.12 **b**	0.462*
Male	**LDL (mmol/L)**	3.12 ± 0.72 **a**	3.20 ± 0.52 **a**	2.74 ± 0.23 **b**	2.47 ± 0.16 **b**	0.478*
Female		2.97 ± 1.07 **a**	3.11 ± 0.75 **a**	3.28 ± 0.42 **a**	3.25 ± 0.63 **a**	0.361 **NS**
Male	**VLDL (mmol/L)**	0.457 ± 0.08 **b**	0.610 ± 0.04 **ab**	0.482 ± 0.07 **b**	0.710 ± 0.44 **a**	0.269*
Female		0.372 ±	0.353 ±	0.505	0.580 ±	0.275

		0.10 **a**	0.04 **a**	± 0.11 **a**	0.22 **a**	**NS**

Mean **±** SE = Mean **±** Slandered error.

LSD = Less significant differences.

Results of Homomcysteine (Hcy) showed significant increases (p<0.05) between diabetic groups among Male and Female ith healthy control group. The result of High sensitive C-Reactive protein (hsCRP) showed significant differences (p<0.05) in Male and Female as compared with control group. Malondialdehyde (MDA) result showed significant decrease (p<0.05) in Male and Female as compared with control group.

Table (4): Explain the effect of Diabetes mellitus type2 on Homocysteine (Hcy), High sensitive CRP(hsCRP) and Malondialdehyde (MDA).

Homocysteine High Sensitive CRP (hsCRP) Malondialdehyde (MDA)		Mean ± SE				LSD value
		Control	group A Oral tab	group B Insulin	group C tab +Insulin	
Male	Hcy (µmol/L)	4.45 ± 0.22 **b**	10.07 ± 0.63 **a**	9.14 ± 0.75 **a**	10.37 ± 0.62 **a**	2.622*
Female		3.79 ± 0.28 **b**	9.59 ± 0.55 **a**	8.99 ± 0.64 **a**	8.00 ± 0.23 **a**	2.705*
Male	hsCRP (mg/dL)	2.001 ± 0.22 **b**	9.05 ± 2.27 **a**	8.60 ± 1.45 **a**	8.03 ± 1.03 **a**	2.308*
Female		2.023 ± 0.17 **c**	9.16 ± 1.07 **a**	6.83 ± 1.23 **b**	5.70 ± 0.64 **b**	2.641*
Male	MDA (µmol/L)	1.08 ± 0.08 **b**	2.60 ± 0.33 **a**	2.57 ± 0.29 **a**	2.24 ± 0.17 **a**	0.533*
Female		1.25 ± 0.11 **c**	2.74 ± 0.23 **ab**	2.48 ± 0.24 **b**	3.00 ± 0.33 **a**	0.427*

Mean ± SE = Mean ± Slandered error.

LSD = Less significant differences.

Results of Glutathione (GSH) showed significant decreases (p>0.05) in Male and Female as compared with control group, Also the results of Vitamin C explained significant decreases (p>0.05) in Male and Female as compared with control group and the results of Vitamin Erevealed significant decreases (p>0.05) in Male and Female as compared with control group.

Glutathion (GSH) Vitamin C (Vit.C) Vitamin E(Vit.E)		Mean ± SE				LSD value
		Control	group A Oral tab	group B Insulin	group C tab +Insulin	
Male	**GSH (µmol/L)**	3.52 ± 0.33 **a**	1.371 ± 0.15 **b**	1.482 ± 0.12 **b**	1.427 ± 0.09 **b**	0.853*
Female		3.69± 0.31 **a**	1.415 ± 0.06 **b**	1.523 ± 0.12 **b**	1.463 ± 0.04 **b**	0.548*
Male	**Vit.C (mg/dL)**	1.71 ± 0.18 **a**	0.691 ± 0.07 **b**	0.735 ± 0.08 **b**	0.642 ± 0.06 **b**	0.304*
Female		1.63 ± 0.21 **a**	0.710 ± 0.08 **b**	0.702 ± 0.08 **b**	0.680 ± 0.05	0.452*

					b	
Male	**Vit.E (mg/dL)**	0.872 ± 0.09 **a**	0.360 ± 0.04 **b**	0.445 ± 0.06 **b**	0.357 ± 0.07 **b**	0.229*
Female		0.987 ± 0.13 **a**	0.396 ± 0.06 **b**	0.490 ± 0.05 **b**	0.340 ± 0.04 **b**	0.317*

4. Discussion

This study was based on the relation between some of physiological and oxidative stress parameters among diabetics patients type 2, In this study there is significant increases observed in glucose concentration in diabetic groups as compared with control group, this results agree withSuwarto et al.[44].The HbA1c has been used as an objective marker of average glycemic control because the levels of HbA1c in the blood reflect the glucose levels which erythrocyte has been exposed during its lifespan[45]. HbA1c concentration in this study showed significant increases in male and female agree

with results obtained byNada& Abdul Jalil[46] . Serum Uric acid is produced by the metabolism of nitrogen bases(Purine) ,and is considered as a risk factor for diabetes complication. In the study there is significant increases in Male and Female this results agree withHayden &Tyagi[47], Increases in the concentration of uric acid is a well-knownabnormality observed in diabetic patient[48].

Patients with type2 diabetes frequently have an abnormal blood lipid profile consisting ofelevated LDL-C, and triglycerides with decrease in HDL-C. The result of the present study showed non-significant differences in total cholesterol concentration,and increases (p<0.05) in Triacylglycerol concentrationwith significant increases (p<0.05) in Low density Lipoprotein (LDL-C) among Male patient compared with control group, but there is non-significant differences among Female patient compared with control group, ,while there is non-significant differences in High Density Lipoprotein (HDL-C) among Male patient compared with control group,), but there is significant decreases (p>0.05) inHigh Density Lipoprotein (HDL-C) among Female patient compared with control group.Very Low Density Lipoprotein (VLDL –C) in Male diabetic patient there was significant increase (p<0.05) as compared with control group, while there was non-significant differences in Female diabetic patient as compared

with control group. This result agreement withFinchet al.[49].Hypercholesterolemia and Hypertriglyceridemia were associated with oxidative modification of LDL-C, protein glycation and glucose auto oxidation, thus leading to excess production of lipid peroxidation products which may cause elevation of oxidative stress in higher lipid and hyperlipidemic subjects[50]. Oxidative stress was indicated by increased free radicals production, the generation of free radicals may lead to lipid peroxidation and the formation of several types of damage in diabetes mellituspatrint. In this study we observed that a lipid peroxidation product, MDA level as a marker of oxidativestress, were elevated significantly in diabetic patients in both Male and Female.Similar result were observed byMahboob[51]. Oxidative stress linked to cardiovascular disease duo to oxidation of LDL –C in vascular endothelium is a precursor to plague formation. Oxidative stress also play a role in the ischemic caused duo to oxygen reperfusion injury following hypoxia that include strokes

and heart attacks, oxidative stress has been implicated in chronic fatigue syndrome[52], that contributes to tissue injury in many condition one of them Diabetes mellitus.

Homocysteine concentration in type2 diabetics patients significantly increase in Male and Female group compared with its concentration in control group this result corresponds with the result ofMoselhy&Demerdash[53]. Hyperglycemia in type2 diabetics patient causes abnormal lipids, carbohydrates and proteins metabolisms which may leads to abnormal elevated homocysteine concentration, also some medications which is used in great number of diabetics is known to cause vitamins B12 and folate deficiencies that causes consequently leads to hyperhomocysteinemia[54]. Many studies have shown that Hcy acts on the cardiovascular system with a direct toxicity on the endothelium and increase in vascular smooth muscle cells inducing its proliferation, enhance collagen production[55].

The study showed significantly increase (p<0.05) in the concentration of High sensitive C-Reactive Protein (hsCRP) among diabetic groupthis result combatable with the result present byAl-Thanoon&Mahmood[56].Oxidative stress play a multiple role in the inflammatory response by Cytokine – related ROS release and by regulation of transcription factors its proinflammatary cytokine especially IL6, IL –1β and TNF –α produced by monocyte and the acute phase reactant CRP protine released in response to ROS and also important in plug formation[57].Homocysteine and hsCRP which both indicates the role of oxidative stress and inflammation in atherosclerosis[58].

Products of lipid peroxidation such as MDA are capable of inactivating many cellular proteins by forming protein cross linkage[59]. Lipid peroxidation products MDA it used as abiomarkersof oxidative stress.This study showed significantly increase in the MDA concentration of diabetics patient

compared with control group.This result agreed with the result submitted byVarashree&Bhat[60].MDA considered as an important indicator for evaluating oxidative stress in degenerative diseases like diabetes mellitus. The elevated in the MDA level indicated that any oxidative stress incurred sufficiently cause of free radical mediated peroxidation of lipid components in cell membrane, which lead to the damage of the cell[61]

The study showed significant decreases (p>0.05) in GSH concentration in Male and Female diabetic patient as compared with control group.

GSH is important for the protection of cell membrane from lipid peroxidation and protect lipids from oxidant attacks[62]. These result agree with our results that the concentration of GSH decreased significantly in diabetic patient, low GSH level in red blood cells reflects generalized decrease in intracellular content of this compound. Similar result was obtained byLivingstone& Davis[63]. These results indicate

that patients with type 2 diabetes have lower concentration of intracellularGSH,which increases thesusceptibility of cells to the damaging effects of RO

The present study showed significant decreases (p>0.05) in vitamin C concentration in diabetic patient as compared with control group in Male and Female, The result obtained in these study agree withTarng et al.[64]. Also the result of vitamin E showedsignificant decreases (p>0.05) in diabetic patient as compared with control group in Male and Female, Vitamin E (Alph –Tocopherol) has a biochemical efficacy in beneficially altering the biomarkers of oxidative stress and in increasing erythropoiesis or reducing the required dose of erythropoietin. Also vitamin E may help stabilize atherosclerotic plug[65].

Conclusion

1. Diabetes mellitus considered as a chronic disease cause they affects the lives of millions of people around the

world, and generally conceder as an oxidative stress disease .

2. Elevated homocysteine level as one of diabetes mellitus type2 complications, that conceder as a risk factor for CVD, also the increase level of (hsCRP)conceder as specific parameters for Heart disease.
3. Increase in the level of some oxidative stress parameters such as(Glucose ,HbA1c, Lipid profile, Uric acid and MDA) as an expected result from diabetic complication.
4. Decreases in the level of antioxidant defense (GSH, Vit C and Vit E) .

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