ESTIMATION OF HOMOCYSTEINE LEVELS IN TYPE 2 NON HYPERTENSIVE AND HYPERTENSIVE DIABETIC PATIENTS

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ABSTRACT

Background: Hyperhomocysteinemia increases risk of morbidity and mortality secondary to cardiovascular diseases such as increased risk of atherosclerosis, ischemic heart disease and peripheral vascular disease and has been associated with microalbuminuria in type II diabetes mellitus. **Subjects and Methods:** 100 patients, 50 hypertensive diabetic patients, 50 normotensive diabetic patients, and 20 patients as control group matched for age, gender and body mass index (BMI) and were clinically assessed and laboratory investigations were done through estimation of plasma homocysteine, lipid profile, fasting and two hours post prandial blood glucose, and serum creatinine. **Results:** Homocysteine was elevated more in hypertensive diabetic group than diabetic group with high significant correlation (P=0.001), moreover homocysteine was highly significant correlated to BMI fasting blood glucose, cholesterol, serum triglyceride (P=0.001), and insignificant correlation to serum creatinine in both goups. **Conclusion:** Homocysteine is a significant risk factor in hypertensive diabetic patients. Using folic acid supplement 0.4mg daily is essential to reduce level of serum homocysteine especially in hypertensive, diabetic patients to decrease risk of cardiovascular events in addition of controling blood pressure, blood sugar and obesity.

Key words: homocysteine, hypertension, type II DM, cardiovascular disease

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INTRODUCTION

Homocysteine is an amino acid contains sulphur and derived from methionine through demethylation of S-adenosylmethionine (SAM) and S-adenosyl homocysteine (SAH).

Methionine is an essential amino acid derived partly from diet and partly from methionine recycle system .[1]

Patients with the inborn metabolic error affecting homocysteine have markedly elevated plasma homocysteine and also in urine which is considered main factor contributing in adulthood and also in childhood occlusive cardiovascular diseases [2]

The theoretical role of homocysteine as a cause of atherosclerosis was demonstrated in 1976 in a published study about the role of homocysteine metabolism in patients with coronary artery disease. Since then many evidences had been gathered on the relation between moderate elevation of plasma homocysteine and the risk of occlusive vascular diseases as coronary, cerebral, and peripheral arteries and also venous thrombosis which is recently discovered.[3, 4]

The mechanism of atherosclerosis related with hyperhomocysteinemia is still unknown many biochemical mechanisms try to explain the vascular pathology including autointoxication through the production reactive oxygen species, hypomethylation by forming SAH, a potent inhibitors of biological transmethylations, nitrosylation by binding to nitric oxide and protein homocysteinylation by incorporating into protein.[5,6,7]

It was found that in patients with metabolic syndrome especially hypertensive patients, plasma homocysteine was elevated and the prevalence of cardiovascular events increases with the increased plasma concentration of homocysteine [8]

Patients with diabetes mellitus are more liable to cardiovascular disease especially when homocyteine concentration in plasma is elevated which may be involved in the athero-embolic process. [9]

This study was performed to estimate homocysteine levels in non hypertensive diabetic subjects in comparison to hypertensive diabetic subjects and analyse any difference between two groups and in comparison to control group.

SUBJECTS AND METHODS

The study included 100 patients, 50 were known to be hypertensive with type II diabetes mellitus, and 50 were diabetics.

Hypertensive patients were on antihypertensive drugs, none was taking vitamin supplementation and none was complicated by ischemic heart disease, myocardial infarction or congestive heart failure.

The age ranges from 30-70 years, males, non pregnant , non lactating females, 20 persons as control group

All were evaluated clinically, ECG was done, BMI, lipid profile, blood glucose and plasma homocysteine , and serum creatinine

Laboratory methods:

Principle of measurement of homocysteine:

Homocysteine was measured by (ELISA) method by commercially available kin from USCAN LIFE from USA.

The micro titer plate has been pre-coated with an antibody specific to homocysteine. Standards and samples were the added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for homocysteine and avidin. Conjugated to Horseradish Peroxidase (HRP) was added to each well. Only those wells that contain homocystein, biotin-conjugated antibody and enzyme-conjugated.

Avidin will exhibit a change in color. The enzyme substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectophotometrically. The concentration of homocysteine in the samples is then determined by comparing the O.D. of the samples to the standard curve.

STATISTICAL ANALYSIS

The clinical data were recorded on a report form. These data were tabulated and analyzed using the computer program SPSS (Statistical package for social science) version 16 to obtain:

Descriptive data

Descriptive statistics were calculated for the data in the form of:

1. Mean and standard deviation $(\pm SD)$. for quantitative data.
2. Frequency and distribution for qualitative data.

Analytical statistics

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests:-

1- Student's *t*-test:- Used to compare mean of two groups of quantitative data.

$$t = \frac{\bar{x}_{1-}\bar{x}_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}}$$

2- ANOVA test (F value):-Used to compare mean of more than two groups of quantitative data.

Inter-group comparison of categorical data was performed by using chi square test (X^2-value) and fisher exact test (FET).

$$x^2 = \frac{\sum(observed - \exp ected)^2}{Expected}$$

$$Expected = \frac{col.total\ x\ row\ total}{Grand\ total}$$

3- Correlation coefficient:- to find relationships between variables.

A *P* value <0.05 was considered statistically significant (S) while >0.05 statistically insignificant P value <0.01 was considered highly significant (HS) in all analyses.

RESULTS

The study included 100 patients, 50 were known to be hypertensive, type II diabetes mellitus group (1), 50 were known to be normotensive, type II diabetes mellitus group (2), and 20 as control group.

Age ranged from 30-70 years, in group (1): 9 (18%) males41 (82%) were females. In group (2): 13 (26%) were

males, 37 (74%) were females, and control group 6 (30%) were males, 14 (70%) were females.

In comparison between group I, II, and control: homocysteine level was more elevated in group I (19.99±5.86) in correlation to group II (17.34±5.91) and control group (10.56±2.1) with high significant relationship. (P=0.001)(Table 1), (Figure 1)

There was high significant correlation between BMI, blood sugar and serum cholesterol between group I, group II, and control group. (P=0.001) (Table 1)

There was insignificant correlation between homocysteine level between male and female patients (Table 2).

Serum triglyceride (TG), was higher in group II (145.46±53.15), in comparison to group (2) (126.94±32.14) with significant correlation (P=0.038), and serum creatinine was higher in group I (1.0±0.43), than in group II (0.84±0.37) with significant correlation (P=0.043) (Table 3).

In group I: there were high significant correlation between homocysteine and BMI, fasting blood glucose, serum cholesterol and serum triglyceride (P=0.001), but the correlation between homocysteine with serum creatinine and prandial blood glucose was insignificant (P>0.05). as high significant relationship between homocysteine, BMI, fasting, prandial blood glucose and cholesterol (P=0.001) and significant correlation with serum triglyceride (P= 0.042) and the correlation with serum creatinine was insignificant. Table (4).

Table (1): Comparison between studied groups.

	Group I	Group II	Control group	F test	P value
Sex					
Male	9(18.0)	13(26.0)	6(30.0)	FET=1.61	0.462
Female	41(82.0)	37(74.0)	14(70.0)		
Homocysteine	19.99±5.86^£	17.34±5.91^	10.56±2.1	16.39	0.001**
BMI	33.24±3.66^	34.48±4.62^	27.35±2.32	24.12	0.001**
FBS	190.64±78.37^	208.48±74.02^	89.2±11.08	21.58	0.001**
2h PP	266.18±117.96^	279.7±99.0^	116.15±16.35	20.77	0.001**
Cholesterol	201.82±37.04^	211.48±35.41^	179.75±19.23	6.21	0.003**
TG	126.94±32.14£	145.46±53.15^	105.5±35.15	6.68	0.002**
Creatinine	1.0±0.43^£	0.84±0.37	0.68±0.14	6.02	0.003**

^=significant with control group £=significant with group II **=highly sig

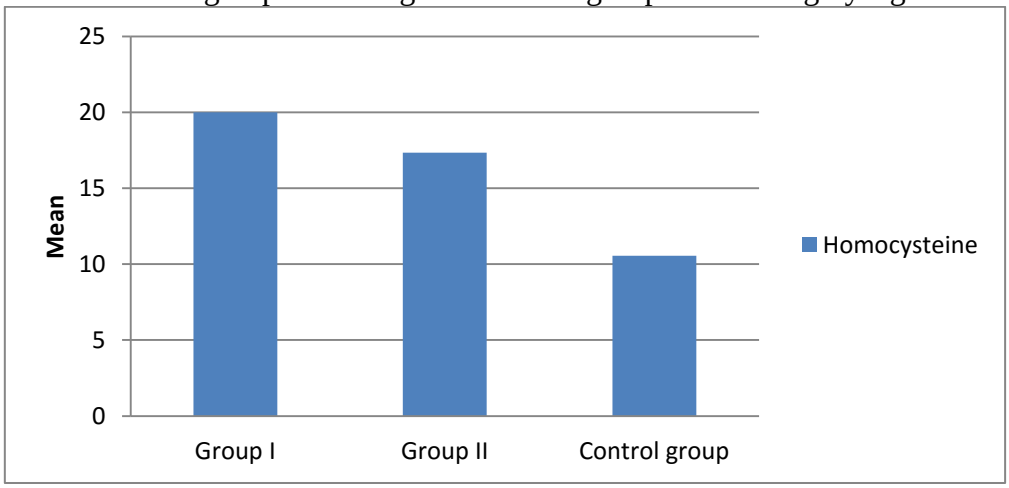

Figure (1): comparison of homocysteine in different groups

Table (2): Sex difference regarding homocysteine.

Homocysteine	Male	Female	St t test	P value
Group I	22.33±4.44	18.11±6.16	1.94	0.058
Group II	19.62±6.33	16.54±5.62	1.65	0.106
Control group	10.9±1.9	10.41±2.22	0.472	0.643

Table (3): Comparison between group I and group II.

	Group I	Group II	St t test	P value
Sex				
Male	9(18.0)	13(26.0)	X^2=0.932	0.334
Female	41(82.0)	37(74.0)		
Homocysteine	19.99±5.86	17.34±5.91	21.44	0.001**
BMI	33.24±3.66	34.48±4.62	1.49	0.14
FBS	190.64±78.37	208.48±74.02	1.17	0.245
2h PP	266.18±117.96	279.7±99.0	0.621	0.536
Cholesterol	201.82±37.04	211.48±35.41	1.33	0.186
TG	126.94±32.14	145.46±53.15	2.11	0.038*
Creatinine	1.0±0.43	0.84±0.37	2.05	0.043

*=significant

Table (4): Correlation between homocysteine and other variables.

Homocysteine	Group I		Group II		Control group	
	r test	P value	r test	P value	r test	P value
BMI	0.506	0.001**	0.72	0.001**	-0.331	0.154
FBS	0.466	0.001**	0.633	0.001**	0.116	0.626
2h PP	0.277	0.051	0.511	0.001**	-0.051	0.832
Cholesterol	0.554	0.001**	0.458	0.001**	0.459	0.042*
TG	0.424	0.002**	0.351	0.012*	0.153	0.519
Creatinine	-0.101	0.486	-0.084	0.561	0.091	0.702

**=highly sig

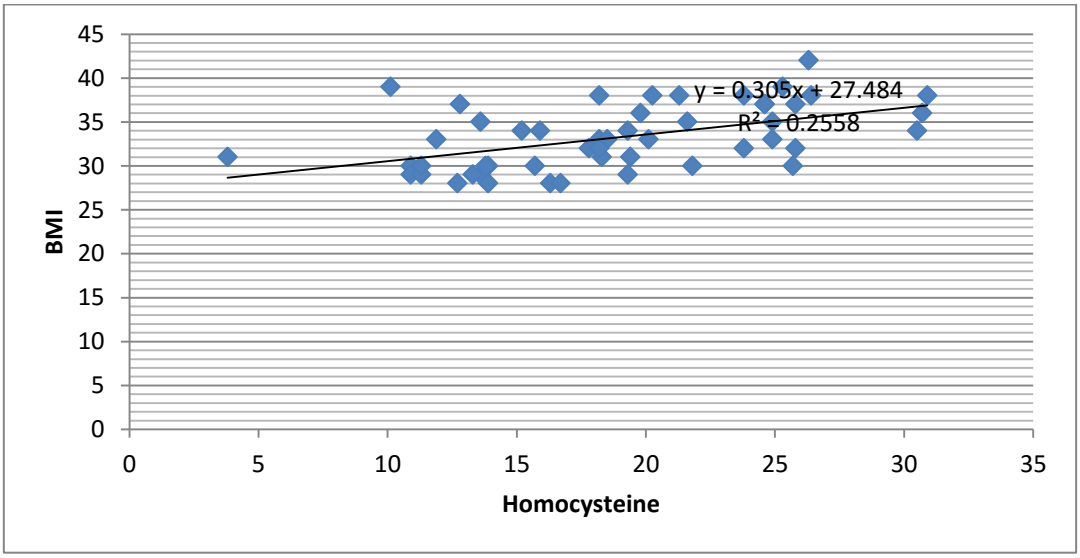

Figure (2): Correlation between homocysteine and BMI in group (1)

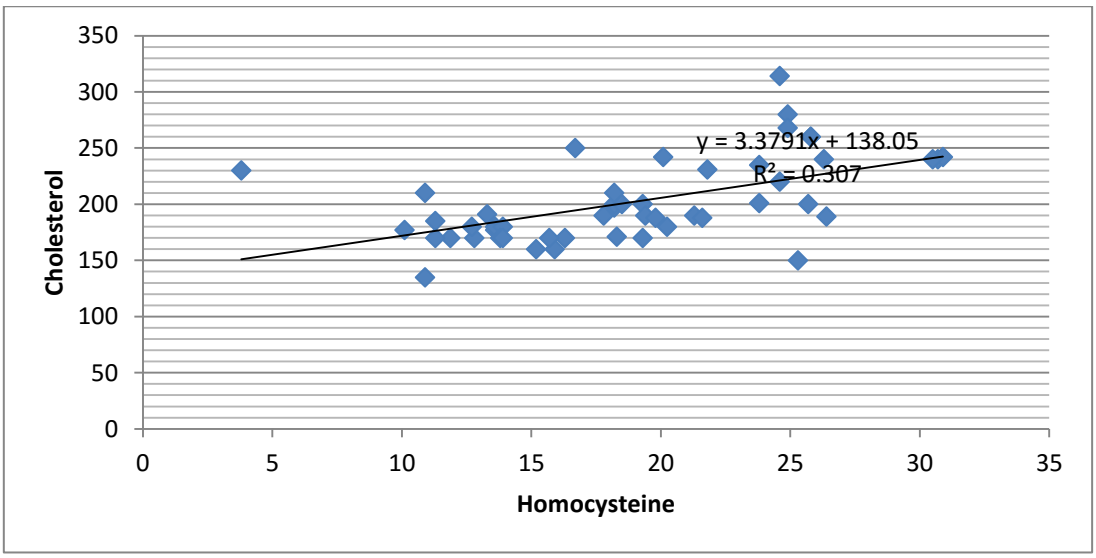

Figure (3): Correlation between homocysteine and cholesterol in group(1)

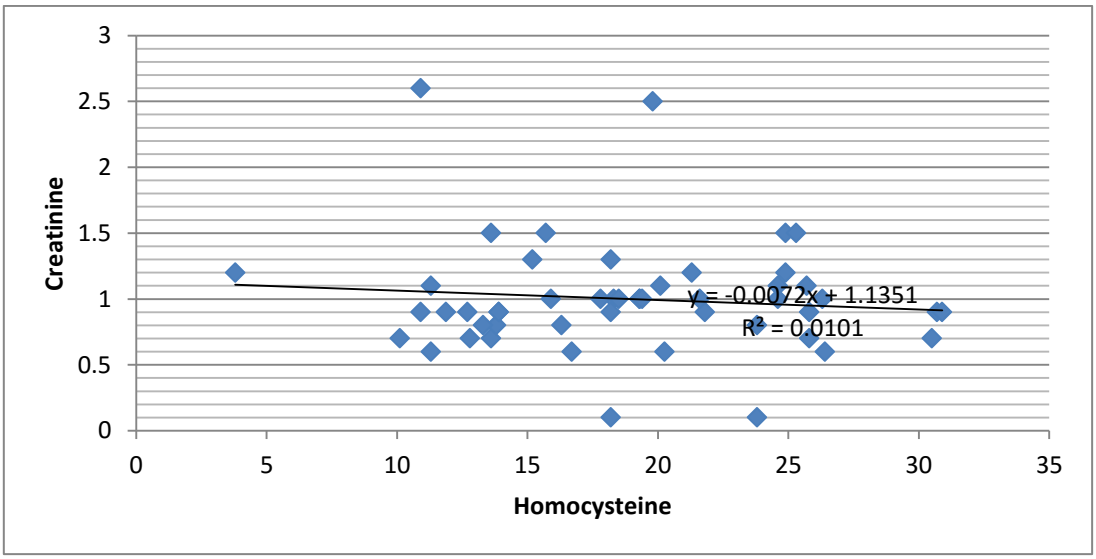

Figure (4): Correlation between homocysteine and serum creatinine in group (1)

DISCUSSION

The causes of hyperhomocysteinemia are multifactorial such as genetic defects, pathological conditions, lifestyle and drugs related hyperhomocysteinemia.[11,12]

Increased level of homocysteine is considered a risk factor in cardiovascular related morbidity and mortality such as the increased risk of atherosclerosis related myocardial infarction, peripheral vascular disease and microalbuminuria and retinopathy in both type I, type II diabetes mellitus (DM).[13,14]

Hyperhomocysteinemia is involved in the plaque formation through damaging endothelial cells of the vascular wall and also interferes with nitric oxide role as a vasodilator derived from endothelial cells, also hyperhomocysteinemia enhances smooth muscle cells hypertrophy and both mechanisms induce vascular occlusion.[15,16]

B vitamins are essential cofactor on the metabolism of homocysteine to methionine via the remethylation-pathway, (vitamin B_{12}, folic acid) and to cystathionine via the trans-sulphuration pathway (vitamin B_6).[17, 18]

In a study on 282 patients showed that increasing age the homocysteine level is increased and also oxidative stress similarly to ypung diabetic hypertensive patients irrespective to their age. [19]

In this study, homocysteine level was more elevated with hypertensive, diabetic group more than diabetic normotensive group in comparison to control group with high significant correlation. (P=0.001). This result is in agreement with (Qureshi et al., 2003) and (Cassone-Fladetta et al 2000) which showed that the levels of plasma homocysteine elevated in hypertensive diabetic patients.[20,21]

It was found that there was a relationship between high homocysteine levels and prevalence of macroangiopathy, retinopathy and nephropathy especially in type I DM.[22]

(Dulex et al., 2005) demonstrated that hyperhomocysteinemia especially in type II DM with coronary heart disease (CHD) and significantly correlated with glycated hemoglobin, serum creatinine and apolipoprotein B.[23,24]

In this study, there was high significant correlation between homocysteine, BMI, blood glucose and lipid profile in hypertensive diabetic patients and diabetic normotensive group (P=0.001) which are considering risk factors of atherosclerosis related cardiovascular diseases.

This is also matched with a study done on 64 diabetic patients and demonstrated that hyperhomocysteinemia was significant in patients with coronary heart disease of the studied group.[6]

In our study, the sex difference was insignificant between both hypertensive diabetic group, diabetic normotensive group in comparison to control group, (P>0.05) and also there was insignificant correlation between homocysteine and renal function in both groups which came in agreement with a study demonstrated that renal function was independent determinants of homocysteine levels in patients with type II diabetes mellitus.[25]

Folic acid and B vitamins required for remethylation of homocysteine to methionine. Daily supplementation typically lowers plasma homocysteine levels.[16,26]

Severe or moderate hyperhomocysteinemia can be treated with vitamin B complex especially vitamin B6 B12 and also folic acid in a daily dose of 0.4mg has also shown reduction in plasma homocysteine level of normal persons. [27]

Moreover the endothelial function also has been improved by lowering plasma homocysteine, so short term homocysteine lowering therapy has some clinical benefits when used for 12 months and was demonstrated that it decreases the incidence of adverse events after percutaneous coronary intervention. [28]

A study done by (Aghamohammade V et al.,2011) was demonstrated that pharmacological doses of folate supplementation lowered plasma homocysteine in patients with type II diabetes mellitus, also in a prospective population-based Cohort study in china was shown that hypermonocysteinemia in chinese hypertensive patients was efficiently reduced by folic acid supplementation.[23,29]

CONCLUSION

hyperhomocysteinemia increases cardiovascular morbidity and mortality in type II diabetes mellitus especially if it was associated with hypertension and more risk appears in obese patients with abnormal lipid profile so reduction of homocysteine level by using folic acid supplementation of 0.4mg daily is essential. In addition to control of body weight and use of antihypertensive drugs to control hypertension.

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