World Applied Sciences Journal 16 (9): 1269-1273, 2012
ISSN 1818-4952

Plasma Homocysteine Level in Patients with *Diabetes mellitus*

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Abstract: This descriptive case series study evaluates the frequency of hyperhomocysteinemia in patients with diabetes mellitus & conducted at Liaquat University Hospital Hyderabad from January 2011 to June 2011. All patients with diabetes mellitus were further evaluated for plasma homocysteine level and also for the macro and microvascular complication of the diabetes mellitus in association with plasma homocysteine. Out of 100 diabetic patients, 70 were type 2 diabetes and 30 patients were type 1 *diabetes mellitus*. The mean age and standard deviation of patients with type 1 and type 2 diabetes mellitus was 18.63±2.51 (SD) and 51.82±4.92 (SD), respectively. The mean ±SD for plasma homocysteine in overall subjects was 87.65±12.94. The mean random blood glucose level was 273.74±10.55 (SD) in hyperhomocysteinemia diabetics whereas it was 135.78±8.73 (SD) in normohomocysteinemia diabetic patients. The hyperhomocysteinemia was observed in 58 patients, of which 48 were in type 2 diabetes and 10 were in type 1 diabetes (p=0.03). Of hyperhomocysteinemic population 44(75.9%) were males and 14(24.1%) were females (p=0.02). The HbA1c was raised in 38(65.5%) hyperhomocysteinemic patients (p=0.04). The duration of diabetes was >5 years in majority of subjects. Regarding severity 14/58 (24%) were in moderate class, 20/58 (35%) were in intermediate class and 24/58(41%) were in severe class of hyperhomocysteinemia (p=0.86). The macrovascular complications observed in 35(60%) and microvascular 23(40%) hyperhomocysteinemic diabetic population (p=0.05). The chronic poor metabolic control of diabetes mellitus is associated with elevation of plasma homocysteine concentration.

Key words: Homocysteine · Hyperhomocysteinemia · Diabetes Mellitus

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by increase blood glucose level resulting from defects in insulin secretion, insulin action, or both [1].The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. The cross sectional survey conducted earlier in rural and urban areas of Pakistan upon 5433 individual which show 19% prevalence of diabetes mellitus [2]. The number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future, is important to allow rational planning and allocation of resources [3]. There are an estimated 23.6 million people in the U.S. (7.8% of the population) with diabetes and 17.9 million being diagnosed, [4] 90% of whom are type 2 [5]. With prevalence rates doubling between 1990 and 2005, CDC has characterized the increase as an epidemic [6]. Pakistan is estimated to have 7 million people with diabetes and presently according to WHO estimation of prevalence of diabetes it is 8th in the world and by the year 2025 is expected to be 4th with 15 million people with diabetes, representing a 2 fold increase in caseload [7]. The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged peoples [8].

The Homocysteine (Hcy) is an amino acid, is a homologue of the amino acid cysteine, differing by an additional methylene (-CH2-) group. It is biosynthesized from methionine by the removal of its terminal methyl group and can be recycled into methionine or converted into cysteine with the aid of B-vitamins [9]. Increased

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circulating levels of homocysteine are seen in patients with homocysteinuria, patients with homozygosity for the thermolabile variant of methylenetetrahydrofolate reductase, or individuals with dietary deficiency of folate and or cyanocobalamin [10].

Diabetes mellitus is a clearly recognized risk factor of the development of atherosclerosis, which is two to three times more frequent than in the normal non-diabetic population [11]. Homocysteine levels in patients with diabetes mellitus have been reported as either low or elevated compared to control non-diabetic groups. By keeping such information in mind the present study was conducted at tertiary care teaching hospital Hyderabad / Jamshoro. The objective of this study is to evaluate the plasma homocysteine levels in patients with diabetes mellitus and the potential role of homocysteine in the macro and microvascular complications in diabetic patients.

MATERIAL AND METHODS

This descriptive case series study was conducted in medical ward at Liaquat University Hospital Hyderabad from January 2011 to June 2011 on the patients with history of *diabetes mellitus* for ≥ 3 years duration attending the medical OPD or admitted in medical ward. The inclusion criteria for the study were patients ≥ 18 years of age present at Liaquat university hospital, known cases of *diabetes mellitus* (type 1 and type 2), of either gender and patients who were agree and ready to give consent for participation in the study; whereas the exclusion criteria of they study were: Patients with acute pancreatitis, pernicious anaemia, severe hepatic impairment, renal impairment, psoriasis, hypothyroidism, systemic lupus erythematosus (SLE), anorexia nervosa, organ transplantation, malignancies of breast, ovary, pancreas and acute lymphoblastic leukaemia, severe skeletal muscle damage or trauma, the patients already on folic acid, pyridoxine and vitamin B12 therapy and who refused to give written consent for participation in the study. A written consent was taken from all patients for participation in the study. The detail history was taken and relevant clinical examination was performed including fundoscopy to evaluate retinopathy. All the baseline investigations with relevant including lipid profile, serum folic acid, vitamin B12 level, 24 hours urinary creatinine clearance, Doppler ultrasound, nerve conduction studies, relevant radiographs, electrocardiography and echocardiography was performed to evaluate the micro and macro vascular complications of diabetes mellitus. All such patients who meet the inclusion criteria were evaluated for their blood glucose level and plasma homocysteine level by taking 3 cc fasting venous blood sample in a disposable syringe and sent to laboratory for analysis. The normal plasma homocysteine level is between 5 to <15 µmol/L. The results of plasma homocysteine level were interpreted as normal, moderate, intermediate and severe according to the reference range (moderate = 15 to 30 µmol/L; intermediate = 30 to 100 µmol/L; severe = >100 µmol/L). The HbA1C estimation was carried out by a modified calorimetric method. The data was collected on pre-designed proforma and all such maneuvers were under medical ethics. The frequency and percentage was calculated for hyperhomocysteinemia in *diabetes mellitus* 1 & 2 as well as for gender distribution. The chi-square test was applied between categorical variables at 95% confidence interval while independent t-test was also applied as far as mean ± SD concerned. The p-value ≤ 0.05 was considered as statistically significant. The stratification was done between hyperhomocysteinemia and duration of diabetes where as the mean ± standard deviation (SD) calculated for age.

RESULTS

Out of 100 diabetic patients, 70 were type 2 diabetes and 30 patients were type 1 *diabetes mellitus*. The mean age and standard deviation of patients with type 1 and type 2 diabetes mellitus was 18.63±2.51 (SD) and 51.82±4.92 (SD), respectively. The mean ±SD for plasma homocysteine in overall subjects was 87.65±12.94. The distribution of diabetic patients participated in the study is shown in table 1 where as the frequency of hyperhomocysteinemia as far as *diabetes mellitus* and gender is concerned is shown in tables 2 and 3. The mean random blood glucose level was 273.74±10.55 (SD) in hyperhomocysteinemia diabetic whereas it was 135.78±8.73 (SD) in normohomocysteinemia diabetic patients. The hyperhomocysteinemia in relation to haemoglobin A1c (HbA1c) is shown in Table 4. The hyperhomocysteinemia in relation to duration of diabetes is shown in table 5. Gender distribution in relation to severity of hyperhomocysteinemia in patients with diabetes mellitus is shown in Table 6. The microvascular and macrovascular complications of diabetes mellitus in relation to severity of hyperhomocysteinemia is shown in table 7. Regarding the demographical distribution majority of the patients (76%) were belonged to rural populations.

Table 1: Gender distribution of diabetic patients

	Diabetes Mellitus			
	Type 2	Type 1	Total	P - Value
Gender				
Male	58 (72.5%)	09 (45.0%)	67 (67.0%)	0.01*
Female	22 (27.5%)	11 (55.0%)	33 (33%)	
Total	80 (100%)	20 (100%)	100 (100%)	

*p-value is statistically not significant
X^2 value = 5.47; df = 1

Table 2: Frequency of hyperhomocysteinemia in patients with diabetes mellitus

	Homocysteine			
	Hyperhomocysteinemia	Normal	Total	P-value
Diabetes				
Type 2	48 (82.8%)	32 (76.2%)	80 (80.0%)	0.03*
Type 1	10 (17.2%)	10 (23.8%)	20 (20.0%)	
Total	58 (100%)	42 (100%)	100 (100%)	

*p-value is statistically significant
X^2 value = 5.63; df = 1

Table 3: Gender distribution in relation to homocysteine level in patients with diabetes mellitus

	Homocysteine			
	Hyperhomocysteinemia	Normal	Total	P-value
Gender				
Male	44 (75.9%)	23 (54.8%)	67 (67.0%)	0.02
Female	14 (24.1%)	19 (45.2%)	33 (33.0%)	
Total	58 (100%)	42 (100%)	100 (100%)	

*p-value is statistically significant
X^2 value = 4.90; df = 1

Table 4: Frequency of hyperhomocysteinemia in relation to glycated hemoglobin (hba1c)

	HOMOCYSTEINE			
	Hyperhomocysteinemia	Normal	Total	P-value
HbA1c				
Raised	38 (65.5%)	19 (45.2%)	57 (57.0%)	0.04*
Normal	20 (34.5%)	23 (54.8%)	43 (43.0%)	
Total	58 (100%)	42 (100%)	100 (100%)	

*p-value is statistically significant
X^2 value = 4.08; df = 1

Table 5: Prevalence of hyperhomocysteinemia and duration of diabetes mellitus

Duration of diabetes	No: of patients	Hyperhomocysteinemia	Prevalence (%)
3-5 years	20	07	35
6-10 years	34	25	74
11-15 years	28	19	68
15-20 years	12	03	25
> 20 years	06	04	67

Table 6: Gender distribution in relation to severity of hyperhomocysteinemia in patients with diabetes mellitus

	Homocysteine				
	Moderate	Intermediate	Severe	Total	P-value
Gender					
Male	10 (71.4%)	15 (75.0%)	19 (79.2%)	44 (75.9%)	0.86*
Female	04 (28.6%)	05 (25.0%)	05 (20.8%)	14 (24.1%)	
Total	14 (100%)	20 (100%)	24 (100%)	58(100%)	

*p-value is statistically non significant
X^2 value = 0.30; df = 2

Table 7: Microvascular and macrovascular complications of diabetes mellitus in relation to severity of hyperhomocysteinemia

	Homocysteine				
	Moderate	Intermediate	Severe	Total	P-value
Complication					
Macrovascular	05 (35.7%)	12 (60.0%)	18 (75.0%)	35 (60.3%)	0.05*
Microvascular	09 (64.3.6%)	08 (40.0%)	06 (25.0%)	23 (39.7%)	
Total	14 (100%)	20 (100%)	24 (100%)	58(100%)	

*p-value is statistically significant
X^2 value = 5.70; df = 2

DISCUSSION

Plasma homocysteine levels are elevated in patients with diabetes, particularly in patients with type 2 diabetes as well as in individuals in prediabetic states who exhibit insulin resistance. The levels of homocysteine in such individuals are also influenced by their insulin concentrations, therapy with insulin and medications such as metformin and glitazones that can either raise or lower homocysteine levels. In present study we found hyperhomocysteinemia in patients of diabetes mellitus with the male predominance and it is consistent with the study of Schalinske [12].

Hyperhomocysteinaemia is known to be a risk factor for vascular occlusive diseases [13]. Elevated levels of plasma homocysteine have been found in patients suffering from peripheral vascular occlusions, such as coronary artery disease, cerebral vascular accidents and deep-vein thrombosis as well as from ocular vascular occlusions such as retinal vein and retinal artery and anterior ischaemic optic neuropathy [13]. In recent prospective studies on large populations, significantly higher concentrations of homocysteine were found in the groups of patients with diabetes [14]. Hoogeven have reported on increased mortality due to CVD in the population of diabetic patients with high Hcy levels in the prospective Hoorn study [15, 16]. Meigs have found that hyperhomocysteinemia is an independent risk factor of CVD incidence in diabetic patients [17].

Strong association between atherosclerosis, hyperhomocysteinemia and diabetes type 2 has been found in the Japanese population [18]. There have been reports of lower than in general population Hcy levels in the group of patients with diabetes contributing to a higher rate CVD mortality.

In the present study higher levels of Hcy in diabetic subjects were noticed. Similarly to other authors, we found a relation between the concentration of Hcy and incidence of macrovascular complication in the groups of diabetic patients in our series. The microvascular disease in diabetes mellitus is largely manifested as retinopathy and nephropathy. Studies that have examined the relationship between homocysteine levels in patients with diabetes mellitus and the presence of microvascular disease have shown correlation between nephropathy and retinopathy [13].

Hyperhomocysteinaemia correlates with both change in glomerular filtration rate (GFR) as well as the presence of microalbuminuria. Homocysteine concentrations have, however, been shown to correlate with the presence of diabetic peripheral neuropathy [19] and have also been associated with the presence of autonomic neuropathy in patients with type 1 diabetes. The relationship between homocysteine levels and the presence or absence of macro and microvascular disease in patients with diabetes shows, that for the most part, homocysteine elevation in patients with diabetes mellitus only occurs when renal function deteriorates. Buysschaert [20] have found a higher incidence of complications such as macroangiopathy and nephropathy in the population of 122 diabetics in the group with hyperhomocysteinemia in comparison to the patients with normal Hcy level.

In our study the HbA1c is raised in patients with hyperhomocysteinemic diabetic population that shows poor diabetic control. The results achieved by Drzewoski and Czupryniak and colleagues indicate that metabolic control of diabetes may influence Hcy levels. Those authors have shown that patients with bad metabolic control of diabetes (HbA1c-9.8%) had significantly higher Hcy levels in comparison to diabetics with normal HbA1c levels (6.6%). Statistically significant correlation between Hcy concentration and HbA1c was also noticed in some studies [21]. However Hoogeven *et al*, did not note such dependence [16]. Determination of the role of hyperhomocysteinemia in macrovascular and microvascular diabetes complications could be of importance in their prevention through dietary and pharmacological modifications of homocysteine levels.

CONCLUSION

The hyperhomocysteinemia is detected in patients with diabetes mellitus which may contribute to the development of chronic complications. The influence of diabetes treatment on Hcy levels requires further advance observations.

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