



FREQUENCY OF ELEVATED PLASMA HOMOCYSTEINE (Hcy) LEVELS AMONG TYPE 2 DIABETES MELLITUS (T2DM) PATIENTS.

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ABSTRACT... Objectives: To determine the frequency of elevated plasma homocysteine (Hcy) levels among type 2 diabetes mellitus (T2DM) patients. **Study Design:** Cross sectional study. **Setting:** Department of Medicine, Abbasi Shaheed Hospital, Karachi. **Period:** From 28 September 2012 to 26 March 2015. **Material & Methods:** Type 2 Diabetic patients fulfilling inclusion criteria were enrolled. Patients with conditions known to cause altered homocysteine levels were excluded. Patients' HbA1c and fasting serum homocysteine levels were obtained. Level $>15 \mu\text{mol/L}$ was labeled as elevated. Data was collected with the help of Performa. Data was analyzed using SPSS version 21. **Results:** (90) ninety patients were enrolled in this study during study period with mean age of 61.5 ± 7.3 years. Of (90) ninety patients, 45 (50%) were male and 45 (50%) were female with male to female ratio of 1:1. Mean duration of diabetes was 6.9 ± 1.7 years. 40 (44.44%) cases were of controlled diabetes and 43 (47.7%) were on treatment. Of 90 patients 36 (40%) patients had Homocysteinemia. Homocysteine levels were found to be significantly raised in males 51.1% v/s 28.8% ($p < 0.03$), older patients (>60 years of age) 55.5% v/s 16.6% ($p < 0.001$), having diabetes for > 7 years, 59.2% v/s 17% ($p < 0.00004$), in 21% v/s 57.4% cases who were and were not on treatment respectively ($p < 0.0004$), in 22.5% patients with controlled diabetes and 54% patients with uncontrolled diabetes respectively ($p < 0.002$). **Conclusion:** Hyperhomocysteinemia is prevalent (40%) in type 2 diabetics with statistically significant raised levels in males, >60 years of age, non-compliant diabetics, have long duration diabetes, and uncontrolled disease.

Key words: Frequency, Homocysteinemia, Stratification, Type 2 Diabetes.

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INTRODUCTION

Diabetes Mellitus is fast growing epidemic. The International Diabetes Federation found, (2007) diabetes affected more than 200 million people worldwide and is expected to affect more than 350 million by 2025. Pakistan is at the seventh position in terms of the highest number of diabetics with 40.9 million diabetic persons.¹ Its prevalence in Pakistan was 5,217,000 in 2000 and is expected to become 13,853,000 by 2030.²

Diabetes is an illness with several numerable complications and early mortality, responsible for around 10% of total expenses of health care in various countries of the world; therefore many researches are done on factors which effect on diabetes control and its complications. Homocysteine is one of these factors.³

Homocysteine (Hcy) is a thiol containing amino acid generated during the metabolism of methionine^{4,5}, occurring in almost all human tissues. Elevated levels of homocysteine are associated with vascular disease (in 28%)^{6,7} by inducing endothelial dysfunction via increased oxidative stress and ADMA (Asymmetric Dimethyl Arginine).⁸⁻¹⁰ In type 2 diabetics, homocysteine levels are significantly increased as compared to healthy subjects¹¹ 31% of the individuals had laboratory values of $>15 \mu\text{mol/l}$.¹² Homocysteine is known to be recognized as an isolated factor of risk for heart disease in 30%¹³ with peripheral arterial disease.¹⁴ A study in diabetic patients undergoing dialysis showed elevated homocysteine levels in 85%.¹⁵ There is also an association between elevated homocysteine and retinopathy in type 1 and 2 diabetics in 16.5%.^{16,17}

A meta-analytical study done by Tao Huang et al. found strong causal association of raised homocysteine (Hcy) values linked with enhanced risk of "Diabetes mellitus Type 2".¹⁸

Work done on this topic internationally shows mixed results; one study shows no difference in homocysteine levels between type 2 diabetics and non-diabetics¹⁹, others showing increased homocysteine levels in 31%¹², 39% of type 2 diabetics vs only 7% in age matched controls²⁰, but to our knowledge there is no study in Pakistan showing the prevalence of hyperhomocysteinemia among type 2 diabetics.

For this reason we plan to do this study in our local population. Thereby strategies could be made to screen such cases and early diagnosis leads to decreased morbidity. The correlation of its level would be most valuable in primary prevention studies.

MATERIAL TOOLS AND METHODOLOGY

This study was conducted in Department of MEDICINE, comprising of three units with 120 beds at Abbasi Shaheed Hospital from 28 September 2012 to 26 March 2015.

90 patients were enrolled. Type 2 Diabetic patients > 18 years of age, either sex, controlled (HbA1C ≤ 7) and uncontrolled (HbA1C > 7) diabetes, diabetes of any duration [long term (> 7 years) and early diabetes (≤ 7 years)] were included in this study after informed consent. Patients with type I diabetes Mellitus, those taking pyridoxine, folic acid/ B-12 treatment, antiepileptic or hormone replacement therapy (HRT), with known history of stroke, cardiovascular disease, end stage renal disease, thyroid illness, autoimmune disorders, megaloblastic anemia, pure vegetarians, pregnant females and addicts were excluded from the study. Information regarding patient's age, sex, duration and control of diabetes and treatment was taken at the time of data collection. Patients' fasting (8 hours overnight) serum homocysteine and HbA1c levels were obtained. Level > 15 $\mu\text{mol/L}$ was labeled as elevated homocysteine level. All this information was noted in Performa.

This was a cross sectional study. Non-probability purposive sampling was used for the study. Sample size calculation was taken using WHO Sample Size Determination in Health Studies calculator with 5% level of significance, 90% power of the test, 10% detectable difference, 31%¹² population proportion and 52% anticipated proportion.

Statistical Analysis

Study data was entered and statistically calculated and assessed using standardized (SPSS) software application 21.0. Frequency along with percentage were computed for all categorical variables like gender, treatment of diabetes, control of diabetes and homocysteinemia. Mean (SD) were computed for all the quantitative variables like age and duration of diabetes. Statistical study test used was Chi-square test which was used to assess significant affiliation of age, gender, duration of diabetes, treatment of diabetes and control of diabetes with homocysteine. P-value < 0.05 was considered significant. Stratification of variables of study was done with age, gender, duration of diabetes, treatment of diabetes and control of diabetes.

RESULT

Total of 90 patients were enrolled in this study during study period. The mean (SD) age of enrolled participants is 61.5 ± 7.3 years. Of 90 patients, 45 (50%) were male and 45 (50%) were female with male to female ratio of 1:1. Mean (SD) duration of diabetes was 6.9 ± 1.7 years (Table-I). Of 90 patients 36 (40%) patients of diabetes had Homocysteinemia.

Age in Years	
Mean (SD)	61.5 \pm 7.3
Min-Max	48-72
Duration of diabetes (years)	
Mean (SD)	6.9 \pm 1.7
Min-Max	4-10
Gender, n (%)	
Male	45 (50)
Female	45 (50)
Male: Female ratio	1 : 1
On treatment, n (%)	43 (47.7)
Controlled diabetes, n (%)	40 (44.4)

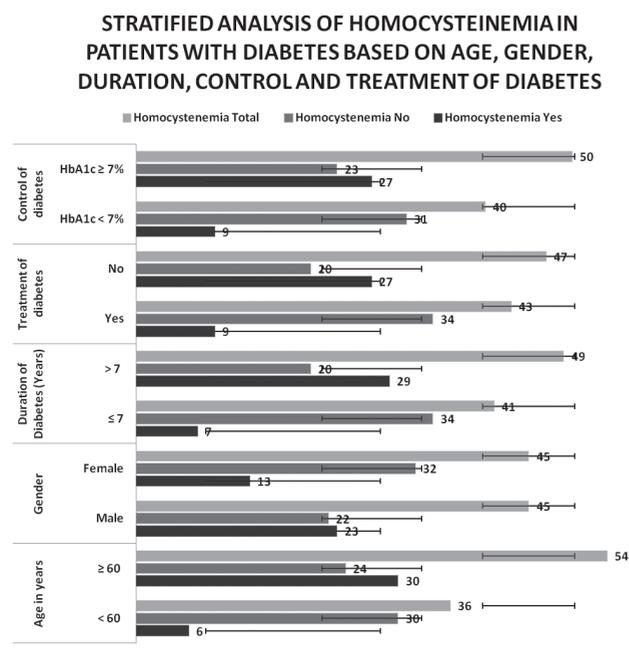
Table-I. Characteristics of study population

Stratified analysis based on age, gender, duration of diabetes, control of diabetes and treatment of diabetes is summarized in Table-II.

Variables	Homocysteniemia		Total n (%)	P- Value
	Yes n (%)	No n (%)		
	Age (years)			
<60	06 (16.6)	30 (83.3)	36 (40)	<0.001
>=60	30 (55.5)	24 (44.4)	54 (60)	
Total	36 (40)	54 (60)	90 (100)	
Gender				
Male	23 (51.1)	22 (48.9)	45 (50)	<0.03
Female	13 (28.8)	32 (71.1)	45 (50)	
Total	36 (40)	54 (63.3)	90 (100)	
Duration of Diabetes (years)				
≤7	07 (17)	34 (83)	41(45.55)	<0.00004
>7	29 (59.2)	20 (40.8)	49(54.44)	
Total	36 (40)	54 (60)	90 (100)	
Treatment of Diabetes				
Yes	09 (21)	34(79)	43 (47.7)	<0.0004
No	27(57.4)	20 (42.6)	47 (52.2)	
Total	36 (40)	54 (60)	90 (100)	
Control of Diabetes				
HbA1c<7	09 (22.5)	31 (77.5)	40 (44.44)	<0.002
HbA1c≥7	27 (54)	23 (46)	50 (55.55)	
Total	36 (40)	54 (60)	90 (100)	

Chi-square test

Table-II. Stratified analysis of homocysteinemia in patients with diabetes based on age, gender, duration, control and treatment of diabetes



DISSCUSSION

Increased homocysteine in blood is a known modifiable risk component for heart disease that is not dependent on common major risk contributors like high blood pressure, diabetes, increased cholesterol levels, and smoking.²⁰⁻²³

Haung et al¹⁸ found that absolute pooled homocysteine level was 0.94 μmol/L (95% CI, 0.40-1.48) values, more than that in control subjects with measured OR associated with T2DM of 1.29 for for 5 μmol/L increased laboratory values in Hcy suggesting a strong causal association between raised homocysteine levels and development of T2DM. Not only this, increased level of this compound is linked to around 5-year mortality not dependent on other common major risk entities and seems to be a more strong (1.9-fold) risk component for mortality in type 2 diabetic patients in Netherland.²⁴

In general population, prevalence data of raised homocysteine levels (>15 μmol/L) ranges between 5% to 30% in Canada, Norway and Boston.²⁵⁻²⁸ In a recent study from Canary Islands, hyperhomocysteinemia in Canarian males was found in 32.2%.²⁹ In Pakistan, a study conducted at multicenter including Civil Hospital Karachi, Aga Khan hospital, Armed Forces Institute of Cardiology and Military Hospital, Rawalpindi and National Institute for Cardiovascular Diseases³⁰, the prevalence of hyperhomocysteinemia was found to be 57.2% in young healthy adult cohort, considerably higher than any of the regions.

Talking about type 2 diabetics, researches by Bussychaert¹² et al, and Hoogeveen et al²⁴, conclude the prevalence of hyperhomocysteinemia to be 31% and 25.8% respectively. In contrast to this, Ramachandran et al³¹ did not find any difference in serum homocysteine levels among diabetic and patients who are in non diabetic control population (levels 2.6886 μgm/ml Vs 2.3037 μgm/ml). Due to very high prevalence in healthy Pakistani population, the prevalence of homocysteniemia was anticipated even higher in our diabetic population, but surprisingly, the result of our study falls within international reported range. Unfortunately, no locally conducted studies

on similar topic are available for comparison with our result. Out of 90 patients enrolled in our study, 36(40%) had hyperhomocysteniemia. The prevalence is slightly higher but is comparable with Bussychaert's results. It was also observed that level of homocysteine was influenced by older age, gender, duration, control and compliance to therapy. Patients who were >60 years of age had homocysteinemia in 30(55.5%) cases ($p<0.001$). Homocysteinemia was present in 13(28.8%) females and 23(52.2%) males ($p<0.03$) this finding is in accordance with results of Bussychaert's et al and Iqbal et al³⁰ where males had higher level (66 v/s 34% and 89.8 v/s 10.2% respectively). Like our study, significant hyperhomocystenemia in patients with high HbA1c has also been observed by Ramachandaran et al ($p<0.003$).

On the other hand, the level was not found to be affected by duration of diabetes in Bussychaert's research, whereas, in Ramachandaran et al and our study, the level was significantly ($p<0.00004$) influenced by duration of diabetes. The discrepancy could be due to the reason that the cohort in our study had overall shorter duration of diabetes 6.9 ± 1.7 yrs (4-10 years) v/s 14 ± 9 years.¹² The higher prevalence in our study could be a reflection of high levels in our general population³⁰ and inclusion of patients who were not taking any treatment.

The difference in prevalence between Pakistani healthy adults and diabetics in our study could be due to factor that we did not include vegetarians, though the criteria to exclude did not include this in the study by Iqbal et al.³⁰ Secondly, their study was conducted in different centers of two cities representing mixed population.

Homocysteine metabolism is dependent upon sufficient proper stores of vitamins found in dietary sources: vitamin B12 (cobalamin), folic acid and vitamin B6 (pyridoxal phosphate). Deficiency of any of these can lead to hyperhomocysteiemia. This highly prevalent (both healthy and diabetics) risk factor, can be treated by simply correcting the levels of vitamins.²⁴⁻³⁰ Failure to recognize the importance of correction of deficiencies leads to added risk of developing diabetic complications.

Simple addition of these vitamins as fortified food and oral/parenteral replacement can reduce the morbidity and mortality²⁴ due to cardiac, renal and vascular complications in diabetics and can reduce the risk of developing of T2DM¹⁸ in normoglycemic individuals.

In future, large scale studies can be done to compare the levels in blood among diabetic and non-diabetic patients, in patients with one v/s multiple confounders, delay in complications with correction of homocysteine levels and preventing the development of homocystenemia in diabetics and decreasing the occurrence of T2DM in normoglycemics.

CONCLUSION

The overall prevalence of increased homocysteine was high in type 2 diabetic individuals; 40% of them had laboratory findings >15 $\mu\text{mol/l}$. Male gender, older age, longer duration, poor control and non-compliance to treatment are associated with increased frequency of elevated homocysteine levels hence making patients more prone to develop complications.

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