

Study of Assessment of Plasma Homocysteine Level in Microvascular Complications of Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: Diabetes mellitus along with its complications bears a pertinent threat to human well being. The macrovascular and microvascular complications of diabetes contributes significantly to the mortality and morbidity caused by this disease. Recently, the elevated levels of plasma homocysteine have been linked with the development of these complications. This study was aimed to assess the plasma homocysteine levels in the microvascular complications of diabetes.

Material and Methods: This case control study evaluates the frequency of hyperhomocysteinemia in patients with diabetes mellitus. A total of 80 randomly selected Type-2 Diabetes Mellitus patients were included in this study after taking informed consent. These patients were grouped into two categories, Group A consisted of those patients who had Diabetes with Microvascular complication, Group B included diabetic patients without any complications and Group C (controls) included 20 healthy subjects. Detailed clinical history was taken and appropriate blood tests were performed.

Results: The mean age group of Group A, Group B and healthy controls were 52.94±10.46, 51.13±7.10, and 49.15±11.75 respectively. The mean serum homocysteine levels in patients with all three microvascular complications were significantly higher than other patients. The mean homocysteine level was 18.596±6.03, 13.74±3.46 and 11.085± 4.7 in Group A, Group B and healthy controls respectively. This difference was found to be statistically significant when analyzed by ANOVA test, F value being 17.28 and $F_{critical}$ being 3.2 and $p=0.00$.

Conclusion: Plasma homocysteine level in diabetic patients with microvascular complications is an important biomarker and hence should be routinely evaluated in these patients.

Keywords: Diabetes, Microvascular, Homocysteine, Nephropathy, Neuropathy, Retinopathy.

There are an estimated 23.6 million people in the United States with diabetes and 17.9 million being diagnosed³, 90% of whom are type-2⁴ With prevalence rates doubling between 1990 and 2005, CDC has characterized the increases as an epidemic.⁵

Multiple studies have shown a positive correlation between glucose intolerance and cardiovascular disease with obesity, dyslipidaemia, hypertension, polycystic ovaries, smoking, sedentary lifestyle, certain ethnic groups, poorly regulated diabetes, and hyperinsulinaemia, due to any reason or risk factors. However, not all of these factors were able to explain the strong association of diabetes with premature atherosclerosis. Recently, it has been suggested that homocysteinaemia could be an important and independent predictor of complications in diabetes mellitus⁶, especially atherothrombotic events.

Over the last years, there has been a great interest in homocysteine, primarily because of the realization that elevated levels of plasma homocysteine is an important risk factor for vascular occlusive diseases such as coronary artery disease^{7,8} cerebral vascular accidents⁹ and deep-vein thrombosis.¹⁰

Homocysteine (Hcy) is a non-essential sulphur containing amino acid whose metabolism stands at the intersection of two pathways:

1. Remethylation to methionine (which requires folate, vitamin B12 and vitamin B6 and 5,10 methylene tetrahydrofolatereductase which is the key rate limiting enzyme required for conversion of dietary folate to 5-methyltetrahydrofolate, the methyl group donor for remethylation of homocysteine to methionine in vivo) and
2. Transsulfuration to cystathionine, which requires pyridoxal-5'-phosphate.

Genetically inherited defects of the enzymes involved in the remethylation or transsulfuration process of methionine or methylene tetrahydrofolatereductase (MTHFR) thermolability are the most important determinants of marked Homocysteinemia.

Elevated Homocysteine level caused by MTHFR genetic variants has been demonstrated to be associated with Insulin resistance.¹¹ Homocysteine exerts detrimental effect on a number of cell lineages including endothelial cells and neuronal

INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that can impose tremendous burden on the individual with Diabetes and on the health care system.¹

The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 382 million in 2013. Based on the current trends, the International Diabetes Federation projects that 592 million individuals will have diabetes by the year 2035.²

India has become the Diabetes Capital of the world with 66.85 million Diabetics between 20-79 years suffering from this dreaded disease and continues to increase day by day. The International Diabetes Federation (IDF) estimates that by 2030, 8.4% of India's adult population will have diabetes and this figure is expected to go up to 101.1 million.²

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How to cite this article: Praneet Ansal Mundu, Bindey Kumar, Jeewan Kumar Mitra, Manish Kumar, Rashmi Sinha. Study of assessment of plasma homocysteine level in microvascular complications of type 2 diabetes mellitus. International Journal of Contemporary Medical Research 2017;4(4):879-883.

cells through production of reactive oxygen species.¹² Both acute and prolonged exposure to Homocysteine has detrimental effect on beta cell glucose metabolism, insulin secretory responsiveness and cell viability.¹³ Homocysteine generates reactive oxygen species in a redox-cycling reaction that explains the decline in viability of insulin secreting cells leading to reduced glucokinase phosphorylating ability, diminished insulin secretory responsiveness and cell death.¹⁴

Various studies suggest that an elevated level of homocysteine in poorly controlled type-2 diabetes mellitus is related to increased risk of atherosclerosis and cardiovascular disease. The increased prevalence of elevated homocysteine levels in which macroangiopathy and nephropathy in type-2 diabetes mellitus have been demonstrated.¹⁵

Elevated homocysteine levels, whether due to nutrient deficiencies or defective genes, can easily be normalized in virtually all cases, simply and inexpensively, using a combination of nutritional supplements. The most effective defense against homocysteine buildup is a combination of vitamins B6, B-12 and folic acid, which convert homocysteine into nontoxic substances.

Homocysteine, has been definitely shown to be associated with increased risk of diabetic cardiovascular disease, but its association with microvascular complications has been inconclusive hence this study has been undertaken to evaluate the association between elevated homocysteine levels with microvascular complications of type 2 DM.

The aim of this study was to examine the relation between serum total homocysteine concentrations and microvascular complications in patients with Type 2 diabetes, to study the relation between serum homocysteine level with glycemic control in diabetic patients and to study the relation of serum homocysteine level with various biochemical parameters in diabetic patients with microvascular complications.

MATERIAL AND METHODS

This observation case-control study was done on 80 randomly selected Type-2 DM in the Department of General Medicine, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand during the period of October 2015–October 2016 after taking informed consent. The approval of institutional ethics committee was taken prior to the commencement of this study.

These patients were grouped into two categories:

Group A: Consisted of those patients who had Diabetes with Microvascular complication (n=50)

Group B: Included diabetic patients without any complication. (n=30)

Group C: Healthy controls included 20 healthy subjects from doctors and paramedical staff of RIMS, with no long standing medical illness or history of drug intake affecting. (n=20)

Inclusion criteria

Type 2 DM patients of age group 20 to 80 years.

Exclusion criteria

1. Cases and controls aged <20 and >80 years.
2. Patients with disease of ovary and pancreas.
3. Patients with a genetic disorder associated with elevated homocysteine levels.

4. Pregnancy
5. Malignancy
6. Patient of Type 1 DM.
7. Severe psoriasis, CHF, or any major invalidating disease and deficiency disorders like anaemia, hypothyroidism

The approval of institutional ethics committee was taken prior to the commencement of this study. The detailed history was taken as per proforma and a thorough clinical examination was done supported by the necessary laboratory tests.

Serum Homocysteine: Serum homocysteine was determined by enzymatic colorimetric method for the quantitative determination of homocysteine (Refsum, 2002), using Globe diagnostics kit, Italy. The normal level of serum homocysteine is 8- 15 $\mu\text{mol/l}$.

Evaluation for Microvascular complications of Diabetes:

Funduscopy was done by experienced ophthalmologist in Dept. of Ophthalmology. Both Direct and Indirect ophthalmoscopy was performed to evaluate retinopathy in diabetic patients.

Microfilament test, Neurological examinations, Nerve conduction velocity test

were done to establish the diagnosis of neuropathy. Blood Urea, Serum Creatinine, Routine examination of urine for proteinuria were done to confirm the presence of diabetic nephropathy.

STATISTICAL ANALYSIS

Data were computer analyzed using MedCalc statistical package. Microsoft Excel program version 11.0 was used for correlation graph plotting. Chi-square (χ^2) was used to identify the significance of the relations, associations, and interactions among various categorical variables. The independent sample t-test procedure was used to analyse nominal variables One way Analysis of variance (ANOVA) was applied to analyze more than two categorical independent variable. Pearson's correlation test was applied to measure the strength of linear association. The results in all the above mentioned procedures were accepted as statistical significant when the p-value was less than 5% ($p < 0.05$).

RESULTS

The mean age group of Group A, Group B and healthy controls were 52.94 ± 10.46 , 51.13 ± 7.10 , and 49.15 ± 11.75 respectively. No significant difference in serum homocysteine levels with relation to gender and ethnicity of the patients was observed.

This study showed that there is a significant correlation between the dietary habit and serum homocysteine levels in diabetic patients. Diabetic patients who followed an exclusive vegetarian diet were observed to have a high serum homocysteine levels than non vegetarians. Mean serum homocysteine amongst the vegetarians was 20.07 ± 6.29 and for non vegetarians it was 14.38 ± 2.004 . The Chi Square value was 5.8212, significance level 0.5 and the p value was 0.015 (Table 2).

The patient's duration of diabetes was observed to be associated with increased serum homocysteine levels 70.37% patients with duration of diabetes from 5-10 yrs and 81.81% of patients with duration of diabetes >10 years had increased serum homocysteine levels. Analyzing the results by chi square test showed that the difference was statistically significant with p value 0.02 (Table 3).

In this study there was no significant correlation between family

history of diabetes and serum homocysteine levels.

The serum homocysteine levels in patients with high random blood sugar was high and the results were statistically significant, however similar relation was not seen with fasting blood sugar level and glycosylated haemoglobin levels.

In this study amongst the patients in Group A i.e. diabetic patients with microvascular complications, 50% patients had only nephropathy, 14% had only neuropathy, 4% had only retinopathy, 4% patients had both nephropathy and neuropathy, 6% patients had both nephropathy and retinopathy and 4% patients had all three microvascular complications of diabetes (Figure 1). The mean serum homocysteine levels in patients with all three microvascular complications was significantly higher than other patients (Table 5).

The mean homocysteine level was 18.596 ± 6.03 , 13.74 ± 3.46 and 11.085 ± 4.7 in Group A, Group B and healthy controls respectively. This difference was found to be statistically significant when analyzed by ANOVA test, F value being 17.28 and $F_{critical}$ being 3.2 and p value 0.00 (Table 4).

DISCUSSION

Over the past three decades, the number of people with diabetes mellitus has more than doubled globally (expected to reach 366 million in the year 2030, Wild et al., 2004),¹⁶ making it one of the most important public health challenges to all nations. Although few studies have recently assessed some early markers of microvascular complications of diabetes yet there is a lack

of comprehensive studies in this subject. Serum homocysteine levels have been linked to macrovascular complications of Diabetes but its relation to microvascular complications still needs to be explored, which was the intent to conduct this study. The personal profiles of the patients were assessed in the study taking into consideration multifactorial causes of both diabetes and its microvascular complications. Factors like age, gender, ethnicity, diet and family history of Type 2 Diabetes mellitus were compared with the serum homocysteine levels in both study and control group (Table 2). The mean age of the patients belonging to all three groups showed no statistical difference. Maximum number of patients belonged to the age group of 41-60 years (Table 1). The age distribution of patients when compared with the serum homocysteine levels in diabetic patients with and without microvascular complications revealed no significant difference (Chi square= 5.9785, p value = 0.0503). The relationship between the ethnicity and the serum homocysteine levels of diabetic patients with and without microvascular complications was tried to be explored (Table 3), considering the ethnic diversity of the Indian State of Jharkhand. Significant proportion of Jharkhand's population consists of tribal people. The lifestyle, diet, disease burden and socioeconomic profile of tribal and non tribal people vary considerably hence any significant result would help in implementation of health care and health education policies in Scheduled areas of Jharkhand. However no significant difference was observed in serum homocysteine levels of diabetic tribal

Age group (Group A+ Group B)	Increased homocysteine	Normal homocysteine	Total
20-40 years	4 (57.14%)	3 (42.85%)	7 (100%)
41-60 years	26 (44.82%)	32 (55.17%)	58 (100%)
61-80 years	12 (80%)	3 (20%)	15 (100%)
Total	42 (52.5%)	38 (47.5%)	80 (100%)

The Chi square value is 5.9785 and p value is 0.05032 hence statistically not significant since the p value is >0.05

Table-1: Plasma homocysteine level in different age groups

Variable	Group A			Group B		
	Increased homocysteine	Normal homocysteine	Total	Increased homocysteine	Normal homocysteine	Total
Male	24	9	33	7	12	19
Female	12	5	17	4	7	11
Total	36	14	50	11	19	30
Chi Square=0.0255, p=0.873			Chi Square =0.0007, p=0.979			
Non-tribal	25	13	38	3	6	9
Tribal	11	1	12	9	12	21
Total	36	14	50	12	18	30
Chi Square =3.029, p=0.08			Chi Square =0.235, p=0.625			
Non-vegetarian	6	7	13	9	14	23
Vegetarian	30	7	37	2	5	7
Total	36	14	50	11	19	30
Chi Square =5.812, p=0.015			Chi Square =0.254, p=0.61			

Table-2: Plasma homocysteine level in correlation with gender, ethnicity and diet

Duration of diabetes	Increased homocysteine	Normal Homocysteine	Total
< 5 yrs	19 (45.23%)	23 (54.76%)	42 (100%)
5-10 yrs	19 (70.37%)	8 (29.62%)	27(100%)
>10 yrs	9 (81.81%)	2 (18.18%)	11 (100%)

Chi square value is 7.0839, significance level 0.5. The p value was calculated to be 0.02895. Hence statistically significant.

Table-3: Correlation of plasma homocysteine level with duration of diabetes

patients with or without microvascular complications (Chi square= 3.029, p = 0.8177).

Patients having an exclusively vegetarian diet showed elevated levels of serum homocysteine (Table 2) and the difference was statistically significant in diabetic patients with microvascular complications (Chi square=5.812 at significance level 0.5 and p value = 0.015 hence statistically significant).

Abraham et al (2006)¹⁷ in their study showed that no significant difference exists between the serum homocysteine levels of the vegetarians and non vegetarians. However Upadhyay et al¹⁸ in their study showed that elevated levels of serum homocysteine are seen in diabetic population who follow strict vegetarian diet. This is attributed to Vitamin B₁₂ deficiency which leads to increased homocysteine levels. Hence Vitamin B₁₂ Supplementation in these patients can be employed to reduce the risk of hyperhomocysteinemia.

In our study we demonstrated that the association between duration of diabetes and serum homocysteine levels (Table 3) was also statistically significant (Chi Square = 7.089 and p value = 0.028). Pearson’s correlation test was applied to test the strength of association between these two variables where the R Score was 0.4095 (Figure 1). Hence there is a moderate correlation between duration of diabetes and Serum homocysteine levels (R is between 0.3 to 0.5). So longer the duration of diabetes higher the serum homocysteine levels. Similar finding was also observed in the study done by Sheikh et al¹⁹ where they showed a positive co relation with duration of diabetes and homocysteine levels.

No significant the relation between the Family history of diabetes and Serum Homocysteine levels was exhibited in our study (Chi square = 0.73, p value=0.38). In contrast to our study Altinova et al²⁰ in their study demonstrated a positive relationship between family history of diabetes and Serum Homocysteine levels.

Analysing the mean homocysteine level in all three groups (table 4) we found that the difference of serum homocysteine levels amongst them was statistically significant (ANOVA test F value =17.28 and $F_{critical}$ was 3.2, since $F > F_{critical}$ and p

value =0.00 hence the difference was statistically significant. Similar findings were also observed in study done by Fahmy et al (2010)²¹ and Ramachandran et al.²²

Out of total 50 patients with Microvascular complications 25 patients (50%) had only nephropathy, 7(18%) had only neuropathy and 4 patients (8%) had only retinopathy. Both Nephropathy and retinopathy was present in 6 patients (12%), nephropathy and neuropathy was present in 4(8%) patients and all three microvascular complications were found in 4 (8%) patients.

The assessment of serum homocysteine levels in all these sets of patients by ANOVA test showed that the difference was statistically significant. The analysis of mean homocysteine amongst various microvascular complication groups showed that the serum homocysteine levels in patients having all three microvascular complications of diabetes had higher mean

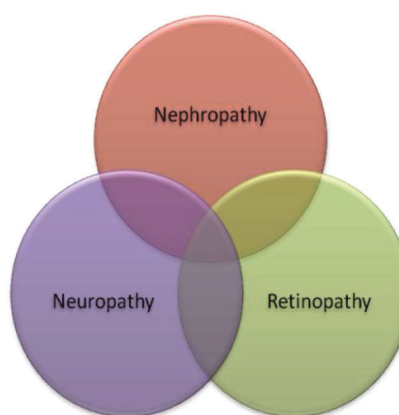


Figure-1: Venn diagram showing distribution of various microvascular complications of diabetes mellitus in study population

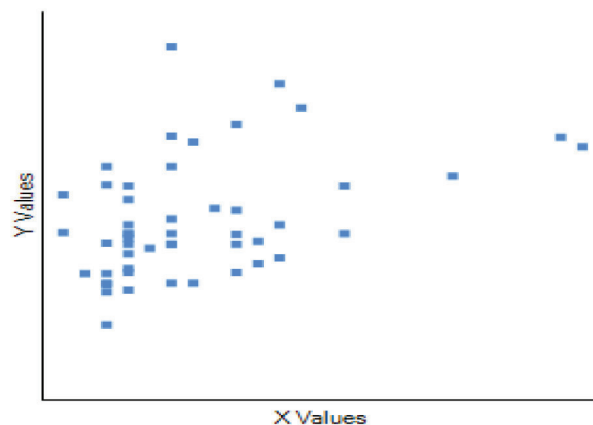


Figure-2: Scatter diagram for Pearson’s correlation between duration of diabetes and plasma homocysteine level

Groups	N	Mean homocysteine	SD
Group A	50	18.596	6.03
Group B	30	13.74	3.46
Group C	20	11.085	4.7
Healthy Controls			
Total	80	16.775	5.7

ANOVA test F value=17.285 and $F_{critical} = 3.093$ and $p=0.00$, Since $F > F_{critical}$ and $p < 0.05$ hence there exists a significant difference between these three groups.

Table-4: Mean homocysteine level in all three groups

Groups	n	Mean of homocysteine levels	SD
Nephropathy	25	15.796	3.52
Neuropathy	7	15.17	2.39
Retinopathy	4	20.47	4.8
Nephropathy + Neuropathy	4	21.65	7.2
Nephropathy + Retinopathy	6	25.91	7.3
Nephropathy + Neuropathy+ Retinopathy	4	26.175	2.25

ANOVA test F value = 8.26, degree of freedom=5, and the p value was 0.000 hence the difference was statistically significant.

Table-5: Mean homocysteine level in correlation with microvascular complications of type 2 diabetes mellitus

homocysteine levels than rest of the patients (ANOVA test $F=8.26$, degree of freedom=5, and the p value = 0.000 hence the difference was statistically significant). Hence it can be inferred that elevated serum homocysteine levels is a risk factor in diabetic patients for development of microvascular complications.

CONCLUSION

Serum Homocysteine is an important biomarker in patients with microvascular complications of Diabetes Mellitus and hence should be routinely monitored in diabetic patients. Serum homocysteine levels are affected by dietary habit of diabetic patients. Vitamin supplementation to reduce folate deficiency which in turn reduces chances of hyperhomocysteinemia is recommended to prevent or delay the appearance of microvascular complications of diabetes

Health education of diabetic patients regarding strict glycaemic control and prevention of microvascular and macrovascular complications through lifestyle alteration and compliance to antidiabetic drugs should be an integral part of diabetic patient care.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 22-03-2017; **Accepted:** 27-04-2017; **Published:** 05-05-2017