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Correlation of serum homocysteine level with microvascular complications in patients of Type 2 diabetes mellitus: cross sectional study at tertiary care centre in Western Uttar Pradesh

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Abstract

Introduction: *Raised serum homocysteine levels have been found to be risk factor for cardiovascular diseases. In the present study we have tried to assess the correlation of serum homocysteine levels with microvascular complications in patients of Type 2 diabetes mellitus.*

Materials and Methods: *A total of 80 randomly selected Type 2 diabetes patients were grouped into two categories: Group A consisted of those patients who had diabetes with microvascular complication and Group B included diabetic patients without any complication. Group C (controls) included 20 healthy age and sex matched subjects. Detailed history was taken and thorough clinical examination was done with relevant investigations.*

Results: *Significant positive correlation was found between duration of diabetes and serum homocysteine levels (Chi square value = 7.0839 with p-value 0.02). Inter –group analysis of mean homocysteine levels showed that homocysteine levels in patients having all the three microvascular complications were highest as compared to rest of the patients (F value = 8.26 and p value = 0.000). Correlation between homocysteine levels and HbA1C levels was not significant.*

Conclusion: *It could be concluded from the present study that a moderate positive correlation exists between duration of diabetes and serum homocysteine levels. The levels of serum homocysteine correlated positively with microvascular complications of diabetes. Thus serum homocysteine could be used as surrogate biomarker for diabetic microvascular complications.*

Keywords: *Type 2 diabetes , serum homocysteine , microvascular complications, diabetes in western UP.*

Introduction

The worldwide prevalence of diabetes mellitus 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 (IDF) projects

that 592 million individuals will have diabetes by the year 2035[1.] India has become the diabetic capital of world with 66.85 million diabetics between 20-79 years of age. IDF estimates that by 2030 8.4% of India's adult population will have diabetes.[1]

Multiple studies have shown a positive correlation between glucose intolerance and cardiovascular disease with obesity, dyslipidemia, hypertension, polycystic ovarian disease, smoking and sedentary lifestyle. However, not all of these factors were able to explain the strong association of Type 2 diabetes mellitus (T2DM) with premature atherosclerosis. Recently, it has been suggested that homocysteinemia could be an important and independent predictor of complications in T2DM[2] , especially atherothrombotic events.

Over the last few 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 (CAD)[3,4], cerebro-vascular accident (CVA)[5] and deep vein thrombosis.[6]

Genetically inherited defects of the enzymes involved in the remethylation or transsulphation process of methionine or methylene-tertrahydrofolate reductase (MTHFR) thermolability are the most important determinants of marked homocysteneimia. In patients of diabetes, elevated homocysteine levels were associated with insulin resistance and nephropathy. Of note, several studies have demonstrated that elevated homocysteine levels predict the risk of death or coronary events in patients of T2DM. Elevated homocysteine level caused by MTHFR genetic variants has been demonstrated to be associated with insulin resistance.7 homocysteine exerts detrimental effect on a number of cell lineages including endothelial cells and neuronal cells through production of reactive oxygen species.8 both acute and prolonged exposure to homocysteine has detrimental effect on beta cell glucose metabolism, insulin secretory responsiveness and cell viability.[9]

The European Union Concerted Action Project "homocysteinemia and vascular disease" indicated that a plasma homocysteine level above 0.162 mg% accelerates the risk of myocardial infarction, CVA and peripheral vascular disease. There are studies suggesting that an elevated level of homocysteine in poorly controlled T2DM patient, is related to increased risk of atherosclerosis and CAD. The increased prevalence of elevated homocysteine levels in which macroangiopathy and nephropathy in T2DM was demonstrated.[10]

One of the studies in India showed normal homocysteine levels in T2DM patients both with and without CAD.[11] However, another study reported higher total homocysteine levels in obese T2DM patients and not in lean ones.[12]

Hyperhomocysteinemia with treatment is a modifiable risk factor to an extent. Therefore, correction of hyperhomocysteinemia may have a beneficial effect in T2DM.

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. 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 correlation between elevated homocysteine levels and microvascular complications of T2DM.

Materials and Methods

The present study was undertaken in Department of Human Metabolism and Endocrinology, Lala Lajpat Rai Memorial Medical College, Meerut, UP during the period of September 2018 to August 2019. A total of 80 randomly selected T2DM patients attending the OPD of Endocrinology department 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 and Group B included diabetic patients without any complication. Detailed history was taken and thorough clinical examination was done with relevant investigations. Group C (controls) included 20 healthy age and sex matched subjects.

Inclusion Criteria: T2DM patients of age group 20 to 80 years were taken as cases.

Exclusion Criteria: i) patients of T1DM, ii) patients with a genetic disorder associated with elevated homocysteine levels condition known to increase the serum level of homocysteine like pregnancy, renal failure, stroke, and myocardial infarction within last three months, iv) malignancy, v) disease of ovary and pancreas, vi) severe psoriasis, CHF, or any other major invalidating disease and deficiency disorders like anemia , hypothyroidism.

Investigations: All the following blood investigations were carried in the department lab : Fasting and post prandial blood glucose, HbA1C, serum creatinine (eGFR was calculated by CKD-EPI formula), urine albumin-creatinine ratio(ACR), serum homocysteine. Fundoscopy was performed by competent ophthalmologist. Nerve conduction velocity (NCV), Microfilament test and Vibration perception threshold (VPT) were conducted to detect neuropathy. Serum homocysteine was estimated by enzymatic colorimetric method for the quantitative determination of homocysteine (Refsum, 2002), using Globe diagnostic kit (Italy). National Institute of Standards and Technology (NIST) standardized study shows 15 mmol/l as the cut-off value for upper normal level of serum homocysteine in adults. Serum glucose was determined by glucose oxidase/glucose peroxide method.

Statistical Analysis: Data were computer analyzed using MedCalc statistical package. Chi-square was used to identify the significance of the relations, associations, and interactions among various categorical variables. The independent sample t-test was used to analyse nominal variables. ANOVA was applied to analyse more than two categorical independent variables. Pearson's correlation test was applied to measure the strength of linear association. The p-value < 0.05 was considered significant.

Results

For correlation between duration of diabetes and levels of homocysteine, Chi square value came to be 7.0839 with p-value 0.02. So there is significant positive correlation between duration of diabetes and serum homocysteine levels.(Table 1)

While assessing the correlation between increased homocysteine levels and various microvascular complications of T2DM, the inter –group analysis of mean homocysteine showed that homocysteine levels in patients having all the three microvascular complications were highest as compared to rest of the patients. (Table 2) Analysing the mean homocysteine of all groups by ANOVA test, the F value came to be 8.26 with degree of freedom=5, and the p value was 0.000; hence the difference was statistically significant. Correlation between increased levels of serum homocysteine levels with glycosylated haemoglobin (HbA1C) was assessed by applying Chi square test. The correlation came to be statistically not significant with p value=0.318. (Table 3).

Table 1: Correlation of duration of diabetes with levels of homocysteine

Duration of diabetes (years)	Subjects with elevated homocysteine levels	Subjects with normal homocysteine levels	Total
<5	19(45.23%	23(54.76%)	42
5-10	19(70.37%)	8(29.62%)	27
>10	9(81.81%)	2(18.18%)	11

Table 2: Correlation between increased homocysteine levels and various microvascular complications of T2DM

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

Table 3: Correlation of increased homocysteine levels with glycosylated hemoblobin (HbA1C)

	Serum homocysteine levels (n=80)		
HbA1C(n=80)	**Raised**	**Normal**	**Total**
Raised (n=47)	32(68.08%)	15(31.92%)	47
Normal (n=33)	18(54.54%)	15(45.46%)	33
Total	50	30	80

Discussion

Over the past three decades, the number of people with T2DM all over the world has increased more than two times, making it one of the most important and grave public health challenges to all the nations. Biochemical tests for diabetes were restricted to monitoring blood glucose levels while the patient visits the doctor. Although few studies have recently assessed some early markers of microvascular complications of diabetes, still there is lack of comprehensive studies in this area. Serum homocysteine levels have been linked to macrovascular complications of diabetes but its correlation with microvascular complications still needs to be explored thoroughly. The present study is a case control study design which comprised of 80 T2DM patients classified into two groups: group A included 50 patients with microvascular complications and group B included 30 diabetes patients without any microvascular complications. Control group comprised of 20 non-diabetic healthy subjects. Serum homocysteine levels were assessed in these subjects along with other parameters related to diabetes including microvascular complications.

Table 1 shows the association between the duration of diabetes and serum homocysteine levels. Subjects were divided into three groups : group 1 with patients having diabetes for less than 5 years, group 2 with patients having diabetes for 5-10 years and group C had patients with diabetes for more than 10 years duration. Significant difference among these three groups were observed. The Chi square value was 7.089 and p-value was 0.028. Pearson's correlation test was applied to test the strength of association between these two variables where R score was 0.4095. Hence there is a moderate positive correlation between duration of diabetes and serum homocysteine levels. Hence longer duration of diabetes has an effect on serum homocysteine levels. Similar finding was observed in the study done by Sheikh et al[13] where they showed a positive correlation between diration of diabetes and homocysteine levels. Table 15 shows the distribution of microvascular complications among the groups and their correlation with serum homocysteine levels. Out of the total 50 patients with microvascular complications, 25 (50%) patients had only nephropathy, 7(18%) had neuropathy and four (8%) patients had retinopathy alone. Both nephropathy and retinopathy co-existed in 6 (12%) patients while neuropathy and nephropathy were found together in four patients. All the three complications were found together in four patients. The assessment of serum homocysteine levels in all these sets of patients by ANOVA method showed that the difference was statistically significant. The inter group analysis of mean homocysteine showed that in patients

having all the three microvascular complications have higher mean homocysteine levels than rest of the patients. Similar findings were observed in study done by Fahmy et al[14] and Ramchandran et al[15]. Finally the serum homocysteine levels were correlated with the glycosylated haemoglobin levels (HbA1C) of the patients (Table 3). However, the results obtained were not statistically significant (Chi square value=0.994 and p value=0.318). Alnajjar et al in their study found that there is a significant positive correlation between homocysteine levels with HbA1C (r=0.465, p=0.000 and r=0.517, p=0.000 respectively). This contrast can be explained on the basis of the fact that HbA1C levels vary significantly with haemoglobin levels and patients with diabetic kidney disease usually have some degree of anemia.

Conclusion

It could be concluded from the present study that a moderate positive correlation exists between duration of diabetes and serum homocysteine levels. The levels of serum homocysteine correlated positively with microvascular complications of diabetes and highest levels were seen in patients having all the three microvascular complications. Thus serum homocysteine could be used as surrogate biomarker for diabetic microvascular complications.

Compliance with Ethical Standards

There is no conflict of interest

Permission from local ethical committee has been taken for the study.

Informed consent was taken from subjects before enrolment

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