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Original Research Article.

Study of Correlation between Serum Homocysteine Levels and Albuminuria in Type 2 Diabetes Mellitus

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ABSTRACT

Background: Diabetes Mellitus is a condition known for its chronic complications such as retinopathy, neuropathy and nephropathy. The objective of this study was to evaluate serum homocysteine levels in patients of diabetes mellitus and to correlate serum homocysteine levels with albuminuria in diabetics.

Methods: The study was single centric study undertaken in the Department of Medicine at SGRD Institute of Medical Sciences and Research, Amritsar. It was a case control study with 100 patients diagnosed as type 2 diabetes mellitus as per the American Diabetic Association guidelines and attending Department of Medicine, SGRDIMSR, Vallah, Sri Amritsar. The patients were divided into two groups. Study group consisted of 50 patients with type 2 diabetes having albuminuria & control group comprised of 50 patients of type 2 diabetes mellitus without albuminuria. Serum homocysteine levels were measured in the patients of both the groups.

Results: It was observed that Serum homocysteine levels were significantly raised in diabetic patients with albuminuria (p value = <0.001). In the study, serum homocysteine levels were increased in patients with macroalbuminuria in comparison to microalbuminuria (p value = 0.001).

Conclusions: Our study demonstrated that a significant elevation in serum homocysteine levels was seen in patients with diabetic nephropathy. The increase in homocysteine levels is proportional to decline in creatinine clearance and to the degree of albuminuria.

Keywords: Diabetes Mellitus, Diabetic Nephropathy, Albuminuria, Homocysteine.

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INTRODUCTION

Diabetes Mellitus is a chronic condition, which refers to a group of common metabolic disorders sharing the phenotype of hyperglycemia.[1] The metabolic dysregulation associated with diabetes secondarily causes pathophysiological changes in multiple organ systems imposing a burden on the affected individual as well as the health care system.[2] International Diabetes Federation has predicted the number of diabetics by 2035 to be 592 million. With 65 million diabetics, India currently ranks second in the list of countries with major disease burden of this disease and India has been dubbed as "Diabetes capital of the World".[3]

Diabetes affects multiple organ systems resulting in coronary artery disease, peripheral vascular disease, cerebral vascular disease, retinopathy, macular edema, nephropathy and gastroparesis among others. Diabetic nephropathy is one of the many microvascular complications associated with diabetes mellitus. Diabetic nephropathy is today world`s leading cause of chronic kidney disease and also the most significant complication in terms of the morbidity and mortality in diabetic patients.[4] The burden of chronic kidney disease (CKD) in India cannot be measured accurately but the most common cause of CKD in India as per the population-based studies is diabetic nephropathy.[5] Diabetic nephropathy is the most common cause of end stage renal disease and patients requiring renal replacement therapy.[6]

The two main manifestations of nephropathy in people with type 2 diabetes mellitus are a reduction in Glomerular Filtration Rate (GFR) or the presence of albuminuria/proteinuria. In type 2 diabetic nephropathy renal impairment and albuminuria are believed to be two different, complimentary manifestations, thus this mandates assessment for renal impairment as well as for albuminuria.[7-9]

Urine albumin is a sensitive marker for CKD.[10] Screening for urine albumin excretion can be performed by urine albumin creatinine ratio (UACR) in a random spot collection or in a 24-hour or timed

urine collections. Albumin creatinine ratio is affected by the level of creatinine excretion in addition to the level of albumin excretion because creatinine excretion reflects creatinine generation by muscle mass and to a lesser extent, dietary intake. Because of the variability, albuminuria is confirmed by high levels in at least 2 out of 3 sample collections, within a period of 3 - 6 months.[11]

Homocysteine is a sulphur containing amino acid formed during the conversion of methionine to cysteine.[12] Serum homocysteine levels have been reported to be higher in diabetics than normal subjects in several studies.[13-15] Increased serum levels of homocysteine were first associated with the presence of arterial disease in the 1960s, when extensive atherosclerosis was described postmortem in individuals with homocysteinuria.[13] While studies supporting hyperhomocysteinemia as a risk factor for macrovascular complications of diabetes are abundant, few studies have been undertaken to evaluate the correlation of serum homocysteine levels with microvascular complications of diabetes. The present study was undertaken to study the correlation between serum homocysteine and albuminuria in diabetics.

MATERIALS AND METHODS

Source of Data

The study was conducted in the Department of Medicine, SGRD Institute of Medical Sciences and Research, Vallah, Sri Amritsar, in close collaboration with Department of Biochemistry of the institution

Study Design

This was a case control study performed in Type 2 Diabetes mellitus patients of both sexes. The study involved 100 subjects.

Exclusion Criteria

1. Dysthyroidism
2. Pregnancy
3. Treatment with methotrexate, carbamazepine, anticonvulsants or other antifolate medication.
4. Taking folic acid supplementation
5. Patients with urinary tract infection.

Inclusion Criteria

1. Adult Type-2 diabetic patients, diagnosed by latest ADA Criteria.[16] According to American Diabetes Association Criteria patients were considered having Diabetes Mellitus if:
   a) Fasting plasma glucose levels were more than or equal to 126mg/dl.
   b) 2 hour plasma glucose was more than or equal to 200 mg/dl during an OGTT.
   c) Patient had symptoms classical of diabetes mellitus or random plasma glucose concentration of >200mg/dl.
   d) HbA1C levels more than 6.5 %.[16]

Complete medical history with emphasis on history pertaining to chronic complications of diabetes and treatment history was taken in all the patients. Detailed general physical examination was carried out in each case. Weight and height of all the patients enrolled in the study were recorded and basal metabolic index (BMI) was calculated for each patient. Fundus examination was carried out as a routine in all diabetic patients. Emphasis was laid on neurological examination, in which patients were evaluated for any evidence of diabetic peripheral neuropathy using 10g monofilament, tuning fork and pinprick sensations.

Albuminuria was determined in all patients by using urine albumin creatinine ratio (UACR). The patients collected random urine samples, of 10-15 ml, in sterile containers and these samples were then processed at the Biochemistry Laboratory at SGRDIMSR, Amritsar, using Siemens Dimensions RXL fully automatic analyzer. UACR was calculated as concentration of urine albumin (in mg)/ concentration of urine creatinine (in gm). Normal cutoff of UACR was considered < 30 mg/g. UACR of >30mg/g was considered albuminuria. Patients with UACR 30 - 300 mg/g were considered as having microalbuminuria and patients with UACR >300 mg/g were labeled as macroalbuminuria.[17] For assessment of serum homocysteine levels, 5 ml of the venous blood sample was taken from patient with dry disposable syringe under aseptic conditions from antecubital vein, in a sterile, dry and acid washed vial for biochemical analysis. The blood was then allowed to stand for half an hour. After the clot formation, the separated serum was centrifuged by remicentrifuge machine at 3000rpm. The serum was then analyzed using homocysteine 2 reagent enzymatic assay with Siemens Immulite 2000 XPi immunoassay analyzer. The normal cutoff value for serum homocysteine was 15 µmol/L in adults. In addition, baseline investigations including ECG, random blood glucose, HbA1C, lipid profile, serum creatinine and blood urea nitrogen were carried out in all the patients.

Results obtained were analyzed statistically, using unpaired Student T Test, Chi square test and one way ANOVA. P values obtained were considered significant if < 0.05.

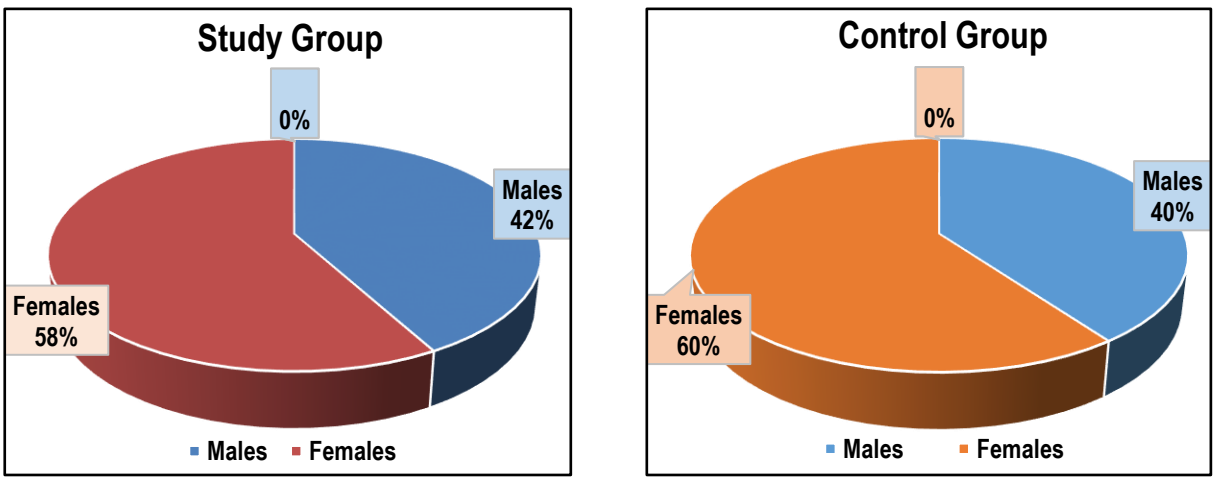

Figure 1: Gender distribution of patients in the study and control groups

Table 1: Clinical characters of all patients enrolled in the study.

Variables	Study group	Control group	p value
Age (in years)	54.60 ± 8.96	56.78 ± 13.18	0.02*
Duration of diabetes (in years)	10.13 ± 4.95	7.22 ± 5.52	0.003*
BMI (kg/m^2)	27.85 ± 4.02	27.17 ± 4.72	0.39
SBP (mm Hg)	133.42 ± 17.69	123.16 ± 16.13	0.004*
DBP (mm Hg)	80.96 ± 10.29	76.58 ± 10.04	0.042*
eGFR (ml/min)	55.37 ± 33.99	73.84 ± 23.60	0.001*
HbA1C (%)	9.26 ± 2.59	8.62 ± 2.46	0.21
Random blood glucose (mg/dl)	248.74 ± 106.65	184.20 ± 57.78	<0.001*
UACR (mg/g)	882.50 ± 492.40	14.48 ± 7.23	
Serum homocysteine levels (µmol/L)	19.53 ± 6.88	8.91 ± 4.32	<0.001*

Table 2: Correlation of duration of diabetes, HbA1C, S. creatinine, estimated glomerular filtration rate, S. homocysteine and lipid parameters with albuminuria in study group.

Variables	Urine Albumin Creatinine Ratio		P value
	Microalbuminuria (35 patients)	Macroalbuminuria (15 patients)	
Duration of Diabetes (in years)	9.02 ± 4.31	13.20 ± 5.27	0.005*
HbA1C (%)	9.50 ± 2.49	8.68 ± 2.79	0.308
S. Creatinine (mg/dl)	2.20 ± 1.94	4.00 ± 3.19	0.023*
eGFR	62.15 ± 36.32	37.60 ± 30.69	0.027*
S. Homocysteine	17.17 ± 3.94	25.03 ± 9.03	0.005*
S. Cholesterol (mg/dl)	156.00 ± 90.93	169.79 ± 61.55	0.269
S. Triglycerides (mg/dl)	141.43 ± 62.94	150.43 ± 61.23	0.319
Serum HDL cholesterol (mg/dl)	34.01 ± 10.66	37.07 ± 6.84	0.312
Serum LDL Cholesterol (mg/dl)	95.03 ± 72.14	109.77 ± 50.62	0.476

Table 3: Correlation between BMI, blood pressure, eGFR, UACR, S. creatinine, lipid profile, HbA1C levels and random blood glucose with homocysteine in study group.

Variable	Pearson Correlation	P value
BMI with S. homocysteine	0.069	0.632
SBP with S. homocysteine	0.298	0.035*
DBP with S. homocysteine	0.176	0.221
eGFR with S. homocysteine	-0.305	0.031*
UACR with S. homocysteine	0.529	0.000*
S. creatinine with S. homocysteine	0.268	0.060
Total serum cholesterol with S. homocysteine	0.121	0.401
Triglycerides with S. homocysteine	-0.139	0.336
HDL with S. homocysteine	-0.342	0.015*
LDL with S. homocysteine	0.104	0.473
HbA1C with S. homocysteine	0.262	0.066
Random Blood Glucose with s. homocysteine	0.280	0.049*

OBSERVATIONS AND RESULTS

In this study, 100 patients of diabetes mellitus were enrolled for the study after taking proper consent. Age of the patients varied from 40 – 90 years. Mean age of the patients was 55.69 years. Majority of the patients in the study as well as the control groups were in age range of 41-50 years (42% and 40% respectively). It was seen that 59% of the total patients were females and 41% were males. Figure 1 shows gender distribution of patients in the study and control groups. Patients in the study group had significantly longer duration of diabetes compared to the control group (10.13 ± 4.95 vs 7.22 ± 5.51 years with p value = 0.003).

General physical examination of the patients that majority of the patients enrolled in the study were overweight (with BMI 23 – 27.9 kg/m^2). 58% of patients in study group were overweight and 30%

were obese, in comparison, 50% patients in control group were overweight and 20% were obese. As detailed in table 1, clinical characters were assessed and compared in the two groups. Hypertension was more common in the study group compared to the control group (40% vs 20%). Systolic and diastolic blood pressures were significantly raised in the study group compared to the control group (p value = 0.004 and 0.042 respectively).

Prevalence of coronary artery disease (assessed based on history, clinical examination and ECG changes) was more in the study group compared to the control group (38% vs 16%, p = 0.013). Prevalence of peripheral vascular disease was also higher in the patients of the study group (52% vs 30%, p = 0.025). Estimated glomerular filtration rate (eGFR) was assessed in all patients using cockroft gault equation. In the study group, 28% (n = 14) patients had eGFR of < 30 ml/min and 72% (n = 35) patients had eGFR > 30 ml/min. In the control group, all the patients had eGFR > 30 ml/min and 50% (n = 25) patients had eGFR 60-89 ml/min. The eGFR of the study group was significantly lower than the control group (55.37 ± 33.99 ml/min vs 73.84 ± 23.60 ml/min, p = 0.001). Random blood glucose levels were significantly raised in the study group compared to the control group (248.74 ± 106.65 mg/dl and 184.20 ± 57.78 mg/dl respectively with p value < 0.001). On comparing HbA1C levels in the two groups, it was found that patients in both the groups had poor glycemic control. The mean HbA1C levels in study group and control groups were 9.26 % and 8.6% respectively. The difference however was not statistically significant (p = 0.21, NS). There was no significant difference in total serum cholesterol, triglyceride or HDL levels in the study or the control group (total cholesterol 160.13 ± 82.82 vs 158.30 ± 55.05 mg/dl respectively, triglycerides 144.13 ± 61.95 vs 142.32 ± 102.75 mg/dl respectively, HDL 34.92 ± 9.70 vs 36.17 ± 15.47 mg/dl respectively) .

Based on albuminuria, patients of the Study Group were divided into two subgroups. Patients with UACR of 30-300 mg/gm were classified into microalbuminuria and patients with UACR of>300 mg/gm were classified as having macroalbuminuria (Table 2). 30% (n = 15) patients in Study Group had macroalbuminuria and 70% (n = 35) patients had microalbuminuria. The patients with macroalbuminuria had significantly longer duration of disease (13.20 ± 5.27 vs 9.02 ± 4.31 years, p = 0.004) and lower eGFR (62.15 ± 36.32 vs 37.60 ± 30.69 ml/min, p=0.027) than patients with microalbuminuria. There was no significant correlation between various lipid parameters and HbA1C between the two subgroups (table 2).

In the present study, it was observed that serum levels of homocysteine were higher in study group than control group (19.53 ± 6.88 µmol/L and 8.91 ± 4.32 µmol/L respectively, Table 1). Implying that the patients with albuminuria had higher homocysteine levels compared to the diabetic patients without albuminuria. This difference was statistically significant with p value <0.001 (Table 1).Serum homocysteine levels were significantly higher in the patients with longer duration of disease. Patients with diabetes < 5 years duration had mean homocysteine levels of 14.16 ± 3.36 µmol/L and patients with duration of diabetes for > 5 years had mean serum homocysteine levels of 20.39 ± 6.83 µmol/L, p < 0.001. Serum homocysteine levels were also significantly higher in the subgroup of patients with macroalbuminuria compared to the patients with microalbuminuria (table 2).

On studying various other parameters in correlation with serum homocysteine, it was observed that systolic blood pressure and random blood glucose had significant positive correlation with serum homocysteine levels. Serum HDL cholesterol and estimated glomerular filtration rate showed significant negative correlation with serum homocysteine levels (Table 3).

DISCUSSION

Diabetic nephropathy is one of the most commonly occurring chronic complications of diabetes mellitus. It is a progressive disease with 5 stages. Stage 1 is the stage of prenephropathy characterized by early hyperfunction and hypertrophy. Stage 2 is the stage of characterized by increased GFR and normal albumin excretion. This stage is characterized by morphologic lesions without signs of clinical disease. Stage 3 is the stage of incipient nephropathy characterized by microalbuminuria (UACR < 30 mg/gm). Stage 4 is the stage of overt nephropathy characterized by macroalbuminuria (UACR > 300 mg/gm). Stage 5 is the end-stage renal failure with uremia due to diabetic nephropathy i.e. any state requiring renal replacement therapy.[18]

Hyperglycemia worsens this complication of diabetic nephropathy as was evident in our study with patients of diabetic nephropathy having higher mean random blood glucose levels. In addition, the risk of diabetic nephropathy also increases with increasing duration of the disease. There has been controversy regarding homocysteine levels in patients with type 2 diabetes mellitus with various studies providing evidence for similar or raised homocysteine levels in diabetic patients compared to the control subjects.[19] The data available on the factors affecting plasma homocysteine concentrations in diabetic patients is limited and still lesser is known about the link between homocysteine and microvascular complications of diabetes mellitus like nephropathy.

Our study demonstrated that a significant elevation in serum homocysteine levels was seen in patients with diabetic nephropathy. It also demonstrated that increase in homocysteine levels is proportional to decline in creatinine clearance and to the degree of albuminuria, with patients of macroalbuminuria having more increase in serum homocysteine levels compared to patients with microalbuminuria.

Serum homocysteine levels are elevated in patients with severe diabetic nephropathy as in other forms of chronic renal failure, perhaps due to decreased GFR and pre- and intrarenal factors. Several mechanisms like the C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) gene, deficiency in any enzyme or co-factor required for the metabolism of homocysteine or diminished elimination of homocysteine have been proposed.[20] Total homocysteine concentration are closely related to GFR according to various studies.[21,22] This has been documented in our study too, where it was observed that serum homocysteine levels had significant negative correlation with eGFR. The chief observation of this study was the significant association observed between serum homocysteine and albuminuria in patients with diabetic nephropathy. Hyperhomocysteinemia is associated with an increased risk of diabetic nephropathy with longer duration of diabetes.

There are numerous factors, which influence the development and progression of diabetic nephropathy. Hyperhomocysteinemia may be another contributing factor to microvascular angiopathy. Hyperhomocysteinemia causes endothelial damage to the

capillaries, which leads to impairment in blood supply to the affected tissue and causes resultant hypoxia. It is a known fact that microvascular complications of diabetes are augmented by hypoxia. Thus, hyperhomocysteinemia augments the microvascular damage in diabetic patients. Serum homocysteine levels should therefore be assessed in all diabetic patients and any existing hyperhomocysteinemia should be treated with the aim of reducing the toxic effect of homocysteine on blood vessels. Treatment of hyperhomocysteinemia is easy, safe, and well tolerated. Folic acid supplement in addition to the usual dietary intake, together with vitamin B6 and B12 may be used to decrease hyperhomocysteinemia and thus delay microvascular complications of type 2 diabetes mellitus.

ETHICAL APPROVAL

The study was approved by Institutional Ethics Committee.

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