Homocysteine levels in patients of type 2 diabetes mellitus with diabetic nephropathy and its clinical implications

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Abstract

Introduction: Diabetic nephropathy is one of the most common and serious complications of long standing type 2 diabetes mellitus. Microalbuminuria is a strong predictor of diabetic nephropathy. Homocysteine level plays an important role in pathogenesis of diabetic microvascular complications, particularly diabetic nephropathy. Vitamin B_{12}, Folic acid and Vitamin B_6 facilitate homocysteine metabolism. **Methods:** This case- control study was carried out at a tertiary care centre. Total 150 subjects were enrolled, which included 60 cases of type 2 diabetes with microalbuminuria, 60 cases of type 2 diabetes without microalbuminuria and 30 healthy controls. Besides routine investigations, fasting blood glucose, glycated haemoglobin, and homocysteine levels in serum were measured. All subjects were screened for microalbuminuria. Statistical analysis was done. **Results:** Homocysteine levels, fasting blood glucose and glycated haemoglobin were significantly higher in patients of type 2 diabetes with microalbuminuria as compared to those without microalbuminuria ($p = 0.00$, $p = 0.01$, $p = 0.01$). Strong positive correlation was observed between the homocysteine levels and degree of microalbuminuria($r = +0.758$, $p = 0.00$), and also between the fasting blood glucose levels and degree of microalbuminuria ($r = +0.259$, $p = 0.02$). **Conclusions:** It would be useful to perform an early screening for raised homocysteine levels and for low vitamin levels in the patients of uncontrolled diabetics. This would help to evaluate the need of folic acid, Vitamin B_{12} and Vitamin B_6 supplements since these supplements can be beneficial for delaying the progress of diabetic nephropathy in these patients.

Keywords: Homocysteine, Diabetic Nephropathy, Microalbuminuria, Type 2 Diabetes Mellitus.
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Introduction

Diabetes mellitus in all its heterogeneity has taken a central stage as one of the ultimate medical challenges all over the world. According to a WHO Reporting group, the total number of people with diabetes is projected to rise from to 366 million by 2030 [1]. Diabetic nephropathy is one of the major long term complications of diabetes mellitus[2]. It is the leading cause of diabetes mellitus related morbidity and mortality.

1.1 Microalbuminuria and its relation to diabetic nephropathy: Microalbuminuria is the hallmark of diabetic nephropathy in its earliest stages [3].

According to a study, diabetic nephropathy can be detected at quite an early stage by detection of microalbuminuria. It is defined as urinary albumin excretion rate(AER) in the range of 30-300 mg/day[4]. Normal value of urinary albumin excretion is less than 20 mg/L [5]. Microalbuminuria is a risk factor for development of overt renal failure [6]. It is an independent risk factor for development of cardiovascular disease in patients with diabetes mellitus, hypertension, and even in the general population.[7].

1.2 Homocysteine metabolism: Homocysteine is a sulphur containing amino acid. It is not present in food, but is generated from methionine via transmethylation reactions [8]. Homocysteine is either converted to cysteine via a series of vitamin B_6 dependent processes,

or it is remethylated to methionine. The first pathway of homocysteine metabolism is catalysed by enzyme cystathione β- synthase which needs vitamin B_6. The second pathway requires enzyme homocysteine methyl transferase, methyl tetrahydrofolate and Vitamin B_{12}. [9] Hence mentainence of normal blood level of these three vitamins i.e. Folic acid, Vitamin B_{12} and Vitamin B_6 is very important to keep homocysteine levels in control.
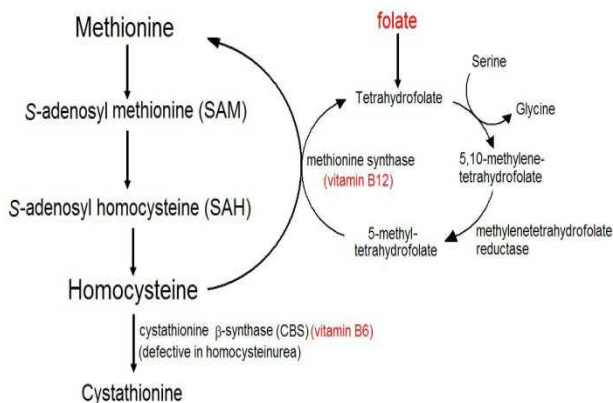
Metabolic pathways showing the relationship between homocysteine, methionine, Vitamin B_6, Vitamin B_{12} and Folic acid

1.3 Diabetes mellitus and homocysteine levels- Relationship to diabetic nephropathy: Increased serum homocysteine level is a risk factor for accelerated atherosclerosis [10]. Patients with homocysteinuria display early onset of arteriosclerosis and venous thrombosis [11,12]. High levels of homocysteine are believed to promote the formation of oxidation products such as homocysteine disulfide and homocysteine thiolactone. These oxidation products damage vascular endothelium by causing excessive sulfation of collagen, which in turn promotes thrombosis and arteriosclerosis. Diabetes mellitus is a clearly recognised risk factor for accelerated atherosclerosis, which is two to three times more common than in normal non-diabetic population [13]. The association between increased homocysteine levels and atherosclerotic vascular disease has been shown to be especially strong in type 2 diabetes [14]. It is one of the major causes for development of diabetic microvascular and macrovascular complications, especially diabetic nephropathy.

Homocysteine levels are closely linked with creatinine clearance since it is also partially excreted by kidneys. Its levels have been found to be elevated in patients with impaired renal function, particularly in patients with end stage renal disease [15,16]. Hyperhomocysteinemia correlates with both change in

glomerular filteration rate (GFR)[17], as well as the presence of microalbuminuria [18,19]. Cross-sectional studies have proved that rise in homocysteine level precedes the development of microalbuminuria, and its level correlates with the degree of microalbuminuria and worsening of other diabetic microvascular complications [20]. So the present study was intended to compare serum homocysteine levels in patients of type 2 diabetes mellitus with and without diabetic nephropathy and to find correlation of its level with severity of the disease.

Materials and Methods

2.1 Study design: This hospital-based case control study was carried out at a tertiary care centre during June 2011 to May 2014. The study was conducted after obtaining clearance from the thesis and ethical committee of the institution. Participants' voluntary consent (oral and written) was obtained after explanation of the purpose, method and course of the study. A total number of 150 subjects were enrolled in the study. These 150 study subjects were grouped as follows:

1. Group A comprised 60 cases of type 2 diabetes with microalbuminuria.
2. Group B comprised 60 cases of type 2 diabetes without microalbuminuria .
3. Group C comprised of 30 healthy controls.

Exclusion criteria was not to consider those patients with liver disease, thyroid disorders, females on oral contraceptives, pregnant and lactating mothers because of the associated hormonal changes which affect body metabolism. Patients on anti-epileptic and anti-cancer drugs were also excluded from the study since these patients show false high levels of homocysteine.

A detailed questionnaire was completed for all patients regarding age, gender, smoking history, history of alcohol consumption, family history, duration of diabetes, history of hypertension and history of any medication. Screening for diabetic nephropathy was done by detection of microalbuminuria using Nycocard Reader i.e. if more than 20 mg/L [5].

Around three millilitres of venous blood sample was collected from the patient on the day of examination. Relevant routine investigations were performed which included haemogram, complete urine analysis, fasting blood glucose, blood urea, serum creatinine, uric acid, bilirubin, AST, ALT, Alkaline phosphatase, electrolytes, calcium and complete lipid profile. All

these investigations were performed on Beckman Coulter AU 480 fully automated analyser. An early morning first-voided spot urine sample was collected for detection of microalbuminuria. Serum homocysteine level was measured on Siemens Immulite 1000 Chemiluminescence, based on the principle of competitive immunoassay [21]. The normal value of homocysteine is 5-12 μ mol/L [22]. Glycated haemoglobin and microalbuminuria were measured on Nycocard Reader, based on the principle of solid phase, sandwich format, immunometric assay [23,24].

Glomerular filteration rate (GFR) was estimated based on the value of serum creatinine by using Cockcroft-Gault formula.[25].

eGFR =[(140-age)×weight×0.85 if female]/(72×S Cr), adjusted for BSA by 1.73m^2/BSA

2.2 Statistical analysis: Statistical Package for Social Sciences (SPSS) version 16 was used to perform statistical analysis. Baseline characteristics of the study subjects were presented as mean $\pm$ standard deviation. ANOVA (Analysis of variance) and Post-Hoc tests were used for multiple comparisons between the groups, and to calculate the level of significance of difference in parameters between different groups(p value). p value < 0.05 was considered as statistically significant. Pearson's correlation coefficient (r value) was calculated between the suggested biomarkers i.e. homocysteine, microalbuminuria, fasting blood glucose and glycated haemoglobin.

Results

For the present case-control study, subjects were taken after age and sex matching. We compared all the important biochemical parameters between the three groups which included serum homocysteine levels, fasting blood glucose and glycated haemoglobin.

As shown in Table 1, all the parameters showed highest levels in patients of type 2 diabetes with microalbuminuria (Group A) in comparison with diabetics without microalbuminuria (Group B) and healthy controls (Group C). Patients of Group A revealed serum homocysteine level in the range of 12.9 - 25.7 µmol/L. In patients of Group B, serum homocysteine level was in the range of 7.1 - 14.4 µmol/L. In patients of Group C comprising healthy controls, serum homocysteine level was in the range of 6.2 - 9.5 µmol/L (normal value of homocysteine is 5 - 12 µmol/L). In comparison with Group B and C subjects, Group A patients had much higher serum homocysteine levels (p=0.00), fasting blood glucose levels (p=0.01), and glycated haemoglobin (p=0.01). Values of these parameters in healthy controls (Group C) were in normal range. These p values established significant difference in the levels of all these parameters among the three groups.

Table 1: Comparison of different relevant parameters amongst the three study groups*

Variables	Group A (Diabetics with microalbuminuria) n=60	Group B (Diabetics without microalbuminuria) n=60	Group C (Healthy controls) n=30	p value†
FBS (mg%) (mmol/L)	182.9 $\pm$ 62.4 (10.1$\pm$3.4)	128.8 $\pm$ 40.9 (7.1$\pm$2.2)	99.4 $\pm$ 8.8 (3.5$\pm$0.5)	0.01 (HS)
HbA1c (%) (mmol/L)	7.51$\pm$2.1% (mean of 58mmol/L)	5.7$\pm$0.8% (mean of 45mmol/L)	5.4$\pm$0.2% (mean of 36 mmol/L)	0.01 (HS)
Hcy(µmol/L)	19.3$\pm$6.5	10.8$\pm$3.7	7.9$\pm$1.7	0.00 (HS)
eGFR (ml/min)	108.1 $\pm$5.3	120.2$\pm$7.4	129.4$\pm$6.2	0.00 (HS)

FBS: Fasting blood glucose, HbA1c: Glycosylated haemoglobin, Hcy: Homocysteine, eGFR: effective Glomerular filteration rate, HS: Highly significant, SD: Standard deviation
*Values expressed as mean $\pm$ 1 S.D; †p value< 0.01: Highly significant
Pearson's correlation coefficient was calculated between the concerned parameters. As depicted in Table 2, patients of type 2 diabetes with microalbuminuria(Group A) showed significant positive correlation between the serum

homocysteine levels and the degree of microalbuminuria(r = +0.758**, p = 0.00) which indicates high level of significance. With increasing degree of microalbuminuria, there was corresponding increase in homocysteine levels in these patients.(Table 2, Figure 1)

Table 2: Pearson's correlation coefficient between relevant parameters in Group A patients

Parameters	Correlation coefficient(r value)*	p value†
μAlb & Hcy	+ 0.758	0.00
FBS & HbA1c	+ 0.586	0.01
FBS & μAlb	+ 0.259	0.02

μAlb: Microalbuminuria, FBS: Fasting blood glucose, HbA1c: Glycosylated haemoglobin, Hcy: Homocysteine

*Positive r value indicates positive correlation;

† p value< 0.01: Highly significant

Figure 1: Scatter plot showing correlation between homocysteine levels and degree of microalbuminuria in Group A type 2 diabetes subjects with diabetic nephropathy.
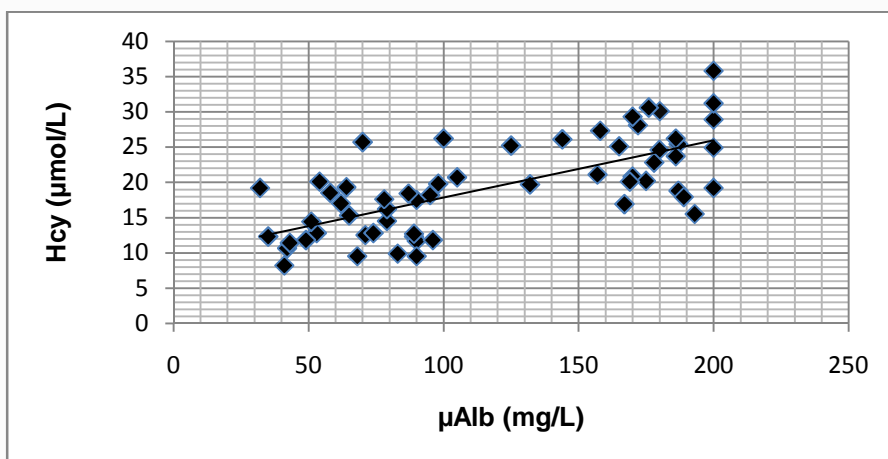
Hcy: Homocysteine, μAlb: Microalbuminuria

Our study results showed strong positive correlation between fasting blood glucose levels and the degree of microalbuminuria(r = +0.259**, p = 0.02) indicating high level of significance. So uncontrolled diabetics had higher degree of diabetic nephropathy.

Both groups of type 2 diabetes(Group A and Group B) revealed strong positive correlation between fasting blood glucose and glycated haemoglobin (r = +0.586**, p = 0.01) indicating high level of significance. Hence glycated haemoglobin is a very good and reliable indicator of blood glucose over a particular period in diabetic patients.

Discussion

Diabetic nephropathy is one of the major causes of diabetes mellitus related morbidity and mortality. In the present case-control study, we compared seum homocysteine levels in cases of type 2 diabetes with microalbuminuria, diabetes without microalbuminuria and healthy controls. All the three groups were age and sex- matched.

Our study results illustrated strong positive correlation between fasting blood glucose levels and the degree of microalbuminuria(r = +0.259**, p=0.02). Poor glycemic control has been a well known risk factor for progression of diabetic nephropathy. Keeping a tight glycemic control prevents the kidney damage in uncontrolled diabetes [26]. This fact is supported by a study which showed that uncontrolled diabetics with nephropathy had strong positive association between glycated haemoglobin and degree of microalbuminuria [27].

In our present study, homocysteine levels were found to be highest in patients of type 2 diabetes with

microalbuminuria in comparison to the patients of type 2 diabetes without microalbuminuria and healthy controls(p = 0.00). The healthy controls had normal homocysteine levels(i.e. 5-12 μ mol/l) [22]. Diabetic patients with microalbuminuria showed strong positive correlation between the homocysteine levels and degree of microalbuminuria (r = +0.758**, p = 0.00). Most of these patients with microalbuminuria more than 180 mg/L had homocysteine levels above 20 μ mol/l. Hence with increasing degree of microalbuminuria, the levels of homocysteine were also more.

According to the Hoorn study by Jager A et al, increased level of homocysteine is an independent determinant of the development of (micro)albuminuria, and the cumulative incidence of (micro)albuminuria increases with categories of increasing homocysteine levels [28]. The Hoorn Hoorn study proved strong association between increased homocysteine level and the degree of microalbuminuria. A similar cross-sectional study proved that increased serum homocysteine levels are correlated with (micro)albuminuria and contribute to worsening microvascular damage in type 2 diabetes [20]. Another similar study established that proteinuria is a predictor of total serum homocysteine levels in patients of type 2 diabetes with diabetic nephropathy [29]. These different studies support our results.

The exact pathogenesis of diabetic nephropathy is multifactorial and remains largely unclear. Intact renal endothelial and mesangial cell function is important for regulation of intraglomerular pressure and glomerular capillary membrane size selectivity. Dysfunction of these cells may increase intraglomerular pressure and/or decrease glomerular capillary membrane size selectivity. In patients of uncontrolled type 2 diabetes, increased serum homocysteine level is associated with vascular endothelial cell dysfunction, chronic inflammation and oxidative stress [30]. Increased levels of homocysteine cause increased generation of free radicals which cause endothelial and mesangial cells dysfunction in glomerular capillaries. This leads to development and progression of microalbuminuria in these patients.

Hyperhomocysteinemia is a well established risk factor for the development of macrovascular disease including coronary artery disease[31,32]. Since it is partially removed from body by renal clearance, its levels are particularly elevated in patients with diabetic nephropathy, and particularly in those with end stage renal disease.

By facilitating the normal metabolism of homocysteine, blood levels of folate, vitamin B_{12} and vitamin B_6 are among the important predictors of homocysteine levels [33,34]. Many cross-sectional studies have shown that circulating levels of homocysteine are increased in individuals with dietary deficiency of folate and/or cyanocobalamin [29,35]. In some studies, folic acid and vitamin B_{12} supplements have been proved to be helpful to decrease homocysteine levels in circulation [35,36].

Key Message and Conclusion

This study proved that high serum homocysteine level is an important independent risk factor for development of diabetic nephropathy in type 2 diabetes patients (as detected by presence of microalbuminuria). In view of the proven relationship between hyperhomocysteinemia and vascular injury, its levels must be kept under control in patients with uncontrolled diabetes. Our study could be of clinical use because increased levels of homocysteine can be effectively controlled by administration of folic acid, Vitamin B_{12} and Vitamin B_6 supplements. So these medications can be beneficial in delaying the progress of diabetic nephropathy in the patients of uncontrolled diabetes who have proven deficiency of these vitamins.

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