Research Article

Comparison of homocysteine levels and deranged lipid profile as a predictor of microalbuminuria in Type 2 diabetic patients with diabetic nephropathy

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Abstract

Introduction: Diabetic nephropathy is one of the major long term complications in patients with Type 2 diabetes. Microalbuminuria is an important marker to diagnose diabetic nephropathy at its earliest stages. It is also a strong indicator of the risk of atherothrombotic diseases and cardiovascular disorders. Deranged lipid profile, particularly increased total cholesterol and LDL-cholesterol are well known to cause atherosclerosis. According to many recent studies, it has been hypothesized that increased homocysteine level promotes atherosclerosis and is independently associated with increased risk of microalbuminuria in patients of diabetic nephropathy.

Aim: This study was intended to establish the role of homocysteine as a marker of microalbuminuria, and to compare the role of homocysteine and deranged lipid profile as a predictor of the degree of microalbuminuria.

Materials and Methods: This case-control study was conducted on a total of 150 subjects which were divided into three groups. Group A consisted of 60 diabetic patients with microalbuminuria. Group B consisted of 60 diabetic patients without microalbuminuria. Group C consisted of 30 healthy controls. Baseline investigations and estimation of glycosylated haemoglobin, microalbuminuria and homocysteine were performed. Statistical analysis of the results was done.

Results: The study documented stronger correlation between increased homocysteine levels and the degree of microalbuminuria (r = 0.401) in comparison to total cholesterol (r = 0.131) and also in comparison to LDL cholesterol (r = 0.246), (p<0.001).

Conclusions: Increased homocysteine level is a better indicator of the degree of microalbuminuria in comparison to total cholesterol and LDL cholesterol.

Keywords: Diabetic nephropathy, microalbuminuria, homocysteine, lipid profile, atherosclerosis, type 2 diabetes.

1. Introduction

Diabetic nephropathy is one of the major long term complications of diabetes mellitus. It is the leading cause of end-stage renal disease worldwide and a major cause of diabetes mellitus related morbidity and mortality.1 This complication is first manifested as increase in urinary albumin excretion i.e. by appearance of microalbuminuria, which is defined as urinary albumin excretion rate in the range of 30-300 mg/day.2 (Normal urinary albumin excretion rate being less than 20 mg/day).

Detection of microalbuminuria is important from a clinical standpoint because it helps in timely identification of subjects at risk of developing overt proteinuria and renal failure in future. Hence microalbuminuria is also called the stage of incipient diabetic nephropathy. It may represent a glomerular reflection of generalized increase in capillary endothelial membrane permeability. It has also been recognized as a significant risk factor for prediction of morbidity and mortality due to cardiovascular diseases in patients with diabetes as well as in general population.3 So determination of its causative factors is of great importance from a clinical view to know about the pathophysiological mechanisms of diabetic nephropathy and necessary therapeutic intervention. Current recommendations from the American Diabetes Association (ADA)4 suggest annual screening for microalbuminuria once duration of diabetes in a subject has crossed five years.

Recent studies in diabetic population have also suggested that proteinuria is positively associated with increased levels of total homocysteine. Homocysteine is a sulphhydril amino acid. It is not present in food but is generated from methionine via transmethylation reactions.5 Homocysteine is an important risk factor for arteriosclerosis and atherothrombosis. Patients with homocysteinuria display early onset of atherosclerosis, and manifest arterial and venous thrombosis.6 There is an established relationship between homocysteine levels and the presence of microvascular disease in diabetic patients. In early diabetic nephropathy, homocysteine levels have been reported to be normal. Increase in homocysteine level correlates with both changes in GFR7 as well as with the presence of microalbuminuria.9

Hyperhomocysteinemia is extremely common in patients with moderate to severe renal failure.10 The association between hyperhomocysteinemia and atherosclerotic vascular disease is especially strong in type 2 diabetes, causing development of microvascular and macrovascular complications, particularly diabetic nephropathy.11 Increased serum levels of total homocysteine is a risk factor for accelerated atherosclerosis, and the associated condition may not necessarily be diabetes.12

Deranged lipid profile, particularly increased total cholesterol and LDL-cholesterol has been well known to be associated with atherosclerosis. LDL particles are particularly atherogenic because of their increased susceptibility to oxidation to form ox-LDL particles.13 These ox-LDL particles cannot be recognized by the LDL receptors. These are taken up by scavenger receptors in macrophages. This leads to foam cell formation and atherosclerotic plaques.14 Particularly, the small sized LDL particles induce peroxidation of capillary endothelial membrane lipids, leading to increased endothelial membrane permeability. In Type 2 diabetes, these small sized LDL particles are associated with development of diabetic nephropathy leading to microalbuminuria.

Many studies have demonstrated high prevalence of deranged lipid profile in Type 2 diabetic patients with nephropathy as compared to Type 2 diabetic patients without nephropathy as well as healthy controls. These studies suggested that an adverse lipid profile might cause nephropathy in both Type
1 and Type 2 diabetic patients through possible mechanisms which include mesangial cell proliferation, recruitment of macrophages and increased matrix deposition.\textsuperscript{13,16}

In type 2 diabetics, there is increased generation of free radicals as a result of glucose auto-oxidation and increased lipid peroxidation. This cause’s oxidative stress in this patients.\textsuperscript{1} A study by Bagchi et al \textsuperscript{1} suggested the central role of oxidative stress in development of diabetic nephropathy. Similar results were obtained in a study by Vasavada et al \textsuperscript{1} which demonstrated that increased oxidative stress plays a major role in development of diabetic nephropathy.

So the study was intended to compare the role of homocysteine and deranged lipid profile in predicting the degree of microalbuminuria in patients of diabetic nephropathy.

1.1 Objectives

The objective of the study was to investigate and compare the role of homocysteine and deranged lipid profile in predicting the degree of microalbuminuria in patients of diabetic nephropathy.

2. Materials and methods

This case-control study was conducted on a total of 150 subjects attending the OPDs or admitted in indoor wards of Medicine Department of G.G.S. Medical College and Hospital Faridkot, Punjab, India. The present work was approved by institutional research and ethical committee. Informed written consent was taken from all the enrolled subjects.

The study subjects were divided into three groups:
- Group A: 60 Diabetic patients with microalbuminuria.
- Group B: 60 Diabetic patients without microalbuminuria.
- Group C: 30 Healthy controls.

Microalbuminuria was defined as urinary albumin excretion in the range of 30-300 mg/day.\textsuperscript{2} All the three groups were age and sex-matched. Detailed present and past history were taken which included the chief complaint of patient, history of present illness, past and family history of diabetes, hypertension and its duration and finally, history of any drug intake. Informed written consent was taken on a printed proforma. Patients with liver disease, thyroid disorders, patients on anti-epileptic and anti-cancer drugs, pregnant and lactating mothers were excluded from the study.

Baseline investigations included fasting blood glucose, blood urea nitrogen, serum creatinine, serum electrolytes, serum triglycerides, total cholesterol and LDL cholesterol. Special investigations included measurement of glycosylated haemoglobin, microalbuminuria and homocysteine levels. Glycosylated haemoglobin and microalbuminuria (from spot urine sample) were measured using Nycocard Reader. Both are solid phase, sandwich format immunometric assay. Homocysteine was measured on Siemens Immulite 1000 Chemiluminescence machine. Homocysteine assay was based on the principle of competitive immunoenzymoassay.\textsuperscript{10} Normal reference range was taken to be 5-12 µmol/L.\textsuperscript{21}

2.1 Statistical Methods

All the results and observations recorded were subjected to appropriate statistical analysis to draw the final conclusions. p value was calculated to know the level of significance of difference in variables among the three groups. Pearson’s correlation coefficient was applied to calculate the degree of correlation between different variables. p value < 0.001 was considered to be highly significant.

3. Results

Comparative analysis was done between the three groups. Descriptive data of all the three groups has been enlisted in Table 1. The values mentioned are in Mean ± 2 S.D.

**Table 1: Baseline characteristics of the three groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A (Mean ± SD)</th>
<th>Group B (Mean ± SD)</th>
<th>Group C (Mean ± SD)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>60</td>
<td>60</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.40 ± 15.00</td>
<td>55.82 ± 13.46</td>
<td>56.33 ± 15.02</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.55 ± 3.70</td>
<td>22.73 ± 2.72</td>
<td>22.73 ± 2.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FBS (mg%)</td>
<td>182.95 ± 62.39</td>
<td>128.82 ± 50.95</td>
<td>99.43 ± 48.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Cholesterol (mg%)</td>
<td>195.28 ± 41.95</td>
<td>165.68 ± 27.25</td>
<td>161.53 ± 23.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg%)</td>
<td>182.03 ± 56.47</td>
<td>140.82 ± 46.04</td>
<td>127.67 ± 43.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL Cholesterol (mg%)</td>
<td>119.25 ± 33.62</td>
<td>98.79 ± 20.86</td>
<td>93.60 ± 20.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.51 ± 2.07</td>
<td>5.68 ± 0.79</td>
<td>5.40 ± 0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microalbuminuria (mg/l)</td>
<td>123.15 ± 71.24</td>
<td>16.12 ± 4.46</td>
<td>13.07 ± 4.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>19.34 ± 6.48</td>
<td>10.76 ± 5.70</td>
<td>7.89 ± 1.67</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

n = No. of subjects in each group
All results are expressed in mean ± Standard Deviation(SD.)
*p value < 0.001; Highly significant.

Stepwise multivariate analysis of variables presented in Table 1 showed that there were statistically significant increased levels of fasting blood glucose, total cholesterol, serum triglycerides, LDL cholesterol, glycosylated haemoglobin and homocysteine levels in Group A subjects when compared to Group B and C subjects.

After controlling for other variables, correlation analysis was performed between microalbuminuria and other variables of Group A as shown in Table 2.

**Table 2: Correlation coefficient between different parameters and level of significance in subjects of Group A**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient (r)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS and HbA1c</td>
<td>+0.375</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FBS and microalbuminuria</td>
<td>+0.201</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total Cholesterol and microalbuminuria</td>
<td>+0.131</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LDL-Cholesterol and microalbuminuria</td>
<td>+0.246</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Triglycerides and microalbuminuria</td>
<td>+0.016</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Homocysteine and microalbuminuria</td>
<td>+0.401</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*p value < 0.001; Highly significant
As shown in Table 2, homocysteine was an independent predictor of microalbuminuria levels in Group A diabetic patients. As is depicted in the table, there was a higher degree of correlation between homocysteine and microalbuminuria levels ($r = +0.401$) in comparison with degree of correlation between deranged lipid profile and microalbuminuria levels ($r = +0.131$ for total cholesterol and $r = +0.246$ for LDL cholesterol).

Figure 1: Scatter plot showing correlation between Fasting blood glucose and microalbuminuria

![Figure 1](image1)

Figure 2: Scatter plot showing correlation between LDL-Cholesterol and microalbuminuria

![Figure 2](image2)

Figure 3: Scatter plot showing correlation between homocysteine levels and microalbuminuria

![Figure 3](image3)

4. Discussion

Analysis of this case-control study showed that homocysteine levels were significantly higher in uncontrolled diabetic patients with microalbuminuria (i.e., 13-25 µmol/L) as compared to diabetic patients without microalbuminuria (i.e., 7-14 µmol/L) as well as healthy controls (i.e., 5-12 µmol/L). All the three groups showed significant difference in homocysteine levels ($p < 0.001$). The healthy controls had normal homocysteine levels. Our results matched with the studies by Elias AN et al.\textsuperscript{22} which showed that in comparison with healthy controls, plasma homocysteine levels are higher in diabetic patients, particularly Type 2 diabetics as well as in pre-diabetic states and in individuals who exhibit insulin resistance. The study by Elias AN et al.\textsuperscript{23} also established that increase in homocysteine levels occurs in diabetics only when their renal function deteriorates.

According to our study, there was a strong positive correlation between the homocysteine levels and the degree of microalbuminuria ($r = +0.401$). Increased homocysteine level is an independent determinant of development of microalbuminuria in type 2 diabetes patients. These findings are in line with the cross-sectional study by Hoogeven et al.\textsuperscript{24} according to which association between increased homocysteine levels and atherosclerotic vascular disease.
was especially strong in Type 2 diabetes. This causes development of microvascular and macrovascular complications, especially diabetic nephropathy. Similar results were obtained in a study by Lanfredini et al. which proved that plasma homocysteine values are correlated with worsening vascular damage and microalbuminuria in type 2 diabetic nephropathy. Our study supports the hypothesis that homocysteine plays an important pathophysiological role in the development of microalbuminuria in Type 2 diabetes patients. When diabetic nephropathy was more severe, the levels of homocysteine were also more (r = 0.401).

The Hoorn study by Jager et al. in 2001 established that in Type 2 diabetes, the subjects who developed (micro) albuminuria in comparison with the subjects who did not develop (micro) albuminuria were more obese, significantly older, had higher blood pressure as well as increased levels of homocysteine. According to this Hoorn study, the cumulative incidence of (micro) albuminuria increases with categories of increasing homocysteine levels. Both of them showed strong positive association (p<0.05). The Hoorn study proved that hyperhomocysteinemia is an independent determinant of the development of microalbuminuria even in non-diabetics. Our study supports these findings.

The link between hyperhomocysteinemia and diabetic microvascular complications has also been elucidated by Hoffman MA et al. in 1997. A study by Chiara A et al. showed that rise in homocysteine levels precedes the development of microalbuminuria in diabetes mellitus. It is an important step towards establishing high homocysteine levels as a causal risk factor for development of microalbuminuria.

Though the study by Friedman A et al. proved no significant association between microalbuminuria and homocysteine levels. But results of the study did confirm previously well documented association between homocysteine and arteriosclerotic outcomes. Also it proved the documented association between homocysteine and deteriorating renal functions in patients of diabetic nephropathy.

Several arteriosclerotic risk factors have been seen to be more frequent in diabetic patients with microalbuminuria, e.g. deranged lipid profile, increased systolic B.P. as well as other markers of cardiovascular disease. According to the present study, diabetic patients with microalbuminuria had more deranged lipid profile as compared to those without microalbuminuria and healthy controls (p < 0.001). Hyperlipidemia is one of the important factors which cause progression of glomerular injury. More rapid decline in renal functions has been observed in patients of diabetic nephropathy with hyperlipidemia than those with normal lipid profile.

A study by Evans et al. showed that deranged lipid profile in diabetics causes increased lipid peroxidation and generation of free radicals. The abnormally high level of free radicals causes damage to cell membrane, including injury to glomerular capillary endothelial membrane. This ultimately leads to various complications of diabetes, including diabetic nephropathy. The results of our study support this hypothesis.

In a recent study on diabetic patients with microalbuminuria, statistically significant correlation was found between increased levels of total and LDL-cholesterol with the degree of microalbuminuria. This study found that total cholesterol and LDL-cholesterol were significantly increased in diabetic patients with microalbuminuria in comparison with those without microalbuminuria as well as healthy controls. Our study is in line with these findings. According to our study, the diabetic patients with more deranged lipid profile, especially with higher total cholesterol and LDL-cholesterol suffered from higher degrees of microalbuminuria. There was a strong correlation between the two parameters. But homocysteine had a stronger correlation with the degree of microalbuminuria (r = 0.401) in comparison to total cholesterol (r = 0.131) and LDL-cholesterol (r = 0.246).

5. Conclusions

 Altogether the present study showed that uncontrolled diabetic patients with high fasting blood glucose and microalbuminuria had higher levels of homocysteine in comparison with diabetics without microalbuminuria and healthy controls. The levels of homocysteine had positive and independent correlation with the levels of microalbuminuria. According to our study, increased homocysteine levels may be a causal determinant of microalbuminuria although further mechanistic work is required to prove this hypothesis. The study showed that homocysteine is a better predictor of microalbuminuria than deranged lipid profile in patients of Type 2 diabetes suffering from diabetic nephropathy. Our findings could have clinical relevance because increased levels of homocysteine can be effectively controlled by adequate supplementation with Folic acid and Vitamin B12. So these medications can be beneficial in patients of diabetic nephropathy.

Acknowledgements

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References


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