کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Interleukin-6 and C-Reactive Protein in Pathogenesis of Diabetic Nephropathy
New Evidence Linking Inflammation, Glycemic Control, and Microalbuminuria

Nikhil Choudhary, Ravinder S Ahlawat

Introduction. Recent studies have shown that subclinical inflammation is a part of type 2 diabetes mellitus. This study was designed to explore the relationships between low-grade inflammation and renal microangiopathy in patients with type 2 diabetes mellitus.

Materials and Methods. Sixty patients with type 2 diabetes mellitus were included in the study and further divided into normoalbuminurics, microalbuminurics, and macroalbuminurics, of 20 patients each. We analyzed serum concentrations of high-sensitivity C-reactive protein (HS-CRP) and interleukin-6 (IL-6) and studied their correlation with proteinuria. The patients and a control group of 20 healthy individuals were followed-up for a period of 6 months and the markers measured again.

Results. A positive correlation was found between urinary albumin excretion and levels of HS-CRP \( r = 0.781, P < .001 \) and IL-6 \( r = 0.708, P < .001 \). The level of glycosylated hemoglobin (HbA1c) showed a significant positive correlation with urinary albumin excretion \( r = 0.630, P < .001 \), CRP \( r = 0.750, P < .001 \), and IL-6 \( r = 0.680, P < .001 \). Levels of HbA1c, HS-CRP, and IL-6 significantly decreased in all three diabetic groups after 6 months of treatment. Also, the percentage of HbA1c decrement correlated well with the decrease percentage in HS-CRP \( r = .277, P = .01 \).

Conclusions. Inflammatory markers in early type 2 diabetic nephropathy are elevated and are independently associated with urinary albumin excretion. It is possible to hypothesize on the participation of locally released cytokines in the development of kidney damage.

INTRODUCTION

Diabetic nephropathy is the leading cause of chronic kidney failure worldwide. It is also one of the most significant long-term complications in terms of morbidity and mortality for individual patients with diabetes. An interaction of metabolic and hemodynamic factors has been considered as traditional aspect in the development of kidney lesions in patients with type 2 diabetes mellitus (DM). Despite improvement in the knowledge of diverse aspects related to DM, the pathogenesis and initial molecular events leading to diabetic nephropathy are still elusive. In 1998, a hypothesis was proposed suggesting that long-term innate immune system activation, resulting in chronic inflammation, elicited disease instead of repair, leading to the development of type 2 DM. In the past few years, numerous studies have shown that
low-grade inflammation is associated with the risk of developing type 2 DM. Several recent studies have also shown that patients with type 2 DM and overt nephropathy exhibit high levels of diverse acute-phase markers of inflammation, including C-reactive protein (CRP), serum amyloid A, fibrinogen, and IL-6. Cytokines promote inflammation by increasing leukocyte infiltration and adherence, in the glomeruli and tubules, along with a marked increase in macrophage infiltration. Interleukin-6 (IL-6) is a pleiotropic cytokine with a key impact on both immunoregulation and nonimmune events in most cell types and tissues outside the immune system. Interleukin-6 affects extracellular matrix dynamics at mesangial and podocyte levels, stimulates mesangial cell proliferation, increases fibronectin expression, and enhances endothelial permeability. These findings strongly involve inflammation in the development of kidney injury in DM. Whether inflammation plays a role in the pathogenesis of early diabetic nephropathy and what the underlying mechanisms are, constitute questions which still have to be answered.

The present study was designed to investigate the role of subclinical inflammation in the pathogenesis of early diabetic nephropathy. We hypothesized that urinary albumin excretion in patients with type 2 DM might be related to markers of chronic inflammation such as CRP and IL-6. Further, we investigated the role of glycemic control on both inflammatory parameters and albuminuria.

MATERIALS AND METHODS

Study Design

A total of 134 patients with type 2 DM attending the diabetic clinic of Lok Nayak Hospital were initially considered for the study. Patients with concurrent acute illnesses including infectious diseases within the past 1 week, malignancy, and active immunological diseases; medical history of clinical cardiovascular disease; confounding factors for proteinuria such as severe uncontrolled hypertension (> 160/100 mm Hg) or renal insufficiency (serum creatinine > 1.5 mg/dL); and smoking history were excluded from the study. We selected 3 groups of 20 patients with normal albuminuria (urinary albumin excretion [UAE] persistently lower than 30 mg/d), microalbuminuria (UAE between 30 mg/d and 300 mg/d), and macroalbuminuria (persistent UAE greater than 300 mg/d). Each group consisted of 10 men and 10 women matched for age with those of the other groups. Twenty healthy individuals matched for age and sex with the patients were included in the study as a control group. The controls were taken from relatives of pregnant patients attending the antenatal clinic of Lok Nayak Hospital who did not have any diagnosed disease or symptoms. All of the participants provided written informed consent.

Data Collection

Blood samples were taken before breakfast in the morning (between 8 AM and 11 AM), after an 8- to 12-hour overnight fast. Samples were collected in sterile tubes, centrifuged at 3000 rpm for 10 minutes at 4°C, and then stored at -80°C until assayed. Fasting plasma glucose (FPG) level was measured by an automated enzymatic method. The glycosylated hemoglobin (HbA1c) concentration was measured by ion exchange chromatography.

Urinary Albumin Excretion

Two 24-hour urine samples were collected from subjects. Urinary albumin excretion (UAE) was confirmed in the 2 samples, and the mean value was calculated. Urinary albumin was quantified by using colorimetric method.

Markers of Inflammation

High-sensitivity CRP (HS-CRP) was measured by means of an ultrasensitive solid-phase enzyme-linked immunosorbent assay (Calbiotech Inc, Spring Valley, California, USA). The procedure had a sensitivity of 0.2 mg/L. Expected values were classified as: low risk (< 1 mg/L), normal (1 mg/L to 3 mg/L), and high risk (> 3 mg/L). Enzyme-linked immunosorbent assay (Diaclone, Stamford, USA) was used for detection of interleukin-6 (IL-6). The intra-assay coefficient of variation was 4.2% and the inter-assay coefficient of variation was 7.7%.

Follow-up

The patients were followed up for a period of 6 months. They were treated either with diabetic diet alone, diabetic diet and oral hypoglycemic drugs, or diabetic diet and insulin with or without oral hypoglycemic drugs. In addition, if required, the patients were administered on an optimum dose of angiotensin-converting enzyme inhibitors or
angiotensin receptor blockers based on proteinuria and blood pressure levels complying with the standard treatment of DM. After a period of 6 months, all the parameters were repeated and analysis done. At the end of the 6-month follow-up period, measurements of all the markers were repeated and the data were analyzed.

**Statistical Analyses**

Continuous variables were presented as mean ± standard deviation or median as appropriate. Statistical analyses of data were performed using the SPSS software (Statistical Package for the Social Sciences, version 11.0, SPSS Inc, Chicago, Ill, USA). Differences in the parameters between the groups were analyzed by means of the *t* test and Mann-Whitney U test when appropriate. Comparisons of the continuous variables between the patients as stratified by albuminuria status were done using 1-way analysis of variance (ANOVA) and Kruskal-Wallis test as appropriate. Correlations between variables were tested using the Pearson and Spearman rho correlation tests. Finally, a forward multiple regression analysis was performed to determine the independent associations between the potential predictor variables (age, sex, duration of diabetes, body mass index [BMI], FPG, systolic and diastolic blood pressures, HbA1c, serum creatinine level, HS-CRP, and serum IL-6 level) and the UAE as the dependent variable. *P* values were considered significant if they were less than .05.

**RESULTS**

**Clinical Characteristics of Participants**

There were no significant differences between the diabetic patients and the control individuals regarding age, sex distribution, BMI, and waist-hip ratio (WHR), but the patients had higher values of FPG, serum creatinine, UAE, and IL-6 (Table 1). Analysis of differences in age and duration of DM between the four groups by ANOVA did not show any statistical significance (*P* = .96 and *P* = .81, respectively; Table 2).

**Correlation Between Proteinuria and Inflammation**

When patients with DM were stratified by the albuminuria status, we observed that those with abnormal albuminuria had greater concentrations of inflammatory parameters than normoalbuminuric patients with DM. The mean levels of HS-CRP were 1.31 ± 0.42 mg/L, 2.73 ± 0.695 mg/L, 5.06 ± 2.18 mg/L, and 5.90 ± 2.16 mg/L in the controls and patients with normal albuminuria, microalbuminuria, and macroalbuminuria, respectively (*P* < .001; ANOVA). Similarly, the mean levels of IL-6 at baseline in the four groups were 0.97 ± 0.86 pg/mL, 2.06 ± 0.61 pg/mL, 2.53 ± 1.06 pg/mL, and 3.62 ± 1.12 pg/mL, respectively (*P* < .001; ANOVA).

Using the Spearman rho test for correlations and taking the 60 diabetic patients as 1 group, a correlation coefficient of 0.781 (*P* < .001) was found between UAE and levels of HS-CRP and
Inflammatory Markers in Diabetic Nephropathy—Choudhary and Ahlawat

Table 2. Characteristics of Patients With Diabetes Mellitus in Relation to Albuminuria*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normoalbuminuria</th>
<th>Microalbuminuria</th>
<th>Macroalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.25 ± 6.48</td>
<td>55.05 ± 7.57</td>
<td>54.8 ± 7.94</td>
</tr>
<tr>
<td>Duration of DM</td>
<td>9.45 ± 3.52</td>
<td>10.15 ± 2.89</td>
<td>10.50 ± 3.49</td>
</tr>
<tr>
<td>Baseline Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.36 ± 2.05</td>
<td>23.69 ± 1.82</td>
<td>23.09 ± 2.77</td>
</tr>
<tr>
<td>WHR</td>
<td>0.85 ± 0.07</td>
<td>0.85 ± 0.08</td>
<td>0.85 ± 0.07</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>123 ± 7</td>
<td>125 ± 8</td>
<td>126 ± 6</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>80 ± 4</td>
<td>83 ± 5</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>UAE, mg/d</td>
<td>122.35 ± 43.94</td>
<td>155.75 ± 49.92</td>
<td>164.1 ± 47.92</td>
</tr>
<tr>
<td>HS-CRP, mg/L</td>
<td>2.73 (1.8 ± 4.1)</td>
<td>5.06 (1.8 ± 8.4)</td>
<td>5.9 (1.3 ± 10.5)</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>2.06 (1.12 ± 3.45)</td>
<td>2.53 (0.95 ± 4.08)</td>
<td>3.62 (0.59 ± 5.42)</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.7 ± 0.5</td>
<td>7.6 ± 0.8</td>
<td>8.1 ± 0.6</td>
</tr>
</tbody>
</table>

Parameters at 6 months
<table>
<thead>
<tr>
<th></th>
<th>Normoalbuminuria</th>
<th>Microalbuminuria</th>
<th>Macroalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAE, mg/d</td>
<td>18.15 (6 ± 26)</td>
<td>181.35 (46 ± 282)</td>
<td>505.70 (346 ± 696)</td>
</tr>
<tr>
<td>HS-CRP, mg/L</td>
<td>2.38 (0.6 ± 4.1)</td>
<td>4.13 (0.90 ± 7.70)</td>
<td>5.36 (0.80 ± 9.50)</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>1.74 (0.86 ± 2.58)</td>
<td>2.14 (0.96 ± 3.15)</td>
<td>5.36 (0.80 ± 9.50)</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>6.47 ± 1.35</td>
<td>6.99 ± 0.46</td>
<td>7.13 ± 0.66</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation or median (range). DM indicates diabetes mellitus; BMI, body mass index; WHR, waist-hip ratio; BP, blood pressure; FPG, fasting plasma glucose; UAE, urinary albumin excretion; HS-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; and HbA1c, glycosylated hemoglobin.

*Values are expressed as mean ± standard deviation or median (range).

A positive significant correlation was also found between HS-CRP and IL-6 (r = 0.728, P < .001). The controls did not show any correlation between levels of proteinuria and inflammatory markers.

**Correlation Between Glycemic Status, Level of Proteinuria, and Markers Of Inflammation**

The level of HbA1c at baseline showed significant a positive correlation with UAE (r = 0.630, P < .001), HS-CRP (r = 0.750, P < .001), and IL-6 (r = 0.680, P < .001) in the diabetic population.

**Follow-up After 6 Months**

After their initial evaluation, the patients were treated in the diabetic clinic for 6 month. Table 3 demonstrates the P values of decreases in the levels of inflammatory markers in the three groups of diabetic patients. At the end of the treatment period, the FPG levels decreased from a mean of 147.60 ± 49.85 mg/dL to 114.90 ± 29.23 mg/dL. Three groups. Postprandial values decreased from 206.90 ± 44.02 mg/dL to 165.6 ± 28.59 mg/dL. The differences were statistically significant in both values (P < .001). Glycosylated hemoglobin increased slightly from 5.26 ± 0.44% to 5.31 ± 0.33% in the controls, while in the diabetic population, it decreased from 7.83 ± 0.57% to 6.85 ± 0.93%. The differences in controls were not significant, in contrast to the diabetic group of patients (P < .001). The 24-hour UAE was repeated after 6 months. Only the group of diabetic patients with macroalbuminuria showed a significant reduction in the level of proteinuria from a median value of 595.7 mg at baseline to 505.7 mg. None of the patients belonging to the normoalbuminuria or microalbuminuria groups showed any significant change of proteinuria level. None of the patients had any change of albumin excretion that would change their group.

None of the patients developed any deterioration of kidney function, and serum creatinine values

**Table 3. P Values of Differences in HS-CRP and IL-6 Between Baseline and Follow-up Values**

<table>
<thead>
<tr>
<th>Inflammatory Markers</th>
<th>Controls</th>
<th>Normoalbuminuric</th>
<th>Microalbuminuric</th>
<th>Macroalbuminuric</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS-CRP, mg/L</td>
<td>.92</td>
<td>.004</td>
<td>.001</td>
<td>.13</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>.94</td>
<td>.001</td>
<td>.005</td>
<td>.048</td>
</tr>
</tbody>
</table>
remained steady throughout the study course. The mean values in each of the three groups of diabetic subjects showed a decline in the levels of IL-6 and HS-CRP. Except for the slight decrease in the level of HS-CRP, all the other decrements were significant with P values less than .01. A strong correlation was found between the UAE and both HS-CRP and IL-6 at 6 months (r = 0.733 and r = 0.729, respectively; P < .001). Glycosylated hemoglobin also showed a significant correlation with the UAE and levels of inflammatory markers (P < .001).

**Glycemic Control With Inflammation**

To see the effect of glycemic control on inflammation and albuminuria in diabetic patients, the percentage of sequential changes in the HbA1c levels was compared with the percentage of changes in UAE, HS-CRP, and IL-6 in all the 3 groups of diabetics (Table 4). The mean values of all parameters showed a significant relative decline except the UAE in microalbuminuric patients which showed a slight rise. The correlations between the percentages of changes were then sought using the Spearman rho test. The relative decrease in the level of HS-CRP showed a significant positive correlation with the decrease in HbA1c (r = 0.277, P = .01), but not with the relative changes in UAE or IL-6 levels.

**Partial Variate Analysis**

Finally, a forward stepwise multiple regression analysis was performed to determine the independent association between potential predictor variables (age, sex, duration of diabetes, BMI, systolic and diastolic blood pressures, HbA1c level, serum creatinine level, HS-CRP level, and IL-6 level at baseline) and the UAE as the dependent variable. After adjusting for the effect of other variables by partial correlation analysis, the previous association between UAE and the levels of inflammatory markers of HS-CRP and IL-6 remained significant (Figures 1 and 2), but the relationship between UAE and HbA1c levels was not significant.

**DISCUSSION**

**Inflammation and Nephropathy**

Although there are now convincing evidence that type 2 DM includes an inflammatory component that has been related to such diabetic complications as retinopathy, to date few studies

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HbA1c, %</th>
<th>UAE, %</th>
<th>HS-CRP, %</th>
<th>IL-6, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoalbuminuric</td>
<td>-15.14</td>
<td>-0.30</td>
<td>-13.41</td>
<td>-13.89</td>
</tr>
<tr>
<td>Microalbuminuric</td>
<td>-11.32</td>
<td>8.34</td>
<td>-17.38</td>
<td>-6.43</td>
</tr>
<tr>
<td>Macroalbuminuric</td>
<td>-10.89</td>
<td>-9.55</td>
<td>-8.03</td>
<td>-8.18</td>
</tr>
</tbody>
</table>

HbA1c indicates glycosylated hemoglobin; UAE, urinary albumin excretion; HS-CRP, high-sensitivity C-reactive protein; and IL-6, interleukin-6.

Figure 1. Correlation between high-sensitivity C-reactive protein (HS-CRP) and urinary albumin excretion in patients with diabetes mellitus at baseline (P < .001).

Figure 2. Correlation between interleukin-6 and urinary albumin excretion in patients with diabetes mellitus at baseline (P < .001).
have reported an association of inflammation with UAE, and the results have been conflicting. Findings of the present study showed a significant association between UAE and inflammatory parameters in patients with type 2 DM at early stages of nephropathy. According to our inclusion criteria, patients with a current acute illness (including infectious diseases), severe proteinuria, hypertension, renal insufficiency, cigarette smoking, and medical history of clinical cardiovascular disease were excluded from the study. Therefore, we avoided potential confounding factors that might interfere with the association between UAE and other variables.

We also found that plasma concentrations of the proinflammatory cytokine IL-6 and HS-CRP were significantly higher in patients with overt albuminuria than in those with normoalbuminuria or microalbuminuria. Interleukin-6 belongs to the IL-6 family of cytokines, including IL-11, oncostatin M, leukemia inhibitory factor, ciliary neurotrophic factor, cardiotrophin-1, and cardiotrophin-like cytokine. As for the origin of IL-6, an in situ hybridization histochemical study of the kidney tissue from patients with diabetic nephropathy demonstrated IL-6 mRNA in glomerular resident cells. When considering the pathogenesis of diabetic nephropathy, the possibility of a glomerular origin of IL-6 cannot be fully excluded. It is well known that macrophages infiltrate the glomeruli and/or interstitium in the kidney tissue in diabetic patients with nephropathy. Therefore, infiltrating macrophages may be responsible for increased levels of IL-6 in whom the infiltration of macrophages may have ceased.

Several studies have confirmed that patients with kidney failure or uremia manifest evidence of chronic inflammation. A considerable amount of IL-6 is synthesized by the adipose tissues, which necessitates caution in attributing differences in IL-6 to differing severity of diabetic nephropathy in the present study. Since BMI did not differ between subgroups defined by such severity, our study supported a relationship between diabetic nephropathy and low-grade inflammation in patients with type 2 DM.

Association of IL-6 levels and the UAE has also been reported in a study by Moriwaki and coworkers. They compared levels of IL-18, tumor necrosis factor-alpha (TNF-alpha), and IL-6 in serum and studied 151 patients with type 2 DM and various degrees of nephropathy, as well as 80 healthy volunteers. Although the level of IL-6 did not differ between the patients and controls, IL-6 showed a linear correlation with UAE. Possible explanations for our finding of an association of markers of inflammation with proteinuria in diabetic nephropathy are threefold. First, elevated levels of inflammatory markers may be the result of pre-existing atherosclerosis in patients with microalbuminuria. In nondiabetic individuals as well as patients with type 2 DM, microalbuminuria is associated with increased cardiovascular morbidity and mortality, suggesting that in individuals with albuminuria, atherosclerotic disease prevails. Second, elevations of acute-phase reactants and/or inflammatory cytokines may directly alter glomerular function and thus be causally involved in the development of albuminuria. In previous studies, the UAE has been elevated in inflammatory diseases as well as in a variety of acute syndromes, such as trauma, burn injury, surgery, and acute myocardial infarction. Third, there is a potential link between inflammatory cytokines and glomerular function. Experimental data suggest that IL-6 induces glomerular infiltration by leukocytes, and it influences the metabolism of glycosaminoglycans, which are components of the vascular endothelium and the glomerular basement membrane and are also involved in the etiology of microalbuminuria and possibly macrovascular disease.

Change in Urinary Albumin Excretion After 6 Months

We measure the 24-hour UAE after 6 months of treatment in diabetic patients. Out of our study population, none of the patients had any change of albumin excretion to change their group. Also none of the patients developed change in serum creatinine level to be classified as kidney failure. One reason for this could be the effect of glycemic control on low-grade inflammation that can ultimately prevent from progression of nephropathy. The small period of follow-up, however, could also contribute to this finding. Also, the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers in these patients could have prevented the further development of nephropathy. These drugs control both systemic hypertension and intraglomerular hypertension by inhibiting the actions of angiotensin...
II on the systemic vasculature and renal efferent arterioles. In addition, these drugs also attenuate the stimulatory effect of angiotensin II on glomerular cell growth and matrix production. A prospective population-based study was performed in Casale Monferrato, Italy, including 1253 patients with type 2 DM, 765 with normoalbuminuria, and 488 with microalbuminuria. They showed that of type 2 diabetic patients, 3.7% progress every year to overt nephropathy. Microalbuminuria is associated with a 42% increased risk of progression to overt nephropathy.

**Glycemic Control and Inflammation**

After the 6-month follow-up period, HbA1c decreased significantly in all the three groups of diabetic patients. In the overall diabetic population, the percentage of change in HbA1c did not correlate with that of the UAE. Furthermore, levels of HS-CRP and IL-6 fell significantly in all the three groups of diabetics. Also, the percentage of decrease in HbA1c correlated well with that for HS-CRP. This further supports our hypothesis that good glycemic control further decreases the levels of inflammatory markers which probably plays a role in both primary and secondary prevention of nephropathy in these patients. In the UK Prospective Diabetes Study, a difference in HbA1c of 0.9% was associated with a reduction in the relative risk for development of microalbuminuria or proteinuria of 30% in the intensively treated group at 9 to 12 years.

**CONCLUSIONS**

Our findings suggest that there may be an additional potential aspect related to the development of kidney damage in DM apart from traditional and metabolic factors. The significant association between inflammatory parameters and UAE indicates that inflammation may be a pathogenetic mechanism of diabetic nephropathy. It is possible to hypothesize on the participation of locally released cytokines such as IL-6 in the development of kidney damage in type 2 diabetics. Further analyses are necessary to confirm the intrarenal production and implication of inflammation in the pathogenesis of diabetic nephropathy. Prevention of obesity, prevention of hyperglycemia, use of antioxidants, and other anti-inflammatory treatments may be beneficial in addressing the early progressive inflammatory response associated with diabetes and microvascular disease and mandate further studies in the area.

**CONFLICT OF INTEREST**

None declared.

**REFERENCES**


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مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله