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# Study of the role of Interleukin-6 and highly sensitive C-reactive protein in diabetic nephropathy in type 1 diabetic patients

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**Abstract. – *Background:*** **Diabetes Mellitus (DM) is becoming an increasingly common disease which is the leading cause of chronic renal failure. The pathogenesis of diabetic nephropathy is still a matter of debate. There are conflicting results regarding the relation of C reactive protein (CRP), interleukin-6 (IL-6) and diabetic nephropathy in type 1 diabetes.**

***Objectives:*** **This study was aiming to determine the association between higly sensitive (Hs)-CRP and IL6 with nephropathy in a sample of type 1 diabetic Egyptian patients.**

***Materials and Methods:*** **The study was conducted on forty type-1 diabetic patients (Group I), who subdivided into three subgroups according to their urine albumin excretion rate (AER); Group IA: 10 patients with AER <20 μg/min, Group IB: 15 patients with AER ranges from 20-200 μg/min. Group IC: 15 patients with AER is > 200 μg/min and 10 healthy subjects as a control (Group II).**

***Results:*** **There were high statistical significant difference ($p < 0.001$) between group I with group II regarding HsCRP (4.39 ± 1.94, 1.32 ± 0.39), and IL-6 (2.82 ± 0.76, 1.95 ± 0.35). In group I, we found a positive significant correlation ($p < 0$ .001) between UAE and levels of Hs-CRP (r = 0.927), and IL-6 (r = 0.838), respectively. Also, a positive significant correlation between Hs-CRP and IL-6 (r = 0.728, $p < 0.001$) was found. Fasting plasma glucose (FPG) level and HbA1c showed a significant positive correlation ($p < 0.001$) with Hs-CRP (r = 0.531) (r = 0.750), and IL-6 (r = 0. 490) (r = 0.680) respectively.**

***Conclusion:*** **Hs-CRP and IL-6 are sensitive markers for diabetic nephropathy predicting its progression and severity in type 1 diabetics.**

*Key Words:*

Type 1 DM, Diabetic nephropathy, Hs-CRP and IL6.

## Introduction

Diabetic nephropathy develops in <50% of patients with type 1 diabetes mellitus who have had diabetes for 20 years. Renal disease is less common in type 2 diabetes mellitus, occurring in 15-20% of individuals. Caucasians have the lowest prevalence of diabetic nephropathy among the major ethnic groups. Diabetic nephropathy represents the most common cause of end-stage renal failure worldwide and accounts for < 40% of all new patients entering end-stage renal disease (ESRD)[1]. Inflammation and more specifically inflammatory cytokines, are determinant in the development of microvascular diabetic complications, including neuropathy, retinopathy, and nephropathy[2]. One of the most important changes is related to the participation of immune-mediated inflammatory processes in the pathophysiology of diabetes mellitus and its complications[3]. IL-6, a pleiotropic circulating cytokine, is reported to have multiple effects ranging from inflammation to host defense and tissue injury. It is secreted by many cell types, including immune cells, fibroblasts, endothelial cells, skeletal muscle and adipose tissue, IL-6 circulates as a glycosylated protein[4]. Elevated IL-6 concentrations have been reported in numerous clinical disorders, where they appear to orchestrate a variety of inflammatory responses[5]. C-reactive protein (CRP) production is part of the nonspecific acute-phase response to most forms of inflammation, infection, and tissue damage. Plasma CRP is produced by hepatocytes predominantly under transcriptional control by the cytokine IL-6, although other sites of local CRP synthesis and possibly secretion have been suggested[6]. Circulating CRP concentrations showed correlation



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with the severity, extent, and progression of much different pathology, and the prognostic significance of these associations, are consistent with CRP not just being a marker of disease but also contributing to pathogenesis. This concept will favor the use of novel drugs that specifically block CRP binding and its proinflammatory effects *in vivo*[7].

## Material and Methods

This study was conducted on 50 subjects, they were divided into two main groups, 40 patients with type 1 diabetes (group I), 18 females and 22 males, their age ranged from 17-33 years with a mean of (25.08 ± 7.94) years and 10 healthy subjects as a control (group II), 5 females and 5 males, with matched age, they were recruited from outpatient clinic of Endocrinology and Metabolism, Ain Shams University Hospital.

Group I were subdivided into 3 subgroups according to their urinary albumin excretion rate (AER):

**Group A:** 10 patients (5 females and 5 males) with AER is < 20 $\mu$g/min,
**Group B:** 15 patients (7 females and 8 males) with AER is ranges from 20-200 $\mu$g/min,
**Group C: 15** patients (6 females and 9 males) with AER is >200 $\mu$g/min.

All subjects were submitted to the following:

**1.** Full clinical history and clinical examination.
**2.** Laboratory investigations including:

- Fasting, postprandial plasma glucose, (AST, ALT).
- Quantitative colorimetric determination of glycated hemoglobin (HbA1c) in blood, using Cation Exchange Resin which is eluted and quantitated photometrically by glycohemoglobin reagent set from Pionte Scientific Inc., USA.
- Lipid profiles (cholesterol, TG, LDL and HDL) by enzyme colorimetric assay on a Ciba Corning Express Plus analyzer (Boehringer Mannheim, Germany), measured after an overnight 14 hours fasting. Normal range for serum cholesterol up to 200 mg/dl[8], and for serum triglycerides: up to 150 mg/dl[9]. LDL-C was calculated according to the Friedewald formula as follows: LDLc = Total cholesterol-TG/5-HDLc[10]. Serum creatinine by using immuno-chromatographic assay (Calbiotech Inc, Spring Valley, CA, USA). Reference values for serum creatinine: adult males: 0.8-1.4 mg/dl, adult females: 0.6-1.1 mg/dl[11].
- Albumin excretion rate (AER): The urine had been collected for quantification of urinary albumin on the same day by using colorimetric method[12].
- Normal range for control subjects: 0-20 $\mu$g/min.
- Highly sensitive C-reactive protein (CRP) by ELISA (Immundiagnostik AG Bensheim; Germany). The normal range for Hs-CRP values: Low risk ≤1 $\mu$g/ml. Normal = 1-3 $\mu$g/ml. High risk ≥3 $\mu$g/ml[13].
- Interleukin-6 (IL-6) by (ELISA) Kits (Medgenix Diagnostics SA, B-6220 Fleurus, Belgium). The normal range for IL-6 values[14]: 0.08-5.0 pg/ml.

### Exclusion Criteria

Patients with smoking habit, acute inflammations, acute infections and chronic liver diseases were excluded from our study.

### Statistical Analysis

It was performed using SPSS software package version 12 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation. The Students t test was used for independent samples. A one-way ANOVA with post hoc tests was used to determine LSD (least significant difference) Spearman's correlation coefficient was used to study correlation between different studied variables.

## Results

On comparing the diabetics group (I) with the control group (II), a highly statistical significant difference ($p < 0.001$) was recorded as regard HsCRP (4.395 ± 1.94 vs 1.32 ± 0.39) $\mu$g/mL), and IL-6 (2.82 ± 0.76 vs 1.95 ± 0.35 pg/mL).

When comparing the diabetic group (IA) with the diabetic group (IB), there was a highly statistical difference ($p < 0.001$) as serum creatinine

(1 ± 0.14 vs 1.1 ± 0.25 mg/dl), UAE (17.9 ± 10.5 vs 178 ± 32.7 μg/min), HS-CRP (2.54 ± 0.86 vs 4.1 ± 0.83 μg/ml) and there was statistical significant difference ($p < 0.05$) as regard fasting plasma glucose(152.3 ± 16.9 vs 170.9 ± 52.7 mg/dl), postprandial plasma glucose (225.4 ± 120.7 vs 310.6 ± 144.8) and IL-6 (2.06 ± 0.42 vs 2.5 ± 0.44 pg/ml).

On comparing the diabetic group (IB) and diabetic group (IC): there was a highly statistical difference ($p < 0.001$) as regard UAE (178 ± 32.28 vs 459.7 ± 98.5 μg/min), Hs-CRP (4.1 ± 0.83 vs 5.9 ± 2.1 μg/ml), and IL-6 (2.06 ± 0.42 vs 3.62 ± 0.36 pg/ml). There was non statistical significant differences ($p > 0.05$) as HbA1c% (7.88 ± 1.58 vs 8.04 ± 0.47). There was significant difference ($p < 0.05$) as regard fasting plasma glucose (170.9 ± 52.7 vs 187.9 ± 34.9 mg/dl), PPG (310.6 ± 144.8 vs 266.4 ± 88.6 mg/dl), serum triglycerides (157.5 ± 67.9 vs 180.5 ± 15.1 mg/dl) and serum creatinine (1.1 ± 0.25 vs 1.42 ± 0.17 mg/dl).

Comparing the diabetic group (IA) and diabetic group (IC): there was a highly statistical difference ($p < 0.001$) as regard FPG (152.3 ± 16.9 vs 187.9 ± 34.9 mg/dl), UAE (17.9 ± 10.5 vs 459.7 ± 98.5 μg/min), serum creatinine (1 ± 0.14 vs 1.42 ± 0.17 mg/dl), Hs-CRP (2.54 ± 0.86 vs 5.9 ± 2.1 μg/ml), and IL-6 (2.06 ± 0.42 vs 3.62 ± 0.36 pg/ml). There was statistical significant difference ($p < 0.05$) recorded as regard HbA1c % (7.41 ± 0.22 vs 8.04 ± 0.47), LDL (170.5 ± 6.16 vs 188.9 ± 8.5, mg/dl) and PPG (225.4 ± 88.6 vs 250.4 ± 120.7 mg/dl).

Comparison between group IA, group IB, and group IC showed that Hs-CRP and IL-6 had a greater concentration in macroalbuminuric than microalbuminuric patients (Table I).

We found in our study a positive significant correlation between Hs-CRP and IL-6 (r = 0.728, $p < 0.001$) in diabetic patients. Also, a positive significant correlation between Hs-CRP and IL-6 with UAE (r = 0.927) (r = 0.838) respectively, ($p < 0.001$) (Figures 1 and 2, Table II).

## Discussion

In our study we found that diabetic patients showed significantly higher levels of Hs-CRP and IL-6 than did the controls ($p < 0.001$). This result is in agreement with Picardi et al[15] who reported that patients with recent onset of type 1 DM had higher levels of Hs-CRP and IL-6 than normal individuals[15]. Also, Coulon et al[16]

**Table I.** Comparison between group IA, group IB, and group IC by student (t) test regarding the different variants using ANOVA test.

| Variables | Group IA | | Group IB | | Group IC | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | ± SD | Mean | ± SD | Mean | ± SD | f | P | Sig |
| Age/years | 25.25 | 3.896 | 25.05 | 5.5 | 25.8 | 5.0 | 1.332 | > 0.05 | NS |
| Duration of DM/year | 9.45 | 1.877 | 10.5 | 1.88 | 15.2 | 1.97 | 3.642 | < 0.001 | HS |
| Waist circumference cm | 104.15 | 11.234 | 111.9 | 10.76 | 94.6 | 8.44 | 4.726 | < 0.001 | HS |
| SBP/mm Hg | 123 | 9.775 | 138 | 19.06 | 147.6 | 7.527 | 1.718 | < 0.05 | Sig |
| DBP/mm Hg | 80.5 | 7.071 | 87.33 | 11.317 | 95.3 | 3.086 | 1.452 | < 0.05 | Sig |
| HbA1c% | 7.41 | 0.228 | 7.88 | 1.586 | 8.04 | 0.477 | 2.27 | < 0.05 | Sig |
| FPG mg/dl | 152.3 | 16.97 | 170.9 | 52.7 | 187.9 | 34.97 | 3.732 | < 0.001 | HS |
| PPG/mg/dl | 225.45 | 120.76 | 310.6 | 144.87 | 266.45 | 88.67 | 2.90 | < 0.001 | HS |
| Cholesterol mg/dl | 190.5 | 10.9 | 194.93 | 57.64 | 200.5 | 12.6 | 1.23 | < 0.05 | Sig |
| Serum triglycerides mg/dl | 180.4 | 8.59 | 157.5 | 67.97 | 180.5 | 15.19 | 1.652 | < 0.05 | Sig |
| HDL mg/dl | 35.9 | 2.514 | 41.8 | 14.16 | 38.7 | 11.24 | 1.977 | > 0.05 | NS |
| LDL mg/dl | 170.5 | 6.16 | 116.5 | 45.76 | 188.9 | 8.57 | 2.95 | < 0.05 | Sig |
| Serum creatinine, mg/dl | 1 | 0.14 | 1.1 | 0.256 | 1.426 | 0.17 | 3.99 | < 0.001 | HS |
| UAE μg/min | 17.9 | 10.59 | 178 | 32.28 | 459.7 | 38.7 | 11.24 | < 0.001 | HS |
| Hs-CRP, μg/ml | 2.54 | 0.86 | 4.1 | 0.837 | 5.9 | 2.1 | 4.627 | < 0.001 | HS |
| IL-6 pg/ml | 2.06 | 0.42 | 2.5 | 0.44 | 3.62 | 0.36 | 3.963 | < 0.001 | HS |

$p > 0.05$ = non-significant (N.S); $p < 0.05$ = significant (Sig) and $p < 0.001$ = highly significant (HS).

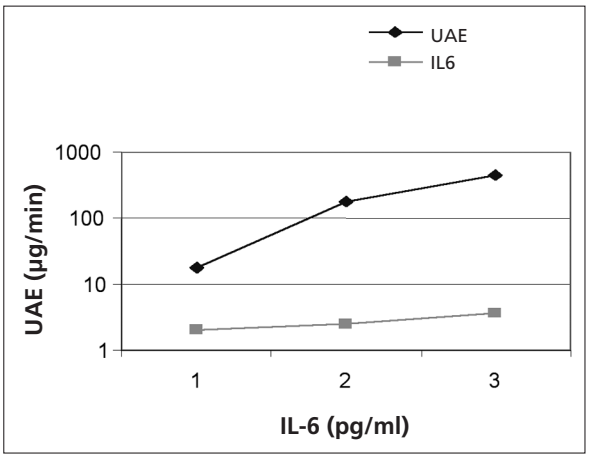


**Figure 1.** Correlation between (IL-6) and urinary albumin excretion (UAE).

proved that diabetic patients have higher levels of cytokines than normal individuals and this elevation might be related to activation of macrophages, increased oxidative stress, or induction of cytokines. So, type 1 DM is now accepted to be a chronic immuno-inflammatory disorder. However, Alexandraki et al[17] did not find any significant difference between 167 type 1 diabetic patients and control group as regards IL-6.

In our study we found that low-grade inflammation was already present in the early stage of microalbuminuria, and it was increased with progressive increase of UAE. In agreement with our results, Picardi et al[15] and Piccirillo et al[18] who observed that low-grade inflammation was already present in the early stage of micro-albuminuria and low-grade inflammatory markers

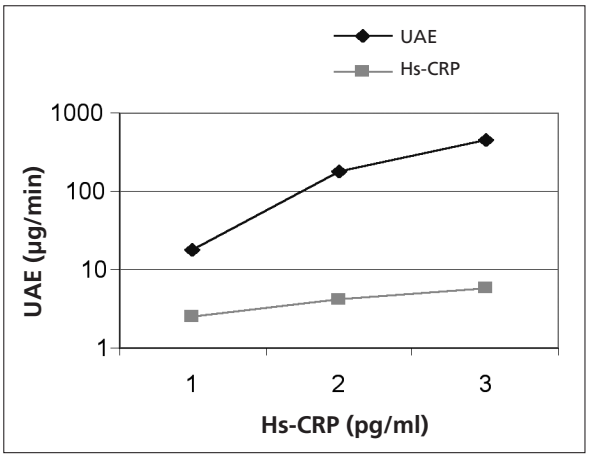


**Figure 2.** Correlation between (Hs-CRP) and urinary albumin excretion (UAE).

**Table II.** Correlation studies between Hs-CRP and IL-6 and other parameters in diabetic patients.

| | | Age/ years | Duration of DM/yr | Waist circumference | SBP | DBP | FBG | HbA1c | s.chol | TG | HDL | LDL | UAE | s.creat. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Hs-CRP** | r | 0.175 | 0.767 | 0.580 | 0.393 | 0.295 | 0.531 | 0.750 | 0.295 | 0.288 | 0.217 | 0.321 | 0.927 | 0.931 |
| | *p* | 0.644 | 0.001 | 0.001 | 0.026 | 0.022 | 0.001 | < 0.001 | 0.129 | 0.26 | 0.14 | 0.05 | 0.001 | 0.001 |
| **IL-6** | r | 0.140 | 0.644 | 0.490 | 0.357 | 0.0398 | 0.434 | 0.680 | 0.266 | 0.242 | 0.225 | 0.327 | 0.838 | 0.931 |
| | *p* | 0.524 | 0.001 | 0.001 | 0.129 | 0.002 | 0.001 | < 0.001 | 0.86 | 0.96 | 0.62 | 0.05 | 0.001 | 0.001 |

$p > 0.05$ = non-significant (N.S); $p < 0.05$ = significant (Sig) and $p < 0.001$ = highly significant (HS).

could serve in predicting initiation, and the progression of diabetic nephropathy. Also Saraheimo et al[19] reported that low-grade inflammatory markers are associated with diabetic nephropathy in type 1 diabetic patients in which C-reactive protein and interleukin-6 were higher compared to normoalbuminuric patients. On the other hand, contradictory study showed higher CRP concentrations in patients with normoalbuminuria than macroalbuminuria[20]. So, the results are conflicting and needs further research to clarify the role of these inflammatory cytokines in diabetic complications.

We found that there were a positive significant correlation between Hs-CRP, IL-6 and duration of DM in type 1 diabetic patients. In agreement with these results, a study conducted on 106 type-1 diabetic patients where duration of diabetes was independently related to inflammatory markers suggested that chronic exposure to glucose could stimulate the production of Hs-CRP and IL-6 [21]. Also a study conducted on 27,358 children, adolescents, and young adults with type-1 diabetes, and revealed that there was a positive significant correlation between Hs-CRP, IL-6 and duration of DM[22].

In our investigation, we found that waist circumference was significantly higher in diabetics group, who in turn had a significant direct correlation with Hs-CRP and IL6. In agreement with this results a study proved that higher waist circumference is associated with higher levels of inflammatory markers which can be explained by increased local adipose tissue inflammation associated with obesity and the secretion by the adipocytes of a number of bioactive proteins collectively termed adipocytokines, one of which is IL-6 which is considered the main stimulus for the hepatic production of CRP[23]. This is consistent with Mahadik et al[24] who conducted a study on Asian Indian diabetic subjects and reported that body mass index and waist circumference are commonly accepted as an indicator of overweight and obesity which are frequently associated with elevated CRP and interleukins (IL6 and IL-8).

In addition, we found that the level of HbA1c at baseline showed a significant positive correlation with Hs-CRP ($r = 0.750$, $p < 0.001$), and IL-6 ($r = 0.68$, $p < 0.001$) in the diabetic group. These results are consistent with a cross –sectional study done on children with type 1 diabetes, in which they found that there is a significant correlation between Hs-CRP and IL6 and glycemic control guided with FPG and HbA1c[25]. Also, Dogan et al[26] conducted a study on population consisted of 27 children with type1 diabetes and 25 healthy controls, and they found that there is a positive significant correlation between level of HbA1c and inflammatory markers, IL-6 and Hs-CRP. Another study reported that patients with high HbA1c% showed a higher level of cytokines and presenting with micro-vascular complications which proceeds to nephropathy in type-1 diabetic patients earlier than have restricted blood glucose level[27].

Also, we found a significant direct correlation between blood pressure and Hs-CRP and IL-6. These results agree with another investigation[28] reported after doing a prospective study on 317 type-1 diabetic patients to research vascular risk factors and markers of endothelial function and the inflammatory markers in type-1 diabetes. The Authors found that inflammatory markers especially CRP are more higher in diabetic hypertensive patients more than normotensive diabetic patients. Also another study[29] reported that systolic blood pressure is positively correlated with C-reactive protein. In addition, a study[20] included 45 consecutive young patients with type 1 diabetes, followed up at a public health assistance center, and 30 healthy subjects matched by age revealed that inflammatory markers especially CRP are more higher in diabetic hypertensive patients more than normotensive diabetic patients.

Our results showed a positive significant correlation between Hs-CRP and IL-6 ($r = 0.728$, $p < 0.001$). This is in agreement with a study[30] which was conducted on 80 patients with new-onset type-1 diabetes. Also consistent with Haller and Schatz[30] who found that there was a positive correlation between elevated plasma level of IL-6 and elevated plasma level of Hs-CRP in young adults with type 1 diabetes.

By doing step-wise multiple regression analysis to determine the independent association between potential predictor variables (age, duration of diabetes, waist circumference, CRP level, and IL-6 level at baseline), the UAE was the dependent variable, after adjusting for the effect of other variables by partial correlation analysis.

Association between UAE and the levels of inflammatory markers of Hs-CRP with $R^2 = 0.927$ ($p < 0.001$) and IL-6 with $R^2 = 0.838$ ($p < 0.001$) was found. These results are consistent with Targer et al[31] who conducted a study on sixty patients with type-1 diabetes mellitus who further divided into normoalbuminurics, mi-

croalbuminurics, and macro-albuminurics. They found that inflammatory markers in early type-1 diabetic nephropathy are elevated and are associated with urinary albumin excretion. It is possible to hypothesize on the participation of locally released cytokines in the development of kidney damage.

Also we agree with Choudhary and Ahlawat[32] who found a high levels of Hs-CRP and IL-6 in children with type 1 diabetes mellitus which were correlated to the albumin excretion in urine. These results are also consistent with a study conducted on 212 type-1 diabetic patients with albuminuria[33], where they found that albuminuria in these patients was associated with elevation of the cytokines levels.

In our study we found there was no significant relationship between UAE and HbA1c levels. Also, another study[34] proved that association between urine albumin excretion and HbA1c are independent, and these suggest that factors other than poor glycemic control may be involved in the pathogenesis of early diabetic nephropathy.

On the other hand, Amine et al[35] reported that urine albumin excretion had a significant association with HbA1c in a cross sectional study of 39 children and adolescents with type 1 diabetes.

In conclusion, Hs-CRP and IL-6 exert an important diversity of actions implicated in diabetic nephropathy, from development of the initial stages of diabetes to progression to renal failure[36].

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