الاعتلال الكلوي السكري وبعض المشعرات الالتهابية (IL-1β, IL-6, IL-8, CRP)

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الملخص

يترافق إنتاج السيتوكينات مع الداء السكري الذي يترافق بدوره بارتشاح جزر لانغرهانس والخلايا اللمفاوية ذاتية التفاعل ومع تخريب نوعي لخلايا ß المنتجة للأنسولين.

لذلك كان هدفنا البحث عن الدور الأساسي لتأثير السيتوكينات خاصة (الانترلوكين-6, الانترلوكين-1 الانترلوكين-8) والبروتين المتفاعل-C (CRP) في المضاعفات الوعائية الصغيرة للداء السكري (الاعتلال الكلوي).

تمت مقارنة 31 مريضاً سكرياً مصاباً بالاعتلال الكلوي, تراوحت الأعمار بين 45-65 سنة. وأخضعوا للديال (التحال) مع مجموعة شاهدة غير سكرية (20سوياً) متقاربة بالعمر والجنس.

وقورنت أيضاً هذه المجموعة مع مجموعة سكرية غير مصابة بالاعتلال الكلوي (n=124).

تمت المقايسات المناعية في المصل بطريقة ELISA.

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كانت تراكيز الانترلوكين-6 والانترلوكين-8 المصلية ما قبل الالتهابية عند مرضى السكري ذوي الإصابة الكلوية أعلى منها في المجموعة الشاهدة:

(وسطي IL-6 عند الكلويين 6.6 pg/ml والمجموعة الشاهدة1.2pg/ml بالترتيب P≤0.05).

(وسطي IL-8 عند الكلـويين 18.3 pg/ml والمجموعـة الشـاهدة8.7 pg/ml بالترتيـب p≤0.05).

(وسطيCRP عنـد الكلـويين 1.3 mg/dl والمجوعـة الشـاهدة 0.35mg/dl بالترتيـب p≤0.05).

دات معلوماتنا على وجود زيادة واضحة في متوسط IL-6 فـي المصـل عنـد المرضـى السكريين ذوي الاعتلال الكلوي مقارنة بالمجموعة السكرية غير المصابة بالاعتلال الكلوي.

أظهرت دراستنا أن هناك فرط إنتاج لبعض الوسائط الالتهابية لدى مرضى الاعتلال الكلوي.

لم تكن المجموعة المدروسة عشوائية وأظهر IL-6 استجابة مترافقة مع هذه المجموعـة أكثر من استجابة IL-8 , IL-1. يعتقد أن مستوىIL-6 بالبول هو مؤشر جيـد للاعـتلال الكلوي السكري ومن ثَم يمكن أن يكونIL-6 المصلي مفيداً في تقيـيم التصـاب العصـيدي المترافق مع الاعتلال الكلوي.

Diabetic Nephropathy with Inflammatory Indicators (IL-1β, IL-6, IL-8, CRP)

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Abstract

cytokines production is associated with diabetes mellitus and in its turn is associated with infiltration of the islets of Langerhans with autoreactive lymphocytes and specific destruction of the insulin-producing B cells. Our aim is therefore to investigate the effect potential role of the effect of the cytokine especially (interleukin-1B, interleukin-6, interleukin-8) and C-reactive protein (C.R.P.) on the microvascular complications of diabetes (nephropathy).

A total of 31 diabetic patients with nephropathy aged 45 to 60 years, undergoing dialysis were compared with non-diabetic control subjects (N=20) after matching for age and sex, and were compared also with diabetic subjects without nephropathy (N=124). Immune parameters were analyzed in serum with rigidly evaluated ELISA.

Serum proinflammatory interleukin-6 (IL-6) and interleukin-8 (IL-8) concentrations were higher in nephropathy diabetic patients than in the control subjects.

(Mean for IL-6 in nephropathy and control subjects 6.9 Pg/ml, 1.2 Pg/ml respectively $P \leq 0.05$).

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(Mean for IL-8 in nephropathy and control subjects 18.3 Pg/ml, 8.7 Pg/ml respectively P≤ 0.05).
{Mean for C.R.P in nephropathy and control subjects 1.3 mg/dl, 0.35mg/dl respectively P≤ 0.05}
Our data showed significant increase in mean plasma Il-6, IL-8, IL-1B (P≤ 0.05) in the diabetic nephropathy compared with diabetic subjects without nephropathy.
our study shows systemic up-regulation of selected inflammatory mediators in patients with nephropathy.
The pattern observed is non- random and fits with IL-6 associated rather than IL-8, IL-1 associated response.
The urinary IL-6 level seems to be good indicator of diabetic nephropathy.
The serum IL-6 may, therefore, be useful in the evaluation of atherosclerosis including nephropathy.
Keywords: interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-1 (IL-1), C.reactive protein {C.R.P}, proinflammatory cytokine

Introduction:

Diabetic nephropathy is one of the main causes of renal and end stage diseases. (1) Morphologically, the development of diabetic nephropathy is characterized by progressive thickening of the glomerular basement membrane and by expression the mesangial Matrix which correlates to glomerular filtration function. (2-3)

Several studies have reported signs of chronic activation of the innate immune system in patients with diabetes mellitus. Abnormalities included small but definite increases of serum or plasma concentrations of several acute-phase proteins, including C-reactive protein (CRP). Acute-phase reactants are primarily produced by hepatocytes and their chief inductor is the pro-inflammatory cytokine interleukin-6 (IL-6). (4)

There was also a contribution by other inflammation- associated cytokines such as IL-8, IL-1.

IL-6 is a multifunctional cytokine produced by many different cell types including immune cells, endothelial cells, and fibroblasts. It promotes the growth of renal cells.

IL-6 may play a major role in such mesangial proliferation, but there has been little research on IL-6 in relation to diabetic nephropathy because of the difficulty in measuring urinary and serum IL-6 levels.

IL-1 mediates aboard array of both local and systemic events; it is belives that it could play an important role in immune- induced damage of Islet B cells. (5, 6)

IL-8 is chemo-tactic factor for Leukocytes, increases access of effectors cells and actives binding by B2 integrins.

IL-8 attracts and activates white blood cells during inflammation.

Subjects and Methods

Subjects: healthy subjects were included in this study absence of any systemic diseases and absence of any infections in the previous month, and matched for sex and ages with thirty-one diabetic nephropathic patients.

Blood biochemistry: Blood samples were drawn and prepared according to the recommendations of the international committee for standardization in hematology. (7)

The following parameters were measured on fresh samples; serum glucose was measured by Hitachi 911 (Trinder, P method. Roch Diagnostics).

Total cholesterol was measured by enzymatic method (Roch diagnostic); serum triglyceride concentrations were analyzed by the GPO-PAP method

Analyses of the cytokines: Plasma CRP concentrations were assessed by high sensitivity latex enhanced nephelometric assay. Serum concentrations of cytokines were measured by ELISA. All ELISAs (Enzyme Linked Immuno Sorbent Assay) were established to meet the following criteria: linearity of signal for standard curve between optical density (OD) 0.05 and 2.0, mean intra assay variation loss of signal after freezing and thawing of sera three times less than 20% .If sera gave signal above OD 2.0,mearsuring were repeated with higher diluted samples. Detection limits of cytokines by ELISA were 0.24 pg/ml for IL-6, 0.16 pg/ml for IL-1 β, 1.45 pg/ml for IL-8.

Statistical analysis: basic variables and serum parameters were descriped by mean ± SD (age, total cholesterol, triglyceride, creatinine) or median and range (all other variables).

For other variable comparisons between continuous variables were done by correlation coefficients. Student t-test was used between group comparison of means of variables and Chi square was used for the correlations between the groups. The level of significance was 5%.

Results:

Basic characteristics of groups studied: A comparison of basic characteristics of this study is given in table (1).

As age and sex were matching variables, no difference was seen, whereas mean or mean values of metabolic parameters differed between diabetic nephropathy and non-diabetic control subjects.

The patients showed statistically significant differences in fasting plasma glucose, triglycerides, and cholesterol concentration.

Cytokines: Mean circulating IL-6 concentrations was increased in diabetic nephropathy group (Fig: 1). There was an overall correlation between IL-6 and incidence of nephropathy ($P< 0.05$, significant).

For serum IL-6 concentrations, 5/31 subjects with nephropathy were elevated (fig: 2).

Mean serum IL-1β concentration were not differ from the control subjects, that was found by student's t-test. But there was a correlation between IL-1β and incidence of nephropathy (chi-square test, $p< 0.05$). (Fig: 3)

Mean serum IL-8 concentration was increased in diabetic nephropathy group (fig: 4). There was an overall correlation between IL-8 and incidence of nephropathy ($p < 0.05$).

For serum IL-8 concentrations,18/31 subjects with nephropathy showed elevated values. (Fig: 5). Acute phase protein: Mean plasma concentrations of CRP were higher in subjects with nephropathy than in the control group, there was an overall correlation between CRP and incidence of nephropathy ($p<0.05$) (fig: 6).

Another component of the inflammatory cytokine network is IL-1β whose actively in turn is regulated, we did not observe any increase of systemic IL-1β concentrations in nephropathy suggesting that IL-1β is not, or is less affected in these subjects, but IL-1β seems to contribute to the rise of circulating IL-6, IL-8 and acute-phase proteins in diabetic nephropathy.

Table 1: Basic characteristics of the study population*

	Diabetic nephropathy N=31	non-diabetic subjects N=20	P
Fasting PG (mg/dl)	**205 ± 8**	**88.7 ± 7.2**	
Triglycerides (TG)(mg/dl)	**233 ±232**	**155.7 ± 104.5**	
Cholesterol (mg/dl)	**218 ±53**	**180.7 ± 41.5**	
Creatinine (mg/dl)	**5.11 ± 0.8**	**0.89 ± 0.12**	
IL-6 (pg/ml)	**6.9 1±2.9**	**1.2 ± 1.5**	**0.0001(S)**
IL-1 β (pg/ml)	**2.03 ± 1.9**	**1.33 ± 1.5**	**0.18(N.S)**
Il-8 (pg/ml)	**18.3 ± 20.9**	**8.56 ± 1.6**	**0.0001(S)**
CRP mg/dl	**1.3 ± 1.4**	**0.35 ± 0.2**	**0.0001(S)**

PG, plasma glucose

Data are means ± SD

* Patients with known history of type II diabetes provided non-fasting blood samples are not included.

*S significant

*N.S non-significant

*N number

Discussion:

The data did not confirm reports on increased systemic IL-1β concentrations in diabetic nephropathy compared with healthy control subjects. Factors accounting for this difference might be the smaller sample size of these studies.

Data from previous studies suggested a complex interaction between IL-6 and features of metabolic syndrome.
IL-6 could affect functions of lipid and muscle cells, IL-6 is also produced by pancreatic beta cells. (8,9,10,11)
Thus, it will be difficult to resolve this complex network of immune and metabolic mediators and to distinguish between primary and responding mediators which can also vary depending on the genetic background.
Correlation between immune Markers:
Both, IL-6 and IL-1β are known to induce the production of acute phase proteins from hepatocytes. Since IL-1β, IL-6 concentrations were increased in diabetes.
It was expected that IL-6, IL-1β concentrations would correlate with concentrations of acute phase proteins.
These data provide clinically important information on systemic immune abnormalities in diabetes.
A key finding is that systemic IL-6 concentrations are increased in diabetic nephropathy. Individual serum concentrations of IL-6 varied considerably, with the normal range covering a 7-fold difference.
We focused on the parallel analysis of immune mediators which are known to constitute an immuno-regulatory network in inflammation. Major components of the IL-6 associated immune network are acute phase proteins for which the chief inducer of productions in hepatocytes is IL-6. (12)
We therefore analysed systemic concentrations of three major acute-phase proteins and found all of them increased in diabetes.
Individual concentrations of acute-phase proteins closely correlated with those of IL-6 and with each other.

Conclusion:

These observations showed the biological relevance of mildly increased concentrations of IL-6 in diabetic nephropathy as these changes are accompanied by considerable increases of acute-phase protein production.

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Fig (1): serum concentrations of IL-6 in diabetic nephropathy and controls

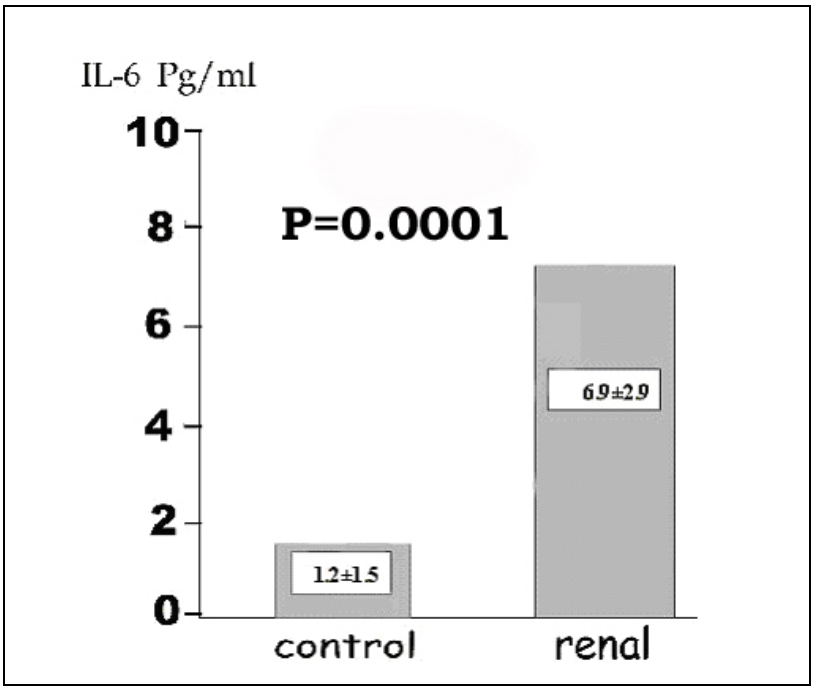


Fig (2): correlation of IL-6 in diabetic nephropathy

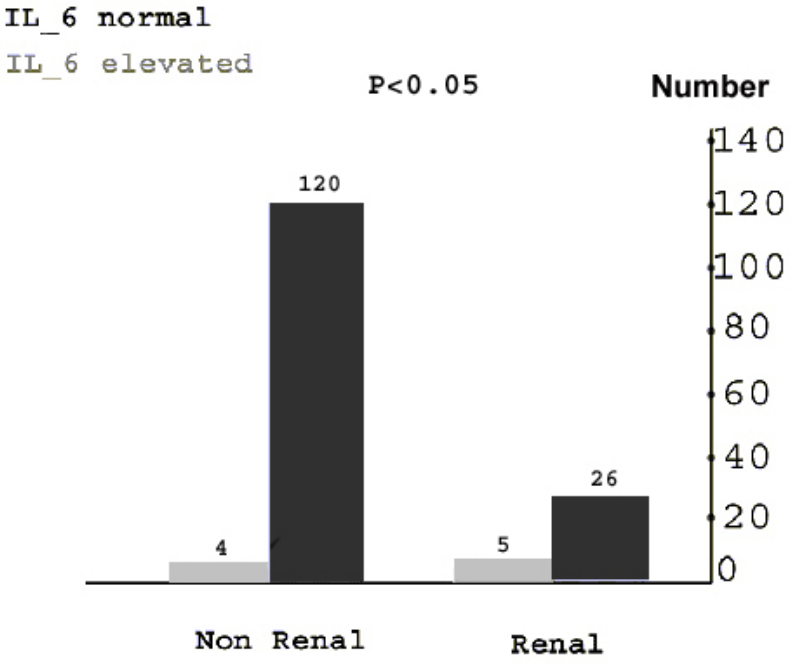


Fig (3): correlation of IL-1β in diabetic nephropathy

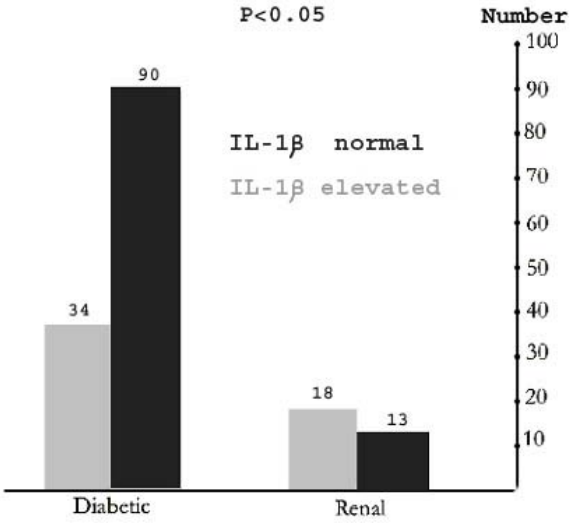


Fig (4): serum concentrations of IL-8 in diabetic nephropathy and controls

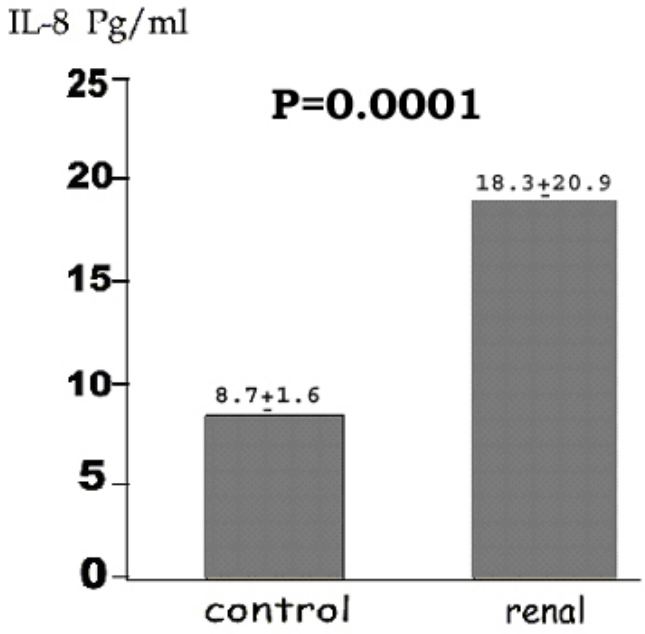
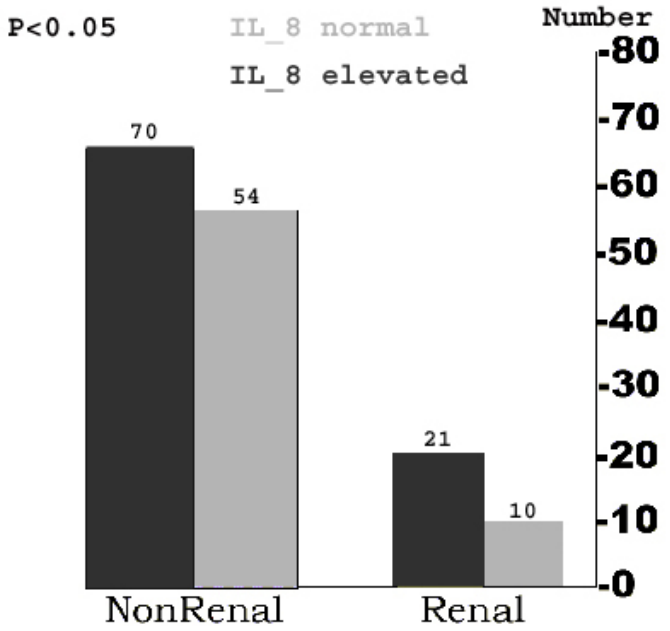
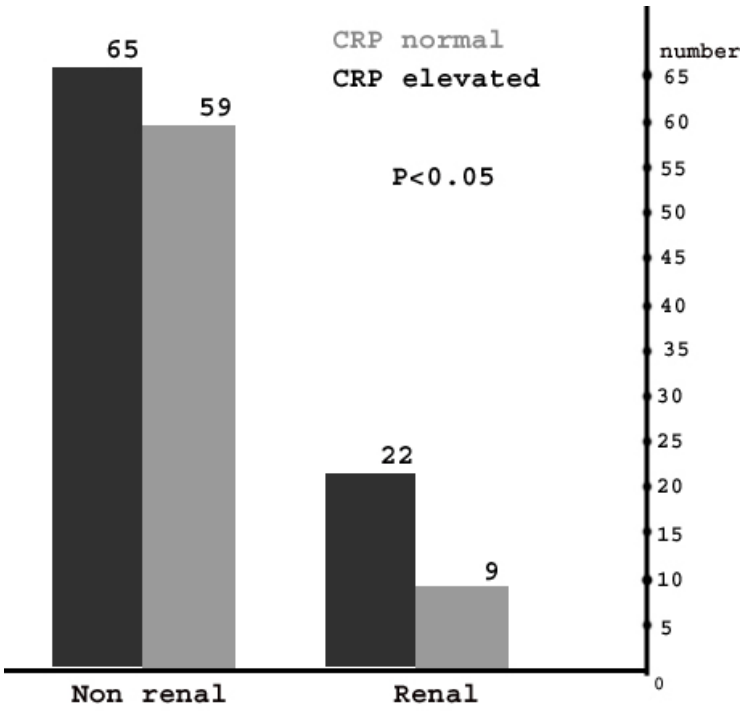


Fig (5): correlation of IL-8 in diabetic nephropathy



(Fig 6): correlation of CRP in diabetic nephropathy



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