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Quantitative Analysis of Cytokines in Diabetic Nephropathy

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Abstract

Diabetes mellitus is the most important public health problems due to its high prevalence and enormous social and economic consequences. Diabetic nephropathy is one of the chronic complications of diabetes mellitus. Inflammatory mediators are believed to play a vital role as predictors of low-grade systemic inflammation in diabetic nephropathy. Pro- and anti-inflammatory mediators relationship between ageing and low-grade systemic inflammation could be determined by measuring pro- and anti-inflammatory mediator's presence in the circulation in subjects of different age groups.

To examine the impact of inflammatory mediators on diabetic nephropathy, we quantified important inflammatory mediators such as CRP, IL-10, IL-6, MPO and TNF-α in diseased and controls using standard ELISA assay. Correlation between the multiplexed assays of ELISA was good for CRP, IL-6, IL-10, TNF-α and myeloperoxidase. Within and between run impression values for the multiplex method was < 15%. As an easier and cheaper test for assessment of diabetic nephropathy, we here with recommend further studies of CRP in diabetic patients.

Materials and Methods

Collection and Storage of Serum

Sufficient blood was collected by venipuncture into a tube (vacutainers) and centrifuged for 15 minutes at 1000× g and 4ºc. Serum was separated and collected into tube, labeled and stored at -20ºc. Hemolysed samples are discarded.

Collection and Storage of Plasma

Sufficient blood was collected by venipuncture into an EDTA venipuncture tube and centrifuged for 15 minutes at 1000× g and 4ºc within 10 minutes after the blood collection. Plasma was separated from the cells and collected into a tube, labeled and stored at -20ºc.

Analysis of Blood Samples

In present study we used Enzyme Linked Immuno Sorbant Assay (ELISA) for the estimation of cytokines CRP, IL-6, IL-10, MPO, and TNF-α in human plasma.

Data analysis

The data are expressed as means ± SEM for normally distributed values, as median with range for non-normally distributed values and percentages. Differences between groups for normally distributed variables were tested using ANOVA and non-parametric data with the Kruskal- Wallis test.

Results and Discussion

The course of diabetic nephropathy is mainly characterized by changes of urinary albumin excretion and glomerular filtration rate. In type 1diabetes, the course is well defined and progresses through five stages. In type 2 diabetes the course of diabetic nephropathy is less well characterized, due to the often unknown date of onset of disease or other factors influencing progression of nephropathy such as hypertension, age or race. Navarro-GJF and Mora-FC (2008), Fornoni et al., (2008) reviewed the role of inflammatory cytokines in diabetic nephropathy.

The present study includes 60 people, these people are divided into four groups, among them 15 normal people come under control group, 15 type 2 diabetic patients group I, 15 type 2 diabetic nephropathy patients having <3mg/dl creatinine group II, and 15 type 2 diabetic nephropathy patients having >3mg/dl creatinine as group III. Cytokines of we tested in diabetic nephropathy are elevated. As per the results we hypothesize that the possibility to predict the diabetic nephropathy status using this elevated cytokine levels.

CRP Profile in Diabetic Nephropathy

The CRP profile is examined for all groups of patients by testing their plasma CRP levels by using Sandwich ELISA technique. The results indicate that the CRP levels are more for Group III patients than Group I and Group II patients since low-grade systemic inflammation of type II diabetes increases the CRP levels. This indicates that type 2 diabetic nephropathy patients having >3mg/dl creatinine have more levels of CRP than the type 2 diabetic patients. See Table 1 and Figure 1.

Recently Mojahedi et al., (2009) reported micro -albuminuria is accompanied by elevated CRP, suggesting activation of inflammatory pathways in progression of renal and cardiovascular atherosclerotic disease.

Group	CRP Mean value (mg/dl)
Control	0.869
Group I	1.26
Group II	1.47
Group III	1.824

Table 1: CRP profile in diabetic nephropathy.

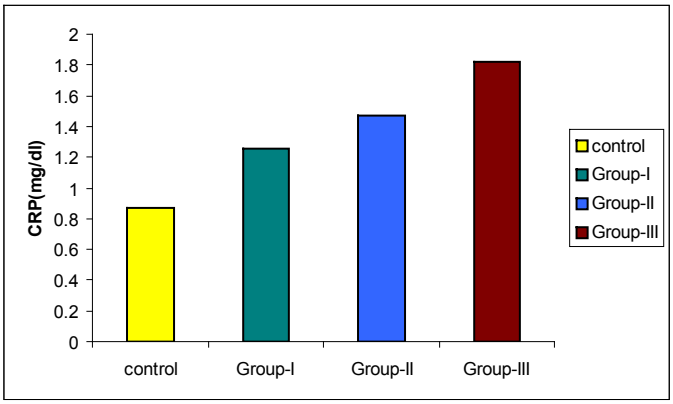

Figure 1: CRP profile in diabetic nephropathy.

Hu et al., (2004) reported that the elevated plasma levels of inflammatory markers, especially CRP, were independent predictors of type 2 diabetes in apparently healthy women. Their findings also supported the hypothesis that low-grade systemic inflammation is an underlying factor in the pathogenesis of type 2 diabetes. Navarro et al., (2005) reported that highest levels of diverse acute phase markers of inflammation, including CRP, fibrinogen in patients with type 2 diabetes and overt nephropathy.

The data analysis indicates that there is significant increase in CRP values between control, type 2 diabetes and diabetic nephropathy groups.

TNF Profile in Diabetic Nephropathy:

The TNF- α profile is examined for all groups of patients by testing their plasma levels by using Sandwich ELISA technique. The results indicate that the TNF- α levels are more for Group III patients than Group I and Group II patients since low-grade systemic inflammation of Type II diabetes increases the TNF- α levels. This indicates that type 2 diabetic nephropathy patients having >3mg/dl creatinine have more levels of TNF- α than the type 2 diabetic patients. See table 2 and figure 2.

Navarro et al., (2009) has reviewed the TNF-a importance as therapeutic target for diabetic nephropathy. In 1999, Navarro et al., reported that urinary protein excretion was strongly related with the TNF- α. Pro-in-

Groups	TNF-α median value levels mg/dl
Control	9.45
Group I	13.669
Group II	35.15
Group III	54.688

Table 2: TNF- α profile in diabetic nephropathy.

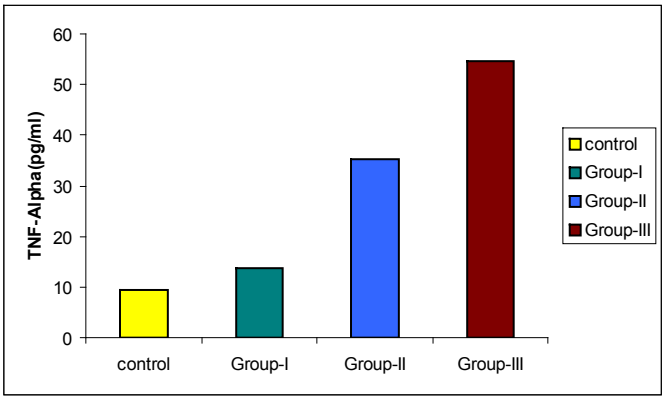

Figure 2: TNF- α profile in diabetic nephropathy.

flammatory cytokines such as TNF-α play a significant role in the renal damage of patients with diabetic nephropathy.

The data analysis indicates that there is significant increase in TNF-α values between control , type 2 diabetes and diabetic nephropathy groups.

IL- 6 Profile in Diabetic Nephropathy

The IL- 6 profile is examined for all groups of patients by testing their plasma IL- 6 levels by using Sandwich ELISA technique. The results indicate that the IL- 6 levels are more for Group III patients than Group I and Group II patients since low-grade systemic inflammation of type 2 diabetes increases the IL- 6 levels. This indicates that type 2 diabetic nephropathy patients having >3mg/dl creatinine have more levels of IL- 6 than the type 2 diabetic patients. See table 3 and figure 3.

In 2000, Pickup et al., reported that type 2 diabetes mellitus is associated with increase circulating concentrations of markers of the acute phase response and IL-6.They confirmed that circulating concentrations of the cytokine

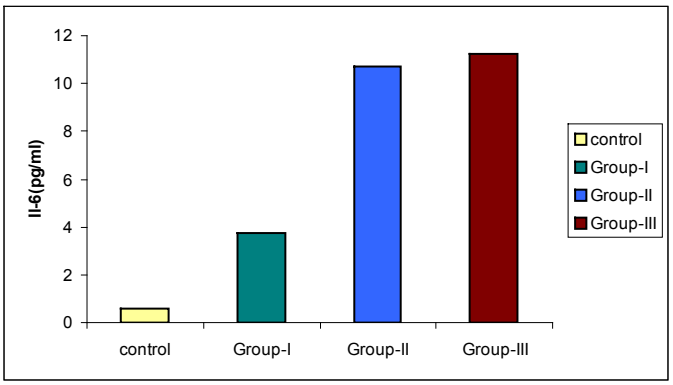

Figure 3: IL- 6 profile in diabetic nephropathy.

acute-phase mediators IL-6 and TNF-α are elevated in type 2 diabetes mellitus. In 2004, Aso et al., investigated diabetic nephropathy relationship with elevated markers for coagulation and inflammation. Plasma concentrations of IL-6 were significantly higher in diabetic patients than in control subjects.

The data analysis indicates that there is significant increase in IL-6 values between control, type 2 diabetes and diabetic nephropathy groups.

IL- 10 Profile in Diabetic Nephropathy

The IL- 10 profile is examined for all groups of patients by testing their plasma IL- 10 levels by using Sandwich ELISA technique. The results indicate that the IL- 10 levels are more for Group III patients than Group I and Group II patients since low-grade systemic inflammation of type 2 diabetes increases the IL- 10 levels. This indicates that type 2 diabetic nephropathy patients having >3mg/dl creatinine have more levels of IL- 10 than the type 2 diabetic patients. The IL-10 levels decreased in Group I patients than the control group. See figure 4 and Table 4.

In 2005, Jolanta Mysliwska et al, examined the level of circulating interleukin-10 (IL-10) and relate it to the grade of albuminuria in patients with diabetic nephropathy (DN) due to type 1 diabetes mellitus (DM). ELISA measured the IL-10 level in serum samples from thirty patients with DN due to type 1 DM, and compared with thirty patients with type 1 DM without DN and a control group of thirty, healthy,

Groups	IL- 6 median value levels mg/dl
Control	0.58
Group I	3.76
Group II	10.733
Group III	11.21

Table 3: IL- 6 profile in diabetic nephropathy.

Groups	IL- 10 median value levels (pg/ml)
Control	1.645
Group I	1.640
Group II	1.665
Group III	1.8

Table 4: IL- 10 profile in diabetic nephropathy.

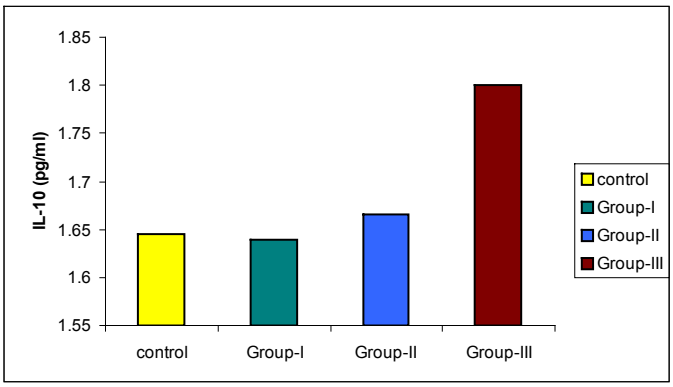

Figure 4: IL- 10 profile in diabetic nephropathy.

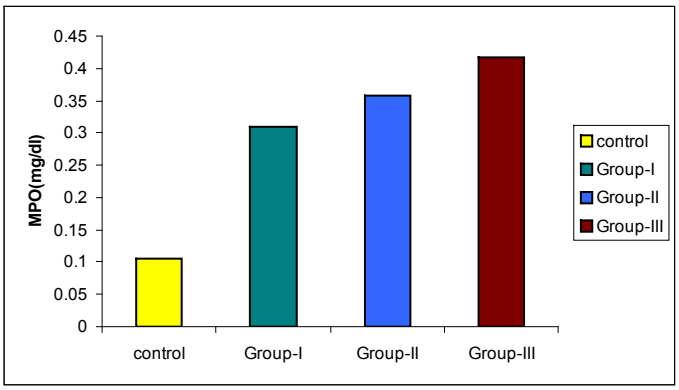

Figure 5: MPO profile in diabetic nephropathy.

age- and sex-matched people. They observed a great-elevated concentration of circulating IL-10 in 30/30 DM patients with DN. The increased concentration of IL-10 in the serum samples from DM patients with DN seems to depend on the severity of the nephropathy. van Exel et al., (2002) reported that the lowest IL-10 production capacity is associated with metabolic syndrome and type 2 diabetes.

The data analysis indicates that there is not a statistically significant difference between control, type 2 diabetes and diabetic nephropathy groups.

MPO Profile in Diabetic Nephropathy

The MPO profile is examined for all groups of patients by testing their plasma MPO levels by using Sandwich ELISA technique. The results indicate that the MPO levels are more for Group III patients than Group I and Group II patients since low-grade systemic inflammation of Type 2 diabetes increases the MPO levels. This indicates that type 2 diabetic nephropathy patients having >3mg/dl creatinine have more levels of MPO than the type 2 diabetic patients. See table 5 and figure 5.

In 2003, Ernst Malle et al reported that MPO-derived oxidants are capable of forming AGEs, a link between the MPO-hydrogen peroxide-chloride systems, the generation of AGEs and subsequent interaction with RAGE may be assumed. MPO expression is up regulated in pathological conditions like diabetic nephropathy. However, increased MPO activity has not been reported in diabetic nephropathy. It is still speculative to regard MPO as a relevant pathogenetic factor for renal complications in diabetes. They hypothesized that MPO may increase in the postprandial state because of postprandial leukocyte or activation, especially in the subject with type 2 diabetes mellitus.

Groups	MPO median value levels mg/dl
Control	0.106
Group I	0.309
Group II	0.358
Group III	0.416

Table 5: MPO profile in diabetic nephropathy.

The data analysis indicates that there is significant increase in MPO between control, type 2 diabetes and diabetic nephropathy groups.

Conclusions & Perspectives

Inflammatory mediators released by islet-infiltrating immune cells play a crucial role in beta-cell dysfunction and apoptotic cell death in the pathogenesis of diabetes. Inflammatory markers in early diabetic nephropathy are elevated and are independently associated. Supporting to the previous studies that have suggested that increased levels of CRP, IL-10, IL-6, MPO and TNF-α are the good predictors of low grade systemic inflammation in diabetes, our study reveals that CRP may be the more efficient inflammatory indicator of low grade systemic inflammation associated diabetic nephropathy. Unfortunately, the ELISA assay results may vary due to the methods used for detection and their sensitivities, interference due to differential drugs used and the effect of concomitant pathologies. Hence, proteomic and bioinformatic studies may do necessary for early stage prediction of the disease, to understand the pathophysiology and new therapeutic interventions of diabetic nephropathy.

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