Significance of Urinary Monocyte Chemoattractant Protein-1 in Early Detection of Nephropathy in Type 2 Diabetic Patients

Mona I Nabih[1], Ahmed El-Mazny[1], Nadia A Mohamed[1] and Amal R El-Shehaby[2]

Departments of Internal Medicine[1] and Medical Biochemistry[2], Faculty of Medicine Cairo University
ahmed_el_mazny@yahoo.com

Abstract: Objective: Monocyte Chemoattractant Protein-1 (MCP-1) is the strongest known monocytes chemotactic factor and has been implicated in the development and progression of diabetic nephropathy. So, measuring urinary MCP-1 would be of great significance in the diagnosis and intervention of diabetic nephropathy. This study aimed at determining the levels of urinary MCP-1 (uMCP-1) at different stages of diabetic nephropathy and to study its correlation with other clinical and laboratory parameters in Egyptian type 2 diabetic subjects. **Materials and methods:** A total of 45 type 2 diabetic subjects were classified into three groups based on their urinary albumin excretion and were compared with non-diabetic controls (Group IV) (n=15). The groups of diabetic subjects were Group I (normoalbuminuria) (n=15), Group II (microalbuminuria) (n=15) and Group III (macroalbuminuria) (n=15). The four groups were age and sex matched. Medical history, clinical examination, anthropometric and biochemical details were recorded for all the subjects. Urinary MCP-1 levels were measured by using solid phase ELISA method. **Results:** The mean level of uMCP-1 in patients with type 2 diabetes was significantly higher than in control subjects ($p<0.0001$) and the mean level of uMCP-1 in the normoalbuminuric group was significantly higher than in the controls ($p<0.0001$). Compared with the normoalbuminuric group, the mean levels of uMCP-1 in the microalbuminuric and macroalbuminuric groups were significantly higher ($p<0.0001$). Also, the mean level of uMCP-1 in the macroalbuminuric group was significantly higher than that in the microalbuminuric group ($P<0.0001$). The levels of uMCP-1 were positively correlated with the levels of albuminuria in all diabetics ($p< 0.0001$) and in the macroalbuminuric group ($p<0.05$). The levels of uMCP-1 were significantly negatively correlated with eGFR in the microalbumnuric group ($p< 0.05$). The levels of uMCP-1 correlated positively with HbA1C in all diabetics ($r=0.6$, $p<0.0001$) and in the macroalbuminuric group ($r = 0.6$, $p < 0.05$) and correlated positively with serum total cholesterol ($r=0.7$, $p<0.0001$) and LDL-C in diabetic patients ($r=0.7$, $p<0.0001$). **Conclusion:** Our study demonstrated that urinary MCP-1 levels increased gradually in type 2 diabetic subjects with increased albuminuria. It is significantly associated with the same risk factors of diabetic nephropathy.

[Mona I Nabih, Ahmed El-Mazny, Nadia A Mohamed and Amal R El-Shehaby. **Significance of Urinary Monocyte Chemoattractant Protein-1 in Early Detection of Nephropathy in Type 2 Diabetic Patients**. *Life Sci J* 2013;10(1):3030-3039]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 372

Key words: Diabetes mellitus, diabetic nephropathy, cytokines, monocyte chemoattractant protein 1.

1. Introduction

The development of diabetes involves metabolic, endocrine, and hemodynamic abnormalities which can promote a state of chronic inflammation and vascular dysfunction in many tissues. In the kidney, this can lead to the development of an innate immune response which is predominantly characterized by the accumulation of kidney macrophages [(1)]. Studies in human and experimental diabetic nephropathy have shown that kidney macrophage accumulation is associated with the progression of diabetes, the development of renal injury (tissue damage and albuminuria), kidney fibrosis and decline in renal function, suggesting that it is an inflammatory mediated disease [(1,2,3)].

Monocyte chemoattractant protein-1 (MCP-1) is a member of the CC chemokine family which is produced by endothelial cells, vascular smooth cells, keratinocytes, fibroblasts, mesangial cells, tubular epithelial cells, lymphocytes and monocytes/macrophages in response to a variety of proinflammatory stimuli. Recent evidence has highlighted the production of MCP-1 by diabetic kidneys as a major factor influencing macrophage accumulation. MCP-1 is a secreted protein which specifically attracts blood monocytes and tissue macrophages to its source, via interaction with its cell surface receptor CCR2 [(4)].

The importance of kidney MCP-1 in the early development of diabetic nephropathy has been determined using both animal and human studies [(5, 6, 7)]. In a model of streptozotocin-induced type 1 diabetic nephropathy, mice genetically deficient in MCP-1 were found to have reduced renal injury compared with wild-type mice with equivalent hyperglycemia [(5)]. Elements of the diabetic milieu induce renal parenchymal cells to secrete MCP-1, which attracts monocytes into the kidney and stimulates myofibroblast-like properties in mesangial cells. Further exposure of kidney macrophages to MCP-1 and the diabetic milieu promotes macrophage activation, resulting in the release of reactive oxygen species (ROS), proinflammatory cytokines (e.g. IL-1, TNF-α, MCP-1) and profibrotic

growth factors (e.g. PDGF, TGF-β).The self-amplifying inflammatory response causes injury and death to parenchymal cells resulting in the development of renal failure [8].

Increased amounts of MCP-1 are detected in the renal biopsies and urine from patients with diabetic nephropathy [9,10,11]. It was also suggested that increased urinary MCP-1 expression appears earlier than microalbuminuria in diabetes [12]. These findings suggest that urine MCP-1 may have significant diagnostic value in evaluating the renal inflammatory response in patients with diabetic nephropathy and provide a strong rationale for developing specific therapies against MCP-1 and inflammation in diabetic nephropathy.

The aim of this work was to evaluate the levels of urinary MCP-1 in different stages of diabetic nephropathy and to correlate the findings with other clinical and laboratory parameters in type 2 diabetes mellitus.

2. Subjects and Methods

Subjects:

This study was conducted on 45 patients with type 2 diabetes mellitus attending the Clinic of Endocrinology, Internal Medicine department, Kasr El Ainy Cairo University Hospital (Diagnosis based on the World Health Organization criteria), as well as 15 healthy volunteers as a control group. Patients were categorized into 3 groups according to urinary albumin excretion (UAE) as following: **Group I**: which included fifteen patients with normoalbuminuria (urinary albumin levels <30 mg/g Cr), **Group II:** which included 15 patients with microalbuminuria (urinary albumin levels from 30 to 300 mg/g Cr) and **Group III:** which included fifteen patients with macroalbuminuria (urinary albumin levels >300 mg/g Cr). In addition to **Group IV,** which included fifteen healthy subjects taken as a control group, who were age and sex matched with the patients (urinary albumin levels less than 30 mg/ g Cr)

Exclusion criteria:

Patients with other kidney, hepatic or rheumatologic disease, current acute illness (including infections), immunologic or neoplastic diseases, or other endocrine diseases. Also, patients with a history of diabetic ketoacidosis or hypoglycemic coma during the last 3 months preceding the study and those with current use of immunomodulatory medications or statins were excluded from the study to avoid potential confounding factors.

Methods:

All subjects gave informed consent to participate in the study. All patients were subjected to complete history taking including duration of diabetes and medications, complete physical examinations including arterial blood pressure and estimation of Body Mass Index (BMI) (weight (kg)/ height (m2) as well as fundus examination.

Patients were also subjected to laboratory investigations which included kidney function tests (Serum creatinine, blood urea nitrogen and Glomerular filtration rate estimated by Cockroft-Gault equation).

Estimated creatinine clearance (ml/min) =

$$\frac{140\text{-age} \times \text{body weight (kg)}}{72 \times \text{PCr (mg/dl)}}$$

(N.B. Multiplied by 0.85 for women) [13]

Also, Serum albumin, Lipid profile including total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) and glycosylated hemoglobin (HbA1C), urinary MCP-1 and levels of urinary albumin were assessed in all patients.

Estimation of lipid profile:

Estimation of serum cholesterol and triglycerides were done by cholesterol enzyme colorimetric assay on a Ciba Corning Express Plus analyzer using commercially available Kit (Boehringer Mannheim, Germany). Estimation of serum HDL was done by precipitation with dextran sulphate Ciba Corning Diagnostics, Frenwald,Germany [14]. LDL-C was calculated according to the Fridwald formula as follows: LDL-C= Total cholesterol-TG/5-HDLC [15].

Quantitative colorimetric determination of glycated hemoglobin (HbA1C) in blood:

Estimation of glycated hemoglobin was done by glycohemoglobin reagent set from Pionte Scientific Inc. Normal range of HbA1C= 4.2- 6.2% [16].

Estimation of urinary MCP-1:

Methods of the assay: Urine samples were drawn from each subject and frozen at -80°C for later analysis of urine albumin/creatinine ratio and urinary MCP-1 by ELISA technique.

Principles of the method: Freshly voided urine samples were collected and centrifuged at 2000 rpm/min for 10 min. Two milliliters supernatant was taken for the estimation of uMCP-1 levels and stored at-80°C until tested. Urinary MCP-1 levels were measured with a solid phase enzyme linked immunosorbent assay (Quantikine MCP-1 ELISA; R&D Systems Inc., Minneapolis, USA). The coefficient of mean variations in the samples were <5%. The minimum detectable MCP-1 level with this kit was less than5 pg/ml. No significant cross-reactivity or interference was observed with this assay kit. Levels of uMCP-1 were expressed as values corrected by the urinary creatinine concentration (mg of creatinine/dl). [17]

Estimation of degree of albumin in urine:

Urine Albumin Creatinine Ratio (UACR) measures albumin excretion in mg albumin/g creatinine. The test is run on a spot urine sample. Urine ACR, which is commonly used in clinical practice, not only correlates with 24-hour urine protein but also is clinically relevant for predicting progression. The normoalbuminuric subjects have urinary albumin by creatinine ratio (ACR) of <30 mg/gm creatinine. The microalbuminuric subjects having urinary albumin by creatinine ratio of 30–300 mg/gm creatinine and the macroalbuminuric subjects have urinary albumin by creatinine ratio of >300 mg/gm creatinine [(18)].

Statistical analysis of the results:

Correlations between urinary MCP-1 were done with different clinical and laboratory data (Age, duration of diabetes, systolic blood pressure (SBP), diastolic blood pressure (DBP), estimated GFR, albuminuria, creatinine in urine, serum albumin, serum creatinine, urea, HbA1C, total cholesterol, HDL-C, LDL-C, and TG) of each group (normoalbuminuric patients, microalbuminuric patients and macroalbuminuric patients). Comparisons were done between clinical and laboratory data of diabetic groups (I, II, III) and control group (IV), as well as between clinical and laboratory data of the three diabetic groups (I, II, III). Data was analyzed using Statistical Package of social science (SPSS) version 9.0. Data was summarized as mean and SD. *t* test was used for analysis of two quantitative data. One way ANOVA was used for analysis of more than two variable followed by post HOCC test for detection of significance.

Simple linear correlation (person's correlation) was done. "r" value was considered weak if <0.25, mild if ≥0.25-<0.5, moderate if ≥0.5-<0.75 and strong if ≥0,75. P value is considered significant if < 0.05.

3. Results

Our study showed there was a high statistically significant difference in u-MCP1 levels between the diabetic group and the control group (P<0.0001) (figure.1). Also, there was a high statistically significant difference in u-MCP1 levels between the diabetic normoalbuminuric group and the control group (P<0.0001). Compared with the normoalbuminuric group, the levels of uMCP-1 in the microalbuminuric and macroalbuminuric groups were significantly higher (P<0.0001). Also, levels of uMCP-1 in the macroalbuminuric group were significantly higher than those in microalbuminuric group (P<0.0001) (table 1 & figure. 2).

There was a highly significant positive correlation between uMCP-1 and albuminuria (r=0.9) (P<0.0001) when all diabetic patients were considered (table 2 & figure 3) and in the macroalbuminuric group (r=0.6) (P<0.05) (Figure 4). However, no correlation was found in the normoalbuminuric or the microalbuminuric group.

When all the diabetic patients were considered, there was a highly significant positive correlation between uMCP-1 and serum creatinine (r=0.48) (P<0.001), HbA1C (r=0.6) (P<0.0001), total cholesterol (r=0.7) (P<0.0001) and LDL-C (r=0.7) (P<0.0001). On the other hand, there was a highly significant negative correlation between uMCP-1 and serum albumin (r=-0.54) (P<0.0001). There was no significant correlation between uMCP-1 and age, duration of disease, SBP, DBP, BMI, e GFR, creatinine in urine, urea, triglyceride or HDL-C.

In normoalbuminuric patients, the correlation study between uMCP-1 and clinical and laboratory data, showed no significant correlation with any of the studied parameters.

In microalbuminuric patients, uMCP-1 showed a highly significant negative correlation with eGFR (r=-0.7) (p<0.05) (Figure 5) and a significant positive correlation with serum creatinine (r=0.6) (p<0.05). There was no significant correlation with age, duration of disease, BMI, SBP, DBP, albuminuria, creatinine in urine, serum albumin, urea, HbA1C, total cholesterol, triglyceride, HDL-C or LDL-C.

In macroalbuminuric patients, uMCP-1 showed a significant positive correlation with HbA1C (r=0.6) (P<0.05). Also, there was a highly significant negative correlation with creatinine in urine (r=-0.8) (P<0.001), but no significant correlation was found with age, duration of disease, BMI, SBP, DBP, eGFR, serum albumin, serum creatinine, urea, HbA1C, total cholesterol, triglyceride, HDL-C, or LDL-C.

Comparison between uMCP-1 in all diabetic patients in relation to retinopathy showed that there was no statistically significant difference between the mean uMCP-1 values in patient with and without retinopathy.

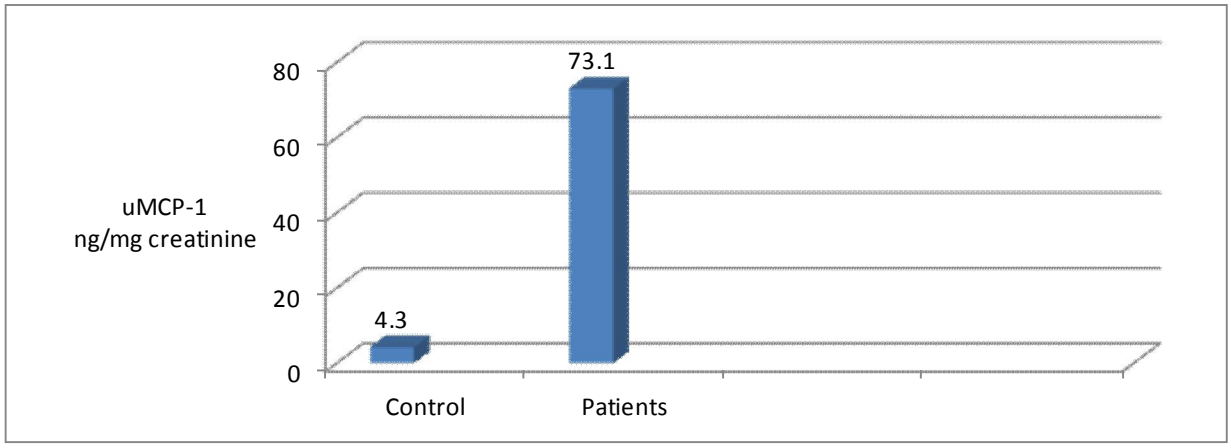

Figure 1: Comparison between uMCP-1 in control subjects and all diabetic patients

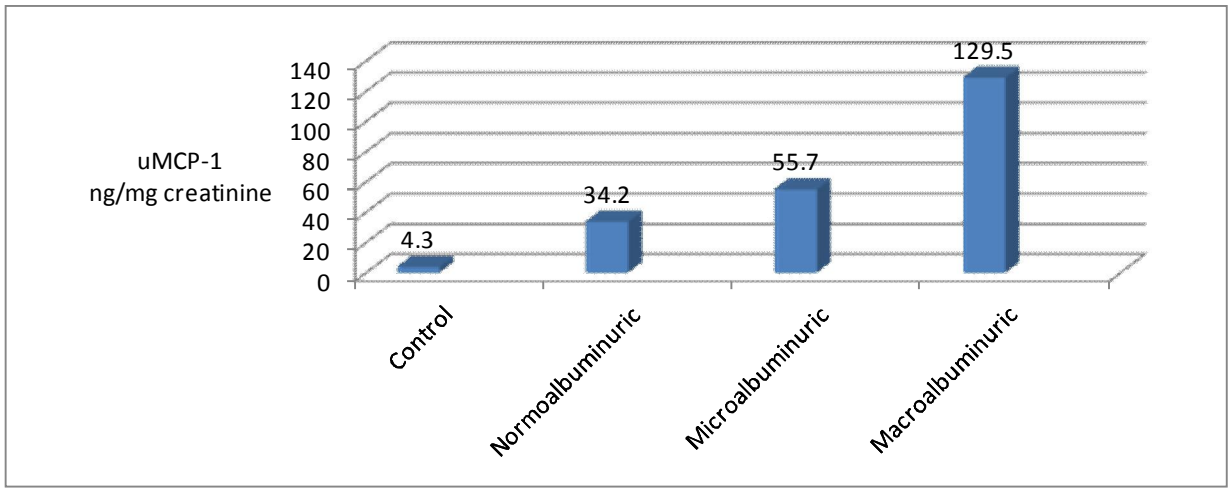

Figure 2: Comparison between uMCP-1 levels in control subjects and the three studied patients groups

Table 1: Comparison between clinical and laboratory data of control subjects, normoalbuminuric, microalbuminuric and macroalbuminuric patients groups

Variables	Control subjects Mean ± SD	Normoalbuminuric patients Mean ± SD	Microalbuminuric patients Mean ± SD	Macroalbuminuric patients Mean ± SD	p-value
Age (yrs)	55.1±7.4	56.5 ± 3.3	55.1 ± 8.5	54 ± 6.0	0.07
Duration of disease (yrs)	-	5.5 ± 3.3	5.1 3.9	4.1 ± 2.5	0.5
BMI (Kg / m^2)	31.8± 6.4	30.7 ± 5.5	32.2 ± 7.6	31.9 ± 3.9	0.08
Systolic blood pressure (mmhg)	121±10.9	129.3 ± 14.4	128.0 ± 12.6	126.0 ± 17.2	0.8
Diastolic blood pressure (mmHg)	75.7± 26.2	77.3 ± 5.9	79.7 ± 7.4	78.7 ± 7.2	0.7
eGFR (ml/min)	-	74.2 ± 21.8[a]	95.2 ± 24.4[b]	71.9 ± 10.2[a]	<0.05*
UACR (mg/g creatinine)	17.7± 4.6[a]	18.3± 6.4[a]	92.7 ± 51.6[b]	460.3 ± 77.8[c]	<0.0001*
Creatinine in urine (mg/dl)	121.1 ± 56.2	154.3 ± 67.7	123.1 ± 67.3	164.8 ± 26.4	0.2
Serum albumin (mg/dl)	4.1 ± 0.3[a]	4.0 ± 0.2[a]	4.0 ± 0.2[a]	3.5 ± 0.5[b]	<0.0001*
Serum creatinine (mg/dl)	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.1	0.09
Urea (mg/dl)	23.4 ± 3.3[a]	29.7 ± 4.3[a]	34.5 ± 7.6[b]	39.2 ± 6.3[c]	<0.001*
HbA1C (%)	6.3 ± 1.2[a]	6.7 ± 1.9[a]	6.5 ± 1.4[a]	9.2 ± 1.9[b]	<0.0001*
Total cholesterol (mg/dl)	193.7± 20.2[a]	183.7 ± 23.7[a]	192.1 ± 32.0[a]	254.7 ± 30.1[b]	<0.0001*
Triglyceride (mg/dl)	117.6±26.2	190.5 ± 81.3	136.7 ± 64.3	181.3 ± 45.5	0.07
HDL-C (mg/dl)	51.3±5.3	44.3 ± 5.5	45.0 ± 4.8	45.6 ± 3.1	0.8
LDL-C (mg/dl)	118.9±18.3[a]	102.9 ± 30.3[a]	115.1 ± 37.0[a]	171.9 ± 33.2[b]	<0.0001*
uMCP-1 (ng/mg creatinine)	4.3±1.5[a]	34.2 ± 18.7[b]	55.7 ± 16.5[c]	129.5 ± 19.4[d]	<0.0001*

*Different symbols indicate statistical significance
UACR = Urine Albumin Creatinine Ratio

Table 2: Correlation between uMCP-1 and clinical and laboratory data in diabetic patients

Variables	r	p-value
Age (yrs)	-0.25	0.06
Duration of disease (yrs)	-0.2	0.2
BMI (Kg / m^2)	-0.16	0.29
Systolic blood pressure (mmhg)	-0.14	0.37
Diastolic blood pressure (mmHg)	0.01	0.9
eGFR	-0.27	0.068
UACR	0.9	<0.0001*
Creatinine in urine (mg/dl)	-0.03	0.8
Serum albumin (mg/dl)	-0.54	<0.0001*
Serum creatinine (mg/dl)	0.48	<0.001*
Urea (mg/dl)	0.02	0.91
HbA1C (%)	0.6	<0.0001*
Total cholesterol (mg/dl)	0.69	<0.0001*
Triglyceride (mg/dl)	-0.02	0.9
HDL-C (mg/dl)	0.06	0.6
LDL-C (mg/dl)	0.67	<0.0001*

UACR = Urine Albumin Creatinine Ratio
*Statistically significant

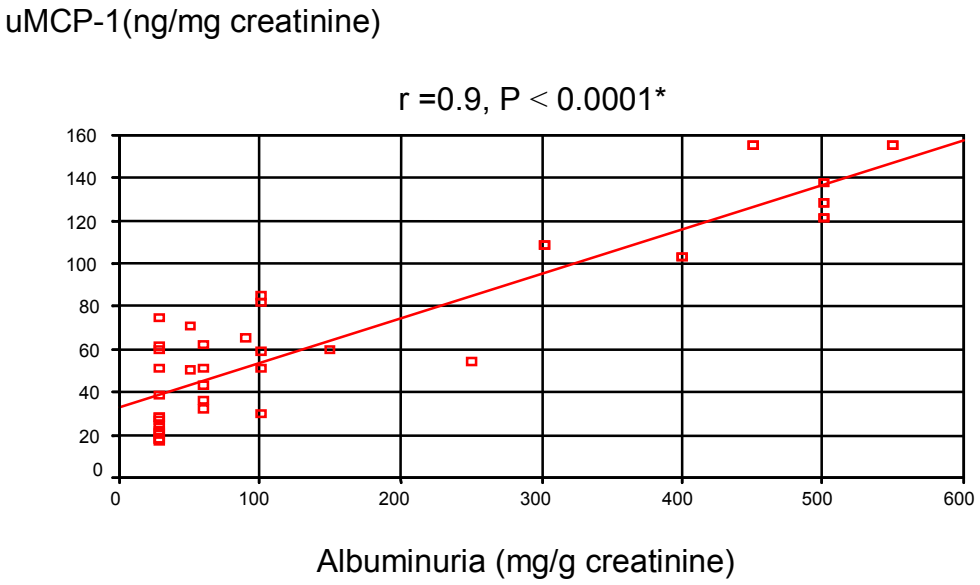

Figure 3: Correlation of u MCP-1 with albuminuria in all patients

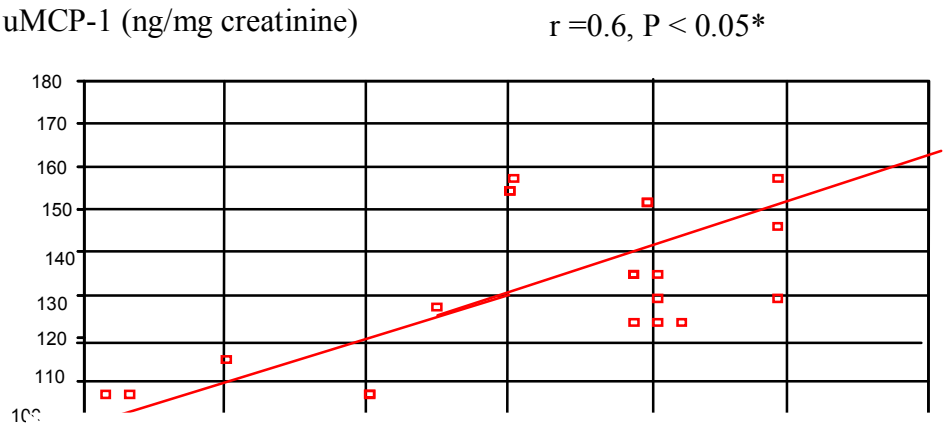

Figure 4: Correlation of uMCP-1 with albuminuria in macroalbuminuric patients

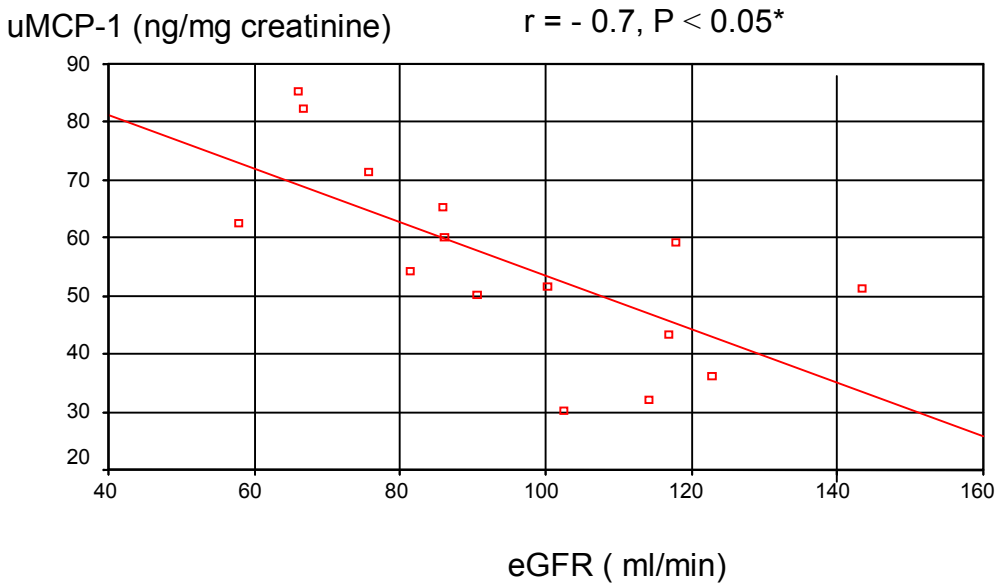

Figure 5: Correlation of u MCP-1 and eGFR in microalbuminuric patients.

4. Discussion

Monocyte Chemoattractant Protein-1 (MCP-1) is the strongest known monocytes chemotactic factor and is upregulated in diabetic nephropathy. So, measuring urinary MCP-1 would be of great significance in the diagnosis and intervention of early diabetic nephropathy. This study aimed at determining the levels of urinary MCP-1 (uMCP-1) at different stages of diabetic nephropathy and to study its correlation with other clinical and laboratory parameters in Egyptian type 2 diabetic patients.

The results of the study showed that concentrations of uMCP-1 are enhanced in diabetic subjects when compared to control subjects and its level is correlated with the level of albuminuria. Compared with normoalbuminuric group, levels of uMCP-1 in microalbuminuric group and macroalbuminuric group were significantly higher. Also, levels of uMCP-1 in macroalbuminuric group were significantly higher than those in

microalbuminuric group. There was also increased level of uMCP-1 in normoalbuminuric patients when compared to control subjects.

A significant positive correlation of uMCP-1 with urine albumin excretion was found in all diabetic patients and in the macroalbuminuric group but not in the normoalbuminuric or microalbuminuric groups.

These results suggest that uMCP-1 may play an important role in progression and development of diabetic nephropathy. Previous studies showed that serum levels of MCP-1 are sometimes elevated in diabetic patients. However, this is not associated with the development of albuminuria, kidney macrophage accumulation or nephropathy [9, 19, 20]. In contrast, urine levels of MCP-1 closely reflect kidney MCP-1 production and correlate significantly with levels of albuminuria, serum glycated albumin and kidney CD68+ macrophages in human and experimental diabetic nephropathy [5,6,9,11, 20] .

It has been suggested that proteinuria during diabetes may itself aggravate tubular injury and accelerate nephropathy by increasing tubular MCP-1 production and the inflammatory response [21, 22]. However, during diabetes, it appears more likely that tubular MCP-1 is initially induced by the diabetic milieu, since increases in tubular MCP-1 and interstitial macrophages coincide with the development of hyperglycemia and precede a rise in albuminuria in type 1 diabetic nephropathy in mice [5]. In comparison, a rat albumin overload model, which develops instant proteinuria, takes 2 weeks to induce an increase in kidney MCP-1 mRNA levels, suggesting that excreted forms of albumin cannot independently promote rapid tubular production of MCP-1 in vivo [23].

Although circulating MCP-1 appears earlier than microalbuminuria in diabetes mellitus [24], levels of uMCP-1 are increased in experimental models and patients with inflammatory renal diseases and diabetic nephropathy while serum MCP-1 levels are normal [19,25].

The increased level of uMCP-1 in the normoalbuminuric group compared to the control group, in our study, suggests that some changes could have occurred early in the pathogenesis of diabetic nephropathy and that uMCP-1 may be of high clinical significance in the early diagnosis and intervention of diabetic nephropathy.

In agreement with our results, previous studies have also shown that the mean levels of uMCP-1 in subjects with type 2 diabetes were significantly higher than healthy subjects. Levels of uMCP-1 were higher in patients with macroalbuminuria as compared with microalbuminuric, normoalbuminuric patients and healthy controls. Levels of uMCP-1 in microalbuminuric patients were significantly higher than normoalbuminuric patients and a significant difference was seen in the uMCP-1 levels between the normoalbuminuric patients and healthy subjects. The levels of uMCP-1 in type 2 diabetic subjects were positively correlated with UAE [12,26].

Eardley *et al*. (2006) studied 215 patients and quantified albumin–creatinine ratio (ACR), urinary MCP-1/CCL2, interstitial macrophage numbers, and in situ damage. ACR correlated with urinary MCP-1/CCL2, interstitial macrophage numbers, and index of chronic damage [27].

However, Morii *et al*. (2003) showed that uMCP-1 excretion levels were not significantly different between the normoalbuminuric group and the microalbuminuric group [11].

In another study, uMCP-1 level was significantly higher in the microalbuminuric and macroalbuminuric patients compared to the normoalbuminuric patients and healthy controls. Patients with macroalbuminuria had significantly higher urinary MCP-1 than microalbuminuric patients. However, normoalbuminuric diabetic patients had normal urinary MCP-1 which is not consistent with our results. The authers assumed that hyperglycemia per se, is necessary but not sufficient in determining increased MCP-1 expression [28]. It is possible that prolonged hyperglycemia, advanced glycation end products (AGE), high oxidative burden and local activation of renin-angiotensin acting together can induce MCP-1 expression in genetically predisposed patients [5].

In diabetic patients, usually an occurrence of metabolic disorder of lipids is observed. In diabetic nephropathy LDL-C is easily oxidized to oxidized LDL (ox-LDL). Both of them, especially the later may stimulate the expression of MCP-1 through mesangial cells [12].

In our study, diabetic patients had significantly higher TG and lower HDL-C than the control group. There was no significant difference between the two groups regarding total cholesterol and LDL-C. Our results showed a significant positive correlation of u MCP-1 with serum total cholesterol and LDL-C, but no significant correlation of uMCP-1 with TG or HDL-C levels was found in diabetic patients. When the three subgroups were considered, no correlation between uMCP-1 and total cholesterol, LDL-C, TG or HDL-C was found in either group. Similar results were obtained by Wang and Chen (2009) [12]. On the contrary, Priyanka *et al*. (2009) showed that uMCP-1 levels correlated positively with serum triglyceride and VLDL–C and no significant correlation was found between uMCP-1 and either total cholesterol or LDL-C [26].

Ha *et al*. (2002) observed that high glucose induced mesangial cells and that high glucose could upregulate the expression of uMCP-1 directly or

through stimulating the generation of ROS which could upregulate the expression of uMCP-1 by activating NF-KB [29].

As high glucose level leads to progression of diabetic nephropathy, we investigated the relation between uMCP-1 and HbA1C in all diabetics and in the three subgroups of diabetes. Levels of uMCP-1 showed a significant positive correlation with HbA1C levels in all diabetic patients and in the macroalbuminuric group, but no significant correlation was found in either the microalbuminuric or normoalbuminuric group.

Our results are in agreement with Banba *et al*.; 2000 who found that urinary levels but not serum levels of MCP-1 increased in accordance with the extent of HbA1C and albuminuria [9]. Also, Kiyici *et al*. (2006) found that uMCP-1 is positively correlated with HbA1C and fasting blood glucose in type 1 diabetic patients with nephropathy [20]. El-Shafey *et al*. (2008) found a positive correlation between HbA1C and both microalbuminuria and u-MCP-1, so that poor glycemic control is correlated with the development of early diabetic nephropathy [30].

On the other hand, Priyanka *et al*.; 2009 found no significant correlation between uMCP-1 and HbA1C [26]. Also, Wang and Chen, 2009, failed to find any significant correlation between uMCP-1 and HbA1C [12].

In our study, microalbuminuric patients had higher eGFR than macroalbuminuric and normoalbuminuric patients. This may be due to the hyperfiltration in early stages of diabetic nephropathy. Although uMCP-1 levels were inversely correlated to eGFR values when all diabetic patients were considered and in the microalbuminuric and macroalbuminuric groups, statistical significance was found only in the microalbuminuric group. The inverse correlation between uMCP-1 and eGFR in the microalbuminuric group defines the use of uMCP-1 as a marker which reflects the degree of kidney damage as estimated by glomerular filtration rate and proves that u MCP-1 is not a simple filtration from serum, but secreted locally.

Ibrahim and Rashed (2008) showed an inverse correlation between uMCP-1 and eGFR in diabetic patients [28]. Also, Priyanka *et al*. (2009) found that uMCP-1 levels correlated negatively with eGFR in diabetic patients. The levels of uMCP-1 were significantly higher in subjects with eGFR value of <60 ml/min compared to the subjects with eGFR values of >60 ml/min [26].

In a study on 40 diabetic nephropathy patients, followed up for 6 years, uMCP-1/Creatinine ratio did not have a significant correlation with baseline eGFR in all patients, but uMCP-1/Creatinine ratio at entry of study correlated with rate of eGFR decline for all patients over a median follow up of 6 years and u MCP-1 levels were better correlated with the rate of deterioration of eGFR than urinary protein /creatinine ratio in the macroalbuminuric group of patients [31].

Our study showed that creatinine level in urine was inversely related to uMCP-1 levels which were of statistical significance only in the macroalbuminuric group.

Heerspink *et al*. (2010) observed that lower urine creatinine concentration or 24-h urine creatinine excretion independently associates with renal progression. They suggest that a lower urine creatinine concentration reflects muscle wasting or poor overall health [32].

In the present study, no significant correlation was found between uMCP-1 and age, duration of diabetes, BMI, systolic BP or diastolic BP. Multiple previous studies showed no significant correlation between uMCP-1 and age, duration of diabetes, BMI, systolic or diastolic blood pressure [26, 28, 31, 33].

Kim *et al*. (2011) assessed serum MCP-1 levels in a case controlled study group of a gender-matched, healthy cohort of 55 patients over the age of 65 and 55 patients under the age of 45. MCP-1 levels were significantly lower in the elderly patients. The partial correlation analysis demonstrating the correlation between cytokine levels when controlled for gender, systolic blood pressure, total cholesterol, HDL cholesterol, triglyceride, and serum creatinine levels further demonstrated that MCP-1 had a significant negative correlation with age [34]. The significant inverse correlation found between uMCP-1 and age, may be explained by age related reduction of circulating monocytes and other leukocytes often observed in aged populations [35].

Shuhei *et al*. (2010), studied serum MCP-1 in type 2 diabetic patients and found that serum MCP-1 level was higher in proliferative retinopathy patients than in non-retinopathy patients. Multiple regression analysis revealed that serum MCP-1 level was correlated with diabetic retinopathy [36].

Our results showed no significant difference in the mean values of uMCP-1 between diabetic patients with and without retinopathy, which could be explained by that uMCP-1 is not a simple outfiltration of serum MCP-1and that increased uMCP-1 observed in patients with diabetic retinopathy is caused by the presence of nephropathy which may or may not associate it.

Prakash *et al*. (2007) conducted a study on 28 proteinuric type 2 diabetic patients, and found that presence or absence of diabetic retinopathy (DR) was a poor predictor of diabetic nephropathy because DN was noted in 50% of patients without DR and 40% of patients with DR had non-diabetic nephropathy either alone or in combination with DN [37].

From the results of our study, we can conclude that increased concentration of uMCP-1 may represent the start of the inflammatory process of diabetic nephropathy. So, it can be used as an early marker that predicts future nephropathy risk in diabetic patients before the appearance of albuminuria. Also, uMCP-1 may be used in the follow up of diabetic patients with nephropathy and to assess the efficacy of treatment in reducing diabetic renal inflammation. New therapeutic approaches targeting uMCP-1 or its receptor may prevent and delay the progression of diabetic nephropathy.

Corresponding author
Ahmed El-Mazny
Departments of Internal Medicine[1] and Medical Biochemistry[2], Faculty of Medicine Cairo University
ahmed_el_mazny@yahoo.com

References:

1. Chow FY, Ozols E, Nikolic-Paterson DJ, Atkins RC, Tesch GH. Macrophages in mouse type 2 diabetic nephropathy: correlation with diabetic state and progressive renal injury. Kidney Int 65: 116–128, 2004.
2. Chow FY, Nikolic-Paterson DJ, Atkins RC, Tesch GH. Macrophages in streptozotocin-induced diabetic nephropathy: potential role in renal fibrosis. Nephrol Dial Transplant 19: 2987–2996, 2004.
3. Nguyen D, Ping F, Mu W, Hill P, Atkins RC, Chadban SJ. Macrophage accumulation in human progressive diabetic nephropathy. Nephrology (Carlton) 11: 226–231, 2006.
4. Boring L, Gosling J, Chensue SW, Kunkel SL, Farese RV, Broxmeyer HE, Charo IF. Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. J Clin Invest 100: 2552–2561, 1997.
5. Chow FY, Nikolic-Paterson DJ, Ozols E, Atkins RC, Rollins BJ, Tesch GH. Monocyte chemoattractant protein-1 promotes diabetic renal injury in streptozotocin-treated mice. Kidney Int 69: 73–80, 2006.
6. Chow FY, Nikolic-Paterson DJ, Ma FY, Ozols E, Rollins BJ, Tesch GH. Monocyte chemoattractant protein-1 induced tissue inflammation is critical for the development of renal injury but not type 2 diabetes in obese db/db mice. Diabetologia 50: 471–80, 2007.
7. Kanamori H, Matsubara T, Mima A, Sumi E, Nagai K, Takahashi T, Abe H, Iehara N, Fukatsu A, Okamoto H, Kita T, Doi T, Arai H. Inhibition of MCP-1/CCR2 pathway ameliorates the development of diabetic nephropathy. Biochem Biophys Res Commun 360: 772–777, 2007.
8. Tesch GH. MCP-1/CCL2: a new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy. AJP-Renal Physiol 294 (4) 697-701, 2008.
9. Banba N, Nakamura T, Matsumura M, Kuroda H, Hattori Y, Kasai K. Possible relationship of monocyte chemoattractant protein-1 with diabetic nephropathy. Kidney Int 58: 684–690, 2000.
10. Mezzano S, Aros C, Droguett A, Burgos ME, Ardiles L, Flores C. NF kappa B activation and overexpression of regulated genes in human diabetic nephropathy. Nephrol Dial Transplant 21:299-313, 2006
11. Morii T, Fujita H, Narita T, Shimotomai T, Fujishima H, Yoshioka N, Imai H, Kakei M, Ito S. Association of monocyte chemoattractant protein-1 with renal tubular damage in diabetic nephropathy. J Diabetes Complications 17: 11–15, 2003.
12. Wang Qui-yue, Chen Fen-qin: Clinical significance and different levels of urinary monocyte chemoattractant protein-1 in type 2 diabetes mellitus. Diab Res Clin Prac.83:215–219, 2009.
13. Sokoll LJ, Russell RM, Sadowski JA, Morrow FD: Establishment of creatinine clearance reference values for older women. Clin Chem; 40:2276-2281, 1994.
14. Lopez-Virella MF: Strategies for the prevention of coronary heart disease: A policy statement of European Atherosclerosis Society. Eur Heart J. 8:77, 1977.
15. Fridwald WT: Calculation of LDL cholesterol.Clin. Chem. 18:499, 1972.
16. Krolewski AS, Laffel LM, Krolewski MJ: Glycosylated hemoglobin and risk of microalbuminuria in patients with IDDM. N Eng J Med. 332: 1251-1255, 1995.
17. Amann B, Tinzmann R, Angelkort B: ACE inhibitors improve diabetic nephropathy through suppression of renal MCP-1. Diabetes Care. 26: 2421-5, 2003.
18. Kestenbaum, B. and Ian H. de Boer: Urine Albumin-to-Creatinine Ratio: What's in a Number? J Am Soc Nephrol 21, 2010.
19. Wada T, Furuichi K, Sakai N, Iwata Y, Yoshimoto K, Shimizu M, Takeda SI, Takasawa K, Yoshimura M, Kida H, Kobayashi KI, Mukaida N, Naito T, Matsushima K, Yokoyama H. Up-regulation of monocyte chemoattractant protein-1 in tubulointerstitial lesions of human diabetic nephropathy. Kidney Int 58: 1492–1499, 2000.

20. Kiyici S, Erturk E, Budak F, Ersoy C, Tuncel E, Duran C, Oral B, Sigirci D, Imamoglu S. Serum monocyte chemoattractant protein-1 and monocyte adhesion molecules in type 1 diabetic patients with nephropathy. Arch Med Res 37: 998–1003, 2006.
21. Eddy AA, Giachelli CM. Renal expression of genes that promote interstitial inflammation and fibrosis in rats with protein-overload proteinuria. Kidney Int 47: 1546–1557, 1995.
22. Takaya K, Koya D, Isono M, Sugimoto T, Sugaya T, Kashiwagi A, Haneda M. Involvement of ERK pathway in albumin-induced MCP-1 expression in mouse proximal tubular cells. Am J Physiol Renal Physiol 284: 1037–1045, 2003.
23. Hasegawa H, Kohno M, Sasaki M, Inoue A, Ito MR, Terada M, Hieshima K, Maruyama H, Miyazaki J, Yoshie O, Nose M, Fujita S. Antagonist of monocyte chemoattractant protein 1 ameliorates the initiation and progression of lupus nephritis and renal vasculitis in MRL/lpr mice. Arthritis Rheum 48: 2555–2566, 2003.
24. Takebayashi K, Sachiko M, Yoshimasa A, and Toshihiko I: Aldosterone Blockade Attenuates Urinary Monocyte Chemoattractant Protein-1 and Oxidative Stress in Patients with Type 2 Diabetes Complicated by Diabetic Nephropathy. J Metab, 91(6):2214–221, 2006.
25. Rovin BH, Yoshimura T, Tan L: Cytokine-induced production of monocyte chemoattractant protein-1 by cultured human mesangial cells. J Immunol 148:2148–2153, 1992.
26. Priyanka T, Zenith K, Satyavani K, Mary B, Vijay V: Clinical significance of urinary Monocyte Chemoattractant Protein-1 (uMCP-1) in Indian type 2 diabetic patients at different stages of diabetic nephropathy. International Journal of Diabetes Mellitus 2: 15–19, 2009.
27. Eardley S, Zehnder D, Quinkler M, Lepenies J, Bates RL, Savage CO, Howie AJ, Adu D and Cockwell P: The relationship between albuminuria, MCP-1/CCL2, and interstitial macrophages in chronic kidney disease. Kidney Int .69: 1189–1197, 2006.
28. Ibrahim S, Rashed L: Correlation of urinary monocyte chemo-attractant protein-1 with other parameters of renal injury in type-II diabetes mellitus. Saudi J Kidney Dis Transpl. 19 (6): 911-7, 2008.
29. Ha H, Yu MR, Choi YJ, Kitamura M, Lee HB. Role of high glucose-induced nuclear factor-kappa B activation in monocyte chemoattractant protein-1 expression by mesangial cells. J Am Soc Nephrol 13: 894–902, 2002.
30. El-Shafey E, El-Nagar GF, Abu Hatab H, Sabry A, and Elbaz H :Upregulation of Monocyte Chemoattractant Protein-1(MCP-1) in Early Diabetic Nephropathy in Patients with Type-1 Diabetes Mellitus. Scholarly Research Exchange. (Article ID 467264):10.3814, 2008.
31. Frederick WK , Bruce LR, Karim M, JoAnn R, Charles DP, Andrew H: Urinary monocyte chemoattractant protein-1 (MCP-1) and connective tissue growth factor (CCN2) as prognostic markers for progression of diabetic nephropathy. Cytokine 47: 37–42, 2009.
32. Heerspink H, Gansevoort R, Brenner B, Shahinfar S, de Zeeuw D: Comparison of different measures of urinary protein excretion for prediction of renal events. J Am Soc Nephrol 21(8): 1355–1360, 2010.
33. Jing-fang L, Bing-yin S, Zhen-jun Z, Mark DP, Bang-lao XU: Urinary excretion of MCP-1, IP-10 and PDGF-BB in type 2 diabetic patients with normo-and microalbuminuria. Journal of Chinese Clinical Medicine 5 (9):1562-90, 2010.
34. Kim Hyun O, Han-Soo K, Jong-Chan Y, Eui-Cheol S and Sungha P: Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassay. Journal of Translational Medicine 9:113, 2011.
35. De Martinis M, Modesti M, Ginaldi L: Phenotypic and functional changes of circulating monocytes and polymorphonuclear leucocytes from elderly persons. Immunol Cell Biol 82:415-420, 2004.
36. Shuhei M, Minoru U, Yoshinori S, Akiko Y, Machi F and Tokio S: Circulating monocyte chemoattractant protein-1 links to diabetic retinopathy in type 2 diabetic patients without renal dysfunction. Diabetology International 2: 78-82, 2010.
37. Prakash J, Lodha M, Singh SK, Rubina V, Raja R: Diabetic Retinopathy is A Poor Predictor of Type of Nephropathy in Proteinuric Type 2 Diabetic Patients. J Assoc Physicians India 55:412-416, 2007.