

Clinical Study

Upregulation of Monocyte Chemoattractant Protein-1 (MCP-1) in Early Diabetic Nephropathy in Patients with Type-1 Diabetes Mellitus

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Background. Monocyte chemoattractant protein-1 (MCP-1) can directly elicit an inflammatory response by inducing cytokine and adhesion molecule expression in the kidney. We investigated the role of MCP-1 in the development of early nephropathy in patients with type-1 diabetes mellitus, in addition to the effect of high-dose vitamin E treatment (8 weeks) on early stages of diabetic nephropathy. **Methods.** This study was carried out on 30 type-1 diabetic patients subdivided into two equal groups according to their urinary albumin excretion, in addition to 10 healthy matched volunteers included as controls. MCP-1, glycated hemoglobin (HbA1c), and albuminuria—before and after vitamin E treatment—were measured in all studied groups. **Results.** Serum MCP-1 and HbA1c were significantly elevated in patients with microalbuminuria and poor glycemic control (941.67 ± 47.03 pg/mL; $16.95 \pm 2.74\%$) compared to normoalbuminuric diabetic patients (622.73 ± 103.23 pg/mL; $7.23 \pm 0.86\%$), and controls (366.60 ± 129.01 ; 3.35 ± 0.66) ($P = .001$), respectively. There was positive correlation between MCP-1 and HbA1c. Both MCP-1 and albuminuria decreased significantly after using high-dose vitamin E treatment, though there was no change in HbA1c in type-1 diabetic patients with early nephropathy. **Conclusion.** These observations suggest that MCP-1 may be involved in the pathogenesis of diabetic nephropathy. High-dose vitamin E may provide a novel form of therapy for the prevention of microvascular complications in type-1 diabetic patients.

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1. Introduction

Diabetic nephropathy occurs in 20–40% of patients with diabetes and is the single leading cause of end-stage renal disease (ESRD). Persistent albuminuria in the range of 30–299 mg/24 h (microalbuminuria) has been shown to be the earliest stage of diabetic nephropathy in type-1 diabetes and a marker for the development of nephropathy in type-2 diabetes. Microalbuminuria is also a well-established marker of increased CVD risk. Patients with microalbuminuria who progress to macroalbuminuria (300 mg/24 h) are likely to progress to ESRD over a period of years. Over the past several years, a number of interventions have been demonstrated to reduce the risk and slow the progression of renal disease [1]. The accumulation of matrix proteins resulting in glomerular sclerosis and interstitial fibrosis is

a prominent feature of human diabetic nephropathy [2]. There may be an interplay of metabolic and hemodynamic pathways in the progression of diabetic nephropathy [3–5]. In addition to these pathways, the infiltration of inflammatory cells such as monocyte/macrophage into the diseased kidneys is a hallmark of the progression of diabetic nephropathy. Infiltrated monocyte/macrophage release lysosomal enzymes, nitric oxide, reactive oxygen intermediates, an transforming growth factor- β , which have reported to play an essential role in renal damage [3, 6].

MCP-1 belongs to chemotactic cytokine that exhibits its most potent chemotactic activity toward monocytes and T-cells, and promotes the transmigration of circulating monocytes into tissues [7]. Recent studies reveal that MCP-1 plays an important role in the pathogenesis of crescentic formation and progressive tubulointerstitial

TABLE 1: Comparison between the studied groups regarding different studied parameters.

Parameter	Patients groups			F	P
	Controls N = 10	Group I N = 15	Group II N = 15		
Age (yrs) Mean \pm SD	13.43 \pm 2.17	14.57 \pm 2.19	15.01 \pm 0.97	2.25	.25
Duration of DM (yrs) Mean \pm SD	—	3.67 \pm 1.32	9.37 \pm 2.65	$t = 754$.001*
HbA1c (%) Mean \pm SD	3.35 \pm 0.60	7.63 \pm 0.86	16.95 \pm 2.74	193.57	.001*
24 hours urine albumin (mg/d) Mean \pm SD	16.80 \pm 5.57	22.23 \pm 5.09	251.8 \pm 43.86	337.77	.001*
MCP-1 (PG/mL) Mean \pm SD	366.60 \pm 29.01	622.73 \pm 103.23	941.67 \pm 47.03	115.41	.001*

lesions via M ϕ recruitment and activation in experimental glomerulonephritis models [8–10] and human nephritis [11–13]. We determined the serum levels of human MCP-1 in type-1 diabetic patients with nephropathy to find out the relationship between poor glycemic control and MCP-1 generation and to evaluate the role of vitamin E on MCP-1 expression.

2. Subjects and Methods

2.1. Subjects. Thirty ambulatory adolescents and young adults (15 males and 15 females) with type-1 diabetes mellitus having a mean age of 12 ± 6 years were included. All were on conventional insulin therapy having two daily subcutaneous injections. They were divided into two equal groups, according to the presence of persistent microalbuminuria. Persistent microalbuminuria was defined as an albuminuria between 30 and 299 mg/24 h: Group I, IDDM patients without nephropathy (8 male and 7 females); Group II, IDDM patients with early nephropathy (7 males and 8 females). In addition 10 apparent healthy volunteers, age- and sex-matched, were enrolled as reference controls. Informed written consent was obtained from all the patients and volunteers.

2.2. Methods. All the patients and control group were subjected to a thorough history taking and complete clinical examination. Fasting and postprandial blood sugar, glycated hemoglobin, (HbA1c), blood urea, serum creatinine, microalbuminuria, and quantitative determination of serum MCP-1 concentrations (by ELISA technique) were determined in patients and controls at the beginning of the study and 8 weeks after using high-dose vitamin E treatment (600 mg/BID).

Quantitative determination of serum MCP-1 levels was determined by an enzyme-linked immunosorbent assay (ELISA), using a specific murine monoclonal antihuman MCP-1 antibody as a capture, and a biotinylated anti-MCP-1 antibody as the second antibody which binds to the solid phase antibody-antigen complex. All assays were performed in duplicate. The detection limits of this ELISA system

were 40 pg/mL for human MCP-1 (Biosource International, Camarillo, Calif, USA).

2.3. Statistics. Statistical analysis was done by SPSS V. 13. The data are expressed as mean and standard deviation. The paired t -test, analysis of variance [ANOVA], and Scheffe test were used wherever applicable. Spearman's rank correlation coefficient (r) was calculated to test the association between two variables; for quantitative data, the number and percent distribution were calculated. Probability (P) values of less than .05 were considered of statistical significance.

3. Results

There was no significant difference between the studied groups regarding the age and sex. However, the duration of DM and HbA1c showed statistically significant difference between Group I (3.67 ± 1.32 ; 7.63 ± 0.86) versus Group II (9.37 ± 2.65 years; $16.95 \pm 2.74\%$), respectively.

Microalbuminuria was significantly higher in Group II (251.80 ± 43.86 mg/d) compared to Group I (22.23 ± 5.09 mg/d) and controls (16.80 ± 5.57 mg/d) ($P = .001$). Serum MCP-1 showed statistically significant higher levels in Group II (941.67 ± 47.03 pg/mL), in comparison to Group I (622.73 ± 103.23 pg/mL) and controls (366.60 ± 129.01 pg/mL) ($P = .001$); see Table 1.

Significant association between MCP-1 and HbA1c in diabetic patients with microalbuminuria ($r = 0.728$, $P < .0001$). Controls and type-1 diabetic patients without nephropathy (Group I) showed no significant difference regarding HbA1c, microalbuminuria, and serum MCP-1 before using vitamin E treatment versus after using it.

Meanwhile, in type-1 diabetic patients with nephropathy (Group II), HbA1c, microalbuminuria, and serum MCP-1 were significantly lower after treatment than before treatment (Table 2; Figures 1, 2, and 3).

4. Discussion

Monocyte infiltration in the mesangium plays an important role in glomerular diseases [14], is associated with fibroblast

TABLE 2: Effects of treatment with vitamin E on different studied parameters among Group I and Group II patients.

Parameter	Patients groups							
	Group I (N = 15)		t	P	Group II (N = 15)		t	P
Before vit. E	After vit. E	Before vit. E			After vit. E			
HbA1c (%) Mean ± SD	7.63 ± 0.86	7.43 ± 0.93	2.82	.14	16.95 ± 2.74	16.95 ± 2.74	2.45	.28
24 hr urine albumin (mg/d) Mean ± SD	22.23 ± 5.09	21.93 ± 4.18	0.90	.38	251.8 ± 43.86	174.80 ± 41.74	50.41	.001*
MCP-1 (PG/mL) Mean ± SD	622.73 ± 103.23	630.80 ± 71.0	0.381	.71	941.67 ± 47.03	685.67 ± 45.74	28.96	.001*

activation and increased extracellular matrix deposition in diabetic rats, and diffuse glomerulosclerosis in patients with diabetic nephropathy [15, 16]. As MCP-1 is a potent chemoattractant for monocytes, it is of interest to know that the increased glomerular expression of MCP-1 has been shown in several glomerular diseases [17] as well as in the mesangium of rats with streptozotocin-induced diabetes [14]. More recently, increased production of MCP-1 by blood mononuclear cells of patients with diabetes has been demonstrated [18]. In this prospective study, we investigated the possible role of MCP-1 in the development of early diabetic nephropathy in patients with type-1 DM. In addition, we studied the relationship between poor glycemic control and MCP-1 generation and the possible role of high-dose vitamin E treatment on microalbuminuria and MCP-1 expression.

The duration of IDDM has generally been mentioned as the strongest risk factor for the development of nephropathy. In the present study, patients with significant microalbuminuria had a mean IDDM duration of (9.3 ± 3.7 years), 2 out of 15 patients in Group II had less than 5 years duration of DM.

Hovind et al. [19] reported significant association between duration of DM and occurrence of diabetic nephropathy, microalbuminuria occurs in 15–40% of patients with type-1 diabetes, with a peak incidence around 15–20 years. Stage II or incipient nephropathy may develop for 2–10 years after the initial changes are noted, microalbuminuria is present at this point and may range between 20–200 µg/m [20]. For patients with type-1 DM, the first screening has been recommended at 5 years after diagnosis, however, the prevalence of microalbuminuria before 5 years can reach 18%, especially in patients with poor glycemic control, lipid control, and high normal blood pressure levels [21]. Repeated measurement of microalbuminuria from the time of diagnosis may be useful in the early detection of patients who will develop microalbuminuria and ultimately overt diabetic nephropathy.

Mean HbA1c values were significantly higher in microalbuminuric diabetic patients than in normoalbuminuric diabetic patients (15.25 ± 4.25 versus 7.75 ± 1.25%, $P = .001$) (Table 1), and a positive correlation was found between HbA1c and microalbuminuria in Group II, so that the poor glycemic control is correlated with the development of early diabetic nephropathy. This is in agreement with Chiarelli et al. [22] who reported that mean HbA1c values were significantly higher in microalbuminuric diabetic

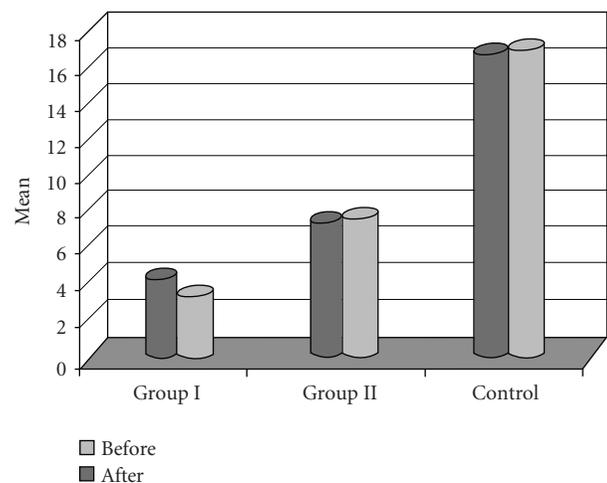


FIGURE 1: HbA1C before and after using vitamin E therapy in different studied groups.

patients than in normoalbuminuric diabetic patients (9.6 ± 1.4 versus 7.3 ± 0.93%, $P < .05$).

In the present study, serum MCP-1 has significantly increased in type-1 diabetic patients with early nephropathy compared to matched patients without nephropathy and controls. This is in agreement with Chiarelli et al. [22] who found that Plasma MCP-1 was significantly higher ($P = .004$) in the microalbuminuric patients with respect to the normoalbuminuric patients and healthy volunteers. In contrast, they found no significant differences between the normoalbuminuric and the control groups. Cipollone et al. [23] observed Bcl-2 downregulation in microalbuminuric diabetic patients as a consequence of increased oxidant burden secondary to persistent hyperglycemia which was associated with enhanced expression of MCP-1, and showed a strong correlation with the albumin excretion rate. This is also in accordance with our observation of the significant association between HbA1c and MCP-1 in diabetic patients with microalbuminuria. However, it is not in accordant with Banba et al. [24] who found that urine levels, but not serum levels, of MCP-1 increased in accordance with the extent of GHb and albuminuria. However, in this study, only a small group of patients (9) had microalbuminuria and so could be correctly compared with our patients. Furthermore, eight of these nine patients had type-2 diabetes. Again, the age (mean

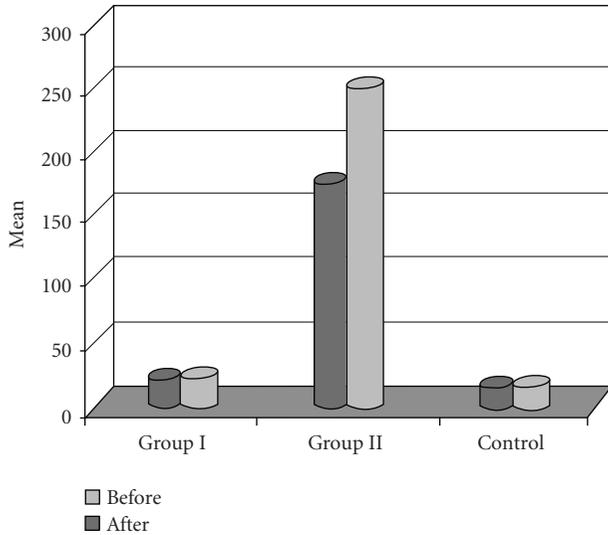


FIGURE 2: Microalbuminuria before and after using vitamin E therapy in different studied groups.

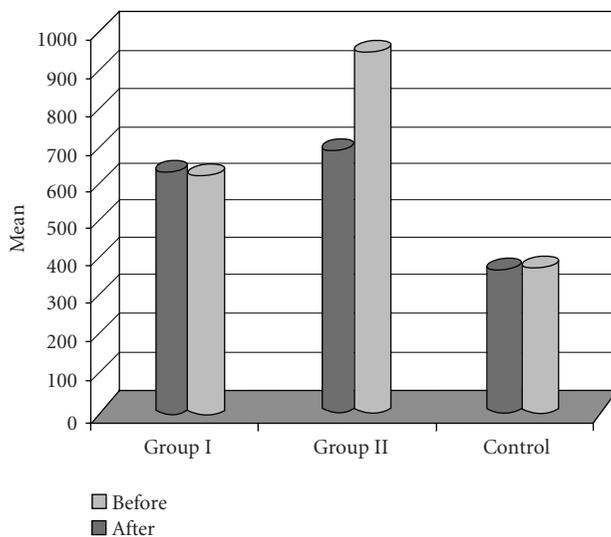


FIGURE 3: MCP-1 before and after using vitamin E therapy in different studied groups.

\pm SD) was 62.210.4 years in the study from Banba et al. but only 15.01 ± 0.97 years in our study. Also, Kiyici et al. [25] reported that there was no difference in serum levels of MCP-1 between type-1 diabetic patients with diabetic nephropathy and without it.

Moreover, in our prospective study glycemic control did not change in diabetic patients after vitamin E treatment, however, the MCP-1 and microalbuminuria were significantly reduced after vitamin E therapy in microalbuminuric patients, thus confirming that MCP-1 reduction after vitamin E was specifically due to vitamin E and not to improved glycemic control. This is in agreement with Chiarelli et al. [22] who observed that both MCP-1 and AER decreased significantly after vitamin E treatment in microalbuminuric patients, despite there were no changes

in HbA1c percentages. In addition to Gaede et al. [26], who reported that treatment with vitamin E plus vitamin C in patients with micro- or macroalbuminuria significantly lowers microalbuminuria though there is no change in HbA1c, Cipollone et al. [23] observed that prolonged hyperglycemia may lead to higher oxidative burden, consumption of endogenous antioxidant buffer, and overexpression of MCP-1. But in contrast, Ishikawa et al. [27] stated that the increased circulating MCP-1 could be merely a secondary effect of diabetic nephropathy, also Cipollone et al. [7] reported that most of the changes observed in diabetic patients after vitamin E therapy were in response to treatment with insulin and the improvement of glycemic control. However, this hypothesis is unlikely, because the direct role of vitamin E in MCP-1 generation is supported by the observation that in vivo generation of MCP-1 was reduced by the administration of high-dose vitamin E in diabetic patients with microalbuminuria [23], and the in vitro MCP-1 generation in monocytes was exclusively downregulated by vitamin E, while insulin or changes in osmolar conditions failed to produce any effect [22]. In contrast, a subgroup analysis of 31654 diabetic patients participating in the heart outcomes prevention evaluation (HOPE) study [28] demonstrated no renal effects in patients receiving low-dose (400 iu) vitamin E. However some characteristics of the HOPE study may contribute to explain this apparent discrepancy, the daily dose of antioxidant administered in the HOPE study is significantly lower with respect to both Gaede et al.'s study [26] and ours. Thus, we can speculate that the simple administration of 400 iu of vitamin E may not sufficiently restore the antioxidant supply to the level necessary to prevent the induction of inflammatory genes, ultimately leading to renal damage and microalbuminuria.

Based on the previous observations, we concluded that upregulation of MCP-1 gene expression by persistent hyperglycemia in type-1 diabetic patients results in the recruitment of monocytes into the kidney, possibly contributing to the development of diabetic nephropathy. Moreover, the causative role of poor glycemic control in diabetic nephropathy is mediated by increased oxidative stress. These findings indicate a pathogenic role for MCP-1 in the evolution of diabetic microvascular complications. High-dose vitamin E may provide a new form of therapy for prevention of microvascular complications in type-1 diabetic patients in which acceptable glycemic control is difficult to achieve despite appropriate insulin therapy.

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