Relationship of Monocyte Chemoattractant Protein-1 with Diabetic Nephropathy in Children with Type -1 Diabetes: Effect of High Dose Vitamin E Therapy

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Abstract:

Monocyte chemoattractant protein-1 (MCP-1) is a specific chemokine that activates monocytes from the circulation to the inflammatory sites. In diabetic nephropathy, similar to other glomerulonephropathies, infiltration and activation of monocytes/macrophages in the glomeruli have been implicated in the development of glomerular injury. The aim of this study was to examine a possible relationship of the MCP-1 with the development of diabetic nephropathy in children with type-1 diabetes before and after treatment with high dose of vitamin E for eight weeks. This study was carried out on thirty diabetic children, group 1; fifteen children with type 1 diabetes mellitus with persistent microalbuminuria, and group 2; fifteen children without microalbuminuria. Fifteen healthy children served as control group. Albumin excretion rate (AER) and glycosylated hemoglobin (HbA1c) were measured, also plasma MCP-1 levels were measured by ELISA before and after treatment with vitamin E for eight weeks.

The results proved that plasma levels of MCP-1 were significantly higher in children with diabetic nephropathy than diabetic children without nephropathy and the control group (P<0.05). There was strong positive correlation between HbA1c and AER and MCP-1 levels (P<0.0001). After treatment with vitamin E, there was a significant decrease in the MCP-1 plasma levels in diabetic children with nephropathy.

Conclusion: This study suggests that facilitated MCP-1 production by the mesangial cells in diabetic children contributes to the initiation and progression of diabetic nephropathy. High-dose vitamin E supplementation may provide an additional benefit, as adjuvant therapy to insulin treatment, in reducing the risks for the development of diabetic nephropathy.

Introduction:

Both metabolic and haemodynamic pathways have impact on the progression of diabetic nephropathy.1 Chronic hyperglycemia, advanced glycation end products, increased sorbitol, activation of protein kinase C, glomerular hypertension and genetic susceptibility have been identified as risk factors in the progression of diabetic nephropathy.2 Moreover, infiltration of the diseased kidney by inflammatory cells such as monocytes/macrophages is a hallmark of diabetic nephropathy. Infiltrated monocytes/macrophages release lysozyme enzymes, nitrous oxide, reactive oxygen, intermediate and transforming growth factor, which play an essential role in renal damage.3

A chemokine; monocyte chemoattractant protein-1 (MCP-1) also termed a monocyte chemotactic and activating factor (MCAF), was found to be secreted by mononuclear cells and various non leukocytic cells, including inflammatory fibroblasts, astrocytes, and renal resident cells, including mesangial cells and tubular epithelial cells.4 Recent studies reveal that MCP-1 plays an important role in the pathogenesis of crescentic formation and tubulointerstitial lesion via monocytes/macrophages recruitment and activation in experimental glomerulonephritis models and human nephritis.5 Thus, the available evidence indicates that MCP-1 is a key factor initiating the inflammatory process of diabetic nephropathy and sustaining the extracellular matrix deposition and cell proliferation.6

Persistent microalbuminuria; albumin excretion rate (AER) > 20 Ug/min, is regarded as the earliest clinical sign of incipient diabetic nephropathy.7 A causal relationship between chronic hyperglycemia and diabetic microvascular disease has now been definitively established from a prospective controlled clinical study.8 In diabetic nephropathy, an increase in both intraglomerular pressure and extracellular matrix protein results in basal membrane thickening, mesangial proliferation and glomerular hypertrophy. These changes reduce glomerular filtration surface and function and can progress to glomerulosclerosis.9 Numerous reports have demonstrated that oxidative stress induced by diabetes plays an important role in
the development and progression of diabetic vascular complications including nephropathy and there is emerging evidence that the formation of reactive oxygen species is a direct consequence of hyperglycemia. Previous studies demonstrated that vitamin E administration may reduce protein glycosylation in diabetic subjects independently of changes in plasma glucose, an effect that may be due to the inhibition of labile glycosylation with prevention of diabetic complications as retinopathy and nephropathy.\textsuperscript{2}

The aim of the present study was to investigate the possible role of MCP-1 in the development of early nephropathy in children with type-1 diabetes mellitus, to correlate between hyperglycemia, AER and MCP-1 level, and to study the effect of high-dose vitamin E therapy for eight weeks on the plasma level of MCP-1 in children with type-1 diabetes.

**Subjects and Methods:**

This study was carried out on thirty diabetic children subdivided into 2 groups according to the presence of microalbuminuria: Group 1; fifteen children aged 6-12 years with mean age 8.7±1.9 years with persistent microalbuminuria and group 2; fifteen children, aged 5-11 years with the mean age 7.9±1.9 years without microalbuminuria. These children were selected from the Pediatric outpatient clinic of Endocrinology, Tanta University Hospital. Persistent microalbuminuria was defined as an AER between 20 and 200 Ug/min in two of three overnight urine collections performed over six months. Fifteen healthy children, aged from 7-13 years with a mean of 8.8±1.8 years, served as a control group (table I).

**Results:** The results of the present study are summarized in tables II and III and figures 1-6.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>MCP-1(Pg/ml) Before Treatment</th>
<th>MCP-1(Pg/ml) After Treatment</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>97.3±6.3</td>
<td>69.4±5.3</td>
<td>p&lt;0.05*</td>
</tr>
<tr>
<td>Group II</td>
<td>71.2±3.3</td>
<td>58.8±6.2</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Control Group</td>
<td>67.8±7.2</td>
<td>55.7±6.3</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

All these children were subjected to the following:

1. Thorough history taking and complete physical examination.
2. Glycosylated hemoglobin levels (HbA\textsubscript{1c} %).
3. Albumin excretion rate (AER).
4. Plasma levels of monocyte chemoattractant protein-1 (MCP-1) before and after eight weeks of vitamin E therapy. These children received vitamin E (\textalpha-tocopherol acetate, 400 mg twice daily for eight weeks).

**MCP-1 Assay:** Venous blood samples were collected after overnight fasting, stored at -20\textdegree C until assayed. Plasma levels of MCP-1 were measured using enzyme-linked immunosorbent assay (ELISA) kits with the detection limit of 0.5pg/ml (Minneapolis, MN, USA).\textsuperscript{10}

**Statistical Analysis:**

Data were expressed as mean ± SD. Statistical comparison among different groups was performed by using ANOVA test. Paired (t) test was used to compare 2 variables before and after treatment. Pearson’s correlation was performed between different biochemical parameters. Calculations were done with the statistical package SPSS for Window, version 10.0. Statistical significance was defined as P<0.05.
Table II and figure 1 show that the mean value of AER in children of group 1 was 87±22.9Ug/min, which was significantly higher than the mean value of AER in children of group 2 which was 15±4.2 Ug/min (p<0.001). This value was also significantly higher than the mean value of AER in children of the control group which was 7.9 ±2.3 Ug/min (p<0.001). There was also a significant difference between the mean value of AER in children of group 2 and the control group (p<0.05).

Table II and figure 2 show that the mean value of HbA1C in children of group 1 was 9.2±0.61%, which was significantly higher than the mean value of HbA1C in children of group 2 which was 7.5±0.4% (p<0.05). Also this value was significantly higher than the mean value of HbA1C in children of the control group which was 4.7±0.7% (p<0.05). The mean value of HbA1C in children of group 2 was significantly higher than the mean value of HbA1C in children of the control group (p<0.05).

Table II and figure 3 show that the mean value of MCP-1 in children of group 1 before treatment with vitamin E, was 97.3±6.3 Pg/ml, which was significantly higher than the mean value of MCP-1 in children of group 2 which was 71.2 ±3.3 Pg/ml (p<0.05). This value was also significantly higher than the mean value of MCP-1 in children of the control group which was 67.8±7.2 Pg/ml (p<0.05). There was no significant difference between the mean value of MCP-1 in children of group 2 and the control group (p >0.05).

Table II and figure 4 show that the mean value of MCP-1 in children of group 1 after treatment with vitamin E was 69.4±5.3Pg/ml, which was significantly higher than the mean value of MCP-1 in children of group 2 which was 58.8 ±6.2 Pg/ml (p<0.05). This value was also significantly higher than the mean value of MCP-1 in children of the control group which was 55.7±6.3 Pg/ml (p<0.05). There was no significant difference between the mean value of MCP-1 in children of group 2 and the control group (p >0.05).

Table III shows a comparison of plasma levels of MCP-1 before and after high-dose vitamin E therapy. It shows that the mean value of MCP-1 after treatment with vitamin E in children of group 1, was significantly lower than the mean value of MCP-1 before treatment (p<0.05). In children of group 2 there was no significant decrease in the plasma levels of MCP-1 after treatment with vitamin E (p >0.05). In children of the control group there was no significant decrease in the plasma levels of MCP-1 after treatment with vitamin E (p >0.05).

Figure 5 shows a highly significant positive correlation between the plasma MCP-1 levels and the AER (r=0.931)(p<0.0001).
In the present study, we found that the plasma levels of MCP-1 were significantly higher in diabetic children with microalbuminuria compared to diabetic children without microalbuminuria and healthy control children. This agrees with the results obtained by Chiarelli et al., who found elevated circulatory MCP-1 in adult patients with type-1 diabetes with nephropathy. These results also agree with Banba et al., who found elevated urinary and serum MCP-1 in patients with type-1 diabetic nephropathy. Lee et al., and Akemi et al. found elevated serum MCP-1 in patients with diabetic nephropathy. Also, our results agree with Hisayuki et al., who reported elevated urinary MCP-1 concentration in patients with diabetic nephropathy than the control subjects.

MCP-1 is believed to play a key role in mediating the infiltration of monocytes into the renal tissues. It is secreted by various cells including monocytes/macrophages, endothelial cells and fibroblasts, and it has found to be produced by mesangial cells and proximal tubular cells. Glomerular infiltration with monocytes is observed in patients with diabetic nephropathy and it may be implicated in the pathogenesis of diabetic nephropathy and directly correlates with the coexisting histopathologic tubulointerstitial changes.

The results of the present study also showed the presence of highly significant correlation between the plasma MCP-1 levels and both AER and HbA1c in diabetic patients with microalbuminuria. This supports the hypothesis that persistent hyperglycemia can induce MCP-1 biosynthesis by increasing oxidative stress, and prolonged hyperglycemia may lead to higher oxidative burden, consumption of endogenous antioxidant buffer (e.g., vitamin E) and overexpression of MCP-1.

The present study also showed decrease in the plasma MCP-1 levels after treatment with high-dose vitamin E, for eight weeks. This finding supports the role of vitamin E in suppression of the MCP-1 generation which was obtained in this study. That in vivo generation of MCP-1 was reduced by administration of high dose both in the diabetic children and the healthy control children. These results agree with Chiarelli et al., who reported decreased MCP-1 plasma levels in diabetic children after high-dose of vitamin E therapy. This finding suggests that reactive oxygen molecule may play a more prominent role in causing renal injury in patients with diabetic nephropathy. Antioxidant therapy may be useful in the treatment in children with diabetic nephropathy. Vitamin E supplementation increased glutathione peroxidase concentration and lowered the malondialdehyde concentrations in the erythrocytes in diabetic patients. Clinical studies have shown that,

**Discussion:**

Hyperglycemia is a major causative factor in the development of endothelial dysfunction in diabetes and the subsequent development of vascular complications. In diabetes, the vascular endothelium demonstrates impaired synthesis or action of vasodilators, and increased vasoconstrictor release resulting in an imbalance of vascular homeostasis. Hyperglycemia can mediate endothelial cell dysfunction through a number of potential pathways, including increased oxidative stress and impaired endothelium-derived factor / nitric oxide.

Figure 6 shows a highly significant positive correlation between the glycosylated hemoglobin (HbA1c%) and the plasma MCP-1 levels (r=0.956) (p<0.0001).

In the present study, we found that the plasma levels of MCP-1 were significantly higher in diabetic children with microalbuminuria compared to diabetic children without microalbuminuria and healthy control children. This agrees with the results obtained by Chiarelli et al., who found elevated circulatory MCP-1 in adult patients with type-1 diabetes with nephropathy. These results also agree with Banba et al., who found elevated urinary and serum MCP-1 in patients with type-1 diabetic nephropathy. Lee et al., and Akemi et al. found elevated serum MCP-1 in patients with diabetic nephropathy. Also, our results agree with Hisayuki et al., who reported elevated urinary MCP-1 concentration in patients with diabetic nephropathy than the control subjects.

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Clinical studies have shown that,
both hyperglycemia and diabetes impair nitric oxide-related endothelium dependent vasodilatation and that antioxidant treatment may ameliorate endothelial cell dysfunction in diabetic patients. Thus, antioxidants as vitamin E received considerable attention with respect to its potential ability to ameliorate diabetes complications. Vitamin E has been proposed to be the major lipid soluble chain breaking antioxidant that protects biologic membranes from lipid peroxidation. In vitro studies have shown that, reduced glutathione can protect against peroxidation of lipids in cytosolic and subfraction component of rat liver and other tissues. Mechanisms that explain glutathione effect include, the removal of species that initiate lipid peroxidation, scavenging of glutathione dependent protein, scavenging of peroxyl radicals by glutathione, and maintenance of membrane protein thiols by glutathione. Thus, vitamin E supplementation can increase cellular glutathione concentration and lower membrane malondialdehyde levels in the erythrocytes of type-1 diabetic children.

**Conclusion:**

1. This study suggests that prolonged hyperglycemia may lead to early renal complications in children with type I diabetes by inducing MCP-1 biosynthesis via enhanced oxidative stress.
2. Long term treatment with high-dose vitamin E significantly decreases MCP-1, thus providing a rationale basis for evaluating vitamin E supplementation as therapy adjuvant to conventional insulin treatment in diabetic children in whom an acceptable glycemic control is difficult to be achieved despite appropriate insulin treatment. This combination may be efficacious in delaying the onset of diabetic nephropathy.

**References:**

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