

Evaluation of Monocyte Chemoattractant Protein-1 (MCP-1) in Type 2 Diabetes Mellitus.

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Abstract: AIM: To investigate the association of serum the chemokine monocyte chemoattractant protein-1 (MCP-1) levels, a major chemoattractant of monocytes and activated lymphocytes, with metabolic parameters like insulin hormone, glycolated hemoglobin and glucose Iraqi patients with type 2 diabetes mellitus.

METHODS: MCP-1, TGF- β 1 and insulin concentrations were measured by enzyme-linked immunosorbent assay (ELISA), while HbA1c and glucose were measured by spectrophotometer technique.

RESULTS: Thirty diabetic patients were compared with 20 healthy subjects to assess the studied parameter. MCP-1 mean concentrations and percentiles were substantially higher in non-diabetic populations. MCP-1 serum levels are related to age, BMI, HbA1c, TGF- β 1, glucose and insulin hormones

CONCLUSIONS: Compared to healthy groups, MCP-1 levels were found to be substantially higher in patients with type 2 diabetes mellitus.

Keywords: MCP-1, hyperglycemia, diabetes mellitus, TGF- β 1.

1. Introduction

Diabetes mellitus is considered one of the most vicious chronic diseases of our time, owing to the fact that, individuals have to endure long years of successive complications, makes it less probable to be controlled. Diabetes mellitus is a metabolic disorder identified by a lingering hyperglycemia as a result of defect in insulin secretion, insulin action or both. Which impairs the body's ability to metabolize sugar, lipid and protein. The effects of diabetes include long-term damage, dysfunction and failure of different organs [1]. Type 2 diabetes comprises 95% of diabetic cases worldwide and its etiology is most probably related obesity and insulin resistance [2].

Chemokines (chemotactic cytokines) are small heparin-binding proteins which direct migration of circulating leukocytes to

sites of inflammation or injury [3]. The largest family is the CC chemokine MCP-1 (monocyte-chemo-attractant protein 1). Two residues of MCP 1, out of four conserved cysteine residues are thoroughly characterized CC chemokine. MCP-1 (monocyte chemo-attractant protein 1), also known as CCL2 – a potent agonist for monocytes, memory T cells and basophils intimal hyperplasia after angioplasty, as well as in vas-culogenesis and thrombosis [4]. MCP-1 is also expressed and secreted by adipocytes, which has been reported to be involved in the recruitment and activation of peripheral blood leukocytes in adipose tissue and in the induction of systemic insulin resistance [5]. Another study has reported the identification of a novel protein; designated MCP-IP (MCP-induced protein), which is expressed in human monocytes and cardiomyocytes after stimulation with MCP-1.

Their results provided a novel molecular pathway, by which MCP-1 signal transduction is linked to transcriptional gene regulation leading to apoptosis [6].

Hyperglycemia is the major cause of diabetic angiopathy. High glucose treatment on endothelial cells isolated from diabetic subjects resulted in a 40-70% increase of MCP-1 release, and a 10-20% increase of the basal expression of vascular cell adhesion molecule-1 (VCAM-1), indicating synergistic enhancement on the monocyte-endothelial cell interaction [7]. In a vitro study demonstrated that advanced glycation end-products; high glucose concentration, glycated albumin and glycoxidized LDL enhanced MCP-1 expression in human endothelial cells [8]. Similarly, high glucose treatment on human aortic smooth muscle cells (SMC) unregulated the expression of MCP-1 and fractalkine leading to increased monocyte-SMC adhesive interactions by a TGF β 1 (DRG diagnostics Company, Germany). insulin (Demedtec Company, Germany) were detected by enzyme-linked immunosorbent (ELISA).

2.1 Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences-version 22). The significance of difference of different means (quantitative data) were tested using Students-t-test or ANOVA test. The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test (χ^2 -test) with application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value for the test of significance was equal or less than 0.05.

3. Results:

mechanism involving activation of MAPK, AP-1 and NF κ B [9]. Consistent with previous reports, exposure of human endothelial ECV304 cells to high glucose for 24 h caused an increase of MCP-1 and intercellular adhesion molecule-1 (ICAM-1), and promoted cell adhesion between monocyte and ECV304 cells[10]. Furthermore, high glucose treatment on human acute monocytic leukemia THP-1 cells increases both mRNA and protein levels of MCP-1, enhanced the adhesion of THP-1 cells to endothelial cells, and the pathways reportedly involved oxidative stress and protein kinase C [11].

The present study is aimed to assess the levels of MCP-1 and other biochemical markers in diabetic subjects compared to a healthy group. Which in turn can be beneficial to clarify the correlations of MCP-1 levels in diabetes mellitus with other clinical parameters.

2. Subject and Methods:

Twenty normal subjects (aged 53.33 ± 6.195 years; mean \pm SD) and 30 diabetic patients were studied (aged 56.27 ± 5.587 years; mean \pm SD). Informed consent was obtained from each subject. Patients with concurrent acute illnesses, malignancy, and active immunological diseases; medical history of clinical cardiovascular disease; medical history included the diseases (hypertension, rheumatoid arthritis, anemia, bronchial asthma) or medications (warfarin, acetylsalicylic acid, alpha-methyldopa, vitamins, tramadol, simvastatin) that interfered with HbA1c % measurements, and smoking history were excluded from the study. The anthropometric measurements and blood pressure were recorded. Biochemical testing included plasma fasting blood glucose (LABKIT, Spain), Glycated hemoglobin HbA1c % (Human Co., German), MCP-1 (Elabscience Biotechnology Co. Japan),

patient and healthy subject, as shown in the table(1) . Table (1) also shown the duration of diabetes in the patients also recorded in years.

Patients and control group were similar in regard to age with no significant difference [P value >0.05] , as well as there were no significant difference between diabetic

Table (1): Means of Age, BMI and duration of disease in T2DM patients and controls.

Parameters (mean ± SD)	Controls (n = 20)	Patients (n = 30)	P.value
Age	48.40±6.53	51.29±7.62	0.48
BMI	29.53 ± 1.552	29.93 ± 1.792	0.45
Duration of disease (year)	4.46±4.69	-	-

Table (2) show the core study analytes. Serum MCP-1 level was significantly higher in diabetic patients than

in control subjects (48.87 ± 12.345 vs. 38.8 ± 8.994 ng/ml respectively, p<0.001), figure (1).

Table (2):- Comparison between biochemical parameters of controls and patients subjects.

Parameters (mean ± SD)	Controls (N =20)	Patients (N = 30)	P.value
MCP1 (ng/ml)	38.8 ± 8.994	48.87 ± 12.345	0.003**
TGF-β1 (pg/ml)	13.6 ± 4.641	19.67 ± 6.586	0.03
Glucose (mg/dl)	83.8 ± 9.689	130.8 ± 16.575	< 0.001 *
Insulin (μIU/ml)	20.93 ± 4.758	41.13 ± 8.132	< 0.001 *
HbA1c	4.68 ± 0.4843	7.993 ± 0.6464	< 0.001 *

* The P-value ≤ 0.001 at degree of freedom 14 is highly significant.

** The P-value ≤ 0.05 at degree of freedom 14 is significant.

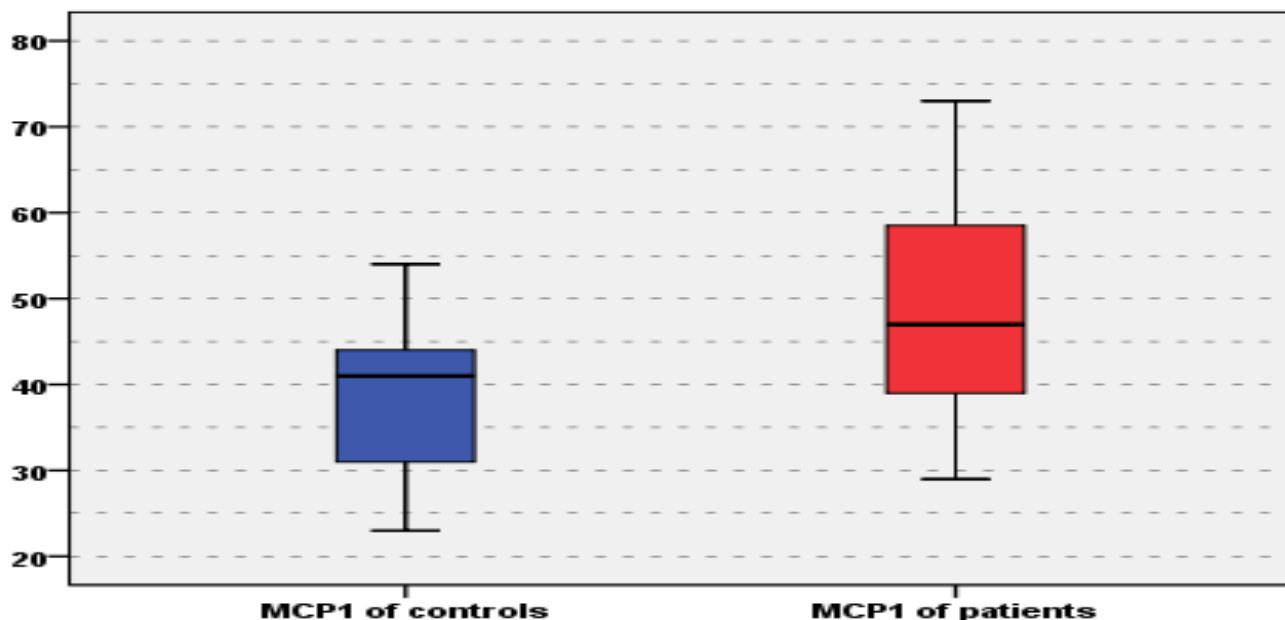


Figure (2):- The Boxplot graph of MCP₁ of controls and patients.

The Boxplot graph (2) shows the difference between the means of TGF of controls and patients. The T2DM patients showed a slightly increased mean of TGF in

comparison with controls (19.67 ± 6.586 vs. 13.6 ± 4.641 pg/ml) and the difference was not significant.

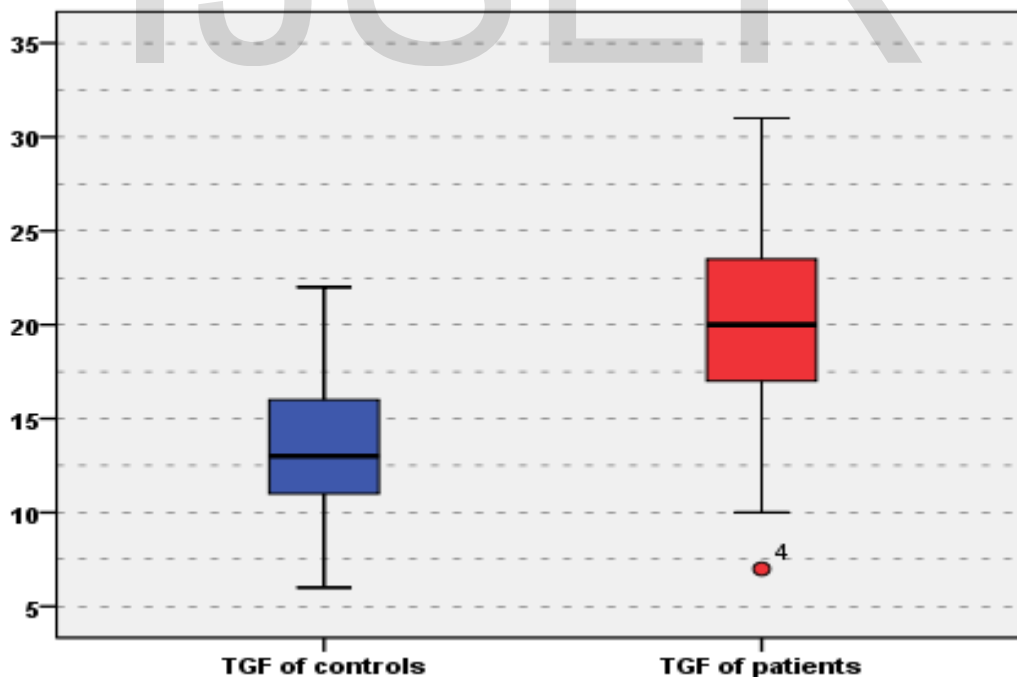


Figure (3):- The Boxplot graph of TGF of controls and patients.

The mean of insulin serum level was highly significantly increased in diabetic patients groups (41.13 ± 8.132 μ IU/ml) as compared to controls (20.93 ± 4.758 μ IU/ml)

. There was a significant difference ($P < 0.001$) in both HbA1c and plasma glucose between studied groups (using t-test), table (2).

In diabetic patients, there were positive highly significant correlation between MCP-1 and age, BMI, HbA_{1c}, TGF- β 1, glucose and insulin ($r = 0.359$, $r = 0.717$,

$r = 0.395$, $r = 0.217$, $r = 0.595$ and $r = 0.558$ respectively, $P < 0.01$), as well as TGF- β 1 also significantly correlated with BMI, HbA_{1c}, MCP-1, glucose and insulin ($r = 0.606$, $r = 0.871$, $r = 0.595$, $r = 0.217$, $r = 0.595$, $r = 0.367$ and $r = 0.178$ respectively), while both MCP-1 and TGF- β 1 were negatively correlated with age ($r = -0.359$ and $r = -0.178$ respectively), table (3).

Table (3):- Correlation between MCP-1 and TGF- β 1 with other biochemical parameters of patient subject.

		Age	BMI	HbA _{1c}	MCP ₁	TGF	FPG	Insulin
	N	30	30	30	30	30	30	30
MCP ₁	Pearson Correlation	-0.359*	0.717***	0.395*	---	0.217*	0.595**	0.558**
	P.value	0.189	0.003	0.145	---	0.437	0.019	0.031
TGF- β 1	Pearson Correlation	-0.178*	0.606**	0.871** *	0.595**	---	0.367*	0.774***
	P.value	0.526	0.017	<0.001	0.019	---	0.178	0.001

4. Discussion

MCP-1 is a potent chemo-attractant for monocytes, after a thorough research has been conducted and successive measurements have been taken, the study showed that; there is a significant increment in diabetic subjects when compared to a healthy group. These findings are corresponded to the well known metabolic changes that occur in normal and diabetic subjects. Healthy individuals (non-diabetic) produce an "accelerated starvation" in the fasting state, with an earlier and more

profound hypoglycemia and an increased fasting insulin level, whereas diabetic patients; first of all, demonstrate an elevated fasting insulin concentrations [4]. Nelson et al. With a significant release of MCP-1 from peripheral blood cultures from pregnant women as compared to non-pregnant women[12].

Moreover, study has shown that; there were a significant positive correlation between MPC-1 and BMI, MCP-1 signaling which has a direct role in the development of obesity. For example, Zue CW et al. has

reported that, MCP-1-induced protein (MCP-1, a zinc finger protein) induced adipogenesis in 3T3-L1 cells independent of PPAR γ activation [13]. Moreover, MCP-1 had angiogenic effect on endothelial cells, and therefore it can contribute to the expansion and remodeling of adipose tissues [14] [15].

One of the studied factors was; transforming growth factor - beta1 (TGF-beta1) - a multifunctional cytokine that exhibits potent immunoregulatory and anti-inflammatory properties and prolongs graft survival [16].

Beata Telejko hypothesize that TGF-beta may be a key factor responsible for the alterations in circulating MCP-1 levels, but the secretion and action of this chemokine in diabetes mellitus need further investigations[4].

In conclusion, the current study's findings suggest that; glycemic status influences serum MCP-1 levels in diabetic patients, where MCP-1 strongly is correlated with glycemic parameter (insulin hormone, HbA1c, glucose). Elevated serum MCP-1 levels could contribute to the onset and progression of several complications in diabetes. Thus, serum MCP-1 may serve as a biomarker of inflammatory activity and helps in early detection and intervention of diabetic complications.

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REFERENCE

1. World Health Organization. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Second Report. Geneva: WHO, 1999. Technical Report Series646.

2. Piemonti L, Calori G, Lattuada G, Mercalli A, Ragona F, Garancini MP, et al. Association between plasma monocyte chemoattractant protein-1 concentration and cardiovascular disease mortality in middle-aged diabetic and nondiabetic individuals. *Diabetes Care*. 2009;32:2105–10.
3. Charo IF, Taubman MB. Chemokines in the pathogenesis of vascular disease. *Circ Res*. 2004;95:858-866.
4. Beata Telejko, Mariusz Kuzmicki, Anna Zonenberg, et al. Circulating monocyte chemoattractant protein-1 in women with gestational diabetes. *FOLIA Histochemica ET Cytobiologica* Vol. 45, Supp. 1, 2007 pp. 153-156]
5. Zaica RE. MCP-1 (monocyte chemotactic protein-1) and harpin molecules in inflammation processes. *Bio Med*, 2014, 44(7);213-217.
6. Zhou L, Azfer A, Niu J, Graham S, Choudhury M, Adamski FM, Younce C, Binkley PF, Kolattukudy PE. Monocyte chemoattractant protein-1 induces a novel transcription factor that causes cardiac myocyte apoptosis and ventricular dysfunction. *Circ Res*.2006;98:1177-1185.
7. Jun Panee. Monocyte Chemoattractant Protein 1 (MCP-1) in Obesity and Diabetes. *Cytokine*. 2012 Oct; 60(1): 1–12.
8. Ihm CG, Park JK, Hong SP, Lee TW, Cho BS, Kim MJ, Cha DR, Ha H. A high glucose concentration stimulates the expression of monocyte chemotactic peptide 1 in human mesangial cells. *Nephron*.1998;79:33-37.
9. Dragomir E, Manduteanu I, Calin M, Gan AM, Stan D, Koenen RR, et al. High glucose conditions induce

- upregulation of fractalkine and monocyte chemotactic protein-1 in human smooth muscle cells. *Thromb Haemost.* 2008;100:1155–65
10. Luo P, Tan ZH, Zhang ZF, Zhang H, Liu XF, Mo ZJ. Scutellarin isolated from *Erigeron multiradiatus* inhibits high glucose-mediated vascular inflammation. *Yakugaku Zasshi.* 2008;128:1293–9
 11. Shanmugam N, Reddy MA, Guha M, Natarajan R. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. *Diabetes.* 2003;52:1256–64.
 12. Nelison B.R, Katiz RW, Anna AA. Differential secretion of chemokines from peripheral blood in pregnant women compared with non - pregnant women. *Australian Journal of diabetes.* 2014;34:125-140
 13. Zue CW, Mohoto A. MCP-1 (monocyte chemotactic protein-1)-induced protein, a recently identified zinc finger protein. *J Biol Chem.* 2011;284:27620–8.
 14. Younce M., Nasif T., Redhah A. Assessment of MCP-1 and VEGF in a study case, *Endo. Bio Jou,* 2014 ; 12(7); 311-313.
 15. kado R et al. Human endothelial cells express CCR2 and respond to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. *Bio. Chem.* 2010;96:34–40
 16. Hong Xie. Effect of the cytokines on successful pregnancy depends upon the control of graft rejection mechanisms. *Transplant Journal.* 2014;39:174-175

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