MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) IS ASSOCIATED WITH PANCREATIC BETA-CELL DYSFUNCTION IN TYPE 2 DIABETES MELLITUS

BY

Miral M. Shehata[a], Mohamed M. Kamal[a], Mohamed H. El-Hefnawy[b] and Hala O. EL-Mesallamy[a].

FROM

[a] Biochemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

[b] National institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt.

ABSTRACT

PURPOSE

Beta-cell dysfunction is the critical determinant for type 2 diabetes. The well-known Monocyte Chemoattractant Protein-1 (MCP-1) has been identified as interesting islet-secreted cytokine that might be involved in beta-cell dysfunction, a role that hasn't been clinically elucidated yet. Therefore, this study was designed to study the clinical association between MCP-1 and beta-cell dysfunction in type 2 diabetes.

METHODS

Anthropometric, routine biochemical markers and serum levels of MCP-1 were measured in 63 diabetic subjects then compared to 16 healthy control volunteers. C-peptide, proinsulin and MCP-1 were measured by ELISA. Beta-cell dysfunction was assessed by proinsulin, proinsulin-to-C-peptide (PI/C-pep) ratio and HOMA2-%β. Relations among various parameters were studied using pearson correlation coefficient**.**

RESULTS

Serum MCP-1 levels were found to be significantly elevated in diabetics as compared to controls. In addition, it was found to be significantly correlated positively to proinsulin and PI/C-pep as well as negatively to HOMA2-%β.

CONCLUSIONS

MCP-1 is associated with beta-cell dysfunction in diabetic patients, indicating a potential role of this cytokine in the pathogenesis of type 2 diabetes by negatively impacting beta-cell function.

Introduction

Type 2 diabetes mellitus is a complex metabolic disorder having a great worldwide concern, being associated with huge economic burden, and decreased quality of life *(Guariguata et al., 2014).* It is widely accepted that β-cell dysfunction, rather than IR, is the critical determinant for type 2 diabetes *(Ashcroft and Rorsman, 2012),* because the disease never develops unless the pancreatic beta-cells fail to produce sufficient amounts of insulin to compensate for the worsening insulin resistance *(Saisho, 2015).* Nowadays, there is an increasing evidence supporting the vital role

played by some islet cells locally produced and secreted cytokines in both regulation and dysfunction of pancreatic β-cells *(Lee and Pervaiz, 2007; Wang et al., 2010).*

Monocyte Chemoattractant Protein-1 (MCP-1), was possibly suggested to be implicated in beta-cell dysfunction ***(Piemonti et al., 2002)***. MCP-1 (CCL2) is a member of C-C chemokine family that is well known to be expressed by a variety of cell types including adipocytes, fibroblasts, smooth muscle, skeletal muscle and endothelial cells upon exposure to proinflammatory stimuli ***(Yoshimura and Leonard, 1990; Pype et al., 1999; Gerhardt et al., 2001)***. Increased circulating levels of MCP-1 have been detected in states of low-grade inflammation as obesity, atherosclerosis, insulin resistance and type 2 diabetes ***(Kim et al., 2006; Harsimran et al., 2009)***. Interestingly, it was discovered that pancreatic beta-cells normally express and secrete MCP-1, even without presence of immune infiltrates ***(Piemonti et al., 2002)***. On the other hand, it was discovered that the failure of transplanted islets to function properly is attributed to increased MCP-1 expression and secretion in beta-cells ***(Sell and Eckel, 2007)***, thus predicting the outcome of islet transplantation ***(Cardozo et al., 2003)***. Therefore, it is expected that MCP-1 may be associated with beta-cell dysfunction in type 2 diabetes, although till today this potential association hasn't been validated in type 2 diabetic patients yet.

Thus, in this study, we aimed to determine the serum levels of MCP-1 in type 2 diabetic patients and its correlation with the other biochemical parameters, to evaluate how it might clinically reflect the beta-cell status in diabetic patients.

Subjects and methods

This study was approved by the ethical committee of National Institute of Diabetes and Endocrinology, Cairo, Egypt. The study was carried out in accordance with the regulations and recommendations of the Declaration of Helsinki. Control group subjects were obtained from healthy volunteers, while all type 2 diabetic patients were recruited from the outpatient clinic of the National Institute of Diabetes and Endocrinology (Cairo, Egypt) and informed consent was obtained from every subject before participating in the study. Seventy-nine subjects were enrolled in the study. The study groups were classified as follows; group I included 16 apparently healthy control subjects, while group II included 63 type 2 diabetic patients with mean diabetes duration of 7 years. A detailed family and medical history were collected from all subjects participating in this study. The characteristics of the studied subjects are summarized in Table 1. Both the diabetic groups and the control group were selected to have matching body mass index (BMI). The following exclusion criteria were used for all subjects: age < 30 or > 70 years, having type 1 diabetes, renal or hepatic diseases, acute or chronic inflammatory diseases, ischemic cardiovascular disease, cancer, thyroid dysfunction, acute or chronic infection, any haematologic disorders, the presence of virus C, alcohol or drug abuse, smoking. Female subjects taking hormonal replacement therapy were also excluded.

Fasting blood glucose (FBG) was measured by commercial kit provided by Spectrum Diagnostics® (GmbH, Schiffgraben, Hannover, Germany). Glycated hemoglobin HbA_{1c} (%) was measured in whole blood with ion exchange high-performance liquid chromatography (HPLC) using the Bio-Rad D-10™ system (Bio-Rad Laboratories, Hercules, CA, USA). All spectrophotometric measurements were carried out according to manufacturer's instructions using a UV⁄ visible 1650

spectrophotometer (Shimatzu, Lenexa, KS, USA). Serum human C-peptide and intact proinsulin (PI) were quantified by the enzyme-linked immunosorbent assay (ELISA) technique using commercially available kits as follows; Chemux BioScience (South Linden, South San Francisco, USA) kits for C-peptide, while DRG International (USA) was used for PI. The updated homeostasis model assessment of beta-cell function (HOMA2-%β) and insulin resistance (HOMA2-IR) were calculated using the HOMA calculator software version 2.2.3, as described previously ***(Wallace et al., 2004)***. Finally, the proinsulin-to-C-peptide (PI/C-pep) ratio was also calculated as sensitive surrogate markers for beta-cell dysfunction ***(Kim et al., 2000; Festa et al., 2008)***, after conversion of necessary units. MCP-1 serum levels were detected using Quantikine® MCP-1 ELISA kit (R&D Systems, Minneapolis, USA).

All data were expressed as mean ± standard error of mean (SEM). Kolmogorov-Smirnov test was done to evaluate the normal distribution of variables. Then, logarithmic transformation was performed to any skewed data to approximate normality. Comparison between different groups was performed by ANOVA using least significant difference (LSD) as a post hoc test. Pearson's correlation coefficient (r) was used to study the association among different parameters after adjustment for the effects of other covariates. All statistical analyses were done using IBM Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp., Chicago, IL, USA). Mean differences were considered statistically significant at p-value< 0.05 and highly significant at p-value<0.01.

Results

Anthropometric and metabolic parameters of studied groups were shown in **table 1.** All groups were matched for their BMI. FBG and HbA_{1c}% showed significantly higher levels in diabetic groups as compared to control group. Regarding C-peptide levels, interestingly they were elevated in group II as compared to group I.

Furthermore, to gain more insight into the beta-cell status of these patients and better assess this function, we measured serum PI level and calculated PI/C-pep ratio. Interestingly, similar to C-peptide, PI showed highly significant elevation in group II (6.69±0.44 pmol/l) as compared to control group (1.48±0.29 pmol/l) **(Figure 1A).** Moreover, PI/C-pep ratio showed highly significant elevation in group II as compared to control group (0.69±0.06 and 0.18±0.04, respectively) **(Figure 1B).** Regarding HOMA2-%β, it showed significant decline in group II as compared to control group (50.10±5.39 and 167.60±16.76, respectively, p<0.01) **(Figure 1C)**.

As for MCP-1, its levels were significantly elevated in the diabetic patients group as compared to control group (289.58±3.46 and 243.46±6.19, respectively, p<0.01) (**Figure 1D)**.

Table 1: Main anthropometric and metabolic parameters of the studied groups.

	Control subjects	Type 2 diabetic patients
N	16	63
Age (y)	47.06±1.5	51.1±0.92[a]
Sex (M/F)	13 / 3	40/23
BMI (Kg/ m^2)	27.14±0.54	27.46±0.21
FBG (mg/dl)	87.69±1.88	246.98± 10.46[a]
HbA_{1c} (%)	5.54± 0.09	10.96±0.32[a]
C-peptide (pmol/l)	930.03±98.65	1258.20±86.87[a]
HOMA**2**-IR	2.03±0.21	4.75±0.41[a]

Results are represented as mean ± SEM.

[a]: Significantly different from control (group I) at $p<0.01$.

BMI: body mass index; FBG: fasting blood glucose; HbA_{1c}: glycated hemoglobin; HOMA: homeostasis model assessment.

Figure 1

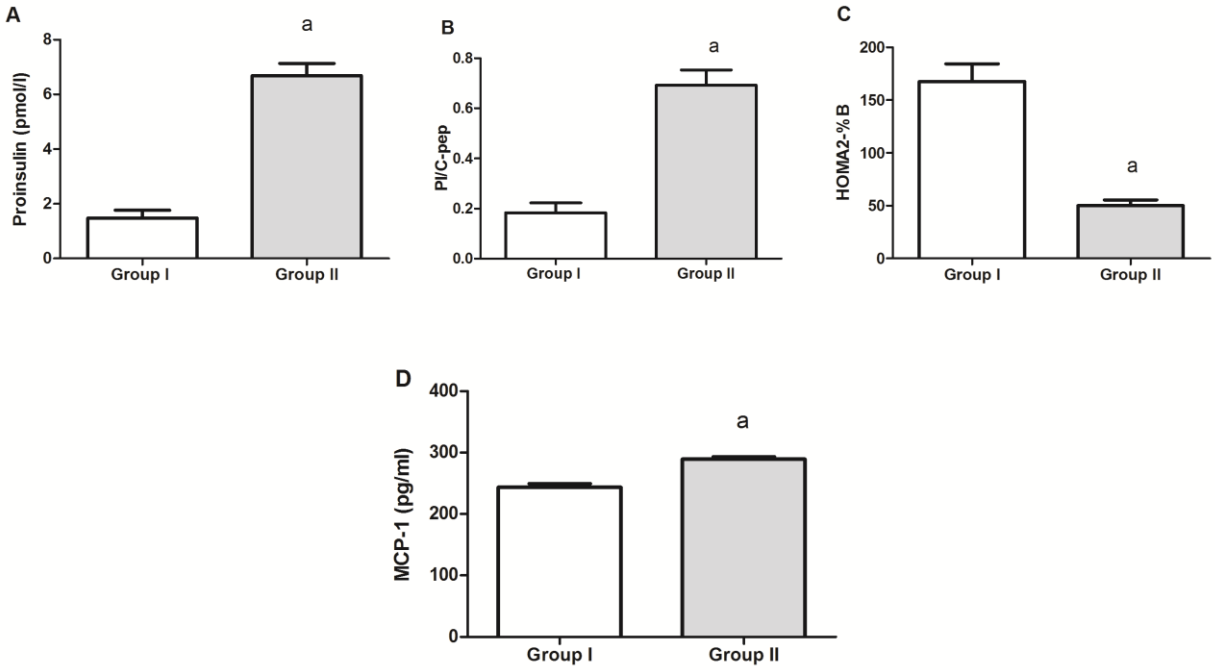

Figure 1: Serum concentrations of Proinsulin (A), PI/C-pep ratio (B), HOMA2-%β (C) and MCP-1 (D) in the studied groups.

Group I: Control subjects; Group II : Type 2 diabetic patients

[a]: Significantly different from control (group I) at $p<0.01$.

Moreover, we studied the association of MCP-1 with the different studied parameters. As shown in **table 2**, MCP-1 was associated with various anthropometric and metabolic parameters. Interestingly, MCP-1 was significantly correlated with FBG, HbA_{1c} % and HOMA2-IR.

Intriguingly, as for its correlation with beta-cell function assessment parameters, MCP-1 showed significant positive correlation with PI (r=0.364, p=0.001) **(Figure 2A)** and PI/C-pep ratio (r=0.370, p=0.001) **(Figure 2B)**, whereas, it showed mild significant negative correlation with HOMA2-%β (r= -0.276, p=0.014) **(Figure 2C).**

Table 2: Association of MCP-1 with other anthropometric and metabolic parameters in the studied groups

Factor	**MCP-1**	
	R	*P*
Age	0.086	0.453
BMI	0.437**	0.000
FBG	0.325**	0.003
HbA_{1c}	0.335**	0.003
C-peptide	0.165	0.147
HOMA**2**-IR[a]	0.249*	0.027

**: Significant at $p<0.01$.

*: Significant at $p<0.05$.

a**:** log transformed values are used.

Figure 2

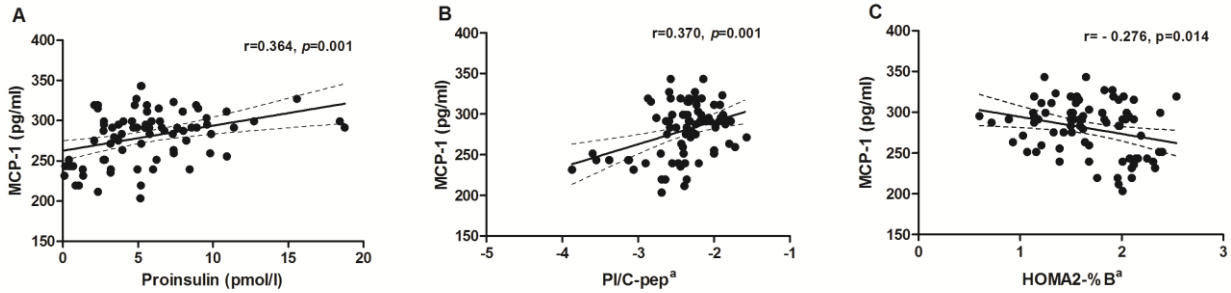

Figure 2: Correlation between MCP-1 and each of the following: Proinsulin (A), PI/C-pep ratio (B) and HOMA2-%β (C) in the studied groups.

[a:] log transformed values were used.

Discussion

It is well known that T2DM is sustained by both insulin resistance and impaired insulin secretion ***(Saisho, 2015)***. In fact, whenever impaired β-cell function appears, it leads to the progressive failure of islet-cells to secrete sufficient amounts of insulin to overcome peripheral IR, ultimately resulting in failure to maintain normal glucose homeostasis over time ***(Cerf, 2013)***. Therefore, it became strongly believed that β-cell dysfunction surpasses IR in inducing the onset and progression of T2DM ***(Cerf, 2013)***, hence being regarded as the critical determinant for T2DM.

Several beta-cell function-assessment markers have been previously proposed including; HOMA model -the widely used tool despite some limitations ***(Wallace et al., 2004)***, as well as PI and PI/C-pep ratio which are recommended recently as more accurate assessment parameters for beta-cell function ***(Pfutzner and Forst, 2011)***. In order to assess the beta-cell function in our patients, we first assessed serum levels of PI. In fact, usually diabetic patients are showing hyperproinsulinemia compared to controls ***(Pfutzner et al., 2004)***. Interestingly, PI showed gradual elevation in the diabetic patients group of this study. Regarding the PI/C-pep, another specific and highly recommended parameters to assess beta-cell functionality nowadays ***(Grill et al., 2002; Loopstra-Masters et al., 2011)***, ENREF_28 results of this study showed that this ratio displayed significant elevation, indicating a deteriorating beta-cell function, in diabetic patients group. Our results were in accordance with other studies showing elevated levels of PI/C-pep ratio in diabetic patients ***(Kim et al., 2000; Loopstra-Masters et al., 2011)***. Moreover, we assessed the HOMA2-%β index. Interestingly, we found that it was progressively declined in the diabetic patients group compared to controls, confirming the clear beta-cell dysfunction in those patients.

After showing the different beta-cell function status in the study groups, we assessed the serum levels of MCP-1. Regarding MCP-1, experimental evidence supports its role in various aspects of human pathophysiology including inflammation, cancer, coronary artery disease, obesity, insulin resistance, and type 2 diabetes ***(Monti et al., 2003; Kim et al., 2006; Harsimran et al., 2009)***. Our results support previous study findings ***(Mine et al., 2006)***, as there was a significant increase in MCP-1 levels in type 2 diabetic patients compared to control subjects. In addition, there was a significant positive correlation between MCP-1 and FBG as well as HbA_{1c}%, thus confirming the underlying link between MCP-1 and type 2 diabetes pathogenesis.

Interestingly, it was discovered that the failure of transplanted islets to function properly is attributed to increased MCP-1 expression and secretion in β-cells under inflammatory conditions ***(Sell and Eckel, 2007)***, thus predicting the outcome of islet transplantation ***(Cardozo et al., 2003)***. Therefore, it is expected that MCP-1 may be associated with β-cell dysfunction in T2DM. In fact, although MCP-1 has been extensively studied in relation to diabetes and/or its complications ***(Wada et al., 2003; Panee, 2012)***, however, until today, very scant data are available on the relationship of MCP-1 specifically to beta-cell dysfunction in type 2 diabetic patients. Therefore, this study is considered the first one to study the specific association between MCP-1 and beta-cell dysfunction in type 2 diabetic patients. Interestingly, our results showed a considerable correlation between MCP-1 and various beta-cell function markers including HOMA2-%β, PI and PI/C-pep. These results present MCP-1 not only as a diabetes-associated cytokine that plays an important role in diabetes pathogenesis, but

also as a beta-cell dysfunction-related cytokine. However, further studies are still needed to elucidate the exact mechanisms through which MCP-1 might be affecting beta-cells.

In conclusion, our results showed that assessment of MCP-1 might not only shed light on potential new mechanisms involved in beta-cell dysfunction, but also might propose promising new therapeutic targets in the future to preserve beta-cell function in diabetic patients.

Conflict of interest

The authors declare that there is no conflict of interest

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ارتباط الجاذب الكيميائي للوحيدات-١ بالخلل الوظيفي لخلايا البنكرياس بيتا في مرض السكري من النوع الثاني

للسادة الدكاترة

ميرال مجدي شحاته*، محمد مصطفي كمال* ، محمد هشام الحفناوي** و هاله عثمان المسلمي*

مـــــن

* قسم الكيمياء الحيويه ، كليه الصيدله، جامعه عين شمس ، القاهرة، مصر.

** المعهد القومي للسكر و الغدد الصماء، القاهرة، مصر.

ان مرض البول السكري وبالاخص النوع الثاني هو اضطراب تمثيل غذائي معقد وهناك لها هتمام كبير في جميع أنحاء العالم، بحيث انه يمثل عبئا اقتصاديا ضخما ويؤدي الي انخفاض جودة الحياة لدي مرضاه. ان الخلل الوظيفي لخلايا البنكرياس بيتا هو عنصر حاسم في التسبب بهذا المرض ولذا يفوق في الاهمية مقاومة هرمون الانسولين التي تم دراستها لفترة طويلة. هذا و يعتبر التهاب خلايا جزر البنكرياس بيتا واحدا من الأسباب التي تساهم في وقت مبكر في الخلل الوظيفي لهذه الخلايا، وبالتالي فإن دور السيتوكينات المسببة للالتهاب التي يتم انتاجها و افرازها من الخلايا بيتا أصبح محل اهتمام مجال البحث العلمي في العقود الماضية.

وفي هذا الصدد، تم اقتراح بعض السيتوكينات التي قد تساهم في الخلل الوظيفي لخلايا بيتا و من بينهم السيتوكين المعروف الجاذب الكيميائي للوحيدات-١(MCP-1) ، الذي تم اثبات تاثيره السلبي علي وظائف الخلايا بيتا من خلال الابحاث التي اجريت علي جزر البنكرياس في الانسان و القوارض ، الا انه حتي الآن لم يتم بعد التحقق من دوره الاكلينيكي في خلل تلك الخلايا في مرض البول السكري من النوع الثاني و كيف يمكن استخدامهم اكلينيكيا لعكس الحاله الفعليه لتلك الخلايا، و بالتالي، تم تصميم الدراسة الحالية كمحاولة لمعرفة مستوى ال MCP-1 في المرضى الذين يعانون من مرض البول السكري من النوع الثاني ، وكذلك للتحقق من ارتباطه مع مختلف دلالات الجسم البشري و الدلالات الكيميائية .