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Expression of Syndecan-1 on Neutrophils in Patients with Type 2 Diabetes Mellitus

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Abstract

***Background*: Type 2 diabetes is a manifestation of an ongoing low-grade inflammation. In diabetes, impairment of neutrophil adhesion to the endothelium and migration to the site of inflammation were detected, which were associated closely with adhesion molecules expressed on neutrophils and endothelial cells. *Patients and Methods*: To determine the expression of syndecan-1 on the neutrophils in diabetic patients, 80 patients with type 2 diabetes mellitus were recruited, in addition to 40 healthy individuals with no history of glucose metabolism disorders as control group. Expression of syndecan-1 was determined using flow-cytometry, and the correlations between syndecan-1 expression and clinical characteristics were analyzed. *Results*: The percentage of neutrophils expressing syndecan-1 was found to be higher in diabetic patients than among the healthy control individuals. In addition, a statistically significant association was found between the percentage of syndecan-1 positive neutrophils and body mass index. A multiple regression analysis found that body mass index is a significant predictor of neutrophils expressing syndecan-1 in type 2 diabetes mellitus patients. *Conclusion*: Surface syndecan-1 on neutrophils is significantly enhanced in subjects with type 2 DM. It is also positively correlated with BMI.**

Keywords: *Syndecan-1, Diabetes mellitus, Neutrophils*

Introduction

Neutrophils play an essential role in the host inflammatory response against infection. It was shown that the chemotactic activity of neutrophils from diabetic patients is significantly lower than in cells from healthy controls. Studies of the phagocytic and microbicidal activities of diabetic patients reveal, with few exceptions, an impairment of these functions(1). Decreased bactericidal activity, impairment of phagocytosis and decreased release of lysosomal enzymes, and reduced production of reactive oxygen species by neutrophils of diabetic patients have been described. Further-more, reduction in leukocyte phagocytosis and bactericidal activity showed a significant correlation with increases in blood glucose levels(2). Endothelial dysfunction caused by neutrophils contributes to the pathogene-sis of both micro- and macro-angiopathy of diabetes. Adhesion molecules express-ed on the surface of leukocytes and endothelial cells are crucial in the accumulation of leukocytes on endothe-lium, such as intercellular adhesion molecule-1, vascular adhesion molecule-1, P-selectin, and E-selectin(3). On neutrophils from patients with diabetes, surface b2-integrin increased and L-selectin decreased(4). The syndecans are a family of cell surface heparan sulfate proteoglycans, which act as adhesion molecules, modula-

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tors of growth factor function, and co-receptors in processes as diverse as morphogenesis, tissue repair, host defense, tumor development, and energy metabolism[5]. Syndecan-1 is involved in the processes of cell growth, differentia-tion, adhesion, lipoprotein physiology, wound healing, and inflammation[6,7]. Protease-mediated cleavage of the intact syndecan ectodomains (shedding) converts the cell-surface molecules into soluble effectors[9]. Increasing evidence suggests an important role for the syndecans in the regulation of inflamma-tions[10]. Some studies found that soluble syndecan-1 was high in type 2 diabetes mellitus patients[8]. However, this present study was done to assess the expression of syndecan-1 on the neutrophils in these patients.

Subjects and Methods

Eighty patients with type 2 diabetes mellitus without any diabetic complica-tions, in addition to 40 healthy individuals (as control), are the subjects of this study. Patients were selected from those who attended the Suez Canal University Hospital outpatient clinics. Exclusion criteria included type 1 diabetes mellitus, a cardiac event, or stroke during the past 6 months, renal failure, malignancy, or hematological disorders, ketosis, inflammatory disease such as infection, connective tissue disease, and patients on immunosuppressive drugs. No participants took any anti-infective or anti-inflammatory medication. To avoid the confounding effect of infection, only subjects with a white blood cell count within the normal range were included. Diabetes mellitus was diagnosed according to WHO criteria[11]. The Ethics Committee of the Suez Canal University approved this study. From all the participants, the following data were collected: age, gender, blood pressure, body mass index (BMI), fasting plasma glucose, triglyceride, cholesterol, high-density lipoprotein, low-density lipoprotein. Venous blood samples were drawn from subjects after 12-14 hours fastness. Height and weight were measured using standard procedures. The body mass index (BMI, kg/m^2) was calculated. Blood pressure was measured in all subjects in a relaxed and sitting position on the right arm with a standard mercury sphygmomanometer. Fasting plasma glucose, triglyceride, cholesterol, high-density lipoprotein, low-density lipoprotein were done using the fully-automated spectrophotometer Hitachi 912 *(Roche Diagnostics, BM, Germany).*

Neutrophilic syndecan-1 expression: Neutrophils were isolated from ethylene diamine tetra acetic acid (EDTA)-anti-coagulated venous blood by the method of Ficoll–Hypaque density gradient centrifugation[12]. Purified neutrophil cell pellets were re-suspended in 100 µl RPMI-1640 5% containing 3 µl fluorescein isothiocyanate (FITC)-conjugated mouse anti-human syndecan-1 *(eBioscience, USA)* or isotype control antibody and incubated for 45 min on ice. The fluorescence of 10^4 cells was measured on a FACSCalibur *(Becton–Dickinson, San Jose, CA)*. The percentage of the syndecan-1-positive neutrophils was evaluated.

Statistical analysis

Results were presented by mean ± SD. One-way ANOVA test was used for between group comparisons of continuous variables, and the chi square test was used for categorical variables. Multiple linear regression analysis was used to determine the association between syndecan-1 and clinical parameters. Statistical significance was considered at level < 0.05.

Results

The study included 80 patients with type 2

diabetes mellitus (45 males and 35 females with a mean age of 55.4 ± 5.5 yrs). In addition, the study included 40 healthy control individuals (23 males and 17 females), with no history of glucose metabolism disorders, whose mean±SD age was 52.1 ± 8.2 years. Table 1 shows the demographic, clinical and laboratory characteristics of both study and healthy control groups. There were statistically significant differences between both groups in fasting blood glucose level, BMI and glycated hemoglobin. The percentage of neutrophils expressing syndecan-1 was significantly higher among diabetic patients (13.592 ± 1.338) than among the healthy individuals (4.342± 0.561) with p value of 0.001. Figure (1) and figure (2) show the histogram of syndecan-1 expression in normal individual and a diabetic patient respectively. When the correlation between patients characteristics and syndecan-1 expression were analyzed, there was a significant correlation between syndecan-1 expression on the neutrophil and BMI ($r = 0.397$, $p = 0.019$) (Figure 3). In addition, syndecan-1 expression was higher among patients with BMI more than 25 kg/ m^2 than among patients with BMI lower than 25 kg/ m^2. Multiple regression analysis showed that BMI ($P = 0.000$, $\beta = 0.681$) was the only significant predictor of percentage of syndecan-1 positive neutrophils in subjects with type 2 diabetes.

Table 1: Demographic, clinical and laboratory characters of both groups

Item	Control	DM	p value
Male no. (%)	23 (57.5%)	45 (56.3%)	
Female no. (%)	17 (42.5%)	35 (43.7%)	
BMI (kg/ m^2)	22.3 ± 0.5	26.7 ± 0.7	0.008
WBCs ($X10^3$ / µl)	7.2 ± 0.4	7.8 ± 0.6	0.562
FBS (mg/ dl)	89 ± 15	197 ± 25	0.000
HbA1c (%)	5.4 ± 0.6	8.2 ± 0.4	0.007
Cholesterol (mg/ dl)	182 ± 7	190 ± 6	0.512
TG (mg/ dl)	133 ± 16	195 ± 32	0.087
HDL (mg/ dl)	50 ± 3	46 ± 4	0.543
LDL (mg/ dl)	112 ± 7	104 ± 8	0.531
Neutrophil-expressed syndecan-1 (%)	4.342±0.561	13.592± 1.338	0.001

BMI: Body mass index, WBCs: White blood cells, FBS: Fasting blood glucose, TG: triglycerides, HDL: High density lipoprotein, LDL: Low density lipoprotein

Discussion

Several aspects have been shown to be impaired during inflammation in diabetes mellitus. These abnormalities might contribute to the increased susceptibility and severity of infections in diabetic patients[13]. The heparin-related glycosaminoglycan heparin sulfate (HS) binds to and modifies the function of several molecules involved in the inflammatory process. On the cell surface, proteoglycans of the syndecan family are the major sources of HS[14]. Syndecans take part as co-receptors in mediating chemokine function by increasing the binding of chemokines to their innate receptors[15]. Syndecan-1 plays an important role in the regulation of inflammation, as suggested by increased leukocyte–endothelial interactions in syndecan-1 null mice[16]. Syndecan-1 is expressed on pre-B cells and plasma cells, and it can be induced

on macrophages. Syndecan-1 is involved in T-cell-independent antigen-stimulated B-cell differentiation and isotype switching which is associated with T-cell-dependent B-cell-differentiation. In peripheral blood, T cells from patients with gingivitis show increased expression of syndecan-1[17]. Although there were few reports about surface syndecan-1 on neutrophils from diabetes, it is confirmed that syndecan-1 plays an important role in chemokine gradient formation for trans-endothelial and transepithelial migration of neutrophils. Transepithelial neutrophil migration is dependent on matrilysin-mediated shedding of syndecan-1 from the mucosal surface of epithelium[10]. In this study, syndecan-1 was found to be highly-expressed on the neutrophils in DM patients than among the healthy control subjects. The up-regulation of syndecan-1 on the neutrophils in diabetic patients may be due to the induction by cytokines and growth factors[18]. Syndecan-1 is increased by basic fibroblast growth factor (bFGF) and platelet-derived growth factor-BB, but decreased in response to tumor growth factor-β1 (TGF-β1), tumor necrosis factor (TNF)-α, interleukin (IL)-1 β, and interferon (IFN)-γ[19]. PR39, an inflammatory cell-derived peptide, up-regulates syndecan-1 expression in vitro and in vivo. Both B and T lymphocytes synthesize syndecan-1, and its expression can be modulated by TGF-β1, IL-2, IL-4, and lipopolysaccharide. Multiple cytokines (including TNF-α, IL-1β, IL-6, IFN-γ) were detected in diabetes, and they were devoted to the B-cells death[20]. It was found that overweight, especially obesity is associated with chronic clinical inflammation[21]. BMI is widely used to represent obesity, and its impact effect on the development of vascular and cardiac complications[22]. Obesity and central obesity are risk factors in type 2 DM[23]. To confirm this notification, weight loss was found to be associated with reduction in inflammatory processes and improvement in endothelial functions[24]. It was documented that neutrophil infiltration and vascular inflammation are increased in obese women, and is correlated with BMI[25]. In this study, it was found that the percentage of neutrophils expressing syndecan-1 is increased and is correlated positively with BMI in diabetic patients, which may be due to increased inflammation in overweight and obese patients. Both previous findings are comparable with the results done on Chinese patients[26]. Many studies documented increased levels of syndecan-1 in diabetics, but, up to the writing of this work, only one study was done to assess syndecan-1 expression on the neutrophils in DM[26]. Syndecan-1 is an important regulator of cell–cell and cell–extracellular matrix interactions as suggested by animal studies[27]. It is reported also that syndecan-1 acts as a negative modulator of leukocyte–endothelial interactions. Rolling, adherence, and migration of neutrophils to endothelium rely on many adhesion molecules, including selectin and integrin. The role of increased syndecan-1 on neutrophils in diabetes remains unclear[28].

Conclusion

Surface syndecan-1 on neutrophils is significantly enhanced in subjects with type 2 DM. It is also positively correlated with BMI. Therefore, decreasing body weight of diabetic patients with type 2 can decrease the inflammatory reactions, which are the main events for the development of diabetic complications.

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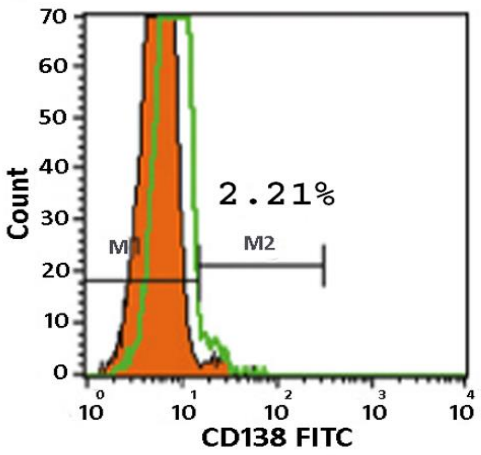

Figure 1: Flow cytometric histogram of syndecan-1 expression on neutrophils in a normal individual

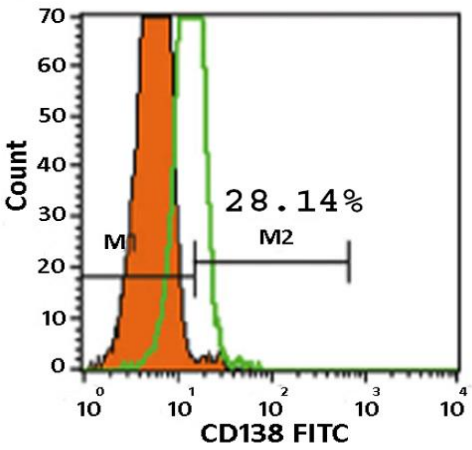

Figure 2: Flow fytometric histogram of syndecan-1 expression on neutrophils in a diabetic patient

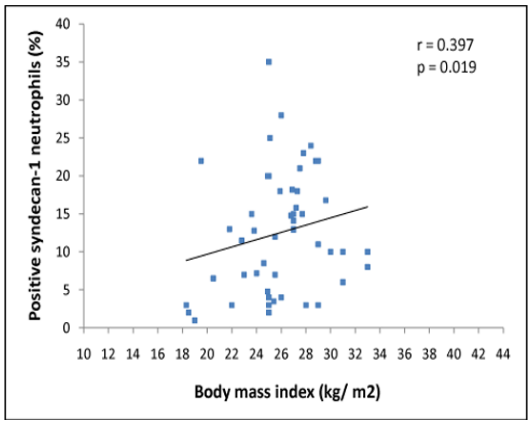

Figure 3: Correlation between body mass index and neutrophilic syndecan-1 expression percentage

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