
Vascular cell adhesion molecule-1 and endothelial leukocyte adhesion molecule-1 as markers of atherosclerosis of NIDDM

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Abstract: Background: Leukocyte adhesion to arterial endothelial cells is thought to be an important step in the development of atherosclerosis, Adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and the endothelial-leukocyte adhesion molecule-1 (ELAM-1) play an essential role in the early stages of atherogenesis of diabetic patients. Materials and Methods: The study was conducted on 80 male divided into two groups 60 of them had diabetes mellitus and 20 subjects were normal healthy individuals served as a control group. Enzyme linked immune sorbent assay (ELISA) was used for the measurement of serum VCAM-1 and ELAM-1 and WBC assay executed by automatic hematology analyzer the information of patients were obtained through a questionnaire consisted Patients with other diseases were excluded from the current investigation. Results: This result revealed elevated level of serum VCAM-1 and ELAM-1 of Diabetes patients compared with healthy group also differentiation count reveal elevated count of WBC, Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil in Diabetes patient as compare with HT group.

Keywords: ELAM-1, VCAM-1, NIDDM

1. Introduction

Accelerated atherosclerosis and microvascular disease are the major vascular complications of diabetes, and constitute the principal cause of morbidity and mortality in this ubiquitous disorder.^[1,2,3] Many underlying factors could contribute to this outcome, including abnormalities in plasma lipoproteins, blood pressure, and renal function. A final common pathway in the development of vascular pathology is the expression of inducible adhesion molecules rendering the vasculature a selective target for circulating peripheral blood cells. In this context, vascular cell adhesion molecule-1 (VCAM-1) is of particular interest as its expression has been linked to the early phase of experimental hypercholesterolemia-induced atherosclerosis^[4,5], and enhanced vascular VCAM-1 expression has been demonstrated in the vasculature of alloxan-treated diabetic rabbits^[6] as well as in human atherosclerotic lesions^[7].

It is well known that soluble intercellular adhesion molecule-1 (sICAM-1), soluble VCAM-1 (sVCAM-1) levels

are elevated in patients with type 2 diabetes^[8,9,10]. Previous studies suggest that hyperglycemia, hyperinsulinemia, or insulin resistance may be responsible for the elevation of adhesion molecules^[11,12]. Adherence of circulating leukocytes to endothelium and their subsequent transmigration in to the arterial intima is an early step in the formation of atherosclerotic lesions^[13]. The recruitment of leukocytes in to tissues is dependent on a cascade of events mediated through a diverse family of cellular adhesion molecules that are expressed on the surface of vascular endothelial cells^[14,15]. Membrane-bound VCAM-1 is expressed mainly on endothelial cells, smooth muscle cells, and tissue macrophages^[16,17], and allows the tethering and rolling of monocytes and lymphocytes, as well as firm attachment and transendothelial migration of leukocytes^[18,19,20]. Endothelial expression of VCAM-1 occurs on human atherosclerotic plaques^[7,21] and has been shown to be an early manifestation of experimental cholesterol-induced atherosclerosis^[4,17]

Soluble forms (sVCAM-1) have been detected in plasma^[22,23]. Secretion of sVCAM-1 is reported to be indicative of the expression of membrane-bound VCAM-1^[24]. Although the physiological role of these soluble forms is unclear, it has been hypothesized that sVCAM-1 levels may serve as a monitor of expression of membrane-bound VCAM-1. Increased levels thus may reflect progressive formation of atherosclerotic lesions^[25]. In addition, recent cross-sectional studies showed sVCAM-1 concentration to be positively associated with carotid artery intima-media thickness^[26,27], and with the severity of peripheral arterial disease assessed by angiography^[28,29,30].

2. Aims of the Study

The aim of the study was to determine the level of Adhesion molecules and possible effect combined with leukocyte in atherogenesis of diabetes mellitus patients.

3. Subjects and Methods

The study was conducted on 80 male divided into two groups 60 of them had diabetes mellitus and the remaining 20 subjects were normal healthy individuals served as a control group. The patients were collected from the diabetic unit in Al-Sadder Medical City /Al-Najaf Al-Ashraf province during the period from July till November, 2013. Diabetes Mellitus was diagnosed by consultant doctors. The information of patients were obtained through a questionnaire consisted of the name, sex, age, weight, height. Patients with renal dysfunction, heart diseases, who were on drugs affect oxidative stress, i.e: antioxidants, antihyperlipidemic agents were excluded from the current investigation. Blood samples were drawn by trained nurses or other health care professionals and divided in two tube first contain anticoagulant for biochemical measurements second tube left at room temperature for one hour to clotting, centrifuged 6000 rpm for 10 minutes, and then serum freezing at -20°C to keep it stable for a few months, Enzyme linked immune sorbent assay (ELISA) was used for the measurement of serum VCAM-1 and ELAM-1.

3.1. Automated Laboratory Methods

3.1.1. Serum Vascular Cell Adhesion Molecule 1 (VCAM-1) and Serum Endothelial Leukocyte Adhesion Molecule 1 (ELAM-1) Estimation

This assessment employs a quantitative sandwich enzyme immunoassay technique, and performed by Automated microtiter plate ELISA reader (HumaReader HS, Cat.No.16670, Semi-automatic, microprocessor-controlled photometer, Wiesbaden. Germany).

3.1.2. WBC Differentiation Count

Differential Count was performed by using CYANHemato analyzer (automatic hematology analyser. Catalog No. CY006, Cypress Diagnostics, Langdorpsesteenweg 160, B-3201 Langdorp, Belgium.)

4. Statistical Analysis

Data were analyzed using the software packages Graphpad prism for Windows (5.04, Graphpad software Inc. USA), Data are presented as the mean \pm standard error (SE). The comparison between the patients and healthy groups were analyzed by one-way ANOVA and t-test. A p-value < 0.05 was considered significant

5. The Result

5.1. Relation between Vascular Cell Adhesion Molecule 1 (VCAM-1) of Diabetes Patients and Healthy Group

Fig.1 shows comparison between Diabetes patients and healthy group. This result revealed the significant increased $P < 0.05$ in serum (VCAM-1) 168 ± 33 (ng/ml) of Diabetes patients compared with healthy group 63 ± 12 (ng/ml).

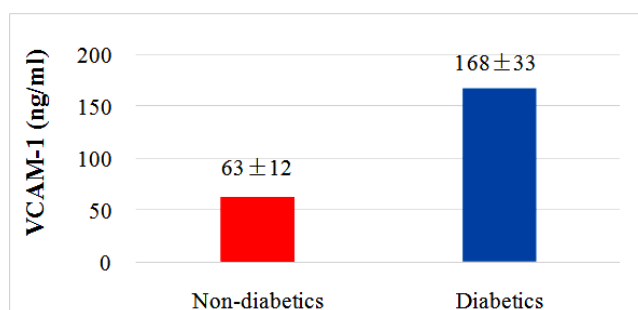


Figure 1. Comparison between VCAM-1 of Diabetes patients and Healthy group.

5.2. Relation between Endothelial Leukocyte Adhesion Molecule 1 (ELAM-1) of Diabetes Patients and Healthy Group

Fig.2 shows comparison between Diabetes patients and healthy group. This result revealed the significant increased $P < 0.05$ in serum (ELAM-1) 53 ± 8 (ng/ml) of Diabetes patients compared with healthy group 37 ± 3 (ng/ml)

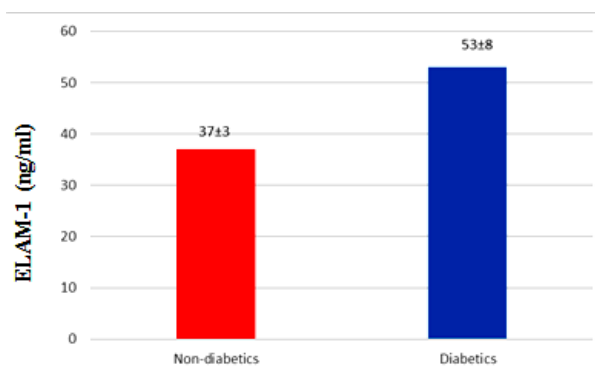


Figure 2. Comparison between ELAM-1 of Diabetes patients and Healthy group.

5.3. Relation between White Blood Cells Counts of Diabetes Patients and Healthy Group

The result in fig.3 shows comparison between Diabetes

patients and healthy group where as significant increased $P < 0.05$ of WBC, Neutrophil count, Lymphocyte count, Monocyte count, Eosinophil count, Basophil count in Diabetes patients 11632 ± 3431 , 5996 ± 565 , 4826 ± 345 , 489 ± 89 , 255 ± 43 , 66 ± 26 (cell/mm³) as compare with HT group 8543 ± 1432 , 4533 ± 139 , 3483 ± 276 , 297 ± 67 , 187 ± 36 , 43 ± 12 (cell/mm³)

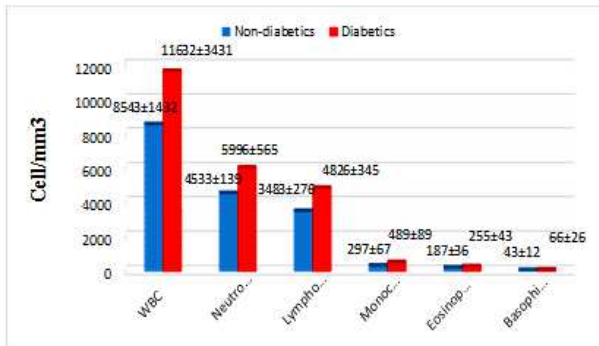


Figure 3. Comparison between White blood cells Counts of Diabetes patients and Healthy group.

5.4. Correlation between Vascular Cell Adhesion Molecule 1 and Endothelial Leukocyte Adhesion Molecule 1 of Diabetes Patients Group

The result of fig.4 mark positive correlation between ELAM-1 and VCAM-1 ($R^2 = 0.97$) with statistical significant.

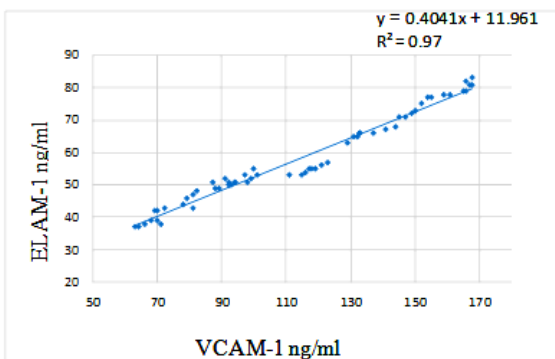


Figure 4. Correlation between VCAM-1 and ELAM-1 of Diabetes patients.

6. Discussion

The results of present study shown elevated level of VCAM-1 and ELAM-1, this study in accordance with [31,24,32] whose said that elevated serum level of VCAM-1, an inducible cell-cell recognition protein on the endothelial cell surface (EC), has been associated with early stages of atherosclerosis. In view of the accelerated vascular disease observed in patients with diabetes, and the enhanced expression of VCAM-1 in diabetic patients, In the early stages of atherogenesis, Leukocyte adhesion to arterial endothelial cells is thought to be an important step in the development of atherosclerosis Adhesion molecules such as ICAM-1, VCAM-1 and the ELAM-1 play an essential role in

this step. [33,34,35] Aggregations of lipid-rich macrophages and T lymphocytes can be demonstrated within the intima. The adhesion of leukocytes on endothelial cells and their transendothelial migration are mediated by adhesion molecules on the endothelial cell membrane that mainly belong to two protein families: the selectins and adhesion molecules of the immunoglobulin superfamily. For two members of the first group (E-selectin [ELAM-1] and P-selectin) and two members of the latter group (ICAM-1 and VCAM-1), expression has been demonstrated in various cell types forming the atherosclerotic plaque, for example, endothelial cells, vascular smooth muscle cells, and macrophages. Especially in intimal neovasculture, the expression of VCAM-1, ICAM-1, and ELAM-1 is upregulated [22,36].

Inflammatory cytokines modulate the homeostatic properties of the endothelium. Local inflammatory cells can generate and release cytokines which have the potential to activate endothelium, transforming its natural anti-adhesive and anti-coagulant properties, The inflammatory response generates cytokines which upregulate the expression of vascular cell adhesion molecules VCAM-1. Several reports support the notion that se- rum levels of VCAM-1 may be useful marker for providing information on atherogenesis , In animal and human models of atherosclerosis, the first sign of disease activity is an up-regulation of adhesion molecules such as VCAM-1. Endothelial dysfunction, a chronic state in which vasoconstrictive stimuli overcome vasodilative stimuli, is associated with insulin resistance from early stages of its development. [37,38,39,40,41]

The present study demonstrated that the total and differential leukocyte counts were significantly altered in patients with hyperglycemia because of peripheral white blood cell (WBC) count has been shown to be associated with insulin resistance, type diabetes, coronary artery disease (CAD), stroke, and diabetes micro- and macrovascular complications. Peripheral blood leukocytes are com- posed of polymorphonuclear cells, including monocytes as well as lymphocytes. Polymorpho-andmononu- clear leukocytes can be activated by advanced glycation end products, oxidative stress, angiotensin II, and cytokines in a state of hyperglycemia. Leukocytes may be activated through the release of cytokines, such as tumor necrosis factor (TNF) [42,43,44,45].

7. Conclusions

This study indicates that elevated serum level of VCAM-1 and ELAM-1 associated with early stages of atherosclerosis in diabetes patients.

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