THE STUDY OF SOLUBLE ADHESION MOLECULES (SICAM-1, SVCAM-1, SELAM-1) IN SERUM AND MEMBRANE FORM OF ADHESION MOLECULES (VCAM-1, ICAM-1) ON THE MONOCYTES IN DIABETIC SUBJECTS TYPE 1

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Abstract

The adhesion molecules are involved in adhesion of leukocytes to endothelial cells and other immune cells. Not only adhesion molecules have membrane form, but they also have soluble form. In this paper, we attempted to investigate the concentration of soluble adhesion molecules in the type 1 diabetic patients (n = 40) and healthy subjects (n = 10). The results indicated that there was an increase in the concentration of soluble adhesion molecules: sICAM-1, sVCAM-1 and sELAM-1, in the sera of diabetic patients rather than the healthy ones (p = 0.00). Increased concentration of sVCAM-1 with the insulin injection dose (p = 0.01) and sICAM-1 with duration of disease (p = 0.05) indicated significant reversal linear correlation in the patients' groups. Also, sELAM-1 with sVCAM-1 and sICAM-1 showed significant direct linear correlation in the same group (p = 0.01). Adhesion molecules were determined by the sandwich ELISA principle.

One of the most important factors in the development of atherosclerosis is the adhesion of monocytes to endothelial cells. Therefore, in this study, the percentage of expression of membrane form of VCAM-1, not ICAM-1, was increased on 10000 monocytes of diabetic patients type-1 (n = 20) in comparison with normal subjects (n = 20, p = 0.05). The expression tensive of membrane form of CAMs on the surface of monocytes was performed by flowcytometry technique.

We concluded that the increase in sICAM-1, sVCAM-1 specially sELAM-1 and also mVCAM-1 indicated endothelial activation, stimulatability of leukocyte cells and increased interaction to endothelial cells. Although, scientists are not aware about the role of elevated CAMs concentration, it can be suggested that the increasing level of sICAM-1, sVCAM-1, sELAM-1 and mVCAM may reflect cellular expression, function of CAMs in autoimmune disease and also provide a potential therapeutic target for human IDDM.

Keywords: Diabetes Mellitus, Atherosclerosis, Adhesion Molecules, Soluble Form of Adhesion Molecules, Membrane Form of Adhesion Molecules.
The Study of Soluble Adhesion

Introduction

Diabetes mellitus type-I or insulin dependent diabetes mellitus (IDDM) is one of the most common chronic endocrine disorders of children and young individuals(1). The disease appears with infiltration of immune cells such as TCD4+, TCD8+ and monocytes to islets of Langerhans, which leads to destruction of β-cells (2,3). Immunohistochemical studies of human and animal tissues have shown that recruitment and localization of effector cells into β cells were performed by cell adhesion molecules (CAM) which were expressed as increased levels on the endothelial cells and even on the pancreatic islets of Langerhans (4-6).

Cell adhesion molecules according to their structures and performances are divided into integrins, selectins and immunoglobulin superfamilies (7-9). ICAM-1 and VCAM-1 belong to immunoglobulin superfamilies and ELAM-1 (E-selectin) to selectin family, and play a critical role in recruitment of leukocytes to normal and inflamed tissues. The expression of CAMs is relatively low in normal cells such as endothelium, monocyte, vascular and is upregulated in response to various stimuli including oxidant and cytokines i.e. IL-1, INF-γ and TNF-α(7, 10-12).

CAMs are also present in the circulation as soluble forms which lack membrane-spanning and cytoplasmic domains that are present in the membrane-bound forms. Although, the origin, metabolism and function of sCAMs are not fully understood, quantitative assessment of the levels of sCAMs is straightforward. These levels have been noted to be elevated in certain pathological conditions such as sepsis, allograft rejection and autoimmune disease (9, 13). Nowadays, scientists believe that elevation in the concentration of soluble adhesion molecules may be indicated to endothelial activation, elevated adhesion of leukocyte to vessel wall or other immune cells (13, 14).

Atherosclerosis is the major cause of death in diabetic patients. Adhesion molecules are involved in the process of atherosclerosis. Increasing evidence indicates that hyperglycemia in IDDM patients accelerates oxidation process and cause endothelial damage by oxidized compounds of plasma (i.e. LDL) and production of AGE-products and foam cells. All these factors lead to activation of NFkB and then increase in the transcription of adhesion molecules: ICAM-1, VCAM-1 and ELAM-1, consequently induce high expression of adhesion molecules on the endothelial and immune cells (15-18). So cell adhesion molecules are involved in chemotaxis and binding of leukocytes and platelets to endothelium. A potential role has been attributed to their initiation and enhancement of atherosclerosis (16,19).

For better understanding of the important role of adhesion molecules, we analyzed the serum concentration of ICAM-1, VCAM-1, ELAM-1 and membrane form of ICAM-1 and VCAM-1 on the monocytes of IDDM patients. Also in this study, we examined the correlation between the dependant factors (CAMs) and other independent factors such as age, sex etc.

Subjects and Methods

Subjects

To examine the soluble adhesion molecules, 40 diabetic patients [(20 males, 20 females) mean age 14.2±4.7 (SD); range: 7-22 yr] were selected from Tehran's Diabetes Institute. The duration of disease was 30.6±36 (range: 2-144 months). The patients received multiple insulin injections (n=2/day). All patients and controls were nonsmokers, free of clinically manifest infections and diabetes-unrelated illnesses and were not taking any medication, specially corticosteroid drugs for several weeks before blood tests. Only 20 patients among 40 patients were chosen [mean age: 15.3±5.06 (SD); range: 7-22 yr] for analyzing mICAM-1 and mVCAM-1 density on the monocytes surface. Ten healthy individuals [mean: 20.2±1.6 (SD); range 18-22], are selected from the students of Iran medical university.

Analytic Methods

To determine the concentration of soluble adhesion molecules, we used serum samples frozen at -70°C. The concentration of sICAM-1, sVCAM-1 and sELAM-1 were determined by the sandwich ELISA technique on the basis of streptavidin-biotin system (Cat.No. 1 742 680, Boehringer Mannheim Ca.).

To determine the membrane form of ICAM-1 and VCAM-1 on the monocytes, EDTA blood was used. So by

*Abbreviation:

AGE = Advanced glycosylation end-product; EDTA = Ethylenediamine tetraacetic acid; ELAM-1 = Endothelial leukocyte adhesion molecule-1; FITC = Fluorescein isothiocyanate; ICAM-1 = Intercellular adhesion molecule-1; IDDM = Insulin-dependent diabetes mellitus; IL = Interleukin; INF-γ = Interferon-γ; LFA-1 = Lymphocyte functional antigen-1; NF = Nuclear factor; NOS = Non- obese diabetic mouse; sCAM = Soluble cell adhesion molecule; TNF-α = Tumor necrosis factor-α; VCAM-1 = Vascular cell adhesion molecule-1; VLA-4 = Very late antigen-4

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lyzing solution (Becton Dickinson Co.) the red blood cells were lysed. At the next stage using monoclonal antibody, i.e. FITC labeled anti ICAM-1 and anti VCAM-1 monoclonal antibody, (Cat.No. 217606, Calbiochem Co.) and by using flow cytometry (from Becton Dickinson Co.) with Simul set program (FSC/SSC), cell groups were shown as dot plots. Then monocytes were gated and 10,000 monocyte cells were selected. The membrane form of the protein was examined according to the degree of florescent light (fig 1).

Statistic Analysis

For all above factors we, considered mean and standard deviation. In order to compare the average degree between diabetics and healthy people, we used t-test, variance analysis, Fisher test; and to determine the correlation between independent factors (such as age, sex, ...) and dependant factors (CAM), we used Pierson test. P values less than 0.05 were considered significant.

Results

CAMs:
The mean and standard deviation of all factors are shown in table -1. In the patients with IDDM the serum concentrations of ICAM-1, VCAM-1 and ELAM-1 (ng/ml)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Diabetic Patients Type I</th>
<th>Healthy Subjects</th>
</tr>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>14.25 ± 4.7 (7 - 22)</td>
<td>20.2 ± 1.6 (18 - 22)</td>
</tr>
<tr>
<td>Sex (female: male)</td>
<td>20 ± 13.7 ± 4.6</td>
<td>6 ± 19.3 ± 1.5</td>
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<tr>
<td>Insulin injection dose (IU)</td>
<td>34.2 ± 16 (4 - 75)</td>
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<tr>
<td>Duration of disease (month)</td>
<td>30.62 ± 36 (2 - 144)</td>
<td>-</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>198.02 ± 108 (61 - 460)</td>
<td>76.4 ± 6 (61 - 110)a</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>327.77 ± 140 (125 - 650)</td>
<td>176 ± 25.19 (125 - 200) a</td>
</tr>
<tr>
<td>sVCAM-1 (ng/ml)</td>
<td>521.27 ± 130 (234 - 768)</td>
<td>444 ± 48 (234 - 500) a</td>
</tr>
<tr>
<td>sELAM-1 (ng/ml)</td>
<td>3.35 ± 1 (1.77 - 6.44)</td>
<td>2.38 ± 0.3 (1.77 - 2.80) a</td>
</tr>
<tr>
<td>mICAM-1 (degree of florescent light)</td>
<td>96.29 ± 31</td>
<td>81.57 ± 28b</td>
</tr>
<tr>
<td>mVCAM-1 (degree of florescent light)</td>
<td>52.45 ± 7</td>
<td>42.02 ± 14c</td>
</tr>
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</table>

Data are given as mean and standard deviation.
Comparing the mean data between diabetic patients and healthy subjects:
a: P=0.00, b: P=0.02, c: P=0.05
FBS= Fasting blood sugar; IU: International unit; sICAM-1=Soluble intercellular adhesion molecule-1; sVCAM-1= Soluble vascular adhesion molecule-1; sELAM-1= Soluble endothelial leukocyte adhesion molecule-1.
were elevated vs their controls. Statistical test showed these differences to be significantly true (P=0.00). Although the concentration of sICAM-1, sVCAM-1 and sELAM-1 in diabetic patients were %12.5, %640 and %35 in normal range, no significant differences were observed in the level of sCAMs in female and male diabetic patients.

According to the intensity of fluorescent light on the monocytes surface of the patients, in comparison with healthy subjects, ICAM-1 and VCAM-1 showed an increase which was significant just in membrane form of VCAM-1 (P=0.05). In healthy subjects, no significant differences were in the expression of these adhesion molecules in monocytes surface of female and male diabetic patients.

Correlations

In the IDDM patients, there was no significant correlation between age with the concentration of sICAM-1 (P=0.23, r =0.19), sVCAM-1 (P=0.1, r =0.51) and sELAM-1 (P=0.11, r =0.25). Also, no significant correlations were found between fasting blood sugar with sICAM-1 (P=0.09, r =-0.26), sVCAM-1 (P=0.21, r =0.20) and sELAM-1 (P=0.92, r =0.01). The insulin injection dose (IU) had significant reverse linear correlation with the concentration of sVCAM-1 (fig 2, P=0.01, r =-0.39), but it didn’t have with sICAM-1 (P=0.16, r =-0.22) and sELAM-1 (P=0.23, r =-0.19). Also, duration of disease had a significant reverse linear correlation with concentration of sICAM-1 (fig 3, P=0.05, r =-0.31) but not with sVCAM-1 (P=0.57, r =-0.90) and sELAM-1 (P=0.35, r =-0.14).

On the other hand, there was a significant correlation among the sCAMs concentration in patients’ group. That is, sICAM-1 with sELAM-1 (fig 4, P=0.01, r =0.39) and sELAM-1 with sVCAM-1 (fig 5, P=0.01, r =0.38) had a significant direct linear correlation. While, in the same group, there was no correlation between sICAM-1 and sVCAM-1 (P=0.44, r =0.12).

In this study, there was no correlation between the concentrations of sCAMs with each other and with independent factors (age, sex, ...) in healthy subjects and also between the membrane forms of ICAM-1 and VCAM-1 with sCAMs and other independent factors in healthy subjects and diabetic patients.

Discussion

In this report, we have shown that sICAM-1, sVCAM-1 and sELAM-1 are significantly increased in the serum of diabetic patients type-1 in comparison with normal control (P=0.00). Also we have shown significant correlation be-

![Graph](image-url)

*Fig 2. Correlation between insulin injection dose and sVCAM-1 in diabetic patients type-1*
between sELAM-1 with sICAM-1 and sVCAM-1 (Fig. 4, 5 respectively, \( P = 0.01 \)) in the same group. We know sELAM-1 and sVCAM-1 are released from activated endothelial cell with inflammatory cytokine such as IL-1 (13) and sICAM-1 from mononuclear and endothelial cells (13, 20). While a physiologic function for circulating form found in human plasma is unknown, we suggest that levels of sICAM-1, sVCAM-1 and specially sELAM-1 in the serum of patients might be markers of endothelial cell activation, elevated endothelial-leukocyte binding and endothelial cells damage in autoimmune disease. On the other hand, since ELAM-1 is expressed as only activated endothelial, the interpretation of changes in levels of sELAM-1 is more indicative of endothelial or vascular damage. Although Cominacini, et. al reported an increase only on the soluble ELAM-1 concentration (21), previous reports by others have shown, elevated levels of circulating sVCAM-1 and sICAM-1, not in the sELAM-1 (22). However, the elevated concentration in one of these markers may indicate an autoimmune process or vascular endothelial damage in IDDM patients.

In multivariable analysis, like the studies of Peter Faching (22), we found no correlation between age and sex with sCAMs in diabetic and healthy subjects. A lack of correlation between sex and elevated level of sCAMs reflected a key role of cytokines but not the role of sex and hormones on the expression of adhesion molecules. Our results support the hypothesis that elevated concentration of inflammatory cytokines such as INF-\( \gamma \) and TNF-\( \alpha \), with association of hormones elevates susceptibility to autoimmune disease. Invitro studies confirm this hypothesis (23).

Invitro studies have shown that hyperglycemia influences adhesion of leukocyte to endothelial cells (15, 19, 22, 24). But in multivariable analysis, we did not find a correlation between fasting blood sugar and soluble form of CAMs. So we supposed that, other unidentified molecules were involved in expression of CAMs, although, some invitro studies by others, have shown correlation between CAMs and glucose (18, 25, 26).

In this report, existence of significant reverse linear correlations between sVCAM-1 and the insulin injection dose (Fig 2, \( P = 0.01 \)) indicate indirect effect of blood sugar on expression of adhesion molecules. In other words, insulin protects monocytes and endothelial cells from the harmful effect of blood sugar by balancing blood sugar (27). It can also be supposed that suitable injection of insulin can decrease the remaining insulin in the rested
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Fig 4. Correlation between sICAM-1 and sELAM-1 in diabetic patients type-1

\[ r = 0.392 \]

Fig 5. Correlation between sVCAM-1 and sELAM-1 in diabetic patients type-1

\[ r = 0.389 \]

cells, which is among the β-cells. Decreasing of released insulin leads to decrease GAD (Glutamic Acid Decarboxylase) expression on the β-cells. GAD is an autoantigen which is recognized by T cytotoxic cells on the β-cells in
IDDM patients (28). But there is no answer to this question as yet as to whether insulin injection is effective in the expression of adhesion molecules, or is not?

β-cells in pancreatic islets of Langerhans in patients (IDDM) is destroyed during 2 years (29). Significant reverse linear correlation in sICAM-1 with duration of disease (fig 3, P= 0.05) may indicate β-cells destruction in those who do not have any disease, symptom or those who are at the first stage of treatment. Immunohistochemical studies have shown that ICAM-1 has had an important role in recruiting immune cells, specially TCD4+ and TCD8+, into β-cell. We assume that serum changes of the marker reflect the risk of disease in susceptible people (30).

The rate of atherosclerosis is accelerated in human with diabetes. The adhesion of monocyte to vascular endothelium is a key factor in the development of atherosclerosis (16, 17). ICAM-1, VCAM-1 and ELAM-1 have been shown to bind monocytes, whereas ELAM-1 and ICAM-1 can bind monocytes, lymphocytes and neutrophils (7, 19). In our study, we observed high expression of mVCAM-1, not ICAM-1, on the monocytes in diabetic patients type-I rather than healthy subjects (P= 0.05), although a study reported a decrease in expression of ICAM-1 on the monocytes of IDDM patients and it was suggested that patients' monocyte cells localized in inflammatory sites did not penetrate the blood (31).

Therefore, based on our results, we suggest that: 1) elevated mVCAM-1 on the monocytes lead to binding to endothelial or other immune cells, which are regulated by leukocyte integrin, including members of β, family (VLA-4). 2) The active monocytes, changes integrin affinity and induce high binding to the endothelium. 3) High binding of monocytes leads signals to cells and it increases the release of inflammatory cytokines. 4) High binding of monocytes to endothelium increases accumulation of monocytes in the endothelium and produces a greater influence on the development of atherosclerosis; as other studies have reported that administration of agonist like sCAMs or anti-adhesion molecules antibody such as anti-ICAM-1 prevent localization of monocytes and T-cells to vascular wall specially islets preventing and reducing autoimmune disease severity. Treatment of diabetic NOD mice with anti-ICAM-1 and anti-LFA-1 indicate idea (32). 5) Elevated mVCAM-1, not mICAM-1, reflect a key role of IL-13 and IL-4 cytokines. We know that these cytokines only affect VCAM-1 expression in surface cells (7, 11). 6) Elevated levels of soluble and membrane forms of VCAM-1 explain releasing of this marker from activated monocyte and endothelial in IDDM disease.

Therefore, we can conclude that elevated concentration of sICAM-1, sVCAM-1, sELAM-1 and mVCAM-1, not mICAM-1, can reflect endothelial activation, enhance leukocyte-endothelial interactions and endothelial damage in autoimmune process. Increase of sELAM-1 indicates release of this molecule from activated endothelial and increasing sICAM-1, VCAM-1 (both of membrane and soluble form) reflect release of these molecules from activated endothelial and monocytes. So, we can suggest that by measuring the soluble form of sCAMs at different intervals, the effectiveness of treatment or even development of vascular complication can be known.

Also, we observed high expression of mVCAM-1 on monocytes of patients representing enhanced leukocyte-endothelial cell interactions and endothelial damage, that may have potential significance in initiating the atherosclerosis process. By using the antibodies of anti-VCAM-1 or other anti-adhesion molecules, we can probably change the inflammatory response in the diabetic patients and also prevent an increase diabetes induced vascular destructions.

References