The appearance of aggregated erythrocytes in the peripheral blood of individuals with insulin resistance

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Abstract

Background Insulin resistance is associated with low-grade inflammatory response. The probability that the acute-phase response is associated with enhanced erythrocyte adhesiveness/aggregation was not explored.

Methods The degree of erythrocyte adhesiveness/aggregation was evaluated by using a simple slide test. The insulin resistance was evaluated by insulin and glucose concentrations after a night of fasting. The inflammatory response was evaluated by variables of acute-phase response.

Results A significant correlation ($r = -0.2$, $p = 0.02$) was noted between insulin resistance expressed as the HOMA index and the degree of erythrocyte adhesiveness/aggregation. This was probably due to the concomitant acute-phase response and the presence of increased amounts of inflammation-sensitive proteins that were found to correlate significantly with the degree of erythrocyte adhesiveness/aggregation. In the multiple linear regression analysis, erythrocyte sedimentation rate and fibrinogen concentration but not HOMA index were found to correlate significantly ($p < 0.0001$ and $p = 0.0007$ respectively) with the degree of red blood cell adhesiveness/aggregation.

Conclusions Insulin resistance is associated with an enhanced degree of erythrocyte adhesiveness/aggregation and this is related to the presence of enhanced inflammation-sensitive plasma proteins that are part of the acute-phase response. These findings might have hemorheological consequences and might contribute to the pathophysiology of the insulin-resistance syndrome. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords insulin resistance; erythrocyte; adhesiveness/aggregation; acute-phase response

Introduction

Vascular complications are common in subjects with ‘metabolic syndrome’, insulin resistance and diabetes mellitus. Recent studies have documented the existence of a low-grade, subclinical and smoldering inflammatory response in these conditions [1–7]. It is assumed that the inflammatory response might have a role in the development of atherosclerosis [8,9].

In addition to the presence of inflammation in diabetic patients, enhanced erythrocyte aggregation has been reported in this disease [10–12]. The increased tendency of red blood cells to aggregate may have an...
unfavourable rheological effect on the microcirculation [13]. The question of whether non-diabetic individuals might present enhanced erythrocyte adhesiveness/aggregation in relation to the presence of insulin resistance has not been addressed. The presence or absence of increased erythrocyte adhesiveness/aggregation in the peripheral blood is not necessarily a theoretical question since it has been shown that therapeutic interventions may attenuate the aggregability of the cells [14–17], thus improving the patient’s hemorheological profile [18].

In the present study, we have evaluated the association between the degree of erythrocyte adhesiveness/aggregation and the concentration of glucose and insulin in the peripheral blood of non-diabetic individuals. The concomitant evaluation of the intensity of the acute-phase response in these individuals enabled us to determine eventual links between insulin resistance, low-grade inflammation as well as enhanced red blood cell adhesiveness/aggregation.

**Subjects and methods**

**Subjects**

This prospective study was performed between November 2001 and April 2002 at the Tel Aviv Medical Center. Enrolled were apparently healthy members of the active and retired medical staff of the Tel Aviv Municipality. All patients gave their written informed consent for participation in the study that was approved by the local ethics committee. We included individuals in various age groups and with various body mass index (BMI). We excluded anyone who had a history of a chronic inflammatory disease (arthritis, inflammatory bowel disease, etc.) as well as individuals with any infection or other inflammatory condition, including infarction, surgery or angiography, during the six months prior to their recruitment into the present study. We also excluded any individual with diabetes mellitus or those treated with steroid or non-steroidal anti-inflammatory medication, except for aspirin (at doses lower then 325 mg/day). Hypertension was defined as blood pressures of >140/90 mmHg or the use of anti-hypertensive medications, while hyperlipidemia was defined as cholesterol or triglyceride concentrations >200 mg/dl or the use of HMG-CoA reductase inhibitors or fibrates. We have presently examined a total of 91 women and 38 men with a mean ± SD age of 55 ± 14.7 years. Of the 91 women, 61 were post-menopausal, 52 individuals were smokers, 43 had hyperlipidemia, 33 had hypertension, while 4 individuals had a documented ischemic heart disease.

**Laboratory variables**

Blood count was performed by using the Coulter STKS (Beckman Coulter, Nyon, Swiss) electronic analyser. The erythrocyte sedimentation rate (ESR) was determined by the method of Westergen [19]. Fibrinogen concentration was assayed by the method of Clauss [20] and a Sysmex 6000 (Sysmex Corporation, Hyaga, Japan) analyser. High-sensitivity C-reactive protein (hs-CRP) concentrations were determined by using the Boering BN II nephelometer (DADE Boering, Marburg, Germany) analyser as previously described by Rifai et al. [21]. Fasting blood glucose was measured by routine biochemical determination. The concentration of fasting insulin was determined by the INSIK-5 (CIS, Gif sur Yvette, France) radioimmunoassay kit. To estimate peripheral insulin sensitivity, we used the homeostasis model assessment (HOMA) defined as the product of fasting plasma insulin (µU/mL) and glucose (mmol/L) divided by 22.5 [22].

**The Erythrocyte Adhesiveness/Aggregation Test (EAAT)**

The EAAT was performed by using a simple slide test [23]. Venous blood from the antecubital vein was obtained between 8 A.M. and 11 A.M. following an overnight fast. Blood was drawn into a syringe containing sodium citrate (1 volume of 3.8% sodium citrate and 3 volumes of whole blood). One drop of the citrated whole blood was trickled onto a slide inclined at an angle of 30° and was allowed to run down by gravity, leaving a fine film. The slides were left to dry in that position at room temperature. A technician who was blinded to the clinical and laboratory results of the patients scanned the slides in a systematic and reproducible way from the upper to the lower part of the slide, by using an image analysis system (INFLAMET™, Inflamet Ltd., Tel Aviv, Israel) [24]. This system consists of a Pentium Win 95 equipped with a Matrox Meteor (Matrox Ltd., Montreal, Canada) color frame grabber, a color charge-coupled device (CDC) camera and a microscope that was operated at 200× magnification, resulting in an image resolution of 0.4 µ/pixel. Nine images were taken from each slide. The fields of view were chosen systematically to sample different regions on the slide. Each image was processed separately and the outputs were then averaged to form the final slide outputs. The nine fields of view covered a total area of 0.6 mm². A variable in the name of erythrocyte percentage (EP) was chosen to represent the degree of red blood cell adhesiveness/aggregation. This is essentially a measurement (in microns) of the area that is covered by the red blood cells. In the absence of aggregation, the area is 100%, whereas with the appearance of enhanced erythrocyte adhesiveness/aggregation, this percentage is lower. Thus, the higher the aggregation degree, the lower is the area covered by the cells and vice versa. This is due to the fact that during cell aggregation, free spaces are formed between the aggregated cells. These free spaces are subtracted from the total slide area covered by the red blood cells. A typical example is represented in Figure 1.
INFLAMET variabilities

The coefficient of variation for the EAAT when one person prepared and read the different slides of a patient with inflammation was 0.14. We repeated this evaluation in five different patients with the same results. When nine different persons prepared slides from the same patient, the coefficient was 0.07. It was 0.1 when the same person read the same slide 10 times. The inter-observer variability of this test was discussed by us in a study performed in 273 individuals with various degrees of infection/inflammation [25]. In addition, we have recently reported the day-to-day variation of EAAT in a group of 30 individuals who had repeated EAAT determinations (7–13 examinations per patient). In that study, we could clearly show that the daily fluctuations in EAAT are comparable with other commonly used variables of the acute-phase response, including hs-CRP, white blood cell count (WBCC), ESR and fibrinogen concentrations [26]. Finally, slides from 50 patients with various degrees of inflammation were read by 2 independent technicians. A highly significant ($p < 0.0001$) correlation was found between the two results obtained by two technicians. We have further examined the degree of erythrocyte adhesiveness/aggregation in the peripheral blood of 20 individuals who were examined twice, 2 weeks apart, and found no significant differences between the results. In fact, the mean ± SD of EP was 88 ± 12 the first time and 84 ± 13 the second time (paired t-test not significant, data to be published elsewhere).

Statistical analysis

All parameters were summarised by means and standard deviations. Pearson correlation coefficients were calculated to study the associations between all variables. A multiple linear regression model was applied to the data to examine the possible association between insulin resistance and EP after adjustment for other parameters. The dependent variable is EP and the variables that were candidates to enter the regression equation were sex, age, BMI, waist-to-hip ratio (WHR), ESR, hs-CRP, fibrinogen, WBCC, cholesterol (LDL and HDL), triglycerides and HOMA. Two model construction methods were performed: forward selection and backward elimination. Both methods resulted in the same model. Statistical analysis was performed with the SAS (SAS, Cary, NC, USA) system for Windows, version 8.02.

Results

The mean ± SD and the range of the laboratory variables obtained in our cohort are reported in Table 1. The age- and gender-adjusted correlation between both BMI and WHR and the laboratory variables as well as the degree of erythrocyte adhesiveness/aggregation are reported in Table 2. A significant correlation exists between the degree of erythrocyte adhesiveness/aggregation and the degree of insulin resistance and that of inflammation. The strongest correlation was noted between EP and ESR, hs-CRP and fibrinogen.

Table 1. The results of the laboratory variables in all 129 participants of our study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
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<tbody>
<tr>
<td>EP (%)</td>
<td>75.9</td>
<td>16.6</td>
<td>31.7</td>
<td>99.9</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>27.3</td>
<td>19.1</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>6.7</td>
<td>7.9</td>
<td>0.2</td>
<td>46.6</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>320.9</td>
<td>67.3</td>
<td>183.5</td>
<td>526.5</td>
</tr>
<tr>
<td>WBCC (cells per cmm × 10⁶)</td>
<td>7.5</td>
<td>2.3</td>
<td>3.6</td>
<td>20.4</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.3</td>
<td>0.7</td>
<td>4.2</td>
<td>7.1</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>27.8</td>
<td>20</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>HOMA index</td>
<td>6.6</td>
<td>5.7</td>
<td>2.1</td>
<td>58.8</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>218.7</td>
<td>35</td>
<td>152</td>
<td>323</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>133.3</td>
<td>32.5</td>
<td>38</td>
<td>228</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>35.2</td>
<td>19.6</td>
<td>27</td>
<td>165</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>150.9</td>
<td>87.8</td>
<td>42</td>
<td>597</td>
</tr>
</tbody>
</table>

EP, erythrocyte percentage; ESR, erythrocyte sedimentation rate; hs-CRP, high-sensitivity C-reactive protein; WBCC, white blood cell count; HOMA, homeostasis model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
We have presently performed an age- and gender-adjusted correlation between both glucose and insulin concentrations and the results of laboratory tests for both erythrocyte adhesiveness/aggregation and inflammatory parameters (Table 3). The results show a significant correlation between the degree of insulin resistance, the intensity of the inflammatory response as reflected by hs-CRP concentration and the elevated WBCC and the erythrocyte adhesiveness/aggregation. Thus, by using a multiple linear regression, and not simple correlations as depicted in Table 2, it turned out that variables of the insulin resistance did not explain the appearance of adhesive and aggregated erythrocytes in the peripheral venous blood of these individuals.

**Discussion**

Insulin resistance is associated with an accelerated atherothrombotic process. An enhanced inflammatory response exists in individuals with the insulin-resistance syndrome [1], which may be related to the development of atherosclerosis [27]. In addition, the low-grade acute-phase response is associated with enhanced synthesis of inflammation-sensitive proteins [28], some of which can be involved in the induction and/or maintenance of increased red blood cell aggregation. In fact, it has been shown that fibrinogen, immunoglobulins, haptoglobin, ceruloplasmin, antitrypsin and even CRP might contribute to enhanced red blood cell aggregability [29–32].

Increased red blood cell aggregability has been reported in individuals with obesity as well as insulin resistance [33–38]. Enhanced cell adhesiveness and aggregation could be detrimental in terms of the patient’s hemorheological profile [39,40]. Yet, the mechanism of increased erythrocyte adhesiveness/aggregation in patients with obesity and insulin resistance has not been clarified. The present findings are therefore significant in that they show a potential link between the presence of an inflammatory response in individuals with obesity and insulin resistance. The connection between inflammation-sensitive proteins, inflammation and increased red blood cell aggregation has been discussed by us in the past [41].
we could show that adhesion macromolecules, including fibrinogen and immunoglobulins, can contribute to the appearance of enhanced in vivo red cell adhesive-ness/aggregation [42,43] and that plasmapheresis is associated with a significant reduction in the degree of cell adhesiveness/aggregation [44]. This finding is related to the reduction of multiple adhesive molecules in the patient's plasma, suggesting that the phenomenon of increased erythrocyte adhesiveness/aggregation is mainly a result of the activity of plasma proteins. The results of the multiple linear regression analysis performed in our study suggest that fibrinogen rather than variables of insulin resistance is a major determinant in the induction and the maintenance of increased red blood cell adhesiveness/aggregation. Yet, the possibility that additional inflammation-sensitive proteins have a contributory effect cannot be ruled out.

Various studies in the past have suggested that increased erythrocyte aggregation is detrimental with respect to the microcirculatory flow [45,46] and tissue oxygenation [13]. We have recently noted that the enhanced red blood cell adhesiveness/aggregation in the peripheral blood is associated with the presence of low-grade inflammation [47]. Therefore, therapeutic interventions, directed at improving this perturbed hemorheological profile, might have a beneficial effect [14–17]. On the basis of the present findings, we assume that the therapeutic intervention should be directed also at attenuating the inflammatory response. Presumably, weightloss [48,49], increasing exercise [50], smoking cessation as well as the administration of statins [51] may attenuate the intensity of the acute-phase response. This attenuation could have a favourable effect on the patient's hemorheological profile [52].

We conclude that individuals with obesity and insulin resistance present increased red blood cell adhesive-ness/aggregation in their peripheral blood, which might have deleterious effects on the individual's hemorheological profile. The association of these abnormalities with the presence of a low-grade acute-phase response suggests that intervention to reduce erythrocyte aggrega-bility might be attempted by reducing the degree of the inflammatory response.

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References


