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RESEARCH ARTICLE

Association of components of the metabolic syndrome with the appearance of aggregated red blood cells in the peripheral blood. An unfavorable hemorheological finding

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Abstract

Background Components of the metabolic syndrome are associated with low-grade inflammation. This can be accompanied by the synthesis of sticky proteins and erythrocyte aggregation.

Methods The degree of erythrocyte aggregation was evaluated by a simple slide test and image analysis along with other markers of the acute-phase response, including the white blood cell count (WBCC), erythrocyte sedimentation rate (ESR), fibrinogen and high sensitivity C-reactive protein (hs-CRP) concentrations. Patients were categorized in four groups according to the absence or presence of 1, 2 and 3 or more components of the metabolic syndrome.

Results We examined a total of 1447 individuals (576 women and 871 men) who gave their informed consent for participation. A significant cardiovascular risk factors, age and hemoglobin adjusted correlation was noted between the degree of erythrocyte aggregation and the number of components of the metabolic syndrome ($r = 0.17$, $p < 0.0005$). This correlation was better than that observed for clottable fibrinogen ($r = 0.13$ $p < 0.0005$), for ESR ($r = 0.11$ $p < 0.0005$) or WBCC ($r = 0.13$ $p < 0.0005$). A somewhat better correlation was noted for hs-CRP ($r = 0.26$ $p < 0.0005$).

Conclusions The multiplicity of components of the metabolic syndrome is associated with enhanced erythrocyte aggregation, probably related to the presence of multiple adhesive macromolecules in the peripheral blood. The enhanced aggregation might contribute to capillary slow flow, tissue deoxygenation as well as vasomotor tone changes in the presence of multiple components of this syndrome. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords erythrocyte aggregation; metabolic syndrome

Introduction

The metabolic syndrome is a significant cause for cardiovascular morbidity and mortality [1,2]. It is associated with the presence of low-grade subclinical and smoldering internal inflammation [3]. It is possible that this inflammatory response takes part in the pathogenesis of atherothrombosis [4].

In a recent work, we have evaluated the possibility that low-grade inflammation and the presence of inflammation sensitive proteins, can be associated with enhanced erythrocyte aggregation in individuals with insulin resistance [5]. A significant correlation was noted between insulin resistance expressed as HOMA index and the degree of erythrocyte aggregation in a group of 129 participants who were also evaluated for the presence of low-grade inflammation.

We focused on the interrelation between components of the metabolic syndrome, the presence of low-grade inflammation and the appearance of aggregated erythrocytes in the peripheral blood. Our working hypothesis was that inflammation is associated with the synthesis of multiple adhesive macromolecules, part of which can be involved in red cell aggregation [6–9]. The presence of aggregated erythrocytes in the microvasculature might be associated with deoxygenation [10,11] as well as reduced vasodilatatory response [12], suggesting a hitherto unexplored pathophysiological mechanism in individuals with the metabolic syndrome.

Subjects and methods

Subjects

Patients attending the Tel Aviv Sourasky Medical Center for a routine health examination between September 2002 and April 2004 were asked to participate in the Tel Aviv Medical Center Inflammation Survey (TAMCIS) [13]. A total of 3038 subjects agreed (1618 men, 1420 women) and showed a compliance rate of 91%. Systematic examination of the reasons for participation yielded no effect of sociodemographic or biomedical variables. An additional 540 subjects were later excluded from the analysis because of known inflammatory diseases (arthritis, inflammatory bowel disease, etc.), pregnancy, steroidal or nonsteroidal treatment (except for aspirin at a dose of $\leq$325 mg/dL), acute infection or invasive procedures (surgery, catheterization, etc.) during the last 6 months. In addition, we excluded 1051 more subjects owing to missing data for any one of the study parameters, any patient with diabetes mellitus, or having fasting glucose $\geq$126 mg/dL, and any patient with triglyceride concentrations $>$400 mg/dL. All participants signed a written informed consent according to the instructions of the local ethics committee.

Definition of the metabolic syndrome components

The components of the metabolic syndrome were identified in conformity with the definition used by the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III [14]).

Laboratory variables

The blood count was determined using the Coulter STKS analyzer, the erythrocyte sedimentation rate (ESR) by the method of Westergren [15], the fibrinogen concentration by the method of Clauss [16] and a Sysmex 6000 autoanalyzer, while the high sensitivity C-reactive protein (hs-CRP) was determined using the Boering BNII Nephelometer according to Rifai [17]. Total serum cholesterol was measured with the Roche/Hitachi 747 Analyzer (Roche Diagnostics, Mannheim, Germany) and the Raichem Kit (Reagents Applications, San Diego, CA, USA) and categorized as $<$200 mg/dL, 200 to 239 mg/dL and $\geq$240 mg/dL. Low-density lipoprotein was assayed on a Roche/Hitachi 747 Analyzer with the Randox Kit (Randox Laboratories, Crumlin, UK) and was used to compute high-density lipoprotein (HDL) levels, selected here as being part of the metabolic syndrome. Fasting glucose was determined with the glucose oxidase method using an autoanalyzer (Beckman Instruments, Fullerton, CA, USA).

The ERYTHROSENSE technology

The aggregation of red blood cells in the peripheral blood was performed by the ERYTHROSENSE technology [5,18,19]. In brief, blood from the antecubital vein was obtained between 8 and 11 AM following an overnight fast. Blood was drawn into a syringe containing sodium citrate (one volume of 3.8% sodium citrate and three volumes of whole blood). One drop of the citrated whole blood was trickled onto a slide inclined at an angle of 30° and 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 by using an image analysis system (INFLAMET™ Inflamet Ltd., Tel Aviv, Israel). For the analysis of the slides, we used an image analysis system (INFLAMET™) [20]. The variable that was used to describe the state of erythrocyte aggregation was the erythrocyte percentage (EP). This is the slide area covered by the erythrocytes. When there is no aggregation, 100% of the slide area is covered with erythrocytes, while during aggregation this percentage is reduced owing to the appearance of clear areas between the groups of aggregated cells. The coefficient of variations of this methodology was depicted elsewhere [21–23].

Statistical analysis

The statistical analysis was performed separately for men and women. All data was summarized and displayed as mean $\pm$ SD for the continuous variables (age, gender, the metabolic syndrome components and the inflammation markers) and as number of patients

plus the percentage in each group for categorical variables (smoking and medication). The cross tabs and descriptive procedures were used to produce frequencies of categorical variables (smoking and medication) and means ± SD of continuous variables (age, gender, the metabolic syndrome components and the inflammation markers). Participants were divided into four groups according to the number of components of the metabolic syndrome: 0, 1, 2 and 3 or more components.

For all continuous variables, a one-way ANOVA analysis was performed to compare the various parameters between the different groups. Hochberg's multiple comparison technique was used for pairwise comparison between patient's categories. Overall tests of significance across the four groups were evaluated by ANOVA and calculated using pairwise comparisons adjusted for multiple comparisons by the Hochberg method. For all categorical variables, the Chi-Square statistics was used for assessing the overall significance across the four groups. Pearson partial correlations for confounding variables were used to evaluate the association between the number of components and the inflammation markers. The hs-CRP has nonnormal distribution; thus, we used a logarithmic transformation, and all the results expressed as hs-CRP are a back-transformed geometric means and SD; for the correlation, the log (hs-CRP) was used. The level of significance used for all of the above analyses was two tailed, $p < 0.05$. The SPSS statistical package was used to perform all statistical evaluation (SSPS Inc., Chicago, IL, USA).

Results

Out of a cohort of 3038 individuals, we excluded 1591 ones for the following reasons: known inflammatory disease (arthritis, inflammatory bowel disease, etc.), pregnancy, steroidal or nonsteroidal treatment (except for aspirin at a dose of ≤325 mg/dL), acute infection or invasive procedures (surgery, catheterization, etc.) during the last 6 months, missing data, known history of diabetes mellitus or fasting glucose ≥126 mg/dL and elevated triglycerides (>400 mg/dL). Our report is therefore based on a total of 1447 individuals (576 women and 871 men). The high rate of individuals who were excluded was needed to guarantee, as much as possible, that reasons other than the components of the metabolic syndrome have an influence on the results. The number of individuals in each category, the mean ± SD of age as well as BMI, and the number of smokers, are reported in Table 1 for both women and men. Seventeen women and 28 men had four or five components and were included in the group of 40 women and 92 men who had three components. The number of individuals who were on medication with a potential anti-inflammatory activity was relatively low. For example, a total of 70 individuals was on a low dose of aspirin, 5 were taking fibrates, while 103 out of the entire cohort were on Coenzyme A reductase inhibitors (statins).

The results of the inflammatory biomarkers as well as the degree of erythrocyte aggregation expressed as the erythrocyte percentage (EP) are reported in Table 2. It can be seen that an increment exists in all of them *pari passu* with the increase in the number of the components of the metabolic syndrome.

Finally we performed an age, cardiovascular risk factors and gender adjusted correlation between the number of metabolic syndrome components and the various inflammatory markers including the degree of erythrocyte aggregation (expressed as the erythrocyte percentage). The correlation with the EP was adjusted also for both hemoglobin and hematocrit (Table 3). The expected intercorrelation between the different variables and themselves is noted. In addition, it can be seen that the correlation between the number of the various components and the erythrocyte percentage is better than

Table 1. Characteristics (*N*, Mean, SD & range) for both women and men, in relation to the number of the components of the metabolic syndrome

Women							
Number of components	0	1	2	≥3	*P*-value	Hochberg	
N	240	177	102	57			
Age (years)	43.5 ± 10.9	48.3 ± 10.1	50.8 ± 9.1	50.9 ± 6.6	<0.0005	0–1,2,3	<0.0005
BMI (kg/m^2)	23.4 ± 2.9	25.7 ± 3.9	28.6 ± 4.5	30.7 ± 4.4	<0.0005	All with all	<0.0005
Smokers (*N*, %)	100 41.5%	77 43.3%	43 41.3%	28 36.4%	N.S.		

Men							
Number of components	0	1	2	≥3	*P*-value	Hochberg	
N	262	297	192	120			
Age (years)	43.0 ± 11.0	46.4 ± 11.9	49.0 ± 10.7	50.1 ± 9.8	<0.0005	0–1 0–2,3 1–3	0.002 <0.0005 0.013
BMI (kg/m^2)	25.2 ± 2.5	26.4 ± 2.7	28.2 ± 3.5	31.1 ± 3.4	<0.0005	All with all	<0.0005
Smokers (*N*, %)	93 35.4%	134 44.2%	102 50.0%	64 43.2%	0.015		

BMI, body mass index.

Table 2. Characteristics (*N*, Mean, SD & range) of the inflammatory biomarkers as well as the degree of erythrocyte aggregation, expressed as the erythrocyte percentage (EP) and the results of the one-way analysis of variance plus the Hochberg test for the entire cohort

	Number of components						
	0	1	2	≥3		Hochberg's	
	$N = 502$	$N = 474$	$N = 294$	$N = 177$	ANOVA	significance	
WBCC (cells per mm)	6.71 ± 1.62	6.86 ± 1.65	7.19 ± 1.71	7.30 ± 1.73	<0.0005	0–2,3	0.001
						1–3	0.027
Hs-CRP (mg/L)	1.1 ± 2.7	1.5 ± 2.9	1.9 ± 2.9	2.5 ± 2.4	<0.0005	0–1,2,3	<0.0005
						1–2	0.011
						1–3	<0.0005
						2–3	0.021
ESR (mm/H)	11.0 ± 8.0	12.7 ± 9.5	13.2 ± 9.0	12.8 ± 9.4	0.002	0–1	0.021
						0–2	0.006
Fibrinogen (mg/dL)	263 ± 51	277 ± 54	287 ± 55	286 ± 52	<0.0005	0–1,2,3	<0.0005
						1–2	0.041
EP (%)	92.1 ± 9.0	91.0 ± 10.7	89.2 ± 12.0	89.6 ± 10.8	0.001	0–2	0.001
						0–3	0.038

WBCC, white blood cell count; hs-CRP, high sensitivity C-reactive protein; EP, erythrocyte percentage.

Table 3. Results of the partial correlation coefficients between the number of components of the metabolic syndrome and the inflammatory variables. The correlations were adjusted for age, gender and cardiovascular risk factors, and in addition to the erythrocyte percentage, adjustment was done to anemia as well, represented by hemoglobin and hematocrit

Entire cohort		WBCC	hs-CRP	ESR	Fibrinogen	EP
Number of components	Correlation coefficient	0.129	0.260	0.111	0.130	−0.169
	Significance	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
WBCC			0.240	−0.011	0.211	−0.102
			<0.0005	N.S.	<0.0005	<0.0005
Hs-CRP				0.351	0.528	−0.346
				<0.0005	<0.0005	<0.0005
ESR					0.497	−0.579
					<0.0005	<0.0005
Fibrinogen						−0.474
						<0.0005

WBCC, white blood cell count; hs-CRP, high sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; EP, erythrocyte percentage.

that noted for fibrinogen, ESR or the white blood cell count (WBCC).

Discussion

The interrelations between components of the metabolic syndrome and the aggregation of red blood cells has not been explored in the past. This interaction might be relevant to the morbid biology of this syndrome due to recent studies that show microcirculatory slow flow and tissue deoxygenation in models of enhanced red cell aggregation.

A clue to the potential interactions between the metabolic syndrome and erythrocyte aggregation comes from the observation that this syndrome is accompanied by a low grade, subclinical and smoldering inflammatory reaction [24–28]. Inflammation is associated with the synthesis of adhesive macromolecules that might be involved in the induction and/or maintenance of increased erythrocyte aggregation [8]. Relevant acute-phase proteins in this context are fibrinogen, ceruloplasmin, immunoglobulins, $\alpha 1$ antitrypsin, haptoglobin and orosomucoid [6–9,29,30].

Instead of looking at the concentration of each of the above-mentioned erythrocyte aggregating proteins, we have developed a concept of using the erythrocyte as a sensor for the presence of these proteins [31]. Thus, the erythrocytes, which are kept in their native milieu, surrounded by the various sticky proteins, are used to sense the effect of this stickiness [32,33]. This is the essence of the ERYTHROSENSE methodology and has been repeatedly shown (although by using another way of measuring the degree of cell adhesiveness/aggregation) to correlate significantly with the degree of inflammation [34] as well as the markers of the acute-phase response [35,36]. Moreover, we have recently shown that the erythrocyte aggregation in our slide test corresponds to significant inter-erythrocyte cohesive forces as examined in a shear-dependent cell flow property analyzer [37].

Another evidence about the interrelation between components of the metabolic syndrome and erythrocyte

aggregation comes from the notion that the aggregation has been observed in part of them. For example, enhanced erythrocyte aggregation has been described in hypertension [38,39], hypercholesterolemia [40,41], hypertriglyceridemia [42], obesity [43] as well as in insulin resistance [5]. Thus, the aggregability of the cells might indeed reflect the presence of low-grade inflammation and enhanced synthesis of adhesive proteins [44]. The significant correlation of erythrocyte aggregation with other markers of the acute-phase response is another support for this observation.

The presence of low-grade inflammation in individuals with multiple components of the metabolic syndrome is not necessarily harmless. In fact, C-reactive protein might be involved in the progression of the disease [45]; hyperfibrinogenemia with increased viscosity [46] and enhanced cytokine production with progression of atherothrombosis [47]. The presence of enhanced erythrocyte aggregation might be another mechanism that contributes to the morbid biology of the metabolic syndrome. In fact, in addition to tissue deoxygenation [10,11], it might also contribute to capillary slow flow [48–54]. Of special interest in this regard is a recent report of Baskurt *et al*. [12]. By using an *in vivo* model of increased erythrocyte aggregation in rats, they could show that enhanced red blood cell aggregation results in suppressed expression of NO-synthesizing mechanism, thereby leading to altered vasomotor tone. The authors raised the possibility that the mechanisms involved in this altered tonus most likely relate to decreased wall shear stress due to decreased blood flow and increased axial accumulation of red blood cells.

We have presently found a better correlation between the number of the metabolic syndrome components and the EP than with clottable fibrinogen. This might be because acute-phase proteins additional to fibrinogen are involved in the induction and/or maintenance of increased erythrocyte aggregation [55,56]. Further studies are performed to reveal what the major determinants are for the appearance of aggregated erythrocytes in the peripheral blood of individuals with multiple components of the metabolic syndrome.

We conclude that the multiplicity of components of the metabolic syndrome is associated with low-grade inflammation and enhanced red blood cell aggregation. On the basis of previously reported *in vivo* studies in animals, the potential pathophysiological consequences of this aggregation might involve capillary slow flow, tissue deoxygenation as well as reduced eNO synthesis and changes in vasomotor tone. These observations might have relevance for the morbid biology of the metabolic syndrome.

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