Clinical Study

Platelet Counts and Platelet Activation Markers in Obese Subjects

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Objective. In this work we studied the correlation between platelet count, platelet activation, and systemic inflammation in overweight, obese, and morbidly obese individuals.

Methods and subjects. A total of 6319 individuals participated in the study. Complete blood counts, high sensitivity C-reactive protein (hs-CRP) serum levels, and body mass index (BMI) were measured during routine checkups. Platelet activation markers were studied among 30 obese (BMI = 41 ± 8 kg/m2) and 35 nonobese (BMI = 24 ± 3 kg/m2) individuals. Platelet activation status was evaluated by flow cytometry using specific antibodies against the activated platelet membrane glycoprotein IIb/IIIa, p-selectin (CD-62 p), and binding of Annexin-V to platelet anionic phospholipids.

Results. Overweight, obese, and morbidly obese females had significantly elevated platelet counts (P < .0001) compared with normal-weight females. No significant elevation of platelet counts was observed in the male subgroups. A significant age adjusted correlation between BMI and platelet counts (P < .0001) was found among females. This correlation was attenuated (P = .001) after adjustment for hs-CRP concentrations. The flow cytometry analysis of platelets showed no significant differences in activation marker expression between nonobese and obese individuals.

Discussion. Obesity may be associated with elevated platelet counts in females with chronic inflammation. Obesity is not associated with increased platelet activation.

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1. INTRODUCTION

Obesity, a major risk factor for cardiovascular disease, is associated with an accelerated atherothrombotic process, resulting in increased morbidity and mortality [1]. Cytokines, such as Interleukin-6 (IL-6), originating from adipose tissue, have a fundamental role in the pathogenesis of atherothrombosis [2]. IL-6 acts synergistically with other interleukins, growth factors, and thrombopoietin in megakaryocytopenia; in-vivo administration of IL-6 to both monkeys and humans reportedly increased circulating platelet counts [3]. Higher platelet counts are associated with adverse clinical outcome in patients with ST-elevation myocardial infarction [4]. The association between increased platelet counts and platelet activation is unclear; in patients with carotid stenosis, increased platelet counts, and leukocyte-platelet complex formation, that is, platelet activation, was associated with the risk of stroke [5]; platelet count and platelet activation are associated in patients with chronic inflammation, such as patients with essential thrombocytosis [6], and patients with inflammatory bowel diseases [7]. Obesity is a chronic inflammation state [2], yet the association between platelet count and platelet activation has never been studied in obese subjects, to the best of our knowledge.

There is an ongoing debate on whether obesity is accompanied by platelet activation. The findings that the leptin receptor is expressed in platelets [8] and that leptin potentiates platelet aggregation by agonists [8, 9] shed light on a possible direct link between obesity and thrombotic complication. Davi et al. demonstrated platelet activation in obese...
individuals by measuring urine excretion of a thrombox-
ane B₂ (TxB₂) metabolite (11-dehydrothromboxane B₂, 11-
dehydro TxB₂) [10]. In contrast, other studies found no evi-
dence of increased platelet activation in obese individuals
[11], in obese and overweight women [12], in overweight

2.1. Patients

Participants were all part of the Tel-Aviv Medical Center
Inflammation Survey (TAMCIS). The TAMCIS is a cross-
sectional study performed on a group of apparently healthy
individuals, consisting of employees of the Tel-Aviv Sourasky
Medical Center (TASMC) and the Tel-Aviv Municipality.
The study was conducted between September 2002 and July
2006. All patients gave their informed consent to partici-
pate in the survey in writing, according to the local ethics
committee instructions. Recruitment for the study was per-
formed through announcements in the monthly salary slips
of the Tel-Aviv Medical Center personnel. Platelet count
was studied initially in 9115 subjects. Excluded were indi-
viduals with an underlying chronic inflammatory disease
[16]. Medical conditions were

2.3. Metabolic syndrome definition

Metabolic syndrome was defined as having at least three
of the following: men with high-density lipoprotein (HDL)
cholesterol ≤ 40 mg/dL, women with HDL cholesterol ≤
50 mg/dL, triglyceride ≥ 150 mg/dL. For both genders, blood
pressure ≥ 130/85 mmHg for both genders, fasting plasma
glucose (FBG) ≥ 110 mg/dL. For both genders, and waist
circumference ≥ 102 cm for men and ≥ 88 cm for women
[15]. Fasting glucose levels and lipid profiles were measured
by routine biochemical determinations. Blood samples for
plasma glucose levels and lipid profile were drawn after an
overnight fast from all individuals.

2.4. Platelet counts and inflammatory markers

Complete blood counts were performed using the Coulter
STKS (Beckman Coulter, Nyon, Switzerland) automatic cell
Analyzer. High-sensitivity C-reactive protein (hs-CRP) was
measured using the Boering BN II nephelometer (DADE
Boering, Marburg, Germany) according to Rifai et al. [17].
Blood samples for complete blood counts and systemic in-
flammation markers were drawn after an overnight fast from
all individuals.

2.5. Platelet activation markers

Platelet activation was studied as previously described [18,
19]. Briefly, blood was collected in citrate-containing sy-
ringes (1 : 10 volume of 3.8% citrate) and processed imme-
diately to prevent possible in-vitro activation of platelets.
Platelet-rich plasma was prepared immediately by standard
slow centrifugation (150× g for 12 minutes) and used for
flow cytometry analysis of platelet activation markers. A 5 μL
platelet suspension (250 × 10⁶ platelets/L) was immediately
incubated with Ca²⁺- and Mg²⁺-free phosphate-buffered
saline (PBS) and monoclonal antibodies (MoAb) in saturat-
ing concentrations, in a total volume of 50 μL. An HBSS-
HEPES buffer containing 2.5 mM CaCl₂ was used for the
incubation with Annexin-V. Following 30 minutes incuba-
tion at 4°C, the samples were diluted 1 : 10 with the same
buffer and analyzed by a FACScalibur flow cytometer (Bec-
ton Dickinson Biosciences, Calif, USA). The samples were
not lysed or fixed and were analyzed immediately. MoAbs
included phycoerythrin (PE)-labeled CD41 against resting
and activated glycoprotein IIb/IIIa (Immunotech, Marseille,
France), fluorescein-isothiocyanate (FITC)-labeled PAC-1
against the activated conformation of glycoprotein IIb/IIIa
(Becton Dickinson Biosciences, CA), FITC-labeled P-selectin
(CD62p), an α-granule membrane glycoprotein expressed
on the platelet surface during secretion (Immunotech, Mar-
seille, France), and FITC-labeled annexin-V, which is known
to react with platelet anionic phospholipids, such as phos-
phatidylserine (PS) exposed on platelets following activa-
tion (R&D Systems, Minneapolis, MN). Isotype-matched
MoAbs (Immunotech, France; DAKO, DK) were used as
negative controls, and platelets treated in-vitro with adeno-
sine diphosphate (ADP) and Ca²⁺-Ionophore A23817 (fi-
nal concentrations 20 μmol/L and 5 μmol/L, resp.) were used
as positive controls. Platelets were identified by light-scatter properties and CD41 expression and analyzed for binding of the specific Ab. All measurements were performed on list mode using logarithmic scales and 10,000 platelets were analyzed in each sample using the Cellquest Pro software (Becton Dickinson). The results are expressed as mean fluorescence intensity (MFI) units for the studied markers of activation.

2.6. Statistical analysis

For platelet count analysis, subjects were divided into four groups based on their BMI: normal weight (BMI < 25), overweight (25 < BMI < 29.9), obese (30 < BMI < 39.9), and morbidly obese (BMI ≥ 40). Data was analyzed separately for males and females due to gender differences in baseline inflammatory profiles and platelet counts [20]. Differences in prevalence of cardiovascular risk factors and cardiovascular disease between the various BMI subgroups were analyzed using the chi-square test for discrete variables, and analysis of variance (ANOVA) with the general linear model for continuous variables. Since hs-CRP had a non-normal distribution to begin with, a logarithmic transformation of hs-CRP was used for all statistical procedures. Differences between the BMI subgroups in terms of platelet counts, hemoglobin levels, and hs-CRP levels were analyzed using ANOVA. Platelet counts, hemoglobin levels and hs-CRP levels for overweight, obese, and morbidly obese subgroups were compared with those of the normal-weight subgroup using the general linear model with post hoc multiple comparisons by the method of Scheffe. The student’s t test was used to evaluate differences in platelet counts between subjects with or without the metabolic syndrome. ANOVA was used to evaluate the association between BMI and platelet counts after age- and hs-CRP-adjustments. For platelet activation analysis, 65 subjects were divided into two groups based on their BMI: nonobese (BMI < 30) and obese (BMI ≥ 30). The student’s t test and Mann-Whitney test were used to evaluate differences in the studied markers between the two groups. BMI and waist-to-hip ratio were normally distributed in both groups. The SPSS statistical package was used (SSPS Inc., Chicago, IL, USA).

3. RESULTS

3.1. Platelet counts and BMI status

Platelet counts were studied among 6319 individuals, 4352 males and 1967 females. The mean age of the cohort was 44.6 ± 10.4 years. Overall, 1234 (19.5%) subjects had hypertension, 246 (3.9) subjects had diabetes mellitus, 1923 (30.4%) had dyslipidemia, 85 (1.3%) had history of ischemic heart disease, and 9 (0.1%) had history of cerebrovascular accident. Overall, 2463 (39.0%) were normal weight, 2749 (43.5%) were overweight, 1058 (17%) were obese, and 49 (0.8%) were morbidly obese. The prevalence of hypertension, diabetes mellitus, and history of myocardial infarction significantly increased with BMI category (Table 1).

Platelet counts increased with BMI in both genders. However, only among females, the platelet counts were significantly elevated in the overweight (P = .015), obese (P < .0001), and morbidly obese (P < .0001) subgroups compared with the normal-weight subgroup after adjustment for age, diabetes mellitus, and hypertension. Using ANCOVA, platelet counts were still associated with BMI among females after adjustment for age and hs-CRP (P = .034). Platelet counts were elevated, though not statistically significant, in the overweight, obese, and morbidly obese male subgroups compared with the normal-weight subgroup. The association between obesity and inflammation was apparent by an increment in hs-CRP concentrations with BMI categories in both males and females (Table 2). Mean platelet counts were significantly higher in obese females with the metabolic syndrome compared with obese females without the metabolic syndrome (P = .032). In the overweight and morbidly obese female subgroups, the platelet counts tended to be higher in women with the metabolic syndrome, however, this tendency did not reach statistic significance (Table 3). Finally, there was a significant age-adjusted correlation between BMI and platelet counts (P < .0001) in females. This correlation was attenuated but remained significant (P = .001) after adjustment for hs-CRP concentrations.

3.2. Platelet activation and BMI status

Platelet activation was studied in 65 individuals, 49 females, and 16 males. They were divided according to their BMI levels into nonobese (BMI < 30, n = 35) and obese (BMI ≥ 30, n = 30) subjects. Their clinical characteristics and laboratory findings are summarized in Table 4. Nine of the obese subjects were on antihypertensive drugs and 3 were on low-dose aspirin therapy. In the nonobese subgroup, 7 subjects were on estrogen treatment (oral contraceptives or hormone replacement therapy), and 4 were on low-dose aspirin therapy. The obese group differed from the nonobese group in fat distribution, as evidenced by larger waist circumferences and larger waist-to-hip ratios. Significant differences between obese and nonobese individuals were also found in the metabolic markers FBG, HDL-cholesterol and serum triglyceride concentrations. Table 4 displays the platelet activation status as measured by the expression of the MoAb studied. No significant differences were found in platelet activation status between the groups. Additionally, no statistically significant correlation was found between BMI or waist-to-hip ratios and indices of platelet activation (Table 5).

4. DISCUSSION

It has been well established that obesity is associated with low-grade subclinical and smoldering inflammation [2, 21, 22]. Both BMI and body fat mass have been shown to correlate with total leukocyte counts [23]. The Atherosclerosis Risk in Community (ARIC) study demonstrated a positive correlation between leukocyte and platelet counts [24], but no significant correlation between obesity and platelet counts. Moreover, it has not yet been established whether elevated platelet counts in obese individuals are associated with platelet activation. The answer to this question is especially
Table 1: Clinical characteristics of subjects for platelet count analysis stratified by BMI.

<table>
<thead>
<tr>
<th>BMI</th>
<th>&lt;25</th>
<th>25–29.9</th>
<th>30–39.9</th>
<th>&gt;40</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2460</td>
<td>2749</td>
<td>1057</td>
<td>49</td>
<td>—</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>41.8 ± 10.6 yrs</td>
<td>45.9 ± 10.1 yrs</td>
<td>47.7 ± 9.0 yrs</td>
<td>45.6 ± 7.6 yrs</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>246 (10.0%)</td>
<td>596 (21.7%)</td>
<td>370 (35%)</td>
<td>22 (44.9%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>38 (15.8%)</td>
<td>91 (3.5%)</td>
<td>106 (10.0%)</td>
<td>11 (22.4%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>451 (18.3%)</td>
<td>911 (33.1%)</td>
<td>451 (51.1%)</td>
<td>20 (40.8%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>30 (1.2%)</td>
<td>35 (1.3%)</td>
<td>19 (1.8%)</td>
<td>1 (2.0%)</td>
<td>.53</td>
</tr>
<tr>
<td>Past CVA</td>
<td>4 (0.2%)</td>
<td>1 (0.0%)</td>
<td>3 (0.3%)</td>
<td>1 (0.02%)</td>
<td>.001</td>
</tr>
</tbody>
</table>

BMI = body mass index; MI = myocardial infarction; CVA = cerebrovascular accident.

Table 2: Age, hypertension, and diabetes mellitus adjusted estimated marginal mean (standard error of the mean) of platelets count, hemoglobin concentration, and CRP stratified by BMI.

<table>
<thead>
<tr>
<th>Body mass index</th>
<th>&lt;25</th>
<th>25–29.9</th>
<th>30–39.9</th>
<th>&gt;40</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1015</td>
<td>585</td>
<td>341</td>
<td>26</td>
<td>—</td>
</tr>
<tr>
<td>Platelet count (×10^9/L)</td>
<td>260 (4)</td>
<td>270 (4)*</td>
<td>281 (4)*</td>
<td>307 (11)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>13.2 (0.08)</td>
<td>13.2 (0.08)</td>
<td>13.2 (0.08)</td>
<td>13.2 (0.21)</td>
<td>.893</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>1.2 (1.1)</td>
<td>2.5 (1.1)*</td>
<td>5.2 (1.1)*</td>
<td>9.3 (1.2)*</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1448</td>
<td>2164</td>
<td>717</td>
<td>23</td>
<td>—</td>
</tr>
<tr>
<td>Platelet count (×10^9/L)</td>
<td>241 (3)</td>
<td>242 (2)</td>
<td>246 (3)</td>
<td>260 (11)</td>
<td>.11</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>14.8 (0.04)</td>
<td>15.0 (0.04)*</td>
<td>15.0 (0.05)*</td>
<td>14.8 (0.20)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>1.1 (1.0)</td>
<td>1.7 (1.0)*</td>
<td>2.6 (1.0)*</td>
<td>6.2 (1.2)*</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

*P < .05 compared with the BMI < 25 group.

Table 3: Platelet counts in overweight, obese, and morbidly obese females with and without the metabolic syndrome.

<table>
<thead>
<tr>
<th>BMI</th>
<th>Metabolic syndrome</th>
<th>No metabolic syndrome</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25–29.9 (n = 585)</td>
<td>270 ± 67 (n = 73)</td>
<td>260 ± 56 (n = 512)</td>
<td>.23</td>
</tr>
<tr>
<td>30–39.9 (n = 341)</td>
<td>283 ± 62 (n = 119)</td>
<td>268 ± 60 (n = 222)</td>
<td>.032</td>
</tr>
<tr>
<td>&gt;40 (n = 26)</td>
<td>314 ± 53 (n = 14)</td>
<td>292 ± 52 (n = 12)</td>
<td>.29</td>
</tr>
</tbody>
</table>

BMI = body mass index.

Table 4: Clinical data, laboratory findings, and platelet activation markers for subjects in the platelet activation analysis.

<table>
<thead>
<tr>
<th>n</th>
<th>Nonobese</th>
<th>Obese</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43 ± 12</td>
<td>41 ± 13</td>
<td>.2</td>
</tr>
<tr>
<td>Body mass index (kg/m^2)</td>
<td>24 ± 2.7</td>
<td>41 ± 7.7</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>83 ± 12</td>
<td>120 ± 14</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>96 ± 6</td>
<td>131 ± 19</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.86 ± 0.13</td>
<td>0.93 ± 0.15</td>
<td>.06</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>82 ± 8</td>
<td>105 ± 26</td>
<td>.003</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>62 ± 17</td>
<td>46 ± 12</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Triglyceride levels (mg/dl)</td>
<td>119 ± 63</td>
<td>170 ± 87</td>
<td>.01</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>232 ± 49</td>
<td>216 ± 47</td>
<td>.2</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>148 ± 47</td>
<td>136 ± 41</td>
<td>.3</td>
</tr>
<tr>
<td>Platelet count (×10^9/L)</td>
<td>259 ± 55</td>
<td>263 ± 55</td>
<td>.7</td>
</tr>
</tbody>
</table>

Platelet activation markers (MFI, range given in parentheses)

| PAC-1 binding | 8.4 ± 5.9 (1.8–24.6) | 6.5 ± 4.7 (1.7–22.7) | .09 |
| P-selectin (CD-62p) | 2.1 ± 0.8 (1.3–4.4) | 2.2 ± 0.6 (1.5–3.6) | .3 |
| Annexin-V binding | 4.4 ± 2.3 (2.1–12.2) | 4.7 ± 1.5 (2.4–8.0) | .15 |

HDL = high-density lipoprotein; LDL = low-density lipoprotein; MFI = mean fluorescence intensity.
reliable when primary prevention by antiplatelet therapy is considered for individuals at risk.

The results of the present study demonstrate an association between obesity and platelet counts in females probably due to higher body fat mass. Yudkin et al. [21] have demonstrated an association between obesity and IL-6 levels. A large proportion of IL-6 in the circulation originates from the adipose tissue that in turn may contribute to atherogenesis and thrombosis, by promoting inflammation. One of the proposed mechanisms for IL-6 contribution to atherogenesis and thrombosis is its effect on platelets, fibrinogen concentrations, and coagulation [2]. IL-6, in addition to other interleukins, plays a crucial role in the proliferation of megakaryocyte progenitors and acts synergistically with thrombopoietin and stem cell factor in stimulating megakaryocytogenesis [3, 25, 26]. Thus it is conceivable that the elevated platelet counts found in obese females in the present study are secondary to the presence of a chronic inflammation as evident by elevated hs-CRP levels. In fact, the correlation between BMI and platelet counts found in females was attenuated once adjusted for hs-CRP levels.

The ambiguity found in the literature regarding the role of platelet activation in obese individuals stems from employment of different markers to evaluate platelet activation in previous studies. Tangorra et al. [27] and Meade et al. [28] used aggregating agents to test platelet susceptibility to aggregation. Both groups showed no correlation between obesity and the susceptibility of platelets to aggregating agents. Davi et al. and Licata et al. used 11-dehydro-TxB2 excreted in urine to evaluate platelet activation. In an earlier (relatively small) study, they found no differences in 11-dehydro-TxB2 excretion between obese and nonobese individuals [11], yet later [10], the same group reported increased levels of excreted 11-dehydro-TxB2 in obese compared to nonobese women associated with android obesity. [12] and with the study published by Marquardt et al. [32] that found no correlation between inflammation markers including leukocyte count, CRP and fibrinogen levels, and platelet membrane P-selectin (CD62p) expression in patients after an ischemic stroke.

**REFERENCES**


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