Original article

A study of β-thromboglobulin and platelet factor-4 plasma levels in steady state sickle cell patients

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Summary. To evaluate the platelet function in sickle cell syndromes we measured the β-thromboglobulin (β-TG) and platelet factor 4 (PF-4) plasma values of 45 patients suffering from homozygous sickle cell anaemia (10) and sickle cell β-thalassaemia (35) in steady state. The results were compared to those of 32 normal controls. Both the β-TG and PF-4 levels were found to be significantly higher in patients than in controls but the β-TG:PF-4 ratio was significantly lower in the patients group. This finding and the absence of any statistical correlation between platelet number and β-TG or PF-4 indicate that platelets seem to be somehow activated in sickle cell syndromes, both in homozygotes and sickle cell/β-thalassaemia heterozygotes. This platelet activation seems to exist even in steady state sickle cell disease patients, regardless of the functional status of the spleen.

Key words: β-Thromboglobulin – Platelet factor-4 – Sickle cell disease

Introduction

The pathophysiology of the various types of crises in sickle cell disease is not yet fully understood. Considerable evidence indicates that coagulation mechanisms may be disturbed in vaso-occlusive sickle cell episodes but the initiating factors and the primarily involved components of the coagulation mechanism have not yet been fully elucidated [9].

Differences in platelet number, structure and function in patients with several forms of sickle cell anaemia have been long recognised but their significance remains elusive and to some extent controversial [10]. These abnormalities may however, have considerable affect on the clinical expression of the disease. On the other hand, interfering with them may considerably alter the clinical course, which may have important therapeutic implications. However, the lack of specific test(s) for a precise evaluation of platelet functional status and the difficult documentation of their disturbance during both steady state conditions and crises of the disease, can explain to some degree the existing inconsistencies in the literature on the role of anti-platelet drugs during and between painful sickling crises [3, 4, 11].

We undertook a survey of platelet function in asymptomatic adults with sickle cell disease who presented for a routine follow-up. As reliable indices of in vivo platelet activation, β-TG (β-thromboglobulin) and PF-4 (platelet factor 4) were utilised [5].

To the best of our knowledge, information of this type for sickle cell patients of European origin is very sparse. Furthermore, there has been increasing recognition of the variability of the disease both at the clinical and the haematological level. This variability seems to be due to the interaction of genetic and environmental factors [10]. Marked geographical differences have also been observed [10].

Materials and methods

We studied a group of 45 consecutive Greek patients suffering from fully documented homozygous sickle cell anaemia or sickle cell β-thalassaemia (10 and 35 patients, respectively). Twelve were males and 33 females ranging from 16 to 65 years in age. All patients were at a steady state of the disease (asymptomatic, at least 6 weeks since transfusion and also since crisis, infection or inflammatory reaction). No patients were under treatment with anti-inflammatory drugs or hormonal preparations.

In these patients, plasma β-TG and PF-4 were measured using radioimmunoassay kits obtained from Abbott (FRG) and Amersham (UK), respectively, according to standard protocols [2]. Platelet counts were also simultaneously measured in EDTA-anticoagulated blood in a Contraves Thrombocell-1000 Counter.

Thirty two Greek healthy subjects, 10 males and 22 females, aged 17–64 years, not under drug therapy of any type, served as a control group. Subjects with a condition known to be associated with increased levels of β-TG and/or PF-4 such as diabetes, hyperlipidaemia, renal failure or clinically profound coronary disease,
atherosclerosis, vein thrombosis were excluded from both patient and control groups.

For statistical evaluation, the Student's t-test and correlation coefficient were applied [7]. Criterion of significance was considered to be P-value less than 0.05.

**Results and discussion**

Table 1 describes the results from the measurements and their statistical analysis. Both the β-TG and PF-4 levels were significantly higher in patients than in controls (P < 0.001). The β-TG:PF-4 ratio was significantly lower in the patients group compared to controls (P < 0.05) as a result of the more-profound increase of plasma PF-4 than β-TG values in sickle cell patients. This ratio is considered a more reliable indicator of in vivo platelet activation than the plasma concentration levels of β-TG and PF-4 [5]. The rise of PF-4 plasma values presumably reflects its diminished clearance by the vascular endothelial cells as normally happens [6].

No correlation was found between β-TG or PF-4 and platelet number, in patients or in normal controls. Correlation coefficient values (r) were as follows: r [Pit. contr., β-TG] = 0.26; r [Pit. contr., PF-4] = 0.17; r [Pit. pat., β-TG] = 0.05; r [Pit. pat., PF-4] = 0.09 with P-values of greater than 0.10. Thus, the increased levels of β-TG and PF-4, according to our results, seem to represent a "true" platelet activation and are not simply the effect of increased platelet number as suggested by other workers [2].

It is of interest to note that between sickle cell homozygotes and sickle cell/β-thalassaemia double heterozygotes no statistical difference was found in β-TG, PF-4 levels or platelet number. The β-TG:PF-4 ratio also did not significantly differ between these two categories of patients (Table 2). However, interpretation of this finding may be difficult due to the small number of homozygotes included in this study (n = 10). Our results provide some evidence that platelets may be hyperactive even in steady state sickle cell syndromes both in homozygotes and sickle cell/β-thalassaemia double heterozygotes.

It has been suggested that platelet hyperactivity in sickle cell syndromes may be due to functional hypersplenism and that similar platelet changes are found in splenectomized subjects without haemoglobinopathy [6].

**Table 1. Levels of plasma β-TG and PF-4, number of blood platelets and β-TG:PF-4 ratio in sickle cell patients and in normal controls**

<table>
<thead>
<tr>
<th></th>
<th>β-TG (ng/ml)</th>
<th>PF-4 (ng/ml)</th>
<th>Plt. no. (x10^11)</th>
<th>β-TG:PF-4 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>72.46</td>
<td>28.06</td>
<td>424.90</td>
<td>2.85</td>
</tr>
<tr>
<td>(n = 45)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>21.46</td>
<td>24.66</td>
<td>116.41</td>
<td>1.92</td>
</tr>
<tr>
<td>Controls</td>
<td>32.35</td>
<td>9.03</td>
<td>271.54</td>
<td>4.17</td>
</tr>
<tr>
<td>(n = 32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>13.07</td>
<td>4.85</td>
<td>62.32</td>
<td>2.33</td>
</tr>
<tr>
<td>T-test</td>
<td>4.6</td>
<td>4.18</td>
<td>6.6</td>
<td>2.6</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

It is worth noting at this point that in our study similar abnormalities were noted in sickle cell/β-thalassaemia double heterozygotes, a condition constantly associated with splenomegaly.

These findings are only in partial agreement with those of other workers in patients of different racial origin and age [1, 2, 8, 11] and indicate the need for a more-detailed evaluation of several parameters of in vivo platelet function in sickle cell syndromes, during both steady state and in the various types of crises of the disease.

As the activity of platelets may affect, or it may be in some way related to, the occurrence of vasoocclusive episodes, and as their activation may be responsible for the vasoocclusive complications in these syndromes, it is apparent that a better understanding of this topic is essential before applying any rational prevention and treatment schedule. For such an approach, a control group employing splenectomized but otherwise healthy subjects (e.g. splenectomy due to accidents) should also be mandatory in evaluating platelet function and involvement of platelets in the pathogenesis of various complications of sickle cell syndromes.

**References**