RELATIONSHIP BETWEEN LIPID PLASMA LEVELS AND SOME VARIABLES OF COAGULATION

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Introduction

Diabetes mellitus is a pathology characterized by a prothrombotic status in which coexist both the chronic activation of the coagulation system and the decrease of the fibrinolitic potential. The studies evaluating the prevalence of the coronary artery disease at the diabetic patients compared with the non-diabetic ones show a 2-3 fold higher prevalence when having the same age, the onset of the clinical manifestation appears 7 to 10 years earlier and the cardiovascular death relative risk 2-3 folds higher in diabetic patients. Acute myocardial infarction has a higher incidence in diabetic patients, lacking in 80% of cases the algic symptomatology (Esposito et al., 2004).

Hyperlipoproteinemias, thrombocytes and fibrinogen play a major role in the progression of the diabetes complications. The existant data regarding the hemostatis alterations in patients with diabetes is not only insufficient, but also contradictory because of the heterogenity of the studied population due to the type of diabetes mellitus, the presence or absence of the cardiovascular complications or of other risk factors, the glycemic status, the type of treatment.

We assessed the influence of the glycemic status on the clotting profile, the lipidic parameters and the inflammatory status.

Methods and materials

Plasma lipids, fibrinogen levels and the number of thrombocytes were measured in 250 patients (135 women and 115 men) with diabetes, some of them suffering from coronary artery disease and others having no ischemic cardiac signs. We also tried to establish a correlation between the fibrinogen levels and the lipidic profile and also between the lipidic profile and the thrombocytes number.

The patients were divided in 6 groups:
1. Diabetic women with clinical or paraclinical (ECG, ecocardiography) manifestations of coronary artery disease.
2. Diabetic women without clinical or paraclinical (ECG, ecocardiography) manifestations of coronary artery disease.
3. Diabetic men with clinical or paraclinical (ECG, ecocardiography) manifestations of coronary artery disease.
4. Diabetic men without clinical or paraclinical (ECG, ecocardiography) manifestations of coronary artery disease.
5. Nondiabetic women with clinical manifestations of coronary artery disease.
The patients were admitted in the Geriatry Clinic of the University Clinical Hospital «C.I. Parhon» and the follow-up period was of 6 months (July 2009-December 2009).

### Results and discussions

The fibrinogen values varied between 289 and 699 mg/dl. 76.7% of the patients had high fibrinogen levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Confidence interval</th>
<th>Min</th>
<th>Max</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD</td>
<td>453.75</td>
<td>106.03</td>
<td>23.71</td>
<td>404.13</td>
<td>503.37</td>
<td>289</td>
<td>699</td>
</tr>
<tr>
<td>Diabetes+CAD</td>
<td>442.70</td>
<td>62.17</td>
<td>13.90</td>
<td>413.60</td>
<td>471.80</td>
<td>324</td>
<td>608</td>
</tr>
<tr>
<td>Diabetes-CAD</td>
<td>416.60</td>
<td>37.28</td>
<td>8.34</td>
<td>399.15</td>
<td>434.05</td>
<td>295</td>
<td>485</td>
</tr>
<tr>
<td>Total</td>
<td>437.68</td>
<td>74.56</td>
<td>9.63</td>
<td>418.42</td>
<td>456.94</td>
<td>289</td>
<td>699</td>
</tr>
</tbody>
</table>

The mean values were higher than the maximum admitted limit in all the studied groups with no significant differences between sexes (p>0.05).

**Table no.1:** Statistical analysis of the fibrinogen profile in the studied groups

The plasmatic fibrinogen levels promote the thrombogenesis, influence the bloodstream rheology, the sanguine viscosity and the platelet clotting (16), proving to be a strong independent cardiovascular risk factor as shown by numerous prospective epidemiologic studies (Kikkawa, 2000). Most of the studies have shown that the plasmatic fibrinogen levels are increased in type 2 diabetic patients whether they have microangiopathy or not (Asakawa et al., 2000).

The analysis of the thrombocytes activity in fasting diabetic patients showed an increase in the sensibility of the aggregation induced by ADP, epinephrine, collagen, arahidonic acid and thrombin. The most important results consist in the release of thrombocytic factors induced by low levels of the aggregant agent. This situation is present in both type 1 and 2 diabetic patients, with or without present vascular complications and in experimental diabetic models.

An important mechanism seems to be the increased thromboxane A2 thrombocytes synthesis in diabetic patients (Davi et al., 1999). The endothelial function changes and the coagulation mechanism modifications may lead to platelet activation causing the subsequent increase of the thrombocytes adesivity and aggregability. The intrinsic platelets metabolism alterations and the modifications of the intrathrombocytic signaling pathways have the same finality: the increase of the platelet reactivity in type 2 diabetic patients (Vinik et al., 2001).

The HDL-cholesterol varied between 28.9 and 71.1 mg/dl having lower values at 80% of the patients.
The mean values were lower in all studied groups (p=0.247) and there were no significant differences between genders (p>0.05). The LDL-Cholesterol varied between 75 and 210 mg/dl having abnormal high values in 81.7% of the patients.

The mean LDL-cholesterol levels were higher in all studied groups. Significant higher values were observed in the nondiabetic group with coronary artery disease (p=0.004). There were no significant differences between sexes (p>0.05). The previous studies showed that the plasmatic LDL-cholesterol level in type I diabetic patients is significantly correlated with the platelet arachidonic acid sensibility (Bădescu et al. 2001).

The triglycerides varied between 46 and 224 mg/dl, having high levels at 86.7% patients.

The mean triglycerides levels were higher in all studied groups, significant higher levels were observed in the diabetic patients with coronary artery disease (p=0.002); there were no significant differences between genders (p>0.05).
The comparative analysis of the monitorized parameters in the diabetic patients shows a longer disease period at the patients who had clinical and paraclinical manifestations of the coronary artery disease (6.30 vs 4.75 years) (p=0.015), the more frequent use of antidiabetic drugs at these patients (92.9% vs 78.6%) (p=0.029) and HbA1C values higher than 7% in 92.9% of the patients having both coronary artery disease and diabetes compared to 66.7% of the diabetic patients without coronary artery disease, (p=0.001).

Table no.V: The comparative analysis of the monitorized parameters in patients with diabetes mellitus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diabetic patients</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ CAD (n=70)</td>
<td>− CAD (n=70)</td>
</tr>
<tr>
<td>Diabetes evolution time (mean±SD)</td>
<td>6.30 ± 1.95</td>
<td>4.75 ± 1.92</td>
</tr>
<tr>
<td>Antidiabetic drugs (n%)</td>
<td>65 (92.9%)</td>
<td>55 (78.6%)</td>
</tr>
<tr>
<td>HbA1c &gt;7% (n%)</td>
<td>65 (92.9%)</td>
<td>40 (66.7%)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>442.70±62.17</td>
<td>416.00±37.28</td>
</tr>
<tr>
<td>Thrombocytes (x10^3/mmc)</td>
<td>595±45</td>
<td>609±62</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>55.78±8.28</td>
<td>58.13±10.13</td>
</tr>
<tr>
<td>LDLc (mg/dl)</td>
<td>140.95±30.75</td>
<td>141.35±33.04</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>189.65±27.78</td>
<td>161.40±29.64</td>
</tr>
</tbody>
</table>

From the lipidic parameters only the triglycerides had significant higher mean values at the diabetic patients with clinic or paraclinic manifestations of coronary artery disease(p=0.004).

The fibrinogen and lipidic plasma levels correlations.

In subjects having only ischemic cardiopathy, HDL concentrations were significantly correlated with fibrinogen in men (r = 0.1406; P < 0.05) but not in women (p > 0.05; not significant); LDL concentrations were significantly correlated with fibrinogen both in men (r=0.1981, P < 0.05) and in women (r = 0.1681, P< 0.05); TG levels were significantly correlated with fibrinogen both in men ( r = 0.1921, P < 0.05) and in women (r = 0.1621, P < 0.05).
In diabetic patients having ischemic cardiopathy, HDL levels were significantly correlated with fibrinogen both in men (\( r = 0.1045, P < 0.05 \)) and in women (\( r = 0.1742, P < 0.05 \)); LDL levels weren’t correlated with fibrinogen levels neither in men (\( r = 0.0035, \text{not significant} \)) nor in women (\( r = 0.002, \text{not significant} \)); TG levels were significantly correlated with fibrinogen in men (\( r = 0.1606, P < 0.05 \)), but not in women (\( r = 0.0118, \text{not significant} \)).

In subjects having only diabetes without ischemic cardiopathy, HDL levels were significantly correlated with fibrinogen in men (\( r = 0.2018, P < 0.05 \)), but weren’t correlated in women (\( P > 0.05, \text{not significant} \)); LDL concentrations weren’t correlated with fibrinogen neither in men (\( r = 0.0022, \text{not significant} \)), nor in women (\( r = 0.0183, \text{not significant} \)); TG concentrations were strongly correlated with fibrinogen in women (\( r = 0.4345, P < 0.05 \)), but weren’t correlated in men (\( r = 0.008, \text{not significant} \)).

Fig 4: The correlations between fibrinogen and lipid plasma levels in diabetic patients with coronary artery disease

Fig 5: The correlations between fibrinogen and lipid plasma levels in diabetic patients without coronary artery disease

The interest regarding the role of fibrinogen in the promotion of the atherosclerosis in diabetic patients increased when it was proved that the fibrinogen is an independent risk factor for the cerebral and heart vascular thrombotic pathology. It was shown that the fibrinogen has a role in increasing the platelets sensibility at aggregant agents. The
fibrinogen binds to platelet receptors and may have an agonist role for in vitro platelet aggregation (Henn et al., 2001). It was proved that in diabetic patients the agonist stimulation leads to an increase in the fibrinogen platelets binding. Therefore, the interaction fibrinogen thrombocytes is present in diabetic patients, thus leading to an increase of the thrombocytes sensitivity to agonist stimulation, that may have a role in the progression of the atherosclerotic process in diabetic patients (Cipollone F, et al. 2004).

A positive correlation was observed between the fibrinogen plasmatic level and the glycemic status (Jain A, et al., 2001), despite the fact that the intensive diabetic treatment did not reduce the fibrinogen plasmatic levels (Becker et al. 2003). This fact could be explained by the direct fibrinogen synthesis stimulation by the antidiabetic treatment, thus contradicting the idea that there is a link between the decrease of the plasmatic fibrinogen level and the increase in the glycemic control. Therefore, while the insulin therapy has no beneficial effect (Fanghanel et al., 2008), the treatment with Metformin significantly decreased the plasmatic fibrinogen levels in type 2 diabetic patients.

**The thrombocytes lipidic plasma levels correlations in the studied groups**

In subjects having only ischemic cardiopathy, HDL concentrations weren’t correlated with the platelets number neither in men (r = 0.0416; not significant) nor in women (r = 0.0348, not significant); LDL concentrations weren’t correlated with the platelet count neither in men (r=0.0341, not significant) nor in women (r = 0.0236, not significant); TG levels weren’t correlated with the platelets number neither in men (r = 0.0074, not significant) nor in women (P > 0.05, not significant).

In diabetic patients having ischemic cardiopathy, HDL levels weren’t correlated with the platelet count neither in men (r = 0.0684, not significant) nor in women (r = 0.0034, not significant); LDL levels weren’t correlated with the platelets number in men (r > 0.05, not significant) but there was a correlation in women (r = 0.3298, P < 0.05); TG levels were significantly correlated with the platelets number in men (r = 0.5798, P < 0.05) and in women (r = 0.1704, P < 0.05).
In subjects having only diabetes without ischemic cardiopathy, HDL levels weren’t correlated with the platelet count in men \( (r = 0.0163, \text{not significant}) \), but were significantly correlated in women \( (r = 0.1627, P < 0.05) \); LDL concentrations were significantly correlated with the number of platelets in men \( (r = 0.4512, P < 0.05) \), but not in women \( (r = 0.0183, \text{not significant}) \); TG concentrations were strongly correlated with the number of platelets both in men \( (r = 0.5767, P < 0.05) \), and in women \( (r = 0.5767, P < 0.05) \).

The thrombocytes function anomalies in diabetic patients have been linked to many intracellular modifications. Thus, the diabetic thrombocytes have a decreased \( \text{Na}^+ / \text{K}^+ \text{ATP-ase} \) activity and an increased \( \text{Ca}^{2+} \text{- ATP-ase} \) activity leading to an increase in the calcium intracellular concentration and to thrombocytic hiperactivity (Li et al., 2003). The intracellular \( \text{Mg}^{2+} \) concentration is decreased in the diabetic thrombocytes, thus leading to an increase in their activity. Even more the hyperglycemia is associated both with an increase in the PKC activity (Mazzanti 2007) and in the superoxid anions production and with a decrease in...
the antioxidants level (glutation) and in the NO-sintase levels (Assert et al., 2001). These modifications may induce oxidative stress and platelet function activation.

Therefore all existing data suggest that the platelets have a proinflammatory action, representing a rich chemokine source (thrombocytic factor 4, RANTES – regulated upon activation, normal T cell expressed and secreted) (Von Hundelshausen et al. 2007) (and inflammatory citokins (interleukine 1b, CD-40L) (5) which are released after the platelet activation thus suggesting new hypothesis regarding the platelet contribution in inflammation and in atherogenesis (Weyrich, et al., 2003).

Conclusions

Although the evaluation of the hemostatic factors isn’t in present a screening test, the physician doctor would much benefit from knowing which factors should be determined and at what extent their correction would improve the hemostatic disfunctions. There is a general consense saying that the diabetic patients should have lower blood pressure values and require multiple therapies for preventing the onset of chronic complications. But the most important therapeutical measure could be the regular diabetes management obtained by repeateded medical controls (Kikkawa 2000). The evaluation of the blood stream, the vascular reactivity and of some endothelial markers were linked to an unfavorable cardiovascular prognosis.

In diabetes mellitus, the hiperglycemia and the metabolic syndrome compounds provoque endothelial disfunction (Hayden et al, 2002). From the lipcidic parameters only the triglycerides had significantly higher mean values at the diabetic patients with clinic or paraclinic manifestations of coronary artery disease. Triglycerides levels were significantly correlated with the increase in fibrinogen levels and in thrombocytes counts, thus leading to a proinflammatory status present at most diabetic patients. The new discoveries regarding the endothelial disfunction mechanisms may lead to new therapeutical strategies. One of the main objectives should be the vascular function improvementas the microvascular endothelial disfunction is linked to the onset of insulin resistance, hypertension and microalbuminuria. The relationship between lipid plasma levels and clotting variables is significantly stronger in diabetic patients than in normoglycemic subjects, providing a compelling argument for accelerated progression of atherothrombosis in these patients.

References


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