UNIVERSITY OF MEDICINE AND PHARMACY
CRAIOVA
MEDICINE FACULTY

A STUDY ON THE ANOMALIES OF HEMOSTASIS ON A GROUP OF DISLIPIDOPROTEINEMIA PATIENTS

Ph.D. THESIS

ABSTRACT

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# CONTENTS

## PART I. GENERAL PART – THE STATE OF KNOWLEDGE

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td></td>
</tr>
<tr>
<td>Content</td>
<td></td>
</tr>
<tr>
<td>Abbreviations</td>
<td></td>
</tr>
<tr>
<td>1. Lipoproteins and Dislipoproteinemia</td>
<td>1</td>
</tr>
<tr>
<td>2. The Alteration of the Clotting-Thrombocyte-Fibrinolysis System in Dislipoproteinemia. Atherogenic Potential</td>
<td>23</td>
</tr>
<tr>
<td>3. Effects on Lipoproteins and Hemostasis in Diabetes Mellitus Type 2</td>
<td>44</td>
</tr>
</tbody>
</table>

## PART II. SPECIAL PART – PERSONAL CONTRIBUTIONS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Personal Contributions</td>
<td></td>
</tr>
<tr>
<td>4.1. Work Hypothesis</td>
<td>56</td>
</tr>
<tr>
<td>4.2. Aim of the Study</td>
<td>57</td>
</tr>
<tr>
<td>4.3 Aims of the research</td>
<td>58</td>
</tr>
<tr>
<td>4.4. Material and Method</td>
<td>58</td>
</tr>
<tr>
<td>5. Results</td>
<td>78</td>
</tr>
<tr>
<td>6. Discussions</td>
<td>126</td>
</tr>
<tr>
<td>7. Conclusions</td>
<td>135</td>
</tr>
<tr>
<td>8. Selective Bibliography</td>
<td>139</td>
</tr>
</tbody>
</table>
Key Words: dyslipoproteinemia, fibrinresistometry, haemostasis, thrombogenesis

INTRODUCTION

The cause-effect relationship between hyperlipoproteinemia-atherogenesis-thrombogenesis is well documented in medical knowledge at this point. Countless experimental studies on animal and human models, as well as a very large number of clinical studies, observational or prospective, indicate a strong link between hyperlipoproteinemia-clotting-fibrinolysis-thromboocyte and the pathogeny of atherosclerosis and its vascular complications. The main complications associated with atherosclerosis, such as miocardic stroke, unstable angina, ischemic stroke have a common physiopathological support, linked to thrombosis. Prothrombotic state is, in turn, linked to the lesion or dysfunction of the endothelium, inflammation, modification of the fibrinolysis, exacerbated hemostasis, as a result of abnormal coagulation factors and a modified platelet function.

While hyperlipoproteinemia is usually associated with atherosclerosis, thrombogenesis is intimately linked to atherogenesis. Hyperlipoproteinemia is associated with increased frequency of thrombotic complications due to heightened thrombotic risk, as result of continued platelet activation, increased generation of thrombin or depressed fibrinolysis.

Classic risk factors, such as hypercholesterolemia, hypertension, smoking and mass index are weak predictors of coronary and cerebral pathology in cohort studies, while thrombotic factors have proven to be strong predictors of these pathologies. It was demonstrated that fibrinogen, plasmatic viscosis, vWF, fibrin, D-dimer, tPA antigen are independent predictors of thrombotic vascular pathology.

Considering these complex connections between thrombocyte-clotting-fibrinolysis and the lipidic sector as a starting point, and especially the implication of these sectors in generating and evolving micro and macro vascular complications in patients with metabolic pathology of the hyperlipoproteinemia type, we consider opportune to approach a segment of this major area for contemporary medicine. We are referring to the highlighting of the anomalies of hemostasis on patients suffering from hyperlipoproteinemia.

This research tackles hyperlipoproteinemia by the interrelations with the hemostatic system, trying to determine the degree to which hemostasis parameters are affected in the presence of heightened concentrations of plasmatic lipids. To evaluate the alterations of hemostasis, apart from determining already established parameters, we introduced a new parameter, called the breakage resistance of the fibrin clot (BRFC), and measured by fibrin-resistometry.

PERSONAL CONTRIBUTIONS

AIM OF THE STUDY

The aim of this research is to find associations between the resistance of the fibrin net and the increase of plasmatic lipids, as well as the value this biological marker has in appreciating hemostasis alterations in this pathology.

The motivation of researching hemostasis anomalies through the fibrinresistometry method

Current evaluation of hemostasis is based on measuring the time necessary to form a fibrin clot, without taking into account the biophysical qualities of it. Theoretically and practically it is possible that, in the same interval of time – normal or pathological – the formed fibrin clot to have different properties and thus different effects on the correlative sectors, especially the endothelial one. The prognostic for future evolutions, in a multitude of situations for the tested subject, as well as the therapy necessary in some situations will have different expressions for the same values, in time, as the temporal tests. The model we propose for evaluating hemostasis does not measure the necessary time to obtain a fibrin clot but rather it evaluates the biophysical properties of it.

The breakage resistance of the fibrin clot measures biophysical qualities of the fibrin net, its adherence to the endothelium and indirectly its aggressiveness towards it. Moreover, it offers direct relations on the measure of activating the hemostatic system, in this case as a response reaction to a modified lipidic specter. Through its capacity to make these connections, the breakage resistance of the fibrin clot can highlight an increased thrombotic status, which we believe confers it the quality of a high utility biological marker for evaluating the thrombogenic potential in patients with hyperlipoproteinemia.
MATERIAL AND METHOD

This study has been a prospective type study. We investigated the anomalies of hemostasis in patients with hyperlipoproteinemia (HLP) by the method of fibrin-resistometry and by established methods of determining hemostasis parameters, at a 6 months interval between the first and second evaluation. The time interval was chosen to observe the modifications of the clot’s resistance following hypolipemiant treatment on some of the studied patients.

The present study complied with the Declaration of Helsinki and was approved by the ethics committee of the University of Medicine and Pharmacy of Craiova. Each patient gave written consent before participation.

Forming study groups. Two study groups of Caucasian patients with HLP were used, the total number being 138 test subjects, of whom 68 were women (49.27%) and 70 men (50.73%), of an average age of 57.17±2.66 and a control group of 113 healthy test subjects with no hemostasis or plasmatic lipids anomalies. In total, the number of test subjects included in the study was 251.

Grouping test subjects:

HLP GROUP Patients with hyperlipoproteinemia and without diabetes mellitus type 2, made up of 86 patients, of an average age of 57.31±13.01 years, of which 43 are men (50%) and 43 women (50%).

DM GROUP Patients with hyperlipoproteinemia and with diabetes mellitus type 2, made up of 52 patients, of an average age of 56.9 ±12.03 years, of which 27 are men (51.9%) and 25 women (48.1%)

Control GROUP Witness group made up of 113 healthy subjects, clinically and anamnestically, with no anomalies of either plasmatic lipids or hemostasis, made up of 57 women (50.44%) and 56 men (49.56%) of an average age of 55.6±13.76 years.

WORK METHODS

The study consisted of determining BRFC through the fibrin-resistometry method on patients with an altered lipidic spectrum, as well as patients with diabetes mellitus type 2. This determination, as well as the clinical and paraclinical evaluation was made when including patients in the 2 study groups (moment 0) and after a period of 6 months, a period in which some of the patients underwent hypolipemiant treatment. For a better evaluation of both the state of health of subjects in the control group and the diabetic illness or complications present in subjects with hyperlipoproteinemia a work protocol was implemented which allowed collecting clinical and paraclinical data used in the study.

Paraclinical and laboratory exploration: we determined: 1. Plasmatic lipids: total cholesterol (TC), triglycerides (TG) and the HDL cholesterol fraction, and the LDL cholesterol was calculated using the Friedewald equation. 2. Breakage resistance of the fibrin clot by fibrinresistometry. This is a method which allows measuring the necessary force to break the fibrin clot. By tractioning the fibrin net elongation and scissoring forces are formed which at some point overwhelm the resistance of the net, which breaks; 3. The number of thrombocytes, plasma fibrinogen, average platelet volume, PT, APTT, INR; 4. Hemoleucogram; 5. Glycemia; anthropometric parameters; 6. Arterial pressure.

Chapter 5. RESULTS

Fibrinresistometry for the HLP group

Results in this subchapter refer to the initial moment of research, when the first measurements were conducted. On patients with HLP, without DM, the breakage resistance of the fibrin clot, determined through fibrin-resistometry, had, at the initial moment of evaluation, an average value of 296.76 F.U. (table 5.I and figure 5.4). Although, apparently this value is within the normal limits of the method, being an average value it can be considered at the upper limit of normal values and the lower limit of pathological values. When compared with the mean value of the witness group, which was of 248.64 F.U. an increase of 19.35 % (p=0.0004) is apparent. We interpret this result as being close to the limits of hypercoagulability, and for most of the patients in this group even within the hypercoagulability interval.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients with increased plasma lipids n = 86</th>
<th>Patients with increased plasma lipids and DM n = 52</th>
<th>Control subjects n = 113</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>57.31 ±13.01 22.70</td>
<td>57.01 ±12.03 21.11</td>
<td>55.66 ±13.76 24.73</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>28.75 ±5.08 17.68</td>
<td>28.99 ±4.96 17.11</td>
<td>23.75 ±2.89 15.63</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>99.20 ±12.8 12.38</td>
<td>100.38 ±13.8 13.75</td>
<td>81.26 ±4.76 11.32</td>
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<tr>
<td>HC (cm)</td>
<td>104.72 ±10.25 9.78</td>
<td>104.38 ±9.48 9.09</td>
<td>92.54 ±5.61 9.54</td>
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<tr>
<td>WHR (cm)</td>
<td>0.94 ±0.09 9.23</td>
<td>0.95 ±0.09 9.99</td>
<td>0.82 ±0.08 8.72</td>
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<tr>
<td>BRFC (FU)</td>
<td>296.76 ±23.37 11.25</td>
<td>323.34 ±19.88 15.43</td>
<td>248.64 ±25.71 10.34</td>
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<tr>
<td>TC (mg/dl)</td>
<td>259.10 ±39.02 15.06</td>
<td>237.11 ±47.26 19.93</td>
<td>188.82 ±12.12 6.42</td>
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<tr>
<td>TG (mg/dl)</td>
<td>133.19 ±38.86 29.18</td>
<td>216.89 ±118.1 54.47</td>
<td>108.53 ±27.70 25.52</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>41.54 ±10.45 25.17</td>
<td>39.73 ±11.38 25.45</td>
<td>52.11 ±7.04 13.51</td>
</tr>
<tr>
<td>LDLc (mg/dl)</td>
<td>150.27 ±22.62 15.05</td>
<td>142.16 ±35.80 25.18</td>
<td>99.47 ±17.97 18.07</td>
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<tr>
<td>Blood glucose (mmol/L)</td>
<td>5.53 ±0.49 8.85</td>
<td>7.34 ±2.79 38.03</td>
<td>5.71 ±0.57 7.43</td>
</tr>
<tr>
<td>Plasma fibrinogen (mg/dl)</td>
<td>370.57 ±52.37 14.13</td>
<td>391.10 ±55.40 24.17</td>
<td>313.47 ±44.62 14.24</td>
</tr>
<tr>
<td>Platelet count -10 x10³/mm³</td>
<td>379.57 ±60.20 11.2</td>
<td>409.23 ±51.07 22.48</td>
<td>297 ±39.32 10.58</td>
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<tr>
<td>MPV*</td>
<td>10.58 ±1.90 18.00</td>
<td>11.45 ±2.09 18.30</td>
<td>9.87 ±0.86 17.91</td>
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<tr>
<td>APTT (sec.)</td>
<td>30.01 ±4.95 17.43</td>
<td>28.85 ±5.64 19.57</td>
<td>32.48 ±3.21 13.73</td>
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<tr>
<td>PT (%)</td>
<td>92.02 ±6.22 6.76</td>
<td>92.63 ±7.32 7.91</td>
<td>86.51 ±4.29 5.43</td>
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<tr>
<td>INR</td>
<td>1.01 ±0.09 8.85</td>
<td>0.99 ±0.09 9.95</td>
<td>1.01 ±0.08 8.34</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>19.71 ±8.70 44.13</td>
<td>22.56 ±9.86 43.71</td>
<td>12.3 ±4.25 19.53</td>
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<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.38 ±1.15 8.01</td>
<td>14.57 ±1.32 9.13</td>
<td>13.92 ±0.96 7.45</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>41.44 ±2.65 6.39</td>
<td>41.67 ±2.97 7.14</td>
<td>40.17 ±2.34 6.41</td>
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<tr>
<td>Leucocytes count (/mm³)</td>
<td>7241.7 ±1320 18.23</td>
<td>7153.5 ±1163 16.27</td>
<td>6493.5 ±1427 17.32</td>
</tr>
</tbody>
</table>

BMI-Body Mass Index; WC-Waist circumference; WHR-Waist-to-hip ratio; BRFC-Breakage Resistance of the Fibrin Clot; FU-Fibrinresistometric Units; TC-total cholesterol; TG- Triglyceride; MPV-Mean Platelet Volume; APTT- Activated Partial Thromboplastin Time; PT-Photrombine Time; INR-International Normalysed Ratio; ESR-Erythrocyte Sedimentation Rate;

Table 5.1 Mean values of evaluated parameters in subjects of the studied groups, at the beginning of the research.

![Figure 5.1. Mean of BRFC values on study groups compared to control group.](image)

We also noticed that BRFC does not vary with sex but is dependent on age. For patients >50 years the average value is 310.1±6.35 FU, versus 285.3±5.23 FU for patients <50 years.

**Fibrinresistometry for the DM group**

On patients with DM, BRFC had, at the initial moment of evaluation, an average value of 323.34 ± 19.88 FU. It is notable that it is the highest measured value for the investigated population. Compared to the witness group an increase of 30.7% is noticeable, and compared to the HLP without DM group, the increase is of 8.9 % UF. The variation coefficient shows a variation of values around the average value of ±15.43%, indicating that the other values are also close enough to this average, which is, obviously, situated in the pathological interval.
Figure 5.2 indicates the distribution of patients of this group in different value intervals, showing that 31 patients (59.61%) have pathological values of above 300 FU. It is noticeable, thus, that more than half of diabetic patients have high values, which definitely indicate an increased resistance of the fibrin clot. We assume that, at least theoretically, such values represent a significant indication towards the tendency of hypercoagulation, and thus practically thrombogenesis. It is also noticeable that values for a further 15 patients (28.85%) fall into the 280-300 FU interval, which constitutes the superior limit of the normal values. Practically, within the limit of average values of the normal are only 6 patients, meaning 11.53 % of the total of the group. By comparing with the HLP group in regards to the distribution of the value interval differences are noticeable, because in that group, the percentage of patients with above normal values is of 40.7 %.

Figure 5.3. Dynamic of BRFC Values in relation to the duration of DM

Results obtained from determining other hemostasis parameters
To find associations between plasmatic lipids and other conventional hemostasis parameters, and also to identify possible correlations between those and BRFC, determined by the fibrin-resistometry method, we considered necessary to investigate: the platelet count, mean platelet volume, plasmatic fibrinogenic concentration, prothrombin time (PT), activated partial thromboplastin time (APTT) and INR. Average values, standard deviation and variation coefficient are shown in table 5.I., where it is noticeable that all averages are within normal limits, with the mention that for the DM group concentration of fibrinogen and the number of platelets have mean values very close to the upper limits of the normal values. When you also consider the variation coefficient around the median (CV%), it becomes obvious that almost 25 % of this group have higher values than the upper limits for the 2 parameters.
Compared to the witness group, the DM group records an increase of the concentration of fibrinogen of 24.76% and a platelet count increase of 37.8% (p>0.05). Compared to the witness group, the HLP group presented an increase in fibrinogen concentration of 18.21%, sizably smaller than the DM versus witness group comparison. The graphic representation of these variations is shown in figure 5.4.

A lot more obvious is the relation between the duration of the diabetic illness, fibrinogen and the platelet count. It is noticeable from tables 5.III and figure 5.4, that the value of fibrinogen increases with the evolution of diabetes in time, the relation having a statistic assurance of p=0.0198 new cases vs DM > 10 years and p=0.0176 for DM<10 years vs DM>10 years.

Results Obtained in Anthropometric Measurements

We considered opportune to measure these parameters as well, due to the frequent references in specialty literature regarding the association of these parameters and the risk of diabetic illness, metabolic syndrome and coronary and cerebral ischemic strokes. If the mass index has a lower, even controversial, association, abdominal fat is more and more often correlated with the above mentioned pathological entities. Table 5. XXVIII shows average values of these parameters on the 3 groups.

HLP and DM groups all have significantly higher averages compared to the witness group (p<0.05) but are also consistently higher than the superior limits of the normal values for these parameters. Highest values were measured on patients of the DM group, which is to be expected, due to the already mentioned association. There are no statistically significant differences (p>0.005) between the HLP and DM groups, the 2 groups being quite similar from this point of view.

Values of fibrin-resistometry, fibrinogen and platelet count, after hypolipemiant treatment

Initial determinations of fibrin-resistometry yielded higher average values for the entire group for this parameter close to the limit that shows a marked tendency for hypercoagulability, which (theoretically) increases the risk of suffering thrombotic vascular complications. At this point it is significant to know whether the treatment influenced these values and if the risk of suffering complications has lessened. From table 5. XXXXI and figure 5.25 it is noticeable that initial average values of fibrin-resistometry for this group were of 309.55 UF and after treatment of 302.26 UF. A decrease of only 2.85% is noticeable, which is statistically insignificant (p>0.05). According to this result it is possible that the resistance of the fibrin clot is not influenced by the hypolipemiant treatment and that the hypercoagulability tendency evident at the first evaluation of the parameter is maintained in time independent of this treatment type.

Table 5.IV. BRFC before and after hypolipemiant treatment.
Fibrinogen had an initial average value of 391±28.87, which dropped significantly after treatment (p<0.05) at 375.91±21.17 mg/dl, significant even though percentually the decrease is of only 3.88%

The other tests utilized to explore hemostasis – platelet count, VTM, APTT, PT and INR had no statistically significant modifications.

Small and statistically irrelevant variations were also recorded for glycemia, erythrocyte sedimentation rate, hemoglobin, hematocrit, and white cell count. Therefore, this group, after undergoing hypolipemiant treatment, with the exception of plasmatic lipids, showed the most obvious modification of fibrinogen concentration, which had a statistically significant drop (p=0.0048).

7. CONCLUSIONS

1. The study aimed at identifying the link between breakage resistance of the fibrin clot investigated through the fibrinresistometry method and the increase in concentration of plasmatic lipids. Research was made on a number of 138 patients with hyperlipoproteinemia, of which 68 women (49.27%) and 70 men (50.73%) and a control group of 113 healthy subjects, without anomalies of either plasmatic lipids or hemostasis.

2. Work protocol included a complex clinical and paraclinical evaluation, which would allow complete diagnosis, as well as the presence of complications, especially those of an aterothrombotic nature. Clinical evaluation included the measurement of anthropometric parameters. Paraclinical evaluation was made in 2 steps, at the initiation of the research and after an interval of 5-6 months and was made up of: determining the breakage resistance of the fibrin clot by the fibrin-resistometry method, establishing the values of plasmatic lipids and glycemia, evaluating hemostasis by other tests: fibrinogen concentration, determining the number of platelets, mean platelet volume, prothrombin time, INR, APTT; determining erythrocyte sedimentation rate and a complete hemoleucogram.

3. Anthropometric parameters of patients from the 2 study groups are consistently modified compared to the witness group and indicate a high percentage (67%) of overweight patients or with an abdominal circumference over normal values. WC is higher by 23 % for patients with DM and higher by 22 % for patients from the HLP group compared to the witness group (p<0.05 in both cases).

4. By analyzing lipidic parameters on studied groups it is noticeable that most alterations of these parameters were recorded on the DM group. Most affected are triglycerides, which have an average value that’s almost double compared to the witness group (increase of 99.8%, p<0.05) and by 62.8 % higher compared to the HLP group (p<0.05). The other lipidic fractions are increased as well for both groups. A marked decrease of HDL for the DM group, of which the average value is 39.73±11.38, a value which is far below the inferior limit of the normal values. Values obtained in this group closely match the characteristics of dyslipidemia of diabetes mellitus

5. Breakage resistance of the fibrin clot at first evaluation took place on the HLP group, an average value of 296.76 FU which we consider the upper limit of normal values. It grew by 19.35 % (p=0.0004) compared to the witness group. 40.7 % of the HLP patients had pathologic values of over 300 UF. Breakage resistance values don’t correlate with sex, but they do correlate with age, patients of over 50 years having a value higher by 8.6 % (p=0.0312) when compared with those <50 years.

For patients from the DM group, the average value of resistance was of 323.34 UF, higher by 30.7 % than the witness group (p=0.015) and by 8.9 % than the HLP group. 31 patients (59.61%) have pathological values of over 300 UF. For this group, the value of resistance increases with the duration of the diabetic illness, at over 10
years being of 355.46 (p=0.0022). Since resistance is higher the longer the evolution of diabetes, we can appreciate that these values indicate an increased thrombogenic potential.

6. Other hemostatic parameters which were investigated did not present significant modifications for either of the 2 study groups, compared to the witness group, with the exception of fibrinogen, which was increased for the DM group by 24.76 %, and of the platelet count, which was increased by 37.78 %, although they remain within normal limits. The value of fibrinogen increases the longer the diabetic illness evolves in time, p=0.0198 for the subgroup >10 years compared to the subgroup of new cases. Mean platelet volume is also increased with the duration of diabetes (p=0.0006). Average values of the investigated coagulation times are normal, both in relation to the witness group and when compared to the accepted normal limits.

7. Other investigated parameters: hemoglobin, hematocrit, white cell count, were all in normal limits. Glycemia was increased for the DM group. Erythrocyte sedimentation rate had an increased average value for the DM group, possibly by relation to the vascular inflammatory processes. Values increase in relation to the duration of the diabetes (p<0.05)

8. Results obtained when reevaluating patients after 6 months. The concentration of plasmatic lipids decreased as response to the administered treatment. The breakage resistance of the clot had a decrease of only 2.85 % (p>0.05). It is possible that the fibrin clot’s resistance be un-influenced by the hypolipemiant treatment, and the hypercoagulability tendency present at the first evaluation of the parameter be maintained in time, independent to this type of treatment. Fibrinogen decreased significantly (p<0.05), although the percentage decrease was of only 3.88%. The other tests utilized for exploring hemostasis – platelet count, VTM, APTT, PT and INR did not have statistically significant modifications.

9. Correlations between investigated parameters highlighted important aspects for the value of the parameter evaluated as a biological marker with prediction value for thrombogenic events.

We noticed a strong positive association between heightened values of breakage resistance and a vascular history for investigated patients. OR has a value of 8.972, meaning a high degree of dependence between high fibrin-resistometry values betraying an increased thrombogenic potential and the presence of coronary strokes in the patients’ history. In case of association with an ischemic stroke, OR has a value of 5.883. RR has shown a risk of 4.2 X greater for miocardic stroke and one of 2.8 X greater of an ischemic stroke compared to patients with normal values. These correlations lead to the conclusion that the breakage resistance of the fibrin clot is a faithful indicator of the tendency for thrombosis installed in investigated patients, representing an efficient marker in evaluating this state.

- We also noticed correlations between the increase in breakage resistance of the fibrin clot and the increase in concentration of the lipidic fractions, especially with the increase of TG (r=0.585) and cholesterol (r=0.574), a stronger association for the DM group, where the TG-BRFC association is stronger (r=0.685). Fibrinogen associates strongly with triglycerides (r=0.507) and with the LDL fraction, r having the value of 0.458. A weaker correlation exists between fibrinogen and cholesterol and a very weak one with the HDL fraction.

- A moderate association was identified between the platelet count and glycemia, as well as between glycemia and the mean platelet volume r=0.449. These connections show the prothrombotic status of DM.

- A large abdominal circumference is strongly correlated with fibrin-resistometry (r=0.576), as well as with heightened levels of fibrinogen, for both groups.

Final Conclusion

The complex study in the present paper, referring to the lipidic profile, hemostasis parameters and especially the breakage resistance of the fibrin net, on groups of patients suffering from hyperlipoproteinemia and diabetes mellitus, is not mentioned in specialty literature.

The fibrinresistometry method, a novelty in literature, allowed the appreciation of the evolution of the breakage resistance of the fibrin clot on studied groups. The breakage resistance of the fibrin clot, increased for both groups of patients, especially those with diabetes mellitus, attracts attention on the thrombogenic potential of investigated patients, the fibrin-resistometry method being an effective marker in establishing this state.
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MAIN INTERNATIONAL AND NATIONAL SCIENTIFIC PAPERS PUBLISHED AND COMUNICATED
