CORRELATIONS BETWEEN THE SERUM ET-1 AND HEMORHEOLOGICAL ABNORMALITIES IN TRAUMATIC SHOCK

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Summary

Nowadays, the most frequent death cause of children in the developed countries is politrauma. The study of hemorheological modifications represents an aspect of the traumatic shock that interests both pathophysicists and practitioners, considering its relative practical accessibility and its value in the diagnosis, therapy control and prognostic evaluation of traumatic shock patients.

The study was performed on two groups of 30 children: a healthy subjects group (control group) and a test group (polytraumatised children with ages between 10 and 16 years, with traumatic shock, admitted in Intensive Care Unit of Constanta Clinical Hospital). The patients group was tested in its evolution, each person being submitted to 2 series of investigations: on admission in the Intensive Care Unit and after 7-days. The link between the data obtained through the determination of sanguine viscosity, serum endotheline-1 and plasmatic proteins, by one side, and the favorable or unfavorable evolution of the politraumatic children, by the other side, proves itself to be extremely useful for the monitoring of trauma children.

It is known that blood viscosity is determined by four important factors: plasma viscosity, hematocrit, aggregation and deformability of the red blood cells. At hospitalization could be observed a significant statistical increase (Pt<0,05) of blood viscosity to polytraumatised children, comparatively with the values of blood viscosity of control group. In all determinations, serum ET-1 values (in patients with traumatic shock) showed statistically significant increased levels in comparison with the serum ET-1 levels of control group.

The importance of serum ET-1 increasement levels in patients with traumatic shock consists in using it as a marker for the treatment and prognostic indicator for the evolution.

Keywords: trauma, shock, viscosity, endotheline-1.

Introduction

The study of hemorheological modifications represents an aspect of the traumatic shock that interests both pathophysicists and practitioners, considering its relative practical accessibility and its value in the diagnosis, therapy control and prognostic evaluation of traumatic shock patients. In terms of rheology, blood is a concentrate suspension of discoidals particles that are deformable in a medium composed of plasma.

Comparing to different models of suspension, blood has a better fluidity. That is because the biggest part of the fluidity is determined by red blood cells capacity of deformation. Blood is a typical non-Newtonian fluid and its viscosity depend on his flow speed.

An important thing that influences the flow of blood is the elastic deformability and this property makes blood to absorb a part of the energy that was applicated before propre begin of flow. This thing is explained by the capacity of red blood cells membrane to suffer reversible deformations because of its viscosity (Sise, 2006). The main cause of tisular transfusion is blood viscosity.
When the tissues are damaged by lack of oxygen their capacity to fix the oxygen metabolism is compromised.

The endothelial cells are on charge for synthetize some peptides with 21 amino-acids, from a group of endothelins family. ET-1 is a major isoform, that has a major part in blood vessels adjustment, by determining a prolonged cerebral vasospasm as a result of the brain injury.

ET-1 is a powerful endogenous vasoconstrictor agent, being a marker in shock. The trauma is usually associated with hypo-perfusion and phagocytosis activation. Ischemic or anoxic tissues injury compromise their capacity to regulate the oxygen metabolism (Beuth, 2001).

**Material and methods**

The study was performed, during 3 years, on a test group of 30 polytraumatised children with ages between 10 and 16 years, with traumatic shock, admitted in Intensive Care Unit of Constanta Clinical Hospital.

All polytraumatised children from the test group had severe brain injury. The patients group was tested in its evolution, each person being submitted to 2 series of investigations:

- on admission in the Intensive Care Unit;
- at 7-days after trauma.

In order to make up a more valid reference system and to compare the results obtained through the investigation of the test group, the measurement of blood viscosity was performed on a group of 30 healthy subjects (control group). Their distribution by sex and age was most similar with that of the patients group.

The determination of blood viscosity was performed using a Brookfield viscometer, type cone/plate (specific features: cone spindle Cp-40, angle 0.8° and ray 2.4 cm)

The use of this type of viscometer has permitted quick and accurately measurements of blood viscosity, using small volume blood samples, in a sample of operation technique and with a minimal risk of operator contamination.

Cone-plate viscometer is made of a plate and an rotating cone above it. The angle of the cone is very small. Cone-plate geometry offers accurately results of viscosity, rate of shearing and shearing force. The sample volume is very small (between 0.5 and 2 ml), and the tube bucket is lining for a precise thermal control.

Work technique: blood samples are taken on the anticoagulant (EDTA-K3–1ml blood/1,5 mg EDTA-K3). It is necessary to use EDTA-K3 (ethylenediaminetetraacetic acid) because the morphology of the blood cells is well kept with EDTA, than by using other anticoagulants. Brandwash slowly with succesive tilts for forestalling blood coagulation.

Samples are processed (in maximum 2 hours from the collection of the blood) with the help of viscometer as it follows:

- the viscometer (tube and spindle) is set;
- the contact between the tube and a water bath with constant temperature, set by us at 37°C is made;
- plug in the viscometer to a 220V source, than start it and wait until the viscometer finishes the autotest;
- move away the tube and pipet 500 µ of blood (after a slowly ruffle);
- remount the tube, fix speed of spindle and start the viscometer;
- the critical viscosity points are posted on the viscometer display or they are printed.

For determination of plasma viscosity, the blood needs to be centrifuged at 3000 rpm for 10 minutes and the steps have to be repeated (but this time the plasma obtained in this way has to be used).

Whole blood endothelin-1 (ET-1) and determinations were conducted in two different evolution periods: T1 – the first 6 hours after hospitalization, T2 – 7 days after injury.

For a better assessment, the ET-1 was also measured on 30 healthy children (healthy subjects group), in the same proportion as test group.
There are several ways for reckon the concentration of ET-1:

1. measuring the average optical density (OD) of each standard and sample by subtraction of average density of the blank from the average density of each standard and sample;
2. it is compared the average optical density of each standard with the concentration of ET-1 in each standard;
3. it is compared the average optical density and its written in the graphic;
4. the data can be used in the graphic programs like Assay Zap.

The standard typical bends are part of primary incubation. This bends must be not used for reckon the concentrations of ET-1, each user must drown a standard bend for each analysis. They were used the National Committee for Clinical Laboratory Standards (NCCLS).

The measure of ET-1 was performed using the DRG kit for ELISA (Enzyme Linked Immunosorbed Assay). This is a full kit for quantitative determination of endothelin-1 in biological fluids. Whole blood ET-1 determination using this method requests the dilution of the samples with a buffer solution. 1:4 is the recommended dilution for the human blood. The kit uses a polyclonal antibody for the sample ET-1. The generated color (read at 450 nm) is in direct proportion with the concentration (pg/ml) of the sample.

### Results and discussions

In order to asses the significance of the values, we used “t”-Student test on the basis of determination of the arithmetical mean and the standard deviation (SD) of the values. The parameter P(t) have been considered statistically significant for a value of 95% (α < 0.05).

The statistic analysis of blood viscosity values in test group (in two moments of posttraumatic evolution) in comparison with control group (Table 1 , figure 1).

<table>
<thead>
<tr>
<th>Rotations / minute</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>60</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>Averagge</td>
<td>3.92</td>
<td>3.55</td>
<td>3.42</td>
<td>3.27</td>
<td>3.19</td>
</tr>
<tr>
<td></td>
<td>Sd</td>
<td>0.23</td>
<td>0.14</td>
<td>0.13</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Test group - 7 days</td>
<td>Averagge</td>
<td>3.63</td>
<td>3.33</td>
<td>3.22</td>
<td>3.07</td>
<td>3.03</td>
</tr>
<tr>
<td></td>
<td>Sd</td>
<td>0.62</td>
<td>0.52</td>
<td>0.45</td>
<td>0.41</td>
<td>0.43</td>
</tr>
<tr>
<td>Pt</td>
<td></td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.007</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Fig.1 The comparative average values analysis of blood viscosity (centipoise) of control group and test group (at hospitalization)

It is known that blood viscosity is determined by four important factors: plasma viscosity, hematocrit, agregation and deformability of the red blood cells. Values of viscosity decrease along with the increase of the number of rotations/minute.

At hospitalization could be observed a significant statistical increase (Pt<0.05) of blood viscosity to polytraumatised children, comparatively with the values of blood viscosity of control group (figure 1).

It is known that blood viscosity increase in cases of great traumatised with shock state.

The severity of traumatic shock consists in the fact that at the begining a hemoconcentration appears with the increase of blood viscosity.

At 7 days post-traumatic, blood viscosity at control group shows an significantly statistical increase comparing to the values of control group.

This decrease of blood viscosity is corelated with the organism answer to intravascular liquid lose, resulting in a fast dilution. Hemodiluation, persuant to
precociously treatment (perfusions with colloidal solution precociously blood albumine solution) mean to decrease the inflammatory answer is benefical in traumatic shock (Mantskava, 2004, Maeda, 2002).

Hyperviscosity syndrome, is obligatory unspecific component of brutal stimulation of a vast receiver field, with a neuro-endocrine generalization of reacational syndrome (Horstick, 2002, Likhovetskaia, 1998 ).

Studied from the dynamic point of view (7 days after hospitalisation) the blood viscosity has a significant increase for the cases with unfavorable evolution compared to the cases with a better evolution (Table2).

The increase of plasma viscosity is associated with the increase of red blood cells aggregation, particulary in microcirculation where the flow is decelerated in sluice of sphincterian capillary device (Khoshyomn, 2004, Koscielny, 2004).

In cases with unfavorable evolution of traumatic shock, severity consists in the fact that from the begining appears a hemoconcentration with blood viscosity increase, despite the activation of fibrinolysis and decrease of fibrinogen level stasis and acidosis in microcirculation space confers blood viscoelasticity of a gel.

The results obtained by ET-1 determination for the study group have been statistically processed and comparatively analyzed taking in consideration the evolution of the children with traumatic shock.

It can be seen an increase of ET-1 level in the serum of the children with trauma shock on both determinations (Table3).This increase is coming soon after the trauma (6 hours after) and is maintained at a high level matching the scientific data (the grown of ET-1 in the first and fifth day after the polytrauma). ET-1 is growing in the plasma of the children after severe skull brain injury.

The level of serum ET-1 in patients with unfavorable evolution under treatment can be a prognostic indicator of evolution and a sign of the good working of treatment.

Seven days after injury, whole blood ET-1 values showed statistically significant increase (P<0.05) for the children with unfavorable evolution after treatment (complications for the severe brain trauma, septic complications), comparing with the patients with a favorable evolution, with an improvement of the clinical state.

Seven days after injury, serum ET-1 values showed statistically insignificant decrease in children with a favorable evolution after treatment, in comparison with serum ET-1 levels optained at admission in the hospital.

In all determinations for the patients with unfavorable evolution after treatment, serum ET-1 values showed statistically insignificant increased levels seven days after injury in comparison with the serum ET-1 levels registered at hospitalization.

Conclusions

The generalized hypoperfusion, the excessive inflammatory reaction, after a latent period following the initial injury, coagulation mechanisms which are also activated leads to tissue injury and extensive endothelial damage, microvascular thrombosis and impaired microvascular circulation, organ ischemia and failure, all of this making the shock irreversible. The metabolic changes of serum ET-1 generally could induce the evolution of the traumatic shock to irreversibility. At the children with favorable evolution, the level of serum ET-1 is going slowly down after 7 days.

As a consequence of the modifications regarding the erythrocyte aggregability and deformability it has to be noticed a sanguine hyperviscosity in test group children, involving changes of tisular oxygenating capacity and of local, microcirculatory metabolic processes. These observations are also valid for the
children with unfavorable evolution, at 7 days after trauma. It is possible to detect this parameter in the acute phase of the syndrome as well as during further evolution, when a good correlation of its dynamics with the case evolution was noticed.

The importance of serum ET-1 increasement levels in patients with traumatic shock consists in using it as a marker for the treatment and prognostic indicator for the evolution. In the future can be used, like a possible therapeutic way, by using the inhibitors of the ET(A) and ET(B) receptors or endothelium-convertase enzyme inhibitors as control methods for decreasing of the persistent vasoconstrictor effect, which leads to tissue hypoperfusion.

Table 2  The analysis of statistics signications between the determinations of blood viscosity at test group (at 7 days) - favorable/unfavorable cases

<table>
<thead>
<tr>
<th>Rotations / minute</th>
<th>Favorable evolution</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>3.35</td>
<td>3.10</td>
<td>3.02</td>
<td>2.90</td>
<td>2.89</td>
</tr>
<tr>
<td></td>
<td>Sd</td>
<td>1.55</td>
<td>3.80</td>
<td>6.04</td>
<td>10.54</td>
<td>12.78</td>
</tr>
<tr>
<td>Favorable evolution</td>
<td>Average</td>
<td>4.12</td>
<td>3.74</td>
<td>3.56</td>
<td>3.36</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td>Sd</td>
<td>0.55</td>
<td>0.50</td>
<td>0.45</td>
<td>0.42</td>
<td>0.39</td>
</tr>
<tr>
<td>Pt</td>
<td>0.0005</td>
<td>0.001</td>
<td>0.002</td>
<td>0.003</td>
<td>0.008</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Table 3 Statistic analysis of ET-1 in test group (hospitalization and 7 days) comparison with healthy subjects group

<table>
<thead>
<tr>
<th>Healthy subjects group</th>
<th>Test group hospitalization</th>
<th>Test group 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>0.017</td>
<td>0.091</td>
</tr>
<tr>
<td>SD</td>
<td>0.016</td>
<td>0.087</td>
</tr>
<tr>
<td>Pt</td>
<td>3.48\times10^{-5}</td>
<td>5.08\times10^{-5}</td>
</tr>
</tbody>
</table>

References


Maeda N., Suzuki Y., Influences of rheological properties of erythrocytes on flow behavior and oxygen release in narrow tube, Acta Physiologica Hungarica, 89(1-3), 73, 2002
