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The Rheological Properties of Blood in Children with Inflammatory Bowel Disease (IBD)

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Abstract

The study included 37 patients aged from 6 to 17 with inflammatory bowel disease (IBD) - 17 with Crohn's Disease (CD) and 20 with Ulcerative Colitis (UC). We investigated: erythrocyte deformability, spontaneous (shear-induced) platelet aggregation, dynamic blood viscosity, morphology of erythrocyte aggregates, the state of erythrocyte cytoskeleton by the method of thermo induction, the condition of hemostasis - using thromboelastograph analyzer TEG 5000 (USA). The results indicate that IBD is accompanied by a significant degradation of hemorheological properties of blood. It is an important factor in the pathogenesis of these diseases and lies at the core of microcirculation disorders in IBD. It was found that in CD the disorders of the majority of studied parameters is not only more significant than in UC, but also much more stable - they remained unchanged even after the treatment. Disorders of the rheological properties of blood are an important factor causing ischemic damage of the intestine. This gives grounds for recommending the use of adjuvant methods to reduce hypoxia and microcirculatory disturbances in IBD.

Keywords: Inflammatory bowel disease; Hemorheology; Blood

Introduction

Up until now there is no single opinion on the causes and mechanisms of the development and progression of Ulcerative Colitis (UC) and Crohn's Disease (CD). Review of research data confirms that the role of microcirculation disorders in pathogenesis of IBD remains the least studied [1]. Therefore, the research of erythrocyte membranes condition in IBD is essential not only for understanding their pathogenesis including development of anaemia but also for assessment of hemorheology as the main part of microcirculation. The study of this problem is also important for assessment of efficacy of therapy during IBD. The goal of the present research was to study the role of disorders of rheological properties of blood in pathogenesis of inflammatory bowel disease in children.

Materials and Methods

The study was approved by the Local Human Subjects Research Ethics Committee. Blood was drawn after the hospitalization of patients and at the end of the treatment before their discharge. The informed consent was obtained from parents of all the children (or from children older than 15 years) for participation in the study. The study included 37 patients of both sexes aged from 6 to 17 with IBD (17 with CD and 20 with UC). The diagnosis was based on a complex examination including clinical and laboratory data, as well as endoscopic examination of the intestinal mucosa with a morphological analysis of biopsies. At the time of the hospitalization, the acute UC was diagnosed in 30% of patients with minimal activity of disease by PUCAI (Pediatric Ulcerative Colitis Activity Index) from 10 to 30 points, 50 percent of patients with medium activity of disease by PUCAI from 35 to 64 points and 20 percent of patients with high activity of disease by PUCAI above 65 points. The acute CD was diagnosed in 71% of patients with medium degree of disease severity by CDAI (Crohn's Disease Activity Index) from 11 to 30 points and 29 percent of patients with severe degree of CD by CDAI from 30 to 100 points. Treatment was conducted with the use of derivatives of 5-aminosalicylic acid, glucocorticosteroids (budenofalk, prednisolone), immunosuppressive drugs (azathioprine, ciclosporin), and genes engineering biological therapy (Infliximab and adalimumab). The research results were compared with the corresponding indexes of 18 conditionally healthy children of both sexes and the same age as a control group. Venous blood was drawn from the antecubital vein following an overnight fast into vacuum tubes containing 3.8 percent sodium citrate (in a ratio 9:1) and K_3 EDTA (for studying of dynamic blood viscosity and

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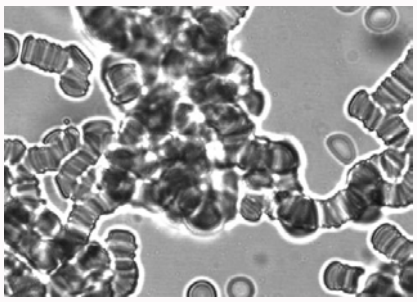

Figure 1A: Aggregation of erythrocytes in CD (hospitalization).

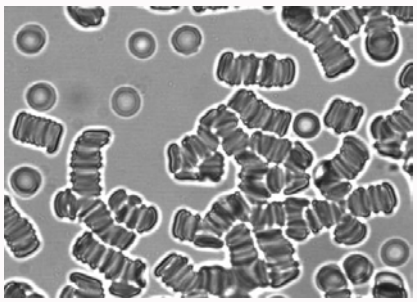

Figure 1B: Aggregation of erythrocytes in CD (discharge).

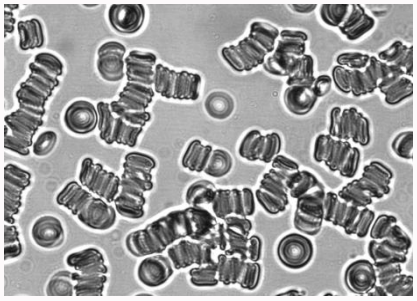

Figure 1C: Aggregation of erythrocytes of a healthy donor.

thrombo elastogram registration). Platelet-rich plasma was prepared by centrifugation of blood stabilized by 3.8 percent sodium citrate (in a ratio 9:1) at 800 g for 7 min. After its separation, the rest of the blood was centrifuged at 3000 g for 20 min and platelet-free plasma and red blood cells were separated. RBCs were washed three times with a physiological solution. In all the patients the following parameters were studied: erythrocyte deformability is using rigido meter [2]. This method is based on the following: erythrocyte suspension is placed between two coaxial cylinders, then the laminar flow is applied in which erythrocytes are deformed (stretched) and fixed in this condition by 0.5% solution of glutaric aldegyde. The number of deformed (stretched) and non-deformed erythrocytes was counted using light microscopy. Spontaneous (induced by shear) platelet aggregation in artificial shear flow using the device of our own design [3]. The device is based on the combination of technique developed by Born [4] and Schmid-Schönbein rheoscope [5]. The device allows studying the spontaneous platelet aggregation in artificial shear flow without the use of exogenous inductors that is in the conditions approximated to the conditions of a human organism. The platelet-rich plasma (number of platelets was preliminary standardized up to the concentration of 200-250×10^9/l) was placed in the device camera in which the flow with shear rate 40 s^{-1} was applied. The video recording of the process of aggregation was made during 400 sec as well as its discretely micro photo shooting with an interval of 20 sec after the beginning of the aggregation process. The micro pictures were processed using a specially developed computer program which calculates the meaning of aggregation amplitudes every 20 sec. and the distribution of aggregates according to the size. The indexes of aggregation degree (maximal amplitude Ma, standard units) and aggregation rate (amplitude at 180s after the beginning of the aggregation process A_{180}, standard units). The dynamic blood viscosity at shear rates 1 s^{-1}, 5 s^{-1}, 7 s^{-1}, 20 s^{-1}, 50 s^{-1}, 100 s^{-1} was determined using viscometer Brookfield DV-II+Pro (USA). The morphology of aggregates in autologous plasma was studied using light microscope Primo Star (Carl Zeiss, Germany) by method developed by the authors [6]. The state of erythrocyte cytoskeleton was analyzed using thermoinduction. The method is based on the fact that at 49°C to 49.5°C the denaturation of spectrin (the main protein of the cytoskeleton) occurs. As a result disc-spherical transformation of erythrocytes takes place discocytes are transformed into spherocytes. The status of the erythrocyte cytoskeleton was determined by the number of spherical forms of cells. The state of hemo stasis was investigated using thromboelastograph TEG 5000 (USA). The results of the study were processed using methods of non-parametric statistics using Mann-Whitney criteria and Wilcoxon matched pairs test. Differences were considered statistically significant at $p<0.05$. Data are expressed as mean ± standard error (SE).

Results and Discussion

The significant change of the character of erythrocyte aggregation in IBD takes place: the clump (pathological) aggregate structures appear along with rouleaux (Figure 1). The character of erythrocyte aggregation changes less in children with UC. Similar difference in the morphology of aggregates at CD and UC takes place after the treatment.

Significant changes of stability of erythrocyte cytoskeleton were observed in all patients. It declared itself in significant increase in the number of spherocytes after thermoinduction in comparison with control group ($p<0.01$). These changes of cytoskeleton proved to be stable since they did not change in the process of treatment both in patients with CD and UC. The significant change of the character of platelet aggregation in IBD also takes place. The spontaneous (induced by shear) platelet aggregation increases in UC ($p<0.05$) and normalizes during treatment. In CD the degree and rate of platelet aggregation increase insignificantly. At the same time, large aggregates are formed in CD, while in the UC small aggregates are formed (Figure 2). This difference is poorly detected by agregometer, but is clearly recorded by microscope. The change of rheological properties of blood and aggregation properties of platelets may be one of the causes of the development of thrombophilia in the children with IBD. The index R of thrombo elastogram reflecting the beginning of blood clotting significantly shortens in IBD ($p<0.01$). However, in UC this index normalizes after treatment, while in CD it remains shortened. Deformability of erythrocytes allowing cells at size 8 micron to pass through capillaries with diameter less than 3 μm is an essential hemorheological index**.** Our findings demonstrate that the IBD is characterized by the significant decrease in erythrocyte deformability and, first of all, the number of the most deformated cells decreases. During treatment, deformability of erythrocytes significantly improves. However, the number of cells most deformated in the artificial shear flow during treatment in UC increase to a greater extent (in mean from 46.1 ± 5.12% to 75.2 ± 8.91%) than in CD (in

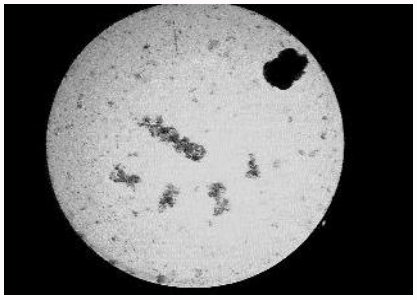

Figure 2A: A patient with CD.

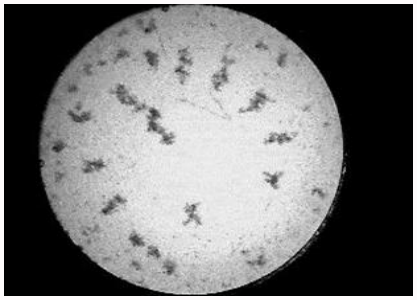

Figure 2B: A patient with UC.

mean from 48.3 ± 6.41% to 56.3 ± 7.25%). It is known that the blood viscosity determines blood fluidity. Blood viscosity depends on three components: erythrocyte concentration (hematocrit), erythrocyte aggregation (at low shear rates) and deformability of erythrocytes (at high shear rates). The results of our research indicate that the decrease in hematocrit occurred almost in all patient with IBD - in mean up to 36.9 ± 4.94 percent. At the same time blood viscosity was higher than normal only at a very low shear rate ($1s^{-1}$) ($p<0.05$). At the rest of shear rates blood viscosity in mean was lower than the norm. It may be inferred that the decrease in blood viscosity in comparison with the norm is associated with decrease in hematocrit that is anemia. The data on dynamics of blood viscosity during treatment gives sufficiently clear evidence to that. The hematocrit before and after treatment is not changed (in mean 36.9% before treatment, and 36.1% after treatment). At the same time, the decrease in blood viscosity after treatment occurs at all shear rates. This dynamics was not dependent on erythrocyte concentration and was dependent only on their aggregation and deformability. At the calculation of blood viscosity to standard hematocrit (40%) the increase in blood viscosity at IBD and its decrease during treatment were observed.

Conclusion

The obtained data suggest that IBD is accompanied by a significant degradation of hemorheological properties of blood. It is an important factor in the pathogenesis of these diseases and lies at the core of microcirculation disorders. Interestingly, the results of the study of the dynamics of hemorheological indexes in the course of treatment demonstrate that during CD disorders of most of the studied indexes are not only more significant than during UC but also much more stable - they remain unchanged even after treatment. It may be inferred that the persistence of disorders of hemorheology in patients with CD after treatment is a predictor of the development of relapses of the disease. Disorders of the rheological properties of blood are an important factor of pathogenesis in IBD leading to ischemic damage of the intestine. This gives grounds to recommend the use of additional (adjuvant) methods of combating hypoxia and microcirculatory disorders during IBD. Further study to check the dynamics of IBD after these methods of treatment will be an important future area of investigation.

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