



Mean Red Cell and Platelet Volume and Blood Cells Aggregation in Children with Inflammatory Bowel Diseases

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Abstract

Background: It has been discussed in literature whether platelet and erythrocyte indexes can serve as biomarkers of activity of Inflammatory Bowel Diseases (IBD). However, how these indexes influence functional properties of platelets and erythrocytes, primarily their aggregation capacity in children with IBD, remains unclear.

Objectives: The objective of the present research was to study the association between the changes in platelet and erythrocyte indexes (MPV, PDW, MCV, and RDW) blood cells aggregation during the course of therapy of children with IBD.

Methods: The study included 50 patients of both sex ages 6 to 17, 25 patients with UC, and 25 patients with CD. The diagnosis was based on a complex examination including endoscopic examination of the intestinal mucosa with a morphological analysis of biopsies. Spontaneous (shear-induced) aggregation of platelets and erythrocytes was studied using a rheoscope designed according to the method.

Results: It was shown that the mean platelet volume and the Platelet Distribution Width (PDW) significantly decrease at IBD, whilst Erythrocyte Distribution Width (RDW) and mean erythrocyte volume increase. A strong correlation between RDW and IBD severity as well as a negative correlation between MPV and IBD severity were revealed. For the first time it has been established that, with a reduced volume, platelets and erythrocytes retain their functional properties, in particular their aggregation activity.

Conclusion: Important reason for microcirculation disorder during IBD is the increase in platelet and erythrocyte aggregation, accompanied by the decrease in their volume and increase in erythrocyte anisocytosis.

Keywords: Erythrocyte and platelet indices; Erythrocyte and platelet aggregation; IBD

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Introduction

Several studies suggest that platelets may play a crucial role in Inflammatory Bowel Disease (IBD), which is sometimes associated with thromboembolic, as well as hemorrhagic complications [1]. In addition to their primary haemostatic function, platelets are involved in the pathogenesis of chronic inflammations. Platelets initiate and support inflammatory processes by secretion of numerous biologically active substances like platelet activation factor, IL-1, platelets factor 4 [2]. Platelet function correlates with their size MPV [3]. The MPV has been investigated as a potential biomarker in IBD. The results of the research remain contradictory. Even though a decrease in MPV has been documented in patients with IBD as compared to healthy controls, the test was not able to distinguish active from inactive Crohn's Disease (CD) patients [4]. In patients with Ulcerative Colitis (UC), a similar decrease in MPV was noted, as compared with healthy controls. However, the difference in MPV dynamics was more prominent in patients with IBD (both active and inactive) compared to CD patients [5]. Whether MPV can be used as a marker in inflammatory bowel diseases is open to question. Many aspects of this problem still need to be determined. It remains unclear what the reason for the change of MPV is and what impact it has on the functional properties of platelets, whether this change reflects response to therapy and whether MPV shows a discriminative value in differentiating between UC and CD patients. In some cases of colitis, definitive differentiation between UC and CD cannot be made, which leads to the diagnosis of indeterminate IBD. That is why further tests, that may be helpful in the diagnosis and differentiation of IBD, are

so important. In this regard not only platelet distribution width may serve as an additional biomarker to assess two main phenotypes of inflammatory bowel diseases (UC and CD), but Red Cell Distribution Width as well (RDW) [6]. In recent years, numerous studies have shown that a higher RDW is associated with several pathologic conditions, including heart failure, atherosclerosis, pulmonary impairment, venous thromboembolism [7]. Some researchers suggested that RDW could be an additional inflammatory marker in inflammatory bowel disease [8]. It was shown that RDW was higher in patients with Crohn's disease compared with ulcerative colitis and significantly correlated with Erythrocyte Sedimentation Rate (ESR), endoscopic activity index (in ulcerative colitis) [9]. However, previous studies demonstrated controversial data on the role of RDW in differentiating between CD and UC and assessing disease activity and treatment efficiency [6,9]. The cell size to a considerable degree determines their functional capacity. Small platelets have lower functional capabilities [10]. This may in part explain the bleeding frequently seen in IBD patients with active disease. Thus, the primary objective of this research was to study spontaneous erythrocyte and platelet aggregation and to assess the correlation between the degree of aggregation and the blood cells size (MPV, RDW). It should be added that prior studies discussing the association of blood cells size with IBD activity and differentiating between UC and CD were performed on adult patients. To our knowledge this association has never been analyzed in children.

Patients and Methods

All patients and healthy donors agreed to participate in the study and signed informed consent forms of Federal State Budgetary Educational Institution of Higher Education "Privolzhsky Research Medical University" of the Ministry of Health of the Russian Federation. The permission of the local ethical committee was obtained for the study. The informed consent was obtained from parents of all children (or from children older than 15 years) for participation in the study. The study included 50 patients of both sexes ages 6 to 17 with IBD (25 with CD and 25 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 a PUCAI (Pediatric Ulcerative Colitis Activity Index) from 10 to 30 points, in 50 percent of patients with medium activity of disease by a PUCAI from 35 to 64 points and in 20% of patients with high activity of disease by a PUCAI above 65 points. The acute CD was diagnosed in 71% of patients with medium degree of disease severity by a PCDAI (Pediatric Crohn's Disease Activity Index) from 11 to 30 points and in 29 percent of patients with severe degree of CD by a PCDAI from 30 to 100 points. Treatment was given with the use of derivatives of 5-aminosalicylic acid, glucocorticosteroids (budenofalk, prednisolone), immunosuppressive drugs (azathioprine, ciclosporin), and antibodies to TNF (infliximab and adalimumab). The results of studies were compared with corresponding indexes of 30 conditionally healthy children of both sexes and same age as the control group. Venous blood was drawn from the antecubital vein following an overnight fast into vacuum tubes containing 3.8% sodium citrate (in a ratio 9:1) and K_3 EDTA for total blood analysis. To obtain serum, blood was collected into vacuum tubes not containing anticoagulant. Blood was drawn after the hospitalization of patients and at the end of the treatment before their discharge. MPV, PDW, MCV, RDW were measured in all the patients (Hematology analyzer

ABX Pentra 60, HORIBA Medical, France). C -Reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR) were determined using automatic devices according to conventional methods. Platelet-rich plasma was separated by centrifugation of citrate blood at 800 g for 7 min. After her separation the rest blood was centrifuged 3000 g for 20 min and platelet-free plasma and red blood cells were separated. Spontaneous (shear-induced) aggregation of erythrocytes was studied using a rheoscope designed according to the method in our modification [11,12]. In this device the blood cells are placed between plane parallel plates rotating in opposite directions. In the centre of the bottom plate there is a cylindrical excavation (0.9 deep to study spontaneous platelet aggregation and 90 μ m to study erythrocyte aggregation). At the contact between each other, the upper and the lower plates create a chamber where erythrocyte or platelet suspension was placed. The process of aggregation was recorded during hydrodynamic mixing and upon its stop. Erythrocyte aggregation was assessed using the following parameters: degree of aggregation according to the maximum amplitude of aggregatogram (mm) – Ma; amplitude of aggregatogram at 40 sec after the start of the aggregation process (mm) – A40; $t_{1/2}$ – time when the half of Ma (s) was reached. The spontaneous platelet aggregation was studied in the artificial shear flow with video recording of the process of aggregation. The platelet-rich plasma (number of platelets was standardized until the concentration $200-250 \times 10^9/l$ before study) was placed in the device camera in which flow with shear rate $40 s^{-1}$ was applied. The video recording of the process of aggregation during 400 s was made and its discretely micro photo shooting with an interval of 20 s after the beginning of the aggregation process was taken place. The micro pictures were treated on a computer with a specially developed program calculating aggregates size distribution, time of the beginning of aggregation (t_{BA} , s), and time of the maximum aggregates appearance (t_{max} , s). The results of the study were processed with methods of non-parametric statistics using Mann-Whitney test and Wilcoxon matched pairs test. The correlation analysis (Spearman's method) was used to study the relationships between the parameters. Differences were considered statistically significant at $p < 0.05$.

Results

The present study revealed that MPV and PDW levels are significantly lower in patients with IBD (Table 1). This decrease is more significant in patients with UC compared to CD. In the process of treatment platelet indexes (MPV and PDW) increase and reach the norm in patients with UC. However, in patients with CD these indexes remain low even upon discharge. Red cell Distribution Width (RDW) also changes during IBD. In contrast to PDW, RDW levels increase in patients with IBD to a greater extent during CD (Table 2). In the process of treatment RDW levels remain elevated in both CD and UC patients. We performed correlation analysis of MPV, PDW, and RDW, disease severity determined by PCDAI (CD) and PUCAI (UC), and inflammatory markers (ESR and CRP). In CD patients RDW correlated with disease severity ($r=0.71$, $p < 0.05$) and CRP ($r=0.40$, $p < 0.05$), while in UC patients with ESR ($r=0.38$, $p < 0.05$). We investigated the correlation between changes of MPV and MCV and their aggregation capacity during IBD. It was revealed that spontaneous platelet aggregation rate increased significantly in both CD and UC. The number of their aggregates increased, as well as the speed of their formation. In the course of treatment, spontaneous platelet aggregation decreased and approached the norm. Platelet aggregation decreased to a lesser extent in patients with CD and decrease of its rate was statistically insignificant. MPV negatively

Table 1: Red blood cell and platelet indices in IBD.

Parameters	Control (n =37)	CD		UC	
		Hospitalization (n =25)	Discharge (n =17)	Hospitalization (n =25)	Discharge (n =22)
MCV, fl	85.69 (4.78)	84.35 (6.71)	86.68 (6.40)	81.84 (5.79)*	83.35 (7.31)
RDW, %	12.00 (0.65)	14.13 (1.61)*	14.44 (2.46)*	13.28 (1.42)#	13.66 (1.58)*
MPV, fl	8.59 (0.46)	8.31 (0.46)*	8.13 (0.54)*	7.75 (0.68)#	8.40 (1.00)\$
PDW, %	14.72 (1.61)	13.87 (1.07)*	13.28 (1.73)*	12.03 (2.06)#	14.45 (3.18)\$

Note: Values expressed as mean (SD).

*p<0.05 – comparison with control, Mann-Whitney test

#p<0.05 – comparison with Crohn's disease, Mann-Whitney test

\$p<0.05 – comparison with hospitalization, Wilcoxon matched pairs test

Table 2: Platelet aggregation in IBD.

Aggregation Indexes	Control (N =15)	CD		UC	
		Hospitalization (N =13)	Discharge (N =13)	Hospitalization (N =10)	Discharge (N =10)
t _{BA} , s	116.00 (32.25)	86.33 (30.13)*	89.23 (15.53)*	86.00 (28.40)*	100.00 (29.80) \$
t max, s	224.00 (68.54)	183.08 (82.40)*	204.62 (65.91)	170.00 (47.40)*	184.00 (63.80)*

correlated to platelet aggregation rate in patients with UC. ($r=-0.50$, $p<0.05$). The study demonstrated significant increase in degree and rate of erythrocyte aggregation during IBD (Table 3). There is no significant difference in erythrocyte aggregation between patients with CD and patients with UC. However, a fundamental difference in the dynamics of erythrocyte aggregation in these diseases in the process of treatment was found. While the rate and degree of erythrocyte aggregation were normalized in patients with UC during treatment, in patients with CD these indexes remained significantly elevated even upon discharge. We did not find any correlation between erythrocyte aggregation indexes and RDW.

Discussion

In recent years some studies have shown that platelets play an important role in pathogenesis of IBD [13]. Activated platelets are a rich source of mediators participating in inflammation and tissue regeneration [14]. CD154, a key molecule in inflammation, is expressed by platelets and is a pathogenic mediator in inflammatory bowel disease. Patients with IBD have an increased risk of thromboembolic complications. It is known that in IBD a significantly higher endogenous potential of thrombin is observed, when thrombin is not only the essential molecule taking part in homeostasis, but also one of the main inductors of platelet aggregation [15]. Both Crohn's Disease (CD) and Ulcerative Colitis (UC) are associated with abnormalities of platelet number and function. There is solid evidence demonstrating that platelets, in addition to their traditional role in hemostasis, can also function as potent proinflammatory cells [13]. Upon activation, platelets secrete a large number of biologically active molecules able to active an inflammatory process. Simultaneously, platelet morphology changes, their Mean Volume (MPV) decreases [5]. Previous studies demonstrated that low MPV can serve as a biomarker of IBD activity [16]. However, these studies did not investigate the correlation between MPV and platelet aggregation. Larger platelets are metabolically and enzymatically more active [17]. Our research revealed that low MPV is not only associated with high aggregation capacity of platelets, but the aggregation even increases. Negative correlation between MPV and platelet aggregation degree in UC was found. One of the reasons for increased platelet aggregation during IBD may be thrombocytopenia [15]. Reasons for lower MPV levels during IBD have not been well investigated. According to one of the hypotheses, the decrease of platelet volume observed in CD and

Table 3: Erythrocyte aggregation in IBD.

Aggregation Indexes		Ma, mm	A ₄₀ , mm	t _{1/2} , s
Control (n =11)		77.82 (7.87)	54.09 (11.87)	17.91 (7.65)
CD	Hospitalization (N =17)	93.82 (15.04)*	70.06 (19.46)*	12.63 (4.11)*
	Discharge (N =10)	93.33 (9.90)*	72.43 (30.00)*	15.22 (3.38)
UC	Hospitalization (N =18)	100.11 (29.10)*	73.67 (20.58)*	16.89 (10.80)
	Discharge (N =13)	87.33 (15.89)\$	65.58 (18.57) \$	15.85 (10.08)

UC could occur due to the presence of a disorder in the regulation of thrombopoiesis often associated with systemic inflammation. Judging by the following data, chronic inflammation during IBD changes platelet morphology [18]. The reduced platelet volume in CD could occur due to quicker consumption of the large activated platelets [19]. However, our findings demonstrate that even with reduced MPV spontaneous platelet aggregation significantly increases, both in CD and UC. Lower levels of MPV during IBD might be explained by enhanced blood cells microvesiculation, which takes place during CD and UC [20]. According to literature, a big amount of Microvesicles (MV) in blood is platelet-derived. The release of MVs is not only accompanied by MPV decrease. MVs themselves play a part in pathogenesis of multiple conditions, especially associated with the risk of thrombosis [21]. Platelet-derived MVs can also induce inflammation. It has been demonstrated that platelet-derived MVs deliver arachidonic acid to endothelial cells, which leads to increased CD54 activity and subsequent monocytes binding [22]. Afterwards leukocytes adhere to the vascular wall; migrate across the vascular intima, where they produce cytokines and growth factors, which stimulate migration and proliferation of smooth muscle cells (myocytes), thus, forming atherosclerotic plaque. It may be assumed that in IBD the process of RBC vesiculation increases as well and it is accompanied by the decrease in their volume (MCV). It is most significant in UC. It allows suggesting that studying the connection between the change in MPV, MCV and release of MV may make an essential impact on understanding IBD pathogenesis.

Conclusion

The increase in platelet and erythrocyte aggregation occurs during IBD in children despite the decrease in MPV and MCV. Platelet and erythrocyte indexes may be useful noninvasive biomarkers for assessment of therapy efficiency and severity of IBD in children.

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