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Assessment of the influence of the inflammatory process on the activation of blood platelets and morphological parameters in patients with ulcerative colitis (*colitis ulcerosa*)

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Abstract: Ulcerative colitis (*colitis ulcerosa*) is a non-specific inflammatory bowel disease of unknown etiology. The symptoms which are observed in the course of ulcerative colitis are: an increase in the number of leukocytes and blood platelets, an increase in the concentration of IL-6 and anemia. Blood platelets are the key element, linking the processes of hemostasis, inflammation and the repair of damaged tissues. Activation of blood platelets is connected with changes in their shape and the occurrence of the reaction of release. P-selectin appears on the surfaces of activated blood platelets and the concentration level of soluble P-selectin increases in the blood plasma. The aim of this study was to define whether the increased number of blood platelets in patients with ulcerative colitis accompanies changes in their activation and morphology. A total of 16 subjects with ulcerative colitis and 32 healthy subjects were studied. Mean platelet count, morphological parameters of platelets and MPC were measured using an ADVIA 120 hematology analyzer. Concentrations of sP-selectin and IL-6 in serum were marked by immunoassay (ELISA). MPC, concentration of sP-selectin and IL-6 were significantly higher in subjects with ulcerative colitis compared to those in the healthy group. There was a decrease of MPV in patients with ulcerative colitis, which is statistically significant. Chronic inflammation in patients with ulcerative colitis causes an increase in the number of blood platelets, a change in their morphology and activation. Decreased MPV value reflects activation and the role blood platelets play in the inflammatory process of the mucous membrane of the colon. A high concentration of sP-selectin, which is a marker of blood platelet activation, demonstrates their part in the inflammatory process. The increase in the concentration of sP-selectin correlated positively with the increase in concentration of IL-6. This is why it may be a useful marker of the activity of *colitis ulcerosa*. (*Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 1, pp. 119–124*)

Key words: blood platelets, soluble P-selectin, IL-6, mean platelet volume (MPV), ulcerative colitis

Introduction

Ulcerative colitis (*colitis ulcerosa*) is an incurable chronic disease which features periods of exacerbation and remission. The many causes of ulcerative colitis include genetic, environmental and bacterial factors as well as disorders of the intestinal immune system. The main roles in the pathogenesis of ulcerative colitis are played by immunological disorders, which are the result of an imbalance between pro- and anti-inflammatory cytokines. A diffused, non-specific inflammatory process in the mucous membrane of the colon starts in the rectum and extends proximately and continuously to the lower part of the ileum. In the active form of *colitis ulcerosa*, a rubicund, granular, swollen, matt bleeding of the mucous membrane is observed in endoscopy. In heavy cases of *colitis ulcerosa* ulceration, pseudopolyps and large amounts of mucus, pus and blood in the inside of the bowel have been observed. The large inflammatory infiltration consists of macrophages,

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neutrophils and plasma cells [1, 2]. In the active forms of non-specific inflammatory bowel disease, the population of lymphocytes T and B and macrophages located in the mucous membrane is increased. There is also an activation of granulocytes and local secretion of cytokines (e.g. IL-1, IL-6, IL-8, TNF-α) and the production of IgG [3, 4]. In laboratory studies, the characteristics of inflammation can be found, such as an increase in the concentration of C-reactive protein (CRP), quicker erythrocyte sedimentation rate (ESR), increased number of blood platelets (PLT), leukocytosis, sideropenic anemia together with hipoalbumin, electrolytic disorders and perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) [5, 6].

An additional marker of the inflammatory process may be interleukin-6 (IL-6), which is a pro-inflammatory cytokine with multidirectional activity [7]. Previous studies have shown that there is an increase in the concentration of IL-6 in ulcerative colitis and its role is to initiate, potentiate and sustain the inflammatory process in the inflamed mucous membrane of the colon [8–10]. Cytokines and interactions between blood cells cause the activation of blood platelets [1, 11].

Blood platelets are the smallest (diameter: 1.5–3.5 μm), discoid in shape, non-nucleated cells in the blood. They are the key element linking the processes of hemostasis, inflammation and the repair of damaged tissue. Blood platelets participate in the complex mechanisms of stopping bleeding where damage to a blood vessel wall has occurred, and they participate in the formation of blood clots. Platelets initiate and support inflammatory processes through: the secretion of numerous biologically active substances stored in their granules, e.g. they release: platelet activation factor (PAF), platelet-derived growth factor (PDGF), platelets factor 4 (PF4), beta-thromboglobulin (β-TG), interleukin-1 (IL-1), leukotriens and prostaglandins [12–16].

The cytotoxic properties of blood platelets are inter alia cytokine-induced — by cytokines such as tumor necrosis factor (TNF), IL-6 and gamma-interferon (γ-IFN). In some inflammatory diseases, after the activation of blood platelets there is a release of alpha granules and fusion of granule membranes with the blood cell membrane. The activation of blood platelets causes the conformation changes to GMP-140 molecule (CD 62P, P-selectin, PADGEM) stored in alpha granules and its integration into blood platelets cell membranes where it functions as a CD62P receptor. P-selectin is recognized as a typical marker of blood platelets activation, as well as the reaction of release of cell granules [17]. P-selectin occurs also in plasma as soluble P-selectin (sP-selectin). Both of these P-selectin forms are a ligand for the Sialyl Levis particle X-CD 15s and sulphoned sequence tyrosine of P-selectin glycoprotein ligand-1 (PSGL-1) which is constantly present on the surface of phagocytes and neutrophils. The interlinking of CD 62P and PSGL-1 activates monocyte, proinflammatory and prothrombose reactions, including the expression of tissue factor. P-selectin takes part in the migration of white blood cells during the inflammatory process causing their adhesion and 'rolling' of the white blood cell on the endothelial cells in blood vessels [1]. An increased concentration of sP-selectin has been found in the course of inflammatory and cancerous processes [16–19].

The aim of our study was to define whether the increase in the number of blood platelets in patients with ulcerative colitis is accompanied by changes in their activation and morphology.

Mean platelet volume (MPV) and large blood platelets (LPLT) indirectly may be an indication of the activation of blood platelets. We investigated the correlation between the inflammatory process and the activation of blood platelets. As an indication of the inflammatory process, the concentration of IL-6 was assayed. In order to define the activation of blood platelets, the concentration of sP-selectin was evaluated and mean platelet component concentration (MPC, a potential marker of activation of blood platelets) was assayed. Our studies were approved by the Local Ethics Commission: Resolution No. R-I-002/42/2007.

Material and methods

Subjects

The subjects in this study comprised 16 patients, nine males and seven females, aged 18–60. The subjects were hospitalized in III Department of General Medicine and Gastroenterology of the Regional General Hospital in Bialystok. All the subjects were diagnosed with ulcerative colitis. At the moment of taking blood for analysis, their condition was in an exacerbation and their clinical status was described as serious. The activity of the disease was classified according to Truelove and Witts's criteria for *colitis ulcerosa* into mild, moderate or severe. In the study group, six subjects were diagnosed with severe *colitis ulcerosa* (hemoglobin concentration less than 10.5 g/dl; frequency of daily bowel movements six or more with substantial rectal bleeding), while in the other ten subjects mild intensification of the disease was observed, without anemia or fever, and with minimal rectal bleeding.

The control group comprised 32 healthy subjects, 11 males and 21 females, who came to different clinics of the University Hospital in Bialystok for periodic check-ups required by their employers under the present Labour Code. These patients were not hospital patients.

Materials

All tests in the study group and the control group were performed simultaneously. Material for analysis included venous blood collected directly into BD Vacutainer vacuum test-tubes onto an anticoagulant K_2EDTA (to determine the PLT and their morphological parameters, as well as MPC) and on clotted blood (to assess the concentration of sP-selectin and IL-6).

Methods

The amount of blood platelets and their morphological parameters (MPV and LPLT), as well as MPC, were determined using a Bayer Advia 120 hematology analyzer. The concentrations of sP-selectin and IL-6 were determined by the immunoassay method using R&D Systems ELISA Kit Human sP-selectin and ELISA Quantikine HS Human IL-6.

ELISA (enzyme-linked immunosorbent assay) employs the quantitative sandwich immunoassay technique, in which monoclonal antibodies specific for sP--selectin are pre-coated onto a microplate and bind sP-selectin present in the blood samples. During incubation with sP-selectin conjugate (second antibody to sP-selectin conjugated with horseradish peroxidase), the immunology complex is created: a monoclonal antibody specific to sP-selectin- sP-selectin conjugate. After adding substrate and terminating the reaction with Stop Solution, the intensity of the color reaction is determined using a spectrophotometer. The color intensity is proportional to the sP-selectin concentration in the plasma.

The quantitative determination of human IL-6 concentration in serum was carried out using R&D Systems ELISA Quantikine HS Human IL-6. This test is based on the quantitative sandwich immunoassay technique. Monoclonal antibodies specific for IL-6 are precoated onto a microplate and bind IL-6 present in the blood samples. After washing any unbound antibodies, a conjugate is added. During the incubation, a 'sandwich structure' is created where the antigen forms a layer between two antibodies. Adding a substrate to the enzyme (alkaline phosphate) joined to the antibody produces a reaction that manifests itself as a color change. Following a period of incubation, an amplifier solution is added to the wells. This enables the color to develop in proportion to the amount of IL-6 bound in the initial step. Color development is then stopped and the intensity of the color measured.

Statistical analysis

The results of the study were statistically analyzed using Statistica 8.0 PL. Arithmetic mean and standard deviations were calculated for the subjects with ulcerative colitis and the control group. Statistical analysis between the study group (B) and the control group (C) were performed using the parametric Student *t*-test for two independent determinants with normal distribution. Results were considered statistically significant at $p < 0.05$. To determine the correlation between the inflammatory process and the activation of blood platelets, a correlation test was performed using determinants for normal distribution applying Pearson's coefficient.

Results

The amount of blood platelets (PLT) in patients with ulcerative colitis (B) was 300.56 ± 110.96 G/L and was significantly higher ($p < 0.001$) compared to the mean platelet count in the control group (C) (248 ± ± 44.74 G/L) (Table 1).

Mean platelet volume (MPV) was significantly lower ($p < 0.05$) in the study group B (8.03 ± 0.83 fl) than the mean value in the control group C (8.68 ± ± 0.64 fl) (Table 1).

The analysis of the amount of large blood platelets (LPLT) in both groups proved to be statistically insignificant ($0.3 < p < 0.4$) being: 4.75 ± 2.21 G/L in group B and 5.34 ± 1.81 G/L in group C (Table 1).

Comparison of the results of the value of MPC in groups B and C showed that average MPC in patients with ulcerative colitis was 26.45 ± 1.45 g/dl; this was slightly higher than in the control group (25.70 ± 1.37 g/dl). The difference between groups B and C was not statistically significant ($0.7 < p < 0.8$) (Table 1).

It was demonstrated that the concentration of sP--selectin was significantly increased ($p < 0.01$) in patients with ulcerative colitis compared to the healthy subjects. Mean concentrations of sP-selectin in group B was 22.33 ± 20.82 ng/ml, which is almost treble the mean concentration of sP-selectin in group C (7.26 ± ± 6.59 ng/ml) (Table 2).

In patients with ulcerative colitis (B) the mean concentration of IL-6 (6.04 ± 4.37 pg/ml) was twice as high as the mean concentration of IL-6 in the control group (C), which was 2.45 ± 1.44 pg/ml. This difference is statistically significant ($p < 0.01$) (Table 2).

The correlation between the concentration of IL-6 and the concentration of sP-selectin was positive at $r = 0.35$, but was not statistically significant ($p < 0.5$).

Table 1. Mean platelet count and their morphological parameters in groups B and C

	Subjects (B) n = 16 X ± SD	Control group (C) n = 32 X ± SD	Statistical significance p
Mean platelet count [G/L]	300.56 ± 110.96	248 ± 44.74	**p < 0.01***
Mean platelet volume [fl]	8.03 ± 0.83	8.68 ± 0.64	**p < 0.05***
Large blood platelets [G/L]	4.75 ± 2.21	5.34 ± 1.81	0.3 < p < 0.4
Mean platelet component concentration [g/dl]	26.45 ± 1.45	25.70 ± 1.37	0.7 < p < 0.8

n — number of cases; X — arithmetic mean; SD — standard deviation; Student's *t*-test; ***p < 0.05** statistically significant differences

Table 2. Concentrations of sP-selectin and IL-6 in both groups (B and C)

	Subjects (B) n = 16 X ± SD	Control group (C) n = 23 X ± SD	Statistical significance p
sP-selectin [ng/ml]	22.33 ± 20.82	7.26 ± 6.59	**p < 0.01***
IL-6 [pg/ml]	6.04 ± 4.37	2.45 ± 1.44	**p < 0.01***

n — number of cases; X — arithmetic mean; SD — standard deviation; Student's *t*-test; ***p < 0.05** statistically significant differences

Discussion

Ulcerative colitis (*colitis ulcerosa*) is a form of chronic non-specific inflammatory bowel disease. Among many factors which contribute to the etiopathogenesis of *colitis ulcerosa* are genetic conditioning, food allergies and bacterial, viral and environmental factors. They affect the mucous membrane of the colon and lead to disorders of the intestinal immune system. IL-6 may be the sensitive but non-specific marker of the inflammatory process in a human body; its concentration in blood plasma can increase up to 100-fold during inflammation. A study by Wędrychowicz et al. showed that the concentration of IL-6 in blood plasma is increased in patients with active ulcerative colitis, whereas it is decreased in patients in the period of remission of ulcerative colitis [20]. Raddatz et al. studied IL-6-mRNA expression in biopsy samples of inflamed mucosa and in the peripheral blood mononuclear cells (PBMNC) from patients with non-specific inflammatory bowel diseases [21]. It was demonstrated that increased IL-6 expression occurred in inflamed mucous membrane of the colon correlating with the extent of the disease process and CRP levels in subjects with *pancolitis ulcerosa*.

Our study demonstrated that the concentration of IL-6 in the ulcerative colitis group of subjects was almost three times higher than the concentration of IL-6 in the healthy group. This shows that IL-6 may be a useful marker of inflammatory activity of *colitis ulcerosa* in inflamed mucosa in subjects suffering from *colitis ulcerosa*. However, the determination of concentration of IL-6 in mucosal biopsies may help to differentiate Crohn's disease from ulcerative colitis.

IL-6 induces many biological effects, such as the stimulation of blood platelets activation. The activation of blood platelets causes the appearance of P-selectin on their surface which, as a receptor protein, contributes to the pathogenesis of inflammation and thrombosis [1]. A part of P-selectin peels from the surface of blood platelets and occurs in plasma in a soluble form. The present study shows that the concentration of sP-selectin in subjects with ulcerative colitis was higher than in the control group, which was statistically significant ($p < 0.01$). Such increases in sP-selectin concentrations prove the activation of blood platelets and their part in the inflammatory process.

An increase in the concentration of IL-6 is a marker of inflammatory process in the mucous membrane of the colon. An increase in the concentration of sP-selectin indicates the activation of blood platelets and their part in the inflammatory process. In the present study, positive correlations between concentrations of IL-6 and sP-selectin were found in subjects with ulcerative colitis, but this was not statistically significant.

An increase in the number of blood platelets has been observed in the course of chronic inflammatory bowel disease [5, 6]. In the present study, morphological parameters of platelets were also analyzed (MPV, LPLT), which may change in relation to their functional state and measurement of the parameters may indirectly indicate the degree of blood platelets

activation [22, 23]. Our study demonstrated that the PLT in subjects with ulcerative colitis (B) was significantly higher compared to the PLT in the control group (C). Previous studies have shown that the blood platelet count was increased in patients with active colitis ulcerosa compared to inactive *colitis ulcerosa* or healthy subjects. Here there was a difference between study groups which was statistically significant [24]. In the present study, the number of metabolically active immature large blood platelets was determined. It was demonstrated that their number was slightly lower in subjects with ulcerative colitis compared to the control group, and that this difference was not statistically significant.

These results suggest the hypothesis that active large blood platelets are used up in the inflammatory process and that smaller platelets influence a decrease of MPV. Other studies have also shown that the number of reticulated platelets was reduced in active ulcerative colitis patients compared to inactive ulcerative colitis patients and healthy subjects [24].

It needs to be remembered that the decrease in MPV in subjects with active ulcerative colitis can be associated with the increase of blood platelets activation in these patients. In the present study, MPV was significantly lower in the ulcerative colitis group (B) compared to the control group (C), something which agreed with the results of other studies [6, 25]. The aim of the study by Yüksel et al. [6] was to determine whether mean platelet volume would be a useful marker of ulcerative colitis, and to analyze its overall accuracy in evaluating disease activity in comparison with other inflammatory markers (leucocytosis, ESR, concentration of CRP). The study showed that MPV was reduced in *colitis ulcerosa* compared to the control group, and that the reduction was statistically significant. MPV values in active *colitis ulcerosa* (8.06 ± ± 1.19 fl) and inactive *colitis ulcerosa* (8.45 ± 0.87 fl) were compared. The differences were shown to be statistically significant. In ulcerative colitis, MPV did not correlate with other markers: leucocytosis, ESR and CRP. On the basis of the results, it appears that decreased MPV may be an indicator for increased disease activity in patients with *colitis ulcerosa*. Parallel dependencies between activity of ulcerative colitis and mean platelet volume were observed in the study by Kayahan et al. [24].

In 2001, Kapsoritakis et al. studied two groups of patients with inflammatory bowel disease, comprising 93 subjects with *colitis ulcerosa* (CU), 66 with Crohn's disease (CD) and 38 healthy subjects [25]. The activity of disease was defined using the Clinical Colitis Activity Index for patients with *colitis ulcerosa* and the Crohn's Disease Activity Index for patients with Crohn's disease. In all groups, blood platelet count and their morphological parameters were measured.

It was shown that the complete blood platelet count was significantly increased in patients with active CU and CD compared to patients with inactive CU and CD or healthy subjects. In the case of MPV, parallel dependencies were demonstrated. MPV was significantly reduced in active inflammatory bowel diseases and correlated negatively with leucocytosis, ESR, concentration of CRP, markers of activation of blood platelets, such as plasma β-TG and PF4. The increase in the concentration of plasma β-TG and PF4 indicate platelets activation and the release of active biological substances, which are stored in the platelet's granules. They initiate and support the inflammatory process in the colon. The increase in the complete blood platelet count and the reduction in MPV reflected this process.

In the study, the usefulness of potential MPC marker of blood platelets activation (degree of their degranulation) was also analyzed; the value of MPC depends on the content of cell granules of blood platelets. Activation of blood platelets and the release of active biological substances outside the blood platelets cause changes in the cytoskeleton of activated platelets and the reduced density of the platelet's components. The indication marker of this process is a decrease in MPC value, although this was not demonstrated in the study.

The examples given above indicate that blood platelets play an important part in the inflammatory process in patients with *colitis ulcerosa*. MPV — similarly to the increase of the number of leukocytes, the concentration of CRP and the quicker ESR — may be an indication of exacerbation of the inflammatory process in the mucous membrane of the colon. A high concentration of IL-6 reflects the inflammatory process, while the increase in sP-selectin concentration reflects the participation of blood platelets in these processes.

Conclusions

Chronic inflammatory process in patients with ulcerative colitis causes an increase in the number of blood platelets and changes in their activation and morphological parameters. The lower value of LPLT in subjects may indicate that large metabolically active blood platelets take part in the inflammatory process in the colon. A decrease in MPV reflects activation and participation of blood platelets in the inflammatory process of the colon mucosa, and because of this, MPV may be a useful marker of active ulcerative colitis. A high concentration of sP-selectin, which is a marker of activation of blood platelets, reflects its partici-

pation in the inflammatory process. The increase in the concentration of sP-selectin correlated positively with the increase in the concentration of IL-6 and this is why sP-selectin may be a useful marker of active ulcerative colitis.

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