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Assessment of Features of Biochemical Factors Associated with the Inflammatory Process and Their Impact on the Count and Morphological Structure of Blood Platelets in Individuals with Intestinal Damages Due to Ulcerative Colitis

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Abstract

Introduction. The cause of ulcerative colitis, also known as colitis ulcerosa, is unclear. It is non-specific inflammatory bowels illness. The critical component that connects the processes of hemostasis, inflammation, and tissue healing is blood platelets.
Purpose. To determine whether changes in ulcerative colitis patients were associated with an increase in blood platelets, alterations in blood platelet shape and activation are associated with elevated platelet counts.
Materials and methods. A total of 65 healthy individuals (as controls) and 35 individuals with UC were included in the study. Using an ADVIA 123 a hematology analyzer, the mean platelet count, MPC, and platelet morphological characteristics were measured. Immunoassay was used to measure the concentrations of Interleukin-6 and soluble P-selectin in serum (ELISA). Subjects with UC had considerably greater MPC, soluble P-selectin, and Interleukin-6 concentrations than those in the healthy group. Venous blood samples for biochemical testing were collected using vacuum tubes, which increased the reliability of the results obtained during the preclinical phase of the study.
Results. Patients with UC had a statistically significant reduction in mean platelet count (MPV). An increase in leukocyte and blood platelet counts, a rise in Interleukin-6 concentrations, and anemia are the symptoms that are seen during UC obtained. Thus, the mean platelet count (MPC) in patients with ulcerative colitis was 301.56±111.95 g/L, whereas in the group of apparently healthy individuals it was 245±44.74 g/L ($p<0.01$), and the mean platelet volume (MPV) was 8.08±1.86 (FL); in the group of essentially healthy individuals, it was 9.34±0.67 (FL), $p<0.05$. The IL-6 level in ulcerative colitis was 7.05±5.37 [pg/ml, g/l], and in apparently healthy individuals 8.26±7.59 [pg/ml], $p<0.001$;

and the concentration of soluble P-selectin (sP-selectin) was 23.44±21.83 and 8.26± 7.59 [ng/ml], respectively ($p<0.001$). High levels of soluble P-selectin are considered an indicator of inflammatory activation and a marker of platelet activation. A positive correlation between increased interleukin-6 concentrations and increased soluble P-selectin levels may be interpreted as an indicator of pathological process activation in ulcerative colitis.

Conclusion. Patients with UC who have chronic inflammation have higher blood platelet counts, altered a modification in their stimulation and morphology. A lower MPV number indicates stimulate and the part blood platelets play in the colon's mucous membrane inflammatory process. High ranks of soluble P-selectin indicate their involvement in the inflammatory process.

Keywords: blood platelet, soluble P-selectin, Interleukin -6, mean platelet volume, UC

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Оценка особенностей состояния биохимических факторов воспалительного процесса и их влияния на количественное содержание и морфологическую структуру тромбоцитов у лиц, страдающих повреждением кишечника при язвенном колите

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Резюме

Введение. Язвенный колит (colitis ulcerosa) – неспецифическое воспалительное заболевание кишечника, причина возникновения которого до конца не ясна. Ключевым морфологическим компонентом, оказывающим сочетанное влияние на процессы гемостаза, воспаления и заживления тканей, являются тромбоциты.
Цель. Установить особенности изменения количества, функционального состояния и морфологической структуры тромбоцитов, а также биохимических факторов воспаления у пациентов, страдающих язвенным колитом.

Материалы и методы. В исследование было включено 65 практически здоровых людей (составивших контрольную группу) и 35 пациентов с язвенным колитом в возрасте от 17 до 65 лет (из них 16 мужчин, 19 женщин). С использованием автоматического гематологического анализатора ADVIA 123 определяли среднее количество тромбоцитов (MPC) и их морфологические характеристики. Для оценки выраженности воспалительного процесса устанавливали концентрацию интерлейкина-6 (IL-6) и растворимого P-селектина в сыворотке крови методами иммуноферментного анализа (ELISA). Взятие венозной крови для выполнения биохимических исследований осуществляли с применением вакутейнеров, что способствовало повышению надежности получаемых результатов на доклиническом этапе исследования.
Результаты. Показано, что у пациентов с язвенным колитом среднее количество тромбоцитов (MPC), растворимого P-селектина и интерлейкина-6 значительно выше, чем у представителей контрольной группы, на фоне снижения при заболевании среднего объема тромбоцитов (MPV). Так, MPC у пациентов с язвенным колитом составило 301,56±111,95 г/л, тогда как в группе практически здоровых лиц – 245±44,74 г/л ($p<0,01$), а MPV – 8,08±1,86 (FL), в группе практически здоровых лиц – 9,34±0,67 (FL), $p<0,05$. Уровень IL-6 при язвенном колите составил 7,05±5,37 г/л, а у практически здоровых лиц – 8,26±7,59 г/л, $p<0,001$; содержание растворимого селектина (sP-selectin) – 23,44±21,83 и 8,26±7,59 нг/л соответственно ($p<0,001$). Высокий уровень sP-selectin расценивается как индикатор активации воспалительного процесса и как маркер активации тромбоцитов крови. Положительная корреляция между увеличением концентрации IL-6 и увеличением содержания sP-selectin может расцениваться как индикатор активации патологического процесса при язвенном колите.
Заключение. У пациентов с язвенным колитом, сопровождаемым хроническим воспалением, установлено статистически значимое увеличение MPC и снижение MPV на фоне увеличения концентрации биохимических факторов воспаления IL-6 и P-селектина, что отражает их участие в патогенезе заболевания.
Ключевые слова: тромбоциты, растворимый P-селектин, интерлейкин-6, среднее количество тромбоцитов (MPC), средний объем тромбоцитов (MPV), язвенный колит (ЯК)

■ INTRODUCTION

Colitis ulcerosa, often known as ulcerative colitis, is a chronic, incurable illness that has flare-ups and remissions. In addition to bacterial, environmental, and genetic factors, abnormalities of the intestinal immune system can also be responsible for ulcerative colitis. Due to a disparity between pro- and anti-inflammatory cytokines, immunological problems play a major role in the pathogenesis of UC [1, 2]. Increased numbers of macrophages and T and B lymphocytes are found in the mucosal membrane in non-specific inflammatory bowel disease that is active. Additionally, granulocytes are activated, and IgG is produced in addition to the local excretion of cytokines (such as Interleukin-8, Interleukin-6, Interleukin-1, and tumor necrotic factor-a) [3, 4]. The features of inflammation that are observed in laboratory studies include elevated CRP concentrations, faster erythrocyte sedimentation rates (ESRs), higher platelet counts (PLTs), leukocytosis, sideropenic anemia along with hipoalbumin, electrolytic disruptors, and (pANCA) [5, 6]. IL-6, a pro-inflammatory cytokine with multidirectional action, may also serve as a marker of the inflammatory process [7].

Previous research has demonstrated that ulcerative colitis is associated with elevated concentrations of IL-6, which is involved in initiating, intensifying, and maintaining the inflammatory process in the colon's inflamed mucous membrane [8–10]. Blood platelets are activated by cytokines and interactions between blood cells [1, 11].

The tiniest (diameter: 2.5–4.5 mm), discoid-shaped, non-nucleated blood cells are called platelets. They play a crucial role in tying together the processes of inflammation, hemostasis, and tissue healing. Blood platelets take role in the production of blood clots as well as the intricate processes that halt bleeding when damage to a blood vessel wall occurs. The release of various biologically active substances from their granules, such as platelet activation factor (PAF), (PDGF), (PF4), (b-TG), (IL-1), leukotriens, and (PNG), is how platelets start and sustain inflammatory processes [12–16].

sP-selectin is acknowledged as a common indicator of blood platelet activation and the response to cell granule release [17]. sP-selectin is also soluble in plasma. Selected P (sP)-selectin is the Sely Levis subdivision X-CD 15s and the sulfur-containing series tyrosine of P-selectin glycoprotein ligand-1 (PSGL-1), which is always present on the surface of phagocytes and neutrophils, are ligands for these two sP-selectin forms. The production of tissue factor is one of the proinflamatory, prothrombogenic, and monocyte responses that are triggered by the interaction of CD 62P and PSGL-1. P-selectin contributes to white blood cell migration during the inflammatory process, which causes the cells to adhere to endothelial cells in blood arteries and "roll" [1]. It has been observed that sP-selectin concentrations rise during inflammatory and malignant processes [16–19].

■ PURPOSE OF THE STUDY

To determine whether changes in ulcerative colitis patients were associated with an increase in blood platelets, alterations in blood platelet shape and activation are associated with elevated platelet counts.

■ MATERIALS AND METHODS

Subjects

Thirty-five patients, ages 17 to 65, were the study's subjects; 16 of them were male and 19 were female. The individuals were admitted to the Al Zahraa teaching Hospital in Wasit province. Ulcerative colitis was identified in all of the individuals. Their clinical state was deemed critical and their condition was in aggravation at the time of blood collection for examination. Using Truelove and Witts' criteria for UC, the disease's activity was categorized as mild, moderate, or severe. Nine of the research group's participants had severe UC (hemoglobin attentiveness less than 10 g/dl; seven or more daily bowel activities with significant rectal bleeding), whereas the remaining ten participants had minor aggravation of the condition. Conversely, in the remaining 26 patients, there was minimal rectal bleeding, no fever or anemia, and a minor worsening of the illness.

The control group consisted of thirty well-being subjects – ten men and twenty women – who visited Al Zahraa teaching Hospital for the periodic physicals mandated by their managers under the current Labor Code. These patient didn't belong in a hospital.

Tools

Every exam was administered concurrently to a control group and the study groups. Venous blood was drawn directly into BD Vacustainer vacuum test tubes, and the material

for analysis included clotting blood (to measure the concentrations of IL-6 and soluble P-selectin) and anticoagulant K2EDTA (to ascertain Platelets and their morphological parameters, in addition to MPC).

Procedures

Using a Bayer Advia 123 hemogram analyzer, the quantity of blood PLT and their morphological properties (MPV and LPLT) in addition to MPC were ascertained. Using the R&D Systems ELISA Kit Human sP-selectin and ELISA Quantikine hs Human IL-6, the immunoassay approach was used to measure the amounts of sP-selectin and IL-6.

The quantitative sandwich immune assay technique, which ELISA (enzyme-linked immunosorbent test) uses, involves pre-coating a microplate with monoclonal antibodies specific for sP-selectin, which then binds sP-selectin found in blood samples. The immunology complex, a MNAC specific to soluble P-selectin conjugation, is formed after incubation with soluble P-selectin conjugate (second antibody to soluble P-selectin linked with horseradish peroxidase). A spectrophotometer is used to measure the color reaction's intensity after the substrate is added and the reaction is stopped with Stop Solution. The amount of sP-selectin in the plasma directly correlates with the color intensity. R&D Systems ELISA Quantikine hs Human IL-6 was used to quantitatively determine the concentration of human IL-6 in serum. The quantitative sandwich immunoassay technique serves as the foundation for this test. Pre-coated microplates containing MAS for IL-6 bind the IL-6 found in blood samples. A conjugating is added after any unbound antibodies have been cleaned. A "sand-wich structure" is formed during incubation, in which a layer of antigen is sandwiched between two antibodies. A color change results from the addition of a substrate (alkaline phosphate) to the enzyme attached to the anti-body. The wells are filled with an amplifier solution after they have been left to incubate. This allows the color to evolve according to the quantity of bound IL-6, in the initial step. Color change is then stationary and the strength of the color measured.

Statistical Analysis

Statistica 9.0 PL was used to statistically assess the study's findings. Both the control group and the ulcerative colitis individuals had their arithmetic means and standard deviations computed. The parametric Student t-test was used for statistical comparison between the study of group (B) and the control group (C) for two independent determinants with a normal distribution. At $p<0.05$, the results were deemed statistically significant. The activation of blood platelets and the inflammatory process were correlated, and this was found by a correlation test that used Pearson's coefficient and normal distribution determinants [20].

■ RESULTS

The mean of platelet count in the control group (C) was 245±44.74 g/L; in contrast, the number of (PLT) in patients with ulcerative colitis (B) was 301.57±111.95 g/L, which was substantially highest ($p<0.01$) (Table 1). The study group B's mean platelet volume (MPV) was 8.08±1.86 fl, considerably lower ($p<0.05$) than the control group C's mean value (9.34±0.67 fl). (Table 1). Table 1 displays the statistically insignificant number of large blood plate-lets (LPLT) in both groups, with 5.75±3.25 g/L in group B and 6.34±1.81 G/L in group C. When the MPC values in groups B and C were compared,

Table 1
Mean platelet count (MPC) and their morphological parameters in groups B and C

	Subjects (B)	Control group (C)	p. value
	n=17	n=30	
	X±SD	X±SD	
Mean platelet count [g/L]	301.56±111.95	245±44.74	<0.01*
Mean platelet volume [FL]	8.08±1.86	9.34±0.67	<0.05*
Large blood platelets [g/L]	5.75±3.25	6.34±1.81	0.4
Mean platelet component concentration [g/dl]	27.47±1.48	26.70±1.37	0.8

Notes: n – No. of cases; X – mean; SD; Novice's t-test; * p<0.05 – statistically significant differences.

Table 2
Concentrations of soluble P-selectin and Interleukin-6 in both groups (B and C)

	Subjects (B)	Control group (C)	p. value
	N=15	N=24	
	X±SD	X±SD	
sP-selectin [ng/ml]	23.44±21.83	8.26±7.59	0.001*
IL-6 [pg/ml]	7.05±5.37	3.48±2.46	0.001*

Notes: n – No. of cases; X – mean; SD; t-test; * p<0.05 – statistically significant differences.

it was found that patients with UC had an average MPC of 26.70±1.37 g/dl, which was marginally higher than the MPC values in the control group (27.47±1.38 g/dl). Table 1 shows that there was no statistically significant difference (0.7<p<0.8) between groups c and b. It was shown that, in comparison to the healthy participants, patients with ulcerative colitis had a considerably higher (p<0.01) concentration of sP-selectin. The average sP-selectin concentrations in group B were 23.44±21.83 ng/ml.) as showed in Table 2.

The mean concentration of IL-6 (7.05±5.37 pg/ml) in ulcerative colitis patients (B) was twice that of the control group (C), where the mean concentration was 3.48±2.46 pg/ml. According to Table 2, this difference is significant statistically (p<0.01). Although there was a positive correlation (r=0.34) between the sP-selectin and IL-6 concentrations, the relationship was not significant statistically (p<0.5).

■ DISCUSSION

Inflammatory bowel illness that is chronic and non-specific is known as ulcerative colitis (colitis ulcerosa). The etio pathogene-sis of UC is caused by a variety of reasons, including environmental, bacterial, viral, and genetic factors; dietary allergies; and genetic conditioning.

They damage the colon's mucosal barrier and cause problems with the intestinal immune system. IL-6 can increase up to 100-fold in blood plasma concentration during inflammation, suggesting that it is a sensitive yet non-specific marker of the inflammatory process in the human body. According to research by Wędry-chowicz et al., individuals

with active ulcerative colitis had higher blood plasma concentrations of IL-6, whereas patients who are experiencing remission have lower concentrations [21].

In biopsy samples of inflamed mucosa and (PBMNC) from individuals with non-specific inflammatory enteric disorders, Raddatz et al., investigated the expression of IL-6-mRNA [22]. It was shown that in individuals with pancolitis ulcerosa, elevated levels of CRP and IL-6 expression were correlated with the severity of the disease process in the colon's inflamed of mucous membrane. The results of our investigation showed that the participants with ulcerative colitis had an almost three-fold greater concentration of Interleukin-6 than the healthy group. This indicates that in patients with UC with inflamed mucosa, Interleukin-6 may be a useful measure of the inflammatory activity of the disease. Nonetheless, separating CD from UC may be made easier by the measurement of Interleukin-6 concentration in mucosal biopsies.

Numerous biological effects, including the stimulation of blood platelet activity, are induced by IL-6. P-selectin, a receptor protein that is surface-exposed during activation of blood platelets, has a role in the pathophysiology of thrombosis and inflammation [1]. The current investigation demonstrates that there was a significant statistically ($p<0.01$) increase in the concentration of sP-selectin in the UC participants related to the control group. These rises in sP-selectin concentrations demonstrate how blood platelets are activated and how they contribute to the inflammatory process.

A rise in IL-6 content in the colon's mucous membrane is indicative of an inflammatory condition. The stimulation of blood platelets and their involvement in the inflammatory process are indicated by a rise in the concentration of sP-selectin. Although not statistically significant, the current study's subjects with ulcerative colitis showed favorable associations between concentrations of Interleukin-6 and sP-selectin. Throughout the course of chronic inflammatory bowel illness, a rise in blood platelets has been noted [5, 6]. The morphological properties of platelets (MPV, LPLT) were also examined in the current study. These parameters may vary depending on how they operate, and measuring them may provide an indirect indication of the quantity of blood platelets activation [23, 24].

This research showed a statistically significant increase in PLT between the ulcerative colitis participants (B) and the control group (C). According to earlier research, patients with active colitis ulcerosa had higher blood platelet counts than either inactive colitis ulcerosa patients or healthy individuals. Here, there was a statistically significant difference between the research groups [25]. The quantity of metabolically active immature big blood platelets was determined in the current investigation. It was shown that, as compared to the control group, the number of participants with ulcerative colitis was somewhat lower, although this difference was not statistically significant.

These findings support the theory that smaller blood platelets contribute to a reduction in MPV while active big blood platelets are depleted throughout the inflammatory process. Additional research has also demonstrated that reticulated platelets were less common in people with active ulcerative colitis as compared to those with inactive colitis and healthy individuals [25]. It is important to keep in mind that participants with active ulcerative colitis may have lower MPV in response to higher blood platelet stimulation. According to the results of previous research [6, 26], MPV was considerably lower in the UC group (B) of the current investigation as compared to the control group (C).

Yüksel et al., study [6] sought to ascertain if mean PLT volume would be a helpful indicator of UC and to assess the measure's overall accuracy in assessing disease activity

relative to other inflammatory indicators (leukocytosis, ESR, and CRP concentration). In comparison to the control group, the study demonstrated that MPV was lower in the CU group and that this difference was statistically significant. The MPV levels of active UC (8.06±0.67 fl) Colitis ulcerosa (8.34±0.86 fl) and inactive colitis (1.18 fl) were contrasted. It was demonstrated that the differences were statistically significant. Leukocytosis, ESR, and CRP were not correlated with MPV in UC.

Based on the findings, it seems that in colitis ulcerosa patients, lower MPV might be a sign of higher disease activity. In the study by Kayahan et al. [25], parall-lel dependencies between mean platelet volume and ulcerative colitis activity were noted. 38 healthy individuals, 66 patients with (CD), and 93 patients with (CU) were the subjects of a 2001 study by Kapsoritakis et al. on inflammatory bowel disease [26]. For colitis ulcerosa patients, the Clinical Colitis Activity Index was used to define the illness's activity, and for Crohn's disease patients, the Crohn's illness Activity Index. Blood platelet counts and their morphological characteristics were assessed in each group.

■ CONCLUSIONS

Patients with ulcerative colitis have elevated blood platelet counts as well as altered activation and morphological properties due to a chronic inflammatory process. The decreased LPLT value in the subjects would suggest that the colon's inflammatory process involves big, metabolically active blood platelets. Because a reduction in MPV indicates that blood platelets are active and participating in the mucosal inflammatory process, MPV can be a valuable indicator of active UC. A high level of soluble P-selectin indicates its involvement in the inflammatory process and is a marker of blood platelet activation. Because of the positive correlation between the rise in IL-6 concentration and the rise in soluble P-selectin concentration may be a useful indicator of active UC.

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