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Abstract

Several publications have pointed out the importance of coagulation and fibrinolysis in the occurrence of chronic urticaria (CU), but only a few indicated the direct role of platelets. We assessed platelet aggregation and evaluated parameters of coagulation and fibrinolysis in patients with CU. Patients ($n = 34$) diagnosed as having CU and 36 healthy controls were enrolled. Platelet aggregation was assayed using an impedance aggregometer and adenosine diphosphate, arachidonic acid, thrombin receptor-activating peptide (TRAP), and ristocetin as agonists. In patients with CU, significantly decreased platelet aggregation to some agonists (ristocetin and TRAP) was observed. The D-dimer levels were elevated, mean platelet volume was decreased, but no alteration was observed in other coagulation assays. Elevated D-dimer levels indicated that coagulation and fibrinolysis are activated in the patients with CU. Evaluation of platelet function may contribute to identify the role of these cells in the pathogenesis of CU.

Keywords

chronic urticaria, D-dimer, platelet, aggregation, coagulation

Introduction

Chronic urticaria (CU) is a relapsing disease associated with itching of swollen skin that lasts for 6 weeks or longer.^{1,2} The prevalence of CU is 0.5% to 3% of the population.³ It is commonly seen in adults,⁴ predominantly in women (2:1 ratio).^{5,6}

The pathophysiology of CU is not fully understood. Studies indicated a role for mast cells, basophils, and hormones⁷ as well as changes in coagulation and fibrinolysis.^{8,9} Activation of the coagulation cascade resulting in thrombin production leads to the exacerbations in CU. Additionally, a chronic inflammatory reaction as evidenced by autoantibodies and high mean platelet volume (MPV) are common pathological changes.¹⁰ Autoantibodies, complement system, mast cell-derived factors, and several cytokines are reported to be responsible for the activation of endothelial cells causing tissue factor expression as well as activation of the extrinsic coagulation pathway.¹¹

Exudation of plasma components into the dermis is one of the major findings in urticaria; this is due to the increased permeability of dermal/cutaneous vasculature.¹² Fibrinogen leakage from blood vessels results in intradermal fibrin formation in skin lesions. The formation of fibrin may affect the levels of coagulation/fibrinolysis parameters in the plasma.¹¹

D-dimer, a fibrin degradation product, is a marker of *in vivo* fibrin formation.¹³ Patients with active CU have the highest levels of D-dimer, when compared with patients with CU under

remission or controls.¹⁴ Previous studies have also shown that plasma D-dimer levels correlate with the severity of the disease.¹⁵⁻¹⁷ Fibrin degradation products were also found to be elevated in CU.¹⁷

At present, no association has been defined between ongoing hypercoagulability state in CU and an increased risk of thrombosis; however, cardiovascular events occurring during episodes of acute urticaria have been previously pointed out.¹⁸ Also, the clinical efficiency of some anticoagulant and antifibrinolytic drugs has been demonstrated.¹⁸ Decreased levels of complement and presence of anti-C1q antibodies

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in circulation are associated with the severity of urticarial vasculitis.^{19,20} High levels of prothrombin fragment 1 + 2 and CRP may also play a role in the severity of CU.^{8,11,15,21-23}

Platelets are involved in many pathophysiological processes including thrombosis, clot retraction, vessel constriction and repair, inflammation (including promotion of atherosclerosis), host defense, and tumor growth/metastasis.^{24,25} Platelets release inflammatory mediators and reactive oxygen species upon activation.²⁶ The role of platelet oxidative stress in CU has been investigated.²⁷ Platelets have also been implicated in the inflammatory process in CU.²⁸ A link between inflammation and coagulation through the interaction of histamine with thrombin has been postulated, which may be involved in the mechanism of urticaria-associated angioedema.¹⁸ Also, psoriasis, an inflammatory disease that mainly affects the skin, is associated with increased vascular risk.²⁹

Mean platelet volume has been used as an inflammatory marker in various diseases associated with inflammation, including CU.³⁰ Decreased MPV in patients with CU has been reported in 1 study³¹ but remains controversial.³²

Data regarding the behavior of platelets and their mediators in different forms of urticaria are scarce. In a recent study, platelet aggregation in patients with CU was examined.³³ In this study, we investigated the response of platelets to several agonists as well as hemostatic assays in patients with CU. We also evaluated vascular thrombosis risk in CU.

Materials and Method

Patients

Patients who were diagnosed with CU by the dermatology outpatient clinic were enrolled. Venous blood samples were obtained from 34 patients with CU (29 women and 5 men; median age: 39.6 years) with active skin lesions and from 36 healthy controls (HCs, 26 women and 10 men; median age: 40.5 years). All cases signed an informed consent form prior to being enrolled. The study protocol was approved by the local ethical committee.

Patients with hereditary/acquired angioedema, anaphylaxis, urticaria-related, or systemic vasculitis were excluded. None of the patients received any oral anticoagulants, had any infections, thromboembolism, hepatic or cardiac diseases, or underwent any surgical treatment in the last 6 months.

Platelet Counts and D-dimer

Blood samples were collected by venipuncture into 1.8 g/L K₂ EDTA vacutainer tubes. Platelet counts were measured using a Micros60 automated hematology autoanalyzer (Horiba Instruments, France), D-dimer levels were measured with Pathfast Immunoanalyzer (Mitsubishi Chemical Medience Corporation, Japan) with a chemiluminescence method.

Platelet Function Analysis

Blood samples were collected into hirudin tubes (Becton Dickinson, New Jersey, USA) with a final concentration of 25 µg hirudin/mL of blood. Platelet function analysis was

performed by the multi-electrode array using Multiplate analyzer (Dynabyte, Munich, Germany). Briefly, 300 µL of pre-heated saline solution was pipetted into 300 µL of blood sample with hirudin and mixed. Following 3 minutes incubation, each aggregating agent was added to the cuvette to stimulate platelet aggregate that forms a platelet monolayer on immersed electrodes, thus increasing the impedance between them. Arbitrary aggregation units are plotted against time and reported as area under the aggregation curve. Platelets were stimulated with adenosine diphosphate (ADP), arachidonic acid (AA), thrombin receptor-activating peptide (TRAP), and ristocetin agonists (20 µL each). The ADP reagent triggers platelet aggregation via ADP receptors. Platelet cyclooxygenase converts AA to thromboxane A₂, which stimulates platelet aggregation. Thrombin receptor-activating peptide stimulates platelet aggregation via the thrombin receptor, while ristocetin forms a complex with GpIb receptor.

Coagulation Assays (Prothrombin Time, Activated Partial Thromboplastin Time, and Fibrinogen)

Blood samples were obtained by venipuncture and collected in 0.105 mol/L trisodium citrate-containing test tubes. The samples were centrifuged at 2000g for 15 minutes. All analytical procedures were carried out on a Sysmex CA-500 (Siemens Healthcare, USA) random access coagulation analyzer, and the reagents were used according to manufacturer's protocol.

Internal quality control and external quality assurance are applied to monitor the accuracy and precision of tests. All tests were performed within 2 hours of blood collection.

Statistics

Statistical analysis was performed with 2-sided Mann-Whitney *U* tests. A *P* < .05 was considered significant.

Results

Results obtained from the study groups are presented in Table 1. In patients with CU, D-dimer levels were significantly higher when compared with HC (*P* = .014). Mean platelet volume in patients with CU was significantly lower than that in HC (*P* = .019). There were no significant differences in platelet distribution width (PDW) and platelet counts between the groups.

Platelet aggregation after ADP and AA stimulation were not different from the HC, but aggregation after ristocetin and TRAP agonists were significantly decreased in patients with CU when compared with HC (*P* < .01 and *P* < .001, respectively).

No significant difference was found in prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen levels between patients with CU and HCs.

Discussion

We investigated platelet aggregation and coagulation parameters in patients with CU. Our findings indicated an altered response of platelets to various stimulants of aggregation in

Table 1. Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), Platelet Counts, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), D-dimer and Fibrinogen Levels, and Aggregation of Platelets After Agonists in Patients With Chronic Urticaria (CU) and Healthy Controls (HCs).^a

	CU (n = 34)	HC (n = 36)	P
Platelets, count/mm ³	275 ± 12.9	282 ± 10.3	.64
MPV, fL	7.44 ± 0.12	7.88 ± 0.13	.01
PDW, %	13.4 ± 0.30	13.2 ± 0.39	.89
AA (AUC)	528 ± 37.4	529 ± 44.9	.97
TRAP (AUC)	559 ± 35.7	756 ± 47.7	.002
ADP (AUC)	427 ± 36.1	463 ± 32.0	.23
Ristocetin (AUC)	322 ± 38.4	631 ± 61.7	.009
PT, sec	13.3 ± 0.18	13.4 ± 0.18	.73
aPTT, sec	30.0 ± 0.51	27.8 ± 0.51	.06
Fibrinogen, mg/dL	337 ± 16.7	316 ± 14.9	.41
D-Dimer, FEU/mL	0.86 ± 0.13	0.38 ± 0.04	.002

Abbreviation: SEM, standard error of the mean.

^aPlatelet aggregation was expressed as area under curve (AUC) after platelet stimulants arachidonic acid (AA), thrombin receptor activating peptide (TRAP), adenosine diphosphate (ADP), and ristocetin (mean ± SEM).

these patients as compared with the HCs. In a previous study, platelet-derived factors were found elevated in the active phase of the disease with a decrease after antihistaminic drug therapy.³⁴ It has also been shown that desloratadine, an antihistaminic agent, is more effective when combined with dipyridamole, a platelet inhibitor, in the treatment of patients with CU.³⁵ Histamine is an important mediator in the appearance of urticaria-related symptoms.^{8,19} Increased levels of histamine have been reported in skin and plasma specimens of patients with CU.³⁶⁻³⁸ Induced activation of mast cell and basophils causes release of histamine and other proinflammatory mediators in CU.³⁹

Association of histamine with platelets in vascular diseases has been reviewed, with the impact of platelet structure and function in the pathogenesis of various vascular diseases.⁴⁰ Histamine uptake by platelets is considered to be an index of platelet behavior in conditions where platelet aggregation is altered (eg, peripheral arterial disease and diabetes mellitus).⁴¹ The synergistic effect between histamine and other monoamines on platelet aggregation may explain some aspects of allergic vasculitis in which platelet aggregation is present.²⁵

In our study, significantly decreased MPV in patients with CU was observed compared with the controls, confirming a previous report,³¹ but in contrast to that study, PDW remained unaltered.

Thrombin, a final product of coagulation cascade, has a role in vessel wound healing, revascularization, and tissue remodeling.⁴² Effects of thrombin both directly on endothelial cells and indirectly through the release of inflammatory mediators have been reported in CU, resulting in increased vascular permeability and edema.^{8,11,39} Thrombin converts fibrinogen to fibrin, also binds to platelet activating receptor (PAR), especially to PAR-1 on platelets, which is followed by aggregation and plug formation. In the study of Asero et al, thrombin formation has been reported to be activated and this activation being triggered

only by the extrinsic pathway in CU.¹¹ In our study, the PT and the aPTT, which are indices of the extrinsic and intrinsic pathways, respectively, remained unchanged in the patients with CU. Also, no difference was observed in fibrinogen levels between the patients with CU and HCs. Previously, Wang et al found no alteration in PT and aPTT in patients with CU and claimed that minor changes in blood coagulation might not be reflected by the coagulation cascade because of a dynamic balance between thrombin generation and secondary fibrinolysis.⁴³ Furthermore, factor XII, a reliable parameter to reflect in vivo activation of the contact system, has been reported to be similar in patients with CU and controls.¹¹ It has been suggested that coagulation factors other than thrombin may be involved in the pathogenesis of CU.⁴³ Various coagulation factors including fibrinogen and fibrin degradation products are known to modulate inflammatory responses by affecting leukocyte migration and cytokine production.⁴⁴ Accordingly, D-dimer levels were significantly increased in our patients with CU, confirming a previous finding.¹⁵ This observation indicates that coagulation and fibrinolytic mechanisms are activated in patients with CU. The controversy with some previous reports demonstrating activated coagulation may be due to the variations in patient selection and the stage of the disease during the laboratory analysis.

Further research may provide a better understanding of the relationship between hemostasis and CU. There is a need to establish whether such a link increases the risk of vascular events.

Authors' Note

This study was presented in European Academy of Allergology and Clinical Immunology (EAACI) Congress held in Geneva, Switzerland, June 16-20, 2012 (poster no: 899, Best poster award in Poster session 34).

Declaration of Conflicting Interests

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