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# Platelet Function and Fibrinolytic Activity in Patients with Bronchial Asthma

Bülent Tutluoglu, MD, Cigdem Bayram Gurel, MSc, Sule Beyhan Ozdas, MSc, Benan Musellim, MD, Serdar Erturan, MD, A. Nihat Anakkaya, MD,* Günseli Kılınc, MD, and Turgut Ulutin, Prof

*Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, and*
**Duzce University Faculty of Medicine Department of Pulmonology, Duzce*

**Summary:** Platelets have the capacity to release mediators with potent inflammatory or anaphylactic properties. Platelet factor-4 (PF4) and beta-thromboglobulin (BTG) are two of these mediators. On the other hand, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) are two important mediators of fibrinolysis. Both mediators are secreted mainly by vascular endothelium. Plasma levels of PF4, BTG, PAI-1, and tPA may show changes in chronic inflammatory diseases such as asthma. This study examined the role of thrombocytes and the function of the endothelium ın asthmatic patients during an attack and during a stable phase. Eighteen patients with known allergic asthma who came to our emergency department with an asthma attack and 14 control subjects were included in the study. Blood samples were taken after starting therapy with salbutamol inhalation. Lung function tests were performed after receiving the first emergency therapy for asthma. Plasma levels of PF4, BTG, PAI-1, tPA were determined before starting steroid therapy and after receiving 1 week of steroid therapy. Plasma levels of PF4 among patients with an asthma attack were significantly higher than those of controls (150.5±8.92 IU/mL vs. 92.5±7.63 IU/mL, $p<0.001$). A further increase in plasma PF4 levels was detected after steroid therapy (163.5±9.16 IU/mL). Plasma BTG levels of patients on admission were not statistically different from those in the control group (140.4±6.34 IU/mL vs. 152.2±8.71 IU/mL). An increase was detected after therapy (171.6±7.27 IU/mL) and post-treatment plasma levels were statistically meaningful versus the controls. Plasma levels of tPA and PAI were statistically higher than those in controls in asthmatic patients on admission (6.01±2.72 vs. 5.4±2.3 ng/mL for tPA and 75.2±27.2 ng/mL vs. 32.7±14.3 ng/mL for PAI-1). Further increases were detected in two parameters after 1 week of therapy with steroids (tPA levels were 6.85±2.96 ng/mL and PAI-1 levels were 83.5±29.6 ng/mL). There seems to be an increased activity of platelets during an asthma attack. Elevated PAI-1 and tPA levels may also indicate the activated endothelium in asthma. Increases of plasma levels of PAI-1 and tPA after steroid therapy need further investigation because elevated PAI-1 levels enhance airway remodeling.
**Key Words:** Asthma attack—PF4—tPA—PAI1—BTG—Platelet.

In the past, studies suggested that platelets are involved in inflammatory phenomena, including bronchial asthma (1–3). Platelets have the capacity to release mediators with potent inflammatory or anaphylactic properties, such as the either-linked phospholipid platelet-activating factor (PAF) or the platelet-specific protein platelet factor-4 (PF4) and, as yet unidentified factors able to induce histamine release from basophils. Alpha granules in the platelet cytoplasm store a variety of proteins, including PF4, platelet-derived growth factor (PDGF), beta-thromboglobulin (BTG), transforming growth factor beta, fibrinogen, and clotting factors V and VII (3).

PF4 posseses many properties that suggest a role in allergy and inflammation. It has been demonstrated to increase the expression of Fc-IgG and FC-IgE receptors (3). PF4 stimulates basophils to release histamine, and is chemotactic for eosinophils and other inflammatory cells. PF4 has the ability to activate eosinophils (4). Although there are negative studies (5,6), in general increased levels of PF4 and BTG in asthmatic patients have been reported in various studies (7–9).

Every inflammatory process including that in the course of bronchial asthma may disturb the balance in the blood coagulation and fibrinolysis

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Address correspondence and reprint requests to Bülent Tutluoglu, MD, Söğütlüçeşme Cad. Bayrak Ap. No: 146/6 81300 Kadıköy/İstanbul; e-mail: bulent@allergist.com.

system. The main proteolytic enzyme in the process of fibrinolysis is plasmin, which is responsible for the degradation of fibrin into fibrin degradation products (FDP) through its effect on blood clots or thrombin. Tissue plasminogen activator (tPA) is produced by endothelial cells and has a role in activating plasminogen to plasmin.

PAI-1 has the main role in the inhibition of fibrinolysis. It is synthesized in endothelium, megakaryocytes, smooth muscle cells of vessels and in the liver, with the help of cytokines, growth factors, cyclic nucleotides, glycocorticosteroids, and bacterial endotoxins. The activation of fibrinolysis are investigated through the assessment of PAP complexes, the concentration of tPA and PAI-1 complexes, and fibrin degradation products, which reflect the effect of plasmin on the thrombus (10).

To examine the role of thrombocytes and function of endothelium in asthmatic patients during an attack and a stable phase, we evaluated the levels of PF4, BTG, PAI-1, and tPA in patients with asthma both during an attack and during the stable phase.

## MATERIAL AND METHODS

Eighteen patients with known allergic asthma who came to our emergency department with an asthma attack and 14 control subjects were included in the study. An asthma attack was defined as an increase in dyspnea, nocturnal awakenings, and exercise intolerance in the previous week, which causes the patient to go to the emergency department.

Ten milliliters of blood was taken into tubes that contained 1 mL of 3.8 % trisodium citrate. Blood was centrifuged at 3000 g for 15 minutes at room tempature and plasma was stored at –70°C until assayed. BTG, PF4, tPA, and PAI-1 levels were determined with enzyme-linked immunosorbent assay (Asniéres Sur Seine, France). Blood samples were obtained after starting therapy with salbutamol inhalation. Lung function tests were performed after the patient received the first emergency therapy for asthma. MIR Spirolab (Roma, Italy ) was used for lung function tests. All the patients were send to their homes after getting better. They received standard therapy, including long acting beta agonists and methyl prednisolone, 40 mg daily. After 1 week of oral steroid treatment, blood samples were taken again from the asthmatic group. A skin prick test was performed during the stable phase by using common allergens. Written informed consent was taken from both the patients and control group before participating in the study.

Characteristics of patients are depicted in Table 1. None of the patients received oral or parenteral steroid therapy at least 1 month before blood samples were withdrawn. The control group included healthy volunteers not receiving any treatment. None of the subjects took medication containing antipyretics or antiplatelet agents that supressed platelet functions during the 2 weeks before blood collection. Neither patients nor the control group were smokers.

### Statistical Analysis

Data were analyzed with the SPSS 10.0 statistical computer program (SPSS, Chicago). The Mann-Whitney test was used to compare the mean values of the two groups. P values less than 0.05 were considered to be statistically significant.

## RESULTS

Mean values and standard deviation of plasma PF4, BTG, PAI-1, and tPA levels both in asthmatic patients and controls are given in Table 2.

Plasma levels of PF4 among patients with an asthma attack were significantly higher than those of controls (150.5±8.92 IU/mL vs. 92.5±7.63 IU/mL, $p<0.001$). A further increase in plasma PF4 levels was detected after steroid therapy—163.5±9.16 IU/mL ($p<0.0001$ compared to control and $p<0.04$ compared to pretreatment phase). Plasma BTG levels of patients on admission were not statistically different from those of the control group 140.4±6.34 IU/mL versus 152.2±8.71 IU/mL ($p>0.05$). An increase was detected after therapy (171.8±7.27 IU/mL) and posttreatment plasma levels were more statistically meaningful than levels in the controls and during the pretreatment period ($p<0.003$ and $p<0.01$).

Plasma levels of tPA and PAI were statistically higher than those in controls in asthmatic patients on admission (6.01±2.72 vs. 5.4±2.3 ng/mL for tPA [$p<0.003$] and 75.2±27.2 ng/mL vs. 32.7±14.3 ng/mL for PAI-1 [$p<0.0001$]). Further increases were detected in two parameters after 1 week of therapy with steroids; tPA levels were 6.85±2.96 ng/mL ($p<0.001$ compared to controls, $p<0.02$ compared to the pretretment period) and PAI-1 levels were 83.5±29.6 ng/mL ($p<0.0001$ compared to controls and $p>0.05$ compared to pretreatment period).

**TABLE 1.** Characteristics of the Patients and the Control Group

| Characteristic | Patients | Control |
|---|---|---|
| Number | 18 | 14 |
| Age | 31.8±8.72 | 33.1±7.9 |
| Gender | 12F/6M | 9F/5 M |
| Predicted $FEV_1$ % | 67±7.4 | 90.3±4.5 |
| Duration of asthma (yr) | 13.3±6.2 | — |
| Skin prick test | + | — |
| Use of inhaled steroids | 15 | — |
| Use of long-acting beta mimetics | 13 | — |
| Use of theophylline | 3 | — |

$FEV_1$, forced expiratory volume in 1 minute.

**TABLE 2.** Plasma PF4, BTG, tPA, and PAI-1 Levels in Patients and Controls

| Parameter | PF4 (IU/mL) | BTG (IU/mL) | tPA (ng/mL) | PAI-1 (ng/mL) |
|---|---|---|---|---|
| Asthma attack (n=18) | 150.5±8.92* | 140.4±6.34 | 6.01±2.72* | 75.2±27.2* |
| After 1 week therapy | 163.5±9.16† | 171.8±7.27* | 6.85±2.96* | 83.5±29.6* |
| Controls (n=14) | 92.5±7.63 | 152.2±8.71 | 5.4±2.3 | 32.7±14.3 |

*$p < 0.01$, †$p < 0.001$.

## DISCUSSION

Platelets are present on the epithelial surface of lung tissue from symptomatic asthmatics (11) within the microvaculature of lung tissue (12) and in BAL fluid (13).

Platelets may play a role in the allergic inflammatory process because they are a rich source of a wide range of biologically active materials capable of inducing or augmenting allergic inflammatory responses. Such materials have been demonstrated to be preformed mediators stored in alfa granules, which are chemokines such as PF4 and BTG (3).

Some evidence for platelet abnormalities in asthmatic patients has been reported. A number of studies demonstrated that circulating platelets are aggregated (14) and platelet-specific proteins PF4 and BTG were released into the circulation (7,15) and BAL fluid (13) during provoked or spontaneous asthmatic attacks. PF4 has also been shown to stimulate human eosinophils or basophils, which are important mediators of inflammation (16). RANTES, which might be released from activated platelets, has an important role in various immune and allergic disorders (16). A positive correlation between elevated RANTES levels and BTG levels suggested that platelet activation might be one of the possible mechanisms for the increase of RANTES in asthmatic patients (17).

The plasminogen activator system (PA) system has an important role in controlling endogeneous fibrosis and regulating extracellular matrix (ECM) proteolysis relevant to tissue remodeling (18). An important mechanism in the regulation of PA activity is PAI-1, which inhibits tPA and has a potential relevance to the process of lung fi-

brosis (19). tPA-1 converts plasminogen to plasmin, which enhances proteolytic degradation of the ECM. Deficiency of PAI-1 is associated with increased fibrinolysis.PAI-1 (20). Detection of high levels of PAI-1 gene expression is strongly correlated with the amount of collagen accumulation within lung tissues, suggesting that the balance of fibrinolytic activity within the lung is an important determinant of the pulmonary response to inflammatory injury (21). Endothelial cells are a major source of tPA and PAIs. Activated macrophages and smooth muscle cells are another source of PAs and PAIs (22,23). Stimulated human mast cells are able to secrete functionally active PAI-1 (24).

Elevated PAI-1 promotes ECM deposition in the asthmatic airway by inhibiting MMP-9 activity and fibrinolysis. In PAI-1 knockout ovalbumin-sensitized mice, collagen deposition was twofold less, fibrin deposition was fourfold less, and MMP-9 activity was threefold higher (21). A significant association was observed between the PAI-1 promoter polymorphism and IgE-mediated allergic diseases (25).

We found high PF4 levels in asthmatic patients both during an attack and during the stable phase. We found higher levels during an attack. Although the PF4 levels decreased after 1 week of treatment, it was still significantly higher than that in the control group. Our results suggested that platelet functions are increased in asthmatic patients. This was more prominent during an attack. Our findings were consistent with the literature.

We couldn't find any significant changes between BTG levels in patients during an asthmatic attack and the control group. This may be due to the relatively small amount of patients who participated in the study. However, after 1 week of prednisone therapy, BTG levels increased to a level that was significantly higher than that in controls.

In this study, higher PAI-1 and tPA levels were found in asthmatic patients. Levels of these parameters were higher during attacks and further increases were seen after treatment.

We assume that increased levels of PAI-1 in asthma may promote fibrin and collagen deposition in the airways. It is well known that fibrin and collagen deposition is the main feature of airway remodeling. In a clinical study, prednisolene treatment has been shown to increase PAI-1 levels (26). This is an interesting issue because it brings up the question of whether frequent usage of oral or parenteral steroids may promote airway remodeling.

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