Platelet Functions in Patients with Allergic Asthma

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ABSTRACT

To investigate platelet functions in patients suffering from allergic diseases including asthma, blood was collected from ten asthmatic patients (five females, five males) and ten healthy controls (five females, five males) and by using whole-blood electrical impedance system; platelet count and platelet aggregation studies (maximum aggregation extent, maximum aggregation rate) were performed. Allergy screening was performed with skin test reactions and with high total and specific immunoglobulin E levels (CAP-Phadiatop system).

Platelet count (333.1 ± 41.1 x 10^9/L), collagen induced the response of platelet aggregation (12.95 ± 4.19) and maximum rate of aggregation (8.00 ± 5.22) in allergic patients were found significantly higher than those of controls (252.1 ± 49.1 x 10^9/L; 8.33 ± 1.19; 4.28 ± 1.31) ($p< 0.05$). Also ADP induced response of platelet aggregation (18.21 ± 3.56) and maximum rate of aggregation (10.64 ± 2.12) in asthmatic patients were higher than controls (12.37 ± 2.63; 7.80 ± 1.64) with statistical significance ($p< 0.01$).

Secretion products of activated platelets such as histamine, serotonin, PGF2α and PAF may play role in bronchial responsiveness in allergic asthma. The results of this study showed that platelet function tests were effected in asthmatic patients. The changes in platelet functions are thought to be related with increased IgE levels and stimulation of platelets by these antibodies.

Key Words: Platelet functions, Allergic asthma, IgE levels.

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INTRODUCTION

Platelets are multifunctional cells, involving in blood coagulation and allergic response via secretion products as leukotriens, prostoglandins, PAF, serotonin, histamine. Especially platelet activating factor (PAF), PGF2α, serotonin and histamine may play a role in bronchial responsiveness in allergic asthma. IgE antibodies by stimulating membranous IgE receptors can activate platelets[1]. With activation of platelets aggregation response occurs as a two-phased action[2]. Epinephrine, ADP, collagen and thrombin are some of aggregation inducers[3].

Platelet functions are altered in patients suffering from atopic diseases including asthma[4]. Such alterations might be related to IgE level. It was shown that platelets of atopic patients differ in alpha-granular content (IgE pool), 10 fold higher compared to nonatopic patients[1]. But some investigators reported that during seasonal changes no correlation was seen between aggregatory responses and IgE titres[5].

Platelet dysfunction has been detected as a reduced aggregatory response to collagen and PAF in asthmatic patients[6]. Analysis of platelet functions is of great importance for diagnosis of atopic and nonatopic forms of bronchial asthma and for a choice of adequate therapy[3].

Another subject is bleeding pattern of atopics. Because of raised plasma heparin levels in atopic patients, thrombin formation was delayed and bleeding times of atopics were longer than normals[5,7].

In some diseases such as Wiskott-Aldrich syndrome, oculocutanous albinism and with some ingested drugs like aspirin, indomethacin, EDTA, alpha adrenergic and beta blocking agents platelet dysfunction can be seen[2].

We planned this study to investigate platelet functions in patients suffering from allergic diseases including asthma.

MATERIALS and METHODS

In this study, platelet functions of ten healthy controls and ten patients who had allergic asthma disease were examined. Blood was collected from all subjects who had not taken any platelet inhibiting drugs for ten days. Blood was carefully withdrawn from a trauma-free venipuncture with rapid flow of blood without venous occlusion from antecubital vein by two-syringe technique[8]. After discarding the first 3-4 mL,10 mL of blood was immediately transferred into polypropylene tubes containing 3.8% sodium citrate (9:1 v/v)[9]. Three additional mL of blood was collected in a tube containing EDTA for platelet count[10].

Whole Blood Electrical Aggregation

All platelet aggregation studies were performed 30-60 minutes after blood collection. Platelet aggregations were performed in citrated blood. 450 µL blood diluted with 450 µL saline were placed in a polypropylene cuvette and incubated in the whole-blood aggregometer (Model NO 560, Chrono-Log Corp., Howertown, PA, USA) equipped with a dual-Channel recorder at 37°C for five minutes by the electrical impedance method[9]. Whole blood stirred by stirrbars at 1000 rpm during the aggregation inducing agent was added. Collagen (Chrono-Par Reagent 385) was used as aggregating agent in whole-blood electrical aggregometry. Volume of 2 µL of the commercial solution of collagen was added to saline diluted blood to reach final concentration of 2 µL/mL[11]. The changes in electrical impedance were registered in a strip chart recorder. After addition of collagen, each aggregation was recorded until the maximal extent of aggregation was reached. The extent of aggregation was determined from maximum height of response in ohms (8 cm = 20 ohm) and the maximum rate of aggregation from the maximum incremental slopes of the individual aggregation waves[8,12]. Because platelet number may affect aggregation response, platelet count was performed before to all samples and all were found in normal ranges[10]. Aggregation was also measured in response to ADP (adenosine diphosphate) at the concentration of 10 µmol/L.

Statistical Analysis

Results were expressed as mean ± standart deviation of mean. The data was analyzed by Mann Whitney U test.

RESULTS

In this study ten asthmatic patients (five females, five males) range of age 35-45 (39.0 ± 2.7 years old) and ten healthy controls (five females, five males) range of age 36-45 (39.5 ± 3.3 years old) were compared. There was no significant difference among ages of gro-

ups. Neither of them had an additional disease effecting platelet function and had taken any platelet inhibiting drugs for the last ten days.

We found that platelet count, collagen and ADP induced maximum extent of platelet aggregation and maximum rate of aggregation in allergic patients were significantly higher than those of controls (Table 1 and 2). All of these results were statistically significant ($p< 0.05$).

DISCUSSION

For many years, the mast cell has been considered the principal cell in bronchial asthma. During the last 2 decades some evidences like induction by PAF, association with airway hyperreactivity suggest that platelets might be involved in asthma[13-15]. In a study comparing the blood parameters in patients with bronchial asthma and pleuritis due to tuberculosis; lymphocyte and eosinophil count were found higher in asthmatic group. Additionally, giant trombocytes in large clumps, reactive lymphocytes, hypersegmentation and toxic granulation in neutrophils were observed in microscopic evaluation[16].

Allergens via IgE can activate platelets. This activation can result in a clinical presentation such as allergic asthma[6]. Platelet aggregation is an index of platelet activation[13]. With platelet activation in atopic patients great amounts of thromboxane A2 are produced which is a potent aggregator[3]. Despite of this reality platelet dysfunction has been detected as a reduced aggregatory response to collagen, PAF, ADP, epinephrine, thrombin in atopic patients[5,6,17,18]. Analysis of platelet functions is valuable in diagnosis of atopic or nonatopic forms of bronchial asthma[3].

Aggregatory response of platelets to epinephrine, ADP, collagen, thrombin exists as a two phased action. In atopic patients especially second phase of aggregation becomes pathologic[5]. Another important point is seasonal variations in response of platelets in atopic patients. In allergic season aggregatory response to epinephrine, collagen, ADP and thrombin becomes pathologic. It was reported that during out season, when res-

Table 1. Platelet count, collagen induced maximum aggregation extent, maximum aggregation rate of allergic patients and controls

	Platelet count x 10^9/L	Maximum Agg. extent (Ohm)	Maximum Agg. rate (Ohm/min)
Allergic patients (n: 10)	333.1 ± 41.1	12.95 ± 4.19	8.00 ± 5.22
Control subjects (n: 10)	252.1 ± 49.1	8.33 ± 1.19	4.28 ± 1.31
p	p< 0.05	p< 0.05	p< 0.05

Agg: Aggregation
(p< 0.05: Statistically significant)

Table 2. Platelet count, ADP induced maximum aggregation extent, maximum aggregation rate of allergic patients and controls

	Platelet count x 10^9/L	Maximum Agg. extent (Ohm)	Maximum Agg. rate (Ohm/min)
Allergic patients (n: 10)	333.1 ± 41.1	18.21 ± 3.56	10.64 ± 2.12
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Agg: Aggregation
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ponse to epineprine improved partly, response to collagen returned completely. ADP and thrombin responses showed no improvement. No correlation was found between improved responses and IgE titers[2]. But conformational changes might occur in platelet membrane IgE receptor during out season, this would explain response improvement and show that this is not a permenant alteration. These findings point that there might be a humoral factor that plays role in platelet responses.

By the activation of platelets, some adhesion molecules (CD62P and CD63) are expressed, these molecules mediate interactions between platelet themselves and platelets and other cells. Reduced platelet aggregatory functions in atopics may be the result of decreased expression of these molecules[19].

In atopic patients bleeding time prolongation also occurs. Although the platelet mass is increased in atopics, it is not enough to compensate depressed aggregatory functions[20,21].

In house dust mite sensitive patients with allergic disease, before treatment with specific immunotherapy, diminished platelet aggregation response (platelet hyposensitiveness) was observed and platelet aggregation partially improved after treatment[22].

The lack of synergistic response to collagen with PAF was found after incubation of the platelets with PAF invitro. In patients with positive inhalative allergen provocation test, a significant reduction of the aggregation response was found[23].

It has been established that a range of molecules mainly associated with the platelet surface or platelet granules, regulate the capacity of platelet interaction with inflammatory cells. Platelet derived growth factor (PDGF) secreted from platelet a-granules, with P-secretin expressed on the platelet surface and with platelet histamine which is secreted from platelets in response to inflammatory stimuli[24].

Formerly, it was commented that, the detection of platelet aggregation responses to additional aggregation agents (thrombin, arachidonic acid etc.) and other platelet functions such as platelet adhesion, secretion, platelet coagulant activities and platelet arachidonate metabolism could be useful for evaluation of alteration in platelet responses of bronchial asthma[25,26].

In our study, platelet count of asthmatic patients were significantly higher than controls. This finding correlates with literature[20,21]. We evaluate platelet functions by using collagen and ADP induced maximum aggregation extent and maximum aggregation rate parameters. Both parameters were higher in asthmatic patients than controls. As a result in atopic patients we found an increased aggregation response to collagen and ADP, it seems as a paradox with literature[2,5,6,20]. First of all in asthmatic patients a platelet activation is present, related to this reality as a index of activation it is normal to find increased aggregation response to collagen and ADP and in other studies, either inducers or doses of epinephrine, thrombin used, might be different. Second this result may be associated with out seasonal response improvement. It was shown that during out seasonal period significant and complete aggregatory improvement to collagen occurred. Third, when platelets of asthmatic patients were seperated from plasma (humoral factor), response to collagen returned to normal. Various factors may affect adhesion and aggregation. Vasoactive mediators work as a network and endothelial injury is also present. For this reason biochemical studies are needed. Fourth, thromboxane A2 is a potent aggregator and its production is increased in atopic patients from PGG2 and PGH2. As a result it is normal to find an increased response to collagen and ADP. Fifth, in this study we did not separate and evaluate aggregation responses into phases. May be if we had done a phased study, we might also had a reduced response to collagen and ADP as a second wave dysfunction. In atopics, especially second wave dysaggregation exists. Finally we point out that it is not a rule to find a decreased aggregatory response of platelets in allergic patients. But it is obvious that a functional alteration occurs.

In clinical practice, most of asthmatic patients exhibit platelet malfunction (especially second phase dysaggregation), similarity is also seen in allergic rhinitis. This platelet abnormality in allergic rhinitis may indicate a predisposition to asthma later in life. Steroid therapy is required for the patients with abnormal platelet aggregation.

During allergy season allergic patients may have longer bleeding time, it is parallel to platelet dysfunction, These patients must be explored for bleeding time prolongation.

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