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Plasma Levels of β -Thromboglobulin – an Indicator of *in vivo* Platelet Activation in Patients with Pollen-Induced Intermittent Allergic Rhinitis During the Pollen Season and out of Season*

Stężenie β -tromboglobuliny w surowicy – wskaźnika aktywacji płytek krwi *in vivo* u chorych na sporadyczny alergiczny nieżyt nosa indukowany pyłkami w okresie naturalnej ekspozycji na alergeny i poza sezonem pylenia

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Abstract

Background. The active participation of platelets in allergic inflammatory response is documented. Increased release of platelet activation markers has been detected in the peripheral blood of patients with allergic diseases.

Objectives. The aim of the study was to investigate changes in platelet activation occurring in the circulation of patients with pollen-induced intermittent allergic rhinitis (IAR). The study was carried out in a dynamic state, during natural allergen exposure and out of season.

Material and Methods. Plasma levels of β -thromboglobulin (β -TG) as an indicator of platelet activation were measured in patients (10 men; median age 24.5 years, range: 18–30 years) suffering from pollen-induced IAR sensitive to grass and/or trees pollens and healthy nonatopic control subjects (15 men; median age was 25 years, range: 19–30 years) by using the enzyme-linked immunosorbent assay (ELISA) kits.

Results. Median levels of β -TG in these patients during the pollen season did not differ significantly compared with out-of-season, nor with controls.

Conclusion. There is no evidence of altered platelet activity (expressed by levels of β -TG) in patients with pollen-induced IAR during natural allergen exposure and symptom-free period (*Adv Clin Exp Med* 2005, 14, 1, 59–62).

Key words: platelet activation, β -thromboglobulin, allergic rhinitis.

Streszczenie

Wprowadzenie. Istnieją dane wskazujące na aktywny udział płytek krwi w procesach alergicznego zapalenia. Wzrost stężenia markerów aktywacji tych komórek wykazano we krwi chorych na choroby alergiczne.

Cel pracy. Ocena stopnia aktywacji płytek krwi u chorych na sporadyczny alergiczny nieżyt nosa (IAR – *intermittent allergic rhinitis*) w czasie objawów choroby (w sezonie pylenia) oraz w okresie remisji (jesiennie-zimowym).

Materiał i metody. Do oceny aktywności płytek krwi stosowano pomiar stężenia β -tromboglobuliny (β -TG) w osoczu metodą immunoenzymatyczną (ELISA). Badaniem objęto 10 mężczyzn chorych na IAR, uczulonych na pyłki traw i/lub drzew (w wieku 18–30 lat; mediana 24,5) oraz 15 zdrowych nieatopowych osób (w wieku 19–30 lat; mediana 25,0).

Wyniki. Nie obserwowano istotnych statystycznie różnic między medianą stężenia β -TG u chorych na IRS w sezonie pylenia i w okresie pełnej remisji objawów choroby w porównaniu z grupą kontrolą.

Wnioski. Naturalna ekspozycja alergenowa (sezon pylenia) pozostaje bez wpływu na aktywność płytek krwi, ocenianą na podstawie stężenia β -TG we krwi obwodowej u chorych na IAR (*Adv Clin Exp Med* 2005, 14, 1, 59–62).

Słowa kluczowe: aktywacja płytek krwi, β -tromboglobulina, alergiczny nieżyt nosa.

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Platelets play well-known role in haemostasis process. In addition, an immune modulatory role of platelet-derived biologic mediators in allergic inflammation is postulated [1, 2].

It is possible that enhancement of platelet activation in response to inflammatory or immune stimuli (i.e. PAF-platelet activating factor and IgE), contribute to the pathogenesis of atopic disorders. In allergic asthmatics, the percentage of platelets that bound IgE was markedly higher compare to normal donors, and these platelets could be directly activated by contact with the corresponding allergen [3]. β -TG is a platelet-specific protein that is released from α -granules of activated platelets and belongs to the family of chemokine molecules, which are considered to be important mediators of inflammation. Moreover, plasma β -TG seems to be a sensitive marker of *in vivo* platelet activation process [4, 5].

Based on earlier results of studies suggesting an important link between alterations in platelet function and respiratory atopy [6, 7], the authors estimated changes in the plasma levels of β -TG – an indicator of platelet activation in patients with pollen-induced intermittent allergic rhinitis (IAR) during the pollen season and out of season.

Material and Methods

The study group consisted of 10 non-smoking patients (10 males; the median age was 24.5 years, range: 18–30 years) with clinical diagnosis of pollen-induced IAR and a positive skin-prick test to grass pollens (four patients), three patients to trees pollens and three both to grass and trees pollens. All patients were symptomatic at the time of the investigation and were studied during a period of natural pollen exposure. All patients with IAR were also examined at a time when they were free of symptoms, out of pollen season.

The control group consisted of 15 non-smoking healthy men (age range 19 to 30 years, median 25.0 years) with no signs of atopy. The controls had negative skin prick tests against a panel of allergens and were studied during the pollen season. All subjects and controls have not been treated with any drug for 2 weeks prior to the examination.

The Local Ethics Commission approved this study and written informed consent was obtained from all participating subjects.

Skin Prick Tests

Allergic status was evaluated by skin prick testing with a panel of 10 allergens: grass pollens, rye pollen, trees pollens, feather, *Derma*thopha-

goides pteronyssinus, cat dander, dog dander and molds (Allergopharma, Hamburg, Germany).

Blood Sampling, Measurement of β -TG

Blood was taken with slight or no stasis from the antecubital vein into Diatube® H tubes (Becton Dickinson) which were immediately placed in the ice/water both for 20 minutes. Then the tubes were centrifuged at 2500 g for 30 minutes at 4°C, and top third the volume of the resultant plasma supernatant were collected and frozen at –20°C for assay. β -TG was assayed by enzyme-linked immunosorbent assay (ELISA) kit (Asserachrom®, Diagnostica Stago, France) according to the manufacturer's instructions.

Immunological Tests

Total IgE were estimated by ELISA (Allergopharma, Hamburg, Germany kit).

Other Laboratory Investigations

Total platelets count was calculated by a direct method.

Nomenclature

The nomenclature for allergic diseases is used according to EAACI recommendation [8].

Statistical Analysis

The levels of platelet activation marker and platelets count were compared between patients and healthy controls using the Mann-Whitney test. Wilcoxon's signed rank test was employed to compare in- and out-of-season the same variables. Data are presented as median and range. *p* values less than 0.05 were considered significant.

Results

β -TG plasma levels in the patients with IAR during the pollen season did not differ significantly compared with out-of-season, nor with controls (Tab. 1). No significant differences in total platelet count were found in patients during the season compared with out of season and control subjects

Table 1. β -TG levels and platelet counts in plasma from patients with IAR during (n = 10) and out of season (n = 10) and controls (n = 15)**Tabela 1.** Stężenie β -tromboglobuliny i liczba płytek krwi w osoczu u pacjentów chorych na IAR w sezonie pylenia (n = 10), poza sezonem pylenia (n = 10) i w grupie kontrolnej (n = 15)

Analyzed parameters (Badane wskaźniki)	Healthy (Osoby zdrowe)	IAR patients (Chorzy na IAR)		Statistical analysis (Analiza statystyczna)
		during season (sezon pylenia)	out of season (poza sezonem pylenia)	
β -TG (β -tromboglobulina) IU/ml				
Median (Mediana)	24.3	19.5	24.25	ns.
Range (Przedział)	10–36.4	6.6–35	8.6–38	
Blood platelets (Płytki krwi) $\times 10^9/l$				
Median (Mediana)	217	240	260	ns.
Range (Przedział)	173–333	185–320	190–350	

ns. – not significant.

ns. – nieistotne statystycznie.

(Tab. 1). In patients with IAR the median total IgE level was 536.5 KU/ml (range 88–622) (the control level of total IgE lower than 50 KU/ml).

Discussion

Platelet dysfunction was observed in respiratory atopy. Some types of platelet abnormalities have been described in patients with nasal allergy and asthma, characterised by prolonged bleeding time, reduced platelet aggregation to several aggregating agents [6, 7], and increased platelet mass [7]. In authors' previous study, platelet aggregation after stimulation by ADP, thrombin and collagen in patients with atopic/eczema dermatitis syndrome (AEDS) were not impaired [9]. Interestingly, the relation of platelet aggregation dysfunction induced by specific aggregating agents to the clinical activity of allergic disease has been demonstrated in patients with allergic rhinitis. Abnormal epinephrine- and collagen-induced aggregation, which was observed during the pollen season improved significantly several months after the end of the allergy season, thereby suggesting cyclic platelet dysfunction in IgE-mediated allergy [6].

Mori et al. [2] reported that platelet-derived endothelial cell growth factor may be involved in the pathogenesis of allergic rhinitis. As mentioned earlier, β -TG is platelet-specific chemokine that is released from α -granules of activated platelet and

may play a role in pathophysiology of allergic inflammation [4].

Yamamoto et al. [10] reported that platelet activation in the circulation was provoked in bronchial asthma, and PAF is likely to be a mediator responsible for the platelet activation. Elevated mean plasma levels of β -TG and platelet factor 4 (PF4) were observed in a certain proportion both in asymptomatic and in symptomatic patients with bronchial asthma [10]. No significant difference was found in the mean values for PF4 and β -TG between normal and unstable asthmatic subjects with nocturnal symptoms of this disease [11]. In this study, a circadian rhythm for β -TG, but not for PF4 plasma levels was detected.

In keeping with their previous study [12], the authors have observed similar plasma level of β -TG in patients with IAR and healthy controls when measurements were performed during the pollen season. Furthermore, when determination of platelet activation was repeated out of pollen season, there were no significant differences in the β -TG plasma levels of IAR patients compared to their β -TG levels during the pollen season.

In summary, these findings suggest that systemic platelet activity (characterised by β -TG) in IAR patients during the pollen season is no different to the platelet activity after period of natural allergen exposure and healthy nonatopic subjects.

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