



Mean Platelet Volume is Increased Only by Subcutaneous Allergen Immunotherapy But not in Allergic Diseases

Ortalama Trombosit Hacmi Allerjik Hastalıklarda Artmamakla Birlikte Subkutan İmmünoterapide Artmaktadır

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ABSTRACT

Objective: Platelets play a role in the pathogenesis of inflammation beside hemostasis. Mean platelet volume (MPV) is used as a biomarker of platelet activation. Although the platelet's role in the pathogenesis of allergic diseases is well known, the MPV value is still controversial in allergic diseases. The objective is to observe the changes of the MPV value in patients with allergic diseases and patients receiving subcutaneous venom and aeroallergen immunotherapy (SCIT).

Materials and Methods: Patients who are followed by our pediatric allergy and immunology clinic were enrolled in the study. The study group included patients with atopic dermatitis, food allergy, chronic urticaria, allergic rhinitis, asthma and obese asthmatics. The control group consisted of age- and gender-matched and otherwise healthy children. The value of MPV was recorded and compared to the control group's value. The value of MPV was evaluated at the beginning and at the second year of SCIT.

Results: Study subgroups included patients with atopic dermatitis (n:132), food allergy (n:47), allergic rhinitis (n:48), chronic urticaria (n:102), asthma (n:22), and obese asthmatics (n:31). The value of MPV was not statistically different between the patient and control groups. The severity of atopic dermatitis, autoimmunity of chronic urticaria and presence of obesity in asthmatic patients did not influence the value of MPV. The beginning and the second year median (IQR) MPV levels of the patients receiving SCIT were

ÖZ

Amaç: Trombositler hemostaz yanında inflamasyonun patogenezinde rol oynar. Ortalama trombosit hacmi (MPV) trombosit aktivasyonunun biyolojik belirteci olarak kullanılır. Allerjik hastalıkların patogenezinde trombositlerin rolünün iyi bilinmesine rağmen MPV değeri allerjik hastalıklarda halen tartışmalıdır. Çalışmanın amacı Allerjik hastalıkları olan hastalarda ve subkutan venom ve aeroallerjen immünoterapisi alan hastalarda MPV değerindeki değişiklikleri izlemektir.

Gereç ve Yöntem: Pediatrik allerji ve immünoloji kliniğimiz tarafından izlenen hastalar çalışmaya alındı. Allerjik hastalıklar grubuna atopik dermatit, besin allerjisi, kronik ürtiker, allerjik rinit, astım ve obez astımlı hastalar dahil edildi. Kontrol grubuna yaş ve cinsiyet uygun sağlıklı kontroller alındı. Allerjik hastalıklar grubu MPV değerleri ve kontrol grubu MPV değerleri karşılaştırıldı. Subkutan immünoterapi grubuna venom ve aeroallerjen immünoterapisi yapılan hastalar alındı. İmmünoterapi öncesi ve immünoterapinin 2. yılındaki MPV değerleri karşılaştırıldı.

Bulgular: Atopik dermatit grubunda 132, besin allerjisi grubunda 47, allerjik rinit grubunda 48, kronik ürtiker grubunda 102, astım grubunda 22, obez astım grubunda 31 hasta mevcuttu. MPV değeri hasta grupları ve kontrol grupları arasında istatistiksel olarak farklı değildi. Astımlı hastalarda obezite eşlik etmesi, atopik dermatitte şiddet skoru sınıflaması, kronik ürtikerde otoimmünite varlığı MPV değerinde istatistiksel anlamlı farklılık oluşturmadı.

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7.5 (7.1-8.1) fl and 8.6 (7.6-9.2) fl respectively in the aeroallergen immunotherapy (IT) group, and 7.9 (7.2-8.4) fl and 8.8 (8.3-9.3) fl respectively in the venom immunotherapy (VIT) group ($p<0.001$).

Conclusion: This study has shown that MPV changes significantly over the years in SCIT.

Key words: Mean platelet volume, immunotherapy, children, venom, aeroallergen, atopic dermatitis, food allergy, asthma, obese asthmatics

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Subkutan immünoterapi alan hastaların immünoterapi öncesi ve ikinci yıl ortanca MPV değerleri aeroallerjen immünoterapi grubunda sırasıyla 7.5 (7.1-8.1), 8.6 (7.6-9.2); venom immünoterapi grubunda sırasıyla 7.9 (7.2-8.4) fl, 8.8 (8.3-9.3) fl idi ($p<0.001$).

Sonuç: Bu çalışma ile subkutan immünoterapide MPV değerinin yıllar içinde anlamlı olarak değiştiği gösterilmiştir.

Anahtar kelimeler: Ortalama trombosit hacmi, immünoterapi, çocuklar, venom, aeroallerjen, atopik dermatit, besin allerjisi, astım, obez astım

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INTRODUCTION

Platelets play an important role in various physiological functions such as haemostasis, wound healing and inflammation (1,2). Platelet activation is associated with a rapid intracellular reorganization of actin and microtubule components of the cytoskeleton. This reorganization produces a considerable enhancement of the platelet surface area. More active platelets therefore tend to exhibit a larger volume than do less active platelets (3). MPV is the indicator of the average size of platelets and is increased after activation (4). MPV is now automatically estimated by the vast majority of commercial hematologic analyzers (5), thus providing an inexpensive, easy, fast and reliable parameter to help studying thrombocyte activation in many diseases. The relevance of MPV has been clearly underlined in studies carried out in patients with chronic inflammatory conditions (6-11). Some of these studies have found that value of MPV is elevated (6,7,10). In contrast, others have reported that the value of MPV are reduced (8,9,11).

Platelets have been shown to express both high-affinity and low-affinity IgE receptors on their surfaces (12). Therefore, they are thought to play a role in allergic inflammation. Alterations of MPV value have been shown in allergic diseases like chronic urticaria and asthma (13,14). Although these studies have contradictory results, the changing of MPV values could indicate inflammation in general. The aim of this study was to investigate the values of MPV in children with atopic dermatitis, food allergy, chronic urticaria, allergic rhinitis and obese asthmatics.

Based on these studies, we hypothesized that MPV levels would change during AIT. AIT, which is the only way of changing the destination of allergy, changes the

response to allergen exposure by inducing immunological tolerance. Allergen-specific T cells are activated, leading to an inflammatory reaction. Therefore, we evaluated whether there was any effect of AIT on the changes of MPV in children with asthma and venom anaphylaxis during IT.

MATERIALS and METHODS

This hospital-based, cross-sectional study was conducted in patients with atopic dermatitis, food allergy, chronic urticaria, allergic rhinitis, and asthma, and on obese asthmatics. The control group included age- and gender-matched healthy controls. We also evaluated the changes in the value of MPV by both venom and aeroallergen IT. The values were compared with those at the beginning and at the second year of SCIT. The study was approved by the local ethics committee.

The patients with atopic dermatitis were grouped as mild, moderate and severe using the "severity scoring of atopic dermatitis" index. All patients with food allergy had IgE-mediated food allergies. Patients with chronic urticaria were evaluated based on a positive and negative autologous serum skin test. All the patients with allergic rhinitis were sensitive only to grass pollens. Blood samples were taken from these patients in season and off season. All the patients with asthma and obese-asthma were under treatment and control.

VIT was performed with the culprit insect venom. Aluminum hydroxide-adsorbed venom extract preparations (Alutard SQ® 100% *A. mellifera* or *Vespula spp.* ALK, Horshølm, Denmark) were used.

Pollen immunotherapy with grass pollen was performed using aluminum hydroxide-adsorbed standard allergen extract preparations (ALK-Abelló, Madrid,

Spain and Allergopharma, Germany). House dust mite immunotherapy was performed using aluminum hydroxide-adsorbed standard allergen extract preparations (Allergopharma, Germany).

A conventional protocol was used for VIT, pollen immunotherapy and house dust mite immunotherapy groups. After reaching the maintenance dose, injection intervals were every 4 weeks throughout the maintenance phase. Injections were administered subcutaneously at the distal lateral aspect of the upper arm.

Venous blood samples were collected in vacuette tubes. Complete blood count analyses were performed using a Coulter MaxM device (Beckman Coulter).

Statistical Analysis

Statistical analyses were performed using the SPSS software, version 20. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they were normally distributed. Descriptive analyses were presented using medians and interquartile range (IQR) for the non-normally distributed and ordinal variables. Since the age and MPV levels were not normally distributed; nonparametric tests were conducted to compare these parameters, as well as to compare the ordinal variables. The Mann-Whitney U test was used to compare MPV levels between the patient and the control groups. The Wilcoxon test was used to compare the change in MPV levels between baseline and second years in the SCIT group. A p-value of less than 0.05 was considered to show a statistically significant result.

RESULTS

The study subgroups includes patients with atopic dermatitis (n:132), food allergy (n:47), allergic rhinitis (n:48), chronic urticaria (n:102), and asthma (n:22), in addition to obese asthmatics (n:31). Median ages (IQR) of patients with atopic dermatitis, food allergy, chronic urticaria, allergic rhinitis, and asthma and the obese asthmatics were 7 (5-12) months, 8 (5-13) months, 9.2 (7.3-11.2) years, 9.7 (7.5-10.9) years, 10.1 (7.7-11.6) years and 9.3 (8.2-10) years, respectively. Median ages (IQR) of patients receiving venom (n=23, ALK specific) and aeroallergen IT [pollen immunotherapy (n=28, ALK specific; n=6, Allergopharma) and house dust mite IT (n=6, Allergopharma)] were 8.2 (6.6-12.4) and 12.5 (10.5-14.6) years, respectively.

Median MPV levels (IQR) of patients with atopic dermatitis, food allergy, chronic urticaria, allergic rhinitis, asthma and asthma obese were 7.4 (7-8) fl, 7.6 (7.2-8.1) fl, 7.8 (7.4-8.6) fl, 8.4 (7.7-8.9) fl, 7.8 (7.5-8.4) fl and 7.7 (7.3-8.1) fl respectively. There was no difference between the patient groups and with the control group ($p>0.05$). Comparisons of the MPV values in allergic diseases and control groups have been shown in Figure 1A-F.

The severity of atopic dermatitis did not affect the values of MPV significantly. The values of MPV was not also significantly different in the patients with chronic urticaria whose autologous serum skin tests were positive or negative [8 (7.4-8.5) fl and 8 (7.5-8.6) fl respectively]. An effect of seasonal differences was not observed in patients with allergic rhinitis and asthma. No difference was detected in MPV levels in season and off season [in season MPV median (IQR) 8.5 (7.7-9.1) fl, off season MPV 8.6 (7.9-9.2) fl].

However, during SCIT, the values of MPV gradually changed. At the beginning and the second year, median (IQR) MPV levels of the patients receiving SCIT were 7.5 (7.1-8.1) fl and 8.6 (7.6-9.2) fl respectively in the aeroallergen IT group (Figure 2A) and 7.9 (7.2-8.4) fl and 8.8 (8.3-9.3) fl respectively in the VIT group (Figure 2B). MPV value in patients who have received SCIT showed an increase in the groups (aeroallergen IT group MPV0-MPV2: $p<0.001$; in VIT group MPV0-MPV2: $p=0.001$).

DISCUSSION

The main finding of the study is that MPV value gradually increased in patients with receiving aeroallergen IT and VIT groups. Secondly, there was no difference observed in values of MPV in patients with allergic diseases.

Recently, MPV value has been studied in various allergic diseases as a marker of activation. The MPV value is accepted as one of the potential indicators of platelet production and consumption (15). It has been suggested that changes in the value of MPV are correlated with the function and activation of platelets (16,17).

There is no consistency between studies showing MPV changes in allergic diseases. Unlike other studies, MPV changes were assessed in patients with various allergic diseases in our study. There is only one study assessing the MPV change in atopic dermatitis in the literature.

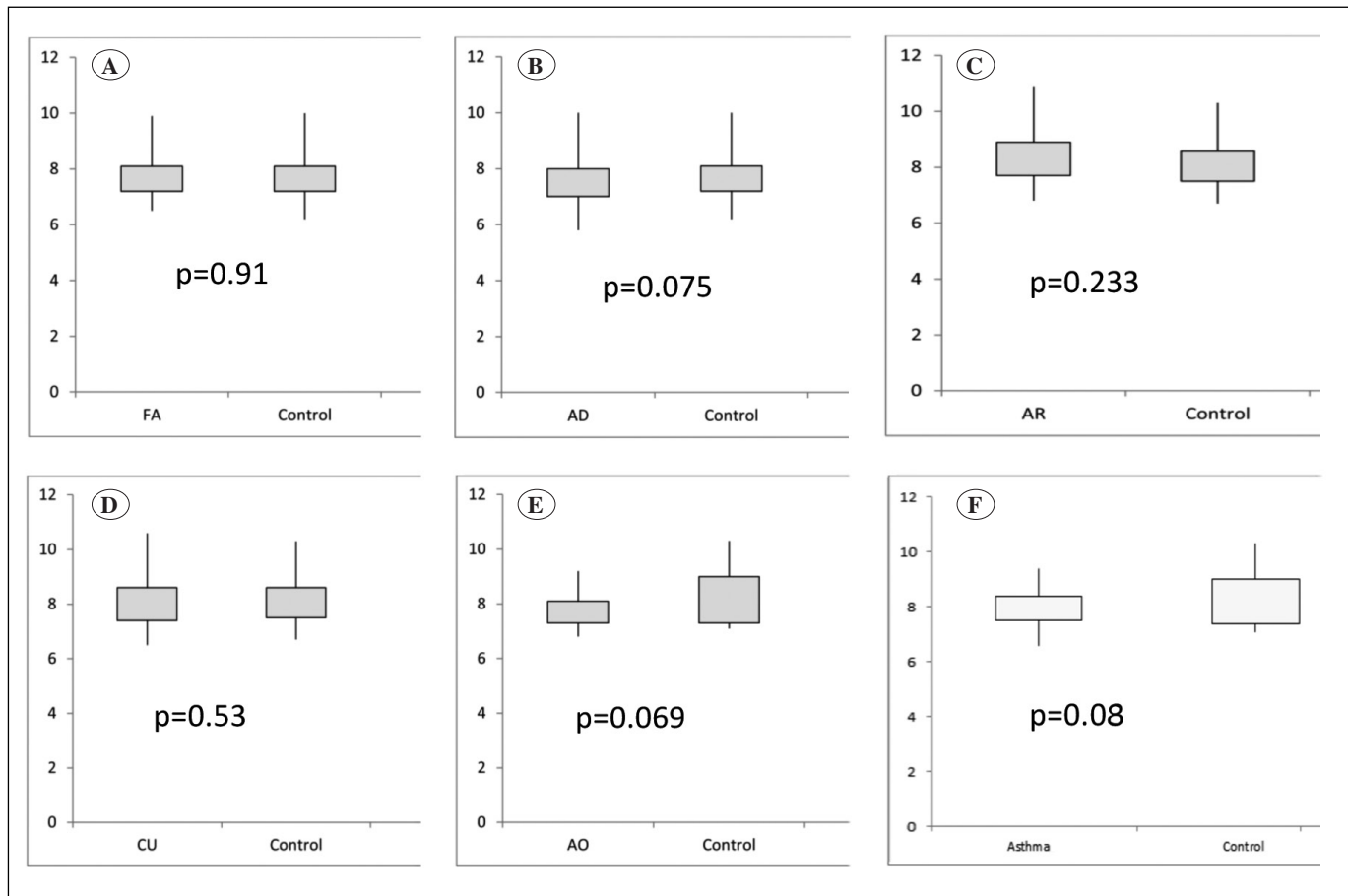


Figure 1. Comparison of the MPV values in allergic diseases and control groups. **A)** Food allergy (n:47) vs. the control group (n:48); **B)** Atopic dermatitis (n:132) vs. the control group (n:48); **C)** Allergic rhinitis (n:48) vs. the control group (n:29); **D)** Chronic urticaria (n:102) vs. the control group (27); **E)** Asthma obese (n:31) vs. the control group (n:17); **F)** Asthma (n:22) vs. the control group (n:17).

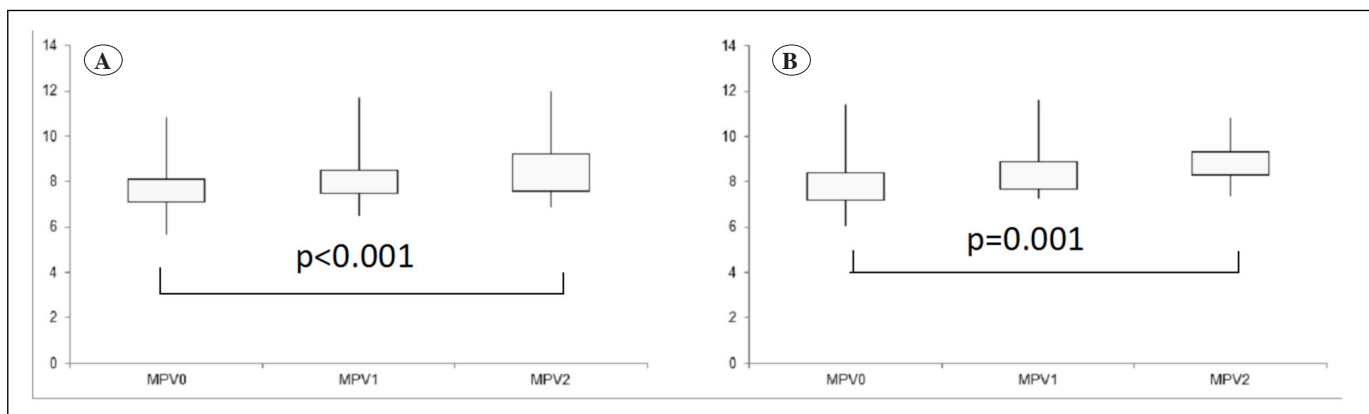


Figure 2. The changes of MPV values during immunotherapy. **A)** Aeroallergen immunotherapy group. **B)** Venom immunotherapy group. **MPV0:** MPV level before IT, **MPV1:** MPV level after 1 year of IT, **MPV2:** MPV level after 2 years of IT.

In this study, while the MPV level was higher in patients with atopic dermatitis compared to the control group, no correlation was found with the disease severity (18). In our study, we found that the disease and its severity did not change the MPV values.

There is no study evaluating the relationship between food allergy and MPV value so far. We have found that there is no difference between the patients with IgE-mediated food allergy and the control group in terms of MPV values in our study.

The studies evaluating MPV changes in allergic diseases have mostly been in patients with chronic urticaria and controversial results have been obtained. Kasperska-Zajac et al. (19) reported no difference between the patients with chronic spontaneous urticaria and control group in terms of MPV values. Chandrashekar et al. have shown that MPV values are higher in the group with chronic spontaneous urticaria (20). Moreover, it has been shown that MPV values are higher in those having a positive autologous serum test compared to those having a negative autologous serum skin test (20). Magen et al. similarly, showed that MPV values in the patients with chronic spontaneous urticaria who have positive autologous serum skin test are higher than the patients with negative autologous serum skin test and the control group (14). In our study, however, there was no difference between the group with chronic spontaneous urticaria and the control group in terms of MPV values and similarly, MPV values were not different between those having a positive autologous serum skin test and those having a negative autologous serum skin test.

MPV value in patients with allergic rhinitis has not been evaluated so far. There was no significant change in terms of MPV value between the patients with allergic rhinitis and the control group. Seasonal differences did not affect the values of MPV in the patients with allergic rhinitis.

A relationship has been shown between obesity and MPV values (21). For the first time, MPV values were studied in the patients with coexistence of asthma and obesity and no difference was detected.

Although MPV values are controversial in allergic diseases, our study has shown that especially the MPV values change over time in patients receiving pollen IT, house dust mite IT and VIT.

Kowal et al. have shown the advent of platelet activation developed by allergen challenge (22). They evaluated platelet counts, plasma levels of beta thromboglobulin, platelet factor-4 and soluble p-selectin in DP sensitive asthmatic patients prior to and after challenge with DP periodically at the 30th minute, and 6th and 24th hours. In this study, eleven patients responded to allergen challenge only in the early phase, and 22 patients responded to the allergen challenge both in the early and late phases. In the early responsive group, platelet counts decreased for the first 30th minute and markers of platelet activation in plasma increased. While markers of platelet activation were found to be high, platelet counts were lower both in the early and late phases in the dual responsive group. Pre-challenge platelet activation status was not different than the control group (22). In a previous study it was shown that circulating platelet activity, the measure of beta-TG, in patients with grass-pollen induced intermittent rhinitis during the course of the dose increase phase of grass pollen SIT did not show any significant changes (23, 24). Besides, a recent study showed that platelet activation is inhibited significantly during sublingual immunotherapy (25). In our study, changes of MPV values and platelet counts could be explained by allergen exposure as it is applied in allergen immunotherapy. Having MPV changes in VIT like allergic inflammation is another interesting result of our study. As mentioned in the studies where an allergen challenge has been applied and platelet activation evaluated, a response 24 hours later than the challenge is considered a late response. The evaluation of MPV in patients receiving immunotherapy has been done before the next dose of immunotherapy and an average of 20 days has passed after applying the last allergen immunotherapy. MPV, markers of activation of platelets, increased over time in patients receiving IT. According to this result, it can be concluded that platelets might have a function in immunomodulation mechanisms.

In conclusion, this study has underlined that MPV changed significantly over the years in SCIT.

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