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Neopterin, homocysteine, and ADMA levels during and after urticaria attack

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Background/aim: Urticaria is a vascular skin reaction characterized with papules and plaques. Neopterin is accepted as an immunologic marker and an indicator of activation of the immune system. Homocysteine and asymmetric dimethylarginine (ADMA) are the markers of increased vascular resistance. Alteration in vascular resistance has a role in the pathogenesis of urticaria. We aimed to investigate whether there is a relationship between urticaria and neopterin, homocysteine, or ADMA.

Materials and methods: The study is designed as a prospective descriptive study and patients with a diagnosis of urticaria in the emergency department were included in the study. Demographic data and characteristics of the disease were recorded. Neopterin, homocysteine, and ADMA levels were measured both during and after urticaria attacks. All data were statistically analyzed.

Results: The differences between neopterin levels measured during and after urticaria attacks were statistically significant ($P < 0.001$). The differences between homocysteine and ADMA levels measured during and after urticaria attacks were not statistically significant ($P > 0.05$).

Conclusion: Our results indicate that neopterin levels in patients with urticaria attacks are increased and the level of neopterin is also a useful parameter in acute urticaria. Further studies should clarify whether homocysteine levels contribute to diagnosis of acute urticaria. However, no relation was found between ADMA and urticaria.

Key words: Acute urticaria, neopterin, homocysteine, ADMA

1. Introduction

Acute allergic syndromes are common problems in primary care and in emergency departments (1). The life-time prevalence for any subtype of urticaria is approximately 20% (2). These are a heterogeneous group of disorders with a large variety of underlying causes. One of them is acute urticaria (AU) (3). Urticaria involves common and histaminergic reactional lesions localized to the superficial dermis of the skin, generally resolved in 24 h (4). These lesions are usually intensely pruritic, erythematous, well circumscribed, and evanescent. Primary care management in AU is symptomatic treatment regardless of the underlying cause. Symptomatic treatments include antihistamines, corticosteroids, or adrenaline (5).

Neopterin is synthesized from guanosine triphosphate and produced preferably by macrophages and monocytes (6). Elevated neopterin concentrations in serum or urine are associated with infection diseases, autoimmune diseases, inflammatory diseases, and organ transplantation rejection (7,8). In addition, it correlates with disease stage and predicts a poor prognosis (8,9). Stimulating the immune system, neopterin is accepted as an immunologic marker and an indicator of activation of the immune system (10). Neopterin may be a useful marker in acute allergic syndromes such as AU.

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Asymmetric dimethylarginine (ADMA) is produced by methylation of arginine residues of the intracellular proteins (11,12). ADMA is an endogenous nitric oxide synthase (NOS) inhibitor that contributes to cardiovascular disease pathogenesis related to endothelial dysfunctions such as atherosclerosis, diabetes mellitus, and chronic renal disease (13,14). It has been presumed that ADMA has an important role in the regulation of the L-arginine/nitric oxide (NO) pathway (15,16). NO has a role in pulmonary physiological regulation of bronchodilation, airway responsiveness, and airway inflammation (16,17). Klein et al. reported that elevated ADMA concentration results in the inhibition of NOS that leads to smooth muscle constriction in the airway (17).

Homocysteine (Hcy) is a sulfur-containing essential amino acid. High plasma Hcy levels have an important role in the pathogenesis of various diseases, especially in the cardiovascular system (18,19). As a prooxidant, homocysteine was found to increase reactive oxygen species, endothelin-1, ADMA, and various adhesion molecules such as ox-LDL and to reduce tetrahydrobiopterin bioavailability, leading to decreased bioavailability of eNOS and endothelial dysfunction (20). In some studies, it was suggested that Hcy may play a role in increased oxidative stress in the damage to vascular endothelial cells (21,22). It was also hypothesized that Hcy-lowering independent mechanisms are linked to a reduction of oxidative stress (20).

In a recent study it was demonstrated that serum neopterin levels are increased in AU as compared with chronic urticaria (23). Homocysteine and ADMA are markers of increased vascular resistance. Alteration in vascular resistance has a role in the pathogenesis of urticaria (24,25). Although serum neopterin, Hcy, and ADMA levels are associated with various clinical syndromes demonstrating similar pathogenesis as AU in the literature, a study including AU in patients with these three parameters analyzed together could not be found. To the best of our knowledge, this is the first study aimed at investigating the associations between urticaria and serum neopterin, plasma total Hcy (tHcy), and ADMA levels.

2. Materials and methods

2.1. Patients

Seventy-seven consecutively admitted patients presenting with AU in the Department of Emergency Medicine, Gülhane Military Medical Academy, Ankara, Turkey, were included in this study. Oral and written consent was obtained from all patients, and authorization was given by the ethics committee of Gülhane Military Medical Academy, Ankara, Turkey (1491-82-11/1539-1564-05.24.2011).

By completing a questionnaire, relevant background information was provided by these volunteers and included medication, presence of allergy and its types, history of atopy, and hereditary and other diseases. History, initial symptoms, physical findings, and treatments were noted. The number and the extent of involvement with urticaria were assessed and recorded. Each urticaria case was evaluated with the urticaria activity score (UAS) as previously described (2). Briefly, the urticarial lesions and pruritus were scored on a scale of 0 to 3 (normal to severe) for a total score of 0–6. All patients were treated with antihistamines and steroids during attack.

2.2. Blood sampling

Blood samples were obtained twice from all the patients: first during the attack of urticaria before any treatment and secondly 15 days after treatment. The blood samples were centrifuged at 2000 rpm for 10 minutes and stored at –80 °C until being analyzed. Because neopterin is slightly sensitive to direct sunlight, samples were protected from light during transport and storage by enveloping the samples in aluminum foil.

2.3. Measurement of ADMA

Measurement of ADMA was accomplished by high-performance liquid chromatography (HPLC) using the method described by Cayci et al. (26). In brief, to 1 mL of serum, 20 mg of 5-sulfosalicylic acid was added, and the mixture was left in an ice bath for 10 min. Samples were centrifuged at 2000 × *g* for 10 min and then 10 µL of supernatant was filtered through a 0.2-µm filter. It was mixed with 100 µL of derivatization reagent (consisting of o-phthaldialdehyde, methanol, borate buffer, and 2-mercaptoethanol) and then injected into the HPLC system. ADMA separation was performed using a 150 × 4 mm C18 Nova-Pak column with 5-µm particle size (Waters, Millipore Corp., Milford, MA, USA), and 50 mmol/L sodium acetate buffer (pH 6.8), methanol, and tetrahydrofuran were used as mobile phases (A, 82:17:1; B, 22:77:1; % v). Flow rate was 1.0 mL/min. The wavelengths of the fluorescence detector were set at 338 nm for excitation and 425 nm for emission. The variability of the method was less than 7%, and the detection limit of the assay was 0.1 µm.

2.4. Measurement of serum neopterin

Serum neopterin levels were measured with an HPLC device (Agilent Technologies 1200 Series System, Santa Clara, CA, USA), using the method defined by Gul et al. (27). Briefly, 100 µL of 2 M trichloroacetic acid was added to 500 µL of serum for protein precipitation. Then samples were centrifuged at 4 °C and 2000 × *g* for 10 min, and then 100 µL of supernatant was filtered and injected into the HPLC system. ADMA separation was performed using a 250 × 4.6 mm C18 Allsphere ODS-2 analytical column with 5-µm particle size (Alltech, Deerfield, IL, USA) and an Allsphere ODS-2 guard column (Alltech),

and 0.015 M phosphate buffer (pH 6.4) was used as the mobile phase (isocratic elution). Flow rate was 0.8 mL/min. The wavelengths of the fluorescence detector were set at 353 nm for excitation and 438 nm for emission. Serum neopterin levels were expressed as nmol/L. The intraassay and interassay coefficients of variations (CV %) were 0.85% and 1.07%, respectively.

2.5. Homocysteine measurement

Plasma tHcy concentrations were measured using a fully automated HPLC system with a fluorescence detector (Shimadzu RF-10AxL) and Hcy kits (ImmuChrom GmbH, Heppenheim, Germany) using the method described by Akgül et al. (28). The intraassay and interassay coefficients of variations (CV %) were 1.83% and 2.94%, respectively.

2.6. Statistical analysis

All statistical analyses were performed by using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Distributions were evaluated using the one-sample Kolmogorov–Smirnov test. Wilcoxon tests and Mann–Whitney U-tests were used for testing differences between groups. The results were expressed as mean ± standard deviation, median (minimum–maximum), and number (frequency). The Spearman rho correlation test was used to indicate relationships between variables. $P < 0.05$ was considered statistically significant.

3. Results

Of 77 consecutively admitted patients presenting with AU in the Department of Emergency Medicine, 48 (62.3%) and 29 (37.7%) were women and men, respectively. The mean age of patients was 42 ± 15 (range: 19–79) years. Forty-nine of the patients had symptoms for no longer than 24 h before being admitted. The rest of patients (n = 28) had symptoms for more than 24 h. Hypertension, diabetes mellitus, acute coronary syndrome, and chronic urticaria were noted in 12, 8, 2, and 1 patients, respectively. There was a history of atopy in 24 patients. Food allergy and drug allergy were noted in 5 and 9 patients, respectively. In the patients' history, there was cold urticaria (n = 1), contact urticaria (n = 1), and demographic urticaria (n = 1). In addition, there were no urticaria types including heat, cholinergic, solar, aquagenic, or exercise-induced. According to UAS evaluation, 15, 15, 15, 10, 12, and 10 of the patients had 6, 5, 4, 3, 2, and 1 points, respectively.

The concentrations of plasma tHcy and ADMA and of serum neopterin are shown in the Table. There was no difference in plasma tHcy or ADMA concentrations in any patients during and after the attacks of urticaria ($P > 0.05$ for both). Serum neopterin levels during attacks were significantly higher than those after attacks ($P < 0.001$).

There was a statistically significant correlation between plasma tHcy levels after attack and serum neopterin levels both during and after attack ($r = 0.424$, $P = 0.009$ and $r = 0.523$, $P = 0.001$, respectively). Moreover, there was a statistically significant correlation between serum neopterin levels during and after attack ($r = 0.483$, $P = 0.002$) (Figure). However, there was no correlation among UAS, serum neopterin, plasma tHcy, and ADMA concentrations during and after attack.

4. Discussion

In this study, we examined the associations between urticaria and serum neopterin, plasma tHcy, and ADMA levels. To our best knowledge, this is the first study to do so.

Many of the patients described in our study had increased UAS. No patients had loss of consciousness or required cardiopulmonary resuscitation or intubation. All patients had elevations of serum neopterin, plasma ADMA, and tHcy during the urticaria attack. There was no statistically significant difference in plasma tHcy or ADMA concentrations of any patients during and after attacks of urticaria. These findings suggest that elevated levels of plasma tHcy and ADMA do not appear to initiate the attack of AU, and increased production of these molecules may potentiate AU. In addition, it has been shown that plasma tHcy may increase in response to immune activation and cell proliferation during type 1 immune response (23). Nevertheless, plasma tHcy was not associated with the attack of AU in our study, suggesting that plasma tHcy may not be the causal factor in AU.

Table. Biochemical parameters.

Parameters	During attack	After attack	P*
Plasma total homocysteine (μmol/L)	10.40 ± 3.88	9.66 ± 1.62	0.330
Serum neopterin (nmol/L)	15.06 ± 5.18	9.27 ± 1.61	<0.001
Plasma ADMA (μmol/L)	0.69 ± 0.43	0.55 ± 0.15	0.090

ADMA: Asymmetric dimethylarginine.
All data are expressed as mean ± standard deviation.
*Mann–Whitney U-test.

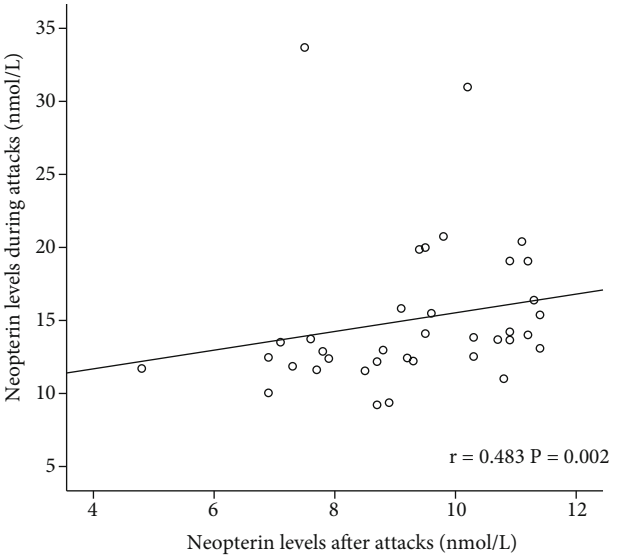

Figure. The correlation of serum neopterin concentrations during urticarial attack and after attack.

During the attack of urticaria serum neopterin levels were statistically higher than after the attack. This predominance of neopterin elevations compared with plasma ADMA and tHcy elevations was observed in all patients.

Ciprandi et al. demonstrated that serum neopterin levels are increased in AU compared with chronic urticaria (10). In our study, we found that serum neopterin levels were increased during the attack of urticaria as compared to after the attack. Therefore, our results were similar with the results of the study performed by Ciprandi et al. (10). In addition, they did not find any correlation between serum neopterin level and severity of disease. In our study, we also showed no correlation between serum neopterin level and severity of urticaria. High serum neopterin levels might show possible infectious diseases and immune system disorders. However, there were no infectious or immune system diseases in our study population. The observed elevation in serum neopterin levels may suggest immune activation and increased monocyte/macrophage activities in AU. Neopterin stimulates the immune system (29). In a recent study, Murr et al. showed that increased neopterin production is associated with inflammation and immune activation (30). As neopterin is a marker of activated monocytes/macrophages, our data suggest that activated monocytes/macrophages may play a role in the pathophysiology of AU. Monocytes and macrophages may be also the primary effectors in the pathogenesis of AU. Some autoimmune and inflammatory diseases such as systemic lupus erythematosus, psoriasis, and dermatomyositis have generally elevated neopterin concentrations (31,32). In a study performed by Reinhold et al., it was shown that the patients with severe atopic dermatitis had elevated serum neopterin levels (33). However, in another study, there was no difference between serum neopterin levels of the patients with chronic urticaria and healthy control subjects (10). Increased serum neopterin levels in AU appear first of all to point toward high activity of the urticarial inflammation.

Our study may have several limitations. We think that there is a lack of measurements of systemic inflammation markers such as IL-6 and CRP during urticarial attacks. Our study group was relatively small in sample size. Another limitation of our study might be the lack of follow-up of patients with AU.

In conclusion, we have shown that serum neopterin concentrations are associated with the attack of urticaria. Our data suggest that neopterin is a marker of activated monocytes/macrophages and activated monocytes/macrophages may play a role in the pathophysiology of AU. These preliminary results demonstrate that serum neopterin levels may be used as a biomarker of immune activation in AU. Further studies should be performed to confirm these findings.

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