

Evaluation of d-dimer, CXCL8, homocysteine, eosinophil cationic peptide, 25(OH)-vitamin D, and immunomodulatory OX-2 levels in allergic patients

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Abstract

Studies on sCD200 (OX-2), 25-hydroxyvitamin-D(25(OH)D), homocysteine (hcy), eosinophil cationic peptide (ECP), d-dimer (DD), CXCL8, and fractional exhale nitric oxide concentrations (FeNO) in allergic patients in Mediterranean regions under various climatic conditions have not been performed. In this report, blood samples were taken in May and June during times of high air pollination. This study was performed to compare serum biomolecule concentrations in allergic patients and matched controls and to evaluate the characteristics of allergic disease. The study participants (n=129) included 25 healthy individuals (controls) and 104 allergic patients. Consecutive patients with managed allergic disease

(Group II, III, IV, and V) above the age of 18 years were included. In the control group, there was a significant positive correlation between ECP level and BMI. Positive correlations among ECP, IgE and OX-2 levels were detected in Group IV. In Group V patients, positive correlations between age and IgE and between BMI and 25(OH) D were identified. Statistical analysis revealed positive correlations among basophil, eosinophil and OX-2 levels, and a negative correlation between ECP and age in Group V. Overall, these data suggest that hcy, 25(OH)D and OX-2 may be useful biomarkers for conventional clinical measurements.



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Abstract

Studies on sCD200 (OX-2), 25-hydroxyvitamin-D(25(OH)D), homocysteine (hcy), eosinophil cationic peptide (ECP), d-dimer (DD), CXCL8, and fractional exhale nitric oxide concentrations (FeNO) in allergic patients in Mediterranean regions under various climatic conditions have not been performed. In this report, blood samples were taken in May and June during times of high air pollination. This study was performed to compare serum biomolecule concentrations in

allergic patients and matched controls and to evaluate the characteristics of allergic disease. The study participants (n=129) included 25 healthy individuals (controls) and 104 allergic patients. Consecutive patients with managed allergic disease (Group II, III, IV, and V) above the age of 18 years were included. In the control group, there was a significant positive correlation between ECP level and BMI. Positive correlations among ECP, IgE and OX-2 levels were detected in Group IV. In Group V patients, positive correlations between age and IgE and between BMI and 25(OH) D were identified. Statistical analysis revealed positive correlations among basophil, eosinophil and OX-2 levels, and a negative correlation between ECP and age in Group V. Overall, these data suggest that hcy, 25(OH)D and OX-2 may be useful biomarkers for conventional clinical measurements.

Key Words: Allergic asthma, allergic rhinitis, severe asthma, soluble CD200, CXCL8, eosinophil cationic peptide (ECP), homocysteine, 25-hydroxyvitamin D (25(OH) D), OX-2, d-dimer.

Introduction

The prevalences of asthma, allergic rhinitis and allergic eye disease in Antalya, a city on the south coast of Turkey, are 8.2%, 10.8% and 7.5%, respectively (1). We investigated the relationships among climate records, pollen in the air and the number of clinical visits. We found previously that profession, building age, building type, and building material affected the sensitivity of patients to allergens (2).

CD200 (OX-2) is a novel immune effector molecule found as both a cell-membrane-bound and soluble form in serum (sCD200), and acts as a pro-inflammatory through its receptor (3). A decrease in serum sOX-2 levels with anti-IgE treatment suggests that sOX-2 may be pro-inflammatory (4, 5). Previous studies have suggested that CD200:CD200R1 regulates allergic inflammatory disease, and that CXCL8 (IL8), d-dimer (DD), eosinophil cationic peptide (ECP) and 25-hydroxyvitamin-D (25(OH)D) play important roles in pulmonary inflammation (4-8).

Alternatively, the development of atopy may be a direct result of elevated homocysteine (Hcy) or metabolite levels, which has several effects on immune function (9). In addition, total homocysteine increases in response to immune activation and cell proliferation during a non-allergic Th1-type immune response (10). Although little is known regarding the health effects of sustained post-load homocysteine, it has negative effects on platelet aggregation and endothelial function (11-13).

Studies on sCD200 (OX-2), hcy, DD, CXCL8, ECP, fractional exhale nitric oxide concentrations (FeNO) and 25(OH)D status among patients with allergies in the Mediterranean region of Turkey under various climatic conditions have not been performed. We previously found that the frequency of hospital admission and the incidence of symptoms increased during times of high air pollution, and collected serum samples during these periods. Hence, we compared the levels of relevant serum biomolecules between allergic patients and matched controls to evaluate disease characteristics.

Materials And Methods

Patients

The study participants (n =129) included 25 healthy individuals (controls) and 104 allergic patients. Consecutive patients with allergic disease (Group II, III, IV, and V) without any other autoimmune and/or chronic disorders (above the age of 18 years) that were managed by the Clinical Immunology and Allergy-Immunology Unit of Internal Medicine Department, Antalya Training and Research Hospital, Antalya, were recruited for the study. Controls were age- and sex-matched patients (n=25, group I; 10 male and 15 female) with no autoimmune disorder or allergic disease and with no history of atopy, cardiac, liver, renal and pulmonary diseases attending the outpatient clinic in the same department.

The second group included 25 newly diagnosed allergic rhinitis patients (n=25, group II; 10 male and 15 female) who did not use any medication. The third group included 22 patients (n=22, group III; 10 male and 12 female) with allergic rhinitis (AR) and nasal polyps who did not use any medication. The fourth group included 32 patients (n=22, group IV; 15 male and 17 female) with severe persistent asthma (SPA)-AR using desloratadin, inhalan steroid (Best Standard Care, following the recommendations of GINA, including inhaled fluticasone 500 mg bid and inhaled salmeterol 50 mg bid), oral methyl-prednisolone, and montelukast therapy. The fifth group included 25 AR + newly diagnosed controlled asthma patients (n=25, group V; 10 male and 15 female) using low-dose inhaled steroids and montelukast therapy. All patients and healthy volunteers provided written, informed content. The study was approved by the local Ethics Committee.

At the time of blood sampling, skin prick test (SPT) and disease activity assessment, all patients discontinued short-acting antihistamines for at least 4 days and long-acting antihistamines and systemic corticosteroids for at least 10 days. SPT on the forearm were performed in all patients using standardized latex extract containing high ammonia natural

rubber latex, as well as a full set of 35 common allergens. Commercial extracts were manufactured by Alyostal ST-IR (Starallergenes S.A., France). The specific IgE levels were also administered within the correlation of SPTs. Blood samples were taken in May and June during times of high air pollution as defined in our previous studies (1, 2). All samples for serum soluble marker measurements were taken in the morning between 8 and 10 am. Due to the effects on inflammatory markers, participants abstained from caffeinated drinks and food for 12 h before testing. Pulmonary function tests and the measurement of FeNO were performed on the same day. FeNO were assessed under the ATS/ERS guidelines (14, 15). The demographics of the patients, including mean age, gender, duration of disease symptoms, and BMI, are provided in Table 1.

Laboratory investigations

Blood samples were obtained after an overnight fast. Serum was then separated by centrifugation at 3000 rpm for 10 min. Serum total and specific IgE and hsCRP were measured immediately. Serum portions were stored at -80°C and subjected to analysis of homocysteine levels. All assays were performed in duplicate.

DD plasma level was measured using enzyme-linked fluorescence assay (ELFA) kits (VIDAS® D-Dimer Exclusion II) from bioMérieux, France. The cut-off level was 500 ng/mL.

Total and specific IgE levels were enumerated using fluoroenzyme immunoassays (ImmunoCAP-FEIA) with the ImmunoCAP (Pharmacia, Uppsala, Sweden) kit. Values above 100 and 0.35 kU/L for total and specific IgE levels were considered abnormal. Serum hs-CRP levels were measured using an hs-CRP assay kit (Behring Latex-Enhanced using the Behring Nephelometer BN-100; Behring Diagnostics, Westwood, MA, USA). The sensitivity of the assay

ranged from 0.04–5.0 mg/L. Serum Hcy levels were measured using an Hcy assay kit (Roche). Concentrations of sCD200 (sOX-2) (Sino Biological Inc., Catalog Number: SEK10886) and CXCL8 (Woburn, MA, USA) in the serum samples were quantified using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Serum levels of 25(OH)D were quantified using a radioimmunoassay (Cobra Quantum, Packard, MN, USA) and categorized into sufficient (≥ 30 ng/ml), deficient (<20 ng/ml) or insufficient ($20 - < 30$ ng/ml) based on previous reports (16). The results are reported as means of duplicate measurements.

Statistical analysis

The laboratory data are presented as means $\pm$ SEM. The pulmonary functional tests and clinical data are presented as means $\pm$ SD. Statistical analyses were performed using the SPSS software (version 13.0; SPSS, Chicago, IL, USA). Comparison of parameters between groups was performed using Student's t-test. Correlations between the variables were analyzed using linear regression analysis. A p-value less than 0.05 was considered to indicate statistical significance.

Results

We found significant differences in d-dimer levels between Group I and Group IV ($p<0.05$) ($p=0.000$); between Group I and Group V ($p<0.05$) ($p=0.005$); between Group II and Group IV ($p<0.05$) ($p=0.001$); between Group II and Group V ($p<0.05$) ($p=0.001$); between Group III and Group IV ($p<0.05$) ($p=0.01$); and between Group-III and Group-IV ($p<0.05$) ($p=0.02$).

Significant differences in OX-2 levels (sCD200) were observed between Group IV and Group V ($p<0.005$) ($p=0.002$) and between Group IV and Group I ($p<0.005$) ($p=0.002$). Significant differences in Hcy plasma levels were observed between Group I and Group IV ($p<0.05$) ($p=0.000$); between Group I and Group V ($p<0.05$) ($p=0.000$); and between Group II and Group

IV ($p < 0.05$) ($p = 0.015$). Significant differences in 25(OH)D levels were observed between Group I and Group IV ($p < 0.05$) ($p = 0.036$) and between Group IV and Group V ($p < 0.05$) ($p = 0.023$). Significant differences in CXCL8 levels were observed between Group II and Group III ($p < 0.05$) ($p = 0.046$) and between Group III and Group IV ($p < 0.05$) ($p = 0.001$). A significant difference in FeNO level was observed between Group IV and Group V ($p < 0.005$) ($p = 0.002$). A significant difference in MPV level was observed between Group I and Group IV ($p < 0.05$) ($p = 0.02$). Significant differences in IgE levels were observed between Group I and Group II ($p < 0.05$) ($p = 0.000$); between Group I and Group IV ($p < 0.05$) ($p = 0.000$); between Group I and Group V ($p < 0.05$) ($p = 0.001$); between Group II and Group IV ($p < 0.05$) ($p = 0.023$); between Group II and Group V ($p < 0.05$) ($p = 0.001$); between Group II and Group V ($p < 0.05$) ($p = 0.001$); and between Group III and Group IV ($p < 0.05$) ($p = 0.001$). Significant differences in HsCRP levels were observed between Group I and Group II ($p < 0.05$) ($p = 0.017$); between Group I and Group III ($p < 0.05$) ($p = 0.005$); between Group I and Group IV ($p < 0.05$) ($p = 0.000$); between Group I and Group V ($p < 0.005$) ($p = 0.002$); and between Group II and Group IV ($p < 0.05$) ($p = 0.002$).

In the control group, there was a significant positive correlation between ECP and BMI ($p = 0.005$, $r = 0.559$). Positive correlations between ECP, IgE and OX-2 were detected in Group IV ($p = 0.001$, $r = 0.647$; $p = 0.035$, $r = 0.374$, respectively). For Group V patients, positive correlations between age and IgE ($p < 0.05$, $p = 0.037$, $r = -0.495$) and between BMI and 25(OH)D ($p < 0.05$, $p = 0.032$, $r = -0.447$) were also detected. Statistical analysis showed a positive correlation between basophil, eosinophil and OX-2 levels ($p = 0.018$, $r = 0.469$; $p = 0.01$, $r = 0.474$, respectively) and a negative correlation between ECP levels and age ($p = 0.032$, $r = -0.430$) in Group V.

Discussion

DD is a fibrin degradation product formed during thrombus lysis. Recent reports have shown that patients with chronic urticaria show signs of thrombin generation and activation of the tissue factor pathways in the coagulation cascade (17). In another study, increased serum levels of activated factor VII, DD, F1+2 and normal factor XII were suggestive of extrinsic pathway activation during chronic urticaria (18). During inflammatory disorders of the lung, related mediators of coagulation (including tissue factors) leak from plasma to the bronchoalveolar space. In addition, during allergic inflammation this leakage initiates and aggravates the allergic reaction. During a severe asthma attack, excess intra-alveolar and distal airway fibrin can cause resistance to treatment, leading to death (19). In this study, we found a significant difference in DD levels between the control group and asthma group and between the allergic rhinitis group and asthma group.

The initiation of coagulation starts with tissue factor on mononuclear, epithelial, and endothelial cells by stimulation. The alveolar epithelium, macrophages, mast cells and eosinophils produce the tissue factors that mediate allergic reactions. Previous studies have reported increased platelet activation during atopic asthma and increased circulating platelet leukocyte aggregates after asthma attacks and allergen challenge (19). High levels of circulating IgE are activated in response to the extrinsic coagulation pathway (20).

In this study, we identified a significant difference in MPV levels between the control group and severe asthma group. More interestingly, one patient had severe persistent asthma and underwent anti-IgE treatment with a history of multiple pulmonary emboli and protein C-S deficiency. This male patient was a heterozygous carrier of factor V Leiden and the prothrombin G20210A mutation (which is related to a high risk of thrombosis) and had decreased DD levels and increased 25-hydroxyvitamin D (25(OH) D), antithrombin III and protein S and C levels during

long-term (3 years) treatment without any pulmonary or intracranial embolism according to the hypertension (5). Fibrinolysis is mediated by plasmin, which converts blood clots into fibrin degradations. Plasminogen is converted to plasmin by the tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA). The main inhibitor of tPA and uPA is plasminogen activator inhibitor type I (PAI-1). In the lung, many cell types produce tPA, uPA and PAI-1. Asthma is related primarily to increased production of PAI-1, which inhibits fibrinolysis in the airways. PAI-1 levels are significantly elevated in the sputum of asthmatic patients (19).

OX-2 may also play a role in the immune response during the pathogenesis of autoimmune, inflammatory skin and pulmonary disorders (4-6, 8). In this study, we identified a significant difference in OX-2 between the severe asthma and controlled asthma groups. In addition, we detected positive correlations between ECP, IgE and OX-2 in Group IV. While positive correlations among basophil, eosinophil and OX-2 levels were found, negative correlations between ECP and age were identified in Group V.

Increased folate levels, along with increased vitamin B12 and lower homocysteine levels, were associated with increased DNA methylation and hypermethylation that may lead to atopic disease (13). Methylenetetrahydrofolate reductase (MTHFR) variants are genetic determinants of folate and hcy status. Specific MTHFR variants may modify the effects of folate and homocysteine on atopic disease [13]. Hcy is a homologue of the amino acid cysteine, and elevated plasma levels of Hcy lead to vascular inflammation (21, 22) and endothelial senescence (23). Furthermore, high Hcy levels are a risk factor for osteoporotic fracture (24). In addition, an *in vitro* study revealed a link between Hcy metabolism and 25(OH)D (25). The fourth group with SPA using inhaled fluticasone 500 mg bid and inhaled salmeterol 50 mg bid steroid doses, oral

methyl-prednisolone, and montelukast therapy showed a high risk of osteoporosis. As shown by our data and in our recent report of the efficacy of anti-IgE treatment in asthmatic patients with cardiovascular complications and its association with inflammatory markers, levels of clinical follow-up markers are strongly correlated with increased levels of immunoregulatory 25(OH)D and decreased levels of FeNO, DD, OX2, hs-CRP, sTRAIL, IL-1 β and ECP (4, 5). This study showed a significant difference in hcy plasma levels between the healthy control groups and allergic asthma groups. A significant difference in 25(OH)D levels was found between Group I and Group IV and between Group IV and Group V. Significant differences in CXCL8 levels were observed between Group II and Group III and between Group III and Group IV. For Groups IV and V patients, the increases in levels of OX2, CXCL8, eosinophil, basophil, IgE, ECP, d-dimer, hs-CRP, FeNO, and Hcy, and the sedimentation rate, as well as the decrease in 25(OH)D, support the possible vascular endothelial protection mechanism. This information on anti-IgE treatment for asthma and other allergic diseases has improved our understanding of the mechanism underlying the effect of the treatment, including its influence on levels of oxidative stress markers and regulation of levels of inflammatory markers, including cytokines, PT, PTT, INR, MPV, platelet count, and albumin (4, 5, 20, 26, 27).

In conclusion, these data suggest that the measurement of hcy, 25(OH)D and OX-2 levels may provide additional information on the pathology of allergic asthma.

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Declaration Of Interest

All authors declare that they have no conflict of interest.

Authors' Contributions

A.D. Yalcin, RM. Gorczynski conceived and designed the study. Clinical follow-up: A. D. Yalcin., analyzed the data: AD. Yalcin, S. Gumuslu Contribution of reagents/materials: A. D. Yalcin., A. Writing of the paper: A.D.Yalcin, ST.Onur, B.Celik, A. Cilli, RM. Gorczynski.

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Table 1.

	Allergic rhinitis	Nasal polyps	Asthma	
			controlled	severe
Group I	–	–	–	–
Group II	+	–	–	–
Group III	+	+	–	–
Group IV	+	–	–	+
Group V	+	–	+	–

Table 1.Groups

Table 2.

Parameters	Groups				
	Group I	Group II	Group III	Group IV	Group V
	(n= 25)	(n= 25)	(n= 22)	(n= 32)	(n= 25)
Smoker (yes/no)	8/17	10/15	6/ 16	10/22	7/18
Eosinophil(mm3)	235.9 ±2.3	333.4 ±3.1	321.2 ±2.4	411.9 ±1.8	418.9 ±1.5
Basophil (%)	0.17±0.12	1.66±0.7	1.75±0.9	1.99±0.6	1.89±0.7
MPV (fL)	9.64 ± 0.12	10.21 ± 3.03	10.11 ± 1.23	12.54 ± 0.19	10.46 ± 0.18
Platelet (mm3)	251.7 ± 11.6	256.10 ± 14.81	260.8 ± 23.2	261.37 ± 13.9	258.9 ± 13.5

Diagnosis- Year	-	6.57 ± 2.7*	7.08 ± 3.1*	22.00 ± 6.9*	2.0 ± 1.1*
FVC (%)	-	-	-	62.9 ± 12.1*	87.8± 7.5*
FEV₁ (%)	-	-	-	58.9± 8.9*	87.1± 6.6*

Group I : Healthy individuals (controls); **Group II**: Allergic rhinitis; **Group III**: Allergic rhinitis + nasal polyps; **Group IV**: Allergic rhinitis + severe asthma , **Group V**: Allergic rhinitis + controlled asthma. **MPV**: Mean platelet volume; **PFT**: Pulmonary function test (FVC, FEV₁).

Table 2. Clinical findings and Laboratory findings allergic patients

Table 3.

Parameters	Groups				
	Group I (n= 25)	Group II (n= 25)	Group III (n= 22)	Group IV (n= 32)	Group IV (n= 32)
Age (years)	41.6 ± 7.8*	41 ± 9.3*	39 ± 8.1*	43.6 ± 12.4*	44.4 ± 8.3*
BMI (kg/m2)	30.6 ± 4.9*	24.5 ± 6.1*	28.8 ± 4.5*	31.8 ± 6.6*	27.1 ± 7.6*
Total IgE (U/L)	21.5 ± 1.9	249 ± 37.7	239.1 ± 24.2	437.3 ± 28	197.7 ± 22
Hs-CRP (mg/L)	2.6 ± 0.2	3.3 ± 0.2	3.8 ± 0.4	5.08 ± 0.5	4.5 ± 0.4
25(OH)D (ng/ml)	14.42 ± 0.58	14.38 ± 0.64	14.83 ± 1.16	12.85 ± 0.63	16.07 ± 1.32
Sedimentation rate(mm/h)	12.20 ± 3.42*	16.85 ± 3.37*	15.08 ± 4.30*	28.36 ± 9.3*	17.20 ± 8.81*
DD (ng/mL)	342 ± 3.4	345.8 ± 9.6	346.7 ± 6.87	498.7 ± 2.57	468.6 ± 2.1

25(OH)D (ng/mL)	14.42 ± 0.58	14.38 ± 0.64	14.83 ± 1.16	11.85 ± 0.63	12.07 ± 1.32
Hcy (mmol/mL)	7.97 ± 0.32	9.19 ± 0.4	8.96 ± 0.39	11.41 ± 0.59	8.66 ± 0.38
OX-2(pg/mL)	29.25 ± 1.96	34.71 ± 2.44	33.36 ± 2.4	36.84 ± 2.05	30.49 ± 2.01
ECP (ng/mL)	-	-	-	67.3±2.6	42.4 ±0.7
CXCL8 (pg/mL)	5.90 ± 0.62	6.40 ± 0.63	5.72 ± 0.48	19.13 ± 3.25	6.04 ± 0.83

*Mean±SD, **ECP**: Eosinophil cationic peptid; **Hs-CRP**: High sensitive-C reactive protein; **25(OH)D**: 25-hydroxyvitamin D; **BMI**: Body mass index ; **Hcy**: Homocystein; **DD**:D-dimer.

Table 3. Clinical findings and Laboratory findings allergic patients