ABSTRACT

Objective: Asthma is a chronic inflammatory lung disease. The renin-angiotensin system (RAS) targets several tissues and maintains fluid homeostasis. It is also known that RAS plays a role in inflammatory processes. Angiotensin-converting enzyme (ACE) and its product angiotensin II (AngII), which are the components of RAS, regulate the known effects of RAS. The other components of RAS, ACE2 and its product Ang 1–7, regulate the counter-effects of RAS. That is why the activation or inhibition of RAS can offer new therapeutic strategies for treating several diseases. We aimed to investigate the serum level of RAS components such as angiotensinogen (AGT), ACE, AngII, ACE2, and Ang 1–7 in asthma and control groups.

Materials and Methods: This study was performed on 27 asthma and 23 healthy individuals. The serum levels of AGT, ACE, AngII, ACE2, and Ang 1–7 were measured by human enzyme-linked immunosorbent assay (ELISA) kits.

Results: There were no statistically significant differences for the serum levels of AGT and ACE between the groups. The serum levels of AngII, ACE2, and Ang 1–7 were significantly higher in the asthma group than those in the control group.

Conclusion: Our results indicate that two tails of RAS, i.e., the ACE and ACE2 pathways, are activated in asthma compared to the control group. We suppose that the activation of ACE2 tail of RAS occurs because of the homeostatic balance requirements of this system. We believe that the activation of ACE2 pathway, in addition to the inhibition of the ACE pathway, can provide clinical benefits in treating asthma.

Keywords: Angiotensinogen (ANG), Angiotensin II (AngII), Angiotensin-converting Enzyme (ACE), ACE2, Ang 1–7

INTRODUCTION

Generally, the renin-angiotensin system (RAS) is described as a main regulatory of fluid homeostasis. It targets several tissues such as the brain, pituitary gland, heart, blood vessels, liver, kidneys, and adrenal glands (1, 2). RAS includes different enzymes that have critical importance in the pathophysiological processes of several diseases (3, 4). Renin is the key enzyme of RAS and converts inactive angiotensinogen (AGT) to angiotensin I (AngI) (5, 6). The effects of RAS show balanced character due to its two cascades. One of these cascades is called ACE/AngII/AT1R, and the other one is called the ACE2/Ang1–7/Mas-receptor axis (7, 8). AngI is cleaved to AngII by the proteolytic activity of ACE, a pulmonary carboxyl dipeptidase. This conversion can occur through non-ACE pathways too (9).

AngII provides main effects of RAS. It acts as a paracrine hormone and also as an autocrine hormone because it exits in circulation as well as in tissues (10). The physiological functions of AngII take place by its two specific receptors, AngII receptor subtype 1 (AT1R) and subtype 2 (AT2R). These receptors reveal the opposite effects of AngII (9). Most known effects of RAS occur via AT1R such as vascular hypertrophy, vasoconstriction, and hypertension (11). The opposite effects of RAS are controlled by ACE2 activity. The major effect of ACE2 is converting AngII to Ang1–7 (12). Although the function of Ang1–7 is still not fully understood, it is known that Ang1–7 is responsible for antiproliferation, natriuresis, and the activation of vasodilatation. Its function is mediated through the G protein-coupled Mas receptor, and it shows antagonist properties against AT1R function (7, 8, 13).

Asthma is a chronic inflammatory lung disease. Disease characteristics are described to be airway inflammation, elevated mucus secretion, and reversible airway obstruction (14-16). It has been shown that the levels of renin and AngII are elevated in acute severe asthma, although the exact reason is not yet clarified (17).

It is known that ACE2 is highly expressed in the heart, kidneys, testes, and also in the lungs, in particular (18, 19). Alterations in the level of AngII can be seen up to the expression level of ACE2 (20). It is thought that the activation of the ACE2 pathway also provides protection against several lung diseases besides the inhibition of the ACE pathway (3). To the best of our knowledge, ACE2 and its product Ang1–7 as counter balanced components of RAS are not defined in asthma, although other RAS components are widely studied. We suppose that...
understanding the role of RAS in diseases needs more information about both tails of this system because only the inhibition of RAS cannot provide a complete benefit, which also needs the activation of the counter-balanced regulators of RAS. For this reason, in this study, we examined the serum levels of the components of the ACE2 pathway, in addition to the serum levels of the components of the ACE pathway of RAS.

MATERIALS and METHODS

Patients
This study was performed on 27 chronic severe asthma patients who were treated at Dumlupınar University, Faculty of Medicine, Department of Chest Diseases, Kütahya, Turkey. The diagnosis of asthma was established on the basis of criteria proposed by 2014 Global Initiative for Asthma (GINA) Guideline (21). The control group comprised 23 healthy age-matched subjects who were treated at Dumlupınar University, Faculty of Medicine, Department of Chest Diseases, Kütahya, Turkey. Written informed consent for all procedures were obtained from each individual. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki and was approved by the ethics committee of Afyon Kocatepe University.

All individuals were assessed by the criteria according to GINA 2014 (21) to evaluate the level of asthma symptom control (ASC). For this purpose, the following questions were asked to the patients: In the past 4 weeks, 1) did you have daytime symptoms more than twice a week? 2) Did you have any night awakenings due to asthma? 3) Did you need a reliever medication more than twice a week? 4) Did you have any activity limitation due to asthma? The patients answered these questions as “Yes” or “No.” If the patient had none of these, the level of ASC was defined as ‘well-controlled’. If the patient had 1 or 2 of these, it was defined as “partly controlled.” If the patient had 3 or 4 of these, it was defined as “uncontrolled”.

Enzyme-Linked Immuno Sorbent Assay (ELISA) Analyses
Peripheral blood samples were collected in tubes without EDTA from all subjects. After centrifugation, the serum of each individual was stored at −80°C until ELISA analysis. The serum concentrations of AGT (Cusabio Biotech, Cat No CSB-E08564h), ACE (Cusabio Biotech, Cat No CSB-E11269h), AngII (Cusabio Biotech, Cat No CSB-E04493h), ACE2 (Cusabio Biotech, Cat No CSB-E04489h), and Ang1–7 (Cusabio Biotech, Cat No CSB-E14242h) were analyzed by rat ELISA assay kits. Chemiluminescence data were analyzed by an ELISA microplate reader (das, Digital and Analog Systems, Vimercate, MI, Italy).

Statistical Analysis
Statistical analyses were performed by SPSS (Statistical Package for Social Sciences; Chicago, IL, USA) 16.0 package program. The serum levels of interested (AGT, ACE, AngII, ACE2, and Ang1–7) parameters were given as mean±standard error of the mean (SEM). Statistical significances between the two groups were analyzed by Mann–Whitney U tests. Differences were considered significant at p<0.05.

RESULTS

The frequencies of the level of asthma symptom control evaluations were found to be 22.2% for partially under control (n=6) and 77.8% for uncontrolled (n=21) in the asthma group and 100% for under control (n=23) in the control group. The distribution of the asthma control evaluations were found to be significantly different between the groups (X²=50,000; df=2; p=0.000) (Table 1). The frequency of the uncontrolled individuals was significantly higher in the patients than in the control (cohort analyses for uncontrolled=0.222, 95% CI=0.11–0.45; p=0.000).

There were no statistically significant differences for the serum levels of AGT (15.94±1.9 ng/mL) and ACE (2.1±0.3 ng/mL) in the asthma group compared with the serum levels of AGT (13.24±1.4 ng/mL) and ACE (1.9±0.3 ng/mL) in the control groups, p=0.259 and p=0.763, respectively (Table 2). The serum levels of AngII (0.11±0.01 ng/mL), ACE2 (0.1±0.01 ng/mL), and Ang1–7 (4.7±0.2 ng/mL) were significantly higher in the asthma groups compared with the serum level of AngII (0.06±0.01 ng/mL), ACE2 (0.08±0.03 ng/mL), and Ang1–7 (3.9±0.3 ng/mL) in the control group; p=0.002, p=0.01, and p=0.01, respectively (Figure 1-3).

DISCUSSION

RAS is the main regulatory system for water and salt homeostasis (1, 2). The counterbalanced action of this system occurs on different tissues via the tails of RAS called as ACE/AngII/AT1R and ACE2/Mas-receptor pathways (7, 8, 13). Activated RAS due to renin indirectly stimulates the synthesis of the components of the ACE pathway of RAS.

Table 1. The frequencies of the asthma symptom control evaluations in asthma and control groups

<table>
<thead>
<tr>
<th>Asthma symptom control evaluation</th>
<th>Well-controlled</th>
<th>Partly controlled</th>
<th>Uncontrolled</th>
</tr>
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<tbody>
<tr>
<td>Asthma</td>
<td>0</td>
<td>6 (22.2%)</td>
<td>21 (77.8%)</td>
</tr>
<tr>
<td>Control</td>
<td>23 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

X²=50,000; df=2; p=0.000

Table 2. The serum levels of angiotensinogen (AGT) and angiotensin-converting enzyme (ACE) in asthma and control groups

<table>
<thead>
<tr>
<th></th>
<th>Asthma</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT (ng/mL)</td>
<td>15.94±1.9</td>
<td>13.24±1.4</td>
<td>0.259</td>
</tr>
<tr>
<td>ACE (ng/mL)</td>
<td>2.1±0.3</td>
<td>1.9±0.3</td>
<td>0.763</td>
</tr>
</tbody>
</table>

P values were evaluated by Mann–Whitney U test
pathophysiological features of asthma (17, 22, 23). Because of the limitations of our study, we could not evaluate the exact reason for the elevated serum levels of AGT and ACE in the asthma group. On the other hand, it is known that the elevated plasma levels of AGT and ACE are related to their homozygote mutant variations (24). We evaluated AGT and ACE polymorphisms in the asthma and control groups. We did not observe any statistically significant difference between the groups for interested polymorphisms (data not shown). This can be one of the explanations for the elevated serum levels of AGT and ACE, which are not significant in the asthma group, compared with those of the control group. According to our results, we suppose that the elevation of the serum levels of AGT and ACE in chronic severe asthmatic patients lays the groundwork for the negative effects of RAS such as the hypertrophy of bronchial smooth muscles and bronchoconstriction.

As mentioned above, the elevated serum level of AngII is determined in acute asthma and acute severe asthma patients due to β-agonist therapy (17, 22). We could not observe any reports about serum level of AngII in chronic severe asthmatic patients. In our study, we found a significant increase for the serum level of AngII in the chronic severe asthma group compared with that of the control group. According to our results, the elevated serum level of AngII in the asthma group is not affected by the serum level of ACE as its product. We could not evaluate the exact reason for the increased serum level of AngII in the asthma group. We suppose that the elevated serum level of AngII can cause adverse effects in asthma treatment such as the hypertrophy of bronchial smooth muscles and bronchoconstriction. We think that pharmacological treatments, which have antagonistic properties against AngII and inhibit AngII production or AT1R activation, provide benefits in addition to β-agonist treatment.

ACE2 is described as an ACE homolog and has carboxypeptidase activity. ACE2 converts AngII to Ang1–7, which regulates the counterbalanced activity of RAS (7, 25). To the best of our knowledge, there is no study indicating the serum levels of ACE2 and Ang1–7 in patients with asthma. Our results have revealed the activation of ACE2/Ang1–7 axis of RAS in chronic severe asthmatic patients. Increased ACE2 level can be induced via the elevated level of AngII. The elevation of both AngII and ACE2 naturally causes an increase in the level of Ang1–7. Unfortunately, we do not have a certain explanation for the mechanism of the increased level of ACE2 and Ang1–7. It has been supposed that the imbalanced action of ACE/AngII/AT1R and ACE2/Ang1–7/Mas-receptor axis of RAS can cause lung diseases. It has been thought that ACE inhibitors or AT1R blockers eliminate the negative effects of RAS during the treatment of lung diseases. However, these treatments could not provide a significant beneficial effect completely for some of them such as pulmonary fibrosis and pulmonary hypertension (3). It is believed that ACE2 activation or ACE2 gene therapies provide more beneficial effects during the treatment of pulmonary diseases besides RAS inhibitory strategies (26). We suppose that ACE2 elevation and Ang1–7 production, which may have occurred to maintain homeostatic balance in asthma patients in our study, by ACE2 activator therapeutics such as XNT, which is described as synthetic ACE2 activator, provides important clinical benefits.

CONCLUSION

We suppose that the activation of the two tails of RAS indicate that RAS acts as a homeostatic regulatory system even in treated asthmatic patients. We suppose that the inhibition of the ACE pathway of RAS and the homeostatic action of RAS, which is the activation
of the ACE2 pathway, besides β-agonist treatment can provide more beneficial outcomes for asthma treatment. We think that getting more knowledge about RAS in asthma helps understand the pathophysiological processes in this disease. For this reason, we believe that further studies are needed to provide the effective combined therapeutic strategies, including the inhibition and/or activation of specific components of RAS, in the treatment of asthma.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Authors’ Contributions: Conceived and designed the experiments or case: CA, ÜT, OG. Performed the experiments or case: CA, ÜT, OG, SŞ. Analyzed the data: CA, ÜT, OG, SŞ, IB, ÖA, MA. Wrote the paper: CA. All authors have read and approved the final manuscript.

Conflict of Interest: No conflict of interest was declared by the authors.

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