Research Article

The role of asymmetric dimethylarginine and ghrelin in asthma patients

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Abstract

Background: Asthma is an important disease that progresses with chronic inflammation and can be accompanied by many comorbidities during its course. Although these patients have different comorbidities, it is often seen that they are accompanied by cardiovascular diseases, obesity and some infections. To date, the role of asymmetric dimethylarginine (ADMA) and ghrelin has not been elucidated in asthma pathogenesis.

Objectives: The aim of the study is to evaluate ADMA and ghrelin parameters in asthmatic patients and to compare these data with healthy individuals.

Material and Methods: In our study, thirty-eight patients diagnosed with asthma and 35 healthy individuals were included. ADMA levels were determined by high performance liquid chromatography (HPLC), while ghrelin levels were determined by enzyme linked immunosorbant assay (ELISA) analysis.

Results: ADMA levels were significantly higher in the patients group compared to controls. In contrast, ghrelin levels were significantly lower in the asthma patients compared to the control.

Conclusion: Asthma is an important global health problem that affects people of all ages. Another global health problem, the presence of obesity in asthma patients can be considered as an important risk factor for the development of cardiovascular diseases. According to the data obtained from our study, increasing ADMA and decreasing ghrelin parameters are thought to be important markers in predicting cardiovascular diseases that may develop in asthma patients. Within the findings of your study, the follow-up of ghrelin/ADMA biomarkers in patients diagnosed with asthma and undergoing treatment is important in terms of predicting cardiovascular diseases for the future. If necessary, the process can be brought into balance with drug supplements that will affect the mechanism.

Keywords: Asthma; Asymmetric dimethylarginine; Endothelial dysfunction; Ghrelin

Abbreviations: Asymmetric dimethylarginine (ADMA); C-reactive protein (CRP); Cardiovascular disease (CVD); Enzyme-linked immunosorbent assay (ELISA); Global Initiative for Asthma (GINA); High-density lipoprotein cholesterol (HDL-C); High-performance liquid chromatography (HPLC); Interleukin (IL); Low-density lipoprotein cholesterol (LDL-C); Nitric oxide (NO); Nitric oxide synthase (NOS); Platelet distribution width (PDW); Red blood cell distribution width (RDW); Tumor necrosis factor-α (TNF-α)

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Introduction

Asthma is a global health problem affecting approximately 300 million people worldwide.[1-3] It is a chronic respiratory disorder defined by airway hypersensitivity and airflow obstruction.[1,3-5] However, more studies are needed to fully clarify this relationship. Studies on endothelial function, a useful prognostic indicator of cardiac events in CVD, may be useful in clarifying this issue.[6,7]

Nitric oxide (NO) and other endothelium-derived substances play important roles in the cardiovascular system. Changes in NO homeostasis due to endothelial dysfunction are generally assumed to result in CVD.[8] In this pathway, endothelial nitric oxide synthase (NOS), which catalyzes NO biosynthesis from L-arginine, plays an important role.[9,10] Studies in asthma and allergic airway inflammation model systems reveal that L-arginine and NO metabolic pathways change, especially in the pathophysiology of asthma.[11,12] In this pathway, Asymmetric dimethylarginine (ADMA) is an effective NOS inhibitor that limits the bioavailability of nitric oxide and can also interfere with various processes associated with the evolution of inflammatory airway disease.[13,14] The increase in endogenous ADMA may contribute directly to the underlying pathology of the decrease in lung function by directly influencing NOS isoforms and/or L-arginine depletion.[15] However, there are few studies on the role of ADMA in asthma pathogenesis.[16,17]

One of the personal risk factors effective in the emergence and development of asthma is obesity. Obesity alone is an important factor influencing the risk and prognosis of asthma.[18] Asthma is more frequently observed and in those with a body mass index>30 kg/m2. Obese Asthmatics have lower respiratory functions and more comorbid diseases compared to normal-weight asthmatics.[18,19] The role of obesity in the development of asthma is not clearly known. In addition to the genetic, hormonal and neurogenic effects of obesity, it is thought to affect respiratory functions via lung mechanics and cause the release of pro-inflammatory cytokines. Various pro-inflammatory mediators are released from adipose tissue, such as IL-6, tumor necrosis factor (TNF) -α and eotaxin.[20] Certain mediators such as leptin, adiponectin and ghrelin may also affect airway function and increase asthma tendency.[20,21] Some studies have shown that the recently discovered pro-inflammatory effects of ghrelin and leptin may cause asthma. Both hormones have some immunomodulating effects and oppose each other in vitro for the production of pro-inflammatory cytokines such as TNF-α and IL-1 on human lymphocytes. On the other hand, inflammation reduces serum ghrelin levels and increases serum leptin levels.[22] Chronic inflammation is suggested to play a significant role in asthma pathogenesis.[4,5] Ghrelin plays a role in varied diseases associated with inflammation.[23,24] It acts on the adaptive and innate immune systems to inhibit pro-inflammatory cytokines including interleukin-1β (IL-1β), IL-6 and TNF-α which are involved in the asthma pathogenesis.[23,25] Ghrelin may have also cardioprotective effects involving decreasing blood pressure, and preventing of atherosclerosis, as well as enhancing vascular

and endothelial function.[26,27] However, the role of ghrelin in asthma mechanisms is still unclear.[24]

The importance of early identification of biomarkers in order to identify the risk of these comorbidities is essential in reducing mortality and limiting morbidity in asthmatics. We therefore designed our study to measure the levels of ADMA and ghrelin in asthmatics so as to identify and minimize the risk of obesity and cardiovascular disease in asthmatics.

Materials and Methods

Study design and population

Thirty eight patients who were admitted to the Chest Diseases Hospital Polyclinic between September 2016 and March 2017 and diagnosed with asthma and 35 healthy controls were included in the study. The study was approved by local Kırıkkale Universty, Clinical Research Ethics Committee (Protocol 01-01/2014).

Patient's inclusion criteria: Asthma patients aged above 18 years were selected consecutively and included in the study. The diagnosis of asthma was made according to Global Initiative for Asthma (GINA). Patients with additional disease in the patient groups were excluded from the study. The control arm was comprised of healthy individuals who had never smoked, were not using corticosteroid and were not on anybody or mind altering medications.

Patient's exclusion criteria: İndividuals with chronic obstructive pulmonary disease, bronchiectasis, tuberculosis, malignancy disease, gastroesophageal reflux symptoms or disease, chronic diseases (diabetes, congestive heart failure, chronic renal failure, irritable bowel syndrome, rheumatoid arthritis and scleroderma inflammatory diseases), acute infection complaints such as fever, shortness of breath and cough and and who were less than 18 years were excluded from the study Control group's inclusion criteria: The control arm was comprised of healthy individuals above 18 yers of age without any of the following diseases; chronic obstructive pulmonary disease, bronchiectasis, tuberculosis, malignancy disease, gastroesophageal reflux symptoms or disease, chronic diseases (diabetes, congestive heart failure, chronic renal failure, irritable bowel syndrome, rheumatoid arthritis and scleroderma inflammatory diseases) in the study.

The preparation of blood samples and methodology

Fasting blood samples were collected in the morning of the examination from the study participants. Fasting blood glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C, Lymphocytes (mm^3), platelet distribution width (PDW), red blood cell distribution width (RDW) (%) and C-reactive protein (CRP) were determined in line with the standard laboratory protocol in the hospital. Appropriate blood samples were collected, centrifuged their serum separated and stored at -86 degrees, protected from light for the determination of ADMA and Ghrelin.

Determination of ADMA: ADMA levels were analyzed by high-performance liquid chromatography (HPLC) as used by Chen et al and developed by Avci et al.[28,29] The areas of peaks detected by fluorescent detector (excitations, 338 nm; emission, 425 nm) were used for quantification. The intra and inter-assay coefficients of variation for ADMA were 2.8% and 4.5%, respectively. Determination of Ghrelin: The determination of human ghrelin in samples according to the manufacturer's protocol used a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (200-12-0973; Sunred Biological Technology Co., Shanghai). Optical density (OD) below 450 nm wavelengths was used in the measurement samples.

Statistical analysis

The findings of the study were evaluated statistically using IBM SPSS Statistic 22.0 (IBM Co., Armonk, NY, USA). Samples number was determined according to power analysis before the study. All data were given as mean ± standard deviation. Statistical significance level was defined as 0.05. Shapiro-Wilk test was used to check whether the data were suitable for normal distribution. Mann Whitney-U test, one of the non-parametric tests, was used due to the lack of normal distribution of data.

Results

The demographic features and biochemical study parameters from patient with asthma and control groups were shown in Table 1. In our study, the asthma patient was consisted of 20 female and 18 male (n=38) with the mean age (57.0 ± 14.0) and control group was consisted of 15 male and 20 female (n=35) with the mean age (51.0 ± 14.0). There was no statistical difference in the gender, age, glucose, total cholesterol, HDL and cholesterol between the asthmatics and the control groups. There was however a statistically significant difference in PDw, RDW and CRP ($p>0.05$), $p<0.05$ respectively) between the asthmatics and the control group.

The ADMA levels were significantly higher among asthmatics than the controls (p=0.014). In contrast, the ghrelin levels were significantly lower in the asthmatics compared to the healthy controls (p=0.039) (Table 2).

Table 1: *The demographic features and biochemical parameters of patient and control groups.*

	Controls (n=35)	Asthma Patients (n=38)	P values
Gender (Male/Female)	15/20	18/20	0.071
Age (years)	51.0 ± 14.0	57.0 ± 14.0	0.097
Fasting Blood Glucose (mg/dL)	99.7 ± 8.1	101.23 ± 5.8	0.129
Total cholesterol (mg/dL)	208.33 ± 37.6	194.2 ± 28.5	0.317
HDL (mg/dL)	50.2 ± 17.5	49.8 ± 7.3	0.283
Triglycerides (mg/dL)	115.2 ± 58.3	119.1 ± 66.4	0.178
Lymphocytes (mm3)	3255 ± 1388	2462 ± 850*	0.149
PDW(GSD)	16.2 ± 1.1	17.6 ± 1.6*	0.044
RDW(%)	12.9 ± 2.8	14.4 ± 1.2*	0.047
CRP (mg/L)	1.17 ± 1.08	5.13 ± 2.80*	0.019
P value was set 0.05 and *, $p<0.05$			

Table 2: ADMA and ghrelin levels in patient and control groups

	Controls (n=35)	Asthma Patients (n=38)	P values
ADMA (μmol/l)	0.47 ± 0.09	1.13 ± 0.24	0.014
Ghrelin (pg/ml)	154.3 ± 21.6	116.24 ± 12.09	0.039
P value was set 0.05			

Discussion

In the present study, the serum ADMA levels in asthma patients were found higher than those in the control group. The results found in our patients agree with those of many other authors regarding increased ADMA values. Scott et al. demonstrated that ADMA levels were higher in human asthma lungs and sputum samples than controls.[10] In a similar investigation, serum ADMA concentrations were evaluated and it was found that ADMA levels were significantly increased in patients with asthma compared with healthy controls.[17] In spite of the fact that Lara et al. did not find significant differences in plasma ADMA levels between subjects with asthma and controls.[11] Grasemann et al. showed that ADMA concentrations were higher in CF airways compared to healthy control subjects and concluded that enhanced ADMA levels may contribute to airway obstruction in CF patients by reducing NO formation.[30] In a previous study, it was found that ADMA demonstrated correlations to airway inflammatory markers in COPD patients, supporting the impaired eNOS function in COPD.[31] Carraro et al. reported a statistically significant increase in ADMA levels of children with asthma compared with controls.16 It was shown that plasma ADMA levels were higher in subjects with late-onset asthma compared to early onset.[32]

Inflammatory pathogenesis is suggested to be one of the underlying cause of asthma and CVD despite different inflammatory pathophysiologies.[33,34] Low-grade systemic inflammation presented in asthma, could influence later risk of CVD.2 Chronic airway inflammation might promote systemic inflammation and increase the risk of cardiovascular diseases.[35] Ghrelin exerts diverse effects including controlling energy homeostasis, attenuating sympathetic nerve activity, increasing cardiac output, and inhibiting inflammation.[25,36] In our study, we found that ghrelin levels were significantly lower in asthma patients compared with healthy subjects. Our finding is in accordance with previous studies that found lower ghrelin levels. Yuksel et al. reported that ghrelin levels were lower in obese children with asthma when compared to non-obese children with asthma or controls, and they also found significant reductions in non-obese children with asthma than in controls. The authors concluded that ghrelin may implicate in the inflammatory pathogenesis of asthma and obesity comorbidity.[37] Tsaroucha et al. demonstrated that asthmatic patients exhibited lower ghrelin concentrations compared to controls, whereas ghrelin levels did not change significantly along with asthma severity.[38] In a study by Matsumoto et al., it was found that ghrelin levels tended to be lower in asthmatic subjects than non-asthmatics.[39] It was shown that there was no significant difference in children with and without asthma in terms of serum ghrelin levels in the literature.[40] In contrast to some studies, for example, Toru et al. observed statistically significant increase in the serum ghrelin levels of asthmatic patients. It was proposed that ghrelin has an anti-inflammatory effect in asthma.[23] Moreover, previous studies on COPD reported that circulating ghrelin levels were significantly higher in COPD patients than in controls.[41-44] It was suggested that ghrelin administration decreased airway inflammation in lungs and increased body weight.45 Within the findings of your study, the follow-up of ghrelin/ADMA biomarkers in patients diagnosed with asthma and undergoing treatment is important in terms of predicting cardiovascular diseases for the future. If necessary, the process can be brought into balance with drug supplements that will affect the mechanism.

Conclusion

Asthma is an important global health problem that affects people of all ages. Another global health problem, the presence of obesity in asthma patients can be considered as an important risk factor for the development of cardiovascular diseases. According to the data obtained from our study, it is predicted that increased ADMA and decreased ghrelin levels may contribute to the pathophysiology of asthma, and these changes lead to cardiovascular diseases. In such asthmatic patients, it is recommended that the process be followed soon and controlled with additional markers.

Conflict of interest

The authors declare that they have no conflicts of interest and we would like to thank Dr. Mesut Arslan, MD, Corum Chest Diseases Hospital, Clinic of Chest Diseases, Corum, Turkey for the clinical evaluation of the patients..

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