CLINICAL STUDY

High serum levels of IGF-I and IGFBP3 may increase comorbidity risk for asthmatic patients

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ABSTRACT

OBJECTIVE: Asthma is known as a chronic inflammatory lung disease which has also systemic features. Insulin-like growth factor I (IGF-I) plays a role for asthma pathogenesis. Controversially, IGF-binding protein 3 (IGFBP3) blocks asthma development. That is why IGF-I and IGFBP3 are targeted for future therapeutic treatments of asthma. We aimed to investigate serum level of IGF-I and IGFBP3 in patients with asthma. This study was performed in 27 asthma and 23 healthy individuals. Serum levels of IGF-I and IGFBP3 were measured by human ELISA assay kits. Serum levels of IGF-I and IGFBP3 were significantly higher in the asthma group than the control group. Significant negative correlation was found between IGF-I and asthma control test (ACT) puan, O₂ saturation, Forced Expiratory Volume in 1 second/ Forced Vital Capacity (FEV1/FVC), Forced Expiratory Flow 25 second/75 second (FEF2575) (%). Significant positive correlation was found between IGFBP3 and IGF-I, systolic blood pressure. Significant negative correlation was found between IGF-I and FEV1 (ml).

RESULTS: Our results indicate that the serum levels of IGF-I and IGFBP3 are significantly elevated in the asthma group. We assume that current treatment strategies are not really good enough for asthma. We suppose further strategies which are seeking to balance IGF-I and IGFBP3 should be developed for more effective and curative treatment of asthma (Tab. 2, Fig. 2, Ref. 22). Text in PDF www.elis.sk.

KEY WORDS: insulin-like growth factor I (IGF-I), IGF-binding protein 3 (IGFBP3), asthma.

Introduction

Synthesis of the insulin-like growth factor I (IGF-I) is induced mainly in the liver by growth hormone (GH) which is secreted under the control of GH-releasing hormone and ghrelin. IGF-I is the major inhibitor of its own secretion and also its secretion is inhibited by somatostatin (Spiess et al, 1983, Kojima et al, 1999, Butler et al, 2001, Brazeau et al, 1973). IGF-I acts as an endocrine factor and plays important role for cell proliferation, differentiation and apoptosis. It interacts with multiple inflammatory mediators. Thus, IGF-I induces subepithelial fibrosis, airway inflammation, hyper-responsiveness and smooth muscle hyperplasia (Lee et al, 2014).

Insulin like growth factor binding proteins (IGFBPs) include six high affinity binding proteins that are responsible for transpor-

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tation of IGF to regulate biological action of IGFs. Most abundant form of IGFBPs is the IGF-binding protein 3 (IGFBP3). It bounds to IGF-I with high affinity and inhibits its effects such as suppression of airway inflammation and airway hyper-responsiveness via IGF-I dependently or independently (Jogie-Brahim et al, 2009, Domené et al, 2005, Yaren et al, 2012).

Asthma is known as a chronic inflammatory lung disease which has systemic features. Airway inflammation, elevated mucus secretion and reversible airway obstruction are considered asthma characteristics (Barnes et al, 2002, Bousquet et al, 2000, Roche et al, 1989).

IGF-I and IGFBP3 are a target for various treatment strategies including allergic airway diseases as well as for the treatment of asthma (Lee et al, 2014). Therefore, we would like to evaluate the effectiveness of current strategies through both of them. For this purpose, we measured serum levels of IGF-I and IGFBP3 in asthma patients who were treated with current therapeutics. We evaluated relationship of clinical parameters and serum levels of IGF-I and IGFBP3.

Materials and methods

Patients

This study was performed in 27 (3 males, 24 females) chronic severe asthma patients who were treated at Dumlupinar University, Faculty of Medicine, Department of Chest Diseases, Kütahya, Turkey. The diagnosis of asthma was established on the ba-
sis of the criteria proposed by 2014 Global Initiative for Asthma (GINA) Guideline (Global Initiative for Asthma Guideline, 2014). The control group consisted of 23 healthy age-matched (4 males, 19 females) subjects. All of the procedures and a written informed consent were obtained from each individual. The study protocol conforms to the ethical guidelines of Declaration of Helsinki, and was approved by the Ethics Committee of Afyon Kocatepe University.

Both groups were evaluated by several clinical parameters such as; asthma control test (ACT) puan, systolic and diastolic blood pressure, pulse, O₂ saturation, FVC (Forced Vital Capacity) (ml, %), FEV1 (Forced Expiratory Volume in 1 second) (ml, %), FEV1/FVC, FEF2575 (Forced Expiratory Flow 25 second/75 second) (%), PEF (Peak Expiratory Flow) (ml, %). All individuals were assessed by the criteria according to the asthma control test to calculate ACT puan. For this purpose, the following questions were asked (in the past 4 weeks): 1) How much of the time did your asthma keep you from getting as much done at work, school or at home? 2) How often have you had shortness of breath? 3) How often did your asthma symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake you up at night or earlier than usual in the morning? 4) How often have you used your rescue inhaler or nebulizer medication (such as albuterol)? 5) How would you rate your asthma control during the past 4 weeks? The patients scored these questions 1 to 5. Asthma was grouped as well-controlled, partly controlled and uncontrolled according to the ACT puan; ≥ 25, = 20–24, and ≤ 19; respectively (Nathan et al, 2004).

ELISA analyses

Peripheral blood samples were collected in tubes without EDTA from all subjects. After centrifugation, serum of each individual was stored at –80°C until ELISA analysis. Serum concentrations of IGF-I (Cusabio Biotech, Cat No CSB-E04580h) and IGFBP3 (Boster Human, Cat No EK0386) were analyzed by human ELISA assay kits. Chemiluminescence data were analyzed by an ELISA microplate reader (das, Digital and Analog Systems, Vimercate, MI, Italy).

Statistical analyses

Statistical analyses were performed by SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) 16.0 package program. Serum levels of IGF-I, IGFBP3 and clinical parameters were given as mean ± standard error of the mean (SEM). Statistical significances between the two groups were analyzed by Mann-Whitney U test. Differences were considered significant at p < 0.05. Pearson correlations were calculated to discern the relationship between clinical parameters and serum levels of proteins of interest.

Results

There were no statistically significant differences for pulse, FVC (ml, %), FEV1 (ml, %) and PEF (ml, %) (Tab. 1). ACT puan, O₂ saturation, FEV1/FVC ratio and FEF2575 (%) were significantly lower in the asthma group compared to the control group, respectively; p = 0.000, p = 0.035, p = 0.002, p = 0.004 (Tab. 1). Systolic and diastolic blood pressures were significantly higher in asthma group compared to the control group, respectively; p = 0.001, p = 0.034 (Tab. 1). Serum levels of IGF-I and IGFBP3 were significantly higher in the asthma group than control group, respectively; p = 0.000, p = 0.035, p = 0.002, p = 0.004 (Tab. 1).

| IGF – I (pg/ml) | Asthma | Control | p       |
|----------------|--------|---------|---------|
| 2.74±0.3       | 0.85±0.3 | 0.000*** |

Tab. 1. The comparisons of characteristics between the patient and control groups.

| IGFBP3 (pg/ml) | Asthma | Control | p       |
|----------------|--------|---------|---------|
| 526.21±9.25    | 459.31±14.90 | 0.006*** |

| ACT puan | Asthma | Control |
|----------|--------|---------|
| 14.68±0.1 | 25.00±0.0 | 0.000*** |

| Systolic Blood Pressure (mmHg) | Asthma | Control |
|--------------------------------|--------|---------|
| 115.93±2.71                   | 99.69±4.94 | 0.001*** |

| Diastolic Blood Pressure (mmHg) | Asthma | Control |
|---------------------------------|--------|---------|
| 76.40±2.38                     | 68.91±2.72 | 0.034*  |

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Tab. 2. Pearson correlations of evaluated parameters.

| IGFBP3 | p     | IGF – I | p   |
|--------|-------|---------|-----|
| 0.442  | 0.001*** | 0.442 | 0.001*** |

| IGF – I | p     |
|---------|-------|
| Ac    | 0.505 | 0.001*** | 0.233 | 0.133 |

| Systolic Blood Pressure (mmHg) | Asthma | Control |
|--------------------------------|--------|---------|
| 0.270                          | 0.058  | 0.363   | 0.010** |

| Diastolic Blood Pressure (mmHg) | Asthma | Control |
|---------------------------------|--------|---------|
| 0.208                          | 0.147  | 0.275   | 0.053  |

| Pulse | Asthma | Control |
|-------|--------|---------|
| 0.080                          | 0.667  | 0.111   | 0.552  |

| O₂ Saturation | Asthma | Control |
|---------------|--------|---------|
| 0.454          | 0.009** | 0.206   | 0.258  |

| FVC (ml) | Asthma | Control |
|----------|--------|---------|
| 0.193                          | 0.260  | 0.326   | 0.052  |

| FVC (%) | Asthma | Control |
|---------|--------|---------|
| 0.044                          | 0.799  | 0.200   | 0.242  |

| FEV1 (%) | Asthma | Control |
|----------|--------|---------|
| 0.314                          | 0.062  | 0.334   | 0.046* |

| FEV1 (%) | Asthma | Control |
|----------|--------|---------|
| 0.236                          | 0.166  | 0.166   | 0.333  |

| FEV1/FVC ratio (%) | Asthma | Control |
|-------------------|--------|---------|
| 0.414                          | 0.012* | 0.088   | 0.610  |

| FEF2575% | Asthma | Control |
|---------|--------|---------|
| 0.062                          | 0.733  | 0.060   | 0.738  |

| FEF2575 (%) | Asthma | Control |
|-------------|--------|---------|
| 0.448                          | 0.010* | 0.108   | 0.556  |

| PEF (%) | Asthma | Control |
|---------|--------|---------|
| 0.122                          | 0.492  | 0.093   | 0.602  |

| PEF (%) | Asthma | Control |
|---------|--------|---------|
| 0.245                          | 0.162  | 0.096   | 0.587  |
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(Abbreviations not defined)

Significantly negative correlation was found between the serum level of IGF-I and ACT puan, O2 saturation, FEV1/FVC, %FEF25-75; p = 0.001, p = 0.009, p = 0.02, p = 0.01; respectively (Tab. 2). Significantly positive correlation was found between the serum level of IGFBP3 and systolic blood pressure. Significantly negative correlation was found between the serum level of IGFBP3 and FEV1 (ml); p = 0.001, p = 0.01, p = 0.046; respectively (Tab. 2).

Discussion

IGF-I has been identified as one of the most important molecules in the pathogenesis of asthma in the manner of inducing fibrosis, airway inflammation and hyper-responsiveness and airway smooth muscle hyperplasia (Lee et al, 2014). IGF-I receptor (IGF-IR) has a high affinity for IGF-I and it mediates actions of IGF-I. Their complex induces pro-inflammatory responses (Lee et al, 2014, Siddle et al, 2001). IGFBP3 is binding more than 70 % circulating IGF-I with higher affinity than IGF-IR. Therefore, IGFBP3 is mainly responsible for inhibition of IGF-I actions by reducing IGF-I/IGF-IR pathway (Jones et al, 1995). On the other hand, it can reserve IGF-I to protect down regulation of IGF-I receptor. That is why it is speculated that IGFBP3 has dual effect on IGF-I action which means high level of IGFBP3 reduces the action of IGF-I and low level of IGFBP3 enhances the action of IGF-I (Conover et al, 1991, Wetterau et al, 1999).

Current strategies for asthma treatment can be inadequate approximately for 10 % patients with asthma. Additionally, they are mostly not curing asthma because they have weak effect on inhibiting airway remodeling (Lee et al, 2014). That is why new treatments are needed for effective asthma treatment. IGF-I and IGFBP3 are possible targets for asthma treatment strategies because of their role in the pathogenesis of asthma. It has been shown that mRNA levels of IGF-I in endobronchial biopsies are significantly higher in asthmatic patients than controls. This elevation was found to be correlated with subepithelial fibrosis (Hoshino et al, 1998). In the literature, we could not recognize any studies that are comparing serum levels of IGF-I between asthmatic patients and healthy individuals. In the present study, serum levels of IGF-I was found significantly higher in asthma group than in the control group. If we consider that this elevation is in significantly negative correlation with ACT puan, O2 saturation, FEV1/FVC and %FEF25-75, we suppose that IGF-I may be involved the pathogenesis of asthma.

IGFBP3 has been found in high levels in tissues and bronchial lavage fluids of asthmatic patients. That is why it is suggested that there is a possible interaction with IGFBP3 and asthma (Veraldi et al, 2009). IGFBP3 takes a role in the pathophysiology of asthma dependent from IGF-I as well as independent from IGF-I. Thus, it is suggested that up-regulation of IGFBP3 can be one of the new strategies in treatment of asthma (Lee et al., 2014). We have found that serum level of IGFBP3 in asthmatic group was significantly higher than in control. It is known that circulating level of IGFBP3 is elevated by IGF-I and glucocorticosteroids (DiGirolamo et al, 2007, Conover et al, 1995). Our results also indicate that serum levels of IGF-I and IGFBP3 are significantly positively correlated with each other. That can be one of the reasons for elevated level of IGFBP3. Additionally, it can be a result of glucocorticosteroid treatment. We did not examine serum levels of IGF-I and IGFBP3 before and after treatment. That is why we can not exactly conclude the reason for the elevation of IGFBP3 in asthmatic patients. Serum level of IGFBP3 is significantly positively correlated with systolic blood pressure. It has been speculated that IGFBP3 has proatherogenic effect (Colangelo et al, 2004). We assume that this effect of IGFBP3 may lead to an increase in systolic blood pressure which can cause the development of comorbidities in asthmatic patients.

Conclusions

Current treatment strategies of asthma do not suppress the production of IGF-I which plays an important role in the pathophysiology of asthma. On the other hand, same treatments cause
elevated serum level of IGFBP3 which is more likely to reduce IGF-I action. Moreover elevated serum level of IGFBP3 can cause high systolic blood pressure. We think that this point should be kept in mind during the review of treatment strategies for asthma.

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