The effects of inhaled Fluticasone on inflammatory markers in asthmatic patients

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Abstract

Inhaled corticosteroids are the most effective anti-inflammation drugs that are used in asthma treatment. This study investigated the effects of inhaled Fluticasone on C-reactive protein (CRP) serum levels and peripheral eosinophils in asthmatic patients. We studied 30 non-smoker adult asthmatic patients before and after using 500 µg twice daily dose of inhaled Fluticasone for 4 weeks. Serum CRP levels were examined by enzyme-linked immunosorbent assay method. Also, peripheral eosinophil percentage was determined using automated cell counter. We used a paired t test to compare serum CRP values and peripheral eosinophils before and after the inhaled Fluticasone usage. Our results showed that serum CRP levels were significantly different before using inhaled Fluticasone (2.39±1.98µg/ml) compared with 4-week treated inhaled Fluticasone (1.58±1.37 µg/ml) (p=0.001). Moreover, there were significant differences between blood eosinophils before (6.3±4.6%) and after (3.4±2.1%) using inhaled Fluticasone (p=0.001). No significant correlations were found between serum CRP levels and percentage of blood eosinophils before and after inhaled Fluticasone treatment. Besides, there was no significant correlation between serum CRP levels and gender before and after using Fluticasone. Our findings indicate that inhaled Fluticasone with decrease serum CRP and number of eosinophils may improve local and systemic inflammation in asthmatic patients.

Keywords: Asthma, CRP, Eosinophil, Fluticasone, Inflammation

INTRODUCTION

Asthma is one of the most common chronic inflammatory airways disease with an estimated 300 million people affected worldwide. Its prevalence is increasing, especially among children. Symptoms include recurring wheezing, breathlessness, coughing and chest tightness particularly at night or in the early morning (GINA, 2014). Asthma is classified as atopic and non-atopic. Many cells have been played an important role in asthma such as mast cells, macrophages, neutrophils, T lymphocytes and eosinophils (Bradley et al., 1991). Eosinophils are granulocytes that develop during haematopoiesis in the bone marrow before migrating into blood. They control mechanisms associated with allergy and asthma. Infiltration of eosinophils showed in airways of asthma patients (Dal Negro et al., 2003; Hoshino and Nakamura, 1996).

C-reactive protein (CRP) mostly synthesizes by hepatocytes and Kupffer cells in liver and is regulated by cytokines such as tumor necrosis factor-alpha and Interleukin (IL)-6. In human, CRP has many important functions that some of them are unknown. (Anderson, 2006). In some situation such as acute phase response,
there is a rapid increase in the production of CRP resulting in the release of elevated quantities into the circulation. The new generation of highly sensitive assay can detect the CRP at levels of 100-fold lower than the earlier assay (Aziz et al., 2003).

Serum levels of the well-known inflammatory marker CRP can be simply and inexpensively measured in order to assess systemic inflammation. Measurement of serum CRP level has suggested the involvement of low-grade systemic inflammation in several disorders, such as cardiovascular disease and diabetes mellitus (Seo et al., 2013). Recent population based studies have showed that associations of increased levels of serum CRP with a high frequency of airway hyper responsiveness and low forced expiratory volume in one second (FEV1) among subjects without heart disease, suggest that systemic inflammation may be associated with respiratory impairment (Takemura et al., 2006).

Inhaled corticosteroids are the most effective anti-inflammation drugs that use in asthma treatment. These drugs decrease the number of inflammation cells and their proceeds. Some studies have showed that there are reverse relationship between the lung behavior and serum CRP value. Also, the relationship between serum CRP levels and the percentage peripheral eosinophils were reported (Takemura et al., 2006; Jeffery and Haahtela, 2006). Regarding that the eosinophils are one of the most effective cells in the inflammation, they can be used as disease diagnose, treatment response and the illness final destination. Despite these, the effect of systemic inflammation process in asthma and relationship between the systematic inflammation and the air ways in this sickness is not obvious. Different methods such as determining of the inflammatory cytokine profile have been used to evaluate the systematic inflammation, but the CRP evaluation is one of the easiest and reachable methods that are efficient and useful.

The main aim of this study was to determine the effect of inhaled Fluticasone, as a synthetic glucocorticoid, on serum CRP levels and peripheral eosinophils percentage in asthmatic patients. We used high sensitive CRP method. However, the study investigated whether there was any correlation between serum CRP levels and percentage of peripheral eosinophils.

MATERIALS AND METHODS

Study population and design

This is quasi experimental study of before and after design. A total of 30 adult patients (15 males and 15 females) referred to Shahid Sadoughi hospital, Yazd, Iran during two years were enrolled in the study. The diagnosis of asthma was performed according to American Thoracic Society Criteria (American Thoracic Society, 1987) and their characteristics were documented by questionnaire. All the subjects were non-smokers and none of them had a history of inhaled steroids or exacerbation of asthma during 4 weeks before enrolment. Subjects with heart disease, diabetes mellitus, malignant diseases, obesity (body mass index (BMI) ≥30 kg.m⁻²); known systemic inflammatory disorders (such as collagen vascular diseases), allergic rhinitis and serum level IgE>200IU/ml were excluded. The Ethical Committee of the Shahid Sadoughi University of Medical Sciences approved the project. Informed consent was obtained from all subjects.

Measurement of serum high-sensitive C-reactive protein levels

Peripheral blood specimens were collected and centrifuged. The sera aliquots were stored at -70°C and then subjects were treated with inhaled Fluticasone (GlaxoSmithKlin Inc, France) for 4 weeks (twice daily dose 500 µg). After using drug, blood samples were collected again and were then centrifuged. Levels of serum CRP were measured using Enzyme-Linked Immunosorbent Assay (ELISA) method according to the manufacturer’s instruction (DRG, International, Inc., USA). The minimal detectable concentration was 0.02 µg/ml.

Determining of peripheral blood eosinophil percentage

For the whole blood and eosinophil count, A sample of peripheral venous blood (2ml) was taken and stored in an Ethylenediaminetetraacetic acid (EDTA)-coated tube at the time of enrolment and analyzed within two hours after blood sampling by automated cell counter (CelltacE, Nihon Kohden, Japan).

Statistical analysis

Statistical analyses were conducted using the SPSS software Ver.15 (SPSS Inc., Chicago, IL, USA). For analytic purposes we used a paired t test to compare serum CRP values and peripheral eosinophils both before and after the inhaled Fluticasone usage. Moreover independent samples t test was used for comparing serum CRP levels before and after the treatment in men and women. Pearson's correlation test was used to test correlation between the serum CRP level and blood eosinophils percentage both before and after the treatment. Data are presented as mean±SD and a p-value of <0.05 was considered significant.
Table 1. Characteristics of asthmatic subjects

<table>
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<th>Maximum</th>
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<td>39</td>
<td>108</td>
<td>78.9</td>
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</tr>
</tbody>
</table>

Figure 1. The serum CRP levels before and after Fluticasone usage in study group

RESULTS

Samples description

In our project 30 asthmatic patients (15 females and 15 males) were considered. Studied samples were among the ages of 18-75 years old (37.7 ±13.1). The patients' weights were in the range of 45-98 Kg, with a mean value of 66.9 Kg and a standard deviation of 11.9Kg. In spirometry, FEV₁ was in a range of 39-108 of the predicted percentage and had a mean of 78.9 and standard deviation of 17.5. The demographic properties of studied patients are presented in Table 1.

The relationship between inhaled Fluticasone and serum CRP levels

In the group of patients, before the Fluticasone usage, the CRP mean of 2.39 µg/ml and standard deviation of 1.98 was achieved and after the steroid usage these values decreased to 1.58 µg/ml and 1.37 in CRP mean and standard deviation respectively. As presented in Figure 1, there were significant differences between the serum CRP levels before and after the Fluticasone usage (p= 0.001).

DISCUSSION

The primary aim of this study was to prove the concept that serum CRP levels could be reduced by inhaled corticosteroid and eosinophils

In asthmatic patients, before the Fluticasone usage the peripheral blood eosinophils mean was 6.3% (375.2 Cell/mm³) and its standard deviation was 4.6% and these results decreased to 3.4% (221.2 Cell/mm³) and 2.1% in mean and standard deviation respectively. The bloods eosinophils mean difference was meaningful (p= 0.001) as shown in Figure 2.

Regarding the results, there was no correlation between the serum CRP levels and blood eosinophils percentage before and after the treatment. There was no significant correlation between serum CRP levels and gender before and after using Fluticasone. In other words, the CRP levels in men’s serum showed no significant correlation before and after using Fluticasone comparing to CRP levels of that of women.
Fluticasone, synthetic glucocorticoid, in asthmatic patients. The results of this research indicated that the inhaled corticosteroids usage for 4 weeks was associated with a decrease in serum CRP levels. In asthma, besides the airway inflammation, systemic inflammation may also exit. CRP is one of the most characteristic markers of the inflammatory process and may play a role in the pathogenesis asthma. Some studies have tried to validate the use of CRP as a surrogate marker of inflammation in bronchial asthma.

Kasayama et al. reported that plasma levels of high-sensitivity CRP were significantly higher in the asthmatic patients than in the non-asthmatic subjects. Also, they found that treatment with inhaled corticosteroids was associated with a significant reduction in plasma CRP levels (Kasayama et al., 2009). Our finding confirms this result. In the study by Takemura et al. it was shown that in patients with steroid-naive asthma, serum levels of CRP were increased compared with healthy controls, and correlated negatively with indices of pulmonary function and positively with numbers of sputum eosinophils. In this study on steroid-inhaling patients with asthma, no significant correlations were found between serum CRP levels and clinical and sputum indices (Takemura et al., 2006). The report of Qian et al. revealed that serum CRP levels are associated with asthma severity (Qian et al., 2008). The relationship between serum levels of CRP and asthma control test in thirty asthmatic patients and thirty healthy controls was determined. The results showed that there was a positive association between CRP and the severity of asthma and a negative association between CRP and asthma control test (Kilic et al., 2012). One study showed that serum amyloid A levels were significantly increased in patients with asthma and patients with allergic rhinitis compared with healthy controls but the mean CRP and fibrinogen values in these groups were not significantly different when compared to the control group (Büyüköztürk et al., 2004).

In another cross-sectional population-based study, levels of acute phase proteins, such as plasma fibrinogen and serum amyloid A, were positively associated with asthma prevalence. (Jousilahi et al., 2002). Decrease in CRP levels using inhaled corticosteroid was reported in other respiratory disorders such as chronic obstructive pulmonary disease (COPD). One study demonstrated that CRP levels were raised in COPD non-smoker patients without clinically relevant ischemic heart disease. The study showed that CRP levels reduced in mentioned patients with COPD using inhaled corticosteroid. It was shown that CRP may be a systemic marker of the inflammatory process that occurs in patients with COPD (Pinto-Plata et al., 2006). This result was confirmed by the findings of Anderson et al. who found that CRP is elevated in clinically stable COPD and asthmatic patients and may be a marker of systemic inflammation (Anderson, 2006). Inhaled corticosteroids may affect CRP production indirectly by down regulating the expression of certain cytokines such as IL-6. In COPD, IL-6 expression in the airways is increased, which causes stimulation of hepatocytes, production of CRP and other acute-phase proteins (Van Snick, 1990). Persistent therapy with inhaled corticosteroids attenuates IL-6 expression in patients with COPD (Patel, 2003). Maybe, Fluticasone down regulates IL-6 productions in the airways, which then reduces CRP production by the liver. These findings showed that in asthmatic patients using inhaled corticosteroids decreased serum CRP and improved local and systemic inflammation.

Also, we found that there was no significant correlation between serum CRP and gender before and after treatment. There is conflicting evidence concerning relative CRP levels in men and women. Reports from

![Figure 2. The blood eosinophil percentage before and after Fluticasone usage](image)
European populations have shown similar CRP concentrations in men and women (Imhof et al., 2003; Garcia-Lorda et al., 2006). Yamada et al. reported that men have higher serum CRP concentrations in Japanese population (Yamada et al., 2001). Also, a report on Korean adult showed that serum CRP levels were higher in men that women (Lee et al., 2009).

Another important finding of this study was that using inhaled Fluticasone decreased peripheral eosinophils. This finding is in agreement with Laviolette’s findings which showed that using inhaled steroids decrease the percentage of eosinophils in patients with moderate asthma (Laviolette et al., 1994). Grutters et al. have shown that treatment with the combination of salmeterol and beclomethasone leads to inhibition of several responses associated with eosinophil priming. This study suggested that priming status of eosinophils in peripheral blood could be used as a means of monitoring inflammatory processes in the lungs before and after treatment (Grutters et al., 1999). Adelroth and co-workers demonstrated that compared with the control group, there was a significant increase in the percentage of eosinophils in asthmatic patients and in patients who were treated with beta-2-receptor agonist or inhaled corticosteroid. Eosinophil cationic protein (ECP) levels in serum and bronchoalveolar lavage were decreased (Adelroth et al., 1990). de Blay et al. reported that under corticosteroid treatment, serum ECP levels decreased. In this study a significant increase in ECP levels was found in periodic asthmatics during the pollen season. According to this finding they suggested that serum ECP is a useful marker of allergen exposure and of acute asthma treatment (de Blay et al., 1998).

The shortcomings of this study were the low number of samples, determining of sputum eosinophils and exhaled nitric oxide levels. Further investigations with higher sample size are needed to examine the effect of Fluticasone and the role of CRP in both the severity and control of asthma.

CONCLUSION

In conclusion, our findings indicate that inhaled Fluticasone decrease serum CRP levels and number of peripheral eosinophils which suggests that inhaled Fluticasone may improve local and systemic inflammation in asthmatic patients.

REFERENCES


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