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Journal of Asthma

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597262>

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Online Publication Date: 01 November 2008

To cite this Article Lee, Ka Young, Lee, Kyung Sun, Park, Seoung Ju, Kim, So Ri, Min, Kyung Hoon, Choe, Yeong Hun and Lee, Yong Chul(2008)'Clinical Significance of Plasma and Serum Vascular Endothelial Growth Factor in Asthma',Journal of Asthma,45:9,735 — 739

To link to this Article: DOI: 10.1080/02770900802216775

URL: <http://dx.doi.org/10.1080/02770900802216775>

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ORIGINAL ARTICLE

Clinical Significance of Plasma and Serum Vascular Endothelial Growth Factor in Asthma

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Background. Vascular endothelial growth factor (VEGF) is a mediator of airway inflammation and remodeling in asthma. **Purpose.** We investigated whether VEGF levels are elevated in plasma and serum obtained from patients with asthma and evaluated whether levels of plasma VEGF correlated with those of serum VEGF. **Methods.** We measured levels of plasma and serum VEGF in patients with stable asthma or with acute asthma and examined the correlation between plasma and serum VEGF concentration with initial forced expiratory volume in 1 second (FEV₁). **Results.** We found that levels of VEGF in plasma or in serum were significantly increased in stable asthmatic patients and even higher in acute asthmatic patients compared with the levels in healthy control subjects. The levels of serum VEGF correlated significantly with those of plasma VEGF. Additionally, the circulating VEGF levels were significantly inversely correlated with the percent predicted FEV₁. **Conclusion.** These results suggest that the overproduction of VEGF is implicated in asthma exacerbation and that measurement of either plasma or serum VEGF level can be a valid index in asthmatic patients. Therefore, the changes in the VEGF levels in peripheral blood of asthmatic patients can be used as a measure for progression of asthma during treatment.

Keywords asthma, plasma, remodeling, serum, vascular endothelial growth factor

INTRODUCTION

Bronchial asthma is a chronic inflammatory disease of the airways that is characterized by airway remodeling, which consists of goblet cell hyperplasia, subepithelial fibrosis, smooth muscle hypertrophy and hyperplasia, and neovascularization (1). Several studies have shown prominent increases in vessel number, vessel size, vascular surface area, and vascular leakage, including important correlations between these alterations and disease severity in asthma (2–12). Increased vascular permeability causes secretion of intravascular components. Exudation of plasma proteins into the airways contributes to the airway obstruction and hyperresponsiveness (3, 13).

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogenic peptide and plays a key role in vasculogenesis and angiogenesis (14). VEGF also increases vascular permeability and leads to airway inflammation (2, 3, 15). VEGF is one of the major determinants of asthma and thus the inhibition of VEGF receptor may be a good therapeutic strategy (2, 3, 7). In addition, VEGF is a mediator of vascular and extravascular remodeling and inflammation that enhances antigen sensitization and is crucial in adaptive T-helper type 2 cell (T_H2)-mediated inflammation (15). Recently, we have reported that levels of VEGF in sputum are increased in asthmatic patients (16). However, there are few data regarding clinical significance of circulating VEGF in asthma.

For VEGF in peripheral blood, serum concentration of VEGF has been examined in patients with various disorders. A relationship between the concentration of VEGF in the serum and the status of diseases has been demonstrated (17–22). Recently, it has been suggested that measurement of VEGF concentration in the plasma is more reliable for evaluation of disease status (23–26), because the serum level is influenced by the levels of VEGF released from platelets and white blood cells during clotting (27). However, there is still considerable controversy as to what is most valuable for estimating peripheral levels of VEGF: measuring VEGF concentration in the serum or in the plasma (28).

The aim of this study was to investigate whether VEGF levels are elevated in plasma and serum obtained from patients with stable and acute asthma. An additional aim of the present study was to determine whether levels of plasma VEGF correlated with those of serum VEGF.

METHODS

Patients

Eight asymptomatic patients with stable asthma (3 women and 5 men, 28 to 75 years of age, mean, 59.6 years of age) and 5 acute asthmatic patients (2 women and 3 men, 27 to 79 years of age, mean, 55.2 years of age) were recruited from the Chonbuk National University Hospital. Asthma was diagnosed according to the criteria of the American Thoracic Society (29). Eight normal healthy subjects (4 women and 4 men, 26 to 79 years of age, mean, 45.4 years of age) were also selected for this study. None of the subjects was a current or previous smoker. Forced expiratory volume in 1 second (FEV₁) was recorded using a computerized pulmonary function sensor (model 2200, Sensor Medics Co. Yorbalinda, CA). Asymptomatic patients were defined with

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stable asthma (defined as no decrease in $FEV_1 < 80\%$ for at least 6 months) and had no symptoms such as episodic wheezing, chest tightness, or dyspnea over a period of 4 weeks. None of the subjects had received inhaled or oral corticosteroids or any other anti-inflammatory drugs in the previous 6 months. The 5 acute asthmatic subjects were admitted to the hospital for more than 7 days because of severe or moderate asthma exacerbation and were treated under the recommendation described by Corbridge et al. (30). The treatment for acute asthma consisted of infusion of methylprednisolone (1–2 mg/kg/day), salbutamol inhalation (5–20 μ g/kg/day), and continuous theophylline drip infusion (400–600 mg/day). This study was approved by the Ethics Committee of Chonbuk National University Medical School, and fully informed, written consent was obtained from each subject.

Collection of Samples

Plasma samples were collected in vacutainer tubes containing heparin (Becton Dickinson, Rutherford, NJ). The samples were centrifuged for 15 minutes at $1000 \times g$ within 30 minutes of collection. Serum samples were collected in vacutainer tubes without additive (Becton Dickinson). The samples were allowed to clot for 30 minutes and centrifuged for 15 minutes at $1000 \times g$. The plasma and serum samples collected were stored at -70°C until use.

Measurements of Plasma and Serum VEGF Levels

Levels of VEGF were determined by enzyme immunoassays according to the manufacturer's protocol (R & D Systems Inc., Minneapolis, MN). Sensitivities for VEGF assays were 9 pg/mL.

Statistical Analysis

Data were expressed as mean $\pm$ SEM. For statistical analysis in asthmatic patients and in control subjects, the Kruskal-Wallis analysis of variance test was used to examine significant inter-group differences and if significant, the Mann-Whitney U test was used for between-group comparisons. Spearman's rank correlation was calculated to assess the correlation between data. Comparisons of the time course of VEGF in plasma and serum were made through an analysis of variance (ANOVA) and Scheffe's test. Statistical significance was set at $p < 0.05$.

RESULTS

FEV_1 Values in Patients with Stable Asthma or Acute Asthma and in Healthy Control Subjects

Initial FEV_1 values of acute asthmatic patients ($33.6 \pm 5.8\%$ of predicted values) were significantly lower than the values of subjects with stable asthma and healthy control subjects (89.5 ± 3.0 and $101.3 \pm 2.4\%$ of predicted values, respectively). FEV_1 values of acute asthmatic patients at days 4, 7, and 28 were 49.8 ± 7.7 , 56.4 ± 7.8 , and $78.4 \pm 5.2\%$ of predicted values, respectively.

VEGF Levels in Serum

The levels of VEGF in serum were significantly increased in stable asthmatic patients and even higher in acute asthmatic patients compared with the levels in healthy control subjects. The serum levels of VEGF in acute asthmatic pa-

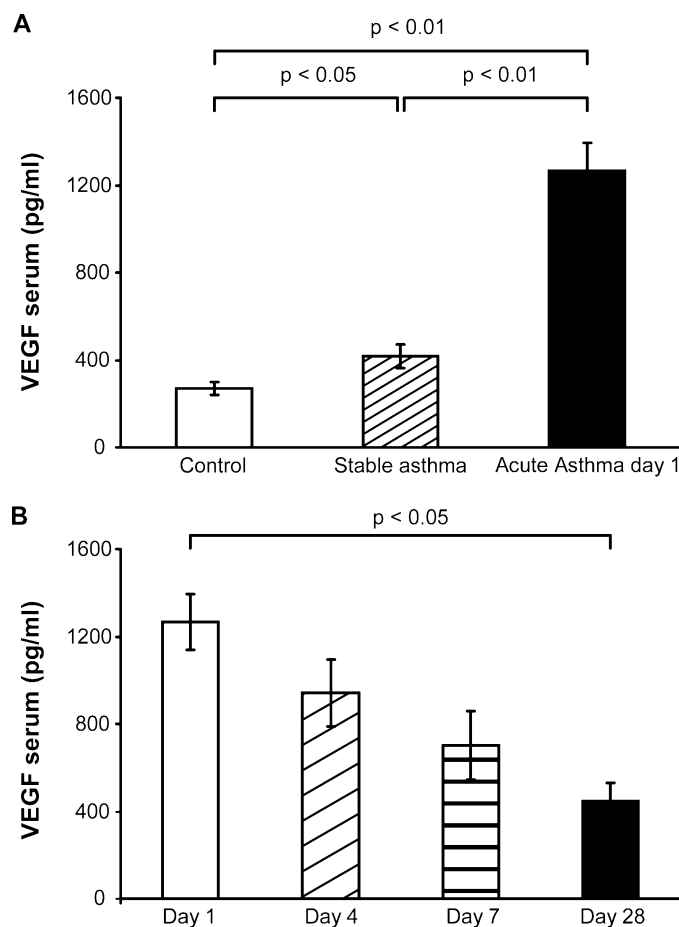


FIGURE 1.—(A) Concentrations of vascular endothelial growth factor (VEGF) in serum obtained from control, stable asthmatic, and acute asthmatic subjects. (B) Time course of VEGF levels in serum obtained from acute asthmatic subjects. Samples were obtained at day 1 of exacerbation, from day 4, day 7, and day 28. Data represent mean $\pm$ SEM.

tients were significantly higher than those in stable asthmatic patients (Figure 1A). Time course of serum VEGF levels in acute asthmatic patients before and during treatment is shown in Figure 1B. The elevated VEGF levels on day 1 had decreased by day 4 and even further by day 7 and day 28.

VEGF Levels in Plasma

The levels of VEGF in plasma were significantly increased in stable asthmatic patients and even higher in acute asthmatic patients compared with the levels in healthy control subjects. The plasma levels of VEGF in acute asthmatic patients were significantly higher than in stable asthmatic patients (Figure 2A). Time course of plasma VEGF levels in acute asthmatic patients before and during treatment is shown in Figure 2B. The elevated VEGF levels on day 1 had decreased by day 4 and even further by day 7 and day 28.

Correlation between the Concentrations of Plasma or Serum VEGF and Percent Predicted FEV_1

The levels of VEGF in plasma or in serum obtained from healthy control subjects and from subjects with asthma were

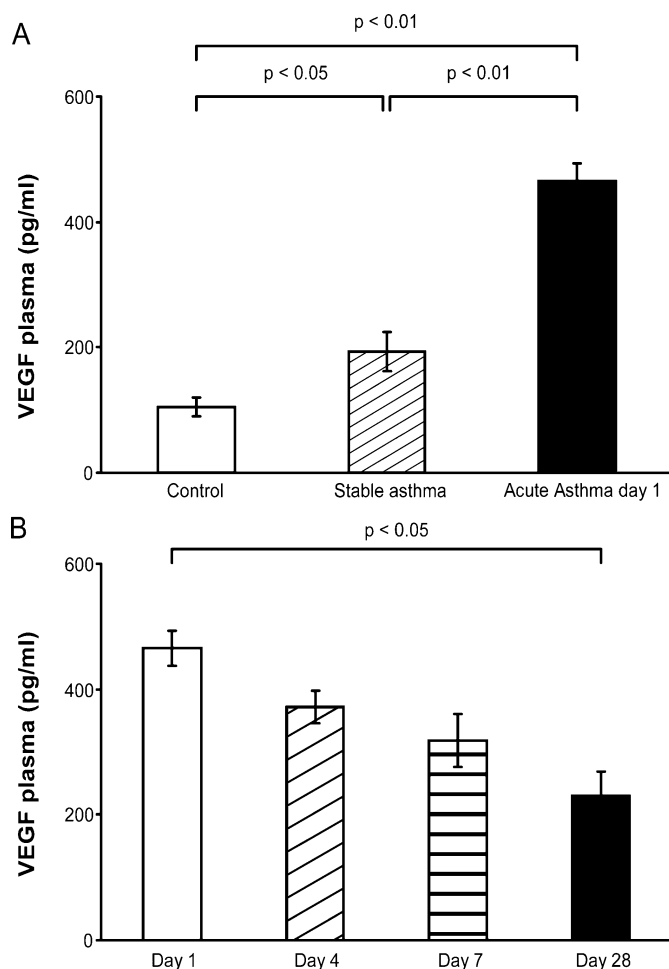


FIGURE 2.—(A) Concentrations of VEGF in plasma obtained from control, stable asthmatic, and acute asthmatic subjects. (B) Time course of VEGF levels in plasma obtained from acute asthmatic subjects. Samples were obtained at day 1 of exacerbation, from day 4, day 7, and day 28. Data represent mean $\pm$ SEM.

significantly inversely correlated with the percent predicted FEV₁ (Figure 3).

Correlation between Plasma and Serum VEGF

In healthy control subjects and in subjects with stable and acute asthma, serum VEGF levels correlated significantly with plasma VEGF levels (Figure 4).

DISCUSSION

Bronchial asthma is characterized by inflammation of the airways, which is usually accompanied by increased vascular permeability resulting in plasma exudation (1). A number of studies have indicated that expression of VEGF is increased in asthma and that overproduction of VEGF can increase vascular permeability so that plasma proteins, including inflammatory mediators, and inflammatory cells leak into the extravascular space, and thereby inflammatory cells migrate into the airways (2, 3, 7, 8, 15, 31–33). The VEGF-mediated microvascular permeability is induced by enhancing the functional activity of vesicular-vacuolar organelles, resulting in leakage of plasma proteins into the extravascular space and

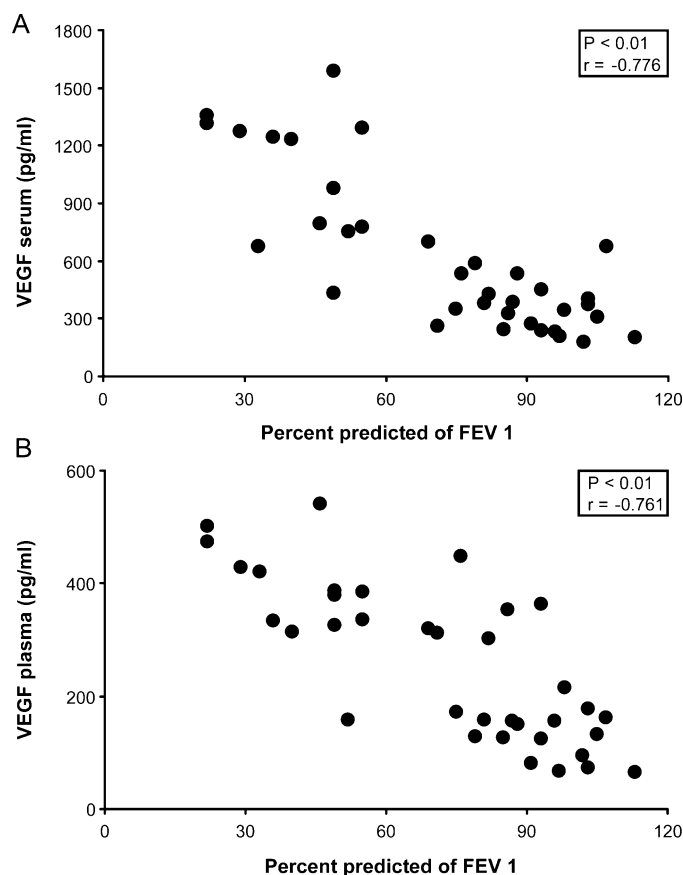


FIGURE 3.—Correlation between the concentrations of VEGF in serum (A) or plasma (B) and percent predicted FEV₁ in healthy control subjects and in subjects with asthma.

leads to edema and profound alterations in the extracellular matrix (14). Plasma protein leakage induces a thickened, engorged, and edematous airway wall, resulting in the narrowing of the airway lumen observed in asthma exacerbations. In addition, plasma proteins may also traverse the epithelium and accumulate in the airway lumen. Plasma exudation may compromise epithelial integrity and its presence in the lumen may slow ciliary function and thus, reduce mucus clearance (34). Plasma proteins may also promote the formation of viscid luminal plugs of exudate mixed with mucus and with inflammatory and epithelial cells. In addition, VEGF is also a potent stimulator of inflammation, airway remodeling, and physiologic dysregulation that augments antigen sensitization and T_H2 cell inflammation (15). Our present study with human subjects has shown that the levels of VEGF in plasma and in serum are greatly increased in acute and stable asthmatic patients compared with those in healthy control subjects. These findings suggest that VEGF plays a crucial role in the pathogenesis of asthmatic patients.

In this study, we have also found that the level of VEGF in plasma and in serum from asthmatic patients during exacerbation was significantly higher than those during remission days and after the therapy for asthma, including methylprednisolone. Mechanisms of the beneficial effects of corticosteroids on asthma include down-regulation of immune responses and stabilization of the microvasculature. These

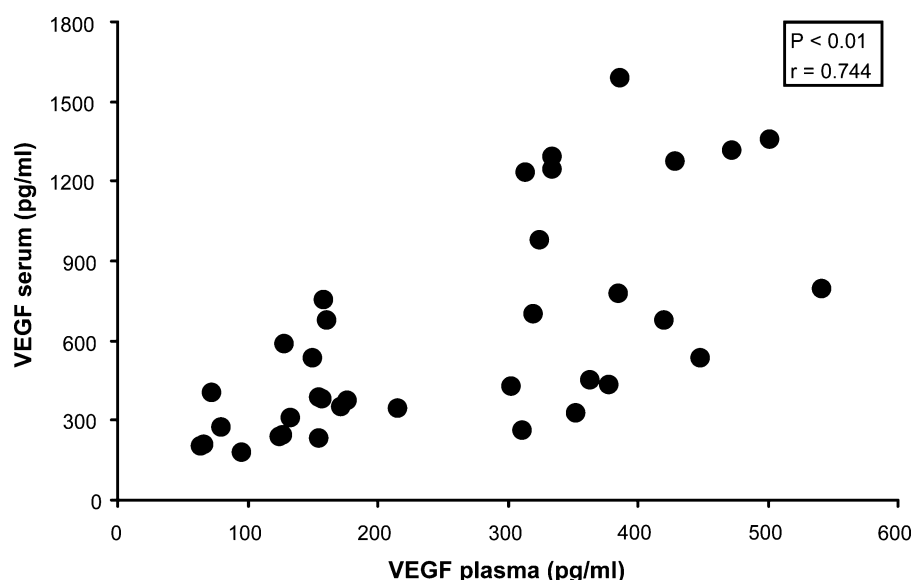


FIGURE 4.—Correlation between the concentrations of serum and plasma VEGF in healthy control subjects, stable asthmatic subjects, and acute asthmatic subjects.

lead to a reduction of plasma extravasation and a reduction of mucosal thickening, thereby reversing airway narrowing and inflammation (35). The anti-edematous effect of corticosteroids is also seen in a variety of other pathologic conditions in addition to lung disorders (36). Taken together, the therapy for asthma including corticosteroids may suppress the overproduction of VEGF.

As both plasma and serum samples are frequently used in clinical studies, we have studied VEGF in both serum and plasma from healthy control, stable asthmatic, and acute asthmatic subjects. In our study, a significant correlation has been found between plasma and serum VEGF levels in healthy control, stable asthmatic, and acute asthmatic subjects. Our results also showed that both plasma and serum VEGF levels were significantly inversely correlated with the percent predicted FEV₁. However, plasma VEGF concentration was lower than the matched serum VEGF concentration in the current study since addition of anticoagulants minimized the degranulation of white blood cells and platelets. VEGF has been reported to be stored in circulating white blood cells and platelets, and several studies have indicated that serum VEGF concentration may reflect degranulation of white blood cells and platelets during clotting after blood sample have been collected (37–41). Although some researchers have recommended that assay of plasma VEGF concentration was as a preferred quantitative evaluation of VEGF in circulating blood (23, 24), our results showed that both plasma and serum VEGF concentrations were significantly correlated with lung function. A positive correlation between plasma and serum VEGF levels was also observed in our study.

Although our data are limited by the small sample size, the statistical analysis showed the significance among the data. Moreover, this study has its own merit in introducing systemic circulating VEGF as a new diagnostic and/or monitoring marker for the asthma irrespective of the process of the blood specimen, that is, serum or plasma. Therefore, we expect that this study will be a cornerstone for the future research related to this topic. However, the large sample-sized

studies in the future are required to support our conclusion and the use of the circulating VEGF as a marker for asthma in clinical practice.

In conclusion, our findings suggest that asthma exacerbation correlates with the overproduction of VEGF, which may contribute to airway remodeling and inflammation, and that both plasma and serum VEGF levels can be used to estimate the circulating VEGF in asthma patients.

ACKNOWLEDGMENT

The authors thank professor Mie-Jae Im for careful reading of the manuscript. This work was supported by a grant of the Korea Science and Engineering Foundation (KOSEF) through the National Research Lab. Program funded by the Ministry of Science and Technology (R0A-2005-000-10052-0(2007)), by Korea Research Foundation Grant funded by Korea Government (MOEHRD, Basic Research Promotion Fund) (KRF-2005-201-E00014), by a grant of the Korea Health 21 R&D project, Ministry of Health & Welfare, Republic of Korea (A060169) (to Y. C. L.), and also by a grant from the Korea Health 21 R&D Project (0412-CR03-0704-0001) (to S. J. P.).

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