A Comparative Study of Two Angiogenic Factors*

Vascular Endothelial Growth Factor and Angiogenin in Induced Sputum From Asthmatic Children in Acute Attack

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Background: **Angiogenesis is a prerequisite for airway remodeling in bronchial asthma. Several factors may play important roles in inflammation and angiogenesis through effects on inflammatory cell infiltration or neovascularization.**

Objectives: **(1) To determine the levels of vascular endothelial growth factor (VEGF) and angiogenin in sputum supernatants of asthmatic children during the acute attack and 6 weeks after start of therapy; and (2) to correlate their levels with the degree of asthma severity.**

Subjects and methods: **Twenty asthmatic children with acute attack (mean age, 9.6 ± 3.5 years [± SD]) and 12 sex- and age-matched healthy control children were enrolled in the study. Sputum supernatants were collected for determination of VEGF and angiogenin levels. Serum samples were withdrawn for IgE measurement. The above tests were performed using an enzyme-linked immunosorbent assay. The FEV_1 was measured using spirometry. VEGF, angiogenin, and FEV_1 estimations were repeated for asthmatic children 6 weeks after start of therapy.**

Results: **During the acute attack, asthmatic children had significantly higher levels of VEGF and angiogenin than in healthy control children ($p < 0.001$). VEGF and angiogenin levels showed more elevation with increase in asthma severity ($p < 0.001$). A significant positive correlation existed between both angiogenic factors ($r = 0.98$, $p < 0.001$). A negative significant correlation was found between FEV_1 percentage of predicted and both VEGF ($r = -0.99$, $p < 0.001$) and angiogenin ($r = -0.97$, $p < 0.001$). A nonsignificant correlation was found between serum IgE and sputum VEGF ($r = 0.09$, $p > 0.05$). Although there was a significant decrease in the levels of both VEGF and angiogenin after 6 weeks of treatment with corticosteroid inhalation therapy, the levels did not reach normal control levels ($p < 0.001$ and $p < 0.05$, respectively).**

Conclusions: **Our results show that both VEGF and angiogenin levels were elevated in children with acute asthma. The study also suggests that increased severity of bronchial asthma in children is associated with the expression of both angiogenic factors, which are implicated in asthma pathogenesis. After 6 weeks of therapy, the levels of both angiogenic factors showed significant decrease.**

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Key words: angiogenesis; angiogenin; asthma; inhaled corticosteroid; vascular endothelial growth factor

Abbreviation: VEGF = vascular endothelial growth factor

Angiogenesis and microvascular remodeling are known features of chronic inflammatory diseases such as asthma and chronic bronchitis.[1] Angiogenesis is the growth of new blood vessels from existing vessels, whereas microvascular remodeling is characterized by hypertrophy and hyperplasia of airway

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smooth muscle, increase in mucous glands, and thickening of the reticular basement membrane as well as structural alterations—usually enlargement—of arterioles, capillaries, or venules, without the formation of new vessels. This is thought to contribute to irreversible airway obstruction, which is one of the factors that make the treatment for asthma difficult.[2]

The mechanism responsible for the formation of new vessels and the remodeling of the existing vessels is unknown. Mediators and inflammatory cells can be involved in different ways such as histamine and bradykinin, which can induce vasodilation, as well as some cytokines present in the airways that could determine the formation of new blood vessels. The increase in the number and size of vessels can contribute to thickening of airway wall, which leads to critical narrowing of bronchial lumen when bronchial smooth-muscle contraction occurs.[3] The formation of new vessels and the remodeling of the existing vessels are likely to be induced by multiple growth factors, including vascular endothelial growth factor (VEGF) and angiogenin.[4]

VEGF is widely expressed within many different highly vascularized organs such as the lungs.[5] VEGF is one of the most potent proangiogenic cytokines, which plays a central role in mediating the process of angiogenesis. VEGF also increases vascular permeability so that plasma proteins can leak into the extravascular space, leading to edema and profound alterations in the extracellular matrix.[6]

Angiogenin, which was first isolated from the conditioned medium of colonic carcinoma cells grown in culture, is a member of the ribonuclease superfamily, which is normally present in the circulation.[7] It is one of the most potent tumor-derived angiogenic factors. Moreover, angiogenin plays a role in a number of nonmalignant vasculoproliferative pathologic conditions. It has been implicated as a mitogen for vascular endothelial cells, an immune modulator with suppressive effects on polymorphonuclear leukocytes, an activator of certain protease cascades, as well as an adhesion molecule.[8] In the present work, our aims were as follows: (1) to determine the levels of both angiogenic factors, VEGF and angiogenin, in the sputum of asthmatic patients during the acute attack and 6 weeks after start of therapy; and (2) to correlate these levels with the degree of asthma severity.

Materials and Methods

Twenty well-documented asthmatic children were enrolled in the present study. They were recruited from the Pediatric Chest Clinic, Ain Shams University Hospital (14 male and 6 female children; age range, 5 to 16 years; mean, 9.6 ± 3.5 years [± SD]). Diagnosis of asthma was made according to the criteria suggested by the American Thoracic Society.[9] All patients had episodic cough, wheezing, dyspnea, blood eosinophilia, and normal chest radiograph results. The patients also exhibited reduced FEV_1 and bronchial hyperresponsiveness to cholinergic agents. Following Global Initiative for Asthma classification,[10] the children were further classified according to severity of asthma: mild persistent ($n = 7$), $FEV_1 \geq 80\%$ of predicted and at least one of the following: asthma symptoms more than once a week but less than once per day, and nighttime asthma more than twice a month but less than once a week; moderate persistent ($n = 9$), $FEV_1 \geq 60\%$ but $< 80\%$ of predicted, daily symptoms, or nighttime asthma more than once a week; and severe persistent ($n = 4$), $FEV_1 < 60\%$ of predicted, or physical activities limited by asthma. The patients were maintained on β_2-agonists (long- and/or short-acting rescue treatment), and none were receiving oral or inhaled corticosteroids for at least 1 month before presentation. All patients presented to hospital with an acute asthmatic attack as defined by National Asthma Education and Prevention Program guidelines[11]: cough, shortness of breath, wheezing, chest tightness, use of accessory muscles, and suprasternal retractions. Patients with lower respiratory tract infections were excluded from the study. Patients received inhaled short-acting β_2-agonists by metered-dose inhaler or nebulizer and oxygen therapy. Patients who did not show improvement with the above regimen received IV methylprednisolone, 1 to 2 mg/kg. After recovery from acute attack, all patients received inhaled corticosteroids (100 to 250 μg of fluticasone propionate) for 6 weeks. β_2-Agonists were added to the treatment regimen for moderate and severe cases.[11]

Twelve age- and sex-matched healthy children attending the Pediatric Clinic, National Research Center for routine follow-up were considered as a control group. Informed consent was obtained from all parents and approved by the ethics committee of Ain Shams University Hospital and National Research Center.

Sputum Induction and Processing

Two sputum samples were obtained from each patient: one immediately after admission to the hospital, and the other 6 weeks after treatment. For healthy children, only one sample containing normal bronchial secretions was collected.

Sputum induction and processing were performed as previously described by Asai et al.[12] All subjects were instructed to wash their mouths thoroughly with water. They then inhaled 3% saline solution nebulized in an ultrasonic nebulizer at maximum output, at room temperature under close medical supervision. They were encouraged to cough deeply at 3-min intervals thereafter. The sputum samples were kept at 4°C for no more than 2 h before further processing. A portion of the sample was diluted with phosphate-buffered saline solution containing 10 mmol/L dithioreitol and gently vortexed at room temperature for 20 min. After centrifugation at 400*g* for 10 min, the supernatant was stored at − 70°C for subsequent assay for VEGF and angiogenin. No material was added to stimulate rupture of cells.

Blood Sample Collection

Three milliliters of whole blood was withdrawn from every subject via venipuncture and was prepared for the different laboratory investigations as appropriate. All of the studied children completed the following: (1) full history taking and thorough clinical examination; (2) chest radiography (posteroanterior and lateral views [for patients only]); (3) pulmonary function testing using spirometry (MedGraphics 1070 series 2E/1085; Medical Graphics; St. Paul, MN) with emphasis on FEV_1 percentage of