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Biomarkers of oxidative stress and antioxidants in severe asthma
A Prospective Case-Control Study

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ABSTRACT

Background: Bronchial airway inflammation is the hallmark of asthma, which may be driven by an imbalance between oxidative stress and antioxidant defenses. Antioxidants deficiency may play a role, but this has remained unconfirmed.
Objective: To evaluate the oxidative stress burden and antioxidants defenses in patients with increasing asthma severity.
Methods: This prospective case-control study compared fractional exhaled nitric oxide (FeNO), exhaled breath condensate nitrite/nitrate (EBC-NOx), spirometry, and serum vitamins and trace elements among patients with and without asthma.
Results: Sixty participants were recruited (30 with severe asthma number; 23 women [76.7%]; mean age, 41.4 years; mean forced expiratory volume in 1 second [FEV_1], 2.2 L [72.2% predicted]; mean inhaled corticosteroid dosage, 2,540 μg/d; 18/30 [60%] receiving maintenance oral corticosteroids; 15 with mild asthma; all corticosteroids naïve; 9 women [60%]; mean age, 34.6 years; mean FEV_1, 3.48 L [100.5% predicted]; 15 healthy controls; 12 women [80%]; mean age, 37.6 years; and mean FEV_1, 3.53 L [111.7% predicted]). The mean FeNO levels increased significantly with increasing asthma severity ($P = .01$), but the EBC-NOx levels did not change significantly ($P = .90$). Paradoxically, vitamin A and vitamin E increased with increased disease severity, with vitamin E levels increasing significantly ($P = .07$ and $P < .001$, respectively). There was no significant difference between groups in the levels of copper ($P = .37$), zinc ($P = .97$), or selenium ($P = .90$).
Conclusion: FeNO but not EBC-NOx is increased significantly with asthma severity with no evidence of vitamins or trace elements deficiency in severe asthma. Impaired oxidative stress defenses in severe asthma may be driven by factors other than vitamins or trace elements deficiency.

Introduction

Asthma is a chronic inflammatory disorder of the airways associated with airway hyperresponsiveness, leading to recurrent episodes of wheezing, breathlessness, chest tightness, and cough. Although airway inflammation is the hallmark of asthma, several phenotypes have been described that may differ in their response to treatment.[1]

Oxidative stress represents increased production of oxidizing species attributable to inflammation and/or a reduction in antioxidant capacity, which can trigger a cascade of events, resulting in oxidant-mediated cellular injury and death. The respiratory tract is at particular risk of increased oxidative stress because of environmental factors alongside physiologic production. It has been suggested that patients with asthma are deficient in antioxidants, with the resultant oxidative stress burden leading to pulmonary dysfunction.[2,3]

Although reactive oxygen species (ROS) play essential roles in maintaining health, increased levels can become damaging.[4] The

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lung is exposed to exogenous ROS, generated from air pollutants and smoking, and endogenous ROS, produced by inflammatory cells, such as activated eosinophils, neutrophils, and macrophages in asthmatic airways. High levels of ROS are generated primarily through the NADPH oxidase–dependent complex and the cytosolic xanthine oxidase system.[5] Reactive nitrogen species are generated through interaction of nitrite and myeloperoxidase or eosinophil peroxidase–derived ROS.[6] ROS are difficult to measure directly because they are highly reactive and short lived; therefore, products of ROS reactions are measured instead to estimate the oxidative stress burden and inflammation. Fractional exhaled nitric oxide (FeNO) has been used as a marker of oxidative stress and eosinophilic airways inflammation. Consequently, it has been used in the assessment of asthma diagnosis and control.[7]

Exhaled breath condensate (EBC) collection has been used to measure nitrate and nitrite (EBC-NOx),[8] which are stable end products of nitric oxide after reaction with oxygen.[9] Previous studies reported correlation between EBC-NOx and airway inflammation,[8] with increased levels in patients with asthma compared with healthy controls.[10] Levels of EBC-NOx decreased after treatment with inhaled corticosteroids and have been found to relate to asthma control.[11] EBC-NOx is closely correlated with other common markers of oxidative stress.[12] However, the EBC-based methods of oxidative stress assessment proved difficult to replicate, and the procedure of EBC collection and analysis suffered from lack of standardization.[13] In contrast, FeNO measurement was robust and reproducible.

Antioxidants counteract the damaging effect of oxidative stress and play a vital role in maintaining the health of the airways. Enzymatic antioxidants, such as the superoxide dismutases, catalase, and glutathione peroxidases, require copper, zinc, selenium, and others to catalyze oxygen radicals to hydrogen peroxide and then to water and oxygen.[14] Other antioxidant defenses include nonenzymatic compounds, such as vitamins A, C, and E, ceruloplasmin, ferritin, and bilirubin, among others, which react directly with oxygen species, thereby reducing their potency as a radical.[15] Vitamin A has a role in promoting proliferation and prolonging survival of T cells, enhancing their antigen-presenting capacity and promoting T_H2 cell response, thereby playing a central role in asthma and allergy.[16] Although it has demonstrable antioxidant properties in vitro, its role in vivo remains uncertain. Observational studies reported no association between asthma and vitamin A intake or a potential benefit.[17] In children, serum vitamin A was negatively correlated with asthma severity.[18] Interventional studies, however, have found no benefit with dietary vitamin A supplementation,[19] and conversely high dietary vitamin A supplementation was associated with increased risk of asthma onset and severity in a mouse model.[20] Similarly, observational studies reported no association between asthma and vitamin E[17] or improvement in asthma symptoms and severity[21] with increased dietary intake. However, dietary supplementation has produced no benefit.[19]

The role played by trace elements in the severity of asthma remains uncertain. Although increased selenium levels in maternal and cord blood were associated with reduced incidence of wheeze, this effect disappeared by 5 years of age.[22] In adults with asthma, selenium levels were lower than in controls,[23] but this was not confirmed in other studies.[24,25] In a mouse model, worse outcomes were associated with both low and high selenium levels when compared with a medium group,[26] suggesting the influence of selenium on asthma may be more complex than a simple dose-response relationship. Zinc studies have been mixed, finding either no effect[27] or an association between lower zinc levels and asthma.[28] Copper studies have found either no effect[27] or increased serum copper levels in asthma.[23]

The association between asthma severity and oxidative-antioxidant balance remains unclear. Studies have reported increased oxidative stress burden with increasing asthma severity; however, the evidence for this is weak, with no clear-cut documented evidence of antioxidants deficiency in severe asthma. This study assessed the oxidative stress and antioxidants defences in a well-characterized severe asthma cohort compared with mild corticosteroid–naive patients with asthma and healthy, nonatopic, nonasthmatic controls.

Methods

This prospective case-control study compared patients with severe asthma (British Thoracic Society/Scottish Intercollegiate Guidelines Network Guidance steps 4/5, representing patients treated with high-dose inhaled corticosteroids [ICSs] and other controller asthma medication or those receiving maintenance oral corticosteroids [OCSs], respectively[29]) with mild corticosteroid-naive patients with asthma and nonatopic healthy controls. The local research and ethics committee approved the study, and all participants who met the study protocol inclusion and exclusion criteria signed a written informed consent form before their inclusion in the study.

Study Participants

All patients and healthy volunteers were screened using predesigned study questionnaire for the following inclusion and exclusion criteria; age range of 18 to 60 years, nonsmokers or ex-smokers with less than 10 pack-years of tobacco smoke, and no other active respiratory disease, autoimmune disease, infection, or malignant tumor. Other exclusion criteria were pregnancy, lactating patients taking corticosteroid-sparing agents (eg, cytotoxic medications), and those taking dietary antioxidant or trace elements supplements.

Thirty patients with uncontrolled severe asthma despite treatment with 2,000 μg/d or greater of beclomethasone dipropionate or equivalent and a second controller medication were recruited from the Birmingham Regional Severe Asthma Service (BRSAS) at Heartlands Hospital. Fifteen atopic and corticosteroid-naive patients with mild asthma were recruited from the local clinics and primary care practices. Atopy was defined as a positive skin prick test result to at least one of the common aeroallergens. All patients with mild asthma were corticosteroid naive and treated with intermittent short-acting β-adrenergic agonist bronchodilator not exceeding 3 doses per week. Patients with mild asthma had a forced expiratory volume in 1 second (FEV_1) of 80% predicted or greater and evidence of reversible airflow obstruction (12% increase in FEV_1 after 400 μg of inhaled salbutamol, diurnal peak expiratory flow variation of $\geq$20% on least 50% of the time during a 2-week period, or positive methacholine bronchial provocation test result). Patients with mild asthma were free from severe asthma attacks or hospital admissions in the preceding 5 years to recruitment.

The 15 healthy controls were never-smokers recruited from the general public through local advertisements in the hospital and university. Healthy controls had no personal or familial history of asthma and had no history of asthma symptoms. Their lung function was normal, including negative peak expiratory flow variability after 2 weeks of recordings. All healthy controls had a negative skin prick test results, confirming their nonatopic status.

Clinical Assessment and Study Visits

All participants attended 3 visits, 3 weeks apart, each lasting 60 minutes, in which they completed a predesigned questionnaire that detailed their asthma and allergy history, symptoms, medical and social history, medications, and use of vitamin and trace elements supplementation. Physiologic measurements conducted included spirometry and reversibility, skin prick testing, FeNO, and EBC.