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Original article

Cellular and plasma oxidative stress biomarkers are raised in adults with bronchiectasis☆

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SUMMARY

Background & aims: Oxidative stress is believed to play an important role in the pathophysiology of bronchiectasis. The aims of this study were to evaluate the oxidative stress status in bronchiectasis patients.

Methods: This cross-sectional study included 90 clinically stable adults with bronchiectasis of any aetiology (36 with cystic fibrosis [CF] and 54 without CF) plus 50 healthy controls. Plasma and serum oxidative stress biomarkers were measured using commercial kits. Cellular oxidative stress biomarkers in white blood cells (mitochondrial membrane potential, intracellular glutathione, superoxide anion and hydrogen peroxide) were analyzed by flow cytometry.

Results: Compared with the control group, the catalase activity and lipid peroxidation (TBARs and 8-isoprostanes) were significantly increased in the patient group and the total antioxidant capacity and the activity of superoxide dismutase were decreased. Intracellular superoxide anion and hydrogen peroxide were significantly elevated in the patients versus the controls in total leukocytes, lymphocytes, monocytes and neutrophils. Compared to the controls, the mitochondrial membrane potential was significantly lower in neutrophils and intracellular glutathione in monocytes. No significant differences were observed between CF and non-CF bronchiectasis patients in the oxidative stress biomarkers studied.

Conclusions: Biomarkers of oxidative stress, both in plasma and intracellular were raised in patients with bronchiectasis compared with controls. No differences were seen in the CF patients compared with the others.

1. Introduction

Bronchiectasis is the end result of several different diseases that share principles of management. As a result of the associated dysfunction of mucociliary clearance, a vicious cycle is established involving persistent bacterial colonization, chronic inflammation of the bronchial mucosa, and progressive tissue destruction. Bronchiectasis causes pulmonary infections and loss of lung function, results in chronic morbidity and worsening of quality of life and may contribute to premature mortality.[1–3] Bronchiectasis is usually divided into non-cystic fibrosis (non-CF) bronchiectasis, which affects a heterogeneous population and has various aetiologies, and bronchiectasis due to cystic fibrosis (CF).

Under pathophysiological conditions, reactive oxygen species (ROS) can be produced at increased rates by numerous different

Abbreviation: CF, cystic fibrosis; Non-CF, non-cystic fibrosis; ROS, reactive oxygen species; HRCT, high resolution computed tomography; SEPAR, Spanish Society of Pulmonary and Thoracic Surgery; BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in one second; WBCs, white blood cells; MFI, mean fluorescence intensity; TAC, total antioxidant capacity; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; 8-iso-PF2α, 8-iso-prostaglandin F2α; ELISA, enzyme-linked immunoassay; TBARs, thiobarbituric acid reactive substances; MDA, malondialdehyde; CFTR, cystic fibrosis transmembrane conductance regulator.

☆ The work was performed at Pneumology and Endocrinology–Nutrition Services at Carlos Haya University Hospital, Malaga, Spain.

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mechanisms. Oxidative stress is a disturbance in the pro-oxidant/antioxidant balance in favour of the former, leading to damage to biomolecules.[4] Oxidative stress is believed to play an important role in the pathophysiology of CF,[5] as well as in the pathophysiology of non-CF bronchiectasis. The chronic upper airway inflammation leads to the release of proinflammatory cytokines[6] which in turn can trigger a prolonged release of ROS and raise the levels of oxidative stress markers.[7,8]

To date, studies of oxidative stress biomarkers have mainly been performed in CF patients, with only a few in non-CF bronchiectasis patients. These studies have measured the levels of biomarkers in plasma, saliva, urine, sputum, cells from bronchoalveolar lavage or in the condensate of exhaled air.[5,7–11] However, very few studies have examined intracellular markers in peripheral blood leukocytes *in vivo* by flow cytometry in this population. These measurements could provide a better approach as they permit direct evaluation of the cell interior and can detect subclinical changes in these patients, thus reflecting the condition of the whole organism.[12] In addition, only a very few studies have combined more than four cell and plasma oxidative stress biomarkers in this population.

The aim, therefore, of this study was to evaluate oxidative stress using different biomarkers (both plasma and intracellular in peripheral blood leukocytes) in a group of clinically stable adult patients with bronchiectasis (CF and non-CF) and compare the results with a group of healthy controls.

2. Material and methods

2.1. Participants and study design

This cross-sectional study included 90 patients with a diagnosis of bronchiectasis with and without CF,[1,2,13] periodically monitored in the adult bronchiectasis/CF unit at a university hospital. A control group comprised 50 healthy subjects, matched with respect to patient sex, age and nutritional status. The study (no: 7/2009) was approved by the Research Ethics Committee of Carlos Haya Hospital, and all the participants provided signed consent after being fully informed of its goal and characteristics.

2.1.1. Inclusion criteria

Patients older than 16 years of age with bronchiectasis of any aetiology (including CF) attending the adult bronchiectasis/CF unit for routine annual review. In all cases, bronchiectasis was diagnosed by high resolution computed tomography (HRCT) of the chest.[13] All the patients underwent a full aetiological study following the diagnostic algorithm of bronchiectasis of the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR).[1]

Patients were excluded if they had not completed puberty, were taking dietary supplements with omega 3 fatty acids, were not in a clinically stable condition during the month prior to the study, or had received a transplant or were on the transplant waiting list (the latter due to the greater degree of chronic hypoxia that could contribute to greater mitocondrial dysfunction and define a different group with even greater oxidative stress). If, at the time of recruitment to the study, they had a respiratory exacerbation or a recent hospital admission their inclusion was postponed at least 30 days until completion of treatment of the acute process.

2.2. Measures

2.2.1. Clinical, analytical, radiological and spirometric variables

Body weight and height were measured in all the participants, and the body mass index (BMI) was calculated.

A prospective dietary questionnaire was administered to all the subjects according to a previously described protocol.[14]

The patients underwent simple and forced spirometry, and measurements were made of the forced vital capacity (FVC) and the forced expiratory volume in one second (FEV1) (Jaeger Oxycon Pro® Erich Jaeger, Würzberg, Germany) expressed as a percentage of the theoretical value in a reference population.

Measurement of the mean amount of sputum produced daily (in millilitres) was evaluated by instructing the patients to collect the sputum during the 3 days prior to the visit in three graded sterile containers (one per day), and marking the amount reached each day on the container. Instructions were given to ensure that sputum collection was as correct as possible, with the percentage of saliva recorded being the lowest possible.

A full clinical history, from diagnosis through to study participation, was recorded in a database: at each visit (every 2–3 months) information was collected systematically on demographic and clinical variables (including respiratory exacerbations), using the SEPAR criteria[1] and a sample of sputum was collected for microbiological study. We analyzed initial colonisation by microorganisms, considering their appearance in sputum (at least 3 positive), regardless of their persistence at the time of the study.

Structural damage was assessed with HRCT using the Bhalla scoring system (lower values indicate worse damage).[15]

Blood samples were collected after a 12-h fast and placed on ice. Some samples were centrifuged at 4000 rpm for 15 min at 4 °C. Plasma and serum were aliquotted and stored at −70 °C until analysis.

Fat-soluble vitamins A and E were analyzed by High Performance Liquid Chromatography (Agilent 1200 of Bio-Rad). Vitamin D was analyzed by electrochemiluminescence immunoassay (Modular E-170, Roche Diagnostics). Zinc was measured by atomic absorption spectrophotometry (AAnalyst200 from Perkin Elmer, Waltham, MS).

2.2.2. Determination of white blood cell oxidative stress biomarkers

Oxidative stress biomarkers were analyzed in white blood cells (WBCs) as total leukocytes, neutrophils, lymphocytes and monocytes. WBCs were isolated from patients by dextran sedimentation followed by density gradient centrifugation with Ficoll–Paque and hypotonic lysis of contaminating RBCs. After purification with two washing steps, 1×10^6 cells/mL WBCs were analyzed on a DAKO cytomation flow cytometer (MoFlo; Beckman Coulter, Fullerton, CA). WBCs were recognized on the basis of forward-angle light scatter and side-angle light scatter, which identified WBCs and excluded dead cells, other cell types, debris and aggregates from the analysis. Propidium iodide and Hoescht stain were also used to discriminate between viable and nonviable leukocytes. The relative distribution of lymphocytes, monocytes and neutrophils (in both patients and controls) was 30–40%, 5–10% and 40–50%, respectively. The mitochondrial membrane potential, superoxide anion and hydrogen peroxide and intracellular glutathione measurements were assessed as previously described.[16] Test standardization, data acquisition and data analysis were performed using the Summit software (DakoCytomation). The levels of oxidative stress markers were shown as the mean fluorescence intensity (MFI) within each gated cell population.

2.2.3. Determination of plasma and serum oxidative stress biomarkers

The total antioxidant capacity (TAC), glutathione peroxidase (GPx) activity, superoxide dismutase (SOD) activity and catalase (CAT) activity were measured in plasma using commercial kits (Cayman Chemical, Ann Arbor, MI). The 8-iso-prostaglandin F2α (8-iso-PF2α) was analyzed by a competitive enzyme-linked