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Research Article

Oxidative Stress, Antioxidant Status and Inflammation in Chronic Bronchitis and Pulmonary Emphysema

Abstract

Background: Chronic Obstructive Pulmonary Disease (COPD) is characterized by a complex range of pathological changes including both pulmonary and systemic effects. Several mechanisms contribute to the variable intermediate and clinically relevant disease phenotypes, such as chronic bronchitis and emphysema, and systemic disease. The molecular mechanisms associated to the pathogenesis of COPD are not yet clearly understood.

Objective: The aim of this study was to evaluate oxidant/antioxidant balance and the systemic inflammation in chronic bronchitis and pulmonary emphysema COPD patients.

Methods: We analyzed COPD patients divided in 2 groups: chronic bronchitis and emphysema. Healthy volunteers without lung disease were used as control group.

Results: We observed a significant ($P < 0,05$) increase in the levels of plasma malondialdehyde (MDA), a marker of oxidative stress, an increase in the circulating C-reactive protein (CRP), used as a biomarker of systemic inflammation, and a decrease in antioxidant defense in COPD patients with emphysema when compared with COPD patients with chronic bronchitis.

Conclusion: Although our results should be regarded as preliminary they indicate a disturbance in oxidant/antioxidant status and systemic inflammatory response associated to COPD patients. The differences observed were more evident in emphysematous phenotype.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by an airflow limitation that is not fully reversible and is usually progressive and associated with an abnormal inflammatory response to noxious particles and gases [1,2]. Although COPD affects the lungs, it also produces significant systemic consequences [2]. The systemic effects associated to COPD are clinically relevant and may contribute to better understanding and management of the disease [3,4]. Several studies demonstrated that an oxidant/antioxidant imbalance play an important role in the pathogenesis of COPD [5-10]. The oxidative stress in patients with COPD derives from the increased of oxidants present in cigarette smoke and/or from the increased amounts of reactive oxygen species released from leucocytes, both in air spaces and in blood [8]. Although there is strong evidence of oxidative stress in the pathogenesis of COPD, the mechanisms associated to the heterogeneity of the disease are not understood. The typical

clinical manifestations of the COPD syndrome include chronic bronchitis, a condition of large-airway inflammation and remodeling, and emphysema, disease of the distal airways and lung parenchyma that manifests as loss of surface area for gas exchange [11]. The majority of the information associated to the pathogenesis of COPD is related to the emphysema. It has been reported that the pathogenesis of COPD, particularly of emphysema, which involves the destruction of small airway structures and alveolar units, is potentiated by interactions between apoptosis, oxidative stress, and protease/antiprotease imbalance [12-14]. It has been suggested that the factors that initiate inflammation and the effects of proteolytic and oxidant-induced damage may be also important in the other COPD conditions, namely chronic bronchitis [6]. There is growing evidence that new markers are needed to provide further insights into the pathogenesis of COPD [15].

The aim of this study was to evaluate oxidant/antioxidant balance and systemic inflammation in chronic bronchitis and pulmonary emphysema.

Methods

Study subjects

The present study assessed the involvement of oxidants/antioxidants balance and systemic inflammation in two groups of subjects with clinically stable COPD and with different phenotypes- chronic bronchitis and emphysema.

Fifty healthy subjects (mean age 41,60±12,31 years; 12 female, 38 male and body mass index, BMI 27,322±4,310 kg/m²) having no history of respiratory or atopic disease and free of any medication were used as control group. Twelve of them were current smokers and thirty eight had never smoked.

Patients with stable COPD were study. COPD was defined according to the criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD). Lung function was assessed with body plethysmography. COPD patients were recruited from the respiratory outpatient consultation. Data from medical history were collected, with focus in smoking history, respiratory symptoms and signs. Lung function were evaluated for diagnosis and classification of severity of COPD, according to the GOLD criteria. Carbon monoxide gas diffusion were also performed. The characteristics of COPD group are shown in Table 1. The patients were grouped in chronic bronchitis and emphysema. The patients were included in Emphysema group if they had emphysema by high resolution computed tomography and dyspnea on exertion. Were exclusion criteria the presence of others respiratory diseases or malignancy and acute exacerbation. The COPD patients were grouped as follows: patients with chronic bronchitis (n=11) and patients with emphysema (n=9). The clinical and physiological characteristics of COPD phenotypes are shown in Table 2.

The study was conducted according to the rules of the declaration of Helsinki. The ethical conduct was ensure in this investigation and informed written consent was obtained from all subjects participating in the study.

Table 1: Characteristics of COPD group.

Clinical and physiological parameters	Values
Age, years	71,30 ±7,68
Male/Female, n	11/9
Body mass index, Kg/m ²	27,918±1,724
Smoking status: ex-smokers/non-smokers, n	15/5
Packs-years in ex-smokers	68,8 ± 10,754
GOLD stage: I/II/IV, n	2/4/14
FVC, % predicted	77,100±5,190
FEV ₁ , % predicted	58,130±5,332
FEV ₁ /FVC, %	59,453±3,805
TLC, % predicted	113,700±5,709
RV, % predicted	169,221±15,514
DLCO, % predicted	68,987±5,736
DLCO/VA, % predicted	74,080±4,459

BMI- body mass index; FVC-forced vital capacity; FEV₁-forced expiratory volume in the first second; TLC-total lung capacity; RV-residual volume; DLCO-carbon monoxide diffusing capacity; VA-alveolar volume. Values are expressed as (mean±SEM).

Table 2: Clinical and physiological characteristics of COPD phenotypes.

	Chronic Bronchitis	Emphysema
Subjects, n	11	9
Age, yrs	71.454±6.861	71.111±9.006
BMI, Kg/m ²	33.305±1.779	21.333±1.000
FVC, % predicted	75.718±5.728	78.811±9.559
FEV ₁ , % predicted	64.582±6.266	50.244±8.712
FEV ₁ /FVC, %	67.191±3.702	47.706±4.875
TLC, % predicted	101,189±6,411	126,211±7,637
RV, % predicted	139,110±14,570	202,678±24,739
DLCO, % predicted	85.714±4.471	49.400±2.952
DLCO/VA, % predicted	93.143±4.484	51.728±2.777

BMI- body mass index; FVC-forced vital capacity; FEV₁-forced expiratory volume in the first second; TLC-total lung capacity; RV-residual volume; DLCO-carbon monoxide diffusing capacity; VA-alveolar volume. Values are expressed as (mean±SEM).

Methods

Oxidative stress

One of the main targets of oxidative stress are the polyunsaturated fatty acids present in cell membranes, leading to lipid peroxidation [16,17]. Malonyldialdehyde (MDA) is a product resulting from lipid peroxidation. The evaluation of MDA in biological samples is considered an indicator of increased lipid peroxidation and is used as an oxidative stress biomarker [16,18,19]. MDA concentrations were measured spectrophotometrically in terms of thiobarbituric acid reactive substances (TBARS) using a spectrophotometric method modified from Okhawa et al. [16]. The absorbances were read at 532 nm corresponding to the colored complex formed between the MDA and thiobarbituric acid (TBA). The concentration of TBARS was calculated using the MDA concentration and using a calibration curve previously prepared. The concentration of MDA was expressed in nmol/mL of plasma.

Antioxidant plasma status

Antioxidant plasma status was evaluated by the quantification of vitamin C and total sulphhydryl groups. Vitamin C was determined based on the method described by Omaye et al. [21]. In brief, the dinitrophenylhydrazine reacts with oxidized vitamin C (oxidized ascorbic acid) to give a colored product. The absorbance was measured at 520 nm and is directly proportional to the vitamin C concentration. The levels of vitamin C were expressed in µg/mL of plasma.

The quantifications of total sulphhydryl groups were performed according to the method described by Rice-Evans et al. [22]. The non-protein sulphhydryl groups are mainly in the form of reduced glutathione (GSH). We evaluated the sulphhydryl group using a spectrophotometric method involving the use of Ellman's Reagent. The 5,5'- Dithiobis(2-nitrobenzoic acid) (DTNB) undergoes disulfide exchange with sulphhydryl groups and occurs the formation of 5-thio-2-nitrobenzoate anion (TNB). The absorbance of the reduced chromogen was measured at 412 nm and is directly proportional to the GSH concentration. The levels of GSH were expressed in µmol/mL of plasma.

Inflammation

Circulating inflammatory mediator was assessed by the measurement of the acute phase protein C-reactive protein (CRP) levels, a marker of systemic inflammation. CRP levels were measured by chemiluminescent immunoassay. The levels of CRP were expressed in mg/L.

Statistical analysis

The results are expressed as mean \pm SEM of the concentrations of plasma parameters evaluated. The appropriate nonparametric test was chosen for data not normally distributed. Comparisons between two groups were tested using unpaired *t*-test or Mann-Whitney U test. A difference with $P < 0,05$ was considered statistically significant.

Results

The plasma concentrations of MDA, vitamin C and GSH of COPD patients with chronic bronchitis and COPD patients with emphysema are shown in Table 3.

The plasma MDA levels found to be significantly ($P < 0,001$) higher in COPD patients with chronic bronchitis and with emphysema when compared with control group ($3,830 \pm 0,310$ nmol/mL). Additionally, our results have shown a significant ($P < 0,05$) increase in the marker of oxidative stress associated to emphysematous COPD patients when compared to bronchitis Figure 1.

The two sub-groups of COPD patients had a significant ($P < 0,001$) decrease in antioxidant status in chronic bronchitis and emphysema when compared with control group (vitamin C $6,230 \pm 0,220$ μ g/mL; GSH $0,275 \pm 0,011$ μ mol/mL). However, no significant difference was observed when compared chronic bronchitis and emphysema. But the emphysematous patients have a decrease in the antioxidant status Figure 1.

In our study, we observed a significant ($P < 0,001$) decrease in Body Mass index (BMI) and a significant ($p < 0,05$) increase in plasma MDA levels in COPD patients with predominant emphysema when compared with COPD patients with predominant chronic bronchitis Figure 2.

There was no significant difference in CRP levels between COPD patients with emphysema and chronic bronchitis. But

we observed a tendency toward an increase associated to emphysematous patients Figure 2.

Although our results should be regarded as preliminary, we observed that low BMI (< 21 kg/m²) could be related with higher levels of CRP and higher MDA levels in COPD patients with emphysema. In addition, our results indicated that this increase in oxidative biomarker and systemic inflammation is more evident in group of emphysema with low BMI and with very severe COPD.

Discussion

We observed differences in plasmatic markers in the 2 typical clinical phenotypes studied. There was an increase in MDA and CRP levels and a decrease in antioxidant status (vitamin C and GSH) associated to emphysematous patients when compared with chronic bronchitis. Additionally, an increase in MDA and CRP levels were associated to patients with emphysematous phenotype with low BMI.

Our results agree with recent studies that have shown that COPD is associated not only with an abnormal inflammatory response of the lung parenchyma but also with a systemic inflammation and a systemic oxidative stress [3]. There is evidence of an increase in circulating CRP levels associated to stable COPD patients and this acute-phase protein has been regarded as a valid biomarker of systemic inflammation in these patients [23–26]. Some authors investigate the relationship between systemic inflammation, oxidative stress, body mass index and lung function. Karadag et al. [23], reported that CRP levels are higher in COPD patients with low BMI. Other authors suggest that low BMI is associated with higher degrees of bronchial obstruction and pulmonary hyperinflation, in association to circulating CRP levels during acute exacerbations of COPD [27]. However, other studies found increases in CRP levels with increasing BMI in stable patients with moderate to severe COPD [28]. In another study there was no correlation between BMI and MDA in patients with COPD [29]. Most studies of markers of inflammation and oxidative stress in COPD have analyzed the results for all types of patients together and the results are inconclusive. In our study, we observed a significant ($P < 0,001$) decrease in Body Mass index (BMI) and a significant ($P < 0,05$) increase in plasma MDA levels in COPD patients with predominant emphysema when compared with COPD patients with predominant chronic bronchitis. Although our results should be regarded as preliminary, we observed that low BMI (< 21 kg/m²) could be related with higher levels of CRP and higher MDA levels in more severe COPD patients with emphysema. Some recent studies demonstrate differences in pulmonary and systemic markers associated to emphysema and chronic bronchitis. In contrast with our results, other authors suggest that in COPD patients with emphysematous phenotype the inflammatory response and oxidative stress were reduced when compared with chronic bronchitis [30]. More studies are needed to clarify the mechanisms involved in inflammatory response and oxidant/antioxidant balance in different clinical phenotypes of COPD patients. In this study, we only evaluate systemic markers and we don't use EBC samples. Another limitation

Table 3: MDA, vitamin C, GSH and CRP concentrations in COPD patients with mainly chronic bronchitis or with mainly emphysema.

	Chronic bronchitis	Emphysema
Marker of oxidative stress		
MDA (nmol/mL)	7.729 \pm 1.208	9.552 \pm 2.343
Antioxidant status		
Vitamin C (μ g/mL)	1.899 \pm 0.431	1.827 \pm 0.609
GSH (μ mol/mL)	0.143 \pm 0.034	0.126 \pm 0.028
Marker systemic inflammation		
CRP (mg/L)	3.830 \pm 0.666	3.996 \pm 1.049

Results are expressed as (mean \pm SEM).

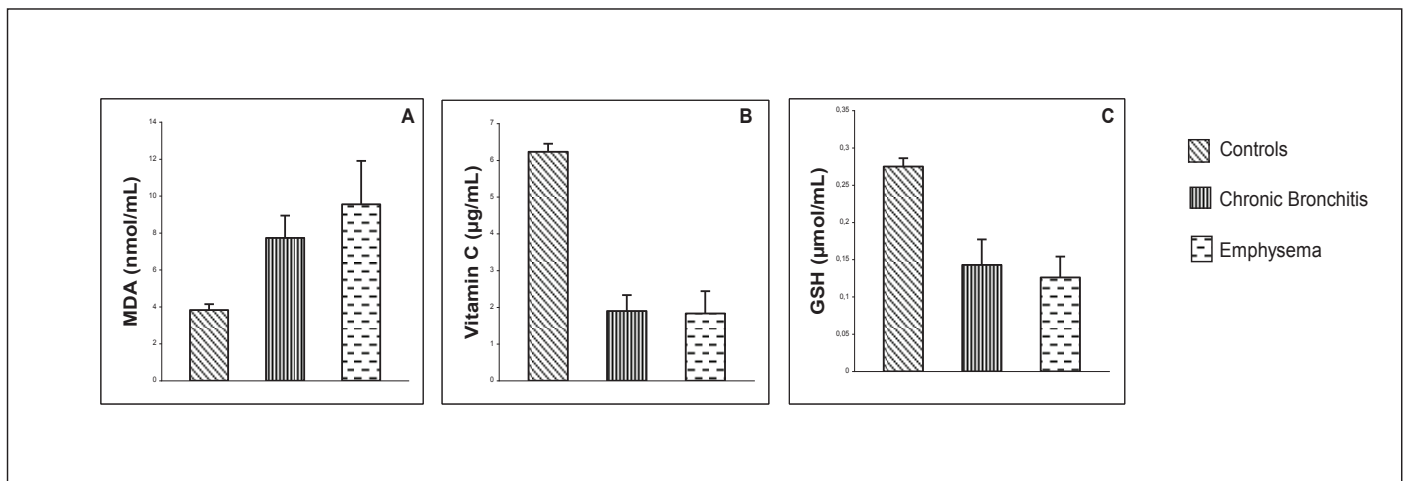


Figure 1: Concentrations of (A) MDA, a marker of oxidative stress, (B) vitamin C and (C) GSH in plasma from controls and COPD patients, mainly chronic bronchitis and mainly emphysema. Results are expressed as mean±SEM. Significant differences ($p<0.001$) were observed between COPD patients and controls. MDA levels were significantly ($p<0.05$) higher in emphysema in comparison with chronic bronchitis. No significant difference was observed between the COPD phenotypes in vitamin C and GSH levels.

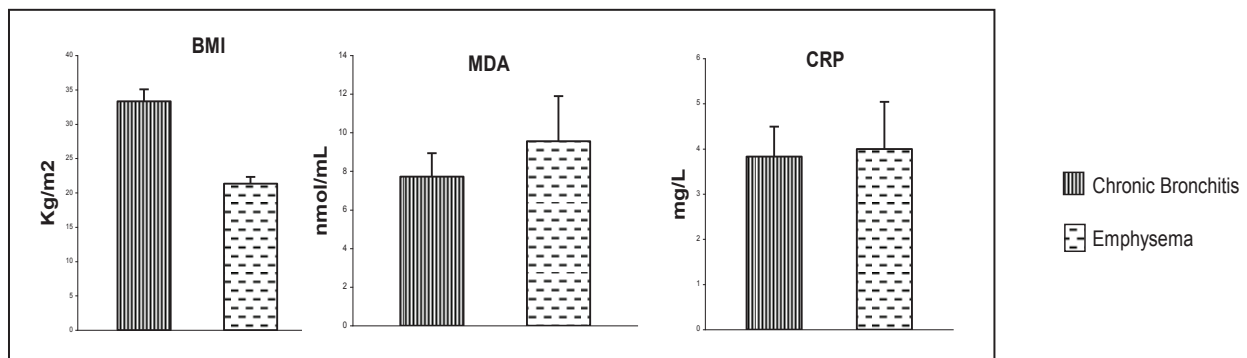


Figure 2: Comparison of BMI, MDA and CRP in COPD phenotypes. A significant decrease ($p<0.001$) in BMI and a significant increase ($p<0.05$) in MDA was observed in emphysema when compared with chronic bronchitis. An increase in CRP levels were associated to emphysema but no significant difference was observed in the two COPD groups.

of our study was the classification of patients by phenotype. Indeed, no well-established cutoffs are currently available. We use the computed tomography images of the thorax and the clinical characteristics to confirm the classification of patients by phenotypes. Additionally, our results should be regarded as preliminary because of the sample size. But the consistency of our results, showing differences in systemic markers between the 2 COPD phenotypes suggests that different clinical phenotypes have different systemic responses and many mechanisms may be involved in the pathogenesis of COPD. Our results, could contribute to consolidate the association between oxidative stress and COPD phenotypes. Most of the recent research has been directed to validated biomarkers to a more complete and clinically characterization of COPD [24,31–33]. The concept of an oxidant/antioxidant imbalance (oxidative stress) in the lung tissue of patients with COPD may be at the center of the pathobiology of these diseases. Oxidative stress may be one mechanism that enhances the inflammatory response [34]. It has been proposed that inflammation is

related to oxidative stress, protease-antiprotease imbalance and apoptosis as destructive processes in emphysema [14,34]. In this study, we observed a statistically ($P<0,05$) significant increase in the biomarker of oxidative stress in patients with emphysema when compared with patients with chronic bronchitis. We didn't find a difference in the antioxidants status (vitamin C and GSH) between the 2 phenotypes but we observe a tendency toward a decrease in antioxidant status associated to COPD patients with mainly emphysema. Other authors found a significant increase in MDA levels and a decrease in vitamin C associated COPD patients and in emphysema but an increase in GSH levels was observed in patients with emphysema [35]. There are a limited number of studies about oxidants/antioxidants in COPD phenotypes. Strong evidence for the role of oxidative stress in the protease/antiprotease imbalance and apoptosis associated to the pathogenesis of emphysema has been provided by studies in animal models of emphysema [13,36]. Other authors reported an association of emphysema with genetic variations in antioxidant genes and a decrease in

the antioxidant capacity [37]. Our results show an association between COPD and insufficiency antioxidant capacity to prevent oxidative damage. More evidence of a disturbance in oxidant/antioxidant status was found in pulmonary emphysema. In addition, our results suggested that an increase in oxidative biomarker and systemic inflammation is more evident in the group of emphysema with low BMI and with very severe COPD. However, since our study population was limited size, these findings should be confirmed in further studies.

Conclusions

Although our results should be regarded as preliminary they indicate a disturbance in oxidant/antioxidant status and systemic inflammatory response associated to COPD patients. The differences observed were more evident in emphysematous phenotype. These results could lead to better understanding of the variability associated to COPD patients and may contribute to the development of novel therapeutic interventions. Further studies are needed to analyze pathophysiological mechanisms involved in the phenotypes of COPD associated to oxidant/antioxidant imbalance.

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