Prooxidant - antioxidant balance in COPD patients

Balanța prooxidant - antioxidant la pacienții cu BPOC

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Abstract

Objective. *Oxidative stress is an important risk factor for the pathogenesis of chronic obstructive pulmonary disease (COPD). Oxidative stress and chronic inflammation are the two major pathogenesis features of COPD. The aim of this study was to assess the role of oxidative stress in the pathogenesis of COPD by using a rapid novel assay of prooxidant-antioxidant balance (PAB).*
Patients and methods. *In the present study, we assessed the extent of oxidative stress in the serum of 60 COPD patients and 60 healthy control subjects using a rapid assay of prooxidant-antioxidant balance (PAB). It is notable that due to non-parametric distribution of quantitative variables, the Mann-Whitney and Kruskal-Wallis tests were performed using SPSS for statistical analysis.*
Results. *Each group consisted of 42 (70%) males and 18 (30%) females. The mean ages of the study and control groups were 58.9±6 and 58.5±6, respectively. Furthermore, the mean rate of smoking was 24.2±9.2 (packet/year) in the study group and 5.1 (packet/year) in the control group. Also, the mean smoking rate (packet/year) was 25.4±8.8 for males and 13.3±5.7 for female COPD patients. Mean serum PAB [Hamidi-Koliakos (H.K.) units] were 193.6±43 and 62.05±40.4 among the patient group and control group, respectively (P<0.001).*
Conclusion. *The current study highlights the key role of oxidative stress in the pathogenesis of COPD.*
Keywords: ***chronic obstructive pulmonary disease, prooxidant, oxidant, balance, PAB method***

Rezumat

Obiectiv. *Stresul oxidativ joacă un rol important în patogeneza bronhopneumopatiei obstructive cronice (BPOC). Inflamația cronică, alături de stresul oxidativ reprezintă două dintre mecanismele patogenice majore ale acestei boli. Scopul studiului a fost de evaluare a rolului stresului oxidativ în patogeneza BPOC prin folosirea unui test rapid de determinare a balanței prooxidanți - antioxidanți (BPA).*
Metodă. *S-a evaluat prezența stresului oxidativ în serul a 60 de pacienți cu BPOC, respectiv în serul a 60 de subiecți sănătoși, prin utilizarea unei testări rapide a balanței prooxidanți - antioxidanți. Datele au fost analizate statistic cu ajutorul programului SPSS, cu aplicarea testelor Mann-Whitney și Kruskal-Wallis pentru variabile cantitative cu distribuție non-parametrică.*
Rezultate. *În ambele loturi au fost incluși 42 de bărbați (70%) și 18 femei (30%). Vârsta medie în lotul de pacienți a fost de 58,9±6 ani, iar în lotul de control, de 58,5±6 ani. Pacienții au avut o rată medie de fumat de 24,1±9,2 pachete-an (PA), iar subiecții sănătoși, de 5,1 PA. De asemenea, indicele PA pentru bărbați a fost 25,4±8,8, iar pentru femei, 13,3±5,7 la pacienții cu BPOC. Valoarea medie a BPA serice exprimate în unități Hamidi-Koliakos (HK) a fost de 193,6±43 în lotul cu pacienți, respectiv 62,05±40,4 în lotul de control (p<0,001).*
Concluzii. *Studiul evaluează rolul-cheie al stresului oxidativ în patogeneza BPOC.*
Cuvinte-cheie: ***bronhopneumopatia obstructivă cronică, prooxidant, oxidant, balanță, metoda balanță prooxidant - antioxidant***

Introduction

Chronic obstructive pulmonary disease (COPD) is a major leading cause of chronic morbidity and mortality worldwide. The most prevalent cause of COPD is tobacco smoking, while genetic background and air pollution are also responsible for the increased risk[1-4]. By exposure to irritant agents, especially smoking, an inflammatory response enhanced in the pulmonary tissue leads to small airways narrowing and damaging pulmonary tissue in COPD patients, so airflow obstruction is considered a main clue for COPD diagnosis[5]. COPD is actually associated with an abnormal inflammatory response of the pulmonary tissue to noxious particles or gases, especially cigarette smoke[6,7]. Two major known features in the pathogenesis of COPD include oxidative stress and chronic inflammation. Increased oxidative stress is an outcome of elevated volume of inhaled oxidants and it is followed by the increment of the amount of reactive oxygen species (ROS) that are produced by various inflammatory, immune, and epithelial cells of the airways[8]. ROS are highly reactive molecules that impair macromolecules such as lipids, proteins, carbohydrates, DNA and so on, that finally lead to pulmonary tissue damage. Also, one of the main roles of oxidative stress has been reported in hypoxemia[9]. Because of the important effects of oxidative stress on the pathogenesis of COPD and the processes relevant to pulmonary physiology, and due to the importance of COPD as a major public health problem and its increasing prevalence and mortality[9-11], in the present study we determined the prooxidant - antioxidant balance (PAB) in COPD patients by means of a rapid novel PAB assay.

Patients and methods

Subjects

In this case-control study, the subjects were COPD patients who presented from 2014 to 2015. The medical history of the patients was recorded and a physical examination was performed by an associated professor of pulmonary disease. The laboratory records, plain radiography of the chest and spirometry were performed and the obtained data were recorded in a questionnaire. The inclusion criteria were: patients with COPD, in which the diagnosis based on the Gold criteria (stage III, IV) was confirmed by an associate professor of pulmonary diseases while considering the patients' medical history, physical examination and laboratory findings. The patients with COPD did not have any cardiovascular comorbidity. All of the COPD patients were past or current smokers. The exclusion criteria were: pregnancy, regular use of systemic corticosteroids within the previous 30 days, presence of infectious diseases, consumption of antioxidants such as vitamins (A, D or E), diseases like diabetes mellitus and renal problems, and patient dissatisfaction. In addition, another questionnaire was filled out containing demographic information, prescribed medicines and cigarette smoking.

Blood sampling

Blood samples were collected from each subject in the morning after an overnight fast. After being allowed to clot, the blood was then centrifuged at 2500 rpm for 15 minutes at room temperature to obtain serum. Hemolyzed samples were excluded from analysis. Serum was stored at -20^{0}C prior to analysis.

Chemicals

TMB powder (3,3´,5,5´-Tetramethylbenzidine, Fluka, Buchs, Switzerland) peroxide enzyme (Applichem: 230 U/mg, A379,0005, Darmastadt, Germany), chloramines T trihydrate (Applichem: A433, Darmstadt, Germany), hydrogen peroxide (30%) (Merk, Darmastadt, Germany). These chemicals and all the other reagents used were reagent grade and were prepared in double distilled water.

Prooxidant - antioxidant balance (PAB) assay:

A modified PAB assay was applied based on a previously described method[11]. The standard solutions were prepared by mixing varying proportions (0-100%) of 250 µM hydrogen peroxide with 3 mM uric acid (in 10 mM NaOH). TMB powder (60 mg) was dissolved in 10 mL Dimetysulfoxide (DMSO). For the preparation of the TMB cation, 400λL of the TMB/DMSO solution was added to 20 mL of acetate buffer (0.05 M buffer, pH 4.5), and then 70λL of fresh chloramines T (100 mM) solution was added. The solution was mixed well and incubated for 2 hours at room temperature in a dark place. Then 25 U of peroxidase enzyme solution was added to 20 mL of TMB cation solution, dispensed in 1 mL and stored at -20^{0}C. In order to prepare the TMB solution, 200λL of TMB/DMSO was added to 10 mL of acetate buffer (0.05 M buffer, pH 5.8) and the working solution was prepared by mixing 1 mL TBM cation with 10 mL of TMB solution. This working solution was incubated for 2 minutes at room temperature in a dark place and then immediately used. Ten microliters of each sample, standard or blank (distilled water), was mixed with 200λL of working solution in each well of a 96-well-plate, which was then incubated in a dark place at 37^{0}C for 12 minutes. At the end of the incubation time, 100 λL of 2 NHCl was added to each well, and the topical density (OD) was measured in an ELIZA reader at 450 nm with a reference wavelength of 620 or 570 nm. A standard curve was provided from the values relative to the standard samples. The values of the PAB are expressed in arbitrary unite (HK), which is the percentage of hydrogen peroxide in the standard solution.

Statistical analysis

Statistical analyses were performed with SPSS version 16 software. Values were expressed as mean±SD. To compare the serum level of oxidant and antioxidant balance, t-student and ANOVA tests were used between the groups. Due to lack of normality of other quantitative variables, we used non-parametric tests such as the Mann-Whitney and Kruskal-Wallis. A two-sided $P<0.05$ was considered significant.

Ethical consideration

This study was approved by the ethical Committee of the Mashhad University of Medical Sciences. Written Consent was obtained from all patients and they were ensured about confidentiality and security of their personal information.

Results

Sixty patients with COPD and 60 healthy individuals in the control group were chosen. In both groups, there were 42 (70%) males and 18 (30%) females. The mean of age in the study and control groups were 58.9±6 and 58.5±6, respectively. T-test and ANOVA did not show a significant difference for mean age between the control and patient groups (P=0.747).

The mean age of males and females were 59.9±6 and 56.7±6 years in the study group, respectively. The mean rate of current smoking based on packet per year is 24.2±9.2 in patient group, while this rate was 5.1 in the control group. The mean rate of smoking (packet/year) was 25.4±8.8 for males and 13.3±5.7 for females in patients. Eight (13.3%) cases of the healthy control group were current smokers. The mean body mass index (BMI) of the study group was calculated at 26.52±3.3 and in the control group it was 25.82±2.9. This rate was 26.64±3.2 for males and 26.25±3.5 for females. The means PAB were 193.6±43 HK and 62.05±40.4 HK in the patient and control group, respectively. Mann-Whitney test was used and significant difference was observed between the study and control groups (P<0.001) (Table1).

Discussion

COPD is the major leading cause of disability and death in the world[2,3]. There are four major mechanisms suggested for the pathologic changes in COPD: oxidative stress, inflammation, protease-anti protease imbalance, and apoptosis[4,8]. Patients with COPD and smoking history presented with higher levels of H_2O_2 in exhaled

breathing compared with non-smokers and former smokers with COPD[12-15]. So, it seems that measuring the oxidative markers in plasma is a good method to show the oxidative stress *in vivo*. In order to discover disease progression, antioxidant agents could play a predictive role by tracking the level of oxidative biomarkers[16]. In the current study, we detected an increased oxidative stress in patients in comparison to control group by increasing PAB value. The increased PAB value indicate that the balance between prooxidants and antioxidants shift in favor of prooxidants such as H_2O_2.

The current findings suggest that oxidative stress plays an important role in COPD pathogenesis as increasing of the oxidative agents and reduction of antioxidant factors is present, which show an imbalance between them. Elevated H_2O_2 rates in the plasma of the patients confirm the presence of oxidative stress in COPD. Our study showed that oxidative stress was more common in the smoker patients comparing with the non-smokers; however, in the control group, we could not find any significant difference between individuals with or without a history of cigarette smoking. Cristovao et al. studied the oxidant and anti-oxidant balance in the pathogenesis of chronic obstructive pulmonary disease[17]. They revealed that oxidative stress was more prevalent in smoker COPD patients than in non-smokers with COPD, this feature being consistent with our findings. Our findings showed that there was a 300% difference between the two groups for oxidative stress, the study group showed 193.6 HK, while for the control group it was 63.05. This indicates that oxidative stress is a very strong hallmark in patients who suffer from COPD. Arja et al. studied the oxidative stress and antioxidant enzyme activity in South Indian male smokers suffering from COPD[18]. Correspondingly, they concluded that there was an oxidant - antioxidant imbalance in their COPD patients. Similar to our findings, Woźniak et al. showed that there was association between oxidative stress and smoking in COPD patients[19]. They also showed that cessation of tobacco for three-months restored the oxidant - antioxidant balance. Joppa et al. examined oxidative stress in patients with COPD and pulmonary hypertension[20]. Similar to our results, they enrolled healthy subjects into their study and they reported the oxidant - antioxidant imbalance in the systemic circulation in COPD patients. Gumral et al. evaluated the antioxidant enzymes and melatonin levels in patients with bronchial asthma and COPD during stable and exacerbation periods. They also showed that an increased oxidative stress existed in patients with bronchial asthma and COPD, associated with reduced rates of antioxidant enzymes and melatonin[21]. The previous studies also confirm our findings. Nadeem et al., Kluchova et al., Rytila et al., Wouters et al., Brindicci et al., Sahin et al., and Ermis et al. have reported systemic oxidant - antioxidant imbalance in COPD patients[22-28]. In almost all the aforementioned studies the increased levels of oxidative stress factors and reduced defensive antioxidant agents are expressed separately. However, due to the specific characteristics of the PAB method in the current study, that evaluates the prooxidant and antioxidant factors when both react with unique substance, the oxidative stress is expressed as the increased PAB value which indicate that the balance between prooxidants and antioxidants shift in favor of prooxidants.

Conclusion

The current study highlights the key role of oxidative stress in the pathogenesis of COPD. The rate of oxidative stress increases the oxidative factors and decreases the antioxidative agents, and in COPD patients the levels of first factors were significantly higher than in the normal and healthy individuals. So, there is a need to provide antioxidants therapy which control the underlying inflammatory and destructive processes of COPD and oxidative stress determine by PAB assay. ■

Competing interests: The authors declare that they have no competing interests.

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Table 1 The comparison of variables between the two groups

Variables	Study group	Control group	P value
Gender	M=28 (70%), F=12 (30%)	M=28 (70%), F=12(30%)	-
Mean age	58.9±6	58.5±6	0.747
Mean current cigarette smoking	24.2±9.2	5.1	P<0.05
Body mass index	26.52±3.3	25.82±2.9	-
PAB (HK unit)	193.6±43	62.05±40.4	0.001

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