

Investigation of Bisphenol A as an endocrine disruptor, total thiol, malondialdehyde, and C-reactive protein levels in chronic obstructive pulmonary disease

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Abstract. – OBJECTIVE: Chronic obstructive pulmonary disease (COPD) is a common health problem and it is associated with oxidant/antioxidant imbalance and systemic inflammation. Bisphenol A (BPA) is an endocrine disruptor agent, exerting a wide variety of metabolic effects. Also, BPA is related with oxidative stress, decreased antioxidant enzymes, and inflammation. The aim of this study is to investigate the relationships between COPD and serum BPA, C-reactive protein (CRP), malondialdehyde (MDA), and total thiol levels.

PATIENTS AND METHODS: This study was enrolled at 83 subjects that they were divided into two groups: control (n = 33), COPD (n = 50). The serum BPA, CRP, MDA, and total thiol levels were analyzed.

RESULTS: The CRP and BPA levels were significantly higher in the COPD patients than control subjects. The total thiol levels were significantly lower in COPD cases than the controls. There is no different between groups for MDA. Also, there had a linear relationship between BPA and CRP in correlation analysis.

CONCLUSIONS: COPD is associated with high serum BPA, CRP and low total thiol levels in comparison with healthy individuals. It is suggested that BPA might have a role in the etiopathogenesis of COPD.

Key Words:

Bisphenol A, Chronic obstructive pulmonary disease, C-reactive protein, Malondialdehyde, Thiols.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major public health problem and it is thought to be due to an enhanced chronic pul-

monary inflammatory reaction to noxious substances, and the disorder is characterized by persistent airflow limitation¹. Smoking is the most important etiological factor for COPD². It is not the only one and there is consistent evidence from epidemiologic researches that nonsmokers may have chronic airflow limitation^{3,4}. There is increasing evidence of nonpulmonary effects in terms of low grade systemic inflammation in COPD patients such as lung cancer, osteoporosis, diabetes mellitus, metabolic syndrome, cachexia, skeletal muscle dysfunction, anemia, cardiovascular disease, and depression⁵.

Previous studies have revealed that circulating markers of systemic inflammation including interleukin-6 (IL-6) and C-reactive protein (CRP) are increased stable COPD patients^{6,7}. Several studies have reported an elevated oxidant burden and consequently increased markers of oxidative stress in the blood, urine, breath and airspaces in smokers and COPD cases. The presence of oxidative stress has important results for the COPD pathogenesis^{8,9}. Malondialdehyde (MDA) is one of the lipid peroxidation products that are increased in lungs of COPD patients and it is negatively correlated with lung function^{10,11}. It is revealed that increased reactive oxygen species (ROS) production and decreased endogenous antioxidants have been showed in COPD^{12,13}. Generally, inflammation, oxidative stress, protease-antiprotease imbalance, and apoptosis are responsible for the pathogenesis of COPD¹⁴.

Bisphenol A (BPA) is used commonly in the manufacture of epoxy resins, polycarbonate plastics, and food and beverage containers¹⁵. In humans, BPA is one of the most causes of environ-

mental exposure¹⁶. BPA exerts a wide variety of metabolic effects and it is also known as an endocrine disruptor agent^{15,17,18}. Previous studies found that the BPA-induced oxidative stress have been performed with laboratory mammals^{19,20}. It is proposed that exposure to BPA throughout intrauterine life and during infancy causes tissue oxidative stress by decreasing the antioxidant enzymes and peroxidation, leading to underdevelopment of the testis, brain and kidney in mice^{19,21}. In a study showed that urinary BPA concentrations were positively related with MDA and CRP levels in the postmenopausal women, and this report revealed that BPA exposure may cause oxidative stress and inflammation²². Therefore, BPA might play a role in the pathogenesis of COPD.

COPD is associated with increased oxidant stress and reduced endogenous antioxidant defense, and low grade systemic inflammation. Also, BPA is related with oxidative stress, decreased antioxidant enzymes, and inflammation.

In this study, our aim is to investigate the relationship between the COPD and serum BPA (nowadays, too popular and restricted all over the world), CRP, MDA, and total thiol levels. To the literature, this is the first report evaluating these relations, especially, in terms of BPA and total thiols in patients with COPD.

Patients and Methods

Study Subjects

This study was approved by Mustafa Kemal University Local Ethics Committee, and the participants signed the written informed consent. The current study was carried out between October 2012 and April 2013. The study had a total of 83 consecutive subjects, including 50 with stable COPD patients (11 non-smokers and 39 ex-smokers) and 33 healthy volunteers (7 non-smokers and 26 ex-smokers). Pulmonary function tests were performed on all the study subjects (Quark PFT, Cosmed Srl, Rome, Italy). The study population was divided into two groups: control group and COPD group. Fifty patients with COPD who fulfilled the criteria of the Global Initiative for Chronic Obstructive Pulmonary Disease were included¹. A total of 33 healthy subjects who were admitted to the Outpatient Clinic of Chest Diseases were enrolled depending on history, physical examination, and spirometric data with a forced expiratory volume in first second/forced vital capacity ratio of >

70%²³. The inclusion criteria for the study, patients had to fulfill the following criteria: no history at least more than 6 months without exacerbation of COPD, respiratory disorders other than COPD, localized or systemic infectious or inflammatory conditions, autoimmune disease, malignancy, chronic liver and renal disease.

Blood Sampling Protocol

Venous blood samples were taken between 08:00 and 09:00 h after fasting overnight. Venous blood samples were immediately centrifuged and the serum was obtained and stored at -80°C until the assay.

Assays

C-Reactive Protein (CRP)

CRP values are determined by nephelometric method (Siemens Nephelometer BNTM II, Eschborn, Germany) according to manufactory recommendations.

Malondialdehyde (MDA)

The MDA level was determined by a method based on its reaction with thiobarbituric acid at $90-100^{\circ}\text{C}$. The concentration of MDA was described as micromoles per liter of plasma or serum²⁴.

Total Thiols

The serum total thiols concentration or sulfhydryl groups were measured by the methods originally described by Ellman and modified by Hu²⁵. This method was adapted to an automated biochemistry analyzer (Architect c8000; Abbott, Abbott Park, IL, USA).

Bisphenol A

BPA standard (minimum purity $\geq 99\%$) was purchased from Sigma-Aldrich (Haverhill Suffolk, UK). All chemicals were obtained from Merck (Darmstadt, Germany). All chemicals used were of analytical-reagent grade and were at least 99.5% pure. 2 mL of serum sample was diluted 1:1 with phosphate-buffered saline (PBS: pH=7.6). If necessary, pH values were adjusted to 7.0 with 1 M NaOH. The sample was applied to the immunoaffinity column by using a 16 port Waters Sep-Pak SPE station (Milford, MA, USA). After the column had been washed with 5 mL of acetonitrile/water (10:90, v/v), BPA was eluted with 4 mL of acetonitrile/water (40:60, v/v). The

Table I. Demographic and laboratory parameters of all the participants.

	Control (n = 33)	COPD (n = 50)	p value
Sex (F/M)	4/29	6/44	0.98
Age (years)	57.6 ± 11.1	61.6 ± 11.2	0.11
BMI (kg/m ²)	27.74 ± 4.56	27.24 ± 5.60	0.67
FVC (% predicted)	87.5 ± 7.54	65.4 ± 18.4	< 0.001
FEV ₁ (% predicted)	86.9 ± 12.8	49.7 ± 16.9	< 0.001
FEV ₁ /FVC ratio	82.1 ± 5.3	61.8 ± 8.7	< 0.001
CRP (mg/L)	3.64 ± 1.22	5.06 ± 3.40	0.03
MDA (μmol/L)	1.06 ± 0.32	1.02 ± 0.32	0.61
Total Thiol (μmol/L)	0.70 ± 0.14	0.64 ± 0.14	0.041
BPA (ng/mL)	0.57 ± 0.19	3.04 ± 3.30	< 0.001

All results are represented as mean ± standard deviation. F: Female, M: Male, BMI: Body mass index, FVC: Forced vital capacity, FEV₁: Forced expiratory volume in one second, CRP: C-reactive protein, MDA: Malondialdehyde, BPA: Bisphenol A.

elute was collected in a 5 mL measuring flask. An aliquot of 100 μL was injected into high-performance liquid chromatography (HPLC) system²⁶. BPA in the standards and sample extracts was determined by HPLC using a column Inertsil ODS-3 5 μm (4.6 × 250 mm) (GL Sciences, Torrance, CA, USA). The diode array detector (SPD-M20A) was used. The mobile phase was acetonitrile/water (40:60, v/v) with a flow rate of 1 mL min⁻¹. The column temperature was 40°C. The calibration curve of HPLC obtained from a linear regression programme by concentrations versus detector responses (peak areas) was in the range 0.05-1 μg mL⁻¹ BPA (r > 0.998).

Statistical Analysis

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as percentages. Kolmogorov-Smirnov test was used to assess the distribution of continuous variables. To compare parametric continuous variables, the Student's *t* test or analysis of variance was used; to compare nonparametric continuous variables, the Mann-Whitney U or Kruskal-Wallis tests was used. To compare categorical variables the chi-square test was used. Correlations between variables were tested by using the Pearson or Spearman correlation tests where appropriate. Two-tailed *p* values < 0.05 were considered to indicate statistical significance. Statistical analyses were performed using SPSS (SPSS Inc, Chicago, IL, USA), version 15.0 for Windows.

Results

Our study consisted of a total of 83 subjects including 33 healthy volunteers (4 female, 29

male) and 50 stable COPD patients (6 female, 44 male). All the demographic and laboratory parameters are represented in Table I. There were no significant differences between COPD group and control group in aspect of sex, age, and body mass index. COPD subjects had statistically lower FEV₁ (% predicted), FVC (% predicted) and FEV₁/FVC ratio in comparison to control subjects (*p* < 0.001).

The mean CRP levels were 3.64 ± 1.22, 5.06 ± 3.40 mg/L for healthy subjects and COPD cases, respectively. CRP levels were significantly higher in the COPD group than the control group (*p* = 0.03) (Figure 1). The mean MDA levels were 1.06 ± 0.32 and 1.02 ± 0.32 μmol/L for control

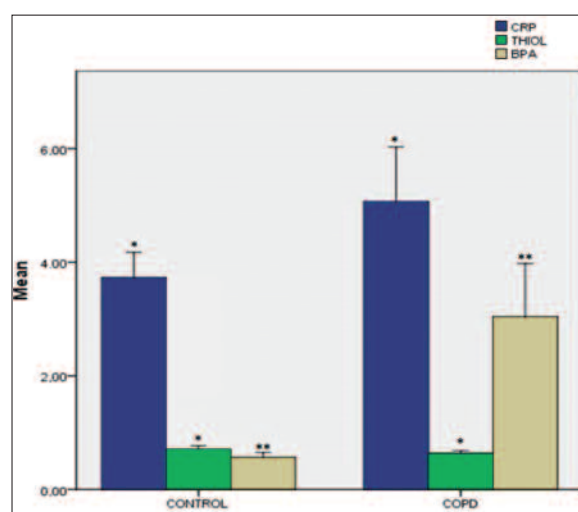


Figure 1. The serum levels of CRP, Thiol, and BPA in control subjects, COPD group. **p* < 0.05, ***p* < 0.001, CRP: C-reactive protein (mg/L), Thiol (μmol/L), BPA: Bisphenol A (ng/mL), COPD: Chronic Obstructive Pulmonary Disease.

group and COPD individuals, respectively. There was no significant difference between control subjects and COPD group in aspect of MDA levels ($p = 0.61$). The mean total thiol levels were 0.70 ± 0.14 , $0.64 \pm 0.14 \mu\text{mol/L}$ for healthy subjects and COPD subjects, respectively. The total thiol levels were significantly lower in the COPD cases than the control group ($p = 0.041$) (Figure 1). The mean BPA levels were 0.57 ± 0.19 , $3.04 \pm 3.30 \text{ ng/mL}$ for control group and COPD group, respectively. BPA levels were significantly higher in the COPD group than the control group ($p < 0.001$) (Figure 1). There was a linear relationship between BPA and CRP in correlation analysis, but it was not statistically significant ($p = 0.07$, $r = 0.20$).

Discussion

In the present study, the serum total thiol levels were found to be lower and CRP levels were significantly higher in COPD patients. The MDA levels in COPD patients were similar to control subjects. The BPA levels were significantly higher in COPD cases.

There is increasing evidence that systemic inflammatory markers including CRP, IL-6 and tumor necrosis factor-alpha (TNF-alpha) are increased in COPD patients^{6,7}. Our findings showed that the levels of CRP were significantly higher in COPD patients than the healthy controls. In a previous research examining CRP levels in patients with moderate to severe COPD reported that CRP levels were found to be high in these patients²⁷. In another work, it was found that CRP was correlated with COPD²⁸. Some investigations have demonstrated increased CRP levels in COPD cases during exacerbations²⁹ and in stable COPD patients^{7,30}.

An imbalance between oxidative stress and antioxidant capacity is suggested to play an important role in the progression and pathogenesis of COPD³¹. Especially, oxidative stress results in epithelial damage and mucus secretion in COPD cases. It causes neutrophil accumulation on alveolar wall and macrophage accumulation in respiratory bronchioles in aspect of smoking in patients with COPD³². MDA which is a lipid peroxidation product can be used as a marker of oxidative stress in COPD^{31,33}. In the current study, there was no significant difference in terms of MDA levels between groups as in the study by Inonu et al³⁴. Karadag et al³⁵ reported that there

was no difference in terms of MDA levels in COPD patients and control group. Additionally, it has been suggested that plasma MDA levels were increased during acute COPD attacks, but might be normal in patients with stable COPD. Increased lipid peroxidation becomes to normal levels when COPD exacerbation was treated³⁶. Previous researches reported a reduction in MDA levels after inhaled and systemic corticosteroid treatment^{37,38}. In the current study, MDA levels were similar between the groups and it may be explained that the majority of the patients had inhaled corticosteroid (ICS) and also, they were stable in terms of COPD. Therapeutic regimens of the COPD patients are shown in Table II.

Thiols have been known as organic molecules and include a sulphhydryl group. Of all the antioxidants, thiols constitute the important part of the total body antioxidants, and they play a major role in the defense against reactive oxygen species (ROS)³⁹. In the current report, the total thiol levels were found statistically lower in COPD patients compared to healthy individuals. Several studies observed decreased total antioxidant capacity in patients with COPD^{40,41}. Raut et al⁴² showed that thiol proteins were low in patients with stable COPD. Investigators revealed that COPD patients had a significant decrease in antioxidant status regarding vitamin C and sulphhydryl groups in comparison to healthy subjects⁴³. N-acetylcysteine (NAC) is a thiol-containing antioxidant that nonenzymatically interacts and detoxifies ROS. Treatment for 1-year with high-dose NAC caused significantly improved small airways function and decreased exacerbation frequency in stable COPD patients⁴⁴.

There is no study investigating the relationship between COPD and BPA regarding the pathophysiology in the literature. In the current study, BPA levels were found to be significantly higher in COPD patients. It was revealed⁴⁵ that BPA cause occupational asthma diagnosed by

Table II. Therapeutic regimens of the COPD patients.

Agent	Number of patient (%)
Short-acting β_2 -agonist (as needed)	35 (70%)
Short-acting anticholinergic (as needed)	9 (18%)
Long-acting β_2 -agonist (regularly)	44 (88%)
Long-acting anticholinergic (regularly)	30 (60%)
Inhaled corticosteroid (regularly)	45 (90%)
Theophylline (regularly)	11 (22%)

specific bronchial provocation test. In the other study⁴⁶, high maternal urine BPA levels at 16 weeks of pregnancy was shown to be related with the increased ratio of wheezing in child's early life. In the another work, Donohue et al⁴⁷ reported that urinary BPA concentrations in children at ages 3, 5, and 7 years were related with asthma, and they suggested that environmental BPA exposure may lead to adverse effects in terms of respiratory system. Therefore, it is suggested that BPA might be associated with COPD in aspect of etiopathogenesis.

One of the logical explanations is that BPA might induce COPD by inflammation. BPA accumulation was shown in many organs, predominantly in the lungs⁴⁸. Researchers revealed that BPA modulates activities of macrophage functions, and stimulates production of cytokines such as interleukin IL-6, tumor necrosis factor⁴⁹. Additionally, urinary BPA concentrations were found to be correlated with CRP levels in the postmenopausal women²². Also, urinary BPA levels are positively associated with metabolic syndrome (MetS), and high sensitivity CRP has been found related to MetS^{50,51}. It is well known that inflammatory cells including neutrophils, macrophages and lymphocytes are increased in the respiratory tract of COPD patients⁵². CRP is a marker of inflammation that it is significantly higher in patients with COPD as in the present study. Therefore, BPA might play a role in the etiopathogenesis of COPD due to increased inflammatory activity. Another explanation is that increased BPA might lead to COPD by disturbing the balance between oxidants and antioxidants. Several experimental studies reported that BPA decreased antioxidant defense systems and induced oxidative stress in liver, kidney and epididymal sperm of laboratory mammals^{53,54}. Many researches showed that urinary BPA levels were positively correlated with the levels of MDA in young women and postmenopausal women^{22,55}. The total thiol is an antioxidant marker which was significantly lower in patients with COPD than the control group, firstly reported in the current study. However, MDA (an oxidant indicator) was similar in both groups and that may be due to ICS treatment of patients with COPD in our work.

Conclusions

COPD is associated with high serum BPA, CRP and low total thiol levels in comparison to

healthy controls. To the best of our knowledge, this is the only report investigating serum BPA (firstly), total thiol (firstly), MDA, and CRP levels in patients with COPD and further researches and large samples are needed to clarify these relations and their roles in COPD patients.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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