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Research Article

Increased Collagen Turnover in Healthy Biomass Smoke Exposed Women; A Cross-Sectional Study

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Abstract

Background: Women with poor socioeconomic status predominantly expose to biomass smoke during cooking and heating. Biomass smoke contains pollutants that causes lung inflammation and parenchyma collagen tissue destruction like COPD. Prolidase is vital for maintenance, degradation, reconstruction of tissue of collagen. Myeloperoxidase (MPO) is in large quantities expressed from neutrophils in inflammation sites.

Objectives: We aimed to asses serum prolidase enzyme activity and MPO levels in healthy biomass exposed women as indicator for increased collagen turnover.

Methods: We evaluated the level of serum prolidase enzyme activity (SPEA) and MPO and association between each other in biomass smoke exposed women with COPD and healthy (Biomass exposed COPD group (BECG) and Biomass exposed healthy Group (BEHG)), and in-non-exposed women (control).

Results: Higher SPEA was found in the BEHG compared with the BECG and the control groups ($p < 0.05$). MPO was higher in BEHG compared with the control group ($p = 0.01$). In the biomass exposed women SPEA had positive correlation with MPO ($r = 0.237$, $p = 0.04$) and MPO had positive correlation with TAC ($r = 0.471$, $p = 0.000$).

Conclusion: Higher SPEA and MPO in BEHG suggests increased collagen turnover associated with increased inflammation. The complex correlation between SPEA, MPO and TAC suggests that collagen maintenance can be associated with oxidant-antioxidant status.

Keywords: *Biomass; Collagen Turnover; Prolidase; Myeloperoxidase; Oxidant-Antioxidant Status*

Introduction

Biomass is a biological firing product that produces energy. It is predicted that just about half population of the world and approximately all the rural population in the non-developed countries uses different kind of biomass fuels like dung and wood [1]. Biomass smoke contains a lot of harmful products for human health such as carbon monoxide (CO), bio-aerosols, organic volatile compounds, aromatic polycyclic hydrocarbons, transition metals, oxidative nitrogen and sulfur, coarse, ultrafine, and fine corpuscles [2]. It is known that the biomass fuel smokes has harmful effects on the lung functions and structure [3]. Chronic inhalation of biomass smoke increases neutrophilic inflammation, neutrophil activation and oxidative stress [4,5].

In the world, chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality [6]. COPD predominantly affects biomass smoke exposured non-smoking women in countryside areas of countries during cooking and heating [1]. Alongside an airway wall inflammation occurring by inflammatory cells, especially by neutrophils, the high levels of myeloperoxidase (MPO), is a characteristic feature for COPD [7]. One of the consequences of this kind of inflammation is increased collagen breakdown and parenchyma tissue destruction like emphysema and small airway fibrosis as occurred in COPD [6].

MPO is profusely expressed in neutrophils at inflammation sites. Recent evidence showed that MPO is complicated in cellular homeostasis and an important factor for both onset and advance of some inflammatory diseases. It is reported that the *in vitro* myeloperoxidase release was not different for both stimulated and unstimulated PMN in healthy and emphysematous subjects [8]. However, sputum MPO levels increases with neutrophilic influx in lung in healthy smokers [9]. Also, it has been shown that the elevated serum MPO levels were associated with a rapid decrease in the pulmonary function and worse cardiovascular consequences in COPD patients [10].

Prolidase is the main enzyme for renovation, degradation, and re-create of collagen tissue, and deterioration in regulation of prolidase activity discloses the disorders in collagen metabolism [11]. The lack of prolidase seriously obstacles the recycling of collagenous tissue. The collagen is an important extracellular matrix (ECM) component and it is necessary for tissue organization and integrity [12]. The increase in the prolidase enzyme activity is associated with elevated collagen recycle [13].

In the our study we investigated the level and association between SPEA, and MPO in biomass smoke exposed healthy women as indicator for increased collagen turnover and inflammation. Also, we examined oxidative status indicators, total oxidant state (TOS), total antioxidant state (TAS) and oxidative stress index (OSI), in these women.

Methods

Subjects

A cross sectional study was used. The 35 women with COPD due to biomass exposured, applied to the outpatients clinic of our university medical center, 45 healthy biomass smoke exposuring (HBE) women, that they were selected randomly a village near the city, and on 35 healthy controls subjects were enrolled in the study. Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines was used for COPD diagnosis on the basis of history, physical examination, and spirometric data, i.e. forced expiratory volume in 1sec (FEV1) and forced vital capacity (FVC) (expressed as percent predicted for both) (6). COPD patients had moderate– severe severity level. We studied samples from patients in the stable state. The patients was taken complete information about the study and requested their consent.

Biomass smoke exposure was recorded as the number of exposure hours per day - the number of days per week and the number of exposure years. The number of years was used the biomass smoke exposure duration. The number of hours per year was calculated for biomass smoke exposure density. If a subject was exposed to biomass smoke for 1 hour per day for a year, her biomass smoke exposure density is 1 hour/year.

Ethical issues

Ethical Board of Institution approved the study by the. The Code of Ethics of the World Medical Association was followed while the work has been carried out. (Declaration of Helsinki).

Samples

Blood samples were obtained after following overnight fasting. The samples were collected into empty tubes and centrifugation at 3,000 rpm for 10 min was used for separated from the cells. And then stored on ice at -80°C. until they were used for measurement of TOS and TAC levels and prolidase activity serum samples were stored at - 80°C.

Detection of prolidase activity

The activity of prolidase was detected by a photometry which the measures the proline values produced by prolidase [14]. The 100 ml specimens were mixed with 100 ml of physiological serum liquid. A total of 25 ml of the admixture was preincubated with a liquor contains the 50 mmol/l Tris HCl buffer, pH 7.0, 1 mmol/l GSH, 50 mmol/l $MnCl_2$ at 371C about thirty minutes. The reaction mix having 144 mmol/l gly-pro, pH 7.8 (100 ml) was approximately incubated with 100 ml of a pre-incubator specimen at 371C within the 5 minutes. One ml of glacial acetic acid was added to terminate the incubation reaction. After the annexation of 300 ml Tris buffer solution with a pH value of 7.8, and 1 ml of ninhydrin liquor (3 g/dl ninhydrin was melted in 0.5 mol/l orthophosphoric acid) the mix was incubated at 901C in 20 minutes and cooled with ice, and thereafter its absorbance was calculated at a wavelength of 515 nm in order to detect the proline level as performed by Myara [15]. This method is the Chinard's modified method [16]. Inter and intraassay coefficients of variability of the assay were under than 10%.

Measuring of myeloperoxidase activity

Depend on the kinetic measurement of the formation rate of the yellowish-orange product of the oxidation of o-dianisidine with MPO in the presence of hydrogen peroxide at 460 nm, the serum MPO activity was proposed by the method of Klebanoff and Clark [17]. A unit of MPO was defined as degrading one µmol of hydrogen peroxide per minute at 25°C. A molar extinction coefficient of $1.3 \times 10^4\ M^{-1}\ cm^{-1}$ of oxidized 0-dianisidine was used for the measurement. MPO activity was described in units per liter of serum (U/L).

Te measure of TAC

The TAC value was detected by using a new automated method, proposed by Erel [18]. With this method, ferrous ion solution and hydroxyl radical, the most potent biological radical is produced, which is present in Reagent-1 is mixed with hydrogen peroxide, present in Reagent-2. The consecutively produced radicals such as brown-colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. The antioxidative effect of the sample against the potent-free radical reactions, launched by the produced hydroxyl radical is calculated by using this method. The analysis has ideal precision values, lower than 3%. The results are described as mmol Trolox Eq./L.

Calculation of TOS

The value of serum TOS was detected by using a new automated method, proposed by Erel [19].

The present Oxidants in the specimen oxidize the ferrous ion–o-dianisidine complex to ferric ion. This reaction is driven up by glycerol molecules, profusely present in the reaction environment. In an acidic environment, the ferric ion makes a colored complex with xylenol orange. The spectrophotometrically measurable color intensity is related to the total amount of oxidant molecules existing in the specimen. The analysis is calibrated with hydrogen peroxide and the results are uttered in micromolar hydrogen peroxide equivalent per liter (e.g. Mmol H_2O_2 Eq/L). Oxidative stress index (OSI remarks the degree of oxidizing stress and is defined as follows: OSI (arbitrary units) = [TOA/TAC]×10014.

Statistical analysis

Results were expressed as means and standard deviations (SD). To determine if the data were normally distributed the one-sample Kolmogorov-Smirnov test was used. According to the distribution pattern of data, the variables to be present between the groups were calculated by using one-way ANOVA or Kruskal- Wallis test for three independent groups and Student's t-test or Mann-Whitney U-test for two independent groups. Spearman correlation analysis was used to determine relationships between variables. The. P values lower than 0.05 were considered as statistically significant.

The statistical package SPSS 18.0 compatible with Windows (IBM Corporation, Armonk, NY) was used to analysis.

Results

A total of 35 with COPD and 45 healthy women, exposed to the biomass smoke, and 35 no exposed matched healthy control subjects were enrolled in this study (Figure 1). The demographic and social data (BMI, sex, age etc.), FEV1 (% predicted), and FEV1/FVC (%) values of the patients are presented in table 1. There were no significant differences among the groups in the condition of anthropometric measurements, gender distribution, and age, ($p > 0.05$) (Table 1). Table 2 summarizes the serum markers of oxidative status (TAC, TOA, OSI), MPO and SPEA, for all groups. BEHG group had significantly higher SPEA compared with the BECG and the control groups ($p < 0.005$ for each) (Table 2 and figure 1). However, for SPEA values there was not a significant difference between the BECG subjects and the controls ($p > 0.05$) (Table 2). MPO levels were significantly higher in BEHG compared with the control subjects ($p = 0,01$) (Table 2). However, There was no significant difference compared with the BECG ($p > 0.05$). Likewise, there was no difference between BECG and the control group ($p > 0.05$).

	Biomass exposed group		Control	p
	COPD HBEG			
Number of patients	35	44	33	
Age	55 ± 6,2	53 ± 4,3	52 ± 5,7	> 0.05
Biomass smoke, h-yr	233 ± 101	248 ± 9		> 0.05
BMI	23.42 ± 2.14	22.11 ± 1.16	22.32 ± 2.21	> 0.05
Mean FEV1 (% predicted)	41 ± 14	87±12	90 ± 9	< 0,05
FEV1/FVC (% predicted)	61 ± 11	89±9	91± 8	< 0,05

Table 1: *Characteristics of the study population.*

	BECG (n = 35)	BEHG (n = 44)	Controls (n = 33)	*p
SPEA (IU/L)	764 ± 301	885 ± 211[1 2]	757 ± 188	0.024[i]
MPO (µm/L)	46,7± 14,1	58,7 ± 19,0[3]	52,0± 17,1	0.01[ii]
TAC (µmol Trolox Eq t/l)	1,40± 0,27	1,55 ± 0,16[4]	1,60 ± 0,28	0.005[i]
TOS (µmolH_2O_2 Eq./L)	90,9± 24,7	95,2 ± 20,1	90,2 ± 21,6	0.460[ii]
OSI (H_2O_2/Trolox)	6,6± 2,1	6,1 ± 1,2	5,8 ± 1,9	0.398[ii]

Table 2: *Prolidase activity and oxidative–antioxidative status in the study groups (mean ± standard deviation).*

[i]: Difference between three groups with one-way ANOVA. Differences between pairwise groups with Student's t-test.

[ii]: *Difference between three groups with Kruskal-Wallis test. Differences between pairwise groups with Mann–Whitney U test.

[1]: Compared with group BECG p = 0,03.

[2]: Compared with control p = 0,016.

[3]: Compared with group BECG p = 0,01.

[4]: Compared with group COPD BECG p = 0,01.

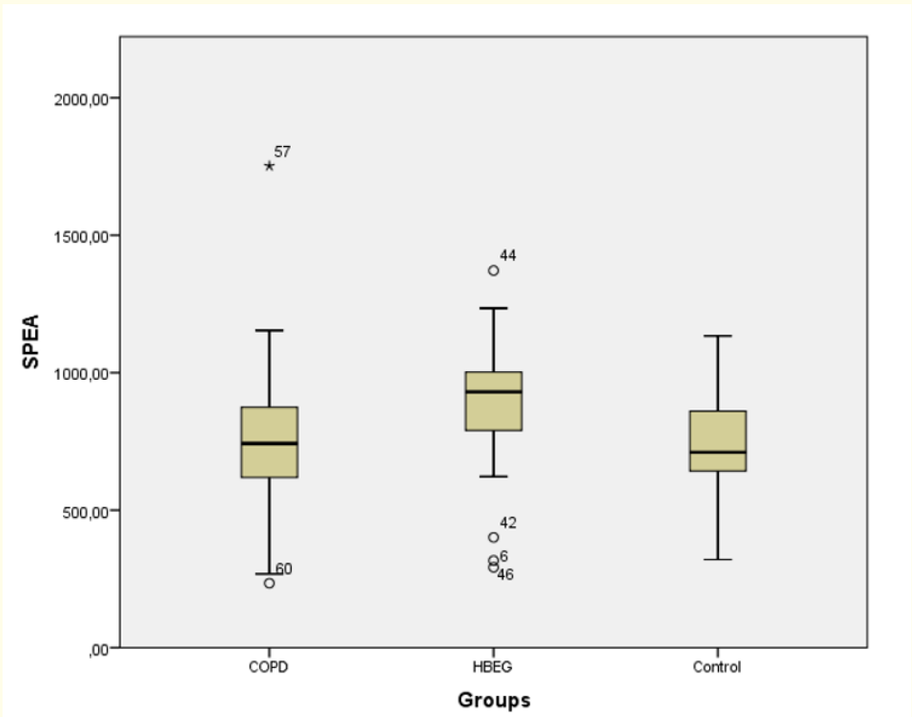

Figure 1: *SPEA levels in groups.*

Compared with the BEHG and the control groups, the TAC values were significantly lower in BECG ($p < 0.005$). Nevertheless, there was no difference between the BEHG and control groups in terms of TAC values ($p > 0.05$). Although the BECG had higher TOS and OSI values, there was no significant difference among the three groups ($p > 0.05$).

In biomass exposed groups, the SPEA had a positive correlations with MPO ($r = 0.237$, $p = 0.04$) (Table 2 and figure 2). The MPO had positive correlations with TAC values ($r = 0.471$, $p = 0.000$). The OSI had positive correlations with TOS ($r = 0.779$, $p = 0.000$), and negative correlation with TAC values ($r = 0.388$, $p = 0.001$).

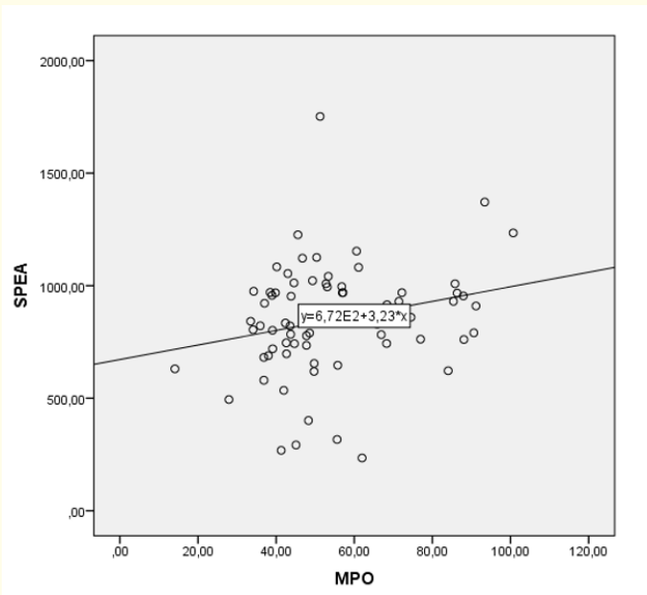

Figure 2: *In the biomass exposured women SPEA levels have positively correlations with MPO levels ($r = 0.237$, $p = 0.04$).*

Discussion

It is known that the large majority women living in undeveloped areas still use the ineffective and very polluting biomass fuel product [20]. Compared with control these women's serum container significantly elevated levels of CRP, IL-6, IL-8 and TNF-α, depleted superoxide dismutase and increased reactive oxygen species generation, sputum's and serum's contain more neutrophils, lymphocytes, eosinophils, and alveolar macrophages [4,5]. Similarly, we found that the TOS and MPO values are higher in the group of BEHG than the other groups. It suggests that biomass exposure causes important level increase of inflammation and oxidants in these cases.

Exposure to biomass smoke was reported to be a clear risk factor for COPD [6,21]. The inflammation of airway is a fundamental pathophysiologic feature in COPD. Neutrophils are the main cause of inflammation in COPD [22]. The inflammation produce oxygen free radicals and release proteolytic enzymes that can cause collagen matrix destruction. In our study, the higher values of MPO in BEHG suggests increased neutrophilic inflammation in these subjects. This inflammation may have triggered collagen destruction of the lungs in HBEG subjects. MPO is known and used as the mediator of neutrophil activity [23]. For a long time, its main function has been known as the generation of the reactive oxygen radicals which contribute to the killing and destruction of engulfed pathogens. Recent evidence has revealed that MPO is also incorporated in cellular homeostasis and has an important influence in the initiation and progression of various inflammatory reactions [25]. It increases in cigarette smokers [24]. Also, this increase continues during the smoking [9]. A recent meta-analysis revealed that the MPO levels were increased in stable COPD patients' sputum when compared with healthy controls, and this increase was especially marked during exacerbations when compared with MPO levels during the stable state. It is reported that sputum MPO value is a promising biomarker to manage the COPD therapy [26]. In Another study showed that an MPO inhibitor stopped small airway remodeling and progression of emphysema in an animal model [27]. Our study showed that HBE group had highest MPO level than COPD and control group respectively. We concluded that active biomass exposure can cause more severe inflammation and collagen destruction than COPD.

Prolidase is crucial for degradation, renewal and maintenance of collagen connective tissue [11]. Prolidase deficiency, a rare autosomal recessive disorder, leads to ineffective degrading some dipeptides in which a proline or hydroxyproline residues located at the C-terminal position that result the loss of proline [28]. Prolidase activity has elevated in cancers including lung cancer. It is thought that there is a relation between enhanced collagen breakdown and cycle with the tumor growing and metastasizes, in patients with cancer. In serum and BAL fluid, both neutrophil elastase and MPO values were found significantly higher in patients with pulmonary carcinoma than in COPD or healthy subjects [30]. One study showed that COPD patients had significantly lower plasma prolidase activity than those in the control subjects and it was correlate with TAC levels. The authors concluded that it can be related to decreasing of collagen recycling [31]. In the current study, we found significantly higher SPEA in BEHG compared with the BECG and the control groups. Also differently from the previous study, our serum prolidase activity was correlated with MPO and MPO was correlated with TAC. Our study suggests collagen break down associated with neutrophilic inflammation. We concluded that collagen break down is repaired by increased SPEA. In Patient with prominent decrease of SPEA, collagen destruction cannot fix like COPD. Further prospective studies should be made to establish our İdea.

Conclusion

Higher SPEA and MPO in BEHG suggests increased collagen turnover associated with increased inflammation. The complex correlation between SPEA, MPO and TAC suggests that collagen maintenance can be associated with oxidant-antioxidant status.

Competing Interests

The authors have no competing interests to disclosure.

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