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C-Reactive Protein Concentrations Among Crop and Dairy Farmers with Stable Chronic Obstructive Pulmonary Disease

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Abstract

AIM: To assess the mean serum C-reactive protein concentrations among farmers and office controls, and further examined its relation to exposure duration, smoking habit, as well as presence or absence of COPD.

METHODS: A cross-sectional survey was performed including examined group (EG), composed of agricultural workers (87 crop - EG1 and 83 dairy farmers - EG2), and control group (CG) composed by 80 office workers. Evaluation of subjects included a questionnaire on chronic respiratory symptoms, lung function tests, COPD diagnostics, and measurement of CRP serum concentrations.

RESULTS: Serum CRP concentrations were raised in stable COPD patients independent of smoking habit or exposure duration. The mean CRP serum concentrations were higher in the subjects of EG1 and EG2 compared with those in CG, but without statistical significance. This count for mean CRP serum concentrations in subjects of EG1 and EG2 exposed more than 20 years and for those in smokers in all three groups as well. The mean CRP serum concentrations were significantly higher in subjects with COPD within EG1 (P=0.049) and EG2 (P=0.040), while those in CG were not.

CONCLUSION: Data obtained suggest that systemic inflammation is present in farmers with COPD and CRP is an important biomarker in COPD in means of reflecting disease severity and prognosis of exposed farmers.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major worldwide health problem with increasing prevalence and incidence. Although cigarette smoking is the most commonly encountered risk factor for COPD worldwide, some genetic and environmental risk factors are also well-identified in the disease pathogenesis. Still, it is well-known that nonsmokers may also develop chronic airflow obstruction [1].

Chronic obstructive pulmonary disease (COPD) is a complex chronic inflammatory disease of the lungs involving several types of inflammatory cells and a variety of inflammatory mediators. The relationship between these cell types, cytokines, and the sequence of events that concludes with progressive airflow limitation and destruction of lung parenchyma remains largely unknown [2]. Although primarily affecting the lungs, the chronic inflammatory process of COPD does have systemic repercussions. Inflammatory markers, including C-reactive protein (CRP), lipopolysaccharide binding protein, the soluble TNF transmembrane receptor 75 (sTNF-R75), and soluble adhesion molecules are increased in the systemic circulation of patients with COPD [3-5].

Systemic inflammation is determined by measurement of the CRP serum concentration and is present in patients with small airways obstruction. Sin et al. [6] reported that the withdrawal of inhaled corticosteroids resulted in a significant increase in CRP levels.

Burning wood, animal dung, crop residues and coal in open fires or improper stoves may lead to serious indoor air pollution. The indoor air pollution resulting from crop or dairy farming is an important risk factor for COPD especially in developing countries worldwide [7]. The chronic inflammation in COPD, orchestrated by multiple inflammatory cells and mediators in the airways and the lung tissues, is induced by inhalation of noxious gases and particulate matter. This persistent inflammatory response in the lung is also associated with a significant systemic inflammatory response yielding adverse clinical outcomes, so-called systemic effects of COPD [8]. Although the origin of systemic inflammation present in COPD remains poorly understood, and correlations in the regulation of inflammation in the pulmonary and systemic compartments are not well-documented yet, it is established that some inflammatory markers are risen in systemic circulation [8,9]. Of the blood-based biomarkers, C-reactive protein (CRP) has shown the greatest promise [10]. In COPD patients increased CRP levels are associated with poor lung function, reduced exercise capacity and worse quality of life as well as being a significant predictor of all-cause mortality [11-14]. As well as COPD itself, smoking, which is the most commonly encountered risk factor for the disease is also responsible for the rise in serum CRP levels [15]. Though to our knowledge the effect of crop and dairy farming exposure, potentially initiating the inflammatory process in the lungs of COPD patients, on serum CRP levels has not been studied in our setting previously. The growing awareness of COPD is a complex disease involving several organs with an established low-grade systemic inflammation, biomarkers have been more focus of interest in clarifying the pathogenesis and progression of COPD as well as in designing new therapeutic targets and models for the disease [14]. Based on the current knowledge that COPD is a multicomponent systemic disease with elevated serum CRP levels and that smoking itself leads to rising in CRP levels, the present study was undertaken. With the study, the authors aim to determine the relationship between serum CRP levels and well-known clinical parameters in COPD considering the impact of smoking behaviour, farming exposure duration and its characteristics.

In the actual study we have assessed the mean CRP serum concentrations among crop and diary farmers and office controls, and further examined its relation to exposure duration, smoking habit, as well as presence or absence of COPD.

Subjects and Methods

Study design and setting

We have performed a cross-sectional research in the Center for Respiratory Functional Diagnostics at the Institute for Occupational Health of Republic of Macedonia, Skopje - WHO Collaborating Center for Occupational Health and GA2LEN Collaborating Center within the period September 2014 and April 2015.

Subjects

The survey included workers employed at agricultural enterprise divided into two groups: examined group (EG), composed of agricultural workers (crop and dairy farmers), and control group (CG) composed by office workers within the same enterprise.

EG consists of 170 subjects, while CG has 80 examinees. For the study purposes, and depending on the main agricultural activity, subjects were divided into two groups, examined group 1 (EG1) and examined group 2 (EG2).

EG1 comprised 87 crop farmers (mean age = 53.4 ± 7.8 years) engaged in crop farming (mean duration of exposure 22.9 ± 7.8 years) with main activities composed of cultivating crops and vegetables, planting, digging, use of mechanized equipment, irrigation, and pesticide handling. They were exposed to various respiratory agents: dust, inappropriate climate, fumes, vapours and pesticides.

EG2 consists of 83 dairy farmers (mean age = 52.6 ± 8.7 years) employed as dairy farmers (mean duration of exposure 23.7 ± 7.6 years), working inside confinement buildings, and exposed to: dust, inappropriate microclimate conditions, chemical hazards, vapors, gases, heavy manual work, animal contact, unfavorable body positions, and repetitive hand movements. Their main occupational activities were: preparation of fodder feeding and animal meals, milking, staying in the barn, preparation of straw, and hay making, cattle rising, as well as taking care of milk hygiene and animal health. Also, a similar group of 80 office workers (mean age = 52.7 ± 8.2 years) with no exposure to respiratory agents, matched for age, duration of employment, daily smoking and socioeconomic status was studied as a control.

The Institute's ethics committee has approved the content of our study protocol, whereas each examined subject was informed and gave written consent before any involvement in the study.

Questionnaire

All study subjects were interviewed by the standardized questionnaire, including questions on work history, respiratory symptoms in the last 12 months, and smoking habit.

Chronic respiratory symptoms in the last 12 months (a cough, phlegm, dyspnea, wheezing, and chest tightness) were obtained using the European Community for Coal and Steel questionnaire (ECCS-

87), and the European Community Respiratory Health Survey (ECRHS) questionnaire [16, 17].

Classification of smoking status was done according to the World Health Organization (WHO) guidelines on definitions of smoking status [18].

Daily smoker was defined as a subject who smoked at the time of the field survey at least once a day, except on days of religious fasting. Among daily smokers, lifetime cigarette smoking and the daily mean of cigarettes smoked were also assessed. Pack-years smoked were calculated according to the actual recommendations [19]. Ex-smoker was defined as a formerly daily smoker, no longer smokes.

Passive smoking or exposure to environmental tobacco smoke (ETS) was defined as the exposure of a person to tobacco combustion products from smoking by others [20].

Spirometry

All study subjects underwent spirometry testing, performed by spirometer Ganshorn SanoScope LF8 (Ganshorn Medizin Electronic GmbH, Germany), measuring forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FVC ratio, and maximal expiratory flow at 50%, 75%, and 25-75% of FVC (MEF50, MEF75, and MEF25-75, respectively), by recording the best result from three measurements of the values of FEV1 within 5% of each other. The results were expressed as percentages of the predicted values according to the European Community for Coal and Steel (ECCS) norms [21].

Diagnostic criteria for COPD

According to the actual recommendations by Global Initiative for Chronic Obstructive Lung Disease (GOLD), COPD is defined by post-bronchodilator FEV1/FVC ratio lower than 0.70 in subjects with dyspnea, chronic cough and/or a cough with phlegm [22]. Measurement of CRP serum concentrations as markers of inflammation. Mean CRP serum concentrations are measured by chemiluminescent immunoassay method with Immulite 1000 in subjects with chronic respiratory symptoms and/or spirometric impairment. CRP serum concentrations < 5 mg/L are within the normal range [23].

Statistical analysis

We have analyzed the data using Statistica for Windows version 7. Continuous variables were expressed as mean values with standard deviation and categorical variables as numbers and percentages. The comparison of mean CRP serum concentrations was performed by independent-samples T-test. A P-value of less than 0.05 was considered statistically significant.

Results

Table 1 gives an overview of the demographic and overall characteristics of the subjects within the examined and control groups.

Table 1: Demographic and overall characteristics of the study subjects

Variable	EG1 (n = 87)	EG2 (n = 83)	CG (n = 80)
Gender / M/F ratio	3.6	2.6	2.7
Age / years	53.4 ± 7.8	52.6 ± 8.7	52.7 ± 8.2
BMI / kg m^{-2}	25.1 ± 3.5	25.4 ± 3.6	26.2 ± 3.7
Duration of employment / years	29.2 ± 8.9	26.3 ± 10.1	25.3 ± 9.8
Exposure duration / years	22.9 ± 7.8	23.7 ± 7.6	/
Active (daily) smokers	45 (51.7%)	39 (46.9%)	39 (48.7%)
Smoking experience / years	19.7 ± 8.1	18.9 ± 7.6	19.2 ± 7.8
Cigarettes / day	15.4 ± 7.3	14.6 ± 6.8	14.8 ± 7.2
Ex-smokers	12 (13.8%)	9 (10.8%)	12 (15%)
Passive smokers	11 (12.6%)	8 (9.6%)	7 (8.7%)

Numerical data are expressed as mean values with standard deviations; frequencies of active, passive, and ex-smokers are given as number and percent of subjects with certain variable.M: males; F: females; BMI: body mass index.

Table 2 shows the frequencies of COPD symptoms (dyspnea, chronic cough and phlegm) negative BD tests, post-bronchodilator FEV1/FVC less than 0.70 and frequency of COPD in subjects of EG1, EG2 and CG.

Table 2: Frequency of COPD symptoms, negative BD tests, post-bronchodilator FEV_1/FVC less than 0.70 and COPD in subjects of EG1, EG2 and CG

Variable	EG1 (n = 87)	EG2 (n = 83)	CG (n = 80)
Respiratory symptoms within the last 12 months	23 (26.4%)	24 (28.9%)	15 (18.8%)
Negative BD test	11 (12.6%)	9 (10.8%)	5 (6.3%)
Post-bronchodilator test FEV_1/FVC < 0.70	7 (8.1%)	8 (9.6%)	4 (5%)
COPD	6 (6.9%)	7 (8.4%)	3 (3.8%)

Data are given as number and percent of subjects with a certain variable.

For the study purposes, we have examined the marker of chronic inflammation - CRP (C-reactive protein) among subjects in the three groups having one or more chronic respiratory symptoms and/or spirometric impairments.

CRP serum concentrations

The mean CRP serum concentrations were higher in EG1 compared to CG, but without statistical significance (Table 3).

Table 3: Mean CRP serum concentrations in subjects of EG1 and CG

	EG1 (n = 35)	CG (n = 15)	*P*-value*
CRP (mg/L)	3.5 ± 2.4	3.1 ± 2.2	0.590

Data are given as means with standard deviation. **Tested with t-test for independent samples.*

The mean CRP serum concentrations were higher in EG2 compared to CG, but statistical significance was not yet reached (Table 4).

Table 4: Mean CRP serum concentrations in subjects of EG2 and CG

	EG2 (n = 32)	CG (n = 15)	*P*-value*
CRP (mg/L)	3.6 ± 2.8	3.1 ± 2.2	0.529

Data are given as means with standard deviation. *Tested with t-test for independent samples.

Table 5 shows the mean CRP serum concentrations in subjects of EG1 with exposure duration less or equal to 20 years and over 20 years.

Table 5: Mean CRP serum concentrations in subjects of EG1 according to job exposure duration

	Exposed > 20 years (n = 24)	Exposed ≤ 20 years (n = 11)	*P*-value*
CRP (mg/L)	3.7 ± 2.8	3.2 ± 2.2	0.646

*Data are given as means with standard deviation. * Tested with t-test for independent samples.*

The mean CRP serum concentrations were higher in subjects of EG1 exposed longer than 20 years, compared to those exposed less than 20 years, but without statistical significance.

Table 6: Mean CRP serum concentrations in subjects of EG2 according to job exposure duration

	Exposed > 20 years (n = 22)	Exposed ≤ 20 years (n = 10)	*P*-value*
CRP (mg/L)	3.9 ± 2.9	3.4 ± 2.2	0.653

Data are given as means with standard deviation. *Tested with t-test for independent samples.

Table 6 gives an overview of mean CRP serum concentrations in subjects of EG2 with duration of exposure less or equal to 20 years and over 20 years. The mean CRP serum concentrations were non-significantly higher in subjects of EG2 exposed longer than 20 years, compared to those exposed less than 20 years. The mean CRP serum concentrations in subjects of all three groups depending on the smoking habit are given in Table 7.

Table 7: Mean CRP serum concentrations in subjects of EG1, EG2, and CG depending on smoking habit

Variable	EG1 (n = 35) 21 vs 14	*P**	EG2 (n = 32) 19 vs 13	*P**	CG (n = 15) Nine vs 6	*P**
CRP in active smokers	3.71 ± 2.6	NS	3.9 ± 3.1	NS	2.9 ± 2.1	NS
CRP in non-smokers	3.3 ± 2.1	0.705	3.4 ± 2.3	0.613	3.3 ± 2.7	0.708

Data are given as means with standard deviation. * Tested with t-test for independent samples.

The mean CRP serum concentrations were higher in smokers within the three groups, but without significant difference between smokers and non-smokers. Mean CRP serum concentrations in all three groups due to the presence or absence of COPD are shown in Table 8.

Table 8: Mean CRP concentrations in subjects of EG1, EG2, and CG due to the presence or absence of COPD

Variable	EG1 (n = 35)	*P**	EG2 (n = 32)	*P**	CG (n = 15)	*P**
	Six vs 29		Seven vs 25		Three vs 12	
CRP in subjects with COPD	6.5 ± 4.9	0.049	6.8 ± 4.9	0.040	5.9 ± 3.9	0.117
CRP in subjects without COPD	3.5 ± 2.8		3.6 ± 2.9		3.1 ± 2.3	

Data are given as means with standard deviation. *Tested with t-test for independent samples.

The mean CRP serum concentrations were significantly higher in subjects with COPD within EG1 and EG2, while those within CG are not.

Discussion

In the actual research within the subjects of all three groups having one or more chronic respiratory symptoms and spirometric impairment, we have measured CRP as a marker of chronic inflammation. The main finding of the present study is that CRP levels were raised in stable COPD patients independent of smoking habit or exposure duration. The mean CRP serum concentrations were higher in the subjects of EG1 and EG2 compared with those in CG, but without reaching statistical significance. This count for mean CRP serum concentrations in subjects of EG1 and EG2 exposed more than 20 years and for those in smokers in all three groups as well. The mean CRP serum concentrations were significantly higher in subjects with COPD within EG1 and EG2, while those in CG were not. This finding is by the results of many studies on COPD and markers of chronic inflammation within the general population, but also in certain groups of exposed workers. Aksu et al. reported a significantly higher CRP serum levels among subjects in the rural areas of Turkey exposed to biomass fuels [7], compared with the control group. The study by Pinto-Plata et al. did not find a correlation between CRP serum levels and smoking, while it significantly decreases in subjects on continuous therapy with inhaled corticosteroids [13].

Several studies on COPD have demonstrated an influx of immune cells, both in the airway wall and lumen, the alveoli and pulmonary vessels [24]. Disease severity is associated with increased airways inflammation [25]. Many studies have shown that COPD patients have higher levels of some inflammatory markers in blood, notably C-reactive protein (CRP) [26–29], fibrinogen [30], and the inflammatory cytokines interleukin (IL)-6 [29, 31] and IL-8 [32, 33], respectively. For tumour necrosis factor (TNF) results are conflicting, as some studies [31, 34] have found higher TNF-a levels among COPD patients, whereas some studies have not [31, 35]. It has been suggested that systemic inflammation may explain part of the heterogeneity of COPD phenotypes, such as loss of lean body mass [36], and the higher prevalence of co-morbid disorders such as coronary heart disease, depression and hypertension [37, 38].

Elevated serum CRP levels indicating a low grade persistent systemic inflammation in COPD patients was first described in early 2000's [39, 40]. Then the positive relationship between CRP levels and important prognostic clinical variables in stable COPD patients was reported in 2006 by de Torres et al. [11]. These results indicate that systemic inflammation is related to the disease severity and physiological parameters should be considered in evaluating the COPD patients instead of focusing only on lung function indices. The airflow limitation in COPD is associated with the abnormal inflammatory response of the lung to noxious particles or gases. Farming exposure to noxious respiratory particles remains still a burning issue in most countries, independently of the degree of economic growth and preventive equipment used. Cigarette smoking, on the other hand, is still the most commonly encountered risk factor [41]. It is probable that such as in the case of smoking, exposure in farming also disturbs the cellular oxidant-antioxidant balance and initiates the inflammatory process in the lungs of COPD patients. To our knowledge, the effect of such exposure on serum CRP levels has not been studied previously. As mentioned above, in the present study no significant difference in serum CRP concentrations was detected between the subjects grouped according to smoking status and to farming exposure duration. About these findings, we confirm that elevated CRP concentrations in subjects with COPD were due to the ongoing inflammatory status and are independent of the smoking habit or farming exposure. High CRP levels have been shown to be associated with all-cause, cardiovascular, and cancer specific causes of mortality [39] thus reflect poor prognosis in COPD patients. Opposed to some previous reports, declaring that the use of inhaled corticosteroids decreases serum CRP levels [6, 13], our study was unable to confirm any significant difference in serum CRP concentrations between subjects with COPD who were on inhaled corticosteroid therapy and those who were not. This is not surprising since the recent reports came along denying any reduction in systemic inflammation in COPD with the use of inhaled corticosteroids [42, 43].

Our present study has some limitations. Namely, relatively small number of the subjects in the study groups may be a limitation, with possible implications on the data obtained and its interpretation, especially in their possible interpolation on the population level for the agricultural workers in R. Macedonia. Also, there is a lack of ambient monitoring and exposure measurement (endotoxin, dust, gases, vapours, and chemicals) in this survey, but the data are based on specially designed questionnaire and job exposure matrices, which were introduced in our country.

Finally, we can conclude that systemic inflammation is present in farmers with COPD and CRP is an important biomarker in COPD in means of reflecting disease severity and prognosis of exposed farmers. Serum CRP levels were raised independently of smoking status and farming exposure duration in COPD patients reflecting that CRP rise was a result of the inflammatory nature of the disease itself. Further trials must be performed to investigate this relationship, but also having in mind exposure duration and its characteristics in farming, as well.

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