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Original research article

Serum levels and gene expression of pentraxin 3 are elevated in COPD

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ABSTRACT

Purpose: Pentraxin 3 (PTX-3) is an acute phase protein that belongs to the pentraxin superfamily. It is synthesized locally at the site of inflammation and its levels are related to the damage of blood vessels. There are only a few studies examining the relationship between PTX-3 and chronic obstructive pulmonary disease (COPD). The aim of this study was to evaluate the serum levels of PTX-3 and relative PTX-3 gene expression in COPD patients and their correlations with cigarette smoking history and lung function.

Materials/methods: A total number of 34 participants were enrolled into this study. Only stable patients without comorbidities were recruited. After obtaining written informed consent all planned procedures were performed (pre- and post-bronchodilator spirometry, blood samples for PTX-3 serum levels and PTX-3 gene expression measurements, demographical data, medical history, COPD patients were also asked for CAT and MMRC questionnaires).

Results: PTX-3 serum levels were significantly higher in the COPD group (29.22 (5.47) ng/ml vs. 14.64 (3.64) ng/ml). PTX-3 gene relative quantification (RQ) values were also significantly higher in the COPD group (0.15 (1.33) vs. -2.80 (1.99)). No differences in CRP serum levels were found between the control group and the COPD group.

Conclusions: Our study demonstrates that serum levels of PTX-3 and the relative expression values of its gene are elevated in COPD, and can be related to cigarette smoking history.

1. Introduction

Pentraxin 3 (PTX-3) is an acute phase protein discovered in the early 1990s. It is a member of the long pentraxin family, which itself exists within the pentraxin superfamily. The protein is encoded by a highly-conserved gene which has almost been left intact by the evolution process [1]. In contrast to C-reactive protein (CRP) which is produced in hepatocytes, PTX-3 is synthesized locally at the site of inflammation and its levels are related to damage of blood vessels [2]. It is known to play a similar role to CRP as a pattern recognition molecule, it can act as a marker of lung cancer, and it can be used in differentiating transudative from exudative pleural effusion [3–5]. However, only a few studies examine the relationship between PTX-3 and chronic obstructive pulmonary disease (COPD), one possible reason being the fact that serum levels of PTX-3 are elevated not only in COPD but also in patients suffering from other diseases, for example acute myocardial infarction (AMI), unstable angina (UA), sepsis, psoriasis and congestive heart failure (CHF) [6–10]. This is an important consideration, as COPD is often accompanied by comorbidities. It has been found that 30% of patients suffer from one additional chronic disease, and another 40% have two or more comorbidities [11].

While the impact of cigarette smoking status and history is an interesting area, only a few studies have examined the role of PTX-3 in COPD patients, and their results are inconsistent. More specifically, while one study [12] suggests that PTX-3 serum levels are significantly higher in patients suffering from COPD than a control group, two other studies found no such differences [13,14].

The aim of our study was to evaluate the PTX-3 serum levels and relative PTX-3 gene expression in COPD patients and their possible correlations with cigarette smoking history and lung function.

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2. Material and methods

2.1. Patient selection

A total number of 34 patients (17 COPD and 17 healthy controls) were enrolled in the study from the lung outpatient clinic of the Barlicki University Hospital, Lodz (Poland). In the COPD group, only stable patients (≥ 3 months free of exacerbation) without comorbidities were recruited. The control group consisted of age-matched healthy volunteers. All participants were ex-smokers or current smokers with a minimum 10 pack-years smoking history. Demographical data such as age, sex, weight, cigarette smoking status and concomitant therapy were collected. The COPD Assessment Test™ (CAT) and Modified Medical Research Council (MMRC) scale were completed for all COPD subjects.

2.2. Spirometry

The main inclusion criterion in the case of the COPD group was a spirometrically confirmed disease, defined as a post-bronchodilator forced expiratory volume in one second to forced vital capacity (FEV_1 : FVC) ratio less than 0.7. In the control group, only patients with a negative post-bronchodilator test result were included. Some patients with an FEV_1 : FVC ratio below 0.7 were included in the control group; however, these were over 60 years old, without any symptoms of COPD or other lung diseases, and their FEV_1 : FVC lower limit of normal was greater than the fifth percentile. Patients who had abstained from cigarette usage for longer than one year were classified into the ex-smokers sub-group.

2.3. Pentraxin 3 measurements

To make a relative quantification of PTX-3 gene expression and PTX-3 serum levels, venous blood samples were taken from every patient. Obtained material was divided into two parts: venous total blood and supernatant serum. Both were stored at -80 °C until the assays were performed.

RNA isolation was performed with the GF-1 Blood Total RNA Extraction Kit (Vivantis, Selangor Darul Ehsan, Malaysia), according to the manufacturer's protocol. The quality and quantity of the isolated RNA was spectrophotometrically assessed (Eppendorf BioPhotometr™ Plus, Eppendorf, Hamburg, Germany). The purity of total RNA (ratio of 16S to 18S fraction) was determined by automated electrophoresis using RNA 6000 Pico LabChip plates on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, California, USA). cDNA was transcribed from total RNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, California, USA). The RT reaction was performed in a personal thermocycler (Eppendorf, Hamburg, Germany) under standard conditions. The relative gene expression analysis was performed in the 7900 H T Fast Real-Time PCR System (Applied Biosystems, Carlsbad, California, USA) using TaqMan probes *PTX3* (Hs00173615_m1). The relative expression levels (RQ values) of the studied gene were calculated using the $^{\Delta\Delta}$CT method, with an adjustment for the β-actin expression level and in relation to the expression level of the calibrator (Human Lung Total RNA, Ambion®), for which the RQ value was equal to 1.

Serum PTX assays were performed using ELISA (Enzyme-linked Immunosorbent Assay Kit For Pentraxin 3, Long (PTX3): Homo sapiens (Human); Cloud-Clone Corp., Katy, Texas, USA), according to the manufacturer's protocol. PTX-3 levels were calculated spectrophotometrically based on a reference curve (EL808 spectrophotometer, BioTek, Winooski, Vermont, USA).

2.4. Ethical issues

The study was conducted in accordance with the 1964 Helsinki declaration and its later amendments, and was approved by the Medical Ethics Committee of the Medical University of Lodz, Poland (approval number: RNN/270/13/KE; KE/957/16). Written informed consent was obtained from each participant.

2.5. Statistical analysis

Statistical analysis was performed using Statistica 12 software (StatSoft, Inc. Tulsa, Oklahoma, USA). Continuous variables were tested for normality by the Shapiro-Wilk test. Values were presented as mean ± standard deviation (SD) or as median with data range (minimum to maximum). In case of non-normally distributed data, a natural logarithm function was used for transformation. To compare results in the control and COPD group, a *t*-test for independent samples was used. To compare the results between the subgroups, we used an analysis of variances (ANOVA). As a *post hoc* test, a Tukey's Honest Significant Difference (HSD) test was chosen. The Pearson's correlation coefficient (PCC) was used to identify correlations and relationships between analyzed variables. A *p*-value of less than 0.05 was considered as statistically significant. All of the test results presented have a power greater than 80%.

3. Results

Both study groups are characterized in Table 1. No differences in CRP serum level were found between the COPD and control group ($p > 0.05$). PTX-3 serum levels were significantly higher in the COPD group (29.22 (5.47) ng/ml vs. 14.64 (3.64) ng/ml) (Fig. 1). The relative quantification values of the PTX-3 mRNA levels in circulating blood cells after logarithmical transformation were also significantly higher in the COPD group (0.15 (1.33) vs. -2.80 (1.99)) (Fig. 2). Mean serum PTX-3 levels in the current smokers COPD subgroup were significantly higher than in the ex-smokers COPD sub-group (31.87 (4.16) ng/ml vs. 24.37 (4.19) ng/ml; $p < 0.01$). Similar observations were made in the control group: mean serum PTX-3 levels were significantly higher in the current smokers sub-group (17.13 (2.75) ng/ml) than in the ex-smokers sub-group (11.83 (2.14) ng/ml); $p < 0.05$) (Fig. 3). No statistically significant differences in PTX-3 RQ values were observed between the presented sub-groups. There were no differences observed in PTX-3 serum levels between male and female individuals and in the relation to patients age.

Correlation analysis performed separately in the COPD group and in the control group did not demonstrate the presence of any dependency

Table 1
Group comparison.

	Control group	COPD group	*p* value
N	17	17	
Sex (M/F)	6/11	10/7	
Median age	{44-79}	67 {57-78}	> 0.05
Mean $\log_n$ CRP (μg/ml)	0.41 [0.97]	0.90 [0.78]	> 0.05
Mean PTX-3 (ng/ml)	14.64 [3.64]	29.22 [5.47]	< 0.0001
Mean $\log_n$ PTX-3 RQ	−2.80 [1.99]	0.15 [1.33]	< 0.0001
Cigarette smoking status (ex/current)	8/9	6/11	> 0.05
Median pack-years	30 {10-50}	40 {10-80}	> 0.05
Mean FEV_1 (l)	2.66 [0.94]	1.16 [0.38]	< 0.001
Mean FEV_1/FVC% (l)	76.2 [5.17]	47.7 [12.23]	< 0.001
GOLD stage (A/B/C/D)		0/2/11/4	
ICS (yes/no)		15/2	
Mode MMRC		4 (9 cases)	
Median CAT		27 {14-39}	

M - male, F - female, CRP - C-reactive protein, PTX-3 – pentraxin 3, RQ - relative quantification, FEV1 - forced expiratory volume in 1 s, FVC - forced vital capacity, GOLD - Global Initiative for Chronic Obstructive Lung Disease, ICS - inhaled corticosteroids, MMRC - Modified Medical Research Council Dyspnea Scale, CAT −COPD Assessment Test™, [] - +/- SD, {} - min-max range.

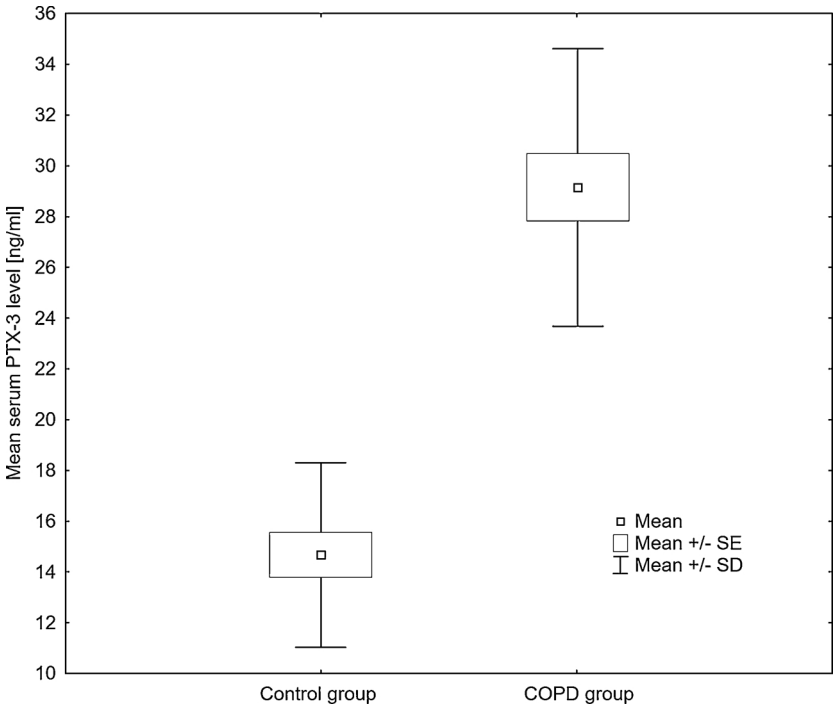

Fig. 1. Mean pentraxin 3 (PTX-3) serum levels in the control and the COPD groups.

between the PTX-3 serum levels, PTX-3 RQ values and other data – especially smoking status, pulmonary function tests, MMRC or CAT questionnaire. As there was no possibility to divide the COPD group according to GOLD guidelines for statistical purposes (i.e. no patients in stage A, only 2 patients in stage B), no calculations were performed with regard to GOLD subgroups [15].

4. Discussion

The study investigated the PTX-3 serum levels in COPD patients and controls, and made a relative quantification of its gene expression. It was found that serum levels of PTX-3 were significantly higher in the COPD group than the control group, which confirms earlier findings [12]. However, as mentioned in the Introduction, our findings also contradict those of two previous studies: Beghé et al. [13] and Van Pottelberge et al. [14]. It should, however, be borne in mind that in contrast to our present study, the Van Pottelberge study was based on a population of end-stage COPD patients and a second population of patients with lung tumors rather than healthy controls. In addition, while Pauwels et al. [16] demonstrated in a mouse model that the

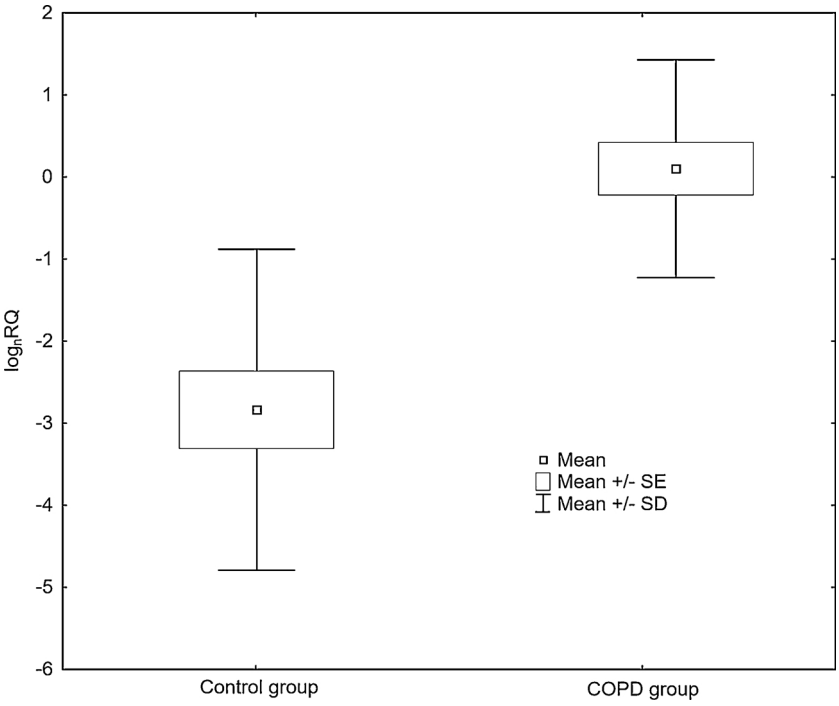

Fig. 2. Mean pentraxin 3 gene relative quantification (RQ) values after logarithmic transformation ($\log_n$RQ) in the control and the COPD groups.

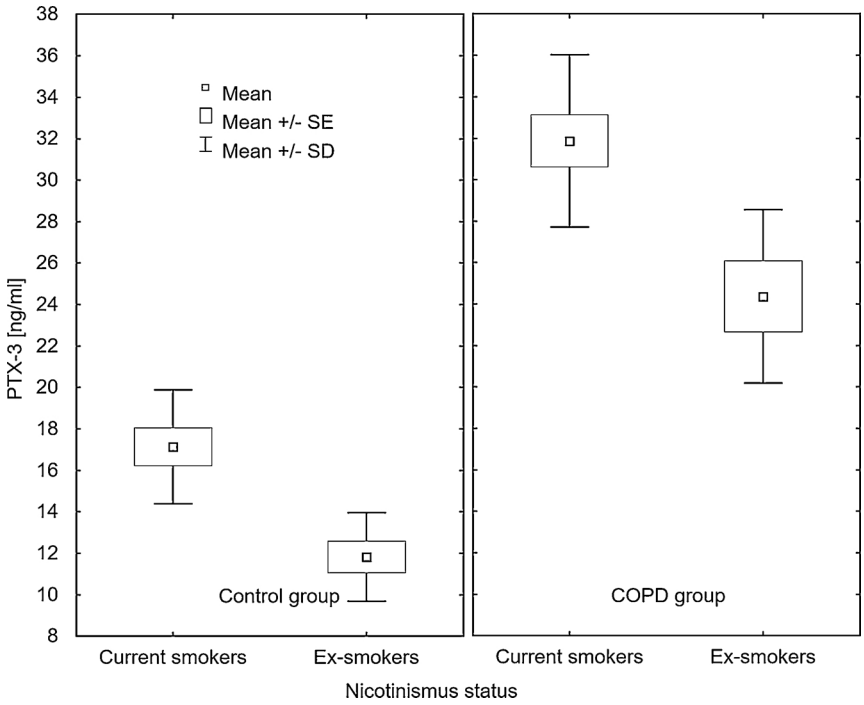

Fig. 3. Mean pentraxin 3 serum levels (PTX-3) after dividing the main groups into current-smoker and ex-smoker subgroups.

expression of PTX-3 in pulmonary veins is induced by cigarette smoke, Beghé et al. [13] did not note any differences in acute-phase inflammatory protein expression between patients with COPD and CHF; this is an unexpected observation, especially in the CHF group, as it has been demonstrated that patients with CHF have higher serum PTX-3 levels than patients in the healthy control groups [9,17–19].

Our results show that the serum PTX-3 levels can be associated with nicotinism (Fig. 3). The influence of cigarette smoke on PTX-3 production can be explained easily by the fact that it induces the production of interleukin 1 (IL-1), which plays a role in stimulation of peripheral mononuclear blood cells (PMBC) for PTX-3 release.

This observation is accompanied by the fact that no differences were observed in the CRP levels between the COPD group and the control group, and that the average CRP levels did not exceed the reference normal value (5 μg/ml) in either group. Similar observation was noticed by Silva et al. [20]. In addition, CRP was not found to correlate with any analyzed variables. Both facts seems to confirm that patients in the COPD group were stable. Furthermore, as COPD is an inflammatory disease, it would be expected that markers of inflammation would be elevated. While inhaled glycocorticosteroids are known to decrease the CRP levels it is not known how they influence PTX-3 levels [21].

The RQ values of the *PTX-3* gene were significantly higher in the COPD group. To the best of our knowledge, this is the first study to examine the RQ expression of the *PTX-3* gene in peripheral blood: whereas previous studies have also quantified *PTX-3* mRNA, they have done so in total lung tissue. Measurements performed in the lungs can provide a picture of the local *PTX-3* gene situation; however, those performed in peripheral blood give more information on a systemic scale. We need to remember that a genetic component can play a role in the regulation of the levels of PTX-3 not only in the inflammatory diseases (i.e. COPD) but also can be important in resistance to some infectious diseases. As an example we can mention two studies on pulmonary aspergillosis. In 2014, Cunha et al. [22] demonstrated that deficiency of PTX-3 can play a role in a decreased antifungal function of neutrophils. This result seems to be confirmed by He et al. [23]. In both studies, genetic origin of decreased PTX-3 plasma levels was observed.

Our results add further weight to the hypothesis that PTX-3 can act as a new marker of inflammation in COPD [24]. Of course, the clinical value of this protein needs to be verified in large-scale trials, and the influence of comorbidities on PTX-3 levels should also be examined.

4.1. Limitations of the study

This study is not free of limitations. The first is the use of relatively small groups. Only patients without comorbidities were included into the study and these are few in number. Similarly, the control group was quite small as it was difficult to recruit age-matched patients who were free of chronic diseases, not only in anamnesis but also presented no symptoms of diseases in physical examination. Nevertheless, all presented results have a statistical power of greater than 0.8 with a probability of making a type I error (α) equal to 0.05.

The second limitation is the fact that biological material was collected at one time point, providing only a cross-sectional rather than a longitudinal view of the sample. Thirdly, the group of the COPD patients is not matched according to the GOLD standards, resulting in a lack of information about the correlations between the stages of the disease and measured parameters. In addition, there are no never-smokers in the control group, which can limit final conclusions.

Finally, interleukin-10 (IL-10) levels were not measured. An in vitro study found that the addition of PTX-3 was associated with an isolated increase of IL-10 concentration in PBMC; this could imply that PTX-3 has inflammation-limiting properties, as IL-10 is also known to play an anti-inflammatory role [25,26]. Therefore, it appears reasonable to assume that the escalation of inflammation, i.e. by cigarette smoke, COPD or CHF, leads to increased concentrations of the proteins responsible for control over this process. However, further studies are needed to examine this hypothesis.

It was mentioned in the Methods section that not every FEV_1 : FVC ratio identified in the patients from the control group was greater than 0.7. The GOLD Report has been strongly criticized for being too conservative when choosing the FEV_1 : FVC ratio for use in the COPD diagnostic process, as it can be inaccurate and cause misclassification: both the under-diagnosis of abnormalities in younger, taller individuals and over-diagnosis in older or shorter patients [27]. A in our present study both groups were age matched and the oldest person in the control group was 79 years old, it is clear that the GOLD criteria should

not be used as the sole basis for enrollment; hence, LLN < 5th percentile was chosen as an indicator of spirometric disorders in the case of some patients.

5. Conclusions

In conclusion, our findings indicate that serum PTX-3 levels are elevated in COPD and can be related to cigarette smoking history. They also show that PTX-3 gene relative expression values are higher in the COPD group. However, repeated measure studies are needed to more precisely explore the role of the PTX-3 in inflammation related to COPD and smoking.

Conflict of interests

The authors declare no conflict of interests

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