Echocardiographic Evaluation of the Relationship Between inflammatory factors (IL6, TNFα, hs-CRP) and Secondary Pulmonary Hypertension in patients with COPD. A Cross sectional study

Evaluarea echografică a relației dintre factorii inflamației (IL6, TNFa, HS.Crp) și hipertensiunea pulmonară la pacienții cu BPOC. Un studiu încrucișat orizontal

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Abstract

Background: *Inflammatory mechanism appears to play a major role in the pathogenesis of various types of human pulmonary hypertension such as idiopathic PAH (IPAH) and PAH associated'' with connective tissue disease. Although we know that inflammatory factors such as IL6 and TNFa have an important role in IPAH, there is limited information about the relationship between acute phase reactants and pulmonary hypertension occurring secondary to pulmonary diseases such as chronic obstructive pulmonary diseases (COPD).*
Methods: *This cross-sectional study was carried out on 94 patients who had COPD. Patients with a recent history of systemic steroid and acetylsalicylic acid (ASA) use, infection, trauma or surgery, gastrointestinal bleeding, coronary artery disease (CAD) and Hypertension were excluded. Body plethysmography and transthoracic echocardiography were done. Blood samples for each patient included were drawn for complete blood count (CBC), IL6, TNFa and highly sensitive C reactive protein (hs-CRP).*
Results: *Twenty patients (28.6%) had pulmonary hypertension. The difference between the mean IL6 and hs-CRP in patients with and without pulmonary hypertension was significant (7pg/ml vs. 4.4pg/ml and 13.04pg/ml vs. 3.31pg/ml) (p= 0.006 and p=0.000). There was a correlation between IL6 and mean pulmonary arterial pressure (r = 0.35, p=0.003). After adjustment for age, sex, serum Hemoglobin, Hematocrit, O2Sat, FEV1, FVC the relationship between the IL6, hs-CRP and the presence of pulmonary hypertension remained significant (p=0.022, p=0.026) .*
Conclusion: *Inflammatory factors such as IL6 and hs-CRP are independent risk factors for pulmonary hypertension in COPD patients.*
Keywords: *COPD, pulmonary hypertension, inflammatory factors, secondary*

Rezumat

Introducere: *Mecanismul inflamator pare sa joace un rol esențial în patogenia diferitelor tipuri de hipertensiune pulmonară (HTP) la om, cum ar fi HTP idiopatică (HTPi) și HTP asociată cu bolile de țesut conjunctiv. Deși știm că moleculele inflamației, cum ar fi IL6 sau TNFa, joaca un rol important în HTPi, există doar informații limitate despre relația dintre reactanții de fază acută și hipertensiunea pulmonară ce apare secundar maladiilor pulmonare, cum ar fi bronhopneumopatia cronică obstructivă (BPOC).*
Metodă: *Acest studiu orizontal încrucișat a fost efectuat pe 94 de pacienți cu BPOC. Au fost excluși pacienții cu tratamente recente cu coricoizi sistemici și acit acetilsalicilic, infecție, traumă sau intervenții chirurgicale, hemoragie digestivă, boală coronariană și hipertensiune arterială. Au fost efectuate body-pletismografie și echocardiografie transtoracică. Au fost prelevate de la toți pacienții probe de sânge, trimise pentru hemoleucogramă, IL6, TNFa și proteina C reactivă înalt senzitivă (hs-CRP).*
Rezultate: *Douăzeci de pacienți (28.6%) aveau hipertensiune pulmonară. Diferența între valoarea medie a IL6 și Hs-CRP la pacienții cu și fără hipertensiune pulmonară a fost semnificativă statistic (7pg/ml vs. 4.4pg/ml și 13.04pg/ml vs. 3.31pg/ml) (p= 0.006 and p=0.000). A existat o corelație între IL6 și presiunea medie în artera pulmonară (r = 0.35, p=0.003). După ajustarea pentru vârstă, sex, hemoglobina serică, hematocrit, saturație în oxigen, VEMS și CVF, relația dintre IL6, hs-CRP și prezența hipertensiunii pulmonare a rămas statistic semnificativă (p=0.022, p=0.026) .*
Concluzie: *Factorii inflamatori ca IL6 și hs-CRP sunt factori de risc independenți pentru hipertensiunea pulmonara la pacienții cu BPOC.*
Cuvinte-cheie: *BPOC, hipertensiune pulmonară, factori inflamatori, secundar*

Introduction

Inflammatory factors are involved in the pathophysiology of specific types of pulmonary artery hypertension (PAH) such as idiopathic pulmonary artery hypertension (IPAH) and PAH associated with connective tissue diseases[1]. Several studies have investigated the role of leukocytes, macrophages and lymphocytes in vessels involved in IPAH[2,3].

PAH is also a common complication of inflammatory diseases, notably scleroderma and systemic lupus erythematosus (SLE). Cole and his colleagues studied patients with PAH secondary to scleroderma and identified mononuclear and inflammatory cells around the vessels[4]. Estimates of the prevalence of PAH in patients with COPD vary widely. Most studies have reported a prevalence of PAH in COPD to be between 30% and 70%[5,6].

It is known that hypoxemia is the cause of PAH in patients with chronic obstructive pulmonary disease (COPD), yet, patients with mild hypoxemia also develop PAH to some degree[7]. Hence, it is hypothesized that other factors may contribute to PAH in the setting of COPD. C-reactive protein (CRP) reduces endothelial nitric oxide synthase (eNOS) expression and bioactivity in aortic endothelium, which may lead to atherosclerosis and systemic hypertension[8], but it is not known if CRP plays the same role in PAH.

Although interleukin 6 (IL6) and the tumor necrosis factor (TNF) are involved in the pathogenesis of IPAH and PAH secondary to connective tissue diseases, limited data is available on the role of these inflammatory factors in development of PAH secondary to COPD.

Materials and Method

This cross-sectional study was conducted after approval of the ethics committee of Tabriz University of Medical Sciences in May 2011 under number 9157. It was carried out from June 2011 to October 2013. The studied population included smokers with post bronchodilator forced expiratory volume in 1 second/forced vital capacity (FEV1/FVC) ratios below a cut-off of 70%, who were referred to the pulmonary disease clinic at Imam Reza General Hospital in Tabriz, Iran.

After signing a written consent form, all patients were interviewed using a structured questionnaire by a trained staff physician. Collected data included age, gender, anthropometrics data, history of smoking, ischemic heart disease, systemic hypertension and steroid use. Height and weight were measured to calculate the participant's BMI.

A blood sample was drawn and sent to the laboratory to determine the levels of IL6, TNF-alpha (TNFα) and high-sensitivity CRP (hs-CRP) levels. Serum samples were centrifuged at 3000 rpm for 15 minutes and collected in a separate tube. They were stored frozen at -70°C. All tests were done by the same technician the same day as the sample collection.

Our exclusion criteria were: 1. history of systemic steroid or aminosalicylic acid (ASA) use. 2. history of malignancy in the past three years. 3. gastrointestinal bleeding, surgery or major trauma in a week prior to sampling, 4. history of systemic hypertension or coronary artery disease. Twenty-four patients were excluded out of 94 and a total of 70 patients were included in this study.

Body Plethysmography

Body plethysmography was performed in all patients. Bronchodilators were discontinued at least one day before the test. All tests were performed at the same time of day and by the same technician and were in accordance with the American Thoracic Society standards for a sitting position. Three technically acceptable maneuvers were performed for all patients and the highest measurement was included in the analysis.

Echocardiography

Echocardiography with Doppler imaging (DE) can provide an estimated right ventricle (RV) systolic pressure, which is believed to reflect pulmonary artery systolic pressure in the absence of RV outflow tract obstruction. However, DE has low sensitivity, specificity, and predictive values in patients with COPD[9] mainly because of technical difficulties in obtaining good windows in these patients. Overall, the success rate for DE in estimating RV systolic pressure in patients with COPD ranges between 26% and 66%[10,11].

Doppler echocardiography including 2-Dimensional (2D), M Mode, Color Doppler, CW and PW Doppler were performed in all patients. Echocardiography was done by the same cardiologist who was blind to the results of the biochemical analysis. Continuous Doppler wave assessment of the peak velocity of the tricuspid regurgitation jet as well as pulsed Doppler recording of the time to peak velocity curves of pulmonary artery blood flow and right ventricular outflow tract were used to assess mean pulmonary arterial pressure (PAP).

Doppler recordings at the maximum systolic pressure gradient between the right ventricle and the right atrium were used to assess peek pulmonary artery systolic pressure. Right ventricular systolic pressure was calculated based on Bernoulli's equation. Patients with no accurate measurement of tricuspid regurgitation jet were excluded.

Jet TR velocity was traced to calculate the average systolic pressure gradient between the right ventricle and the right atrium. Systolic pulmonary artery pressure (sPAP) was calculated as the highest systolic pressure gradient between the right ventricle and the right atrium plus right atrial pressure. The diameter of the inferior vena cava and its respiratory variations were used to estimate right atrial pressure.

To estimate the mean pulmonary artery pressure (mPAP), we used the time to peak velocity curves of systolic blood flow in the pulmonary artery and right ventricle outflow tract[12].

The accuracy of Doppler echocardiography in the assessment of PAP is not high in patients with COPD, but due to lack of other measurement techniques, we divided the patients into those without pulmonary hypertension (mPAP<25 mmhg) and those with pulmonary hypertension (mPAP≥25 mmhg).

Statistical Analysis

SPSS statistical software package (SPSS Inc., Chicago, IL) was used for statistical analysis. Statistical significance was set at p value£0.05. The distribution of age, sex, laboratory values, lung volume and mPAP were analyzed by means and standard deviation for categorical variables. Chi-square test was used to find whether patients with PAH were different in terms of sex or smoking history. Student *t*-test was used to compare means of TNFα, IL6, white blood cell count (WBC), hs-CRP and lung volumes in patients with and without PAH.

To estimate and adjust for confounding variables, multivariable stepwise logistic regression tests were used. For multivariate analysis, we entered all risk factors for PAH and covariates with p value<0.05 in univariate analysis.

Results

Twenty-four patients were excluded and the remaining 70 patients were analyzed. Fourty-six patients (65.7%) were male and 34 patients (34.3%) were female. The mean age of patients was 65 years (Table 1). Overall, 20 (28.6%) patients had PAH and 50 (71.4%) patients had normal pulmonary artery pressures. Seventeen (85%) patients who had PAH were male and 3 (15%) patients were female. Mean mPAP was 26.6 mmHg.

The two groups showed a significant difference in their mean hs-CRP and IL6 levels (13.04 mg/dl vs. 3.31 mg/dl and 7 pg/dl vs 4.4pg/dl) (p value=0.000 and p value=0.006) respectively (Table 2). Mean haematocrit levels were 49.6%±8.7% in patients with PAH and 44.1%±5.4% in patients with normal pulmonary artery pressure, which is significantly different (p value=0.002). Also mean oxygen saturation (SaO2) was 89.3%+-5.56% in patients with PAH and 94.2%±2.56% in patients with normal pulmonary artery pressure, which is significantly different (p value=0.000). TNFα levels were not different between the two groups (23.1pg/dl vs 24/7pg/dl) (p=0.8). A positive correlation was observed between mPAP and IL6 (r=0.35, p value= 0.003) (Figure 1). The negative correlation between Oxygen saturation (SaO_2) and mPAP was found to be significant (r=-0.42 , p value=000) (Table 4). There was no correlation between inflammatory factors (hs-CRP, IL6, TNFα) and SaO_2.

After adjusting for age, sex, FVC, FEV1, haemoglobin, haematocrit and SaO2 in logistic regression analysis, IL6 and hs-CRP were found to be significantly related to PAH (p value=0.022, p value=0.026) (Table 3).

Discussion

Our study shows that hs-CRP and IL6 levels in patients with COPD who have PAH are different from those with normal pulmonary artery pressure. Since these differences remained significant in the multivariate analysis, they are independent risk factors for PAH in patients with COPD. TNFα levels were not different in patients with PAH compared to those with normal pulmonary artery pressure. Also, we showed that circulating IL6 was correlated with mPAP.

A previous study has shown inflammatory infiltrates in the pulmonary artery of patients with COPD[13]. It is also

Table 1 Baseline characteristics of patients

Age	65 (40 to 87)
Male	46 (65.7%)
Female	24 (34.3%)
mPAP ≥ 25 mmhg	24 (34.3%)
FVC % predicted	77.21±17.7
FEV1 %predicted	56.8±18.8
TNFα (pg/ml)	23.6±23.9
IL6 (pg/ml)	5.2±3.5
Hs-CRP (mg/dl)	5.4±8.4
BMI (kg/m²)	26.6±6.02
FEV1/FVC	56.9±11.8
RV % predicted	144.9±58.5
RV/TLC %	138.4±32.38
mPAP (mmHg)	26.8±9.5
Hg (g/dl)	14.5±1.97
Htc %	45.7±6.9

BMI = Body Mass Index; **mPap** = Mean pulmonary artery pressure; **RV** = Residual volume; **TLC** = Total lung capacity

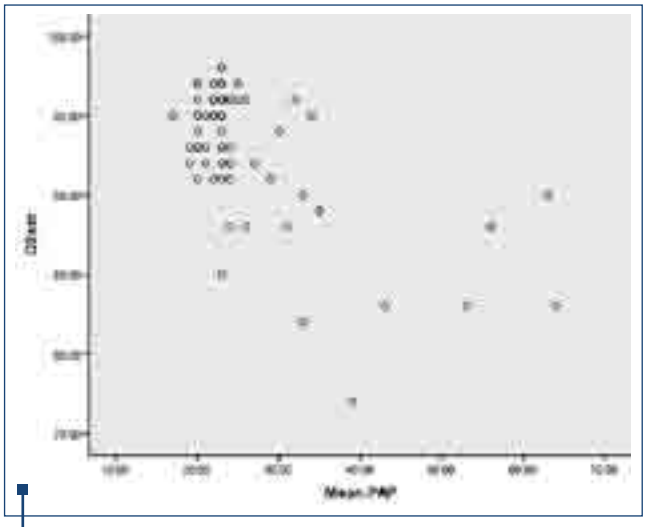

Figure 1 *- Correlation between mPAP and IL6*

shown that several inflammatory proteins are involved in the regulation of pulmonary artery pressure[14]. Over the past decade, the hypothesis that explains the role of hypoxia and emphysema in development of PAH in COPD has been challenged[15].

An earlier study has shown that IL6 is involved in the development of chronic thromboembolic PAH and PAH due to collagen vascular diseases. It is suggested that IL6 contributes to PAH development via release of serotonin from platelets[16]. A piece of contrasting evidence is the work of Ari and his colleagues that show the role of IL6 in PAH secondary to COPD[17]. In our study, not only IL6 levels are different between the two groups but also it is significantly correlated with mPAP. An explanation for the contrasting findings on the role of IL6 in pathogenesis of PAH in the setting of COPD might be genetic sensitivity to inflamma-

Table 2 Univariable analysis

	m.Ppa >=25	m.Ppa <25	OR (95%CI)	*P* value
Male	17(85%)	29(58%)	0.24 (0.6-0.9)	0.032
Age	69.7+-9.1	63.8+-10	—	0.025
BMI Kg/m²	26.4+-7.2	26.6+-5.2	—	0.84
Height cm	163.6+-9.7	161+-8.6	—	0.47
Weight kg	70.5+-18.7	69.7+-14.8	—	0.84
HsCRP mg/dl	13.04+-16.4	3.31+-3.6	—	0.000
IL6 pg/dl	7+-4.01	4.4+-3.1	—	0.006
TNFa pg/dl	24.7+-31	23.1+-20.4	—	0.8
FEV1, % predicted	50.27+-18.8	59.48+-18.3	—	0.064
FVC, % predicted	67.62+-15.8	81.04+-17.1	—	0.004
FEV!/FVC ratio,%	55.34+-10.4	57.56+-12.4	—	0.48
TLC, % predicted	101.25+-33	102.38+-19.5	—	0.86
RV/TLC ratio, % predicted	144.3+-35.4	136.1+-31.1	—	0.34
RV, % predicted	152.7+-77.8	141.8+-49.4	—	0.48
HG g/dl	15.12+-2.5	14.12+-1.6	—	0.105
HTC%	49.6+-8.7	44.1+-5.4	—	0.002
%O_2sat	89.3+-5.5	94.2+-2.5	—	0.000

Table 3 Multivariable analysis and adjust for confounding variable

Variables	*P* value (before adjust)	*P* value (after adjust)
Age	0.025	0.75
Sex	0.032	0.39
Hg gr/dl	0.105	0.017
Htc%	0.002	0.016
IL6 pg/dl	0.006	0.022
HsCRP mg/dl	0.000	0.026
FEV1% predict	0.064	0.19
FVC% predict	0.004	0.17
O_2sat%	0.000	0.014

Table 4 Correlation between inflammatory factors and mean PAP

	R	*P* value
IL6 pg/dl	0.353	0.003
Hs.CRP	0.19	0.106
TNFa	0.103	0.39
FVC% predict	-0.203	0.092
FEV1% predict	-0.087	0.47
HG g/dl	0.029	0.81
Htc%	0.13	0.25
O_2Sat	-0.42	000

tory factors. An important stimulus for IL6 production in COPD is hypoxia and pieces of evidence show that hypoxia can trigger IL6 expression in different cell types[18]. In our study, the possible role of hypoxia on IL6 production could not be addressed.

TNFα is a pro-inflammatory cytokine with a modulatory effect on the pulmonary artery circulation. An animal study has shown the effects of TNFα in increasing pulmonary vascular reactivity[19], which might decrease prostacyclin production in the smooth muscle cells of the pulmonary artery[20]. These studies support the suggested role of TNFα in pulmonary vascular pathophysiology. Clinical studies show inconsistent results on the role of TNFα in the regulation of pulmonary circulation. High serum TNFα levels were found in patients with PAH secondary to chronic thromboembolism[21].

In a recent study conducted by Pavol and his colleagues on 43 patients, the mean TNFα level in COPD patients with PAH was higher than patients without PAH, however, in multivariate analysis, this difference was not observed and there was no correlation between TNFα and PAH[22]. A few years following this study, Jiang et al. conducted a research on the association between TNFα and PAH in COPD but no correlation was found. Though, CRP levels in the two groups were significantly different[23]. We did not find an association between TNFα and PAH in our study. Despite the inconsistencies for the role of TNFα in the pathogenesis of PAH in the setting of COPD, its role is well-studied in IPAH and PAH secondary to collagen vascular diseases. This shows the need for further studies with more patients.

Some studies have shown that CRP has modulatory effects on endothelial cells[23,24] and that CRP reduced eNOS expression in aortic endothelium[24]. These studies indicate that CRP contributes to endothelial dysfunction and may be a potential cause of increased systemic vascular resistance. Another study has investigated the role of CRP in the development of PAH in COPD[22] suggesting an independent role for CRP in PAH pathophysiology. The results of this study were later confirmed by Yang and colleagues[25]. They also showed that CRP levels are associated with smoking rates along with an independent role of CRP in PAH development in the setting of COPD. Similar results were observed by Jiang and colleagues[23]. In our study, the mean CRP levels between the two groups were significant and remained significant after multivariate analysis. Because some of our patients were not smokers and had a history of working near bakery ovens, we could not evaluate the relationship between cigarette smoking and blood inflammatory factors.

Study Limitations

BODE index might have strengthened our findings but we did not perform the 6-minute walking test in our patients and were unable to use this index. Gold standard for measurement of mPAP is based on right heart catheterization and we had some limitation for accurate estimation of mPAP by using echocardiography in our study.

Conclusion

We found that the pro-inflammatory cytokines are involved in the development of PAH in the setting of COPD. Some of these factors such as hs-CRP and IL6 play independent roles in PAH pathogenesis. ■

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