# **Elevated Plasma Hyaluronan Levels in Pulmonary Hypertension**

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Pulmonary arterial hypertension (PAH) is a progressive disease, with a poor prognosis. The pathophysiologic mechanism of PAH is unknown, but may involve both tissue remodeling and inflammatory processes. Hyaluronan (HA) is a large glycosaminoglycan polymer and a major component of the extracellular matrix. In the present study, we measured plasma HA levels in PAH associated with systolic congestive heart failure (CHF, n = 16) or chronic obstructive pulmonary disease (COPD, n = 18). The control group was consisted of 14 healthy individuals without pulmonary or cardiovascular disease. Plasma HA levels (ng/mL) were determined in all patients by an enzyme linked HA binding assay. Pulmonary arterial pressure (PAP) was calculated in echocardiography (mmHg). Pulmonary arterial pressures were significantly higher in CHF and COPD (CHF: 55.0  $\pm$  11 mmHg and COPD: 62.5  $\pm$  21 mmHg, p < 0.001 for each), compared to the control group (25.4 ± 5.9 mmHg). Plasma HA levels were significantly higher in CHF (73.0 ± 37.5 ng/ml, p = 0.007) and COPD (87.3 ± 53.2 ng/ml, p = 0.001) compared to control patients (26.2 ± 8.4 ng/ml). There was no significant difference in plasma HA levels between the CFH and COPD groups (p = 0.690). In COPD, plasma HA levels were significantly correlated with PAP, left atrium diameter. There was no significant correlation between plasma HA levels and age or with echocardiography parameters in CHF. Both CHF and COPD are associated with increased plasma HA levels. Elevated plasma HA may contribute to the development of PAH.

**Keywords:** chronic obstructive pulmonary disease; extracellular matrix; heart failure; hyaluronan; pulmonary arterial hypertension

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# Introduction

Pulmonary arterial hypertension (PAH) is a progressive disease. The pathogenesis of PAH is poorly understood. Several processes are believed to lead to PAH including genetic predisposition, vasoconstriction, cellular proliferation, *in situ* thrombosis, inflammation, and vascular remodeling. It is well recognized that the extracellular matrix (ECM) has important roles in cell proliferation and migration and vascular remodeling (Humbert et al. 2004; Aldred et al. 2006; McLaughlin and McGoon 2006; Heresi et al. 2012).

Hyaluronan (HA) is a large glycosaminoglycan and a major component of the ECM. It has a role in inflammatory processes, cell proliferation and angiogenesis (Laurent and Fraser 1992; Evanko et al. 1999; Aytekin and Caylak 2009). Circulating HA levels are associated with pulmonary pathology (Teder et al. 2002).

Secondary PAH is a common and important issue in

clinical practice. Previous data have shown that the prevalence of PAH is 38% among congestive heart failure (CHF) patients and 50% among individuals with chronic obstructive pulmonary disease (COPD) (Thabut et al. 2005; Kjaergaard et al. 2007). The presence of PAH is also a strong predictor of mortality in patients with CHF or COPD (Wright et al. 2005; Szwejkowski et al. 2012). Despite a clinically important and frequent complication of pulmonary disease, the pathogenesis of PAH in CHF and COPD is unknown. Possible mechanisms include vascular remodeling (Moraes et al. 2000) and increased extracellular matrix synthesis.

Increased plasma HA concentrations and the accumulation of HA within pulmonary arteries have been demonstrated in patients with idiopathic pulmonary arterial hypertension (IPAH) (Aytekin et al. 2008). However, there are no data regarding plasma HA levels in patients with secondary PAH associated with CHF or COPD. Our hypothesis is that plasma HA concentration may correlate with the devel-

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opment of secondary PAH, in a manner similar to IPAH. The aims of this study are to evaluate HA levels in PAH in the setting of CHF and COPD.

## Methods

#### Study population

A prospective and cross-sectional study design was utilized. Inclusion criteria are PAH caused by CHF or COPD. A total of 48 subjects were enrolled in the study. Among these subjects, 16 patients had systolic CHF (13 patients with ischemic cardiomyopathy and 3 patients with non-ischemic cardiomyopathy) and 18 patients were diagnosed with COPD. The control group consisted of 14 healthy subjects who had no pulmonary or cardiovascular disease. Patients with both cardiac and pulmonary disease, any pulmonary or cardiac disease other than CHF or COPD that may cause PAH, atrial fibrillation, inflammatory disease or active inflammation, renal failure (creatinine levels > 1.3 mg /dl), liver disease, morbid obesity or malignancy were excluded from the study.

PAH was defined as a systolic pulmonary arterial pressure (PAP) of greater than 40 mm Hg at rest by echocardiography. The diagnosis of systolic CHF was established with clinical evaluation and echocardiographic examination. COPD was established according to GOLD criteria (Abdool-Gaffar et al. 2011). The study was approved by the ethics committee of Erciyes University of Medicine faculty. Signed informed consent was obtained from all patients.

#### Echocardiography

Echocardiographic examination was performed by a trained cardiologist using a Vingmed system V ultrasound device with 3.5 MHz probe. Following a 5 min resting period M-Mode, 2-dimensional and Doppler echocardiograms were obtained in the left lateral decubitus position. Left ventricular and left atrial dimensions were measured in the parasternal long axis view. Left ventricular end diastolic and end systolic dimensions were measured using M mode echocardiography. Left ventricular ejection fraction (LVEF) was obtained by means of the Teichholz equation (Teichholz et al. 1976). PAP was estimated by continuous wave Doppler echocardiography using the modified Bernoulli equation (4 + [peak tricuspid velocity] with 10 mmHg added for the estimated right atrial pressure (Borgeson et al. 1996; Baumgartner et al. 2009).

#### Biochemical and hematological parameters

Blood samples were drawn in the morning after a fasting period of 12 hours. The concentrations of glucose, blood urea nitrogen, creatinine, lipid profile, liver function tests, and uric acid were determined by Thermo Clinical Lab Systems (Thermo Clinical Labsystems, Vantaa, Finland). Hemoglobin, hematocrit, white blood cell, neutrophil and lymphocyte percentage, mean platelet volume (MPV), and platelet count were determined using a blood counter (Sysmex K-1000, Sysmex Medica Co. Japan).

#### Measurement of hyaluronan

The concentration of HA in the plasma was determined by an enzyme linked HA binding assay (Corgenics Inc., Broomfield, CO, USA) (Haserodt et al. 2011). The system uses a capture molecule known as HA binding protein. The optical densities were measured at 450 nm. All the test procedures were carried out according to the manufacturer's instructions.

#### Statistical analysis

Data are presented as a mean  $\pm$  standard deviation (s.D.). Categorical variables are presented as numbers and percentages. The Shapiro-Wilk test was used to evaluate normality of variables. Variables between groups were compared with parametric (ANOVA) or nonparametric (Kruskal-Wallis) tests. Categorical variables were compared with a chi-square test. After adjusting for PAP and age covariates, analysis was performed to evaluate the difference in plasma HA levels between control, CFH and COPD groups. Pearson or Spearman tests were used to analyze correlation between variable. P values < 0.05 were considered statistically significant. All reported p-values are two-sided.

# Results

#### **Demographics**

Demographic properties of patients are presented in Table 1. Age, PAP and hemoglobin levels were significantly increased in the COPD group relative to other groups. Ejection fraction was significantly decreased in the CHF group in comparison to the other groups (p < 0.001). None of the patients had severe valve disease. None of the patients had severe valve disease. HA levels were not statistically different between male and female patients [Male:  $86.8 \pm 42.2 \text{ ng/mL}$ ; female:  $87.7 \pm 35 \text{ ng/mL} p = 0.973$ ].

# Plasma HA concentration among patients with COPD or *CHF and healthy individuals*

Plasma HA concentration was significantly higher in COPD and CHF compared to the control group [COPD:  $87.3 \pm 53.2$  ng/mL, vs. control:  $26.2 \pm 8.4$  ng/mL; p = 0.001and CHF:  $73.0 \pm 37.5$  ng/mL vs. control:  $26.2 \pm 8.4$  ng/mL; p = 0.007]. There was no significant difference between the CFH and COPD groups [CHF:  $73.0 \pm 37.5$  ng/mL, COPD:  $87.3 \pm 53.2 \text{ ng/mL}; p = 0.690$ ] (Table 2).

After adjusting for the covariates PAP (46.3 mmHg) and age (57.1 year), HA levels were  $14.4 \pm 14.5$  ng/mL in the control group,  $77.4 \pm 11.4$  ng/mL in the CHF group and 96.6  $\pm$  13.4 ng/mL in the COPD group (p < 0.001). Covariate adjustment analysis demonstrated that the plasma HA are significantly higher in the COPD and CHF groups compared to the control group (Table 2).

Plasma HA concentration was significantly correlated with age (r = -0.52, p = 0.001), PAP (r = 0.32, p = 0.03), left atrium diameter (r = 0.62, p = 0.001), neutrophil count (r = 0.79, p = 0.006) and lymphocyte percentage (r = -0.77, p = 0.006)p = 0.008), MPV (r = 0.58, p = 0.07) and platelet count (r =-0.87, p = 0.001) in COPD. However, there was no significant correlation between HA concentration and age or LVEF in the CHF group. There was no statistically significant correlation between PAP and HA levels in control group (r = 0.126, p = 0.668).

## Discussion

PAH is typified by smooth muscle proliferation and vascular remodeling. The processes by which pulmonary artery smooth muscle cells affect vascular proliferation and

	Control group $(n = 14)$	CHF group $(n = 16)$	$\begin{array}{c} \text{COPD group} \\ (n = 18) \end{array}$	р
Age (years)	53.1 ± 15.7	59.3 ± 10.4	63.1 ± 6.6	0.023
Sex (female) $n$ (%)	6 (42.9)	7 (43.8)	11 (61.1)	0.491
Hypertension <i>n</i> (%)	2 (14.3)	4 (25)	4 (22.2)	0.758
Smoking <i>n</i> (%)	2 (14.3)	3 (18.8)	8 (44.4)	0.107
Blood fasting glucose (mg/dl)	$92 \pm 27$	$104 \pm 41$	$98 \pm 43$	0.443
Blood urea nitrogen (mg/dl)	$16.2 \pm 6.8$	$32.7 \pm 12.8$	$17.6 \pm 4.1$	< 0.001
Creatinine (mg/dl)	$0.9 \pm 0.3$	$1.2 \pm 0.4$	$1.0 \pm 0.3$	0.564
Hemoglobin (g/dL)	$13.1 \pm 1.9$	$11.5 \pm 1.7$	$13.9 \pm 2.0$	0.015
Total cholesterol (mg/dl)	$171 \pm 26$	$165 \pm 35$	$177 \pm 46$	0.222
LDL-cholesterol (mg/dl)	$101 \pm 29$	$102 \pm 35$	$108 \pm 38$	0.494
Triglyceride (mg/dl)	$133\pm102$	$127 \pm 121$	$147\pm126$	0.543
Echocardiography				
PAP (mmHg)	$25.4\pm5.9$	$55.0 \pm 11$	$62.5\pm20.7$	< 0.001
LV ejection fraction (%)	$61.9\pm7.2$	$32.4 \pm 13.1$	$58.3\pm10$	< 0.001
LV diastolic diameters (mm)	$4.7 \pm 0.4$	$58.8 \pm 8.8$	$45.6 \pm 7.2$	< 0.001
LV systolic diameters (mm)	$3.1 \pm 0.5$	$44.5 \pm 11.7$	$27.4 \pm 6.7$	< 0.001
AST (U/L)	$20 \pm 2.3$	$23.3 \pm 7.9$	$17 \pm 1.4$	0.355
ALT (U/L)	$19.9\pm8.2$	$21.8 \pm 14.4$	$13 \pm 2.8$	0.611
LDH	$210.5 \pm 16.3$	$228.5\pm29$	$245\pm32$	0.184
Uric acid (mg/dl)	$6.1 \pm 3.2$	$8.5 \pm 4.8$	$6.4 \pm 2.4$	0.165
Hematocrit (%)	$39.7 \pm 4.5$	$39.2 \pm 3.3$	$45.5 \pm 7$	0.063
MCV	$82.7\pm4.9$	$84.2 \pm 7.4$	$83.7 \pm 7$	0.877
MCHC	$32.7 \pm 1.8$	$31.7 \pm 1.6$	$32.1\pm0.9$	0.467
МСН	$27.1 \pm 2.6$	$26.7 \pm 2.7$	$26.9\pm2.4$	0.961
Platelets ( $\times 10^{9}/L$ )	$244 \pm 96$	$285 \pm 146$	$230 \pm 90$	0.464

Table 1. Demographics and clinical characteristics of the groups, Control, CHF and COPD.

LDL, Low density lipoprotein; HDL, High density lipoprotein; AST, Aspartate transaminase; ALT, Alanine transaminase; PAP, Pulmonary artery pressure; EF, Ejection fraction. p < 0.05 accepted as statistically significant.

Table 2.	Plasma	HA	levels	in	COPD	and	CHF.

Hyaluronan levels (ng/mL)			
	Unadjusted	Adjusted	р
Control group	$26.2 \pm 8.4$	$14.4 \pm 14.5^{a}$	
CHF group	$73.0\pm37.5$	$77.4 \pm 11.4$ <sup>a</sup>	0.001
COPD group	87.3 ± 53.2	$96.6 \pm 13.4^{\text{a}}$	

Data expressed as ng/ml. Covariates for adjustment for multiple comparisons: PAP 46.3 mmHg and age = 57.1 years. <sup>a</sup>Significant difference between control and other groups. The difference between CHF and COPD groups was not significant.

remodeling are not clear. It is well recognized that the extracellular matrix (EMC) has important roles in cell proliferation and migration. EMC components are important for normal lung function and response to lung injury. In the present study, we investigated the relationship between plasma HA concentration and PAH due to COPD and CHF. Our results demonstrated that patients with COPD and CHF have increased plasma HA compared to healthy control subjects. These results suggest that HA may be an important molecule in the pathophysiology of PAH, as has been previously demonstrated in IPAH. Identifying EMC abnormalities in other forms of PAH can improve understanding of the underlying disease pathobiology.

Previously studies demonstrated that patients with idiopathic have histopathologic changes in vascular structure that are similar to other types of PAH (Ghamra and Dweik 2003; Pietra et al. 2004; Stewart and Rassl 2009). These pathological changes are the result of long-standing hypertension regardless of underlying etiology. Characteristic plexogenic lesions are seen in the lungs of patients with all types of PAH. This suggests that IPAH may share a common pathophysiologic basis with other forms of PAH. Recently it was reported that patients with idiopathic PAH have higher circulating HA concentration compared to controls (Aytekin et al. 2008; Papakonstantinou et al. 2008). Increased tissue HA production and increased binding of inflammatory cells suggest a major role for HA in remodeling and inflammation in idiopathic PAH (Aytekin et al. 2008).

The present study suggests that HA abnormalities may occur in other forms of PAH as well. Our results demonstrate that patients with COPD have increased HA concentration compared to controls. There was also a significant but weak positive correlation between PAP and HA in COPD. After covariate adjustment, we observed that HA concentration is increased in COPD independent of PAP and age. Dentener et al. (2005) showed a relationship between HA concentration and local inflammation according to COPD severity, suggesting that HA may have an active role in COPD pathogenesis. In addition, we found that plasma HA concentration was correlated with neutrophil and lymphocyte percentage and MPV. Both neutrophil and lymphocyte percentage and MPV are indicators of systemic inflammatory process. HA is also correlated with systemic inflammation (de la Motte et al. 2009; de la Motte 2011). Therefore, results in the present study support the relationship between systemic inflammation and hyaluronan concentration.

Systolic heart failure is the most common cause of PAH. To our knowledge, there are no previous reports describing HA concentration in patients with PAH due to CHF. We found that CHF was associated with high concentrations of HA. Plasma HA concentration was related to PAP. In addition, there was no significant relationship between plasma HA concentration and echocardiography parameters (LVEF, systolic and diastolic diameters). Interestingly, plasma HA concentrations in CHF were comparable to patients with COPD.

The cause of increased plasma HA concentration in patients with CHF and COPD is unknown and requires further study. Numerous studies suggest a pathogenic inflammation and oxidative stress in CFH and COPD (Drost et al. 2005; White et al. 2006; Xu et al. 2011). Inflammation may be a cause of elevated HA in both of these diseases.

Limitation: The main limitation of the present study is a limited number of patients. We suggest that oxidative stress and chronic inflammation in CHF and COPD may contribute to increased plasma HA concentration. Significant correlation between hematologic parameters and HA concentration supports this hypothesis.

# Conclusion

Both CHF and COPD are associated with increased plasma HA concentration. Further studies are needed to demonstrate the cause of elevated plasma HA in these patients and the patholphysiologic role of HA in PH.

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### **Conflict of Interest**

The authors declare no conflict of interest.

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