ORIGINAL ARTICLE

Endothelin-1 Levels in Interstitial Lung Disease Patients During Sleep

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ABSTRACT

Background: Hypoxemia stimulates endothelin-1 (ET-1) secretion. The reduction in alveolar ventilation during sleep is considered sufficient to account for the hypoxemia observed in patients with respiratory diseases. Objective: The aim of this study was to evaluate the arterial ET-1 levels and their relationship with pulmonary hypertension in patients with interstitial lung disease (ILD) during sleep. Methods: We examined 38 patients with ILD using formal polysomnography (electroencephalogram, electrocardiogram, airflow, respiratory muscle movement, oximeter) to detect the presence of nocturnal, nonapneic, oxyhemoglobin desaturation. All patients desaturated below a baseline sleep saturation of 90% for 5 minutes or more, reaching a nadir saturation of at least 85%. Each patient had already undergone right heart catheterization with a Swan-Ganz catheter for measuring hemodynamic parameters. Sampling of arterial blood from a radial artery line for determination of blood gases and ET-1 values was performed simultaneously, after 5 minutes of the first desaturation. Results: At rest, arterial ET-1 levels were higher in ILD patients (1.73 ± 0.37 mgr/mL) than in controls (1.22 ± 0.15 mgr/mL) ($p < 0.001$). Also, the patients with pulmonary hypertension (Pa > 20 mm Hg) presented significantly higher arterial ET-1 levels (1.86 ± 0.32 mgr/mL) than those without pulmonary hypertension (1.31 ± 0.13 mgr/mL) ($p < 0.001$). Arterial ET-1 levels were significantly correlated with mean pulmonary arterial pressure (PAP) ($r = 0.749$, $p < 0.001$), and arterial oxygen partial pressure (PaO_2) ($r = 0.79$, $p < 0.001$). At sleep, during desaturation, arterial ET-1 levels significantly increased in all patients (2.46 ± 0.13 mgr/mL) as compared with resting values ($p < 0.001$). Arterial ET-1 levels were significantly correlated with PAP ($r = 0.657$, $p < 0.001$) and PaO_2 ($r = 0.93$, $p < 0.001$). Conclusions: Ac-

Sleep and Breathing, volume 7, number 3, 2003. Address for correspondence and reprint requests: K. Spiropoulos, M.D., University of Patras Medical School, Division of Pulmonology, Patras, 26500, Greece. E-mail: gtrakpmd@med.upatras.gr. [1]University of Patras Medical School, Division of Pulmonology, Laboratory of Sleep, Patras, Greece; [2]Laboratory of Immunology, Onashion Cardiosurgery Hospital, Athens, Greece; [3]Department of Hematological Diseases, Agios Sabas Hospital, Athens, Greece. 1520-9512,p;2003,07,03,111,118,ftx, en;sbr00236x.

cording to our study, arterial ET-1 is markedly increased in ILD patients, especially in those with pulmonary hypertension.

KEYWORDS: Interstitial lung disease, sleep, endothelin-1 (ET-1), hypoxemia

The endothelins are peptides of 21 amino acids that are produced in a wide variety of cells. Endothelin-1 (ET-1) is produced in endothelial cells, in vascular smooth-muscle cells, and in other cells of the bronchial epithelium.[1] High levels of ET-1 mRNA and ET-1 are present in the lungs of patients with cryptogenic fibrosing alveolitis[2] and in the vascular endothelial cells of patients with primary pulmonary hypertension.[3] Acute pulmonary alveolar hypoxia increases lung and plasma ET-1 in conscious experimental animals and the peptide increase is parallel to the severity of arterial hypoxemia.[4] Nonapneic oxyhemoglobin desaturation associated with sleep has been described in patients with chronic obstructive pulmonary disease (COPD),[5–8] cystic fibrosis,[9,10] interstitial lung disease[11,12] (ILD), and neuromuscular or skeletal diseases affecting the thorax.[13,14] These often profound decreases in oxyhemoglobin saturation (SaO_2) may be accompanied by marked elevation of pulmonary arterial pressure.[15–17] Repetitive, transient episodes of nocturnal hypoxemia have been proposed as one mechanism by which chronic pulmonary hypertension can develop in patients with advanced lung disease.[18,19]

We hypothesized that circulating levels of ET-1 might play an important role in the exacerbation of pulmonary hypertension during sleep in patients with chronic hypoxemia. To test this hypothesis we evaluated the changes in the arterial ET-1 levels and their relation with pulmonary hemodynamics and blood gases in patients with advanced ILD.

MATERIALS AND METHODS

We examined 38 patients with ILD (21 males, 17 females: mean age, 61.3 ± 3.5 years) admitted to our hospital between February 1999 and November 2001 because of dyspnea on excretion. The diagnosis of ILD was performed on the basis of histological findings of a biopsy specimen or after fine needle aspiration cytological examination. Spirometry was performed using a Morgan Flexiflo RS23 C Interface Spirometer. DLCO (diffusing lung capacity for carbon monoxide) measurement was performed using a Multilab LFT-3000 Analyser. Right heart catheterization was also performed using a Swan-Ganz catheter (BIH-7F; Baxter Health Care Co, Irvine, CA, USA) inserted to a basilic vein and placed in the pulmonary artery under local anesthesia. The patients were in the supine position under electrocardiographic and fluoroscopic monitoring. Each patient slept at least 1 night in our laboratory of sleep. Electroencephalographic (C3A2 and C4A1), bitemporal, electro-ocylographic, submental electromyographic, and electrocardiographic leads were placed appropriately. Nasal and oral airflow was detected by a thermistor analyzer attached to a loose face mask. Thoracic and abdominal pneumobelts connected to pressure transducers detected changes in chest and abdominal wall circumference. SaO_2 was continuously monitored by a finger oximeter. All data were recorded on polygraphic recorders (Somnostar-a Series, Sensor Medics Company). Sleep stages and SaO_2 were scored by a trained physician according to standard criteria.[20] Sampling of arterial blood from a radial artery line for determination of blood gases and ET-1 levels were performed simultaneously after 5 minutes of the first desaturation. Measurement of hemodynamic parameters and sampling of arterial blood for determination of blood gases and ET-1 values were performed simultaneously, both during the day and during the night.

Nocturnal desaturation was defined as a baseline awake saturation $\geq$ 90% with a fall below this value for a period of 5 minutes or more. During the period of desaturation, the nadir value had

to reach 85% or lower, but was not required to remain below 85% for 5 or more minutes.

Arterial blood for measuring ET-1 levels was collected in chilled vacutainers containing disodium dihydrogen ethylenediamine tetra-acetate dihydrate, and then centrifuged at 3000 × g for 20 minutes, to obtain plasma. The plasma was aliquoted and frozen at −80°C until analysis. Arterial ET-1 levels were measured by radioimmunoassay. The samples were extracted using 18 cartridges (SepPak, Waters, Missisanga, Ontario, Canada) activated by methanol. Samples and standards (synthetic endothelin-1; Peptide Institute, Osaka, Japan) were reconstituted in assay buffer and incubated with rabbit antiendothelin-1 antiserum (Peninsula Laboratories, Belmont, CA, USA) at 4°C for 24 hours. This was followed by the addition of I125-labeled ET-1 lumen (Amersham International, Amersham, UK) and a second 24-hour incubation. Bound and free radioactivity were separated by the second antibody method and the gamma emission from the precipitate of antibody ET-1 complexes was counted using a gamma counter. The intra- and interassay coefficients of variation were 10% and 13%, respectively. Arterial ET-1 levels in 34 healthy subjects at rest were available for comparison.

All data were expressed as the mean ± standard deviation, unless otherwise specified. The difference among the means of various variables between controls and patients was assessed by the

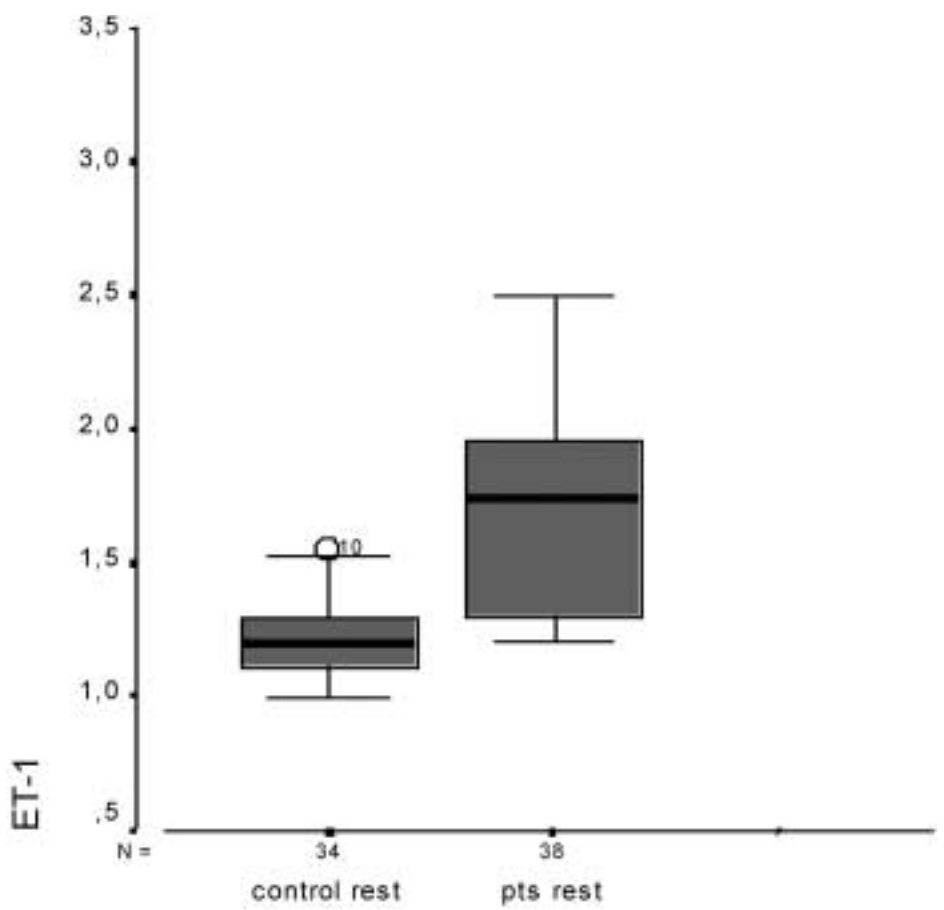

Figure 1 At rest, increased levels of arterial ET-1 were observed in patients with ILD (1.73 ± 0.37 mgr/mL) as compared with normal controls (1.22 ± 0.15 mgr/mL) ($p < 0.001$).

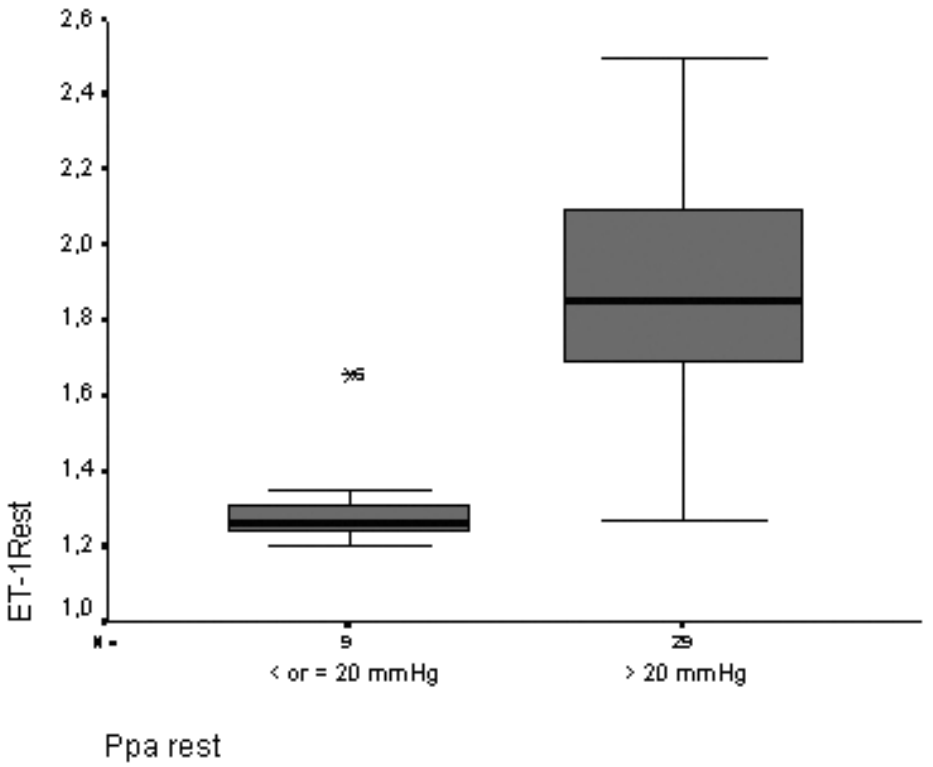

Figure 2 ILD patients with pulmonary hypertension (Pa > 20 mm Hg) presented significantly higher arterial concentration of ET-1 (1.86 ± 0.32 mgr/mL) as compared with those without this vascular complication (1.31 ± 0.13 mgr/mL) ($p <$ 0.001).

Mann-Whitney test. For multiple related variables we used the Kendall's test. The strength of correlation between variables was analyzed by the Pearson product-moment correlation.

RESULTS

At rest, increased levels of arterial ET-1 were observed in patients with ILD (1.73 ± 0.37 mgr/mL) as compared with normal controls (1.22 ± 0.15 mgr/mL) ($p < 0.001$) (Fig. 1).

ILD patients with pulmonary hypertension (Pa > 20 mm Hg) presented significantly higher arterial concentration of ET-1 (1.86 ± 0.32 mgr/mL) as compared with those without this vascular complication (1.31 ± 0.13 mgr/mL) ($p < 0.001$) (Fig. 2).

Arterial ET-1 values at rest correlated significantly with the resting values of PaO_2 ($p < 0.001$; r = 0.79) and Ppa ($p < 0.001$; r = 0.749) (Figs. 3 and 4, respectively).

No significantly statistical correlation between arterial ET-1 values and $PaCO_2$, cardiac frequency, and blood pressure was observed.

At sleep during desaturation, arterial ET-1 levels significantly increased in all patients (2.46 ± 0.12 mgr/mL) as compared with resting values (1.73 ± 0.37 mgr/mL) ($p < 0.001$).

Arterial ET-1 values at sleep also correlated significantly with PaO_2 ($p < 0.001$; r = 0.93) and Ppa ($p < 0.001$; r = 0.657) (Figs. 5 and 6, respectively).

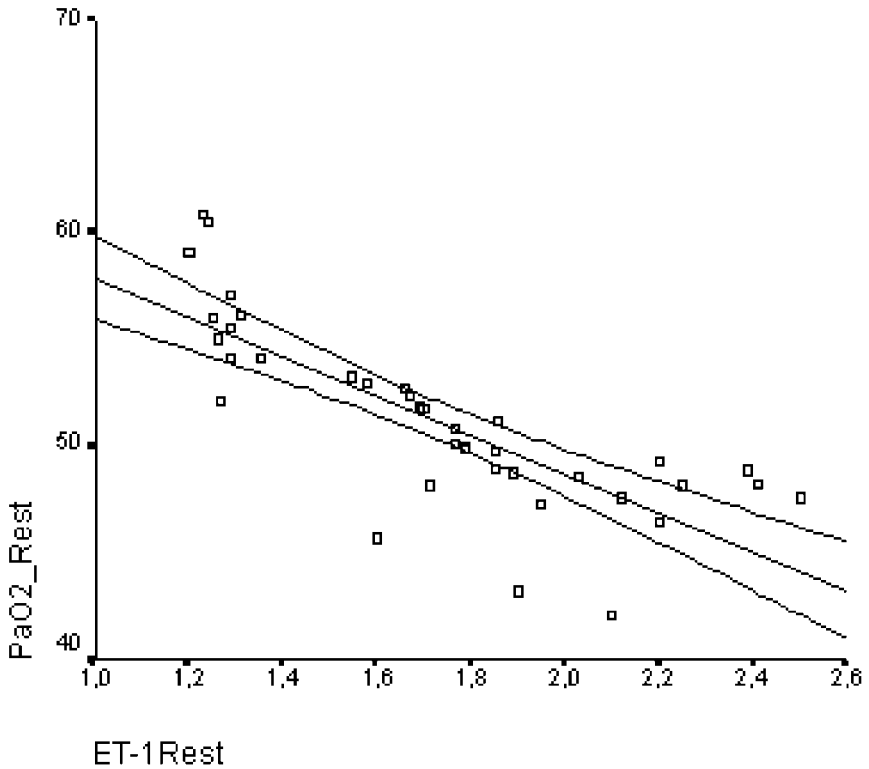

Figure 3 Arterial ET-1 values at rest correlated significantly with the resting values of PaO_2 ($p < 0.001$; r = 0.79).

DISCUSSION

Endothelin-1 was first described as a potent and long-lasting vasoconstrictor derived from endothelial cells.[21] ET-1 is produced in the vascular or nonvascular tissues of the lung and in peripheral circulation.[22]

Increased pulmonary and peripheral blood levels of ET-1 have been described in the pathophysiology of several lung diseases such as primary pulmonary hypertension,[3] bronchial asthma,[23] acute lung injury,[24] and cryptogenic fibrosing alveolitis.[24] In our study, the arterial levels of ET-1 were also found to be significantly elevated in ILD patients as compared with normal subjects.

In the lung, ET-1 can be produced by endothelial, tracheal, bronchial, and alveolar epithelial cells and by tissue macrophages.[22] Once released the peptide can act locally to elicit sustained pulmonary artery vasoconstriction, bronchoconstriction, and activation of alveolar macrophages leading to the release of eicosanoids and increased superoxide production.[25,26] ET-1 can also exert proliferative activity on fibroblast smooth muscle and endothelial cells.[27] The biological activity of ET-1 on the pulmonary vasculature is believed to play an essential role in the pathogenesis of secondary pulmonary hypertension including that associated with cryptogenic fibrosing alveolitis.[2] Also, according to a previous study of our pulmonology division,[28] ET-1 levels during exercise in ILD patients were significantly correlated with mean pulmonary arterial pressure. In this study, a significant increase in the circulating levels of ET-1 was observed in ILD patients with pulmonary hypertension as compared with those without this vascular complication.

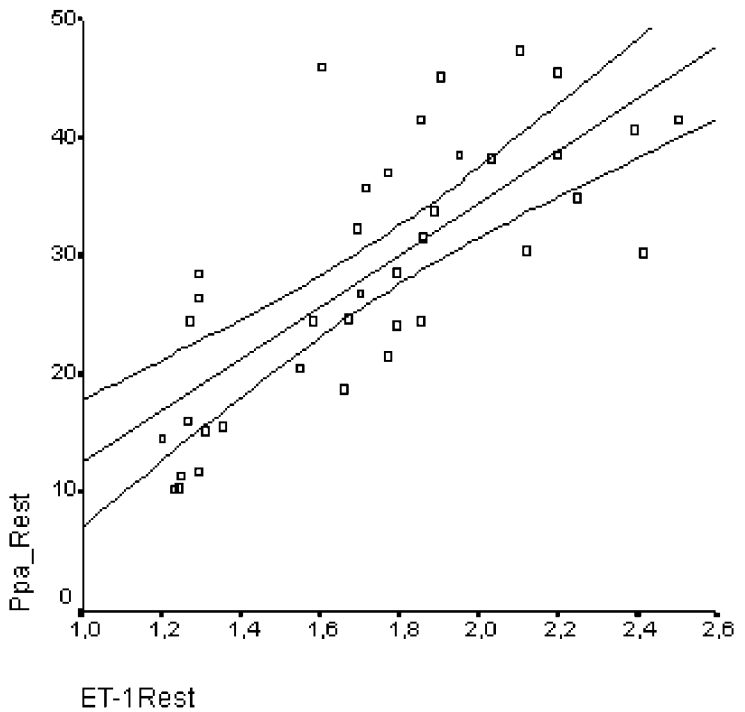

Figure 4 Arterial ET-1 values at rest correlated significantly with the resting values of Ppa ($p < 0.001$; r = 0.749).

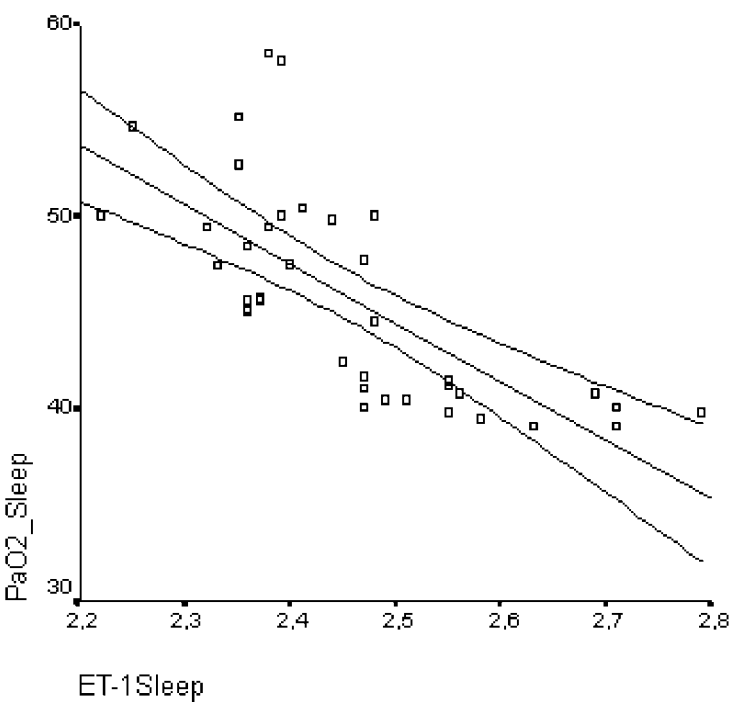

Figure 5 Arterial ET-1 values at sleep correlated significantly with PaO_2 ($p < 0.001$; r = 0.93).

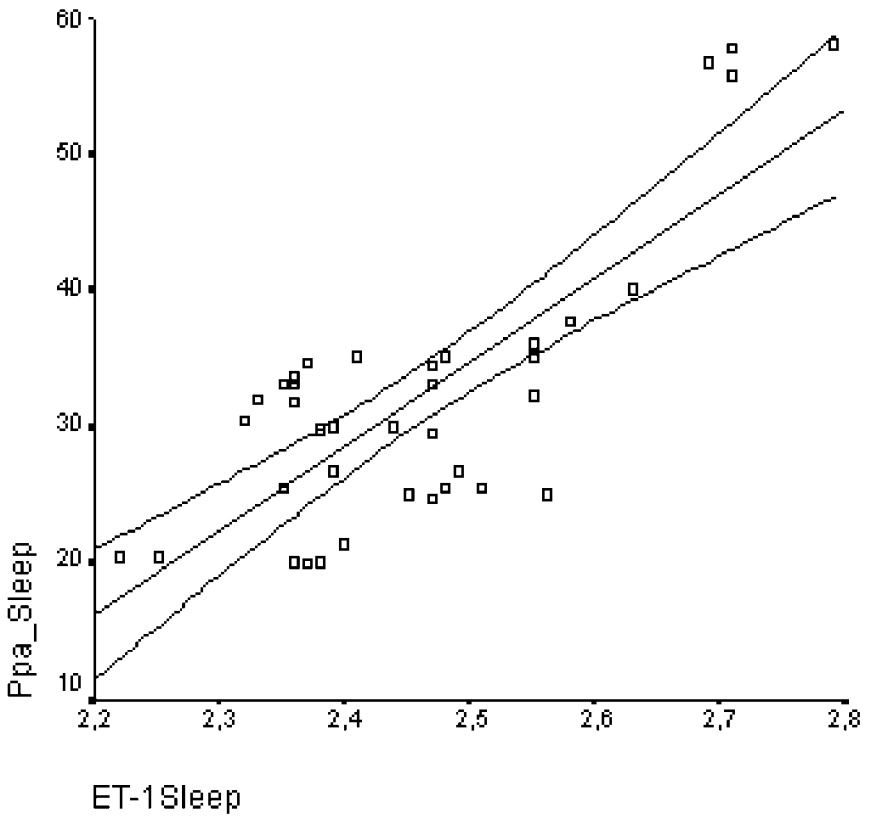

Figure 6 Arterial ET-1 values at sleep correlated significantly with Ppa ($p < 0.001$; r = 0.657).

Ventilation is reduced during sleep, compared with wakefulness, both in normal subjects[29,30] and in patients with respiratory failure.[31,32] The reduction in alveolar ventilation during sleep is considered sufficient to account for the hypoxemia observed in patients with respiratory diseases. Hypoxemia stimulates ET-1 secretion.[4] According to a previous study of our pulmonology division, ET-1 levels were significantly higher in <desaturators> COPD patients as compared with <nondesaturators> COPD patients.[8] In this study, arterial ET-1 levels increased significantly during desaturation at sleep in ILD patients and correlated negatively with PaO_2.

According to our study, ILD patients had significantly higher levels of ET-1 at rest and during sleep desaturation periods. Further long-term studies are necessary to elucidate the exact pathophysiological role of ET-1 in the pulmonary circulation and the clinical significance of the plasma ET-1 levels in ILD patients.

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