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Endothelin-1 levels in the pathophysiology of chronic obstructive pulmonary disease and bronchial asthma

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Summary *Background*: Endothelin-1 (ET-1) has been implicated in the pathogenesis of asthma and chronic obstructive pulmonary disease (COPD). The ET-1 levels are elevated during exacerbations of asthma and COPD in bronchoalveolar lavage, serum, and sputum and fails with treatment of the exacerbations. Hypoxemia stimulates ET-1 secretion. *Objective*: The aim of this study was to examine the serum ET-1 levels in stable asthmatic and COPD patients. *Materials and methods*: We examined 48 COPD and 26 asthmatic patients and 34 normal subjects. We collected arterial samples to measure baseline ET-1 levels in all patients and in the control group, during the day. All the patients underwent formal polysomnography (EEG, ECG, airflow, respiratory muscle movement, oximeter) to detect the presence of nocturnal, nonapneic, and oxyhemoglobin desaturation. Twelve of the COPD patients and six of the asthmatic patients were disqualified because of inadequate sleep or sleep apnea syndrome. Nineteen of the COPD patients desaturated below a baseline sleep saturation of 90% for 5 min or more, reaching a nadir saturation of at least 85%. We collected arterial samples to measure ET-1 levels, 5 min after the first period of desaturation in each of the 19 desaturators COPD patients. None of the 20 asthmatic patients exhibited oxyhemoglobin desaturation during sleep. *Results*: Baseline arterial ET-1 levels during the day were significantly higher in "desaturators" COPD patients (2.08 ± 0.28 pg/ml) compared to "non-desaturators" COPD patients (1.38 ± 0.16 pg/ml) ($P < 0.001$) and to asthmatics (0.72 ± 0.85 pg/ml) ($P < 0.001$). ET-1 levels in normal subjects were 1.221 ± 0.02 pg/ml. In "desaturators" COPD patients ET-1 levels during the night, 5 min after the first oxyhemoglobin desaturation, were significantly higher (4.28 ± 1.10 pg/ml) compared to those during the day (2.08 ± 0.28 pg/ml) ($P < 0.001$). A significant negative correlation was observed between ET-1 levels and degree of desaturation during the day ($P = 0.005$, $r = 0.632$) and during the night ($P < 0.001$, $r = 0.930$) in "desaturators" COPD patients. *Conclusion*: According to our results: (1) ET-1 levels were significantly higher in "desaturators" COPD patients than in "non-desaturators" COPD and in asthmatics; (2) ET-1 levels were significantly higher during the night than during the day in "desaturators" COPD patients; (3) the degree of desaturation correlated negatively with the ET-1 levels in "desaturators" COPD patients, both during daytime and nighttime. These findings are consistent with the hypothesis that ET-1 is implicated in the pathophysiology of asthma and COPD, especially if nocturnal, nonapneic, oxyhemoglobin desaturation exists.

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Introduction

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] Bronchial epithelial cells of asthmatic patients show increased expression of ET-1.[2] ET-1 is raised in the airways of asthmatic subjects and has been shown to stimulate mucus secretion, airway edema, smooth-muscle mitogenesis and bronchial hyper-responsiveness.[3] ET-1 produces prolonged and potent contraction of animal and human airways in vitro[4] and in animal studies in vivo by aerosol administration.[5] It is also thought to have proinflammatory effect in the airways, being both a chemoattractant and upregulator of other inflammatory mediators such as interleukins IL-6 and IL-8.[6,7] Plasma and bronchoalveolar lavage levels of ET-1 have been reported to be elevated in acute exacerbation of asthma falling with treatment of the exacerbation and to correlate with clinical severity of asthma.[8,9]

ET-1 is elevated by hypoxia, which may act both to increase ET-1 expression by increasing the binding of transcription factors such as AP-1 to the ET-1 gene and by increasing the activity of endothelin-converting enzymes.[10–12] ET-1 is believed to be one of the factors mediating pulmonary hypertension, at altitude and with congestive heart failure, for example and it has been implicated in cor pulmonale.[13] Hyperoxia also increases ET-1 levels and the action is believed to be mediated by the formation of reactive oxygen species.[13] Intermittent hypoxia has been shown to produce the same effects as chronic hypoxia and at times even more rapidly.[14] It may be that repetitive episodes of hypoxia and normoxia cause oxygen radical formation similar to ischemia followed by reperfusion.[14] If this is true, it is possible that adverse effects depend on the clustering of hypoxic/normoxic episodes and not just the duration of hypoxia.[12,14]

Nonapneic, oxyhemoglobin desaturation associated with sleep has been described in patients with chronic obstructive pulmonary disease (COPD),[15–18] cystic fibrosis,[19,20] interstitial lung disease[21,22] and neuromuscular or skeletal diseases affecting the thorax.[23,24] These often profound, decreases in oxyhemoglobin saturation ($SatO_2$) may be accompanied by marked elevation of pulmonary arterial pressure.[25–27] 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.[28,29]

The aim of this study was to examine the ET-1 serum levels in stable asthmatic and COPD patients, with and without nocturnal, nonapneic, oxyhemoglobin desaturation. According to our knowledge this is the first study, which examines the day, and night variability of hemoglobin saturation ($SatO_2$) in correlation with ET-1 serum levels in asthmatic and COPD patients in remission.

Materials and methods

We examined 48 COPD and 26 asthmatic patients who met the requirements and agreed to participate, in this study. We also examined 34 normal controls. The study was approved from the Patras University Hospital Ethics committee and written informed consent was obtained from each patient. Twelve of COPD and six of asthmatics patients were disqualified because of inadequate sleep or sleep apnea syndrome.

The inclusion criteria for the COPD patients were a clinical diagnosis of COPD according to American Thoracic Society recommendations and a spirometry consistent with moderate COPD (stage IIa) (50%< forced expiratory volume—FEV1 < 80% of predicted, FEV1/forced vital capacity—FVC < 0.70).[30] Forced spirometry was performed before the day of nocturnal polysomnography. Three average daytime resting PaO_2 were up to 60 mmHg with samples spread over 3–6 months preceding polysomnography. Daytime PaO_2 values were taken as the average of three blood gas levels drawn with the patient seated. All patients were estimated to be clinically stable at the time of the study. All the asthmatics were outpatients and had moderate asthma in remission (50% < FEV1 < 80%).[31] They underwent skin-prick testing and measurement of their bronchial reactivity by a modification of the method of Chai et al.[32] For this visit patients with asthma were asked to withhold short-acting b2-agonist therapy and inhaled corticosteroids for a minimum of 6 h.

None of the patients was receiving systematic corticosteroids. The atopic status was determined using the following common aeroallergens: mixed grass, pollens, dermatophagoids, pterenyssinus, mixed leathers, cat fur and dog hair. The asthmatics were all atopic with wheal reaction of 2 mm diameter or greater, in response to one or more of the above aeroallergens in the presence of negative saline and positive histamine controls.

Their mean PC20 methylcholine was 0.56 + 0.27 mg/ml. Five of the COPD patients were atopic to one or more aeroallergens.

All the patients underwent standard full-night polysomnography performed in a quite, partially soundproof room, with stable humidity and temperature. Each subject slept at least one night in our laboratory. Electroencephalographic (C3A2 and C4A1) bitemporal electrooculographic, submental electromyographic leads were placed appropriately. Nasal and oral airflow were detected by a thermistor analyzer attached to a loose-fitting facemask. Thoracic and abdominal pneumobelts connected to pressure transducers detected changes in chest and abdominal wall circumference. Oxygen saturation (SaO_2) was continuously monitored by an oximeter. All parameters were recorded simultaneously on polygraphic records (Somnostar-a. Series Sensor Medics Company). Sleep stages were scored by a trained physician according to standard criteria.[33]

Nocturnal desaturation was defined as a baseline awake saturation $\geqslant$90% with a fall below this value for a period of 5 min 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 at least 5 min. These periods usually coincided with but were not limited to REM sleep. Not all patients exhibited oxyhemoglobin desaturation during sleep.

We collected arterial samples from the 19 "desaturators" COPD patients, 5 min after the first desaturation, from the radial artery through a Becton–Dickinson catheter (arterial canulla with Flow Switch 20 G/10 × 45 mm, Ohmeda Swindon, UK) to measure ET-1 levels. Only one sample per "desaturator" patient was collected during the polysomnography. None of the asthmatic patients exhibited desaturation. The observer also collected arterial samples in the morning before the study to measure baseline ET-1 levels in both groups.

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 min, 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 (Sep-Pak, Waters, Missisanga, Ont., Canada) activated by methanol. Samples and standards (synthetic ET-1; Peptide Institute, Osaka, Japan) were reconstituted in assay buffer and incubated with rabbit andiendothelin-1 antiserum (Peninsula Laboratories, Belmont, CA, USA) at 4°C for 24 h. This was followed by the addition of I-125-labeled ET-1 (Amersham International, Amersham, UK) and a second 24 h incubation. Bound and free radioactivity was 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 inter-assay coefficients of variation were 10% and 13%, respectively. Data following normal distribution have been presented as mean + standard deviation (SD). When the distribution was not normal, median range has been used. We used Kruskal–Wallis test for differences between unrelated groups more than two. For differences between two related groups Wilcoxon sign ranks test has been used. For differences between two unrelated groups Mann–Whitney test has been used. One way ANOVA was used for differences in age. A *P* value of less than 0.05 two tailed was considered statistically significant.

Results

Asthmatic patients were statistically significant younger than the "non-desaturators" and "desaturators" COPD patients ($P<0.001$) (Table 1). There was no statistically significant difference among blood hemoglobin (mg/ml), total sleeptime (min), REM sleep (min) and NREM sleep (min) in all three groups of patients (Table 1).

The FEV1 (in 1 s)/FVC ratio of our asthmatic patients in remission, was statistically significantly higher than that of our "non-desaturators" ($P = 0.02$) and of our "desaturators" COPD patients ($P<0.001$) (Table 1, Fig. 1). The FEV1/FVC ratio between "desaturators" and "non-desaturators" COPD patients was not statistically significant ($P = 0.09$) (Table 1). "Desaturators" had significantly lower FEV1 and FVC, but the overlap between groups was sufficient to make these parameters alone of little help in separating individual "desaturator".

The daytime $SatO_2$ level of our asthmatics was statistically significantly higher than that of our "non-desaturators" ($P<0.001$) and of our "desaturators" COPD patients ($P = 0.005$) (Fig. 2). No statistically significant difference was observed in the daytime $SatO_2$ level between "non-desaturators" and "desaturators" COPD patients ($P = 0.8$) (Table 1). The daytime baseline ET-1 levels in asthmatic patients were statistically significantly lower than those observed in "non-desaturators" COPD patients ($P<0.004$) and in "desaturators" COPD patients ($P = 0.01$). The daytime ET-1 levels in asthmatic, "non-desaturators" and

Table 1 Age, blood hemoglobin, sleep stages, arterial gases and spirometric values in asthmatic and COPD ''non-desaturators'' and ''desaturators'' patients (mean ± SD).

Asthmatic patients	COPD non-desaturators patients	COPD desaturators patients	
Age (years)	55.70 ± 12.80	60.29 ± 4.80	63.00 ± 3.16
Blood hemoglobin (mg/ml)	14.30 ± 1.10	15.13 ± 1.00	15.06 ± 0.98
Total sleep time (min)	317.30 ± 71.96	299.22 ± 44.10	294.19 ± 57.94
REM sleep (min)	49.62 ± 14.22	38.62 ± 13.94	44.79 ± 14.68
NREM sleep (min)	282.37 ± 36.18	260.67 ± 34.08	250.56 ± 48.08
$SatO_2$ (%)	95.16 ± 3.18	93.54 ± 1.07	91.60 ± 1.08
PaO_2 (mmHg)	87.08 ± 5.49	73.99 ± 5.28	68.05 ± 4.70
$PaCO_2$ (mmHg)	36.80 ± 6.50	41.41 ± 2.98	42.38 ± 2.98
FEV_1 (l)	1.42 ± 0.14	1.33 ± 0.29	1.06 ± 0.11
FVC (l)	2.12 ± 1.00	3.28 ± 0.43	2.90 ± 0.47
FEV_1/FVC	67.95 ± 12.85	41.05 ± 9.08	37.04 ± 5.25
N	20	17	19

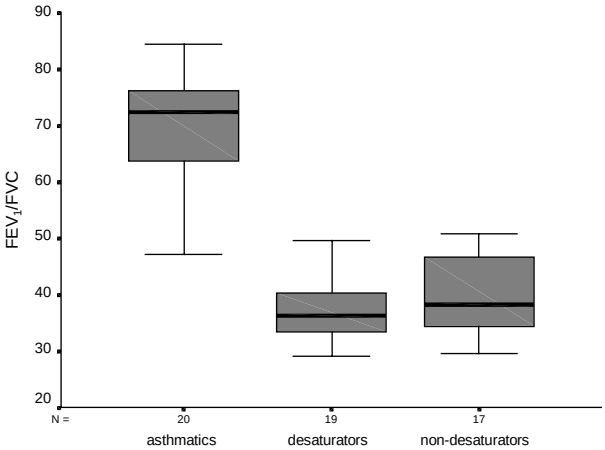

Figure 1 FEV1/FVC ratio in asthmatic and COPD ''non-desaturators'' and ''desaturators'' patients.

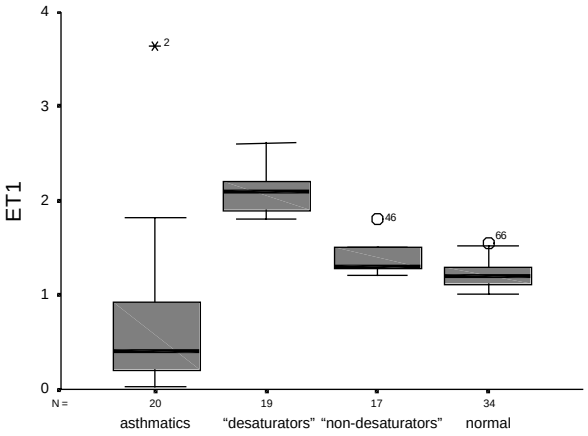

Figure 3 ET-1 in asthmatic and COPD ''non-desaturators'' and ''desaturators'' patients and normal controls during daytime.

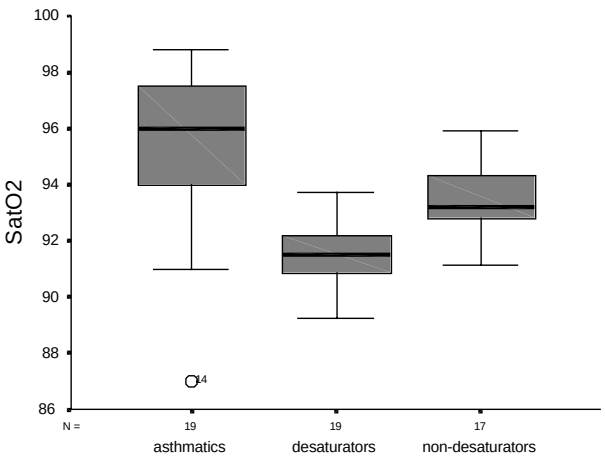

Figure 2 $SatO_2$ in asthmatic and COPD ''non-desaturators'' and ''desaturators'' patients during daytime.

''desaturators'' COPD patients and in normal controls are presented in Fig. 3. The daytime baseline ET-1 levels in ''desaturators'' COPD patients was statistically significantly higher than that of ''non-desaturators'' COPD patients ($P = 0.005$). The ''desaturators'' COPD patients had statistically significant higher levels of ET-1 during the night than during the day ($P < 0.001$) (Fig. 4; Table 2).

In ''desaturators'' COPD patients existed a relationship between ET-1 blood levels and $SatO_2$. ET-1 levels were negatively statistically significantly correlated with nadir values of oxyhemoglobin desaturation during sleep ($P < 0.001$, $r = -0.939$) (Fig. 5) and during daytime ($P = 0.005$, $r = -0.632$) (Fig. 6). No statistically significant correlation between ET-1 blood levels and SaO_2 was observed in the other groups.

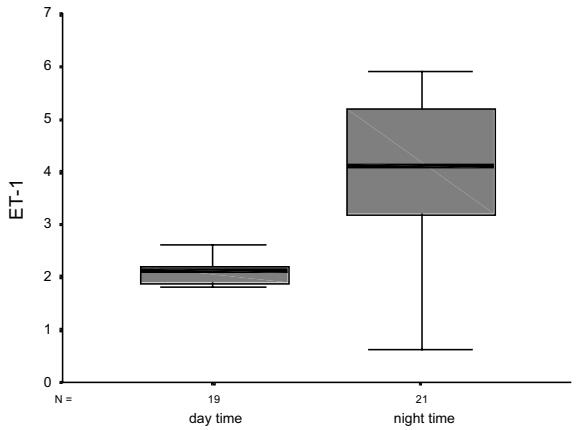

Figure 4 ET-1 in "desaturators" COPD patients during daytime and during sleep.

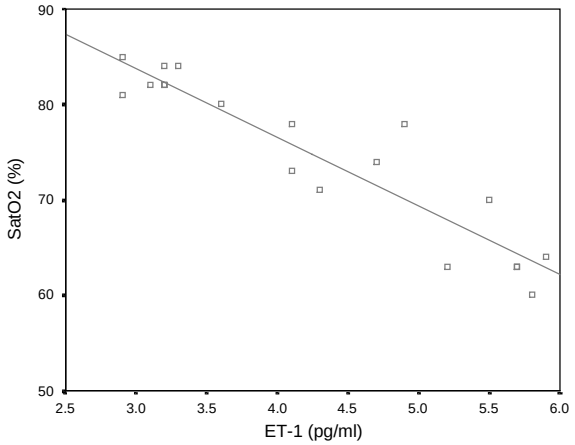

Figure 5 Correlation of $SatO_2$ and ET-1 levels in "desaturators" COPD patients during sleep ($P<0.001$, $r=-0.939$).

Table 2 ET-1 levels in asthmatic and COPD "non-desaturators" and "desaturators" patients during daytime.

	Asthmatic patients	COPD non-desaturators patients	COPD desaturators patients
1	1.817	1.30	1.80
2	3.640	1.20	2.40
3	0.200	1.20	2.10
4	1.234	1.50	2.20
5	0.484	1.50	2.50
6	0.210	1.50	1.80
7	0.320	1.80	1.80
8	0.320	1.40	2.10
9	0.316	1.50	1.90
10	0.420	1.40	1.90
11	0.646	1.40	1.90
12	0.205	1.50	1.80
13	0.083	1.30	2.10
14	1.607	1.30	2.60
15	0.024	1.20	2.30
16	0.368	1.20	2.20
17	0.192	1.30	2.00
18	0.474		1.90
19	0.836		1.80
20	1.011		

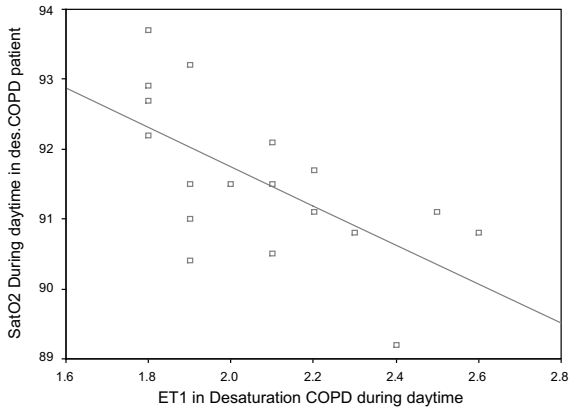

Figure 6 Correlation of $SatO_2$ and ET-1 levels in "desaturators" COPD patients during daytime ($P=0.005$, $r=-0.632$).

Discussion

In this study, we examined ET-1 plasma levels both in asthmatics and in COPD patients in remission, in relation with nocturnal, nonapneic, oxyhemoglobin desaturation.

The main site of ET-1 production is considered to be the bronchial epithelium and the endothelium (ETB-2) of pulmonary arteries.[2,34] ET-1 is a member of a family of 21-amino-acid peptides.[10,11,14] It is formed by a gene located on chromosome 6 whose activation leads to the formation of a 208-amino-acid prepo-endothelin peptide which is cleaved by the enzyme furin convertase to a 38-amino-acid endothelin peptide (big endothelin) and subsequently mainly by endothelin-converting enzyme to the 21-amino-acid form that circulates in the plasma.[10,11,14] ET-1 binds to two different kinds of receptors, ETA and ETB. ETA is found on smooth-muscle cells and mediates vasoconstriction via an increase in intracellular calcium.[10,11,14] ETB receptors are found both in the ETB-2 and in smooth muscle (ETB-1).[10,11,14] ETB-1 receptors produce relaxation and ETB-2 constriction.[10,11,14]

Hypoxia is an important stimulus for ET-1 production[10–12] and it has been implicated in the pathogenesis of pulmonary hypertension and cor pulmonale.[35] ET-1 is elevated in primary and secondary pulmonary hypertension[10,14,36] and in a

number of diseases including some cases of systemic hypertension (particularly among Afro-Americans), renal ischemia and congestive heart failure.[14] Also, ET-1 is a mitogen and a leucocyte attractant and promotes the formation of several growth factors like vascular endothelial growth factor.[14]

In our asthmatic patients, nocturnal oxyhemoglobin desaturation, and elevated ET-1 plasma levels during the night were not observed. Sleep apnea or oxyhemoglobin desaturation are not more frequent in asthmatics than in healthy subjects.[37] In accordance with this finding, all our asthmatic patients did not exhibit sleep apnea or oxyhemoglobin desaturation It seems that asthmatics in remission are not prone to exhibit oxyhemoglobin desaturation during sleep and ET-1 production as a consequence of this mechanism.

In our COPD patients, nocturnal oxyhemoglobin desaturation and elevated ET-1 plasma levels during sleep were observed in 19 subjects. Sleep-related hypoventilation, airway obstruction, hyperinflation, respiratory muscle dysfunction, blunted ventilatory responses to hypercapnia and/or hypoxia, ventilation–perfusion mismatching and medications can all contribute to this sleep oxygen desaturation in COPD patients, observed also in previous studies.[38–40] In agreement with previous studies, our "non-desaturators" COPD patients had lower levels of ET-1 than the "desaturators" when awake.[41,42] Our patients had chronic and severe hypoxia and elevated ET-1 levels. Chronic and severe hypoxemia may be associated with the increase in the plasma ET-1 levels in COPD patients via an increase in local production and an increment in the systemic concentration.[43] Also, the clustering of hypoxic/normoxic episodes during sleep, and not just the duration of hypoxemia, may be associated with the increase in the plasma ET-1 levels in COPD patients.[12,14]

According to our study, ET-1 levels were significantly higher in "desaturators" COPD patients than in "non-desaturators" COPD patients and in asthmatic patients during the daytime. The degree of oxyhemoglobin desaturation correlated negatively with the ET-1 levels in "desaturators" COPD patients both during daytime and sleep. Our findings are consistent with the hypothesis that ET-1 plays an important role in the pathophysiological manifestations of bronchial asthma and COPD. "Desaturators" COPD patients might be in higher risk to develop cardiovascular complications than "non-desaturators" COPD patients because of the nonapneic, oxyhemoglobin desaturation associated with sleep.

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