Eur Respir J 2001; 17: 728–732
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European Respiratory Journal
ISSN 0903-1936

Angiotensin converting enzyme in patients with sleep apnoea syndrome: plasma activity and gene polymorphisms

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ABSTRACT: The prevalence of several cardiovascular diseases is increased with obstructive sleep apnoea syndrome (OSAS), due to, as yet, unclear reasons. Angiotensin converting enzyme (ACE) abnormalities have been implicated in the pathogenesis of various cardiovascular diseases. In this study, plasma ACE activity and the distribution of an insertion (I)/deletion (D) polymorphism of the ACE gene were determined in OSAS patients and in healthy controls.

A total of 63 patients with OSAS (mean±SEM 54.5±2.5 apnoea/hypopnoeas·h^{-1}) and 32 healthy subjects were studied. To avoid potential confounding factors, patients treated with ACE inhibitors or continuous positive airway pressure were excluded, as well as controls in whom a blood sample was not obtained early in the morning. ACE activity was determined spectrophotometrically in 46 OSAS patients and 25 controls. The I/D ACE polymorphism was determined by polymerase chain reaction in 44 patients and 32 controls.

ACE activity was higher in OSAS patients (53.9±2.5 IU·L^{-1}) than in healthy controls (42.4±3.1 IU·L^{-1}, p<0.01). This was independent of the presence of arterial hypertension. The frequency distribution of the DD, II and ID genotypes in OSAS patients (30%, 16%, 54%, respectively) was not significantly different from that seen in healthy subjects (31%, 28%, 41%, respectively, p=0.356).

These results indicate that ACE plasma activity is increased in untreated OSAS patients. This increased activity may contribute to the pathogenesis of the cardiovascular disease in these patients.

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Keywords: ACE
atherosclerosis
cardiovascular diseases
genetics
sleep apnoea

Received: June 6 2000
Accepted after revision December 22 2000

This work was supported, in part, by ABEMAR, FUCAP 1995, Fondo de Investigaciones Sanitarias (FIS 96/1718), Academia de Ciencias Médicas de Cataluña y Baleares (1995) and Carburos Metálicos

The prevalence of several cardiovascular diseases, including arterial hypertension, acute myocardial infarction and stroke, is increased among patients with obstructive sleep apnoea syndrome (OSAS) [1–3]. However, the mechanisms underlying this association are unclear [4, 5].

The angiotensin converting enzyme (ACE) is a zinc metallo-peptidase, whose main functions are to convert angiotensin I into angiotensin II (a vasoactive peptide) and to inactivate bradykinin [6]. Because of its effects upon vascular tone and the formation of the atherosclerotic plaque, alterations in ACE production have been implicated in the pathogenesis of various cardiovascular diseases [6, 7]. Potential abnormalities of ACE metabolism have not been previously reported in OSAS patients. Accordingly, this investigation sought to compare the plasma ACE activity in OSAS patients with and without arterial hypertension and in healthy subjects. Likewise, because several cardiovascular disorders have been linked to the presence of an insertion (I)/deletion (D) polymorphism of the ACE gene [8–12], the prevalence of this particular genotype in these individuals was also determined.

Methods

Subjects and ethics

OSAS patients were recruited from the Sleep Unit of Hospital Universitari Son Dureta. Male subjects, younger than 65 yrs of age, with day-time somnolence and a number of apnoeas plus hypopnoeas per hour of sleep (apnoea-hypopnea index (AHI)) >20 were included in the study. AHI score was established by full polysomnography (Ultrasom Nicolett, Wisconsin, USA) which included recording of oronasal flow (thermocouples), thoraco-abdominal movements (strain gauges), electrocardiogram, submental electromyogram, electrooculogram, electroencephalogram (C_4-A_1, C_3-A_2) and transcutaneous S_{a,O_2} (Criticare Systems Inc, USA). Study patients with sarcoidosis or any other chronic disease, such as chronic obstructive pulmonary disease (COPD), diabetes mellitus, liver cirrhosis, thyroid dysfunction, rheumatoid arthritis, chronic renal failure and/or psychiatric disorders were excluded. OSAS patients were grouped according to the presence or absence of arterial hypertension. Arterial

hypertension was defined as requiring antihypertensive treatment or having a systolic blood pressure (SBP) $\geqslant$140 mmHg or a diastolic blood pressure (DBP) >90 mmHg.

As a control group, healthy, nonsmoking, non-obese volunteers matched for sex and age (±5 yrs) were studied. None had a personal or familial history of cardiovascular disease or diabetes mellitus and none were receiving any type of medication. In these individuals, OSAS was excluded clinically by the criteria of KAPUNIAI *et al.* [13]. None reported habitual snoring or excessive daytime sleepiness. All participants signed an informed consent form, after being made fully aware of the nature of the study. This investigation was approved by the Ethics Committee of the Son Dureta University Hospital.

Measurements

Blood samples were obtained by venipuncture using an antecubital vein, and were collected in tubes without anticoagulant (for ACE activity) and with ethylene/diamine tetraacetic acid (EDTA) (for deoxyribonucleic acid (DNA) analysis).

Plasma angiotensin converting enzyme activity. After sampling, tubes were immediately centrifuged (Jouan, CR422, Saint-Herblain, France) at 1,750 × *g* for 15 min after which the serum was stored at -80°C until assay. ACE activity was determined with a commercial spectrophotometric ACE assay system (SIGMA Diagnostics®, St Louis, USA), following the directions of the manufacturer. This method was based on the hydrolysis of the synthetic tripeptide substrate N-[3-(2-furyl)acrylolyl]-L-phenylalanylglycylglycine (FAPGG) to furylacryloylphenylalanine (FAP) and glycylglycine. ACE activity was determined by comparing the sample reaction rate to that obtained with the ACE reference.

Extraction and amplification of genomic DNA. DNA was extracted from leukocytes according to standard protocols [14]. Samples were preserved at -80°C until analysis. The I and D alleles were identified using polymerase chain reaction (PCR) of intron 16 (DNA fragment) of the ACE gene. PCR conditions were those published by RIGAT *et al.* [15]: 10 pmol of each primer, sense oligo 5′ CTGGAGACCACTCCCATC CTTTCT 3′ and anti-sense oligo 5′ GATGGTGG CCATCACATTCGTCAGAT 3′ in a final volume of 50 µL, containing 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 8.4, 5% dimethyl sulphoxide (DMSO), 0.5 mM of each deoxyribonucleoside triphosphate (dNTP), and 1 unit of Taq polymerase (Pharmacia. Uppsala, Sweden). The DNA was amplified for 30 cycles with denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min using a PCR Termocycler (Omnigen, Hybaid, UK). PCR products were separated by electrophoresis on 2% agarose gel and identified by ethidium bromide staining. The PCR product is a 190 base pair (bp) fragment in the absence of the insertion and a 490 bp fragment in the presence of the insertion (fig. 1).

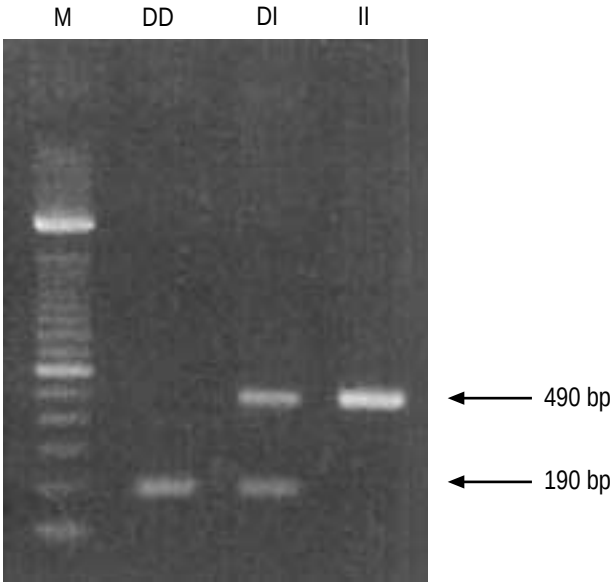

Fig. 1. – Representative agarose gel showing the polymerase chain reaction (PCR) amplified fragments of deoxyribonucleic acid (DNA) corresponding to the different angiotensin converting enzyme (ACE) genotypes (DD: 190 bp, DI: 190/490 bp, II: 490 bp). M: DNA molecular weight marker.

Statistical analysis

In the authors′ laboratory, the mean±SD value of ACE activity in healthy subjects is 40±10 $IU \cdot L^{-1}$. Taking this value into account, and accepting an α error of 0.05, a β error of 0.1, 10% missing data and the need to take into account potential confounding factors such as the presence of arterial hypertension, it was calculated that 45 patients with OSAS and 25 controls would be needed. Results are presented as mean±SEM. Because data were normally distributed (Kolgomorov-Smirnov test), parametric statistics (ANOVA, t-test) were used to assess the significance of differences for continuous variables. Genotype distributions were compared by the Chi-squared test. Correlations between variables of interest were explored using the Spearman-rank test. A p-value <0.05 was considered statistically significant.

Results

Population studied

A total of 63 OSAS patients and 32 healthy controls were studied. Table 1 shows their main clinical characteristics. OSAS patients were more obese and had higher blood pressure than control subjects. All patients had severe OSAS with a mean AHI of 54.5±2.5 h^{-1} and frequent and profound episodes of arterial desaturation (mean nocturnal Sa,O_2, 92.5±0.6%; mean lowest Sa,O_2 during sleep 63.8±3.0%). Thirty patients were smokers, 23 had systemic hypertension and 2 had had an acute myocardial infarction in the past.

Table 1. – Mean±SEM age, and body mass index (BMI) and systolic (SBP) and diastolic blood pressure (DBP) in patients with obstructive sleep apnoea syndrome (OSAS) and healthy subjects

	OSAS patients	Healthy subjects
Subjects n	63	32
Age yrs	50±1	49±1
BMI $kg \cdot m^{-2}$	32.8±0.6***	25.6±0.6
SBP mmHg	140±2***	117±2
DBP mmHg	87±1***	72±1

***: p<0.001 *versus* healthy controls.

ACE activity and genotype distribution were not investigated in all individuals. The former was determined in 46 OSAS patients and 25 controls. For this analysis, 3 patients receiving treatment with ACE inhibitors were excluded, as were 14 patients undergoing continuous positive airway pressure (CPAP) treatment and 7 control subjects (to avoid a potential circadian influence on ACE activity because their blood samples were obtained in the evening). Patients treated with antihypertensive drugs other than ACE inhibitors (n=3), histamine H_2 antagonists (n=2) and theophylline (n=1), were not excluded. On the other hand, the distribution of ACE polymorphisms was determined in 44 patients (19 samples were not analysed due to technical reasons) and 32 controls.

Angiotensin converting enzyme activity

Figure 2 shows the values of ACE activity determined in the serum of healthy controls and OSAS patients (grouped according to the presence or absence of arterial hypertension). Despite the presence of substantial scatter, ACE activity was lower in healthy subjects (42.4±3.1 $IU \cdot L^{-1}$) than in patients with OSAS (53.9±2.5 $IU \cdot L^{-1}$, p<0.01), irrespective of the presence (54.3±3.6 $IU \cdot L^{-1}$, p<0.05) or absence (53.5±3.4 $IU \cdot L^{-1}$

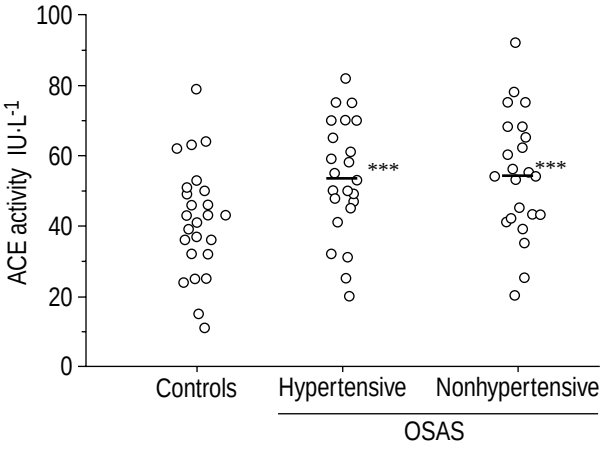

Fig. 2. – Individual and mean (bars) values of angiotensin converting enzyme (ACE) activity in control subjects and obstructive sleep apnoea syndrome (OSAS) patients, grouped according to the presence or absence of arterial hypertension. ***: p<0.001 *versus* control subjects; there was not statistically significant difference between hypertensive and nonhypertensive patients.

Table 2. – Frequency distribution of genotypes and alleles in obstructive sleep apnoea syndrome (OSAS) patients and healthy subjects

	OSAS patients	Healthy subjects
Subjects n	44	32
Genotype distribution		
Genotype II	7 (16)	9 (28)
Genotype ID	24 (54)	13 (41)
Genotype DD	13 (30)	10 (31)
Allelic distribution		
Allele I	38 (43)	31 (48)
Allele D	50 (57)	33 (52)

Data are presented as number of patients or subjects (percentage of patients or subjects).

p<0.05) of arterial hypertension. Likewise, ACE activity in OSAS patients was not influenced by the presence or absence of active smoking (54.1±3.1 $IU \cdot L^{-1}$ (n=30) *versus* 53.5±4.3 $IU \cdot L^{-1}$ (n=16), respectively; p=0.92). No significant correlation between ACE activity and several clinical or biological variables of interest was found in OSAS patients (age, r=-0.008, p=0.96; body mass index (BMI), r=0.141, p=0.36; AHI, r=-0.021, p=0.89; SBP r=-0.099, p=0.52; DBP, r=-0.088, p=0.56).

Genotype distribution and allele frequency

Table 2 shows the frequency distribution of the DD, II and ID genotypes in OSAS patients (n=44) and in healthy subjects (n=32). No significant difference between the two groups was found (p=0.356). Table 2 also presents the allelic distribution (I/D) in these individuals, which were not significantly different between OSAS patients and control subjects (p=0.293).

Discussion

To the authors' knowledge, this is the first study investigating ACE in patients with OSAS. The results indicate that: 1) compared to healthy subjects, ACE activity is significantly increased in OSAS (fig. 2); 2) these differences are independent of the presence of arterial hypertension (fig. 2) and; 3) the distribution of ACE genotypes in OSAS patients does not seem to be different from that determined in healthy subjects (table 2).

The prevalence of cardiovascular disease is higher in patients with untreated OSAS than in the general population [1, 2, 16–18]. The mechanisms underlying this association are unclear [3, 5, 19–23]. Disturbances in ACE production have been implicated in the pathogenesis of various cardiovascular diseases [6, 7]. In this study, it was hypothesized that ACE disturbances may also occur in OSAS patients. The results support this hypothesis in that ACE activity was higher in OSAS patients than in healthy subjects (fig. 2). Further, analysis excluded arterial hypertension as a potential confounding factor because differences between OSAS patients and healthy subjects remained, irrespective of its presence or absence (fig. 2). Several potential

mechanisms may contribute to explain these observations. Firstly, different genotype distributions can result in different ACE activity levels [24]. The present results seem to rule out this possibility because no different distribution or allelic frequency was found between OSAS patients and healthy subjects (table 2). However, it should be acknowledged that the size of the sample studied for this purpose may be limited. Therefore, further studies will be required to confirm or refute this possibility. Secondly, as expected [25], patients were more obese than healthy subjects (table 1). This is unlikely to explain the present findings because obesity does not seem to influence ACE activity on its own [26, 27], and also because no relationship between BMI and ACE activity was found in OSAS patients. Finally, it is conceivable that, irrespective of the particular polymorphism of the ACE gene present in a given individual, the episodes of hypoxia/reoxygenation that occur during each episode of apnoea in OSAS patients can stimulate the synthesis of ACE in endothelial cells [28, 29]. If so, a higher ACE activity would have the potential to jeopardise endothelial function and vascular structure [7] and to contribute to the increased prevalence of cardiovascular diseases reported in OSAS patients [2, 3]. However, no significant correlation between the AHI and ACE activity was found in OSAS patients. This might be explained by the narrow range of disease severity of the patients studied here (all of whom had severe OSAS). Therefore, in order to confirm or refute this potential mechanism, further studies are required to determine ACE activity in patients with less severe disease, as well as to investigate the effect of CPAP treatment on ACE activity in OSAS patients.

In summary, this study indicates that the plasma activity of angiotensin converting enzyme is increased in patients with untreated obstructive sleep apnoea syndrome. This may play a role in the pathogenesis of the cardiovascular diseases that often occur in these patients.

Acknowledgements. The authors gratefully acknowledge the patients who volunteered to participate in this study and the helpful comments and advise of Dr. Xavier Busquets (UNI).

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