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Article

The Curious Role of PAI-1 in Severe OSA

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Abstract: Plasminogen activator inhibitor-1 (PAI-1) has a significant role in fibrinolysis, atherogenesis, cellular senescence, and chronic inflammation. OSA (obstructive sleep apnea) leads to increased PAI-1 levels and the development of cardiovascular disease (CVD). The aim of this study was to determine the effects of CPAP therapy in patients with severe OSA on coagulation parameters and PAI-1. This prospective, controlled study enrolled 57 patients who were newly diagnosed with severe OSA, 37 of whom had had a good CPAP adherence after 6 months of therapy (usage of the device for at least 4 h per night), and their data were analyzed. The analysis showed a statistically significant increase in D-dimer values (415 (316.5-537.5) before CPAP therapy vs. 499 (327-652) after therapy, p = 0.0282) and a decrease in fibrinogen values (3.665 ± 0.752 before CPAP therapy vs. 3.365 ± 0.771) after therapy, p = 0.0075)). The PAI-1 concentration values before and after CPAP therapy did not differ significantly (17.35±7.01 ng/ml before CPAP therapy vs. 17.42±6.99 ng/ml after therapy, p = 0.9367). This study shows a tendency to improve the fibrinolytic capacity in patients with OSA after CPAP therapy although PAI-1 levels did not differ significantly.

Keywords: plasminogen activator inhibitor-1; obstructive sleep apnea; continuous positive airway pressure; atherogenesis; fibrinolytic capacity

1. Introduction

Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor which has a significant role in fibrinolysis as it interferes with plasminogen activation by inhibiting tissue-type PA (tPA) and urokinase-type PA (uPA) [1].

PAI-1 is synthesized in an active form, but is spontaneously converted to a latent form with a half-life of two to three hours and excreted via the liver. The concentration of circulating PAI-1 in its active form is 5-50 ng/ml with large intra- and interpersonal differences and circadian fluctuations [2].

Research conducted over the past two decades had shown that PAI-1 plays a role in regulating mechanisms and participating in processes that are not directly related to antifibrinolytic activity. These include regulation of atherosclerotic processes, neointimal hyperplasia, cell migration, myoendothelial junction, skeletal muscle injury response, insulin signaling, obesity, Alzheimer's disease, multiple sclerosis, cellular senescence, cancers, and the inflammatory response to ischemia and reperfusion [3]. Elevated PAI-1 levels have been found in patients with myocardial infarction, stroke, type 2 diabetes, insulin resistance and metabolic syndrome [4,5].

Recent research has shown that PAI-1 is not only expressed in endothelial and smooth muscle cells (vasculature), but also in skeletal muscle cells, immune cells, heart, liver, kidney, adipose tissue and some cancers. The transduction of Serpine1 (the gene encoding PAI-1) and release of PAI-1 is induced by profibrotic (TGF-β), proinflammatory (TNF-α, interleukins) and hormonal signals (insulin, IGF-1, glucagon, cortisol) [6]. In addition, the promoter region of PAI-1 has an element that is sensitive to glucocorticoids and a site that responds to the presence of aldosterone by increasing the expression of PAI-1 [4]. Reactive oxygen species (ROS) influence the transcription of the Serpine1

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gene by signaling via TGF and simultaneously stimulating activator protein-1 (AP-1), hypoxia-inducing factor-1 (HIF-1) and p53. TGF signaling also leads to a longer half-life of the mRNA for the Serpine1 gene and thus increases the translation of PAI-1 [6].

Obstructive sleep apnea (OSA) is a sleep-related breathing disorder resulting from repetitive upper airway collapse leading to intermittent hypoxemia, intrathoracic pressure fluctuations, and arousal states that result in autonomic nervous system (ANS) dysregulation, oxidative stress, hypercoagulability, and metabolic changes that promote endothelial dysfunction and ultimately lead to an increased risk of vascular events [7].

OSA leads to increased PAI-1 levels and the development of cardiovascular disease (CVD) via multiple mechanisms, including increased ROS levels, stimulation of the inflammatory milieu, concomitant insulin resistance and obesity, hypoxia, fibrosis and arterial hypertension. The increased synthesis and secretion of PAI-1 leads to endothelial dysfunction and atherogenesis via mechanisms of further stimulation of inflammation, inhibition of endothelial nitric oxide synthase (eNOS), neointimal hyperplasia, senescence and hypofibrinolysis [2].

Although patients with OSA have chronically elevated PAI-1 levels, PAI-1 as a biomarker or promoter of CVD in these patients has not been adequately studied in clinical practice. Considering that elevated PAI-1 levels are an independent risk factor for CVD and that PAI-1 levels are elevated in patients with OSA, as well as the fact that current treatment of OSA with CPAP has not resulted in a reduction in cardiovascular risk according to large studies [8], it is challenging to investigate the impact of CPAP therapy on the PAI-1 values, especially long term. Previous studies have not provided clear results on PAI-1 levels after CPAP therapy [9–11]. Could PAI-1 be the missing piece of the puzzle between OSA, CVD and CPAP therapy?

2. Materials and Methods

2.1. Patient Selection and Study Design

This study was designed as prospective, controlled and cohort-based.

All patients who were newly diagnosed with severe OSA (AHI≥30) after an overnight hospital polysomnography (PSG) at a clinic for sleep disorders during the period from July 2019 to November 2020 were included in the study if they did not meet any of the exclusion criteria (pregnancy, severe chronic obstructive pulmonary disease (COPD), severe chronic renal insufficiency, atrial fibrillation, acute heart failure, cerebrovascular insult or transient ischemic attack in the last 6 months, acute coronary syndrome in the last 6 months, chronic anticoagulant therapy). PSG was performed with the device Respironics Alice® 6: Sleepware G3 3.7.3, Philips, Murrysville, PA, USA. Results were interpreted according to American Academy of Sleep Medicine rules for scoring respiratory events [12]. All data were stored on a computer, manually scored, and evaluated by a certified sleep physician and technician. Apnea was considered as a complete cessation of breathing, i.e., a signal reduction by ≥90% for ≥10 s. Hypopnea was considered as a partial respiratory arrest, i.e., a signal reduction ≥30% lasting ≥10 s with a drop in oxygen saturation by ≥3% or arousal.

The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. All patients signed a previously explained informed consent in order to participate in the study. The first study visit was done before patients started using CPAP therapy. At the first visit patient's medical history and status were taken, as well as anthropometric measurements (height, weight, waist and neck circumference) and an Epworth sleepiness scale (ESS) questionnaire. From the 58 patients who initially entered, 57 were enrolled, i.e., did not have any of the exclusion criteria after the initial cardiovascular assessment (one had newly diagnosed atrial fibrillation). Blood sampling for laboratory analysis was carried out in the morning (between 7 and 11 am), on an empty stomach, after at least 12 hours of fasting. Samples were analyzed within 30 minutes of venipuncture, except for the samples for PAI-1 analysis, which were centrifuged (1000 revolutions/min, for 15 minutes) and frozen at a temperature of ≤ -20°C. Analysis of D-dimer was done by immunoturbidimetric analysis (BCS XP - Siemens Healthcare GmbH, Erlangen, Germany), fibrinogen levels with the modified Clauss method (BCS XP (Siemens Healthcare, Erlangen,

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Germany), PV, INR and APTT by coagulometry (BCS XP - Siemens Healthcare GmbH, Erlangen, Germany).

CPAP devices from two manufacturers were used (prisma Smart, Loewenstein Medical Technology GmbH Co. KG, Hamburg, Germany; S9 Escape, ResMed, San Diego, CA, USA). CPAP treatment was administered during hospitalization, and the titration pressure assumed to be effective in treating the majority of events was defined at the 95% percentile. The follow-up study visit was after a minimum of 6 months of CPAP device usage and included the same assessment as the first visit. Of 57 enrolled patients, 37 had a good CPAP adherence (usage of the device for at least 4 h per night, data obtained from the device's memory card) and their data were evaluated in this study. After the collection of all samples, an enzyme-linked immunosorbent assay (ELISA) Human Serpin E1/PAI-1 kit (Quantikine ELISA, Catalog Number DSE100) was performed to determine the value of PAI-1 according to the manufacturer's instructions. This study is an extension of a previously published study, using the same group of patients [13].

2.2. Statistical Analysis

The required number of subjects, determined using the paired-samples t-test with a test power of 80% and a significance level of 0.05, was 34 with an expected 25% decrease in PAI-1 after 6 months of therapy with the CPAP device compared to baseline values (analysis was performed using G*Power for Windows, version 3.1.9.2).

Normality analysis of the distribution of numerical data was performed using the Shapiro-Wilk test, and appropriate parametric and/or non-parametric statistical methods were applied depending on the results obtained. Quantitative data are represented by ranges, arithmetic means and standard deviations in the case of a parametric distribution or by medians and interquartile ranges in the case of a non-parametric distribution. Categorical data is represented by numbers (percentages). Differences in the quantitative values between the individual measurements were analyzed using the dependent t-test or the Wilcoxon test. All *p*-values less than 0.05 were taken into account. Python version 3.8 programming language was used for the analysis.

3. Results

The data of 37 patients who had satisfactory adherence, i.e. average use of the device for more than 4 hours during the night after at least 6 months of CPAP therapy, were included in the further analysis. The demographic and clinical characteristics of the subjects are listed in Table 1.

Table 1. Demographic and clinical characteristics of the study group.

Age (years)	53 ± 10
Male/female gender	29/8
BMI (kg/m^2)	34.4 ± 6.1
Smoker (number/%)	10/27
Comorbidities	
Hypertension (number/%)	19/51
CVD	1 *
COPD	2
Diabetes mellitus (number/%)	6/16
CKD	1*

BMI—body mass index, CKD—chronic kidney disease, CVD—cardiovascular disease including acute coronary syndrome, stroke/transient ischemic attack/heart failure, COPD—chronic obstructive pulmonary disease. * Only one patient had a history of heart failure and mild CKD.

The average age of the patients was 53 ± 10 years with the BMI in the obese range (average 34.4 ± 6.1 kg/m^2). The majority were male (78%), and 27% of them were smokers. The most common

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comorbidity was arterial hypertension (51%), followed by diabetes (16%). The patients generally had no previous CVD.

The polysomnographic parameters, assessment of sleepiness and the duration of CPAP therapy are listed in Table 2.

Table 2. Polysomnographic parameters, assessment of sleepiness and duration of CPAP therapy of the study group.

Polysomnographic parameters	
AHI (events/h)	58.4 ± 22
CA (events/h)	1.3
OA (events/h)	33.35
Hypopnea (events/h)	11.15
Average apnea duration (s)	24.2 ± 6.1
Minimum O^2 saturation (%)	74.03 ± 11.36
ESS	10.6 ± 5.2
Average time of CPAP therapy per night (min)	322.3 ± 51.3
Duration of CPAP therapy (days)	290.49 ± 56.7

AHI—apnea–hypopnea index, CA—central apnea, CPAP—continuous positive airway pressure, ESS—Epworth sleepiness scale, OA—obstructive apnea.

The average AHI was 58.4 ± 22. The polysomnography showed a predominantly obstructive component in the apnea episodes (OA 33.35 vs. CA 1.3). The mean duration of apnea was 24.2 ± 6.1 seconds, and the mean lowest measured oxygen saturation during sleep was 74.03 ± 11.36%. The mean Epworth Sleepiness Scale score was 10.6 ± 5.2, and the mean duration of CPAP therapy was 290.49 ± 56.7 days. The average time of CPAP therapy during the night was (322.3 ± 51.3 min) and the AHI after therapy was 4 (2.9–5).

Coagulogram and PAI-1 parameters before and after CPAP therapy are shown in Table 3.

Table 3. Coagulogram and PAI-1 parameters before and after CPAP therapy.

	Before CPAP Therapy	**After CPAP Therapy**	***p* Value**
Coagulogram			
PT	1.15 (1.08-1.22)	1.12 (1.06-1.19	0.3337
INR	0.94 (0.91-0.97)	0.98 (0.93-1)	0.0033
APTT	23.15 (21.95-24.6)	23.45 (22.5-24.85)	0.4511
D-dimer	415 (316.5-537.5)	499 (327-652)	0.0282
Fibrinogen (g/L)	3.665 ± 0.752	3.365 ± 0.771	0.0075
PAI-1			
PAI-1 (ng/mL)	17.35±7.01	17.42±6.99	0.9367
PAI-1 norm. protein	0.24±0.1	0.24±0.1	0.6638
PAI-1 norm. albumin	0.41±0.17	0.4±0.16	0.6376

APTT - activated partial thromboplastin time, CPAP - continuous positive airway pressure, INR –international normalized ratio, PAI-1 - plasminogen activator inhibitor-1, PT – prothrombin time.

The analysis of the values of the coagulation components showed a statistically significant increase in INR (0.94 (0.91-0.97) before CPAP therapy vs. 0.98 (0.93-1) after therapy, p = 0.0033) and D-dimer values (415 (316.5-537.5) before CPAP therapy vs. 499 (327-652) after therapy, p = 0.0282) and a decrease in fibrinogen values (3.665 ± 0.752 before CPAP therapy vs. 3.365 ± 0.771) after therapy, p = 0.0075)). The PAI-1 concentration values before and after CPAP therapy did not differ significantly (17.35±7.01 ng/ml before CPAP therapy vs. 17.42±6.99 ng/ml after therapy, p = 0.9367). Normalization of the PAI-1 concentration with total proteins and albumins (PAI-1 concentration/total

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proteins, i.e. PAI-1 concentration/albumin) was also performed in order to eliminate the possible impact of hypovolaemia or hypervolaemia on the results, but there was still no statistically significant difference (normalization with total proteins 0.24±0.1 before and after therapy, p = 0.6638; normalization with albumin 0.41±0.17 vs. 0.4±0.16, p = 0.6376).

4. Discussion

The link between OSA and CVD is well known and has been confirmed by numerous studies over several decades. The pathophysiological mechanisms leading to the development and increased incidence of CVD in patients with OSA involve several interrelated cascade processes. The primary event that triggers all cascade processes is a complete or partial collapse of the upper airway leading to gas exchange disturbances, changes in intrathoracic pressure and sleep fragmentation. The basis of treatment for severe OSA is the use of CPAP, which suppresses this primary event and allows unobstructed airway passage. Since the treatment eliminates the primary cause, it would be logical to assume that the treatment also reduces the risk of CVD, i.e. events. Observational studies have confirmed a statistically significant reduction in endothelial dysfunction [14] as well as a lower incidence of death, myocardial infarction and stroke in patients with OSA treated with CPAP [15]. However, randomized clinical trials, which are the gold standard of evidence-based medicine, showed no significant improvement in cardiovascular outcomes following the use of CPAP therapy **[8,16,17**]. There are several possible reasons that have led to statistically insignificant results in large randomized trials. One limitation common to all studies is treatment adherence, which may not have been sufficient to detect differences in outcomes. A meta-analysis that included 4 randomized trials with more than 3700 subjects showed a statistically significant reduction in the risk of MACE in the subgroup of subjects who used CPAP for an average of more than 4 hours during the night, suggesting that adherence to CPAP therapy is crucial for improving cardiovascular outcomes [18].

The aim of this study was to determine the effects of CPAP therapy on coagulation parameters and PAI-1. In the previously published studies comparing the concentration of PAI-1 before and after CPAP therapy, the follow-up period was relatively short, i.e. a few weeks, and this study is the first to follow the change in PAI-1 concentration over a longer period of at least 6 months **[9–11,19]**. In addition, only the results of patients who had good adherence to therapy were included in this study, as adherence to therapy was one of the main limitations in previous studies.

Several intermediary mechanisms involved in the development of CVD in OSA are closely intertwined with the regulation of the fibrinolysis system, in which PAI-1 is an important carrier. This study hypothesized at least a 25% decrease in PAI-1 levels after the use of CPAP. Statistical analysis of the data obtained showed that the difference in PAI-1 concentration before and after CPAP therapy was not statistically significant, even after values were normalized by the concentrations of total proteins and albumin. The coagulation parameters that achieved a statistically significant difference were the values of D-dimer (increase) and fibrinogen (decrease).

Phillips et al. were also unable to demonstrate a statistically significant difference in PAI-1 levels, fibrinogen and D-dimer in 28 subjects with severe OSA after two months of CPAP therapy compared to the placebo group [9]. However, another group of authors showed in a post-hoc analysis a significant decrease in PAI-1 concentration after 2 weeks of CPAP therapy in 44 subjects [11], but without a significant difference in the values of other parameters, e.g. D-dimer. The results of a study by Steffanina et al. showed a statistically significant reduction in PAI-1 levels after one month of CPAP therapy in patients with OSA compared to the control group [10].

One of the possible explanations of the results of this study may be that the short-term beneficial effect of CPAP on PAI-1 values are reversed by homeostatic factors in the long term [7].

The results of this study showed a statistically significant increase in D-dimer levels after CPAP therapy. The reduced fibrinolytic capacity in patients with OSA is partly a combination of increased levels of PAI-1 and decreased levels of D-dimer. The role of D-dimer is usually understood as a marker of fibrin formation leading to atherothrombotic events, but D-dimer levels also reflect fibrin degradation suggesting that reduced D-dimer levels could reflect a reduced fibrinolytic potential [7,20].

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The level of fibrinogen decreased statistically significantly after CPAP therapy, suggesting a possible reduction in coagulability, but also in the intensity of chronic inflammation, considering the role of fibrinogen in inflammatory events. Elevated fibrinogen levels increase the risk of thrombosis due to increased blood viscosity, which leads to slower blood flow and aggregation of erythrocytes and platelets [21]. Studies have shown that elevated fibrinogen levels are associated with the risk of developing CVD [22].

Vascular senescence, characterized by growth arrest and alteration of the gene expression profile in endothelial cells and vascular smooth muscle cells, is another pathological mechanism by which PAI-1 affects the development of CVD [23]. Thus, raising the question if PAI-1 and vascular senescence could be the answer why CPAP therapy is not improving cardiovascular outcomes. Also, senolytic PAI-1 targeted therapy may be beneficial in CVD development.

Taking into account the significant increase in D-dimer levels and the decrease in fibrinogen levels in patients with OSA after CPAP therapy, this study shows a tendency to improve fibrinolytic capacity, although PAI-1 levels did not differ significantly. The reason for this could be the multiple association of PAI-1 with different inflammatory and fibrinolytic mechanisms. Thus, the change of PAI-1 perhaps requires a longer duration of therapy. Also, due to the strong intertwining of PAI-1 with other mechanisms of the coagulation cascade and chronic inflammatory response, the acute change could be reversed by homeostatic factors in the long term. It is possible that PAI-1 plays a greater role in advanced atherosclerosis and that its levels did not decrease due to the characteristics of the patients, who were relatively young compared to the general population and had a low number of comorbidities. PAI-1 levels are different in serum and plasma, considering that a significant portion of PAI-1 is bound to platelets. Other pre-analytical factors that could have influenced the PAI-1 concentration and led to this result, but which were not taken into account, are smoking, physical activity and the fat content of the diet.

5. Conclusions

OSA leads to increased PAI-1 levels and the development of cardiovascular disease through multiple mechanism, including changes in coagulation cascade and supporting the chronic inflammatory response. This study shows a tendency to improve the fibrinolytic capacity in patients with OSA after CPAP therapy.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

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