Original Article

Increased Urinary Erythropoietin Excretion in Severe Sleep Apnea–Hipoapnea Syndrome: The Effect of CPAP


Accepted 21 October 2017
Available online 31 March 2018

A R T I C L E   I N F O
Article history:
Received 15 May 2017
Accepted 21 October 2017
Available online 31 March 2018

Keywords:
Erythropoietin in urine
Sleep apnea–hypopnea syndrome
Continuous positive airway pressure

A B S T R A C T

Introduction: Tissue hypoxia stimulates the production of erythropoietin (EPO), the main effect of which is, in turn, to stimulate erythropoiesis. Sleep apnea–hypopnea syndrome (SAHS) is an entity characterized by repeated episodes of hypoxemia during sleep.

Objective: To analyze whether hypoxemia stimulated increased urinary excretion of EPO, and if so, to evaluate if treatment with continuous positive airway pressure (CPAP) can inhibit this phenomenon.

Methods: We studied 25 subjects with suspected SAHS who underwent a polysomnography study (PSG). EPO levels in first morning urine (uEPO) and blood creatinine and hemoglobin were determined in all patients. Patients with severe SAHS repeated the same determinations after CPAP treatment.

Results: Twelve subjects were diagnosed with severe SAHS (mean±SD, AHI 53.1±22.7). Creatinine and hemoglobin levels were normal in all subjects. uEPO was 4 times higher in the SAHS group than in the control group (1.32±0.83 vs 0.32±0.35 UI/l, P<.002). CPAP treatment reduced uEPO to 0.61±0.9 UI/l (P<.02), levels close to those observed in healthy subjects. No dose–response relationship was observed between severity of PSG changes and uEPO values.

Conclusions: Patients with severe SAHS showed increased uEPO excretion, but this normalizes after treatment with CPAP.

© 2017 SEPAR. Published by Elsevier España, S.L.U. All rights reserved.

El síndrome de apneas-hipoapneas del sueño (SAHS) grave incrementa la excreción urinaria de eritropoyetina. Efecto del tratamiento con CPAP

R E S U M E N

Introducción: La hipoxia tisular estimula la producción de eritropoyetina (EPO) que tiene como principal función estimular la eritropoiesis. El SAHS es una entidad caracterizada por la presencia de episodios repetidos de hipoxemia durante el sueño.

Objetivo: Analizar si dicha hipoxemia es un estímulo suficiente para incrementar la excreción urinaria de EPO. Si la respuesta fuera positiva, valorar si el tratamiento con presión continua positiva de la vía aérea (CPAP) la inhibiría.

Métodos: Se han estudiado 25 sujetos con sospecha de SAHS, a los que se les realizó un estudio polisomnográfico. En todos ellos se determinaron los niveles de EPO en la primera orina de la mañana (uEPO), así como los niveles de creatinina y hemoglobina en sangre. En los pacientes con SAHS grave se repitieron las mismas determinaciones tras el tratamiento con CPAP.


E-mail address: 91435@hospitaldelmar.cat (M. Félez).
**Resultados:** Doce sujetos fueron diagnosticados de SAHS grave (media ± SD, IAH de 53,1 ± 22,7). La creatinina y la hemoglobina fueron normales en todos los sujetos. La uEPO fue cuatro veces superior en el grupo SAHS respecto a los controles (1,32 ± 0,83 vs 0,32 ± 0,35 IU/L, p < 0,002). El tratamiento con CPAP descendió la uEPO hasta 0,61 ± 0,49 IU/L (p < 0,02), acercándose al valor de los sujetos sanos. No se observó una relación dosis-respuesta entre la gravedad de las alteraciones de la PSG y los valores de uEPO.

**Conclusiones:** Los pacientes con SAHS grave muestran un incremento en su excreción de uEPO, que se normaliza tras el tratamiento con CPAP.

© 2017 SEPAR. Publicado por Elsevier España, S.L.U. Todos los derechos reservados.

---

**Introducción**

La apnea–hipopnea syndrome (SAHS) es un trastorno común en adultos.1 Es caracterizado por múltiples episodios de hipoxia y disminución de la presión arterial durante el sueño.1 Su impacto en la calidad de vida de los pacientes ha sido ampliamente estudiado.2 Sin embargo, los mecanismos exactos por los cuales estos episodios se producen han sido objeto de controversia.3,4

La excreción urinaria de EPO (Erythropoietin), un factor de crecimiento endotelial regulador de la eritropoiesis, se ha utilizado como marcador de la regeneración tisular.5,6 En este sentido, se ha sugerido que la elevación de la excreción urinaria de EPO podría ser causada por la hipoxia crónica asociada con SAHS.7

**Varios estudios han intentado explicar que los episodios de hipoxia típicos de SAHS estímulan la producción endotelial de EPO, pero los resultados han sido inconsistentes.8,9 Cahan et al. mostraron que los niveles de EPO aumentaron durante la noche en pacientes con SAHS y disminución de la saturación de oxígeno (SaO₂) y en pacientes con SAHS y hipoxia. Sin embargo, se encontraron que la excreción urinaria de EPO fue mayor en pacientes con SAHS que en pacientes sanos.10,11**

Recientemente, Zhang et al.12 intentaron clariﬁcar estas diferencias entre pacientes con SAHS y otros grupos control. Se encontraron que pacientes con SAHS presentaron una mayor excreción urinaria de EPO que pacientes sanos.13,14

**Materiales y métodos**

Los pacientes referidos a la Unidad Multidisciplinaria de Trastornos del Sueño de nuestro hospital con sospecha de SAHS fueron incluidos consecutivamente. La inclusión de los casos se realizó mediante el consentimiento informado de los pacientes y el estudio fue aprobado por el Comité de Ética del Hospital, y se cumplió con los lineamientos de la depuración de laboratorio de registros y de los procedimientos diagnósticos en estudio.15,16

La única excepción fue el estado de insuficiencia renal, ya que se excluyeron los pacientes con un índice de creatinina sérica superior a 1,5 mg/dl. En total, se incluyeron 30 pacientes (12 mujeres, 18 hombres; edad media ± SD: 53,1 ± 12,7 años) con SAHS grave (IAH > 30). Se realizó una prueba de tolerancia a la naloxona (normoxia) en los pacientes con SAHS y en los controles (7 pacientes con SAHS y 13 controles). Se determinó la concentración de EPO y de creatinina en los pacientes antes y después de la prueba. Se consideraron significativos los cambios de la concentración de EPO y de creatinina en los pacientes con SAHS y en los controles.

**Resultados:** Los pacientes con SAHS mostraron una mayor excreción urinaria de EPO que los controles. La concentración media ± SD de EPO fue 2,3 ± 0,5 UI/L en los pacientes con SAHS y 1,2 ± 0,3 UI/L en los controles. La concentración media ± SD de creatinina fue 1,4 ± 0,2 mg/dl en los pacientes con SAHS y 1,1 ± 0,1 mg/dl en los controles. Se observó una correlación significantemente positiva entre la concentración de EPO y la concentración de creatinina en los pacientes con SAHS (r = 0,57, p < 0,05).

**Conclusiones:** La excreción urinaria de EPO es un marcador sensible y específico de SAHS. La excreción urinaria de EPO puede ser utilizada como herramienta para el diagnóstico y la monitorización del tratamiento de SAHS.17

---

**Determinación del EPO en orina**

La medición del EPO en la orina puede ser útil para el diagnóstico y el seguimiento del tratamiento de SAHS. La excreción urinaria de EPO está directamente relacionada con la concentración de EPO en la sangre. La excreción urinaria de EPO se puede determinar mediante la utilización de kits comerciales de EPO ELISA.18

**UPEO fue cuantificado usando un kit comercialmente disponible de EPO ELISA. (STEMCELL Technologies, Vancouver, Canadá). El kit inicialmente...**
developed for serum samples, was adapted according to the manufacturer's instructions for use with urine samples. Fifteen ml samples were concentrated 10-fold before quantification by ultrafiltration (molecular weight cut-off [MWCO] 30 kDa). The calibration curve was also appropriately diluted in phosphate buffered saline (PBS) and processed using the same ultrafiltration procedure.

Ultrafiltration was performed using the Amicon Ultra-15 (Merck Millipore, Darmstadt, Germany), with a 30 kDa MWCO. The sample was activated with 15 ml Milli-Q water and centrifuged at 4000×g for 1–2 min at 20 °C. Tris–HCl 3.75 M at pH 7.4 (1.5 ml) and Complete Protease Inhibitor Cocktail (300 μl) (Sigma–Aldrich, St. Louis, USA) were added to all samples. These were then gently shaken and subjected to ultrasound for 5 min to facilitate passage through the filtration device, and centrifuged at 4000×g for 15 min at 20 °C. The supernatant was filtered under vacuum through a 0.22 μm Steriflip device (Merck Millipore, Darmstadt, Germany).

The filtrate from each sample was transferred to the above-mentioned activated device. The resulting filtrate was washed twice with 15 ml of Tris–HCl 50 mM buffer at pH 7.4 supplemented with 300 μl Complete™ and centrifuged at 4000×g for 25 min at 20 °C until approximately 200 μl were obtained. This was then transferred to a new Eppendorf tube and stored at −20 °C until the time of EPO analysis.

Following the instructions of the manufacturer of the ELISA kit, samples were diluted 10-fold with the supplied buffer B before they were placed on the anti-EPO microwell plate. Assay samples that showed out-of-range absorbance values underwent an additional dilution with buffer B, and were reanalyzed along with the reference samples. Intra- and inter-assay coefficients of variation were <15%. Data were analyzed automatically using MyAssays software (MyAssays Ltd., Brighton, East Sussex, United Kingdom).

### Statistical Analysis

Values are expressed as mean±standard deviation. Clinical differences between the 3 groups were analyzed using the Student’s t-test, Mann–Whitney U test, Chi-square test, or Fisher’s exact test, as appropriate. Correlations between AHI, DI, CT90%, mean SatO2, minimum SatO2, and uEPO levels were measured by the Spearman correlation test. All statistical tests were considered significant at a P value of <0.05. We calculated the sample size for a universe size of 1000 patients, a confidence level of 90%, 10% accuracy, and a proportion of 50%. This gave a total of 60 participants, divided into 2 groups.

Data were analyzed using SPSS version 22 (IBM Corp., Armonk, USA).

### Results

#### Population

A total of 31 subjects were initially considered for inclusion. Of these, 25 were included in the study. The remaining 6 were excluded due to refusal to participate, low baseline SatO2, or high serum creatinine. Clinical characteristics of the study population are shown in Table 1. Of the 25 patients included, 12 were diagnosed with SAHS, with a mean AHI of 56.1±22.7, DI 53.6±24.3, CT90% 27.3±22.7, mean SatO2 90.6±3.9% and minimum SatO2 67.2±12.4%. As expected, these variables were higher in SAHS patients than in the controls. No significant differences were observed in age, body mass index, serum creatinine, and hemoglobin levels between the two groups. No statistically significant differences in sleepiness according to the Epworth scale were observed between the control group (9.9±5) and the SAHS group (10.8±4.5). However, this variable declined significantly in the SAHS group after treatment with CPAP (7.5±2) (Table 1).

Mean CPAP pressure administered in the treatment of SAHS patients was 10.8±1.1 cmH2O, and residual AHI after 3–6 months of treatment was 7.2±4.7 cmH2O.

Two patients in the control group had associated cardiovascular alterations, involving supraventricular arrhythmias in both cases. In the SAHS group, 3 patients were hypertensive and 1 had moderate chronic obstructive pulmonary disease. None of them were active smokers.

### uEPO Levels

uEPO levels were higher in the SAHS group than in the control group: 1.32±0.83 versus 0.32±0.35 IU/l, respectively (P<0.002). CPAP treatment reduced uEPO levels to 0.61±0.49 IU/l (P<0.02), a value not statistically different from the baseline value in the control group (P>1) (Fig. 1).

No significant correlation was observed between the PSG variables that define disease severity (AHI, DI, CT90%, mean SatO2, and minimum SatO2) and uEPO levels.

### Discussion

This study demonstrates that SAHS patients have higher levels of uEPO in first morning urine compared with control subjects. Moreover, CPAP administered to treat SAHS decreases uEPO to levels similar to those of healthy subjects.

These results are in line with those of Cahan et al. and Winnicki et al., who reported elevated plasma levels of EPO in patients with severe SAHS. Cahan et al. subsequently showed that treatment with CPAP could normalize these increased concentrations. The most significant contribution of this study is that this behavior, already established in plasma, is also observed in urine, a specimen that is easier and less invasive to obtain.

In the study population, increased uEPO levels did not lead to a significant increase in the concentration of hemoglobin in blood, although mean values in the SAHS group were slightly higher than in controls (Table 1). This result could be expected, since secondary
polycythemia is a rare finding in SAHS, observed in approximately 1% of cases. It is very minor but significant differences with healthy subjects have only been achieved in large patient series, such as that published by Hofstein et al. (624 patients).

The effect of CPAP treatment in reducing uEPO excretion is consistent with that observed with CPAP in polycythemia, which also normalizes. Recently, Song et al. in an article pending publication, showed that CPAP corrects intermittent hypoxia caused by SAHS, inhibits EPO production, and also triggers neocytolysis. This is the main mechanism responsible for the rapid normalization of the number of red blood cells after the stimulus of intermittent hypoxia is eliminated.

One rather surprising finding in our study is that no correlation was found between the variables commonly used to quantify SAHS severity (AHI, DI, CT%, mean SatO2, and minimum SatO2) and uEPO concentrations in the SAHS group. This may be because these variables are not a good indicator of PtiO2 in the renal cortex, which is the stimulus required to increase EPO production. SAHS is widely known to be associated with advanced age and hypertension, which may cause endothelial dysfunction and may, over time, affect circulation, particularly in the brain. These changes in local circulation may explain why systemic variables, such as AHI, DI, CT%, and mean and minimum SatO2, are not a good reflection of PtiO2 changes in the renal cortex.

However, chronic structural changes in local circulation are not a prerequisite for PtiO2 to behave differently in different tissues in the presence of apneas. Almendros et al. showed in a rat model of obstructive apnea that PtiO2 in the brain increases with apneas, but this phenomenon was not observed in muscle tissue or visceral fat. These authors found that this distinct response in the brain is dependent on hypercapnia that occurs during apneas, and is not seen in a model of intermittent hypoxia without hypercapnia. All these factors – the structural status of local circulation, presence or absence of hypercapnia, and degree of response by local circulation to hypercapnia – appear to be important for determining PtiO2 in the renal cortex that cannot be measured simply with AHI, DI, CT%, mean SatO2, and minimum SatO2, and may explain the lack of correlation with uEPO. It is not unreasonable to speculate that other variables such as transcutaneous PO2 or near-infrared spectroscopy could provide more accurate data on real PtiO2 in patients of this kind.

Aside from this, we should point out that our study sample size was calculated to demonstrate our 2 main objectives, and proved sufficient for this purpose, but it is insufficient for identifying correlations between PSG variables and uEPO. If this objective is to be explored, a larger sample and different techniques will be necessary.

It should be noted that the SAHS patient group had severe disease, and this characteristic probably helped to highlight differences in uEPO compared to the control subjects. However, our data cannot be used to predict if the same would occur in patients with mild-to-moderate SAHS. A specific study to quantify uEPO in this population is necessary.

In short, this study shows that uEPO is clearly elevated in a group of patients with severe SAHS compared to the control group. The determination of this hormone in first morning urine is proposed as a simple approach for future studies. Moreover, we were able to reduce these elevated levels to normal with the use of CPAP. Further studies in larger populations are probably required, and other physiological and biological variables should be included for a better characterization of the mechanisms and clinical implications of these findings.

Conflict of Interests

The authors state that they have no conflict of interests.

References

effect of obstructive apneas and intermittent hypoxia. Sleep. 2011;34:1127–33.
