ORIGINAL ARTICLE
SLEEP MEDICINE AND SURGERY
DOI: 10.5152/B-ENT.2021.21473

# Decreased galectin-1 levels in obstructive sleep apnea: a novel biomarker

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**Cite this article as:** Düzlü M, İriz A, Cebeci S, Şemsi R, Şahin MM, Sepici Dinçel A. Decreased galectin-1 levels in obstructive sleep apnea: a novel biomarker. B-ENT 2021; 17(3): 169-73.

## ABSTRACT

**Objective:** Obstructive sleep apnea (OSA) is a common disease, which may cause oxidative stress because of intermittent apnea/hypopnea attacks occurring during sleep. In this study, we aimed to investigate the relation between oxidative stress and OSA by examining the serum levels of paraoxonase-1 (PON1), arylesterase (ARES), and oxidized low-density lipoprotein (LDL) as well as galectin-1 (gal-1) in patients with OSA.

**Methods:** A consecutive 100 patients undergoing polysomnography (PSG) between 2018 and 2019 in our sleep laboratory were enrolled in the study. Their demographic data and body mass index were recorded. On the morning after PSG, peripheral venous blood samples were collected from the fasting subjects. The patients were divided into OSA (apnea-hypopnea index [AHI] ≥ 5) and non-OSA groups (AHI < 5) according to their AHI scores. The serum levels of PON1, ARES, oxidized LDL, and gal-1 were compared between the groups.

**Results:** There were 67 (67%) men and 33 (33%) women with the mean age of 47.2 ± 11.8 years and a mean BMI of 29.8 ± 3.9. The mean AHI and oxygen desaturation indices were found to be 19.7 ± 20.7 and 19.2 ± 24.8, respectively.

No significant difference was revealed in serum PON1, ARES, and oxidized LDL levels between the OSA and non-OSA groups (PON1 p = 0.678, ARES p = 0.589, and oxidized LDL p = 0.512). Gal-1 levels were observed to be significantly downregulated in patients with OSA (p = 0.014).

**Conclusion:** No statistically significant difference was found in the serum levels of PON1, ARES, and oxidized-LDL in patients with OSA. Only gal-1 levels were found to be significantly decreased in these patients. Therefore, serum gal-1 levels can be considered a novel biomarker for OSA after further studies confirming its correlation with OSA.

**Keywords:** Arylesterase, galectin-1, obstructive sleep apnea, oxidized LDL, paraoxonase-1

## Introduction

Obstructive sleep apnea (OSA) is a common disease affecting 6% and 4% of men and women, respectively (1). It is characterized by snoring and repetitive apnea/hypopnea attacks during sleep and increased daytime sleepiness (2). Those repetitive apnea/hypopnea attacks may result in hypoxia/reoxygenation cycles, which in turn may cause oxidative stress with the formation of free or reactive oxygen species (ROS) from mitochondria. ROS is known to damage the vascular endothelium, which may lead to cardiovascular complications of OSA (3).

There are also antioxidants in the human serum maintaining a balance with oxidative stress. Antioxidant capacity was shown to be negatively correlated with OSA severity (4). Therefore, several biomarkers showing the status of oxidative stress and antioxidants were studied in patients with OSA previously (3, 5).

Paraoxonase-1 (PON1) is an antioxidant synthesized in the liver, which hydrolyzes oxidized lipids (6, 7). It is found in high density lipoproteins (HDL). Serum PON1 levels are known to decrease in oxidative stress and atherosclerosis (8). Similarly, PON1 levels were shown to be significantly decreased in patients with OSA (5, 9). Arylesterase (ARES) is coded by the same gene as PON1 (10) and has similar antioxidant effects (11).

Oxidative modifications in low density lipoprotein (LDL) are known to be responsible for atherosclerosis and coronary artery disease (12, 13). Oxidized LDL levels were also shown to be increased in patients with OSA (14, 15).

Galectin-1 (Gal-1) is a member of lectins (carbohydrate binding proteins), encoded by the lectin galactoside binding soluble 1 gene located on chromosome 22q12 (16). It shows a high affinity for binding β-galactoside containing glycans with its carbo-

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Received: June 3, 2021 Accepted: October 16, 2021
Available online at www.b-ent.be

hydrate recognition domain (17). Galectins are expressed in various cell types and known to be involved in immune reactions, inflammation, and cell growth as well as neoplastic transformation and cancer progression (18-20). In addition, galectins are known to be associated with oxidative stress (21-23).

In this study, we aimed to investigate the relation between oxidative stress and OSA disease by examining the serum levels of PON1, ARES, oxidized LDL, and gal-1 in patients with OSA.

## Methods

A total of 100 consecutive patients undergoing polysomnography (PSG) between 2018 and 2019 in our sleep laboratory were enrolled in the study after obtaining approval from Gazi University local ethics committee (ethical approval/445-11.06.2018). The study was conducted in accordance with the principles of the Helsinki Declaration. Written informed consent was obtained from all the participants. They were all administered the Epworth sleepiness scale, and their demographic data and body mass index (BMI) were recorded. Patients with coronary artery disease, congestive heart failure, chronic renal insufficiency, diabetes mellitus, and maxillofacial anomaly were excluded from the study. The patients who were included were divided into groups according to their apnea-hypopnea index (AHI) scores.

### Sleep study

Standard overnight PSG was performed on all the patients using the Noxturnal A1 system, version 2.0 (Nox Medical ehf. Katrinartuni 2 IS - 105 Reykjavík, Iceland). PSG included six electroencephalogram channels, electrooculogram, electromyograms, and position sensors. In addition to simultaneous video recording, respiratory monitoring included nasal and oral airflow measures, tracheal microphone, and thoracic and abdominal breathing efforts. At the same time, finger pulse oximetry and an electrocardiogram were performed. Evaluation of the sleep study was performed according to standard criteria recommended by the American Academy of Sleep Medicine. AHI was defined as the number of apnea and hypopnea events per sleep hour.

### Blood sampling

Peripheral venous blood samples were collected from the fasting participants on the morning after the sleep study. The serum was separated by centrifugation at 3,200 g for 10 minutes, and 500 µL of samples were aliquoted in sterile Eppendorf tubes. All the samples were snap frozen and stored at −80°C until laboratory analysis. Analysis of serum PON1 levels were performed in all the patients; however, analysis of ARES, oxidized LDL, and gal-1 levels were done for 80, 92, and 86 patients, respectively. The quantity of a few blood samples were inadequate to perform all the tests.

**Main Points:**

- Repetitive apnea/hypopnea attacks during sleep in patients with obstructive sleep apnea (OSA) is known to cause oxidative stress with the formation of free or reactive oxygen species (ROS).
- ROS is known to damage the vascular endothelium, which may cause cardiovascular complications of OSA. The relationship between oxidative stress and OSA was examined and shown in many clinical studies examining several biomarkers.
- In this study, we evaluated serum paraoxonase-1, arylesterase, and oxidized low-density lipoprotein levels that were already analyzed in previous studies on OSA. In addition, we investigated serum galectin-1 (gal-1) levels in patients with OSA for the first time. Gal-1 levels were found to be significantly decreased in these patients.

### Measurement of serum galectin-1, paraoxonase-1, arylesterase, and oxidized LDL concentrations

Specific ELISA kits were used to determine the concentrations of gal-1 (YLbiont -Catalog No. YLA3564HU, Lot: 20180117046), paraoxonase-1 (PON1-Rel Assay Diagnostics, Lot: EL17050), arylesterase (ARES-Rel Assay Diagnostics, Lot: ST18024R), and oxidized LDL (Oxidized LDL, Biomedica Gruppe Cat No: Bi-20042, Lot 063) in the serum samples. The guidelines of the manufacturers were followed thoroughly. The mean absorbance readings of the samples were compared with the standard curve concentrations. The results of gal-1 and oxidized LDL were expressed as ng/mL and the concentrations of PON1 and arylesterase as U/L.

### Statistical analysis

The Statistical Package for Social Sciences version 22.0 (IBM Corp. Armonk, NY, USA) was used for statistical analysis. The continuous variables were tested for normality with the Shapiro-Wilk test and histograms. Descriptive data were presented as mean ± standard deviation. One-way analysis of variance and independent samples t test were used to compare the groups. Correlation analysis was performed with the Pearson test. A *P* value < .05 was considered as being statistically significant.

## Results

A total of 100 consecutive patients undergoing sleep study were included in this study. There were 67 (67%) men and 33 (33%) women with a mean age of 47.2 ± 11.8 years and a mean BMI of 29.8 ± 3.9. The mean AHI and oxygen desaturation indices (ODI) were found to be 19.7 ± 20.7 and 19.2 ± 24.8, respectively. Demographic data and patient characteristics are given in Table 1. The mean AHI scores for men and women were found to be 23.7 ± 22.8 and 11.3 ±12.3, respectively (p = 0.002). The patients were divided into OSA and non-OSA groups according to their AHI results. There were 25 (25%) patients having AHI < 5 included in the non-OSA group. In addition, comparisons were repeated according to the degree of AHI (AHI < 5, AHI between 5 and 15, AHI > 15). BMI was

**Table 1. Demographic data and patient characteristics**

| | Mean | SD |
|---|---|---|
| Age | 47.2 | 11.8 |
| Sex | M: 67, F: 33 | - |
| BMI | 29.8 | 3.9 |
| AHI | 19.7 | 20.7 |
| ODI | 19.2 | 24.8 |

SD: standard deviation, M: male, F: female, BMI: body mass index, AHI: apnea-hypopnea index, ODI: oxidative desaturation index

**Table 2. Comparison of biomarkers between the OSA and non-OSA groups**

| Parameters | OSA | N | Mean | SD | p |
|---|---|---|---|---|---|
| PON1 (U/L) | - | 25 | 195.25 | 47.72 | 0.678* |
| | + | 75 | 190.46 | 50.36 | |
| | Total | 100 | 191.66 | 49.52 | |
| ARES (U/L) | - | 21 | 156.04 | 46.03 | 0.589* |
| | + | 59 | 149.30 | 49.86 | |
| | Total | 80 | 151.07 | 48.69 | |
| Ox-LDL (ng/mL) | - | 21 | 71.39 | 60.41 | 0.512* |
| | + | 71 | 83.98 | 81.23 | |
| | Total | 92 | 81.11 | 76.85 | |
| Gal-1 (ng/mL) | - | 19 | 1.71 | 0.67 | 0.014* |
| | + | 67 | 1.40 | 0.41 | |
| | Total | 86 | 1.47 | 0.49 | |

SD: standard deviation, OSA: Obstructive sleep apnea, PON1: paraoxonase 1, ARES: arylesterase, Ox-LDL: oxidized low-density lipoprotein, Gal-1: galectin 1
– : absent (AHI <5), + : present (AHI ≥5), * Independent samples t test

**Table 3. Comparison of biomarkers according to different AHI degrees**

| Parameters | AHI | N | Mean | SD | p |
|---|---|---|---|---|---|
| PON1 (U/L) | <5 | 25 | 195.25 | 47.72 | 0.868* |
| | 5–15 | 31 | 188.17 | 31.50 | |
| | ≥15 | 44 | 192.07 | 60.55 | |
| | Total | 100 | 191.66 | 49.52 | |
| ARES (U/L) | <5 | 21 | 156.04 | 46.03 | 0.823* |
| | 5–15 | 25 | 146.94 | 50.24 | |
| | ≥15 | 34 | 151.04 | 50.26 | |
| | Total | 80 | 151.07 | 48.69 | |
| Ox-LDL (ng/mL) | <5 | 21 | 71.39 | 60.41 | 0.768* |
| | 5–15 | 28 | 80.36 | 67.05 | |
| | ≥15 | 43 | 86.34 | 89.96 | |
| | Total | 92 | 81.11 | 76.85 | |
| Gal-1 (ng/mL) | <5 | 19 | 1.71 | 0.67 | 0.027* |
| | 5–15 | 27 | 1.48 | 0.29 | |
| | ≥15 | 40 | 1.35 | 0.47 | |
| | Total | 86 | 1.47 | 0.49 | |

PON1: paraoxonase-1, ARES: arylesterase, Ox-LDL: oxidized low-density lipoprotein, Gal-1: galectin-1, AHI: apnea-hypopnea index
*One-way analysis of variance test

also compared between the OSA and non-OSA groups. The mean BMIs for the OSA and non-OSA groups were found to be 30.40 ± 3.89 and 28.02 ± 3.54, respectively. There was a significant difference (p = 0.008). The mean oxidized LDL levels were found to be 83.9 ± 81.2 ng/mL and 71.4 ± 60.4 ng/mL in the OSA and non-OSA group, respectively, with no statistically significant difference (p = 0.512). Antioxidant enzymes PON1 and ARES were found to be decreased in the OSA group (Table 2) without statistical significance (p = 0.678 and p = 0.589, respectively). Gal-1 levels were significantly decreased in patients with OSA than in the non-OSA group as shown in Table 2 (p = 0.014). Similarly, gal-1 levels were significantly different between non-OSA, mild OSA, and intermediate to severe OSA groups as seen in Table 3 (p = 0.027). In general, there was a decrease in gal-1 levels as OSA severity increased (Table 3). Pearson correlation analysis was performed between AHI and gal-1 levels; however, no significant correlation was found (r = −0.088, p = 0.421, Figure 1).

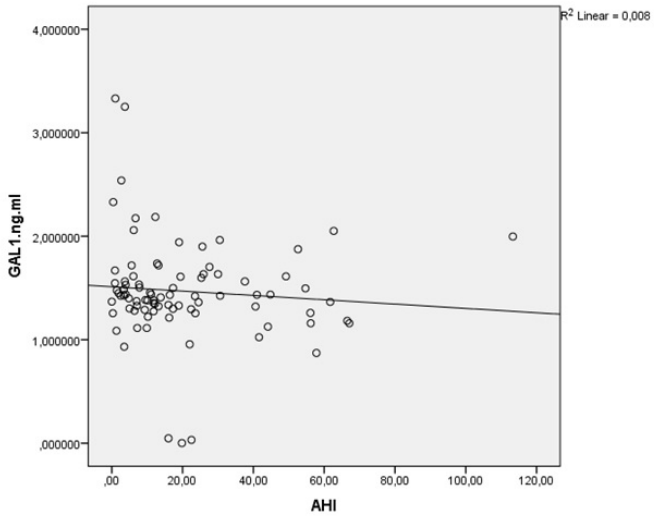


**Figure 1.** Correlation analysis graphic between the apnea-hypopnea index and galectin-1 levels

## Discussion

Repetitive apnea/hypopnea attacks during sleep are responsible for oxidative stress in patients with OSA. The association between oxidative stress, antioxidant status, and OSA as well as atherosclerotic cardiovascular disease was examined in various studies (3-5, 12, 15, 24, 25).

Oxidized LDL is a well-known biomarker for oxidative stress related to various disease statuses in humans (26). Kizawa et al. (15) examined the pathogenic role of angiotensin II and oxidized LDL levels in patients with OSA. They suggested that significantly increased serum levels of angiotensin II and oxidized LDL were responsible for endothelial injury in these patients. Similarly, serum oxidized LDL levels were found to be increased in patients with OSA compared with those in patients without OSA in our study. In addition, the increase in serum levels of oxidized LDL was compatible with increasing AHI scores. However, these findings were not statistically significant. In a meta-analysis by Fadei et al.,[14] the difference in serum oxidized LDL levels between the healthy control group and the OSA group was not statistically significant when age and BMI matching subgroups were analyzed.

PON1 and ARES proteins considered as antioxidant enzymes were previously studied in patients with OSA. Kotani et al. (25) reported decreased serum PON1 and ARES levels in patients with OSA compared with those in the non-OSA group. In addition, PON1 and ARES levels were shown to be increased after

nCPAP treatment. In a study by Baysal et al. (5), the serum levels of PON1 and ARES were shown to be significantly decreased in patients with OSA compared with those of healthy controls. We also observed a statistically non-significant decrease in the serum levels of PON1 and ARES in patients with OSA. Andaku D K and colleagues (24) reported that no significant difference in the serum levels of PON1 and ARES between OSA and control groups.

Gal-1 protein is known to be involved in immune reactions, cell growth, and neoplastic transformation. In addition, galectins are also involved in oxidative stress. Vinnai et al. (21) examined the association between oxidative stress induced galectins and human promyelocytic HL-60 cell differentiation. They observed oxidant dependent expression patterns of galectins. The anti-inflammatory and antioxidant effects of gal-1 were shown in a mouse model of ulcerative colitis (27). Gal-1 was shown to prevent lipopolysaccharide induced lung injury in mice (28). In our study, we also observed significant differentiation in gal-1 serum levels of patients with OSA. Serum gal-1 levels were observed to be significantly downregulated in these patients and also significantly associated with severity of disease; however, there was no significant correlation with AHI scores ($p > 0.05$). We believe that the antioxidant capacity of gal-1 decreased in patients with OSA exposed to increased oxidative stress.

Male sex and obesity are well-known risk factors for OSA. AHI scores of men with OSA were shown to be significantly higher than those in women with OSA in our study. The BMI of the OSA group was significantly high compared with the control group. These findings are compatible with the literature data (29).

To the best of our knowledge, this is the first study examining gal-1 levels in patients with OSA, which were significantly decreased compared with those in the non-OSA group ($p = 0.014$). In addition, gal-1 levels were observed to decrease as OSA severity increased. We believe that gal-1 exhibits an antioxidant protective effect against oxidative stress occurring during apnea/hypopnea attacks in OSA. Therefore, serum gal-1 level may be considered as a novel biomarker for OSA.

The major limitation of our study was the control group selection. Because the consecutive patients who were sent to PSG were prospectively enrolled in the study. Twenty-five patients having AHI < 5 were considered as the non-OSA group; however, comparison with healthy controls with AHI < 5 would be more valuable. In addition, biochemical analysis with the four enzymes were not available in all the patients.

No statistically significant difference was observed in the serum levels of PON1, ARES, and oxidized LDL in patients with OSA. Only gal-1 levels were found to be significantly decreased in these patients. Therefore, serum galectin-1 level can be considered as a novel biomarker for OSA. However, further studies with larger sample sizes including healthy control groups are needed to ascertain its relationship with OSA.

**Ethics Committee Approval:** This study was approved by Ethics Committee of Gazi University, Faculty of Medicine (Approval No: 445-11.06.2018.

**Informed Consent:** Written informed consent was obtained from the patients who agreed to take part in the study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – M.D., A.İ., AS.D.; Design – M.D., A.İ., S.C., R.Ş., AS.D.; Supervision – A.İ., AS.D.; Materials –M.D., A.İ., R.Ş.; Data Collection and/or Processing – M.D., A.İ., S.C., R.Ş.; Analysis and/or Interpretation – M.D., A.İ., S.C., R.Ş., MM.Ş., AS.D.; Literature Search – M.D., S.C., R.Ş.; Writing Manuscript – M.D., A.İ., S.C., R.Ş., MM.Ş., AS.D. ; Critical Review –M.D., A.İ., S.C., R.Ş., MM.Ş., AS.D.

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

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