Serum level of GDF-15 in obstructive sleep apnea syndrome

Nivelul seric al GDF-15 în sindromul de apnee obstructivă în somn

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Abstract

Background. *Growth differentiation factor-15 (GDF-15) is a stress-induced cytokine in (hypoxia, oxidative stress) that has emerged as a novel biomarker of cardiac remodeling used to assess the evolution and prognostic of metabolic and cardiovascular diseases. Obstructive sleep apnea (OSAS) patients are well known to be associated with several cardiometabolic comorbidities. We hypothesized that there is an association between sleep parameters and GDF-15 level.*
Aim. *To investigate the relationship between serum GDF-15 level and OSAS severity.*
Methods. *We enrolled 81 subjects who underwent overnight cardiorespiratory sleep study because of clinical suspicion for obstructive sleep apnea. The patients were classified according to disease severity using the apnea-hypopnea index (AHI): non-OSAS group (AHI<5; n=28), mild-moderate OSAS (AHI: 5-29.9; n=23) and severe OSAS (AHI≥30; n=30). All patients underwent detailed history and physical examination, laboratory tests and respiratory polygraphy. The correlation between clinical and paraclinical parameters was assessed.*
Results. *Serum level of GDF-15 was significantly higher in OSAS group than those in non-OSAS group (p<0.05) and increased with OSAS severity. There was a significant positive association between GDF-15 level and AHI (r2=0.34, p=0.02) and oxygen desaturation index [ODI (r2=0.37, p=0.01)]. The GDF-15 level was associated with ODI, independent of age and BMI (p<0.05). In severe OSAS group we found positive correlation between GDF-15 level and total cholesterol (r2=0.57, p=0.02), lowest oxygen saturation (r2=0.64, p=0.009), average oxygen level (r2=0.53, p=0.03) and AHI (r2=0.71, p=0.003) and a negative correlation with HDL (r2=-0.57, p=0.02).*
Conclusions. *Our findings revealed that GDF-15 levels increased with OSAS severity and correlated with ODI and lowest oxygen saturation.*
Keywords: ***GDF-15, obstructive sleep apnea, oxygen desaturation index, oxidative stress***

Rezumat

Introducere. *Factorul de creștere și diferențiere 15 (GDF-15) este o citokină produsă în condiții de stress (hipoxie, stres oxidativ), biomarker implicat în remodelarea cardiacă, cu rol prognostic în monitorizarea evoluției bolilor metabolice și cardiovasculare. Asocierea comorbidităților cardiometabolice la pacienții cu sindrom de apnee obstructivă în somn (OSAS) este bine cunoscută. Am pornit de la ipoteza că există o asociere între parametrii fiziologici înregistrați în timpul somnului și nivelul seric al GDF-15.*
Scop. *Investigarea corelației dintre nivelul seric al GDF-15 și severitatea OSAS.*
Metode. *Au fost incluși în studiu 81 de pacienți cu suspiciune de apnee obstructivă în somn, care au fost evaluați cu ajutorul poligrafiei cardiorespiratorii. Subiecții au fost împărțiți, în funcție de severitatea OSAS, în trei grupuri: grupul fără OSAS (AHI<5; n=28), grupul cu OSAS ușor-moderat (AHI: 5-29,9; n=23) și grupul cu OSAS sever (AHI≥30; n=30). Pacienții au fost evaluați anamnestic, clinic și paraclinic, prin investigații de laborator și poligrafie cardiorespiratorie. S-a investigat relația dintre parametrii clinici și paraclinici.*
Rezultate. *Nivelul seric al GDF-15 a fost semnificativ mai mare în grupul OSAS față de grupul non-OSAS (p<0,05) și a crescut odată cu severitatea OSAS. Am observat o corelație pozitivă și semnificativă statistic între nivelul GDF-15, AHI (r2=0,34, p=0,02) și indexul de desaturare [ODI (r2=0,37, p=0,01)]. Nivelul GDF-15 a fost corelat cu ODI, independent de vârstă și de IMC (p<0,05). În cazul grupului cu OSAS sever, am identificat o corelație pozitivă între nivelul GDF-15 și colesterolul total (r2=0,57, p=0,02), valorile medii ale saturației minime în oxigen (r2=0,64, p=0,009), valorile medii ale saturației medii (r2=0.53, p=0,03) și AHI (r2=0,71, p=0,003) și o corelație negativă cu HDL (r2=-0,57, p=0,02).*
Concluzii. *S-a demonstrat că nivelul seric al GDF-15 a crescut odată cu severitatea OSAS și valorile acestuia s-au corelat cu ODI și cu media saturației minime în oxigen.*
Cuvinte-cheie: ***GDF-15, apnee obstructivă în somn, index de desaturare, stres oxidativ***

Introduction

Obstructive sleep apnea syndrome (OSAS) is the most common sleep-disordered breathing, highly prevalent among the male population, inducing intermittent hypoxia and sleep fragmentation due to repeated episodes of apnea and hypopnea[1]. Over time, these lead to autonomic nervous system dysfunction with sympathetic dominance pattern, endothelial dysfunction, systemic inflammation, oxidative stress and metabolic disorders[2]. Chronic intermittent hypoxia, a distinctive feature of OSAS, along with oxidative stress, are known to have additional bidirectional interactions and are strongly associated with the development of cardiovascular diseases in OSAS patients[3,4]. Numerous studies have associated OSAS with fatal and non-fatal cardiovascular disease (ischemic heart disease, myocardial infarction, heart failure, arrhythmias, stroke)[5-9]. OSAS was defined as an independent risk factor for cardiovascular morbidity and mortality[10,11]. Growth Differentiation Factor 15 (GDF-15) is a pleiotropic protein with autocrine and paracrine regulation, member of the superfamily of transforming growth factor beta (TGF-beta) cytokines[12]. GDF-15 is expressed under stressful conditions (tissue injury, hypoxia, oxidative stress) in macrophages and many cardiovascular cells (cardiomyocytes, vascular smooth muscle cells, endothelial cells)[13]. GDF-15 is a cardioprotective cytokine due to its anti-inflammatory, anti-hypertrophic and anti-apoptotic properties[14]. High circulating levels of GDF-15 are a strong independent predictor of mortality and

prognostic for cardiometabolic diseases in patients with atherosclerosis, cardiac hypertrophy, arterial hypertension, myocardial infarction, heart failure, atrial fibrillation, stroke, insulin resistance, diabetes[13,15,16]. Recent studies have shown that GDF-15 might provide better understanding than N-terminal pro-brain natriuretic peptide levels (NT-proBNP) in patients with cardiovascular diseases[17,18]. To date, there are no studies available on the association between OSAS and GDF-15 level in general population. Only one study was found that showed similar GDF-15 levels in OSAS patients when compared them with healthy controls[19].

The aim of our study was to evaluate the association between OSAS severity during night and GDF-15 levels the morning after. We hypothesized that GDF-15 levels are influenced by OSAS severity due to additive effect of intermittent hypoxia.

Materials and methods

Study design. This is an ongoing, case-control study, conducted over a one-year period, from January 2016 to February 2017, and included Caucasian male patients referred for sleep studies because of the clinical suspicion of obstructive sleep apnea.

Study population. We recruited 81 male subjects who underwent overnight cardiorespiratory sleep study because of high clinical suspicion for obstructive sleep apnea. The patients were classified according to disease severity using the apnea-hypopnea index (AHI): non-OSAS group (AHI<5; n=28), mild-moderate OSAS (AHI: 5-29.9; n=23) and severe OSAS (AHI≥30; n=30). The subjects with history of hypo- and hyperthyroidism, chronic liver and renal disease, acute or inflammatory disorders, recent cardiovascular or neurological events (<3 months), sleep disorders other than OSAS, chronic corticosteroid use, non-steroidal anti-inflammatory, sedative or hypnotic medications and prior CPAP treatment were excluded from the study. All patients underwent detailed history and physical examination, laboratory tests and respiratory polygraphy.

Data collection. Physical examination included body mass index (BMI, kg/m^2), neck circumference (NC), waist circumference (WC) and blood pressure measurement. Three blood pressure measurements in the right arm were performed after resting in sitting position in a quiet room for at least 5 minutes at an interval of 2 minutes. The mean value of the measurements was calculated and used in the study. Obstructive sleep apnea diagnosis was established by a single overnight cardiorespiratory sleep study at the hospital using a portable multichannel device (Alice PDX; Philips Respironics) that recorded nasal and oral flow, chest and abdominal movements, oxygen saturation, heart rate, snoring and body position.

Blood sampling. Blood samples were obtained from all patients in the morning after overnight polygraphy (PG), after 12 hours of fasting for measurements of blood glucose, glycated hemoglobin (HbA1c), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complete blood count (CBC) analysis and growth differentiation factor 15 (GDF-15) levels. The monocyte count was assessed part of the routine hemogram with a reference value of 2-10%.

GDF-15 measurements. Blood samples obtained for measuring GDF-15 level used a serum separator tube and were centrifuged at 1000 g for 15 minutes and were stored at -80°C until analysis. GDF-15 was measured by ELISA method using Quantikine Human GDF-15 Immunoassay R&D System kit. GDF-15 serum levels were expressed as pg/mL.

Definitions. In order to diagnose OSAS, the respiratory events were scored per hour of recording and were classified as follows: mild OSAS (AHI: 5-14.99/h), moderate OSAS (AHI: 15-29.9/h) and severe OSAS (AHI≥30/h). Apnea was validated in the absence of respiratory airflow for at least 10 seconds. Hypopnea was reported when flow dropped≥30% from baseline for at least 10 seconds and the event was followed by a decrease in saturation by 3% (cut-off value). Oxygen desaturation index (ODI) was defined by average number of desaturation calculated per hour of recording.

Statistical analysis. Data were analyzed by using SPSS version 20.0 software. Shapiro-Wilk test was used to test for a normal distribution of continuous data. Non-normally distributed data were expressed as median and interquartile range and also transformed to natural logarithm for regression analyses. If normally distributed, the results for continuous data were presented as mean±SD and categorical data as proportions. Differences between groups were evaluated by using Student's t test and one-way ANOVA for normally distributed data, Mann-Whitney U test and Kruskal-Wallis for nonparametric variables and chi-square test for proportions. The correlation analyses were performed using Pearson and Spearman tests. Using GDF-15 values as dependent variable, regression analyses were performed in order to evaluate the correlations between clinical and paraclinical data. A p value of <0.05 was considered statistically significant.

Results

Eighty-one subjects were enrolled in the study and divided into three groups according to disease severity using the apnea-hypopnea index (AHI): non-OSAS group (AHI<5; n=28), mild-moderate OSAS (AHI: 5-29.9; n=23) and severe OSAS (AHI≥30; n=30). Demographic, clinical, paraclinical characteristics and the differences between the three groups according to OSAS severity were detailed in Table 1. Non-OSAS and OSAS groups were similar in age (52.4±1.5 versus 51.4±1, p=0.590), had different BMI (32.5±0.6 versus 36.8±1, p=0.006) and had different anthropometric features in terms of neck circumference (41.6±0.4 versus 46.6±0.7, p<0.001) and waist size (99±0.7 versus 122.8±2.4, p<0.001). High density lipoprotein cholesterol (HDL) level was markedly lower in patients with OSAS compared to non-OSAS

group (57.9±1.9 versus 39.5±0.9, p<0.001). We compared the three groups and found that subjects with increased number of respiratory events during sleep tended to have a higher BMI (32.5±0.6 versus 35.5±1.0 versus 37.8±1.5, p=0.01), lower HDL levels (57.9±1.9 versus 43.9 ± 0.9 versus 36.3±0.6, p<0.001) and increased MHR (Monocyte to High density lipo-proteins Ratio) (0.008±0.0003 versus 0.011±0.0008 versus 0.019±0.0015, p<0.001). As expected, sleep study parameters were significantly higher (p<0.0001) in severe OSAS subjects compared to non-OSAS and mild-moderate groups. The GDF-15 levels ranged from 195.2 to 3588.1 pg/mL, with a median value of 621.2 pg/mL for all study participants. Higher GDF-15 levels were observed in patients with OSAS compared to those in non-OSAS group (757.6 [745.9] pg/mL versus 421.6 [287.5] pg/mL, p=0.001). Serum GDF-15 levels were also statistically significant different between the three groups (p=0.02) (Figure 1), as it increased with OSAS severity. Pearson's and Spearman's correlation coefficients between the differences in GDF-15 concentration and the variations in all parameters were calculated for all study population, OSAS group and severe OSAS group. In all study population, significant correlation has been found between GDF-15 levels and total cholesterol (r^2=0.33, p=0.03), plasma glucose level (r^2=0.37, p=0.01), MHR (r^2=0.34, p=0.02), AHI (r^2=0.34, p=0.02) and ODI (r^2=0.37, p=0.01). Similar correlation for total cholesterol was seen in the OSAS group (r^2=0.40, p=0.03). A positive correlation was found in severe OSAS group between GDF-15 level and total cholesterol (r^2=0.57, p=0.02), lowest oxygen saturation (r^2=0.64, p=0.009), average oxygen level (r^2=0.53, p=0.03) and AHI (r^2=0.71, p=0.003). A negative correlation was found between GDF-15 level and HDL (r^2=-0.57, p=0.02). In addition, in OSAS patients, we found significant correlations between AHI and glycated hemoglobin (r^2=0.43, p=0.02). The strongest predictors of variation in GDF-15 levels for severe OSAS were the lowest arterial oxygen saturation (r^2=0.70, p=0.005) that accounted for 70% of the variation, and the average arterial oxygen saturation (r^2=0.59, p=0.02) that represented 59% of the variation. GDF-15 level did not correlate with nonspecific inflammation markers in any of the groups. The linear regression models with dependent variable GDF-15 found that the presence of ODI and lowest saturation were the strongest predictors for OSAS in men, even after adjusting for age and BMI.

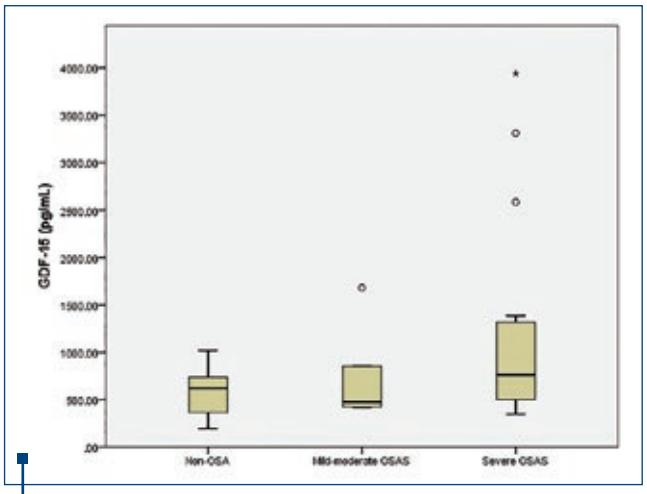

Figure 1. *The distribution of GDF-15 by AHI group. The middle horizontal line in the box is the median. The bottom and top of the box indicate the 25th and 75th percentiles. The bars outside the box are the maximum and minimum values of GDF-15*

Discussions

OSAS is known to be a major public health issue[20,21] associated with high proinflammatory state burden that adds to global cardiovascular disease risk estimated by conventional risk factors[4]. Intermittent hypoxia in OSAS enables a cascade of hemodynamic, autonomic and inflammatory events with cardiometabolic consequences[22]. Recurrent hypoxic apnea episodes trigger a change in autonomic system that allow the sympathetic nervous system to predominate and in turn to be responsible of peripheral vasoconstriction, release of inflammatory cytokines, endothelium dysfunction and oxidative stress[20]. Hypoxia-reoxygenation alternation pattern in OSAS is similar to alternating ischemia-reperfusion model, a well-known way of generating endogenous reactive oxygen species (ROS) during reperfusion periods. ROS exerts contradictory effects depending on concentration, so that in large amounts contributes to inflammation and in small amounts is involved in repairing and healing processes[21,22]. Sustained hypoxia of the cells promotes transcriptional activation mediated by hypoxia-inducible factor 1 (HIF-1) in order to increase the expression of several genes which encode proteins that promote vessel growth and erythropoiesis. This is an adaptive response to hypoxia in order to increase tissue perfusion and oxygenation so that the initial hypoxic event may be overcomed[22-24]. Physiologically, intermittent hypoxia may be beneficial using ROS to modulate the signaling pathways in order to have antioxidant effects, but severity and pattern of hypoxia, as well as individual variability may be involved in the thresholds of these adaptive or harmful mechanisms[25]. Recent experimental studies showed that chronic intermittent hypoxia and sleep fragmentation induced by OSAS may cause visceral adipose tissue inflammation and alterations characterized by adipolysis, enlarged adipocytes, microvessel rarefaction and impaired angiogenesis[26], results that might play a key role in promoting metabolic and cardiovascular diseases[27,28]. GDF-15 has emerged as a novel biomarker of cardiac remodeling[29] used to assess the evolution and prognostic of metabolic and cardiovascular diseases such as heart failure, coronary syndromes, stroke, metabolic syndrome and diabetes[30-33]. The role of GDF-15 and its regulation is not fully known in humans. However, recent studies have associated increased GDF-15 level with high inflammatory states. The fact that high concentrations of GDF-15 are found in both cachectic and

Table 1 Baseline characteristics of study population and differences between the groups according to OSAS severity

Parameter	Non-OSAS group AHI<5 (n=28)	Mild-moderate OSAS AHI: 5-29.9 (n=23)	Severe OSAS AHI≥30 (n=30)	p value
Age, years	52.4 ± 1.5	49.4 ± 1.8	52.9 ±0.9	0.44
BMI, kg/m^2	32.5 ± 0.6	35.5 ± 1	37.8 ± 1.5	0.01
NC, cm	41.6 ± 0.4	46.6 ± 1.5	46.6 ± 0.8	0.42
WC, cm	99 ± 0.7	112.7± 2	130.2 ± 2.7	0.16
History of smoking, n (%)	12 (42.8%)	12 (54.5%)	16 (53.3%)	0.68
SBP, mmHg	135.7 ± 3	144.0 ± 5.3	145.3 ± 3.8	0.18
DBP, mmHg	82.5 ± 2.9	84.5 ± 3.5	86.2 ± 2.1	0.62
HDL, mg/dL	57.9 ± 1.9	43.9 ± 0.9	36.3 ± 0.6	p<0.001
TC, mg/dL	241.6 ± 8.6	248.2 ± 9.4	249.9 ± 5.6	0.71
TG, mg/dL	160.6 (107.1)	188.7 (74.9)	193.6 (149.7)	0.20
Glucose, mg/dL	100.5 (16.5)	104.3 (23)	105.9 (23)	0.11
HbA1c, %	5.3 (0.6)	5.7 (0.7)	6.1 (0.7)	0.23
Monocyte (10^{3}µL)	0.58 ± 0.04	0.63 ± 0.05	0.66 ± 0.04	0.71
MHR	0.008 ± 0.0003	0.011 ± 0.0008	0.019 ± 0.0015	p<0.001
ESR, mm/h	15.5 ± 2.9	18.1 ± 2.4	24.3 ± 5	0.24
CRP, mg/dL	2.9 ± 0.4	5.4 ± 1.3	10.4 ± 5.2	0.27
GDF-15, pg/mL	421.6 (287.5)	740.7 (565.2)	763.2 (908.3)	0.02
AHI, events/h	3.8 (1.2)	12.3 (10.1)	44.5 (24.1)	p<0.001
ODI, events/h	4.2 (1.5)	15.1 (13.7)	50 (28.3)	p<0.001
Lowest SaO_2, %	85.7 (3.5)	82 (4)	61 (20)	p<0.001
Average SaO_2	91.5 (2.2)	87 (5)	77 (6)	p<0.001

Normally distributed data is shown as mean±standard deviation, non-parametric values are expressed as median (interquartile range) and categorical variables as number and percentage (%).

Abbreviations: BMI = Body mass index; NC = Neck circumference; WC = Waist circumference; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; HDL = High density lipoprotein cholesterol; TC= Total cholesterol; TG = Triglycerides; HbA1c = Glycated hemoglobin; MHR = Monocyte-high density lipoprotein ratio; ESR = Erythrocyte sedimentation rate; CRP = C-reactive protein; GDF-15 = Growth Differentiation Factor 15; AHI= Apnea hypopnea index; ODI = Oxygen desaturation Index, SaO_2 = Arterial oxygen saturation.

obese subjects suggests that oxidative stress may play a pivotal role in explaining its opposite effects[34,35]. It is upregulated by several cytokines and growth factors like IL1β, TNFa, IL2, MCSF, TGFβ and is a direct gene of p53 pathway[13]. Under hypoxic conditions, the p53 pathway is activated in a dependent or independent manner of HIF-1, and regulates the expression of different genes involved in senescence, apoptosis, repair and cell cycle arrest[36]. HIF-1 increases the expression of GDF-15 concentration, promoting angiogenesis, suggested by the hypothesis that GDF-15 may inhibit p53 pathway in order to modulate HIF-1 expression[37].

In the present study, we found that GDF-15 levels are detectable in OSAS subjects and increase with OSA severity defined by AHI or nocturnal oxygen desaturation indices. This dose-response relation between OSAS severity and GDF-15 level might be explained by the shared characteristics of GDF-15 levels and OSAS that increase with age, BMI, body adiposity, blood pressure and glucose levels. In our study, we did not find a significant statistical correlation between age and GDF-15 concentration. There was a significant association between ODI, the lowest oxigen saturation and GDF-15 even after adjustment for age and BMI in multivariate regression analysis, but no correlation between AHI and GDF-15 levels was found after linear regression method.

Conclusions

Our study confirms that GDF-15 levels increase with OSAS severity. The relationship between ODI, lowest oxigen saturation and morning levels of GDF-15 might be explained by intermittent hypoxia found in OSAS patients. ■

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