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Posted Date: 16 August 2023

doi: 10.20944/preprints202308.1185.v1

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Significance of Plasma Irisin, Adiponectin and Retinol Binding Protein-4 Levels as Biomarkers for Obstructive Sleep Apnea Syndrome Severity

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Abstract: Objective: Obstructive sleep apnea syndrome (OSAS) is a common sleep disorder that is caused by the reduction or cessation of airflow in the upper airway. Irisin, retinol-binding protein-4 (RBP-4), and adiponectin are the three significant factors in the metabolic process of the human body. The objective of this study was to investigate whether plasma irisin, RBP-4, and adiponectin levels are associated with the severity of OSAS. **Methods:** According to inclusion and exclusion criteria, 125 patients with OSAS and 46 healthy, gender matched controls were included to the study. The patients were classified according to the apnea hypopnea index (AHI) as 14 mild cases (5<AHI<15), 23 moderate OSAS cases (15<AHI<30) and 88 severe OSAS cases (AHI>30). The plasma irisin, RBP-4 and adiponectin levels were measured and compared between groups. **Results:** RBP-4 levels were higher in severe OSAS compared to moderate ($p<0.05$) and mild ($p<0.001$) OSAS groups, irisin levels were significantly lower compared to moderate ($p<0.05$) and mild ($p<0.001$) OSAS groups. There was a negative correlation between irisin and RBP-4 ($r=-0.379$; $p<0.001$) and AHI ($r=-0.834$; $p<0.001$) and a positive correlation with minimum oxygen saturation (MOS) ($r=0.341$; $p<0.001$) in the patient group. In the patient group, there was a negative correlation between adiponectin and BMI ($r=-0.682$; $p<0.001$), RBP-4 ($r=-0.292$; $p<0.01$) and AHI ($r=-0.179$; $p<0.05$) and a positive correlation with MOS ($r=0.397$; $p<0.001$). In the patient group, there was a positive correlation between RBP-4 and BMI ($r=0.406$; $p<0.001$) and AHI ($r=0.473$; $p<0.001$) and a negative correlation with MOS ($r=-0.805$; $p<0.001$). As a predictor of OSAS, adiponectin showed the highest specificity (84.8%) and RBP-4 the highest sensitivity (92.0%). **Conclusion:** The results of the present study demonstrated that the severity of OSAS causes more metabolic problems, including decreased plasma irisin and adiponectin levels and an increased plasma RBP-4 level. As a result, patients with an AHI score greater than thirty should be closely monitored for metabolic and cardiovascular abnormalities.

Keywords: obstructive sleep apnea; irisin; retinol-binding protein-4; adiponectin; apnea hypopnea index

Introduction

Obstructive sleep apnea syndrome (OSAS) is a common sleep disorder that is caused by the reducing or cessation of airflow in the upper airway. The symptoms of OSAS are presented by significant fragmentation of sleep, the existence of hypoxemia and hypercapnia, increased daytime sleepiness, as well as marked disruption of functionalities in numerous aspects of daily life [1]. The prevelance of OSAS is estimated around 6–13 % of among Western countries [2]. The hallmark symptoms of OSAS are daytime sleepiness, which reduces the quality of life because of disturbed sleep. There are numerous other symptoms, such as depression, headaches, memory problems, and

 doi:10.20944/preprints202308.1185.v1

concentration problems. Furthermore, OSAS is strongly linked to cardiovascular events such as hypertension (HT), rtym problems, myocardial infarctions, stroke, and sudden death [1].

Irisin is produced during exercise from myokines and released into the body's circulation. The recent consideration of irisin suggests that it is also produced in adipose tissue and is also accepted as an adipokine [3,4]. Irisin is reported to reverse diet associated obesity by promoting the adipocyte-like cells [4]. It is also well established that serum irisin level correlates negatively with body mass index (BMI) and the level of irisin is reported to be lower in individuals with obesity compared with lean individuals [5]. Although, irisin is a critical factor in obesity, there is restricted numbers of studies which investigated the irisin in patients with OSAS [3,4]. Irisin is also considered to have a place in the pathophysiology of OSAS depending on its anti-inflammation effects.

Retinol-binding protein-4 (RBP-4) is an adipokine that has been shown to affect glucose metabolism and regulate insulin resistance in peripheral tissues. [6] It has been reported that the increase in RBP-4 levels was closely associated with obesity and impaired glucose tolerance. Moreover, the level of RBP-4 was shown to decrease after regular exercise and the weight loss associated with bariatric surgery [7]. As a result, RBP-4 can be used to predict diabetes risk as well as cardiovascular events. Regarding OSAS, it can be said that the number of studies is limited in terms of exploring the association between OSAS and plasma RBP-4. There have been two previous studies that mentioned this issue and reported no significant correlations between apnea-related parameters and RBP-4 [8,9].

Adiponectin is a 30 kDa fat protein hormone that is produced in adipose tissue and secreted into the systemic circulation [10]. Adiponectin was shown to have benefits for metabolic parameters as well as the cardiovascular system. It has also been shown that adiponectin has anti-inflammatory effects [11]. Thus, adiponectin is currently considered to have protective effects against obesity-related outcomes [12]. Several studies indicated no difference in plasma adiponectin level in patients with OSAS; however, some of them reported decreased levels of adiponectin in patients with OSAS compared with individuals without OSAS [13–16].

This study was investigated whether plasma irisin, RBP-4, and adiponectin levels are associated with the severity of OSAS in patients with obesity and type 2 diabetes mellitus (type 2 DM).

Materials and Methods

Informed consent

The protocol for sample collection was approved by Acibadem Mehmet Ali Aydinlar University, Medical Faculty Ethics Committee (2016-8/32) and was carried out according to the requirements of the Declaration of Helsinki. All patients were fully informed of the study procedures before they gave their consent.

Study population

We conducted our study in a single-center and prospective study between June 2016 and May 2017 on participants who applied to our sleep laboratory for the first time and underwent sleep testing. According to the American Academy of Sleep Medicine (AASM) guidelines, 14 mild cases (5<AHI<15), 23 moderate OSAS cases (15<AHI<30) and 88 severe OSAS cases (AHI>30) were included.

The control group consisted of 46 healthy individuals who were admitted to our sleep laboratory with suspicion of OSAS and confirmed to have OSAS by polysomnography (PSG; AHI <5/h). Controls were matched according to age, body mass index (BMI) and gender. They were subjected to the same exclusion criteria as patients with OSAS.

Exclusion Criteria

Patients previously diagnosed with OSAS and using continuous positive airway pressure (CPAP) therapy were excluded from the study. We excluded patients with known cancer, chronic inflammatory disease, any systemic infection, a known acute coronary syndrome, valvular heart

 doi:10.20944/preprints202308.1185.v1

disease, thyroid, renal or hepatic dysfunction, and patients taking glucocorticoids or nonsteroidal anti-inflammatory drugs.

Polysomnography

All participants underwent PSG (Embla N 700 sleep system; Natus Medical Incorporated, Pleasanton, California) testing. At least 6 hours of PSG data were recorded. PSG recordings included 6-channel electroencephalography, 2-channel electrooculography, 2-channel submental electromyography, oxygen saturation via an oximeter finger probe, respiratory movements via chest and abdominal belts, airflow via both nasal pressure sensor and oronasal thermistor, electrocardiography, and leg movements via both tibial anterolateral electrodes.

Sleep stages were scored in 30-second periods by a registered sleep physician certified according to AASM criteria. Scoring as apnea was based on a ≥90% decrease in the respiratory signal (obtained with an oronasal thermal sensor in the diagnostic test) during sleep compared to baseline and a ≥90% signal loss lasting ≥10 seconds. In order to be scored as hypopnea, the respiratory signal during sleep (obtained with a nasal cannula in the diagnostic test) decreased by ≥30% compared to the baseline value, the ≥30% signal loss lasted for ≥10 seconds, and the pre-event basal oxygen saturation decreased by ≥3% or the event ended with arousal. The number of apneas and hypopneas per hour of sleep was calculated to obtain AHI. OSAS severity was evaluated as mild, moderate and severe according to AHI values of 5-14, 15-29 and >30, respectively.

Sample collection and measurements

Blood samples were collected, in EDTA containing tubes and anticoagulant-free tubes in the morning after 12-14 hours of fasting. After centrifugation at 2500 x g for 5 min, the plasma and serum seperated at least in 30 minutes. Each sample was separated into four aliquots and samples were stored at -80°C until biochemical analysis.

Measurement of plasma irisin levels

Plasma irisin levels were measured by competitive enzyme-linked immunosorbent assay (ELISA) method using commercially available kit (Irisin ELISA, Biovendor, Cat. No: RAG018R, Czech Republic), according to the manufacturer's directions. The coefficients of intra- and inter-assay variations were 4.2% (n=20) and 4.9.0% (n=20), respectively.

Measurements of the plasma RBP-4 levels

Plasma RBP-4 levels were measured by a sandwich ELISA kit (Human RBP4 Immunoassay, Quantikine® ELISA, Cat. No. DRB400, USA), according to the manufacturer's directions. The coefficients of intra- and inter-assay variations were 4.3% (n=20) and 5.4% (n=20), respectively.

Measurement of plasma adiponectin levels

Plasma adiponectin levels were measured by sandwich ELISA method using commercially available kit (Human Adiponectin ELISA Kit, Assaypro LLC, Cat. No. EA2500-1, USA), according to the manufacturer's directions. The coefficients of intra- and inter-assay variations were 4.5% (n=20), and 5.4% (n=20), respectively.

Statistical Analysis

Histogram, q-q plots and Shapiro-Wilk's test were applied to assess the data normality. Levene test was used to test the homogeneity of variances. To compare the differences among AHI groups, one-way analysis of variance (ANOVA) and Kruskal-Wallis H tests were performed for quantitative variables, while Chi-square analysis were performed for qualitative variables. Tukey HSD and Siegel-Castellan were applied for multiple comparisons. Pearson correlation coefficients were found to determine existence of the accepted relationship, magnitude and direction of the relationships

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between the variables. To evaluate the relationship between plasma adiponectin, irisinn and RBP-4 levels and development of OSAS, cut-off values for these parameters were determined by the receiver operating characteristic (ROC) analysis. All analyses were conducted using TURCOSA (Turcosa Analytics Ltd. Co. Turkey, http://www.turcosa.com.tr) statistical software. A *p* value less than 5% was considered as statistically significant.

Results

Comparison of General Characteristics

Height (p=0.228), gender (p=0.431), obesity (p=0.137), and mean oxygen saturation (p=0.070) did not statistically differ between groups. A significant difference was found between the mean ages of AHI groups (p<0.001). The difference in age was due to the difference between AHI<5 and AHI>30 groups. The lowest age (41.67±11.04) was observed in the control group with an AHI value less than 5, while the highest mean age (52.57±10.62) was observed in the group with an AHI value greater than 30. A statistically significant difference was found between the mean weight of the AHI groups (p<0.001). The control group with an AHI of less than 5 had the lowest mean weight (74.61±13.91), while the group with an AHI of greater than 30 had the highest mean weight (93.55±16.37). A statistically significant difference was also found between the mean body mass indexes of the AHI groups (p<0.001). This difference in body mass index was due to the AHI<5 group being different from the 15<AHI<30 and AHI>30 groups on average. The lowest mean body mass index (25.47±3.26) was observed in AHI<5 group, while the highest mean body mass index (33.32±6.53) was observed in the group with an AHI value greater than 30. While 100% of the AHI<5 and 5<AHI<15 groups did not have DM, 91.3% of the 15<AHI<30 group and 78.4% of the AHI>30 group did not have DM. There is a significant relationship between AHI groups and HT variable (p<0.001). While 100% of the AHI<5 group had no HT, 76.9% of the 5<AHI<15 group, 47.8% of the 15<AHI<30 group and 56.3% of the AHI>30 group had no HT (Table 1).

Table 1. Demographic, sleep recording variables and laboratory findings of the groups.

Variables	AHI Groups				*p*
	Control AHI<5 (n:46)	Mild OSAS (5<AHI<15) (n:14)	Moderate OSAS (15<AHI<30) (n:23)	Severe OSAS (AHI>30) (n:88)	
Age (Year)	41.67±11.04[a]	49.92±11.25[ab]	48.55±13.97[ab]	52.57±10.62[b]	**<0,001**
Height (m)	170.13±9.84	165.00±9.58	167.09±10.23	167.93±7.95	0.228
Weight (kg)	74.61±13.91[a]	80.50±8.28[ac]	91.13±19.20[bc]	93.55±16.37[b]	**<0.001**
BMI (kg/m²)	25.47±3.26[a]	29.76±4.18[ac]	32.90±8.12[bc]	33.32±6.53[bc]	**<0.001**
MOS (%)	93.00(90.00-96.75)[a]	90.00(85.00-90.00)[a]	81.00(75.00-87.00)[ab]	76.00(66.25-83.00)[b]	**<0.001**
SpO₂ (%)	95.50(92.00-97.00)	94.50(91.00-95.25)	93.00(91.00-94.00)	92.00(90.00-94.00)	0.083
Irisin (µg/ml)	3.20±0.79[a]	3.52±0.67[a]	2.96±0.23[a]	2.34±0.76[b]	**<0.001**
RBP-4 (ng/mL)	28.00±9.58[a]	34.64±5.96[ab]	42.81±11.72[b]	51.24±11.13[c]	**<0.001**
Adiponectin (µg/mL)	8.74(7.58-10.57)[a]	6.34(5.73-7.40)[b]	5.99(5.49-7.94)[b]	6.67(5.43-8.92)[b]	**<0.001**
Gender	n(%)	n(%)	n(%)	n(%)	
Female	15(32.6)	6(42.9)	7(30.4)	21(23.9)	0.431
Male	31(67.4)	8(57.1)	16(69.6)	67(76.1)	
Obesity					
No	22(47.8)	8(57.1)	8(34.8)	28(31.8)	0.137
Yes	24(52.2)	6(42.9)	15(65.2)	60(68.2)	
DM					
No	46(100.0)[a]	13(100.0)[ab]	21(91.3)[ab]	69(78.4)[b]	**0.001**
Yes	0(0.0)[a]	0(0.0)[ab]	2(8.7)[ab]	19(21.6)[b]	
HT					

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No	46(100.0)[a]	10(76.9)[b]	11(47.8)[b]	49(56.3)[b]	**<0.001**
Yes	0(0.0)[a]	3(23.1)[b]	12(52.2)[b]	38(43.7)[b]	

AHI, apnea hypopnea ındex**; MOS,** minimum oxygen saturation; **SpO_2:** oxygen saturation calculated by pulse oxymeter**; RBP-4,** retinol binding protein**; DM,** diabetes mellitus; **HT,** hypertension. *Values are shown as mean±standard deviation or median (1st quarter-3rd quarter) and n(%). p<0.05 values are shown in bold. Differences between groups in terms of mean according to multiple comparison test are indicated with different letters.*

Comparisons of plasma irisin, RBP-4 and adiponectin levels between groups

A statistically significant difference was found between the mean irisin of AHI groups (p<0.001). The mean irisin of the AHI>30 group was statistically significantly lower than the other groups (p<0.05). Irisin levels were significantly lower (p<0.001) compared to moderate (p<0.05) and mild (p<0.001) OSAS. A statistically significant difference was found between the mean adiponectin levels of AHI groups (p<0.001). AHI<5 group significantly differed from the other groups in terms of adiponectin level. There was no statistically significant difference in adiponectin levels between patient groups. The plasma RBP-4 level was found to be significantly different between groups. RBP-4 levels were higher in severe OSAS compared to moderate (p<0.05) and mild (p<0.001) OSAS group (p<0.001) (Table 1).

Correlation Analysis

Table 2 shows the correlation coefficients between the variables. The correlation coefficient between irisin and RBP-4 variables is statistically significant and shows a moderate negative relationship (r=-0.421, p<0.05). The correlation coefficient between irisin and adiponetctin variables is statistically significant and shows a weak positive relationship (r=0.240, p<0.05). The correlation coefficient between irisin and AHI variables shows a statistically significant negative strong relationship (r=-0.834, p<0.05). The correlation coefficient between irisin and BMI variables shows a statistically significant negative weak relationship (r=-0.249, p<0.05). The correlation coefficient between RBP-4 and Adiponectin variables shows a statistically significant negative and moderate relationship (r=-0.507, p<0.05). The correlation coefficient between RBP-4 and AHI variables shows a statistically significant positive moderate relationship (r=0.473, p<0.05). The correlation coefficient between RBP-4 and BMI variables shows a statistically significant positive and moderate relationship (r=0.546, p<0.05). The correlation coefficient between adiponectin and AHI variables shows a statistically significant negative weak relationship (r=-0.118, p<0.05). The correlation coefficient between adiponectin and BMI variables shows a statistically significant negative strong relationship (r=-0.777, p<0.05). The correlation coefficient between AHI and BMI variables was not statistically significant (p>0.05) (Table 2).

Table 2. Pearson correlation coefficients between plasma irisin, adiponectin, RBP-4, and BMI.

Variable	Irisin (µg/ml)	RBP-4 (ng/mL)	Adiponectin (µg/mL)	AHI/h	BMI (kg/m^2)
Irisin (µg/ml)	1	-0.421*	0.240*	-0.834*	-0.249*
RBP-4 (ng/mL)	-0.421*	1	-0.507*	0.473*	0.546*
Adiponectin (µg/mL)	0.240*	-0.507*	1	-0.118	-0.777*
AHI/h	-0.834*	0.473*	-0.118	1	0.203*
BMI (kg/m^2)	-0.249*	0.546*	-0.777*	0.203*	1

AHI, Apnea Hypopnea Index; **BMI,** Body mas index; **RBP-4**, Retinol binding protein-4**.** Values with p<0.05 are indicated with *.

ROC and regression Analysis

The results of univariate regression analysis for the possible confounding parameters (type 2 diabetes mellitus, HT, hyperlipidemia, BMI) on plasma adiponectin, RBP-4, and irisin cut-off values predicted by ROC analysis are displayed in Tables 3 and 4.

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Table 3. ROC analysis for adiponectin, RBP-4 and irisin levels of all patients for the OSAS.

Variables	AUC	(CI)	*p*	Cut of point	Sensitivity	Specificity
Adiponektin	0.826	(0.761-0.879)	<0.001	7.42	0.744	0.848
RBP-4	0.893	(0.837-0.935)	<0.001	32.34	0.920	0.673
Irisin	0.690	0.615-0.758	<0.001	3.42	0.896	0.435
BMI	0.854	0.797-0.907	<0.001	29.8	0.978	0.887

AUC, area under the curve; **CI,** confidence interval; **RBP-4**, Retinol binding protein-4**; BMI,** body mas index.

Table 4. Univariate and multiple binary logistic regression regression analysis for parameters predicted adiponectin, RBP-4, irisin, age, BMI in patients with OSAS.

Variables	Univariate Binary Logistic Regression			Multiple Binary Logistic Regression (Model Backward: Wald)		
	OR	*(CI)*	*p*	*OR*	*(CI)*	*p*
Adiponektin	0.546	(0.444-0.673)	<0.001			
RBP-4	1.202	(1.129-1.281)	<0.001	1.171	(1.097-1.249)	<0.001
Irisin	0.366	(0.224-0.596)	<0.001			
Age	1.082	(1.045-1.121)	<0.001			
BMI	1.433	(1.265-1.624)	<0.001	1.338	(1.145-1.563)	<0.001
Gender (Ref: Female)	1.295	(0.623-2.692)	0.488			

OR, odds ratio; **CI,** confidence interval; **RBP-4**, Retinol binding protein-4**; BMI,** body mas index.

Discussion

In the current study, we were able to show that plasma irisin and RBP-4 levels difference between OSAS groups classified according to AHI. The plasma adiponectin levels was found to be significantly higher in the control group; however, the adiponectin levels in the OSAS groups were comparable. As a predictor of OSAS, adiponectin showed the highest specificity (84.8%) and RBP-4 the highest sensitivity (92.0%). Furthermore, significant correlations were discovered between irisin, adiponectin, and RBP-4, as well as AHI and BMI.

Irisin is a myokine and its secretion is considered to be associated with exercise. Because it is also secreted from adipose tissue, it is also accepted as an adipokine [16,17]. Irisin is reported to increase energy without food intake in an experimental model [18]. It was also reported that irisin could reverse the obesity via effecting adipose cells [19]. The human studies also showed that irisin levels correlated negatively with BMI and its level was found to be lower in obese individuals compared with non-obese ones [20]. Regarding the association between irisin and OSAS, there are two previous studies which mentioned this issue. In a previous study, serum irisin concentration was found to be significantly lower in OSAS patients compared to the control group. It was also shown that the serum irisin level decreased more in patients with severe OSAS compared with those with mild and moderate OSAS. A significant negative correlation was found between serum irisin level and OSAS severity [4]. The second study found no significant difference in serum irisin levels between the mild-to-moderate OSAS group and the severe OSAS group. The authors concluded that the irisin-BDNF axis affected daytime sleepiness [21]. The current study's findings supported the literature in terms of a lower irisin level in severe OSAS compared to mild type. Furthermore, we discovered that plasma irisin levels were significantly and negatively correlated with plasma RBP-4 levels while positively correlated with plasma adiponectin levels. The results of the correlation analysis are the first in literature and show the interaction between plasmairisin, RBP-4 and adiponectin in OSAS patients.

Adiponectin has a key role in insulin resistance and binds to the adipose tissue of numerous organ systems. There have been several studies which investigated the adiponectin levels in patients who suffered from OSAS. In general, serum adiponectin level was reported to be lower in OSAS patients compared with healthy subjects [13,22–24]. Additionally, severity of OSAS which was

determined with AHI was reported to be associated with decreased serum levels of adiponectin [24]. A recent meta-analysis reported significantly decreased plasma/serum levels of adiponectin in OSAS patients and it was concluded that adiponectin had a potential role in the pathogenesis of OSAS [16]. In the present study, we found that the control group had significantly higher serum adiponectin levels compared with other groups. Furthermore, significant correlations were discovered between serum adiponectin levels and serum irisin and RBP-4 levels, as well as BMI. Thus, our results confirmed the previous data and showed novel findings of a correlation between serum adiponectin, irisin, and RBP-4 in patients with OSAS.

RBP-4 is primarily produced in the liver and adipose tissue and is secreted into the circulation. RBP4 is a transporter that moves retinol from the liver to peripheral tissues for the production of retinoic acid (RA). Beside its transporter duty, RBP4 causes the secretion of proinflammatory cytokines and is responsible for the activation of antigen-presenting cells in adipose tissue [25,26]. There have been strong evidences which showed that high level of RBP4 have significant roles in development of metabolic diseases and inducing oxidative stress and inflammation [27–30]. There have been limited data sets that investigated the role of RBP4 in OSAS patients. Makino and his colleagues reported that plasma RBP4 levels in moderate-to-severe OSAS patients were higher than in control subjects. They also concluded that visceral obesity was associated with higher levels of RBP4 in OSAS patients. [9]. Nena and her coworkers reported that serum RBP-4 level was not associated with OSAS-related parameters and demonstrated that serum RBP-4 level decreased under continuous positive airway pressure treatment [8]. In present study, we demonstrated that plasma RBP-4 levels were signifinatly higher in 15<AHI<30 group and AHİ>30 group compared with other groups and supports the literature. As a predictor of OSAS, adiponectin showed the highest specificity (84.8%) and RBP-4 the highest sensitivity (92.0%). Furthermore, RBP-4 levels were found to be significantly and negatively correlated with plasma irisin and adiponectin levels, and this is the novel finding of our study.

The present study has several limitations. Firstly, we did not evaluate other metabolic parameters such as serum lipid levels. This issue is considered a limitation. Secondly, the sample sizes of groups can be considered too small for drawing a general conclusion, and this is another limitation of the study.

The results of the present study demonstrated that the severity of OSAS causes more metabolic problems, including decreased plasma irisin and adiponectin, and an increased plasma RBP-4 levels. As a result, patients with moderate or severe OSAS should be closely monitored for metabolic and cardiovascular abnormalities. Additionally, this research showed that there would be significant associations between plasma irisin, adiponectin, and RBP-4 in patients with OSAS. This study will help to clarify the formation mechanism of OSAS; we think that it will provide important information in terms of both curative and preventive medicine and will facilitate the recognition of this often overlooked health problem, even if it is very common. However, more comprehensive studies are required to be able to confirm this issue.

Author Contributions: Conceptualization, N.F., P.U. and H.U.; methodology, N.F. and H.U.; software, S.D.; validation, formal analysis, N.F., P.U., S.D., S.Y., R.G. and H.U.; investigation, S.D. and R.G.; resources, N.F., P.U. and H.U.; data curation, S.D. and H.U.; writing—original draft preparation, N.F., P.U., S.Y. and H.U.; writing—review and editing, N.F., P.U., S.D., S.Y., R.G. and H.U.; visualization, N.F. and H.U.; supervision, H.U.; project administration, N.F., P.U. and H.U.; funding acquisition, N.F. and H.U.; All authors have read and agreed to the published version of the manuscript.

Funding Statement: This research received no external funding.

Institutional Review Board Statement: The protocol for sample collection was approved by Acibadem Mehmet Ali Aydinlar University, Medical Faculty Ethics Committee (2016-8/32) and was carried out according to the requirements of the Declaration of Helsinki.

Informed Consent Statement: The informed consent was obtained from all volunteer in the study.

Data Availability Statement: Participant-level data are available from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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