Clinical Factors and Biomarkers Associated with the Non-Dipping Profile in Obstructive Sleep Apnea Patients with Metabolic Syndrome

Radostina Vlaeva Cherneva 1*, Ognian Borisov Georgiev 1, Daniela Stoichkova Petrova 1, Emil Ivanov Mano 2, Adelina Dimitrova Tsakova 3 and Julia Ivanova Petrova 4

1Department of Internal Medicine, Division of Pulmonary Medicine, Medical University, Sofia, Georgi Sofiiski 1str, Sofia, 1431, Bulgaria
2Department of Internal Medicine, Division of Cardiology, Medical University, Sofia, Georgi Sofiiski 1str, Sofia, 1431, Bulgaria
3Department of Medical Chemistry and Biochemistry, Laboratory of Synthesis and Analysis of Bioactive Substances, Medical University, Sofia, Zdrave 2str, Sofia, 1431, Bulgaria
4Department of Neurology, Medical University, Sofia, Georgi Sofiiski 1str, Sofia, 1431, Bulgaria

Abstract

**Background:** Patients with obstructive sleep apnea are more likely to have a non-dipping blood pressure profile than hypertensive patients without sleep apnea. The study of clinical factors and biomarkers affecting the nocturnal dip of blood pressure is of practical value.

**Aim:** To determine the role of glucometabolic, sleep study parameters and biomarkers in the detection of dipper and non-dipper obstructive sleep apnea patients.

**Materials and methods:** A total of 87 patients participated in the study. Obstructive sleep apnea was verified by a standard polysomnography. Metabolic syndrome was diagnosed according to IDF, 2005. Anthropological parameters, glucometabolic and sleep study characteristics were assessed. An ambulatory 24-hour blood pressure monitoring was performed. Resistin and free fatty acids plasma levels were measured. Urinary 8-isoprostanes were determined.

**Results:** Nondippers did not differ significantly from dippers regarding the anthropometric measurements. From the sleep study parameters only the duration of sleep at SaO2<90% was longer in non-dippers (63.17 ± 30.32 vs 39.92 ± 34.89). Glucometabolic markers could not differentiate dippers from non-dippers, except for HbA1C (5.9 ± 0.5 vs 6.4 ± 1.05; p=0.005). The biomarkers – resistin, free fatty acids and isoprostanes were of similar range in both groups. The multivariate regression analysis, however, showed that HbA1C lost its value as an independent predictor of non-dipping after adjustment for duration of sleep at SaO2<90%.

**Conclusions:** According to our study the average duration of sleep with SaO2<90% could be of clinical value in the prediction of the non-dipping phenomenon in patients with obstructive sleep apnea and metabolic syndrome.

**Keywords:** Dipper; Nondipper; Blood pressure profile; Obstructive sleep apnea; Metabolic syndrome; Markers

**Introduction**

Blood Pressure (BP) is a haemodynamic parameter, whose precise prediction and management requires much more than a single office measurement. Thanks to ambulatory blood pressure monitoring (ABPM) it is now clear that 24-hour blood pressure shows diurnal rhythm and considerable variability, a substantial component of which may be related to changes in physical activity [1]. Most of the physiological mechanisms responsible for BP variability and the diurnal rhythm, however, are not completely understood [2].

The physiological decrease in nocturnal BP relative to daytime BP is referred to as ‘nocturnal BP dipping’. Although arbitrary, a decrease of 10–20% in nocturnal BP relative to daytime BP is considered normal despite the application of various definitions, several cross-sectional studies have revealed that cardiac morbidity and mortality are more common in non-dippers than in dippers [3]. Furthermore, certain prospective studies have shown that 5% attenuation in the nocturnal BP decline conferred a 20% increase in the risk of cardiovascular mortality in the general or hypertensive population [4]. This explains the need of a research dealing with the mechanisms and clinical parameters related to the ‘dipper’ or ‘non-dipper’ pattern of ambulatory BP profiles.

Obstructive sleep apnea (OSA) is a good pathophysiological model of the non-dipping phenomenon. A ‘non-dipping’ pattern was found in 48–84% of OSA patients and the frequency increases with its severity [5]. The nocturnal BP profile in non-dipper hypertensive patients is strikingly similar to that described in studies of 24h-BP measurements in patients with OSA [6]. OSA itself is assumed as a new cardiovascular disease, complicating both the negative consequences of sympathetic over-activation, sleep disruption, autonomic dysregulation and metabolic derangements [7]. Under such conditions the detection of non-dippers and the strict management of their blood pressure control is undoubtedly pursued.

The aim of this study was to explore the role of some clinical and biological markers, associated with the pathogenesis of OSA in the prediction of the non-dipping BP pattern.

**Patients and Methods**

**Study protocol**

During the study period (January-December, 2011) 256 patients with newly diagnosed OSA were referred to the Clinic of Internal Medicine, Division of Pulmonology, University Hospital “Alexandrovska”. Of them 142 met the inclusion criteria – metabolic syndrome (MetS) and treated hypertension. Ninety-seven patients gave informed consent to undergo

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*Corresponding author: Radostina Vlaeva Cherneva, Division of Pulmonary Medicine, Medical University, Sofia, Georgi Sofiiski 1str, Sofia, 1431, Bulgaria, Tel: 3598885129402; E-mail: cherneva_radost@yahoo.com

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ABPM. Due to insufficient number of measurements, eleven patients were excluded. The necessary number of measurements was considered according to the recommendations of the British Hypertension Society. Patients' characteristics are presented in Table 1.

The protocol of the study was approved by the Ethics Committee of the University Hospital "Alexandrovska" and all participants signed informed consent.

The definition of OSA was based on a combination of clinical symptoms (i.e. daytime excessive sleepiness) and a standard polysomnography. Metabolic syndrome was determined according to the International Diabetes Federation criteria, 2005. None of them had undergone any treatment for OSA.

After polysomnography, fasting blood samples were taken between 7 and 8 a.m. to examine plasma levels of resistin, and free fatty acids. A sample of overnight urine was taken to define the levels of urinary isoprostanes. Following admission, smoking habits, medical history and regular medication use were recorded. Anthropometric data (height, weight, waist circumference) were recorded and BMI was then calculated as body weight (kg) divided by height squared (m²).

In all subjects, an X-ray, electrocardiogram and pulmonary function test were performed. Routine blood examinations included: peripheral blood cell counts; hormones - TSH, FT3, FT4, morning and night cortisol; basic biochemistry - fasting plasma glucose, fasting immunoreactive insulin (IRI), creatinine, liver enzymes, fasting serum triglyceride, low density, very low density and high-density-lipoprotein cholesterol. Insulin resistance was calculated using the HOMA index: plasma glucose (mmol/l) x serum insulin (U/ml)/22.5.

An oral glucose tolerance test was performed to define the patients with impairments in glucose metabolism. The test was performed as described by World Health Organization (WHO) – subjects were fasting for at least 10 hours. After the sampling of fasting glucose they were loaded with 75g glucose. Blood glucose and IRI were measured within 2 hours.

Exclusion criteria were as follows: 1) age > 80 years; 2) current prescription of anti-inflammatory drugs, steroids; 3) the presence of any of the medical conditions, leading to secondary hypertension - chronic kidney diseases, reno-vascular hypertension, primary heart disease (insufficiency of the aorta, coarctation of the aorta, etc) endocrine disorders (Cushing's syndrome or disease, hyper/hypothyroidism, acromegaly, hyper/hypoparathyroidism), connective tissue diseases (panarteritis nodosa), polycythaemia, neoplasm.

**Polysomnography**

Full polysomnography was performed in all the patients (Compumedics, E-series, Australia). Continuous recordings were taken with electrode positions C3/A2-C4/A1-Cz/01 of the international 10–20 Electrode Placement System. Eye movements, chin electromyogram and ECG modified V2 lead. Sleep was scored manually according to standard criteria. Airflow was measured using nasal pressure associated with the sum of buccal and nasal thermistor signals. Respiratory efforts were monitored with abdominal and thoracic bands. Arterial oxygen saturation (SatO2) was measured using a pulse oximeter (Medair, Hudskvall, Sweden). An apnea was defined as a complete cessation of airflow for >10s, and a hypopnea as a >50% reduction in the nasal pressure signal or a 30–50% decrease, associated with either oxygen desaturation of >3% or an arousal both lasting for >10 s. Apneas were classified as obstructive, central or mixed according to the presence or absence of respiratory efforts. The classification of hypopneas as obstructive or central was based upon the shape of the inspiratory part of the nasal pressure curve. In our study, diagnosis of OSA was retained if AHI >15 h⁻¹.

**24-h Ambulatory BP Monitoring (ABPM)**

Non-invasive 24-h ABPM was performed on the non-dominant arm using BOSO TM2420/TM 2480 Profile manager (Bosh & Sohns, Germany). The device was programmed to obtain BP readings at 20-min intervals during the day (07:00–22:00 hours) and at 30-min intervals during the night (22.00–07.00 hours). The ABPM was always performed during a working day. The recording was then analysed to obtain 24-h daytime and nighttime average systolic BP (SBP), diastolic (DBP), mean arterial pressure and heart rates. When the readings exceeded at least 80% of the total readings programmed for the testing period, the recording was considered as valid and satisfactory. The nocturnal dipping was defined as a reduction in average SBP and DBP at night, which was >10% and <20%, respectively, compared with average daytime values; non-dippers had a nocturnal reduction <10%. Nocturnal hypertension was defined as SBP>120 mmHg and/or DBP>70 mmHg.
Definition of dippers and non-dippers

Night-time blood pressure was calculated assuming the measurements performed (between 22pm and 7 am). Measurements performed during the remainder of the 24h period were used for the calculation of the daytime blood pressure. The percentage decline in night-time blood pressure was calculated as follows: (average daytime blood (systolic/diastolic) pressure – average nighttime (systolic/diastolic) blood pressure/average daytime (systolic/diastolic) blood pressure. 100). Patients with a decline of less than 10% were non-dipper hypertensive patients.

Laboratory assays

Blood samples were centrifuged immediately after collection and isolated plasma was stored in vials at ~80°C until assayed. Resistin and free fatty acids were measured by commercial kits, following the procedure protocol.

Resistin was determined by an ELISA kit (RayBiocell Human Resistin ELISA Kit Protocol (Cat#:ELH-Restin-001) The intra- and interassay coefficients of variation in this assay kit ranged from 10 to 12%. Plasma resistin levels were measured in ng/ml.

The concentration of free fatty acids (FFA) was determined by an enzymatic colorimetric method assay for the quantification of non-esterified free fatty acids – (WAKO NEFA-HR2). Results are given in mmol/l.

HRAM determination of 8-isoprostane in urine samples

The levels of 8-isoprostane in urine samples were determined by HRAM (high resolution accurate mass) mass spectrometry on LTQ Orbitrap® Discovery (ThermoScientific Co, USA) mass spectrometer, equipped with Surveyor® Plus HPLC system and IonMax® electrospray ionization module. The analyses were carried out by stable isotope dilution method in negative ionization mode using HESI II (heated electrospray ionization) source type. The concentration and purification of 8-isoprostane from urine samples was processed by affinity sorbent (Cayman Chemical, USA), following the producer’s protocol with some modification. The urinary isoprostane levels were standardized to the levels of urinary creatinine. Creatinine was measured applying the enzyme method - Creatinine plus version 2 Cobas Integra (Roche). Results are given in pg/mkmol/creatinine.

Statistical Analysis

Statistical analysis was performed using SPSS (version 14.0; SPSS) P <0.05 was considered of statistical significance. Descriptive statistics is presented as means ± SD. Kolmogorov-Smirnov was used to find if normal distribution existed. Group comparison (dippers vs. non-dippers) was performed applying one-way analysis of variance (ANOVA) if normal distribution; or Mann-Whitney tests if non-parametric data was found. Correlations between paired variables were performed using Pearson (normal data) or Spearman (nonparametric data) correlation analysis. Multivariate regression analysis was performed to find the independent markers for the non-dipping pattern. The diagnostic (predictive) efficiency was studied by constructing the receiver operating characteristic (ROC) curves and calculating the area under the curve.

Results

General characteristics of the dippers and non-dippers

In the studied group 33.7% of the patients were dippers; 66.2% non-dippers. Anthropometric, haemodynamic and clinical characteristics are given in Table 1 Regarding the anthropometric parameters no large discrepancies existed between groups – Table 1. Dippers and non-dippers did not differ much when analysis of the sleep study characteristics was performed.

The cardiovascular risk factors (diabetes, dyslipidemia, obesity, and smoking) were comparable in both groups. All the patients received antihypertensive therapy. The data presented in Table 1 shows that dippers and non-dippers did not differ substantially when analysis of the antihypertensive medication in each group was performed.

Sleep study characteristics between dippers and non-dippers

The total sleep time was similar in patients with the dipping and non-dipping pattern. Except for the stage of rapid eye movement (REM) the duration of each of the sleep stages in the hypnogram did not present large discrepancies in the two groups (Table 2). The arousal index was much higher in the non-dippers but not of statistical value (36.90 ± 23.29 vs. 45.28 ± 26.29; p-0.212). Parameters, characterizing the severity of OSA were comparable (Table 2). Only the time of sleep at saturation <90% was much larger in patients with the non-dipping profile (39.92% ± 34.89% vs. 63.17% ± 30.32%; p-0.020).

Glucometabolic parameters, characterising the metabolic syndrome in OSA dippers/non-dippers

The parameters, associated with the glucometabolic characteristics of the metabolic syndrome among dippers and non-dippers are presented in Table 3. They did not differ significantly among the two groups except for the glycated haemoglobin. Though higher the average BMI and waist circumference in non-dippers were not substantially distinctive from those in dippers (Table 3). The lipid profiles were of similar range.

Biomarkers of oxidative stress and insulin resistance

Resistin plasma levels were significantly higher in non-dippers (Table 4). Similar are the results, regarding markers of oxidative stress. In dippers urinary isoprostanes showed the same ranges, as those in

<table>
<thead>
<tr>
<th>Sleep study characteristics</th>
<th>Dippers (29)</th>
<th>Non-dippers (57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild to moderate OSA</td>
<td>12/29(41.4%)</td>
<td>25/57 (43.8%)</td>
</tr>
<tr>
<td>Severe OSA</td>
<td>17/29 (58.6%)</td>
<td>32/57 (56.2%)</td>
</tr>
<tr>
<td>AH1, events/h</td>
<td>46.37 ± 25.55</td>
<td>55.37 ± 32.10</td>
</tr>
<tr>
<td>Average desaturation index</td>
<td>14.29 ± 13.53</td>
<td>13.22 ± 14.73</td>
</tr>
<tr>
<td>Sleep duration, min</td>
<td>229.96 ± 43.08</td>
<td>204.41 ± 65.22</td>
</tr>
<tr>
<td>Time with SpO2 &lt;90%, %</td>
<td>39.92 ± 34.89</td>
<td>63.17 ± 30.32</td>
</tr>
<tr>
<td>Hypnogram</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I, min</td>
<td>15.09 ± 21.19</td>
<td>14.85 ± 22.82</td>
</tr>
<tr>
<td>Stage II, min</td>
<td>42.35 ± 14.25</td>
<td>36.89 ± 17.14</td>
</tr>
<tr>
<td>Stage III, min</td>
<td>32.75 ± 21.78</td>
<td>31.53 ± 19.35</td>
</tr>
<tr>
<td>Stage IV, min</td>
<td>9.30 ± 15.56</td>
<td>8.0 ± 12.71</td>
</tr>
<tr>
<td>REM, %</td>
<td>9.95 ± 20.3</td>
<td>5.8 ± 11.96</td>
</tr>
<tr>
<td>Arousal index, events/h</td>
<td>36.90 ± 23.29</td>
<td>45.28 ± 26.29</td>
</tr>
</tbody>
</table>

Table 2: Sleep study characteristics in dippers/non-dippers.
non-dippers. Plasma levels of FFA could not be distinctive when non-dippers from dippers should be discerned.

**Clinical implication of the investigated markers**

ROC analysis was performed to analyse the clinical utility of the parameters that differed significantly between dippers and non-dippers. The ROC analysis of HbA1C and percentage of total sleep time, spent under SatO2<90% is presented in Table 5 (Figure 1 and 2).

The ROC analysis shows that HbA1C ≥5.9 % has poor clinical application - sensitivity (61%) and specificity (62%) in distinguishing non-dippers from dippers. The sleep time at SatO2<90% ≥48.25% has a sensitivity (73%) and specificity (75%) in detecting non-dippers.

The multivariate regression analysis showed that from all the markers that were studied (age, sex, BMI, waist circumference, AHI, AI, duration of nocturnal hypoxia, total sleep time, IRI, HbA1c, FFA, resistin, isoprostanes) only the duration of sleep under SatO2<90% was an independent predictor. HbA1C lost its significance after adjusting for sleep time under SatO2<90%.

**Discussion**

Although controversial the diurnal pattern of blood pressure is an important factor in determining cardiovascular complications in hypertensive patients. Impaired nocturnal blood pressure fall is encountered in 25% of essential hypertensive patients and is associated with a high risk of developing target organ damage and cardiovascular morbidity [8]. In our study we tried to analyse whether the clinical parameters, characterizing the main pathophysiological triggers in OSA patients would be useful in discerning the two groups in accordance to their circadian blood pressure variability.

**Intermittent hypoxia and non-dipping in OSA**

Despite the good knowledge about the events taking place in
OSA, little can be transferred into clinical application. Epinephrine and norepinephrine in overnight plasma or their urinary levels are quite unstable to become indicators of sympathetic activity [9]. The measurement of peripheral neural motor activity is also of little application. As intermittent hypoxia is the main trigger of the increased sympathetic activity and of the pathophysiological events that follow in OSA, biochemical markers of oxidative stress have become target for research [10]. Isoprostanes are a well validated marker of oxidative stress, responsible for cardiovascular damage [11,12]. In our study we tried to discern dippers from non-dippers, contemplating that patients with a lack of nocturnal blood pressure fall are imposed to hypoxia and oxidative stress for a longer period of time. We analysed both clinical and biochemical parameters to evaluate the role of hypoxia. From the sleep study characteristics only the time spent with a saturation <90% is significantly longer in non-dippers. These observations stay true even when the relative presence of patients with mild-to-moderate and severe apnea in each of the groups is taken in consideration. Urinary isoprostanes were almost similar in both groups. These data is in controversy to what was previously established [13,14]. Lavie et al. [15], reported that BP during sleep correlated significantly with the apnea hypopnea index. Sforza et al. [14] found a significant relationship between evening-to-morning BP difference and AHI only in men. Both studies however relate to patients free of any therapy. As our patients have already been on medication its influence on the sympathetic activity, RAAS and the generation of oxidative stress may have affected the results.

Our results show that the duration of sleep at SatO2 <90% remains a reliable and easily determined clinical parameter that may be useful for the recognition of the non-dipping pattern. It confirms the general fact that intermittent hypoxia, accompanying apneas and hypopneas, in OSA is responsible for much of the cardiovascular events. Both apneas and hypopneas provoke sympathetic overexcitation and nighttime BP surges. In addition apnoic episodes end with an arousal which further enhances the sympathetic nerve activity [15-17]. The overall effect is sympathetic nervous system stimulation that remains not only during the night, but also at daytime. The BP profile is changed to higher daytime and nighttime BP values with a loss of the physiological nocturnal dip. The longer the periods of hypoxia, the continuous the sympathetic nervous system excitation and BP dysregulation.

Sleep quality and non-dipping in OSA

Blood pressure changes during sleep are closely associated with sleep quality. Sleep disturbance and sleep deprivation are described as independent factors, responsible for the non-dipping phenomenon. Degree of nighttime activity, measured by actigraphy or various sleep quality scales were usually applied. In healthy volunteers, non-dippers have been reported to have greater sleep activity [18]. In hypertensives, however, actigraphy, alone has no relationship with the dipping pattern. It is generally assumed that in hypertensive non-dippers sleep quality is better determined by Electroencephalography (EEG) [19]. We could not find statistically significant difference between dippers and non-dippers in regards to the total duration of sleep or the separate sleep stages (Table 2) except for the REM stage. Arousals are provoked by apneas and hypopneas, which usually take place in REM, that may be the explanation for the shorter duration of the REM in OSA non-dippers. Our findings are similar to those of Loredo et al. [9] who also could not establish a significant association between sleep quality and dipping.

Glucometabolic derangements and non-dipping in OSA

From the view point of the metabolic syndrome, arterial hypertension is an insulin resistant state. In MetS chronic hyperinsulinemia often precedes the clinical presentation of impaired glucose tolerance and overt diabetes. Simultaneously it causes vascular smooth muscle proliferation and impaired vascular relaxation - underlying mechanisms for prehypertension and non-dipping pattern. In our study of OSA patients with MetS no large discrepancies existed between dippers and non-dippers, when assessing the presence of the components of the MetS. The only glucometabolic parameter that was of significance, while discerning dippers from non-dippers in OSA was HbA1C. This stays true after adjusting for BMI, waist circumference and weight and corresponds to the findings of Lammertyn et al. [20]. They reported that elevated levels of HbA1C correspond to the lack of nocturnal dip and correlate positively to nighttime (00:00- 04:00) SBP. The association between the non-dipping pattern and HbA1C confirms the fact that a moderate increase in the HbA1C level can increase the risk for cardiovascular disease even when fasting blood glucose is below the diabetic threshold [21]. Thus the prevention of cardiovascular diseases in OSA patients imposes the performance of an oral glucose tolerance test and the early detection of impaired glucose metabolism.

According to our results chronic hyperglycaemia, determined by the elevated plasma levels of HbA1C is not only an indicator of impaired glucose metabolism, but was also significantly higher in non-dipping OSA patients. This however lost its importance after adjusting for confounders, especially for duration of sleep at SatO2<90% . This may be due to the fact that intermittent hypoxia and sympathetic activation lead to elevated blood glucose in OSA patients, especially at night. As an indicator for chronic hyperglycaemia HbA1c may be a mediator rather than a marker of the non-dipping phenomenon. It is an advanced-stage glycedated endproduct that may contribute to smooth muscle proliferation, impaired vessel wall relaxation and fixed BP values. In regards to this the duration of sleep at SatO2<90% remains the major marker of the non-dipping phenomenon in OSA that provokes sympathetic nervous system stimulation and all the glucometabolic and haemodynamic consequences that follow.

Adipokines and non-dipping in OSA

Intermittent hypoxia, that follows the apneas and hypopneas enhances the local hypoxia in adipose tissue and leads to adipose tissue dysfunction in a greater degree than in the general population. It is therefore assumed that in OSA obesity, the imbalanced secretion of different adipokines is much important for both metabolic and cardiovascular derangements [22]. Resistin has been implicated in the development of insulin resistance and hypertension. Several small studies have reported that circulating resistin levels are increased in human obesity and diabetes, although not all reports have been consistent [23,24]. Papadopouls et al. [25] established that in prehypertension [25] and masked hypertension [26] resistin plasma levels are much higher and could be of clinical implication. We could not find an association between resistin plasma levels and the non-dipping pattern. Before taking this into practice it should emphasize that our patients are much more obese than those of Papadopoulus (average BMI - 37 vs. BMI-23). It can be then speculated that the secretion of resistin may be blunted in extreme obesity which deter the detection of the slight difference in resistin plasma levels between OSA dippers/non-dippers, if any.

Limitations

The limitations of our study are that: 1) our patients were very obese, which complicates the applicability of data to the general OSA population; 2) they were predominantly men and results cannot be translated to women with OSA; 3) ABPM was performed once and does not allow the reproducibility of the results; 4) patients were already on...
antihypertensive medication, that might have influenced the results; 5) last but not least we performed the relatively small number of subjects with various comorbidities does not allow the establishment of any causality, but only the propositions of certain hypothesis.

Conclusions

In OSA patients with MetS the duration of hypoxaemia at night may be predictive of the non-dipping pattern. The average duration of sleep at SaO2<90% could be of clinical value in the stratification of patients, suitable for ABPM performance.

References