Obstructive Sleep Apnea in Acute Stroke
A Role for Systemic Inflammation

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Background and Purpose—Sleep-disordered breathing is common among patients with stroke resulting in 4- to 6-fold higher prevalence of obstructive sleep apnea (OSA). We prospectively evaluated clinical characteristics and laboratory markers of inflammation and coagulability associated with OSA severity during the acute post stroke period.

Methods—Consecutive patients admitted to the department of Neurology after an acute ischemic stroke were evaluated during the first 48 hours of symptom onset using Watch peripheral arterial tonometry, a wrist-worn ambulatory sleep study device that utilizes peripheral arterial tonometry. Morning blood samples of the patient were tested for tumor necrosis factor, interleukin-6, and plasminogen activator inhibitor-1 levels.

Results—A total of 43 patients with acute stroke were admitted during the study period, 22 (51%) of which have been found to have moderate sleep apnea (apnea hypopnea index [AHI]≥15), AHI≥5 was found in 86% of the patients, and severe OSA (AHI≥30) in 32.5%. Patients with OSA (AHI≥15) did not differ from the rest in stroke severity or symptoms, yet they had higher prevalence of recurrent stroke and atrial fibrillation. All 3 biomarkers levels were higher among patients with AHI≥15: tumor necrosis factor (6.39 versus 3.57 pg/mL), interleukin-6 (6.64 versus 3.14 pg/mL), and plasminogen activator inhibitor-1 (17.64 versus 98.48 pg/mL). After the stratification of AHI into 3 groups (AHI<5, 5–14, and ≥15), the analysis showed that only the highest AHI group differed from the other 2 groups in biomarkers levels.

Conclusions—Use of bed-side somnography technology revealed that in an unselected sample of patients with acute ischemic stroke, almost 90% had sleep-disordered breathing with third having severe form of the disorder. Sleep-disordered breathing was associated with significantly increased levels of inflammatory biomarkers, providing possible pathophysiological explanation of OSA-associated stroke risk. These results warrant prospective screening of patients with stroke for the presence of sleep-disordered breathing and lay the rationale for an interventional trial. (Stroke. 2016;47:1207-1212. DOI: 10.1161/STROKEAHA.115.011749.)

Key Words: apnea ■ emergency department ■ ischemia ■ stroke ■ tumor necrosis factor

Stroke, a leading cause of death and disability worldwide, requires effective therapeutic measures to ameliorate its clinical and socioeconomic burden. Increased knowledge of the pathogenetic mechanisms involved in cerebral ischemia demonstrates that therapies should be administered early and targeted at establishing reperfusion and inhibiting damage mediators.

Sleep-disordered breathing (SDB) is a wide spectrum of sleep-related respiratory abnormalities including both central and obstructive forms. The most common form of SDB, obstructive sleep apnea (OSA), is an independent risk factor for stroke. The exact mechanism remains unknown, the primary reason described in the literature is: episodes of obstructive apnea can cause intermittent hypoxemia. This can lead to surges in blood pressure because of sympathetic activation and release of vasoconstrictive substances, such as endothelin.

SDB is common among patients with stroke, who have a 4- to 6-fold higher prevalence of OSA. In the acute post stroke period, patients with OSA have a greater functional impairment and higher mortality rates than patients without OSA. The use of continuous positive airway pressure treatment is associated with decreased mortality and better functional recovery after stroke. Recently, treatment of SDB during the acute post stroke period was suggested as an additional measure for improving stroke outcome. However, the mechanisms in which SDB affect stroke outcome are not clear, and SDB screening and treatment is not yet a routine practice. Early detection and treatment of OSA may benefit patient’s life, and this early management of OSA may lead to fewer cardiovascular events.

In this prospective study, we evaluated clinical characteristics and laboratory markers of inflammation and coagulability...
associated with OSA severity during the acute post stroke period. We hypothesized that patients with stroke have high prevalence of OSA associated with increased levels of inflammatory cytokines.

Methods

Study Population

The study was conducted at Soroka University Medical Center, a tertiary 1000 beds teaching hospital providing acute neurological care to the population of Southern Israel (700,000). Annual volume of acute stroke admissions to the hospital is ≈500 patients.

We enrolled all consecutive patients admitted to the department of Neurology after an acute ischemic stroke within 24 hours from the hospitalization. We excluded subjects with previous diagnosis of SDB, <18 years of age, or those who are unable to give an informed consent. Patients with chronic pulmonary disorders were excluded as well to avoid possible misinterpretation of somnographic studies. The study was approved by the Soroka Medical Center Internal Review Board.

Clinical Evaluation

All the patients were evaluated and managed according to the American Heart Association guidelines. \(^1\) The clinical data were extracted from the patients’ records and included functional status before the acute event (modified Rankin score), stroke symptoms and stroke severity (National Institutes of Health Stroke Scale [NIHSS]) on admission and discharge, medical history, medical treatment before the stroke, and noncontrast computed tomographic scan. The NIHSS is a systematic assessment tool that provides a quantitative measure of stroke-related neurological deficit. \(^8\)

After enrollment, the patients were also evaluated using the Epworth sleepiness scale \(^9\) and the Pittsburgh sleep quality index. \(^10\)

Nocturnal Respiratory Assessment

All the patients underwent a nocturnal respiratory assessment during the first 48 hours after stroke symptoms onset. The assessment was performed using Watch peripheral arterial tonometry (PAT), \(^11\) a wrist-worn ambulatory sleep study device that utilizes PAT in conjunction with pulse oximetry and actigraphy. The WatchPAT device indirectly detects apnea and hypopnea via selectively measuring peripheral arterial volume changes (mediated by \( \alpha \)-adrenergic receptors of vascular smooth muscle) using a finger-mounted plethysmograph. The information is collated with pulse oximetry in conjunction with respiratory indexes calculated using PAT-based portable devices were found to be positively correlated with those calculated from the scoring of polysomnography. \(^12\)–\(^15\)

Sleep apnea was diagnosed in the patients with apnea hypopnea index (AHI) \( \geq 15 \). Additional information recorded during the sleep study included mean \( \text{SpO}_2 \) (oxygen saturation), % time spent in \( \text{SpO}_2 \geq 90\% \) (T90 score), and desaturation index—the number of desaturations of \( \geq 4\% \) from baseline. T90 score >1 was considered pathological.

Biomarkers

Serum samples were obtained from all the study participants at the first morning after their admission. Blood samples were drawn specifically between 7 and 10 in the morning from all patients as a routine to avoid the well-known night to day variability in the measured circulating factors (such as tumor necrosis factor [TNF]). The samples were immediately delivered to the research laboratory (located close to the ward) to assure homogeneity. Ten milliliter of whole blood was drawn and collected in appropriate tubes, the serum was divided (200 \( \mu \)L aliquots) and stored at \(-70^\circ \text{C}\) in Eppendorf tubes.

All consecutively collected samples were assayed for TNF, interleukin-6 (IL-6), and plasminogen activator inhibitor-1 (PAI-1) levels using commercially available enzyme immunoassorbance kits, as follows: TNF, eBioscience, San Diego, CA; IL-6, eBioscience, San Diego, CA; and PAI-1, Abcam, Cambridge, MA. Samples were processed in duplicates and assayed at least in 2 dilutions, and plate reader absorbance results were analyzed with a 4-parameter logistic curve fit. The intra- and interassay variability for TNF, IL-6, and PAI-1 were <10%. The specificity of TNF, IL-6, and PAI-1 assays was 100% (except for PAI-1 which was 67%). The detection limit of the assays was 0.13 pg/mL for TNF, 0.13 pg/mL for IL-6, and 8 pg/mL for PAI-1.

Statistical Analysis

Primary outcomes of the analysis were the prevalence of sleep apnea among patients with stroke and levels of proinflammatory cytokines during acute stroke period. Secondary outcomes included NIHSS

<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics of the Patient Population Stratified by the Presence of Obstructive Sleep Apnea</th>
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<tbody>
<tr>
<td><strong>Variables</strong></td>
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<tr>
<td>Demographics</td>
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<tr>
<td>Male</td>
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<tr>
<td>Female</td>
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<tr>
<td>Age</td>
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<tr>
<td>BMI</td>
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<td>Medical history</td>
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<td>HTN</td>
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<td>DM</td>
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<td>Dyslipidemia</td>
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<td>AF</td>
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<tr>
<td>MI</td>
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<tr>
<td>Stroke or TIA</td>
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<tr>
<td>Chronic medications</td>
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<tr>
<td>Antiplatelets</td>
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<tr>
<td>Anticoagulants</td>
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<tr>
<td>Statin</td>
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<tr>
<td>Blood pressure</td>
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<tr>
<td>SBP admission</td>
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<tr>
<td>DBP admission</td>
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<tr>
<td>SBP morning second day</td>
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<td>DBP morning second day</td>
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<td>Blood tests</td>
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<td>LDL</td>
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<td>WBC</td>
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<tr>
<td>Na</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Platelets</td>
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<tr>
<td>Hemoglobin</td>
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AF indicates atrial fibrillation; AH, apnea hypopnea index; BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; HTN, hypertension; LDL, low-density lipoprotein; MI, myocardial infarction; SBP, systolic blood pressure; TIA, transient ischemic stroke; and WBC, white blood pressure.
at admission and discharge and hospitalization length compared between the groups of patients with and without OSA.

The data on continuous variables with normal distribution were presented as mean±SD and compared between study groups using Student t test. We used Bonferroni correction for the post hoc analysis. Continuous variables not normally distributed and ordinal variables were presented as median with interquartile range (IQR) and statistical analysis was done using Mann–Whitney test. Categorical data were shown in counts and percentages, and the differences were assessed by χ², Fisher exact test was used when appropriate.

We used Pearson correlation test for normally distributed continuous variables. Not normally distributed variables were correlated using Spearman test.

Two-sided P value of <0.05 was considered significant. Statistical analyses were performed using IBM SPSS Statistics 20 (IBM Corp).

**Results**

A total of 43 patients with acute stroke were admitted during the study period, 22 (51%) of which have been found to have sleep apnea (AHI≥15). AHI≥5 was found in 86% of the patients, and severe OSA (AHI≥30) in 32.5%. Median admission NIHSS of the study population was 4 with IQR of 2 to 5.

**Patient Population and Stroke Characteristics**

The baseline characteristics of the study population stratified by OSA status are presented in Table 1. Proportion of males was numerically higher in OSA group (40% versus 22.7%, P=0.22), OSA patients tended to be heavier (body mass index 29.6±4.8 versus 26.8±4.3, P=0.05). Of 11 patients with previous stroke, 8 (72.7%) had OSA. Similarly, OSA was diagnosed in 5 of 6 patients with AF. Moreover, blood pressure measurements at admission and discharge and hospitalization length compared between the groups with and without OSA.

At discharge, NIHSS increased in 12.5% in OSA group (25.0% versus 4.5%, P=0.003 (Figure 1A). IL-6 (pg/mL, 6.64±5.27 versus 3.14±2.05, P=0.006, Figure 2B) and TNF (pg/mL, 6.39±5.00 versus 3.57±1.87, P=0.022, Figure 2C) were similar.

**Biomarkers**

We found that AHI is correlated with indicators of inflammation and coagulability: IL-6 (ρ=0.37, P=0.02) and PAI-1 (ρ=0.31, P=0.07). PAI-1 was negatively correlated with a saturation nadir (ρ=−0.47, P=0.005) and positively correlated with a desaturation index (ρ=0.41, P=0.02).

We then stratified AHI into 3 groups: normal, mild, and moderate DSB using AHI<5, 5 to 15, and ≥15 cut-off points, respectively. Comparison of the biomarkers levels showed that only the highest AHI group (≥15) differed from the other 2 groups.

PAI-1 (pg/mL) was significantly higher in patients with an AHI≥15; mean of 176.64±74.52 versus 98.48±52.58 pg/mL, P=0.003 (Figure 2A). IL-6 (pg/mL, 6.64±5.27 versus 3.14±2.05, P=0.006, Figure 2B) and TNF (pg/mL, 6.39±5.00 versus 3.57±1.87, P=0.022, Figure 2C) were similar.

**Table 2. Stroke Symptoms and Severity Characteristics**

<table>
<thead>
<tr>
<th>Variables</th>
<th>AHI&lt;15, n=22</th>
<th>AHI≥15, n=21</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS on admission, median (IQR)</td>
<td>4 (2–5)</td>
<td>4 (2–5)</td>
<td>0.79</td>
</tr>
<tr>
<td>mRS baseline</td>
<td>0 (0–0)</td>
<td>0 (0–1)</td>
<td>0.25</td>
</tr>
<tr>
<td>Stroke symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysarthria</td>
<td>3 (14.3%)</td>
<td>6 (30.0%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>1 (4.5%)</td>
<td>0 (0.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Aphasia</td>
<td>7 (31.8%)</td>
<td>6 (30.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Motor</td>
<td>18 (81.8%)</td>
<td>14 (70.0%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Sensor</td>
<td>8 (36.4%)</td>
<td>3 (15.0%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Visual fields</td>
<td>0 (0.0%)</td>
<td>2 (10.0%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Ataxia</td>
<td>3 (13.6%)</td>
<td>1 (5.0%)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

AHI indicates apnoea hypopnoea index; IQR, Interquartile range; mRS, modified Rankin score; and NIHSS, National Institutes of Health stroke scale.
Discussion

We have hypothesized that patients with stroke will have a high prevalence of SDB that, in turn, will be associated with the increased levels of proinflammatory cytokines. We have found that in an unselected sample of patients experiencing mild stroke, almost 90% had SDB with third having severe form of the disorder. Furthermore, we have shown that SDB is associated with significantly increased levels of PAI-1, IL-6, and TNF.

SDB is increasingly recognized as an important risk factor for arrhythmogenesis. Epidemiological and clinical studies have suggested a strong association between OSA and AF. The prevalence of moderate to severe SDB in our cohort was higher among patients with cardioembolic stroke (all of which were associated with AF). AF and a history of cerebrovascular events were strong predictors of SDB. Similar finding was not found in previous studies. It seems possible that AF is an important factor in SDB-associated stroke pathogenesis.

OSA is associated with increased levels of inflammation biomarkers possibly providing an evidence for the cause of the disease. The proinflammatory cytokine IL-6 is secreted from several different cells, including activated macrophages and lymphocytes. Patients with OSA tend to have higher IL-6 and show a strong and positive correlation between OSA severity and IL-6 levels. Similarly, TNF-α is considered a pleiotropic cytokine with regulatory functions and was found to be involved in several diseases with inflammation as a possible cause, such as atherosclerosis, septic shock, and autoimmune disease. Inflammatory cytokines (eg, IL-6), T-cell activation, and TNF-α itself induces soluble form of TNF-α that affect regulation of immune processes.

An important meta-analysis reviewed 512 with 51 studies included into the final analysis, found a significant increase in levels of inflammatory markers in subjects with OSA, including TNF-α and IL-6. Meta-regression showed a significant effect of AHI increase adjusted for age and body mass index.

Our results demonstrate an association between the levels of circulatory proinflammatory mediators to the severity of SDB in the acute post stroke period. The association of
inflammatory markers and mediators and SDB in the acute post stroke period was previously evaluated. C-reactive protein, fibrinogen, TNF-R1, and TNF-R2 were found to be elevated in post stroke patients with AHI≥10 evaluated 72 post admission.45 TNF-R1 and TNF-R2 were found to be correlated with AHI.22 In another study,23 which evaluated 50 patients with stroke during the first week after admission, IL-6 levels were found to be higher among patients with AHI≥30 compared with patients with AHI<30. IL-6 levels were not correlated with AHI, but patients with AHI≥30 were negatively correlated with minimal oxygen saturation levels. Such correlations were not found for levels of IL-1 or TNF-α. Nevertheless, inflammatory cytokines levels seem to be elevated in OSA patients without a stroke as well, as repeatedly demonstrated,24 especially after an acute ischemic event.25 The role of inflammation in the pathophysiology of an acute cerebrovascular event was extensively studied and is considered to be a possible target for acute stroke management.25 Possibly, the contribution of SDB to the inflammatory response is at list partially responsible to its detrimental effect on patients with stroke.

PAI-1 is a major plasma inhibitor of tissue-type plasminogen activator. Increased levels of PAI-1 lead to impaired fibrinolytic function and were found to be associated with ischemic events.26 Increased activity of PAI-1 was associated with SDB, possibly contributing to the increased vascular risk associated with it.7,28 In our study, increased PAI-1 levels were associated with SDB in patients with acute stroke. This association was not reported previously. Although little is known about the influence of increase post stroke levels of PAI-1 on the natural history of ischemic stroke, it was previously associated with increased prevalence of thrombolysis failure.29

In this study, we have used a bed-side technology for sleep evaluation. The validity of the diagnosis of OSA was studied in the past, and the WatchPAT was found to be a reliable alternative to polysomnography (PSG) for confirmation of clinically suspected sleep apnea.10 Nevertheless, this technology was never evaluated on patients with stroke. However, we think that the assessment of the SDB indexes (eg, AHI) is accurate. A meta-analysis11 assessing 909 patients for the correlation between PAT and PSG as measured by AHI found that respiratory indexes calculated using PAT-based portable devices positively correlated with those calculated from the scoring of PSG. Studies comparing the AHI between PAT and PSG had a combined correlation of 0.893 (95% confidence interval, 0.857–0.920; P<0.001). Moreover, we have found that 51% of our study population had AHI≥15. Other studies evaluating SDB in the acute period showed the prevalence of AHI≥15 to be 32% to 66%,10,31 thus comparable with our results. The use of bed-side PAT technology was convenient and easily used as a bed-side measure in the acute post stroke period and enabled rapid diagnosis and therapeutic recommendations as part of the secondary prevention program.

Our study had several limitations. It was a single-institute study with a relatively small sample size. The question of causality or even temporality between OSA and stroke cannot be answered in this study because we have not formally assessed the presence of the SDB before the stroke hospitalization. We were not able to show that SDB presence or its severity was associated with severity of the stroke or was translated into the worsening clinical outcomes possibly because we studied patients with relatively mild strokes. Yet, the finding of a high prevalence of SDB together with evidence of the associate elevation of the inflammatory and procoagulation biomarkers stress the importance of this pathophysiological pathway in stroke development.

Despite aforementioned limitations, we think that the association between proinflammatory and procoagulant factors and SDB we have found in the acute post stroke period demonstrates the importance of larger interventional trials which will address the efficacy of early SDB directed therapy in improving both short- and long-term outcomes of stroke. It is reasonable to assume that such interventions may improve secondary prevention, alongside more conventional modalities. Large interventional trials in the acute post stroke period will need a reliable, simple bed-side measure for SDB diagnosis. The use of WatchPAT for the bed-side diagnosis of SDB immediately after the stroke, in the stroke unit, as presented in this study, will make such studies possible.

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Disclosures
None.

References


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