

Title: Risk factors for cardiovascular, cognitive and metabolic dysfunction in adult OSA patients: Identification of biomarkers and impact of working schedules

Background

Obstructive sleep apnea (OSA) is a common disorder caused by the loss of upper airway dilating muscle activity during sleep superimposed on a narrow upper airway resulting in recurrent upper airway obstruction, intermittent hypoxemia and disturbed sleep (1). Sleep fragmentation and hypoxemia induced by OSA lead to poor sleep quality, excessive daytime sleepiness, neurocognitive dysfunction, and an increased risk of cardiovascular disease (eg hypertension), cerebrovascular disease (eg stroke), and metabolic dysfunction (eg diabetes and chronic kidney disease) (2, 3-7). Potential mechanisms for these sequelae of OSA include activation of the sympathetic nervous system and renin-angiotensin system, systemic inflammation, endothelial dysfunction and oxidative stress (8-14). Treatment of OSA with continuous positive airway pressure (CPAP) has been shown to improve many of these complications (15), highlighting the potential benefits of treatment.

Importance of biomarkers to identify patients with OSA at high risk for cardiovascular disease and other complications: It is currently difficult to identify which patients are at higher risk for cardiovascular events and other complications of OSA based solely on clinical criteria. One possible solution is to use a panel of serum and genetic biomarkers to identify those patients who have the highest risk of these complications. Blood markers of inflammation such as CRP, IL-6, and other inflammatory cytokines have already been linked to increased risk of cardiovascular events independently of OSA, such that individuals with OSA and the greatest elevations of these biomarkers are likely at highest risk of cardiovascular complication (16,17). In addition, OSA is heritable (18-20) and there is evidence for single nucleotide polymorphisms (SNPs) within the inflammatory genes themselves that modify the expression or function of these proteins (21,22) as well as SNPs that modify risk or severity of OSA (23,24) and cardiovascular disease (25) such that interactions among specific genetic profiles in patients with OSA is worth exploring. It is unclear, however, how these markers work together and in conjunction with other clinical criteria, to confer risks of adverse outcomes in individual patients. If useful biomarkers could be found to identify a group of patients at highest risk of adverse clinical outcomes, this would help direct patient care significantly. In particular, if a patient did fit a high-risk biomarker profile, more effort towards encouraging adherence to OSA treatments and modifying other important risk factors (e.g. more aggressive treatment of hypertension) may prove helpful.

Cognitive dysfunction, OSA and inflammatory markers: Cognitive dysfunction is increasingly recognized as an important potential sequela of OSA. Some of the cognitive dysfunction does not appear to reverse with OSA treatment, suggesting that OSA may result in permanent neurologic damage and lead to dementia (26,27). Whether OSA can cause enough cognitive deficits to warrant a diagnosis of mild cognitive impairment (MCI), which can be a precursor of dementia, is currently unknown. However, preliminary data among 100 individuals aged > 55 years showed that at least 35% of individuals with mild to severe OSA have MCI (28). Animal studies suggest that the inflammation associated with intermittent hypoxia may play a role in causing neuronal damage (29). In fact, OSA causes an inflammatory and metabolic cascade that includes oxidative stress (30), inflammation (high level of C-reactive protein), sympathetic and platelet activation, and vascular, endothelial and metabolic deregulation (31-33). These inflammatory and metabolic markers have all been reported to increase the risk of MCI (34) and recently, dementia has been referred to as “type 3 diabetes” since metabolic dysfunctions play a pivotal role in the progression to dementia (35). The relationship between these biomarkers, the occurrence of MCI in OSA patients, and the progression to dementia needs to be investigated.

OSA and chronic kidney disease: Obstructive sleep apnea occurs in up to 40% of chronic kidney disease (CKD) patients who are not dialysis dependent (6). Furthermore, nocturnal hypoxemia due to OSA has been associated with abnormal renal hemodynamics and accelerated deterioration in kidney function (5). Potential mechanisms for this association include both direct effects of hypoxia on the kidney and indirect mechanisms through oxidative stress, endothelial dysfunction, inflammation, sympathetic nervous system activation and hypertension, all of which are associated with OSA and have been proposed to reduce kidney function. Recently, our research group reported that increased renin angiotensin system (RAS) activity within the kidney in OSA patients was significantly improved with CPAP therapy (14). Consequently, the notion that OSA contributes to the progression of kidney failure is biologically plausible and clinically relevant. However, it is unlikely that all patients with OSA develop this complication. It would be helpful to identify biomarkers in the urine that would identify which OSA patients are suffering renal injury from nocturnal hypoxia so that risk factor can be managed appropriately.

Shift Work, OSA and Risk: More than 10% of Canadians regularly work on rotating shifts and 2% regularly work night shifts (36). One of the major issues with shiftwork is that activity is not in synchrony with the circadian or internal pacemaker. Individuals who work shifts on a long-term basis are at increased risk of developing cardiovascular disease, stroke, cancer, and weight gain (37, 38). Given the high prevalence of OSA, many shift workers will have OSA. Indeed, there may be a greater prevalence of OSA in shift workers given that rotating shifts predispose to weight gain, the major risk factor for OSA (39). In one study of shift workers with OSA, the severity of sleep-disordered breathing (reflected by the apnea hypopnea index and hypoxemia) was greater during daytime sleep after night shift work compared to nocturnal sleep (40). This suggests that shiftwork may intensify the adverse health effects of OSA. The identification of cardiovascular risk factors associated with shift work is important to prioritize access to treatment for this working population. This research will impact how occupational policy makers consider rotating schedules and OSA as important and potentially synergistic determinants of adverse health outcomes.

Hypothesis

OSA patients who have increased levels of inflammatory markers and/or specific genetic markers will have higher risks for cardiovascular and cerebrovascular complications, metabolic abnormalities such as diabetes and chronic kidney disease, and poor cognitive outcomes even after controlling for a variety of potential confounders. Further, these complications will be more prevalent in shift workers.

Objectives

Through the collection of serum and urine samples, we hope to identify inflammatory, metabolic and genetic biomarkers to detect OSA patients at high risk for adverse cardiovascular (myocardial infarction, angioplasty, cardiac death), cerebrovascular (stroke), metabolic (diabetes and chronic kidney disease), and cognitive outcomes. Identification of work schedules will enable us to determine the prevalence of these complications in shift workers.

Methods

Overview: We will recruit a prospective cohort of patients referred to the Foothills Medical Centre Sleep Centre for investigation of OSA. We will collect the following: 1) Questionnaires that will include

demographic data (e.g., age, gender, body mass index), medical history including co-existing medical disorders and medications, family history, sleep/health habits, and work schedule; 2) Diagnostic sleep testing (described below); 3) Medical tests that have been done as part of their routine medical care (details below) 4) Cognitive function, based on 3 tests (described below); and 5) Blood and urine samples for analysis of biomarkers (details below);

6) Administrative and laboratory data from provincial databases that will identify patients in our cohort who have developed any of the adverse cardiovascular, cognitive and metabolic outcomes that may be attributed to OSA.

1) *Questionnaire*: The questionnaire (attached) will be completed by the patient and reviewed by a research coordinator. If there are any errors or omissions, the coordinator will follow up with the patient to clarify their answers.

2) *Diagnostic sleep tests*: These are standard diagnostic tests that are performed to assess the presence and severity of OSA. These tests will be done for clinical purposes and not for the research study per se. However, we will request consent from the patient to include the results of these tests in our research data. There are 2 diagnostic sleep tests that are used at our sleep centre: 1) Ambulatory sleep test, called a “Snoresat” (SSAT): Patients are given a SSAT monitor to take home and wear overnight to assess their breathing while they sleep. The monitor is returned the following day and the data is downloaded electronically. 2) Polysomnography (PSG): Patients sleep overnight in the sleep laboratory at the Foothills Medical Centre during which their sleep, respiration, EKG and leg movements are recorded non-invasively with a PSG technologist in attendance. If clinically indicated, the patient’s assessment may include an arterial blood gas to measure the level of oxygen and carbon dioxide in the blood. Both the SSAT and PSG will provide physiologic data that will identify the severity of OSA, including the apnea frequency and the degree of associated hypoxemia.

3) *Medical tests that are part of routine medical care*: If patients have had medical tests that would provide further information about their OSA and its complications, we will include the results of these tests in our data collection. These tests include previous arterial blood gases, pulmonary function test, echocardiography, EKG, hemoglobin level, blood sugar and hemoglobin A1C (which reflect control of diabetes) serum creatinine and protein levels in the urine (which reflect kidney function). Since we have a particular interest in the potential for OSA to injure the kidneys, we will add those tests (ie serum creatinine and protein levels in the urine) to the blood and urine samples we request from the patient (see #5 below).

4) *Cognitive function*: We will use three tests: 1) Montreal Cognitive Assessment test. This test assesses global cognition and requires about 10 min to complete. It is widely used in neurology, geriatric and family practice. 2) WAIS Digit Symbol Substitution test. This test assesses processing speed, visuo-spatial and working memory and takes 3-5 minutes to complete. 3) Rey Auditory Verbal Learning test. This test assesses verbal learning and memory and takes 5-8 minutes to complete. These tests will be administered by a research coordinator under the supervision of Dr Eric Smith, who is a co-investigator and an expert in neurocognitive assessment.

5) *Blood and urine samples for analysis of biomarkers*: All patients will have blood and urine samples taken following their diagnostic sleep study.

Blood sample: A venous blood sample (50 ml = 3 tablespoons) will be drawn at the Heritage Medical Research Clinic. Samples will be centrifuged, serum and plasma will be aliquoted and genetic material obtained from the buffy coat. Samples will be kept in a freezer (-80° C) at the Heritage Medical Research Clinic temporarily (1-3 months) until they are shipped to the Center for Advanced Research in Sleep Medicine, a bio-bank located in the Hospital of Sacre-Coeur and part of the University of Montreal. The standard operating procedures for this blood collection and shipping are attached (Protocol B - Blood collection, processing and shipping of frozen samples). Once at the bio-bank, the samples will be placed in a freezer (-80° C) and subsequently processed for genetic markers, mRNA, and protein levels associated with a variety of inflammatory/cardiovascular markers including CRP, IL-6, and other cytokines. If research subjects consent, these samples will be kept at the bio-bank in Montreal to allow future studies to investigate regulation and consequences of sleep/circadian disorders. The standard operating procedures and consents for the bio-banking have been included. Substantial safeguards for confidentiality are in place. However, if subjects do not consent for the samples to be kept at the biobank for future studies, any unused samples will be discarded after the current project is completed. The details of the options for consent are outlined in detail below.

As outlined in #3) above, some of the venous blood sample (approx. 5ml) will be sent to Calgary Lab Services for measurement of serum creatinine.

Urine specimen: A midstream urine sample (50 ml) will be collected. The urine will be centrifuged at 4 degrees Celsius, 1500xg for 10 minutes and then aliquoted and placed in a freezer (-80° C) at the Biobank for the Molecular Classification of Kidney Disease, at the University of Calgary (REB15-1026), for later analysis of biomarkers of kidney injury. We are currently performing an exploratory study on urine samples collected in a previous research study to identify the specific biomarkers we wish to measure in this study. If research subjects consent, these samples will be kept at the Bio-bank in the University of Calgary for research future studies to investigate biomarkers of kidney injury.

As outlined in #3) above, some of the urine sample (approx. 5ml) will be sent to Calgary Lab Services for measurement of the albumin:creatinine ratio (ACR) which reflects the protein level in the urine.

Approximately 2-3 years following recruitment, patients will be invited to provide another blood and urine sample for the same analysis and to repeat the questionnaire and cognitive tests.

6) *Administrative and Laboratory Data and Data Linkage:* Data from the Alberta Ministry of Health and Wellness and Alberta Health Services (AHS-Analytics and Reporting) will be linked using the personal health number (PHN) recorded for the patients at the time of their diagnostic sleep test (SSAT or PSG). This will include both laboratory and administrative data.

Laboratory data source:

Province-wide laboratory data will be accessed through *AHS - Analytics and Reporting (DIMR)*. Specifications of laboratory test results that will be used for analysis will be outlined within the DIMR Request Management Tool

Administrative data sources:

A. Alberta Health Care Insurance Plan (AHCIP) Registry File

The Alberta registry file identifies virtually all residents in the provincial health insurance plan except a small proportion of special population groups (i.e. Canadian Armed Forces /RCMP, federal inmates – approximately 1% of the total population). The registry contains information on date of birth, gender, First Nations status, postal code, and socioeconomic status by fiscal year.

B. Hospital Discharge Abstracts

The hospital inpatient data source contains details regarding hospitalizations including admission date, discharge date, length of stay in hospital, diagnostic codes (ICD-10-CA) and procedure codes for each admission.

C. Ambulatory Care Classification System File (ACCS)

The ambulatory care file includes emergency department encounters and day procedures (ICD-10-CA)

D. Fee for Service Physician Claims File

The physician claims registry contains information on physician services including dates and location of the visits, up to three diagnostic codes (ICD-9-CM) and provider specialty.

The study index date for each patient will be the date of each patient's diagnostic sleep test. Patients will be followed forward in time to capture relevant cardiovascular, cerebrovascular, cognitive and metabolic outcomes of interests within the proposed administrative data sources. These outcomes include:

Cardiovascular outcomes: Acute coronary syndrome, acute myocardial infarction, angioplasty, coronary artery stent, coronary bypass surgery, heart failure, hypertension etc

Cerebrovascular outcomes: Transient ischemic attack, stroke etc

Cognitive outcomes: Dementia etc

Metabolic outcomes: Diabetes mellitus, chronic kidney disease, proteinuria etc

The data collected will be entered in REDCap which is a secure, browser-based application designed to support electronic data capture (EDC) for research studies. This EDC software is used to facilitate clinical and translational research databases and used widely in the academic research community. The Clinical Research Unit (CRU) in the Faculty of Medicine at the University of Calgary is a local REDCap host and offers the support and use of the service to Faculty of Medicine and Alberta Health Services personnel.

Subjects

Adult patients with OSA will be invited to participate. They will be identified from patients referred to the Foothills Medical Centre Sleep Centre for diagnostic sleep testing (SSAT or PSG) as described above.

Inclusion Criteria: 1) Men and women aged 18-80 years; 2) Patients referred to the FMC Sleep Centre for evaluation of sleep disordered breathing.

Exclusion Criteria: 1) Failure to meet inclusion criteria; 2) Current therapy with continuous positive airway pressure (CPAP) or supplemental oxygen; 3) Unable to provide informed consent; 4) Receiving treatment with warfarin or direct oral anticoagulants (DOACs); 5) Unable to complete the questionnaire due to language or comprehension barrier.

Diagnostic Sleep Tests

- 1) **Snoresat (SSAT):** This is an ambulatory sleep test that has been used for 30 years in our sleep centre to diagnose OSA. It records arterial oxyhemoglobin saturation (SaO_2) and heart rate via a finger pulse oximeter, nasal airflow via a nasal cannula pressure transducer, snoring sounds via a microphone attached to the subjects' neck, and sleep position (supine/non-supine) from a sensor within the microphone housing. The SaO_2 signal is recorded at 1 Hz and analyzed using a proprietary scoring algorithm. The respiratory disturbance index (RDI) is calculated as the number of times SaO_2 decreases by 4% or more divided by the total recording time; this is used as a surrogate measure of the frequency of apnea during sleep. The scoring algorithm also quantifies the severity of hypoxemia. Raw data from the sleep recorder is reviewed by a sleep medicine physician to confirm the presence or absence of OSA.
- 2) **Polysomnography (PSG):** This is a standard noninvasive procedure commonly used in clinical practice to diagnose and quantify the severity of OSA. The test is performed overnight in the sleep laboratory with an experienced PSG technologist in attendance. The PSGs include continuous recording of sleep from two central and one occipital EEG signals, two electro-oculograms, a chin electromyogram; an electrocardiogram, movement of the chest and abdomen by respiratory inductance plethysmography bands, airflow by recording of nasal pressure and oro-nasal thermister, oxyhemoglobin saturation from a finger pulse oximeter, and a microphone to record snoring. These data are collected for approx. 6 hours, manually scored by a PSG technologist and interpreted by a sleep medicine physician. The severity of OSA is reflected by the AHI (apnea-hypopnea index) and the degree of associated hypoxemia. The apnea-hypopnea index is a measure of the frequency of apnea during sleep.

Positive airway pressure therapy

Some patients who are diagnosed with OSA will be treated with positive airway pressure therapy, which will either be CPAP (continuous positive airway pressure) or BPAP (bilevel positive airway pressure) therapy. Adherence with CPAP or BPAP may alter the outcomes we are measuring; consequently, a measurement of adherence will be important. This can be measured accurately by electronic download from the CPAP or BPAP unit. This is done routinely in our out-patient sleep clinic and by respiratory homecare providers in the community for the clinical management of patients with OSA. Consequently, we will ask patients' for their consent to obtain this data either directly from their CPAP or BPAP unit when they attend the sleep clinic at Foothills Medical Centre or from their respiratory homecare provider in the community.

Consent

Two consent forms will be used:

- 1) Consent to participate in the study outlined in this proposal. This consent will include the option for urine samples to be stored in the Biobank for the Molecular Classification of Kidney Disease, at the University of Calgary, for future research studies.
- 2) Consent for the blood samples that are sent to the Center for Advanced Research in Sleep Medicine bio-bank in the University of Montreal to be kept for participation in subsequent research studies.

Statistical Analysis/Sample Size

This is a multi-centre study, which will be done in collaboration with colleagues at the University of British Columbia and the University of Saskatchewan. Our goal is to recruit approximately 300 patients per year from each of the three sites for 5 years. Samples will be processed for genetic markers, mRNA, and protein levels associated with a variety of inflammatory/cardiovascular markers including CRP, IL-6, and other cytokines. In the first analysis, we will determine whether levels of these markers are associated (in cross sectional analyses) with sleep apnea severity and cognitive function after controlling for a number of relevant confounders including BMI, age, and gender. Eventually (approximately 2-3 years from the start of recruitment), we will link our database with provincial databases to ascertain relevant cardiovascular, cerebrovascular and metabolic outcomes (listed above). Multivariable logistic regression analyses will be used to identify key clinical and genetic markers associated with the outcomes of interest adjusting for important confounders such as comorbidity, age, and sex.

Significance

Major gaps in knowledge still exist in providing best treatments for patients with OSA and reducing adverse outcomes. The proposed study will help to address some of these important gaps in knowledge. First, the development of biomarkers of disease consequences will be important in identifying a high-risk group for clinically relevant outcomes such as metabolic and cardiovascular diseases, and cognitive decline. It is expected that those identified as high-risk would need more aggressive OSA treatment and benefit from risk factor reduction interventions. Second, given the enormous challenges and costs associated with neurodegenerative disease in Canada, early identification of OSA patients with cognitive decline and identification of the cerebral mechanisms underlying progression to dementia in OSA is needed to advance the development and evaluation of interventions to slow down dementia progression. Finally, the prevalence of diabetes and chronic kidney disease is increasing which has significant implications for individual patients and the healthcare system. Determining the contribution of OSA to this issue will guide new and innovative strategies to reduce the burden of these metabolic disorders.

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