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POINCARÉ PLOT ANALYSIS OF DIFFERENCES IN HEART RATE VARIABILITY BETWEEN PATIENTS WITH MODERATE OBSTRUCTIVE SLEEP APNEA AND WITHOUT OBSTRUCTIVE SLEEP APNEA

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Master of Science in Oral Biology

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CERTIFICATE OF APPROVAL

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MASTER'S THESIS

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ABSTRACT

POINCARÉ PLOT ANALYSIS OF DIFFERENCES IN HEART RATE VARIABILITY BETWEEN PATIENTS WITH MODERATE OBSTRUCTIVE SLEEP APNEA AND WITHOUT OBSTRUCTIVE SLEEP APNEA

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Introduction: Heart rate variability (HRV) is the variation in the interval between consecutive heart beats. This allows the cardiovascular system to respond to sudden changes in physical demands, which is required for cardiovascular balance. Obstructive sleep apnea (OSA) occurs when pharyngeal muscles relax during sleep causing upper airway collapse, restricting airflow. Low blood oxygenation (hypoxemia) is a result. Repeated hypoxemias trigger autonomic nervous system (ANS) imbalance, causing systemic inflammation, endothelial dysfunction and altered metabolism. The ANS has direct modulating effects on HRV. HRV is reduced in OSA patients; many agree this ANS imbalance contributes to cardiovascular disease, a recognized long-term sequela of OSA. Time-domain and frequency-domain analysis of HRV can be difficult and require specialized skill. Poincaré plot analysis has been proposed as a simplified alternative method. The aim of this study is to evaluate differences in HRV between patients with moderate OSA and without OSA using Poincaré plots.

Methods: Polysomnography data was gathered retrospectively of 20 patients with moderate OSA and 20 without OSA. Poincaré plots were generated and three data points of each collected; length, width and area. Data were analyzed using single and multiple linear regression models.

Results: Standard deviation (SD) regression coefficients revealed no significant difference in all HRV parameters ($p>0.05$). When age, sex and BMI were controlled for, the adjusted difference in SD was significant for all three parameters ($p<0.05$).

Conclusions: There is a significant difference in HRV when controlling for confounding factors such as age, sex and BMI. Previous research evaluating HRV in OSA using Poincaré plots is limited. This study adds evidence that HRV is altered in patients with moderate OSA compared to those without OSA. It also highlights the Poincaré plot as simple and potentially valuable in measuring treatment outcomes which could be useful in multi-disciplinary care since OSA is commonly treated by both physicians and dentists.

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LIST OF ABBREVIATIONS

AADSM	American Academy of Dental Sleep Medicine
AASM	American Academy of Sleep Medicine
AHI	Apnea-Hypopnea Index
ANS	Autonomic Nervous System
BMI	Body Mass Index
CPAP	Continuous Positive Airway Pressure
CVD	Cardiovascular Disease
ECG	Electrocardiogram
h	Hour
HF	High Frequency
HRV	Heart Rate Variability
LF	Low Frequency
MAD	Mandibular Advancement Device
ms	millisecond
NN	Normal-to-normal interval
NO	Nitric Oxide
OAT	Oral Appliance Therapy
OSA	Obstructive Sleep Apnea
PNS	Parasympathetic Nervous System
PSG	Polysomnography

RDI	Respiratory Disturbance Index
RERA	Respiratory Effort-Related Arousal
RMSSD	Root Mean Square of Successive Differences of RR Intervals
RR	R-to-R Interval
SD	Standard Deviation
SDB	Sleep-Disordered Breathing
SDNN	Standard Deviation of all NN Intervals
SNS	Sympathetic Nervous System

CHAPTER I: REVIEW OF THE LITERATURE

OBSTRUCTIVE SLEEP APNEA

Definition

Obstructive Sleep Apnea (OSA) is one of multiple disorders that fall under the category of Sleep-Related Breathing Disorders. The other disorders included are central sleep apnea syndromes, sleep-related hypoventilation disorders, and sleep-related hypoxemia disorder (Sateia, 2014). According to the American Academy of Sleep Medicine (AASM), OSA is defined as a sleep-related breathing disorder that involves a decrease or complete halt in airflow despite an ongoing effort to breathe. This occurs when pharyngeal muscles around the throat relax and allow soft tissue to collapse, blocking the upper airway. Subsequently, airflow is reduced for seconds to even minutes, resulting in a decrease in blood oxygen saturation (AASM OSA Fact Sheet; Myers et al., 2013). Diagnosis of OSA largely centers around obstructive respiratory events observed on an electrocardiogram (ECG) during overnight polysomnography (PSG). These events are summarized as follows:

- Apnea: a cessation of breathing for greater than 10 seconds
- Hypopnea: a 30% reduced respiratory airflow for greater than 10 seconds with an accompanied 4% reduction in oxygen saturation
- Respiratory effort-related arousal (RERA): a reduction in airflow from an obstructed upper airway that is neither an apnea or hypopnea but is associated with an increase in respiratory effort and ends with an arousal

- Apnea-Hypopnea Index (AHI): the number of apneic and/or hypopnea events during an hour of sleep
- Respiratory Disturbance Index (RDI): the number of obstructive respiratory events during an hour of sleep, to include apneas, hypopneas and RERAs

AASM outlines the diagnosis of OSA as 5 or more obstructive respiratory events per hour (AHI or RDI) accompanied by other symptoms and/or disorders (Sateia, 2014). In the absence of other symptoms or disorders, OSA can be diagnosed with an AHI or RDI of 15 alone (Sateia, 2014). Throughout many articles, AHI and RDI are used somewhat interchangeably with AHI being the most dominant. For simplicity, AHI will be used for the remainder of this paper. Detailed diagnosis of OSA including level of severity is included in **Table 1.**

Obstructive Sleep Apnea – Diagnosis
1. PSG with ≥ 5 obstructive respiratory events per hour of sleep *Plus,* the presence of one or more of: - Daytime sleepiness, non-restorative sleep, fatigue, insomnia per patient report - Awakenings with breath holding, choking or gasping per patient report - Observed snoring, breathing interruptions or apneas by a bed partner - Diagnosis of hypertension, coronary artery disease, stroke, congestive heart failure, atrial fibrillation, diabetes type 2, cognitive dysfunction or a mood disorder
2. PSG with ≥ 15 obstructive respiratory events per hour of sleep
Obstructive Sleep Apnea – Severity
Mild: AHI 5 – 15/h
Moderate: AHI 15 – 30/h
Severe: AHI ≥ 30/h

Table 1. Obstructive sleep apnea definition. Obstructive sleep apnea definition, diagnosis and severity level, per the International Classification of Sleep Disorders by the American Academy of Sleep Medicine (Sateia, 2014).

Risk Factors

There is a preponderance of evidence that cites the same common risk factors and demographics time and time again. The current most commonly used OSA screening questionnaire, the STOP questionnaire, was developed and validated over a decade ago (Chung et al., 2008). This questionnaire highlights these common signs, risks and demographics of one who has the potential for OSA, with the acronym standing for Snoring, Tiredness, Observed apnea, and high blood Pressure. While this questionnaire proved to have moderate sensitivity for predicting OSA, the authors recommended four additional questions that included other risk factors. These were whether the patient had a body mass index (BMI) over 35, an age over 50, a high neck circumference and whether they were male. The screening test consists of yes or no questions only, with a value of "1" assigned to each yes answer and "0" for each no answer. The addition of these resulted in the STOP-Bang questionnaire. This questionnaire has proven to have high sensitivity, where when a person scores ≥ 3, it correlates to an accurate diagnosis of moderate to severe OSA at a rate of 93% and 100% respectively (Chung et al., 2008). Another study found sensitivity rates at 95% and above for all levels of severity of OSA (Luo et al., 2014).

The additional risk factors concerning gender and age have been proven multiple times to be highly linked and predictive of OSA. A review article evaluated multiple population-based epidemiology studies and concluded that males were more likely to have OSA with ratios of male to female ranging from 2:1 to 8:1 (Young et al., 2004). They also found that advanced age has a 2 – 3 fold increase in OSA diagnosis, particularly for those over 65 years old. Eikermann et al. (2007) demonstrated that

upper airway resistance and pharyngeal collapsibility both increase with age independent of both gender and BMI. They believe this is the primary mechanism for the development of OSA. Browne et al. (2001) compared young and elderly patients with and without OSA and also found that upper airway resistance increased with age in healthy elderly subjects, but they suggested that aging alone does not contribute to the instability of breathing during sleep. For example, they found that arousal threshold was not impacted by age. One can conclude that it is still controversial as to whether age is the cause of higher rates of OSA or if OSA is higher in the elderly due to the likelihood of higher rates of other comorbidities.

All these studies and review articles, as well as nearly any literature related to OSA, highlight the fact that obesity is considered a major risk factor for the development and progression of OSA. Obesity is associated with a nearly two-fold increase in OSA diagnosis (Romero-Corral et al., 2010). Being overweight or obese has a substantial impact on the diagnosis of OSA, to the point that some authors cite this as a causal factor. An 11-year long-term prospective study of 690 participants showed that there was a positive relationship with weight and OSA severity (Peppard et al., 2000). These authors found that a 10% increase in weight predicted an increase in AHI of 32% in patients. They also noted that a 10% gain in weight also showed a 6-fold increase in the chance that a patient would receive a new diagnosis of moderate to severe OSA.

Prevalence

In an epidemiology study that pooled data from 1,520 participants enrolled in a community-based sleep study, the estimated prevalence of sleep-disordered breathing

(SDB) with an AHI > 5 was 33% (Peppard et al., 2013). These researchers also found that in adults 30 to 49 years old, the prevalence ranged from 36.1% in males to 11.4% in females. Furthermore, the prevalence was over 60% and 36%, respectively, in adults between 50 and 70 years of age. In a systematic review exploring the accuracy of diagnosing OSA, Myers and colleagues (2013) noted a prevalence ranging from 2 – 14%, with rates higher in older men and those with a higher BMI (Myers et al., 2013). Although there is a wide range of prevalence seen among all these articles, the trend is clear that OSA is a very common condition. With a current U.S. population of over 300 million, even at the lowest reported prevalence rates result in dramatically high numbers. Of note, the greatest percentage is found in older men with higher BMIs. More importantly, this prevalence has steadily increased over the last decade (Paskhover, 2016; Peppard et al., 2013). Multiple authors have also stated that an alarming percentage of people with OSA go undiagnosed and untreated, as high as 80-90% (Chung et al., 2016; Young et al., 2004).

OSA Pathogenesis

There is an increasing amount of evidence that OSA can lead to cardiovascular disease (CVD) rather than the reverse. Many authors cite that the pathogenesis of OSA is multifactorial and that the mechanisms involved are complex with some not yet fully understood, but many agree that there is an associated and causal link.

Effect on the Autonomic Nervous System

The primary pathogenesis centers around the effect chronic intermittent hypoxia has on the autonomic nervous system (ANS). The initial insult to the body is the collapse

of the airway causing repeated episodes of hypoxemia which is a low concentration of blood oxygen and hypercapnia, or excess carbon dioxide in the blood. These hypoxemic events have been cited as the primary trigger for a sympathetic nervous system (SNS) response in that the decreased oxygen level activates the chemoreflex system, increasing muscle sympathetic nerve activity (Cutler et al., 2004; Narkiewicz et al., 1998). Increased muscle sympathetic nerve activity is responsible for vasoconstriction, hence an increase in cardiac heart rate and blood pressure. The widely recognized Joint National Committee on Prevention, Detection, Evaluation, and Treatment of Hypertension recognized that repeated hypoxemias resulted in a strong SNS reaction that directly increased blood pressure, and cited this as an identifiable cause of hypertension (Chobanian et al., 2003). There is also evidence that this overactivation of SNS has shown to remain elevated for prolonged periods of time, even hours (Cutler et al., 2004). This adds further support to the notion that higher daytime levels of SNS activity seen in OSA patients is a result of the hypoxemic events they experience at night (Carlson et al., 1993; Spaak et al., 2005). Chemical mediators are also altered with activation of the SNS. Norepinephrine is released at higher levels in patients with OSA (Bao et al., 2002; Chaidas et al., 2014) and like the effect from hypoxemia, this directly increases heart rate and blood pressure. Arousals alone are cited in OSA pathogenesis. There has been a long-held belief that arousals were responsible for waking the patient enough to allow the airway to open and ventilation to resume, ending an apneic or hypoxic event. In a review article by Eckert and Younes (2014), they discussed how this protective mechanism alleviates a severe apneic event but that the physiological changes from the arousal could also have negative effects. They explained that although an arousal can terminate an

apneic event early, this could lead to a higher frequency of events per hour, therefore a higher AHI, or in other words more severe OSA. This leads to prevention of deeper sleep stages and all the associated complications related to sleep fragmentation.

Inflammation and endothelial dysfunction

Endothelial dysfunction is characterized by impaired or reduced vasodilation, enhanced vasoconstriction, and a state of proinflammation and thrombotic activity. Production of nitric oxide (NO) is key to proper vascular function and homeostasis while decreased NO availability is pathological (Tousoulis et al., 2012). This inflammation and vascular impairment contribute to the development of atherosclerosis and cardiovascular disease. Jelic and colleagues (2008) explored the specifics of endothelial dysfunction in OSA and how it contributes to CVD. In their prospective cohort study, they found that specific proteins that regulate NO production and inflammation were altered in OSA patients and that continuous positive air pressure (CPAP) therapy reverses this. They concluded that the hypoxia of OSA induces inflammation and oxidative stress which damages vascular endothelium while also reducing NO availability and repair capacity (Jelic et al., 2008).

Increased levels of a variety of inflammatory markers have been found in OSA patients in many other studies. A protein named pentraxin-3 is from the same family as c-reactive protein has been identified as a biomarker of CVD. Kanbay and colleagues (2015) demonstrated in a cross-sectional study that endothelial dysfunction declined with the increase in OSA severity and that OSA patients had elevated levels of pentraxin-3. Another study also showed elevated levels of pentraxin-3 in OSA patients and that this biomarker could be related with OSA severity (Sozer et al., 2018). Tumor necrosis

factor-alpha and interleukin-6 are classic inflammatory markers that have been repeatedly studied and found to promote vascular inflammation and dysfunction. Elevated levels of both of these biomarkers have been observed in OSA patients (Bielicki et al., 2015; Motamedi et al., 2018). A recent comprehensive review on these specific markers concluded that both are commonly elevated and confirm presence of inflammation in OSA patients, but that due to an abundance of heterogeneity in study findings it should be noted that there are many modulating factors such as genetics, environment and lifestyle elements (Kheirandish-Gozal & Gozal, 2019).

Altered metabolism

OSA and obesity are highly correlated, so it is no surprise that metabolic dysfunction is commonly found in both diagnoses. The exact mechanistic details are still not clear, but a recent review has shown strong evidence from animal studies that there is evidence that OSA leads to metabolic dysfunction separate but similar to that of obesity (Ryan, 2017). The leading proposal is that intermittent hypoxia is the key underlying player. Intermittent hypoxia induces sympathetic activity as well as inflammation in visceral adipose tissue. Both activities impair the insulin-signaling pathway, decreasing insulin resistance and altering adipose lipolysis.

OSA Comorbidities & Complications

OSA is associated with numerous deleterious effects on the body, with the prevalence of OSA extremely high in patients with cardiac and/or metabolic disorders. While CVD is perhaps the most serious comorbidity with OSA, other afflictions readily present at higher levels in OSA patients compared to a healthy individual such as

cognitive impairments, daytime sleepiness and reduced quality of life (Lacasse et al, 2002).

Cardiovascular Disease

In a large cross-sectional study of over 6,000 participants from the Sleep Heart Healthy Study, a linear association was found between AHI and blood pressure, even after adjusting for BMI (Nieto et al., 2000). Taking it further, they controlled for other potential confounders such as age, sex, neck circumference, waist-to-hip ratio, smoking and alcohol consumption and they were still able to report a dose-response relationship between high levels of AHI or sleep time below a 90% SpO2 and hypertension. They also noted that there were more men, increased age (> 65 years), obesity and higher neck circumference in the participants that had more severe SDB, which is consistent in previously mentioned epidemiology studies. A similar study showed that the presence of sleep-disordered breathing was predictive of the presence of hypertension four years later (Peppard et al., 2000). A large observational study of more than 1,000 patients found that OSA independently increased the risk of stroke or death, even in patients with hypertension (Yaggi et al., 2005). In a more recent comprehensive review article, the authors reported strong evidence of causality for elevated blood pressure (Bauters et al., 2016), and they noted that this result is partly caused by sympathetic activation from OSA. Bauters et al. (2016) also cited evidence that OSA contributes to the progression of atherosclerosis, that there was a high prevalence of OSA in stroke patients and that there was evidence that OSA increased the risk and recurrence rate of atrial fibrillation. These findings, in particular OSA's causal association with hypertension, are of utmost

importance in the fact that hypertension is a known risk factor for CVD and is treatable by a variety of means.

Metabolic Disease

Aside from CVD, links between OSA, metabolic disease, and diabetes have also been observed. Multiple studies have shown that OSA is independently associated with metabolic disease such as systemic inflammation and insulin resistance (Ip et al., 2002; Luciene da Silva Araújo et al., 2015; Romero-Corral et al., 2010). Moreover, Akishita and colleagues demonstrated that OSA can worsen endothelial dysfunction in patients already diagnosed with metabolic syndrome (Akishita et al., 2011). A recent meta-analysis of nine studies that included over 64,000 participants showed that OSA is associated with diabetes (Reutrakul & Mokhlesi, 2017).

Other Complications

The constant arousals in OSA result in sleep fragmentation leading to excessive daytime sleepiness, which is attributed to a myriad of consequences. A study examining quality of life with OSA interviewed one hundred OSA patients and found that quality of life was impacted not only by excessive daytime sleepiness, but that participants reported feeling anxious, impatient, and depressed: additionally, their interpersonal relationships were affected and work performance was diminished (Lacasse et al., 2002). A psychiatric review article evaluated evidence focused on cognitive deficits associated with OSA and found that executive functions such as working memory, problem solving and planning were impaired and that attention deficits were commonly found in OSA (Krysta et al., 2017).

Another interesting finding is the association between OSA and pain. A systematic review concluded that there was an overall association of OSA to decreased pain tolerance and higher experienced pain intensity (Charokopos et al., 2018). They found that some studies conflicted in these findings which were likely complicated from other comorbidities and types of pain, but that it was likely due to hypoxemia and sleep fragmentation. This goes along with other disciplines of research that have reported a strong bi-directional relationship between poor sleep and chronic pain disorders (Finan et al., 2013).

Sleep apnea also has an indirect influence on health in that it is linked to a higher risk of postoperative complications. In a large study of over 530,000 patients receiving hip or knee arthroplasty, multiple adverse outcomes were identified in higher rates among those with sleep apnea (Memtsoudis et al., 2014). Pulmonary (1.86 times) and cardiac (1.59 times) complications were more likely, and the mortality rate increased by 1.27 times. Longer stays in the hospital, use of the intensive care unit and higher rates of resources were also found.

Lastly, another indirect consequence is the association of OSA with motor vehicle crash risk. A review article that covered 18 different studies reported that 'individuals with OSA are clearly at increased risk for crash' (Tregear et al., 2009). They found that the average crash risk for a driver was 0.08 crashes per person-year but that if the driver had OSA, this crash risk increased to approximately 0.19.

OSA Treatment

Therapeutic goals

According to the AASM, OSA is a chronic disorder that requires long-term treatment, management, follow-up and a multi-disciplinary approach (Epstein et al., 2009). The overarching therapeutic goal in the treatment of OSA is to reduce CVD risk and to improve quality of life (Aurora et al., 2015). For OSA treatment, effectiveness measures are centered around how OSA is diagnosed. As discussed previously, OSA is diagnosed by a sleep physician who evaluates symptoms, risk factors and uses the aid of an overnight PSG. Objective measurements for therapeutic effectiveness are a normalization of AHI and increase in oxygen desaturation index. Subjective measurements often focus on resolution of daytime sleepiness but should also include assessment of therapy adherence, sleep hygiene, weight loss, and quality of life changes (Epstein et al., 2009).

Positive Airway Pressure

Positive airway pressure (PAP) has long been considered the first line of treatment for OSA(Kushida et al., 2006). This is a device that forces air into the patient's lungs under low pressure which aids in maintaining an open airway and ventilation. The most recent clinical guidelines developed by an AASM Task Force in 2019 recommend CPAP or auto-adjusting PAP for the ongoing treatment of OSA (Patil et al., 2019), particularly for moderate and severe levels. This Task Force cited evidence that sleep-related quality of life and hypertension could be improved with PAP therapy and that in particular they found strong evidence for resolution of excessive sleepiness. PAP devices fit the patient with an attachment that ranges from a full-face mask to a small nasal

attachment. These devices often record pressure and rate of usage to allow for titration and follow-up adjustments with managed care.

While CPAP is considered the gold standard for OSA of all severity levels, patient compliance rates are low. A systematic literature review pulled data on CPAP non-adherence rates from 82 articles and found that 34.1% of patients were not using their CPAP device (Rotenberg et al., 2016). They reported nearly 11% of participants across all included studies stopped using their CPAP completely due to inability to tolerate the device, and the mean average of nightly use for all others was 4.7 hours per night. This tells us that there is a substantial amount of people that do not even use their CPAP long enough to gain the therapeutic benefits. It should be noted that many of the included studies were short-term and included 6 months or less of follow-up. The authors felt strongly that this calls into question whether CPAP should remain the gold standard for OSA treatment if compliance rates are so low. There are few long-term studies for CPAP use and many are subjective, but they also report increasing rates of non-adherence after multiple years in the range of 36 - 51% (Campos-Rodriguez et al., 2016; Chang et al., 2019; Wolkove et al., 2008).

Oral Appliance Therapy

Oral appliance therapy (OAT) has been used regularly as an alternative treatment method. The AASM and American Academy of Dental Sleep Medicine (AADSM) both agree that as a standard of care, OAT should be used in OSA patients who are unable to tolerate CPAP therapy and/or request an alternative mode of treatment (Ramar et al., 2015).

Oral appliance therapy aims to open the oropharyngeal airway and prevent it from collapsing during sleep. This is accomplished by moving the tongue forward, allowing the upper airway soft tissues to remain patent. Most oral appliances accomplish this by advancing the mandible in a protrusive or forward position. These are broadly categorized according to how they are made, to include whether they are custom-made and whether they are titratable. The use of a custom, titratable appliance is recommended over other non-custom devices (Ramar et al., 2015). These appliances are referred to by a multitude of names in the literature to include mandibular advancement appliance/splint/device, mandibular repositioning appliance, and oral jaw-positioning appliance. The most commonly encountered is the mandibular advancement device (MAD). A far less common oral appliance is the tongue-retaining device which advances the tongue forward to open the airway and is sometimes combined with mandibular advancement. Although this has shown to be effective, its use and patient compliance is far less than with MADs (Lazard et al., 2009). While diagnosis of OSA is performed by a physician, fabrication of an oral appliance is done by a dentist.

The AASM and AADSM clinical guidelines for OAT use include in their supporting evidence that OAT had more hours per night usage than CPAP on average (Ramar et al., 2015). More recent studies have shown the non-adherence rate for OAT is lower than CPAP even when measured objectively, in the range of 2 – 18% (de Vries et al., 2013). As with CPAP, OAT non-??adherence rates tend climb with time, with two large long-term studies reporting a non-adherence rate between 14 – 68% after 3 – 4 years of OAT use (Bachour et al., 2016; Saglam-Aydinatay & Taner, 2018).

Surgery

In pediatric OSA, an adenotonsillectomy is commonly prescribed as first-line treatment (Farber, 2002) but in the adult population surgery is rarely the first choice. When indicated, the surgery performed is uvulopalatopharyngoplasty. In addition to removal of the tonsils, the uvula and soft palate are removed, and the anterior and posterior pillars are modified. Sometimes this procedure is done in combination with other nasopharyngeal or oropharyngeal procedures. Surgery is often not indicated due to the fact that many patients will still need to use CPAP therapy after surgical intervention to obtain a resolution of <5 AHI (Khan et al., 2009).

Other

In the clinical guidelines for managing OSA, the AASM outlines other treatment modalities which can be alternatives or adjunctive (Epstein et al., 2009). Examples include other types of surgical approaches such as bariatric surgery or other nasal and oropharyngeal surgeries. Behavior modification is heavily emphasized and often used in conjunction with any of the devices. Examples are sleep hygiene, reduction in tobacco and alcohol use, positional therapy and weight loss (Epstein et al., 2009).

HEART RATE VARIABILITY

Definition

Heart rate variability (HRV) is defined as the change in the interval between consecutive heartbeats as well as the change between consecutive instantaneous heart rates ("Heart Rate Variability," 1996). In simple terms, it is the variation in the interval between consecutive heart beats over a designated period of time. The 1996 Task Force

of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology explains that sympathetic and parasympathetic activities of the autonomic nervous system have direct modulating effects on the sinus node of the heart represented as rhythmic fluctuations ("Heart Rate Variability," 1996).

HRV Analysis - Conventional

The 1996 Task Force article provides guidelines on how HRV is measured and analyzed and explains how HRV reflects the sympathetic and parasympathetic balance within the body. An electrocardiogram (ECG) is commonly used to record the electrical activity of the heart, where the QRS complex is a universally recognized representation of a heartbeat. The time elapsed between two successive R waves of the QRS signal is called the RR Interval, measured in milliseconds (ms) and represents the instantaneous heart rate. In other words, instantaneous heart rate is the exact heart rate at a specific point in time. Some authors use the term NN Interval in place of RR interval, referring to the NN as normal-to-normal RR intervals in which artifacts have been removed.

Analyzing the RR intervals of an ECG record over time is called a time-domain method. There are multiple measurements taken in this method that are cited extensively in literature focused on cardiac monitoring. Examples are summarized in **Table 2**. The most common and valid statistical analysis within a time domain method is measuring the root mean square of successive differences (RMSSD) of RR intervals ("Heart Rate Variability," 1996). The RMSSD estimates the short-term components of HRV and gives us a measure of parasympathetic activity (Carrasco et al. , 2001; Ciccone et al., 2017; Shaffer & Ginsberg, 2017).

Frequency-domain analysis is also commonly seen in HRV literature and is used to describe how variance is distributed as a function of frequency ("Heart Rate Variability," 1996). Parameters used in this method are summarized in **Table 2** with low frequency (LF), high frequency (HF) and LF/HF ratio commonly cited in articles pertaining to this study. Power in the LF range is a combination of both Parasympathetic Nervous System (PNS) and SNS activity, while power in the HF range is attributable to only PNS activity. It is important to note that LF, HF and therefore LF/HF ratios are not always reliable in that the actions of the PNS and SNS are not completely reciprocal (Shaffer & Ginsberg, 2017) and this may account for conflicting results across studies.

HRV Time-Domain Parameters		
Parameter	Definition	Significance
SDNN	Standard deviation of NN intervals	Valuable for 24h recordings to measure cardiac risk - expresses SNS activity. Values predict morbidity and mortality. e.g. Higher value = lower mortality risk
SDANN	Standard deviation of NN interval averages in all 5 min segments	Used in research but does not provide more information as it is a redundant correlation of SDNN.
pNN50	Number of pairs of adjacent NN intervals differing by more than 50 ms	Useful with short recording periods (< 5min), correlated with PNS activity.
RMSSD	Root mean square of successive differences	Measures beat-to-beat variance in heartrate therefore an estimate of short-term HRV. Estimates PNS mediated changes. Identical to SD1.
HRV Frequency-Domain Parameters		
Parameter	Definition	Significance
ULF	Power of ultra-low frequency	24h recordings. Correlated to SDANN
VLF	Power of very low frequency	24h recordings. Has been associated strongly to all-cause mortality e.g. Low VLF power = higher mortality
LF	Power of low frequency	~ 2min recordings. PNS and SNS modulation, can be affected by respiration.
HF	Power of high frequency	~ 1-2min recordings. Predominately PNS modulation, can be affected by respiration therefore not completely representative of vagal tone. Correlated to pNN50 and RMSSD.
LF/HF	Ratio of LF-to-HF	Measure of ANS balance, used with caution as PNS and SNS relationship is not reciprocal.
HRV Poincaré Plot Parameters		
Parameter	Definition	Significance
Width (SD1, T, ΔRRt)	Standard deviation of the distance of each point from the x=y axis	Estimate of Short-term HRV that describes PNS modulation. Correlated to HF power, identical to RMSSD.
Length (SD2, L, ΔRRr)	Standard deviation along the line of identity	Short and long-term HRV, correlated to LF power.
Area	Total area of plot	Overall HRV
SD1/SD2	Ratio of SD1-to-SD2	Measure of ANS balance, used with longer recording periods. Some correlation to LF/HF. e.g. when evaluated as SD2/SD1

Table 2. Summary of HRV analysis parameters. Common HRV parameters measured using time-domain, frequency-domain and non-linear (Poincaré plot) analytic methods. ANS – autonomic nervous system, PNS – parasympathetic nervous system, SNS – sympathetic nervous system (Carrasco et al., 2001; "Heart rate variability," 1996; Guzik et al., 2007; Mermigkis et al., 2009; Shaffer & Ginsberg, 2017).

HRV analysis – by Poincaré Plot

While the time domain method is a linear measurement and can sometimes be complex, there is a non-linear or geometric technique that allows for visualization and calculation of HRV called the Poincaré plot. This was introduced by Raetz and colleagues (1991) where RR intervals during sleep and wake cycles were analyzed via Poincaré plots. They were one of the first to highlight the potential of this method in assessing cardiac changes from various syndromes including sleep apnea. Kamen and Tonkin (1995) noted that heart rate variability does not always fall into a normal distribution making it more difficult to use summary statistical measures, so they proposed that the Poincaré plot had the advantage of being simple and able to provide 'real time' visualization of heart rate data which is difficult when using traditional spectral analysis. The 1996 Task Force that provides guidelines in analyzing HRV has verified geometrical methods as valid and recommends the ECG recordings used be at a minimum of 20 minutes in duration and that all recordings being compared be of equal duration ("Heart Rate Variability," 1996). A Poincaré plot is represented on a simple two-dimensional Cartesian graph with x and y axes. The RR interval is plotted as a function of the subsequent RR interval where RR(n) is plotted on the x-axis over time and RR(n+1) is plotted on the y-axis as demonstrated in **Figure 1.** To orient the reader, lower RR intervals in the lower left of the plot represent faster heart rates and higher RR intervals represent slower heart rates in the upper right. Points located close to the line of identity (y=x) show low variability, while points farther dispersed from the line show higher variability.

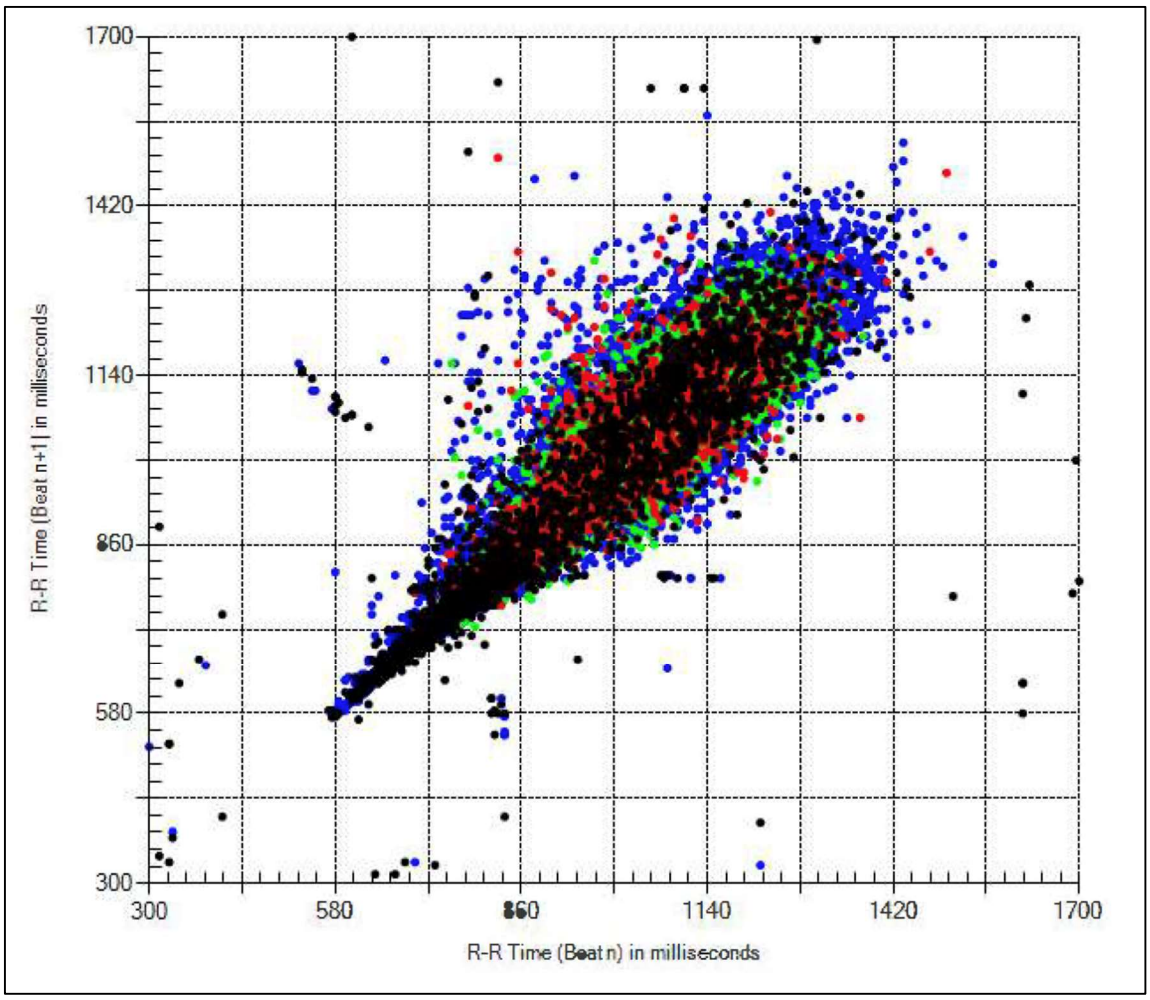


Figure 1. Poincaré plot example. Poincaré plot displaying heart rate variability over time. The RR interval, RR(n) is plotted over time as a function of the subsequent RR interval RR (n+1).

Quantitative assessment of the Poincaré plot is useful when statistical analysis or comparison is desired. Multiple authors have used non-linear techniques to evaluate HRV and their analysis of the plot features are summarized by Carrasco et al. (2001) who also studied the correlation between Poincaré plot analysis and traditional time and frequency domain analysis. As mentioned previously, the most significant measurement taken from an ECG to measure HRV is the time-domain parameter RMSSD which provides a measure of parasympathetic activity. Kamen et al. (1996) conducted a study using Poincaré plots for HRV assessment and was one of the first to suggest that the

width of the plot was a measure of parasympathetic activity and that the entire plot displayed overall HRV. These researchers demonstrated that the width of the Poincaré plot was mathematically equivalent to RMSSD. Poincaré plots for analysis of HRV have continued to be considered equivalent to the time domain method (Carrasco et al., 2001) with the width confirmed as an identical metric to RMSSD (Ciccone et al., 2017). Another interesting finding in the Carrasco study was that visual patterns were found to be useful in reflecting sympathetic activity. For example, plots tended to be significantly more condensed and narrower when SNS activity was high, such as in standing or during exercise. Conversely, the plots tended to have greater dispersion with higher RR intervals when a participant was in a supine and resting position, indicating a predominance of PNS activity. Visual models such as this have previously been described by Brennan et al. (2002) who created HRV Poincaré plot models to represent various sympathovagal balance scenarios.

In summary, the width, length and overall area of the plot are useful measurements for evaluating HRV. Various authors have identified these measurements by different names. Tulppo et al. (1996) identified the width and length as SD1 and SD2 respectively, while Carrasco et al. (2001) used 'T' and 'L' and Mermigkis et al. (2009) used ΔRRt and ΔRRr. In many of these methods, the length and width are obtained by fitting an ellipse over the plot (Carrasco et al., 2001; Copie et al., 1996; Tulppo et al., 1996). Others used a modified technique in which parallel and transverse lines were placed according to the line of identity around the bulk of the plot in order to obtain measurements (Chaidas et al., 2014; Mermigkis et al., 2009). This second method is

simpler than the first in that it requires no special software or training to complete and therefore was used in this study. All parameters are summarized in **Table 2.**

HRV significance in Health and Disease

HRV reflects a myriad of regulatory processes with complex interactions involving neuronal input, chemical messengers, chemo- and baroreceptors and gas exchange that culminate into ANS balance, vascular tone and blood pressure. The constant changes in intervals between consecutive heart beats, or HRV, are key in cardiovascular balance. They allow the cardiovascular system to respond quickly to any sudden changes in physical or psychological events. This flexibility represents health, and HRV typically drops in a diseased state. This is because many physiologic and psychologic disorders induce increased SNS activity. To review, SNS activity is commonly known as the 'fight or flight' response represented by physiological changes such as increased heart rate and myocardial contraction, bronchiole dilation, and blood vessel constriction (Gordan et al., 2015). The SNS overactivity seen in many diseased states applies stress to the body leading to chronicity that propagates an autonomic imbalance. Autonomic imbalance is the underlying factor for poor health and low HRV is a strong indicator of this (Shaffer & Ginsberg, 2017).

A recent multi-center prospective study was conducted in which 1,043 patients without previously diagnosed or known coronary artery disease were given short-term HRV testing conjunction with cardiac stress testing to measure outcomes (Goldenberg et al., 2019). They found that low HRV was independently associated with a 2 – fold increase in the risk of myocardial infarction. In a meta-analysis by Fang, Wu and Tsai,

they found that in patients with pre-existing CVD, a reduced HRV had more than twice the risk of all-cause death (Fang et al., 2020).

A prospective study was conducted on 139 participants to evaluate HRV in adults with varying degrees of risk for type 2 diabetes (Silva et al., 2017). Using both linear and non-linear methods of HRV analysis, they found reduced HRV parameters among all groups that were at higher risk of type 2 diabetes. This was in line with a large population-based study that examined cardiac autonomic nervous dysfunction in diabetes and glucose intolerance in 1,332 participants (Ziegler et al., 2015). They reported that the prevalence of cardiac dysfunction was more prevalent in both patients with diabetes and impaired glucose intolerance. More importantly, they were able to show the association between reduced HRV and modifiable cardiovascular risk factors, not to mention higher mortality.

As mentioned previously, psychological strain can also have a profound impact on cardiovascular health, represented as changes in HRV. A 2019 meta-analysis was conducted of 21 studies centered around major depression and its association with CVD (Koch et al., 2019). They found that all HRV parameters were lower in patients with major depression compared to healthy controls and that this is a good marker of overall health and well-being. Another meta-analysis by Kim et al. (2018) found that psychological stress induces changes in HRV measures and that the most common finding was a reduction in PNS activity.

Heart Rate Variability in OSA

Narkiewicz et al. (1998) conducted one of the earliest studies to explore whether alterations of HRV in OSA existed in the absence of CVD. They compared 16 healthy controls to 18 patients with mild OSA and 15 patients with moderate to severe OSA. All patients were similar in age, BMI and had normotensive blood pressure. None took medications or had been previously diagnosed with any other disease. HRV was evaluated while patients were awake but in a resting state in a supine position. They found that HRV was reduced in patients with OSA or in other words there was 'high sympathetic neural traffic' in this group. They also felt that the degree of 'derangement' may be linked to the OSA severity because alterations were seen predominantly in the moderate to severe OSA group.

Subsequently, another study emerged that evaluated HRV alterations around respiratory events of OSA patients at night (Dingli et al., 2003). Fourteen patients diagnosed with OSA but who were otherwise healthy were compared to seven healthy controls. All were matched for age and BMI. An analysis of HRV during 2-minute intervals around apneic/hyponeic events found that HRV decreased compared to undisturbed sleep, which reflected an increase in sympathetic activity. The authors concluded that the recurrent fluctuations in autonomic balance and the overall increase in sympathetic activity at night might be of importance in the development of CVD in OSA patients.

Aytemir et al. (2007) conducted a prospective study on 80 OSA patients against 55 OSA negative patients to evaluate myocardial vulnerability in the OSA group. They found that HRV, among other parameters tested, was significantly worsened in the OSA

group at night but that it was not linked to severity of OSA. This latter finding conflicts with that of Narkiewicz et al. (1998).

Another interesting study was one by Lado et al. (2012) that described HRV parameter trends of 46 patients with and without OSA during overnight PSG. They showed that OSA patients had consistently lower HRV parameters compared to non-OSA patients. They were also able to show that OSA patients had HRV alterations even during normal breathing, not just during apneas or hypopneas.

A retrospective study of 164 patients comparing an OSA group to a normal control group analyzed HRV by multiple methods including time-domain, frequency-domain and geometric methods (Kim et al., 2015). The participants included had no history of other systemic disease who were matched closely for BMI and all participants were male. Overall results found that an autonomic imbalance could be detected in OSA patients. However, certain HRV parameters expected to decline did not do so as in other studies, such as RMSSD which reflects parasympathetic tone. The authors still were able to report a trend of increased sympathetic activity as responsible for the autonomic imbalance.

In summary, a recent systematic review included two of the above authors in addition to ten other studies with the aim of elucidating autonomic impairment in OSA as measured by HRV (Sequeira et al., 2019). Even with some heterogeneity among studies, they were able to conclude that the majority of the evidence supports that OSA patients have decreased vagal tone and increased SNS activity.

Effect of OSA Treatment on HRV

Both CPAP and OAT effectiveness on HRV have been explored in the literature. Many studies have shown that CPAP can be significantly impactful on improving HRV with long-term use as well as even short-term use. A recent meta-analysis evaluated 11 prospective cohort studies of good quality and found that CPAP with long-term use could decrease the LF and LF/HF parameters of HRV (Guo et al., 2018). They stated that the improved autonomic balance was one way in which the risk of CVD might be reduced with CPAP therapy. Another study demonstrated that HRV can be improved in as little as one night (Kufoy et al., 2012).

We know that OAT has proven to be effective in treating patients diagnosed with mild to moderate OSA, in that it has demonstrated the ability to reduce AHI comparable to CPAP therapy (Aarab et al., 2011; Gagnadoux et al., 2009). On the other hand, there is very little published data on the effect of oral appliance therapy on HRV specifically. Only a handful of studies have been conducted on the effect of oral appliances on HRV, specifically mandibular advancement devices. A recent systematic review and meta-analysis concluded that these studies had small patient samples, were heterogenous and inconclusive (de Vries et al., 2018). Coruzzi and colleagues (2006) concluded that using an MAD may improve cardiac autonomic modulation during wakefulness in healthy patients with mild degree of OSA. In this study, patients diagnosed with mild OSA but who were otherwise healthy were compared to healthy individuals without OSA. Both groups were treated with a MAD for 3 months with their autonomic function measured before and during treatment. They found that treatment resulted in a reduced AHI, an increase in the HRV-HF component and a non-significant decrease in LF/HF. In a

randomized control trial that compared CPAP to MAD therapy in 29 patients diagnosed with moderate to severe OSA, the authors concluded that autonomic modulation during sleep was improved in patients treated with MAD. They also concluded that CPAP therapy was more effective overall in treating OSA than MAD therapy but that compliance with MADs were higher (Dal-Fabbro et al., 2014). In a similar randomized controlled clinical trial, CPAP and MAD therapy were both found to decrease AHI and had similar beneficial changes in daytime cardiac autonomic function, particularly as measured by blood pressure changes. Three months of MAD use had a minor effect on HRV-HF, the parameter for vagal autonomic activity, but overall both CPAP and MAD had no effects on autonomic activity in the frequency-domain (Glos et al., 2016).

Time-domain and frequency-domain analysis of HRV can be difficult and require specialized skill, therefore Poincaré plot analysis has been proposed as a simplified alternative method (Chaidas et al., 2014; Guzik et al., 2007; Kamen and Tonkin, 1995; Mermigkis et al., 2009). OSA has been associated with reduced HRV by various analytic methods (Ayetemir et al., 2007; Dingli et al., 2003; Kim et al., 2015; Lado et al, 2012; Narkewicz et al., 1995; Sequiera et al., 2019) but evidence with the Poincaré Plot method is limited (Aljadeff et al., 1997; Chaidas et al., 2014; Mermigkis et al., 2009). The aim of this study is to evaluate differences in HRV between patients with moderate OSA and without OSA using Poincaré plots.

CHAPTER II: MATERIALS AND METHODS

Study Protocol

This project was reviewed and determined to be research not involving human subjects by the Determination Official at Walter Reed National Military Medical Center, IRB# WRNMMC-EDO-2020-0458, Reference# 923785. Sleep study data were gathered retrospectively from the Walter Reed National Military Medical Center Sleep Disorder Clinic in a randomized fashion and de-identified for this study. All patients included in the study had received a baseline overnight PSG at this clinic which was recorded and analyzed using an AASM-compliant sleep diagnostic system (Somnostar ^{TM}, CareFusion version 11.2). Evaluation and diagnosis of each patients' data was done by a Sleep Medicine certified physician. A sleep technician not involved in the study identified 20 patients to serve as a control that had received overnight polysomnography but were not diagnosed with OSA. The same technician identified 20 additional patients to serve as the experimental group who had also received an overnight PSG in the same clinic during the same timeframe but who had been diagnosed with moderate OSA as defined by an AHI of 15 – 30/h. The technician compiled patient demographic data as listed in **Table 3.** The technician generated and exported five HRV Poincaré plots from the PSG ECG recordings of each patient to include sleep stage N1 – N2, stage N3, REM, wake and entire night plots. All data was de-identified with each patient case being identified as control 1, control 2, control 3 etc., or OSA1, OSA 2, OSA3 etc. These data were then submitted to our research team for analysis.

Patient Demographics, n = 40	Poincaré Plot HRV Parameters
- Age - Gender - BMI - AHI - Sleep efficiency - Sleep distribution (%N1, N2, N3, REM) - Arousal Index - Mean blood oxygen saturation (SpO2) - Epworth sleepiness score (ESS)	- Width: Estimate of Short-term HRV that describes PNS modulation. - Length: Estimate of short- and long-term variability - Area: Total HRV

Table 3. Data Collection. Patient demographics, polysomnography data and resultant Poincaré plot measurements recorded.

Poincaré plot measurements

The Poincaré plots were analyzed for each patient that was created from the entire overnight PSG, which is comprised of all RR intervals recorded during wake, sleep stages N1, N2, N3 and rapid eye movement (REM). Poincaré plots for individual sleep stages or wakeful periods were not analyzed.

Analysis of Poincaré plots was conducted in a similar manner as outlined by Mermigkis, 2009 and Chaidas, 2014 and illustrated in **Figure 2**. This involved first identifying the line of identity on the plot, also known as the x = y line. Next, the maximal and minimal heart rate frequencies were identified. The maximal heart rate (HRmax) frequency is the location of the lowest RR interval while the minimal heart rate (HRmin) frequency is the location of the highest RR interval. From here, parallel and perpendicular lines to the line of identity were drawn to encompass the bulk of the plot to aid in estimating the length and width. The length of the plot was calculated as the difference between HRmax and HRmin. The width was calculated from the

perpendicular lines drawn across the line of identity and the total area of the plot was then obtained.

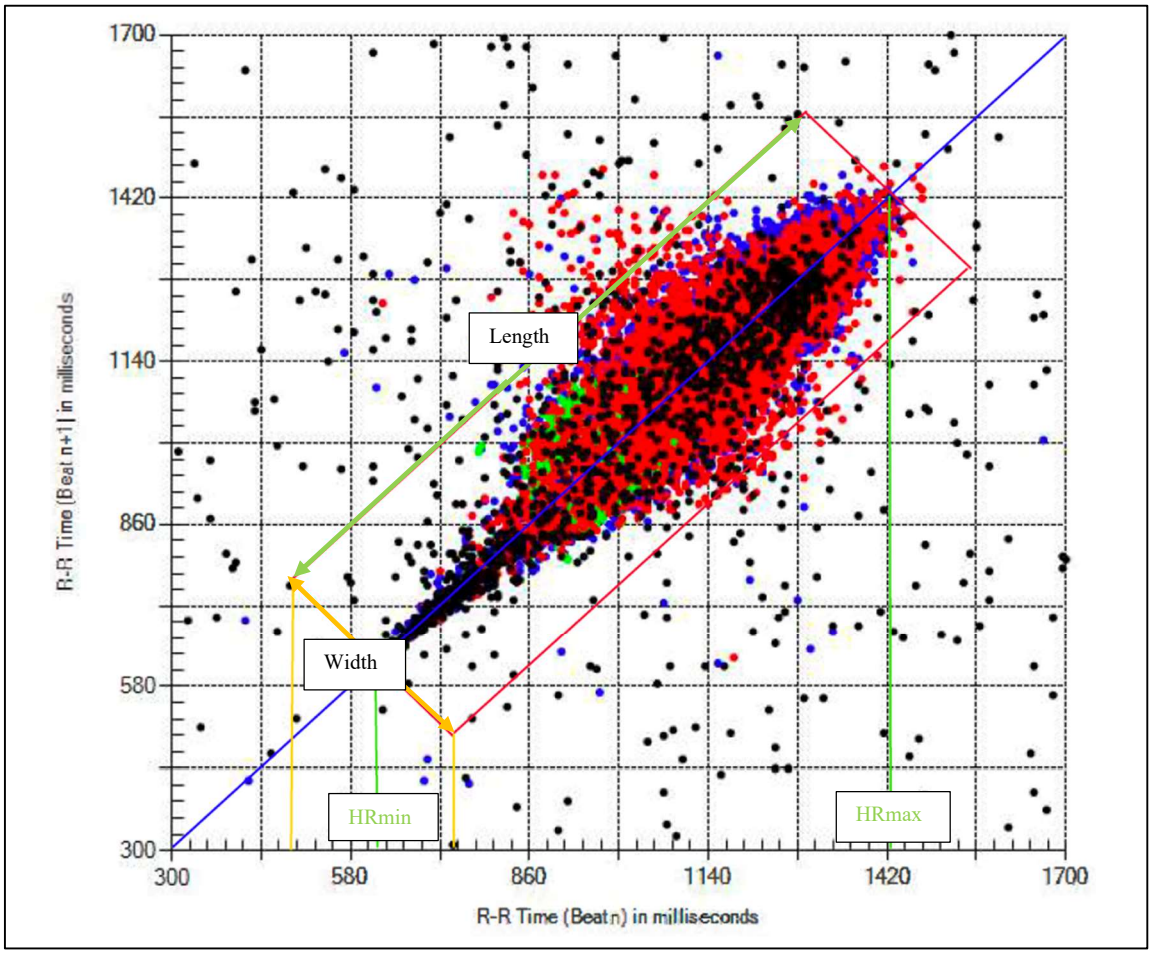


Figure 2. Poincaré plot analysis example. Example shown is a typical plot seen in the control group. Line of identity (blue). Length of the plot is measured along the line of identity (green) and width of the plot is measured from the perpendicular line crossing the line of identity (yellow). Square of the plot (red). Method adapted from (Chaidas et al., 2014; Mermigkis et al., 2009)

Data Analysis

Demographic descriptive data was analyzed using the mean and standard deviation for each independent variable. Box plots were generated for the variables with the most extreme differences between groups to illustrate their roles as confounding factors.

The three Poincaré plot data points; length, width and area, were compiled for each patient in the control group and the moderate OSA group. Initially, these dependent variables were analyzed using single linear regression models. To control for confounding factors, multiple linear regression models were used. The unit of measurement used was ms for the length and width, and for the area it was ms squared. For the area measurement, this created very large numerical values compared to the length and width values. Therefore, analysis was conducted using standard deviation (SD) regression coefficients. The magnitude in differences between sleep apnea cases and controls for length, width, and area were found to be the same; the only difference found was in scale (i.e. -1 to 1 on the x-axis instead of 100,000 to 1,000,000). Having the same scale for all measurements aided in keeping the values small and easily comparable.

CHAPTER III: RESULTS

Descriptive Data

Median, mean, SD and variance of independent and dependent variables were extracted and compared between groups. Large variations were particularly noted in BMI, age, sex, arousal index, mean SpO2 and AHI. Patients in the OSA group were

generally male, older and had a higher BMI than that of the non-OSA group. Findings are summarized in **Table 4** and **Figure 3.** Examples of various plot patterns seen among participants are shown in **Figure 4.**

	OSA (AHI 15 – 30)	Non-OSA (AHI < 5)
Age	43.7 +/- 11.4	36.5 +/- 12.6
Gender (male)	80%	45%
BMI	30.0 +/- 4.3	27.1 +/- 4.5
AHI	22.9 +/- 4.9	1.0 +/- 1
Sleep efficiency	74.2 +/- 17.8	81.7 +/- 12.6
Sleep distribution		
N1%	26.0 +/- 15.9	16.8 +/- 10.5
N2%	46.4 +/- 11.2	48.0 +/- 11.6
N3%	15.1 +/-13.5	19.0 +/- 11.7
REM%	12.6 +/ 17.1	16.2 +/- 8.0
Arousal Index	34.4 +/- 18.1	17.0 +/- 9.3
Mean SpO2	92.9 +/- 1.4	95.6 +/- 2.1
ESS	10.6 +/- 5.2	8.8 +/- 4.2

Table 4. Demographic data. BMI: body mass index; AHI: apnea-hypopnea index; N1: sleep stage1, non-REM; N2: sleep stage 2, non-REM; N3: sleep stage 3, non-REM; REM: sleep stage rapid-eye movement; SpO2: blood oxygen saturation; ESS: Epworth Sleepiness Scale score. Mean +/- SD are shown for each measure.

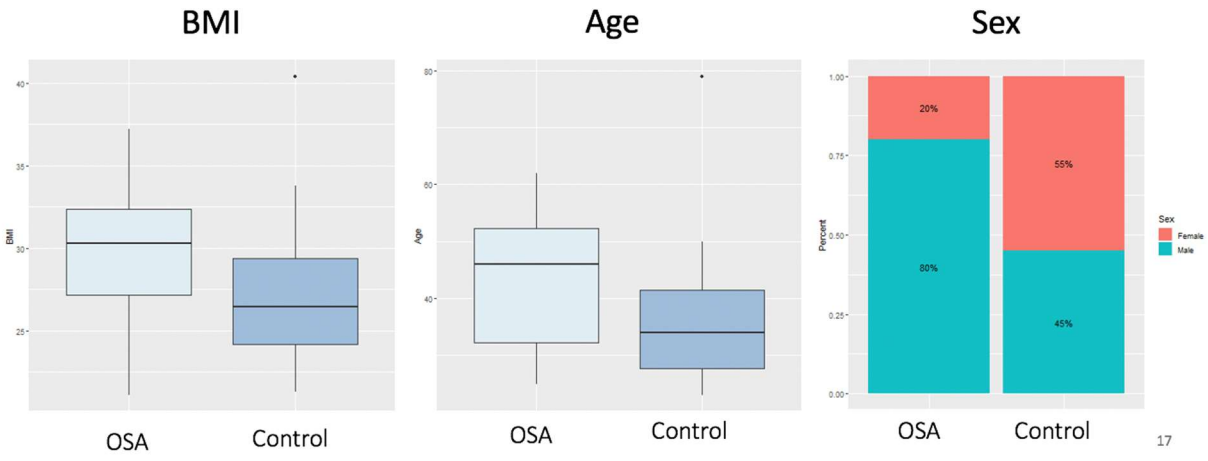


Figure 3. Descriptive data graphs. The horizontal line of each box plot represents the mean and the vertical lines represent the standard deviations of BMI (body mass index), Age and Sex distribution between the OSA and Control groups.

	OSA (AHI 15 – 30)	Non-OSA (AHI < 5)
Width, ms	336.6 +/- 241.5	249.5 +/- 104.5*
Length, ms	874.1 +/- 227.7	866.2 +/- 218.9*
Area, ms^2	330,828 +/- 343,394.4	232,162 +/- 126,014.1**

Table 5. Poincaré plot measurements. Mean (+/- Standard deviation) of length, width and area for OSA group and non-OSA control group measured in ms.
* $p \leq 0.05$ when controlling for age, BMI and sex.
** $p \leq 0.001$ when controlling for age, BMI and sex.

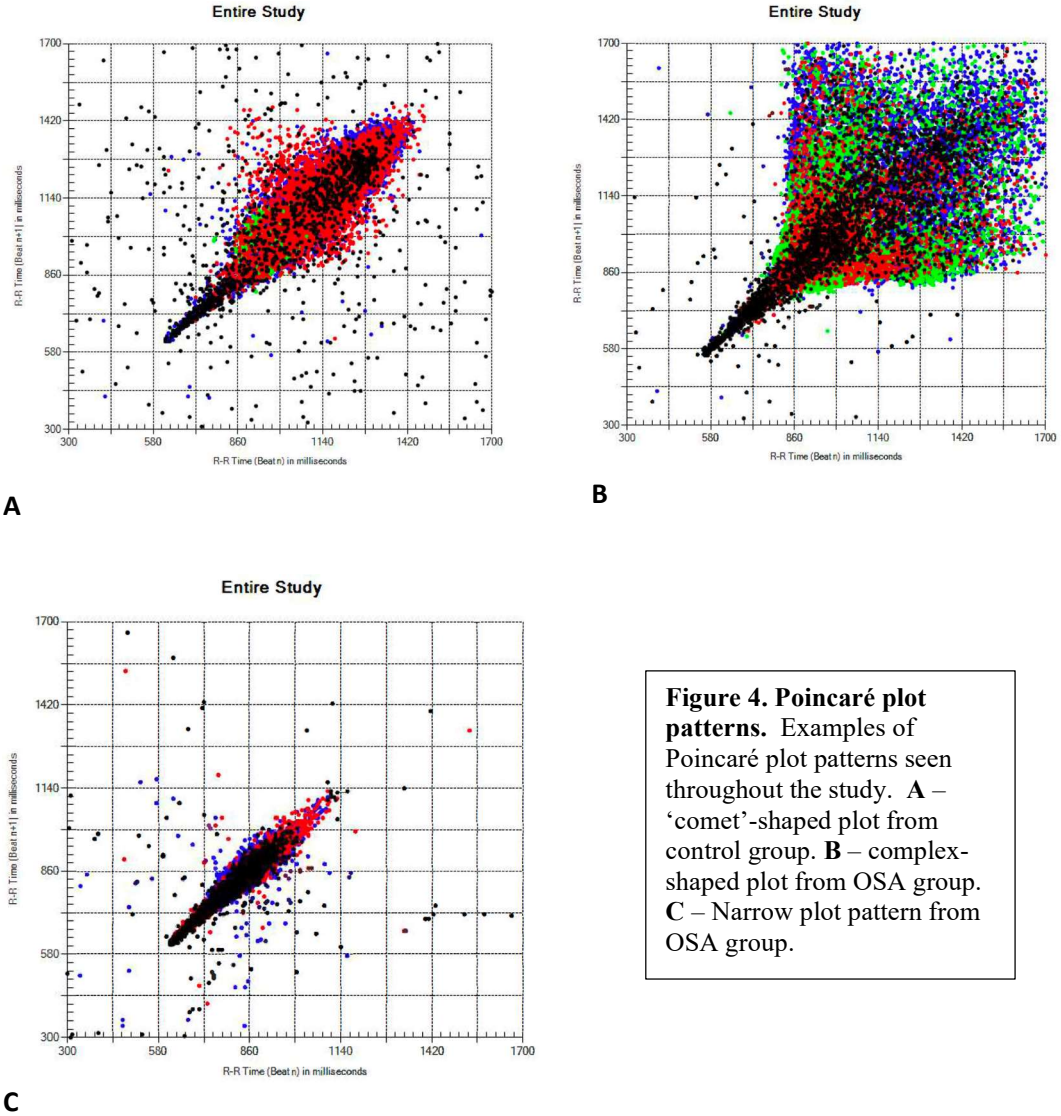
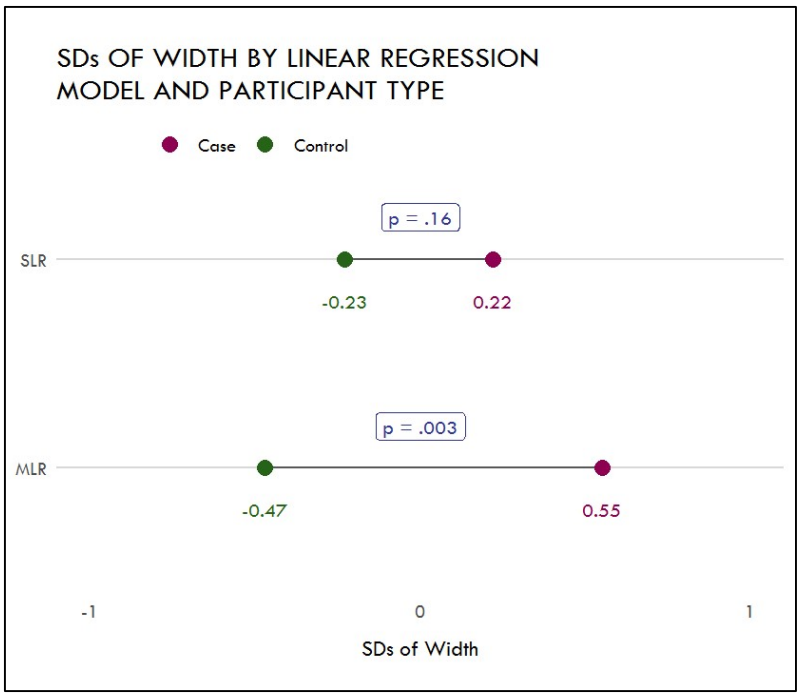


Figure 4. Poincaré plot patterns. Examples of Poincaré plot patterns seen throughout the study. **A** – 'comet'-shaped plot from control group. **B** – complex-shaped plot from OSA group. **C** – Narrow plot pattern from OSA group.

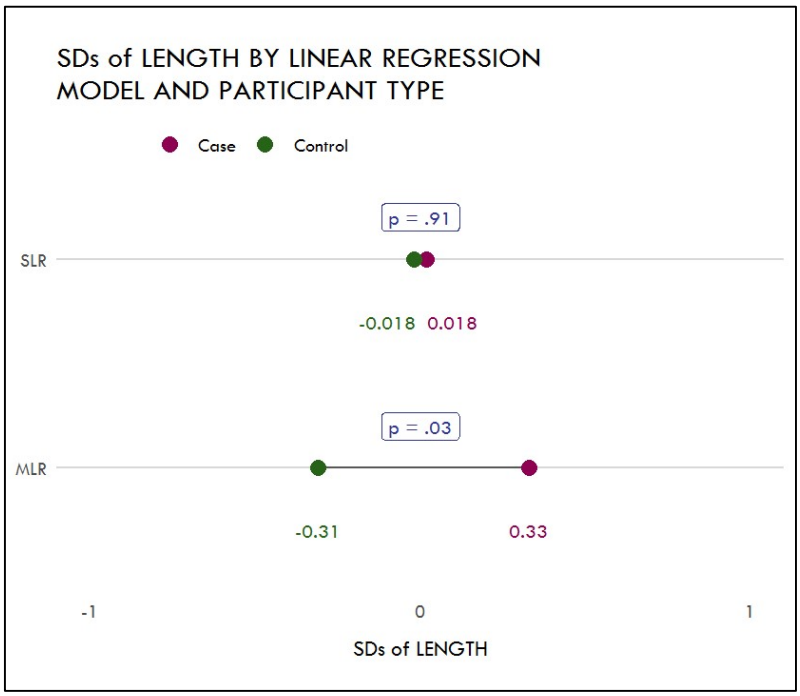
Poincaré Plot Statistical Analysis

To identify if there were statistically reliable differences between the two groups, we tested models that were both unadjusted and adjusted for the demographic differences of BMI, age and sex between the groups. When using unadjusted models, width (Beta=0.45 [-0.18, 1.08], p=0.16), length (p=0.91), and area (p=0.25) were similar across the groups. However, when using adjusted models, width (Beta=1.02 [0.38, 1.66], p=0.003), length (p=0.03), and area (p=0.001) all showed significant differences. Graphical representations of differences between unadjusted and adjusted comparisons are shown in **Figure 5.**

These modeling differences owe largely to the fact that in each model, age and to a lesser extent BMI, were important determinants of the extracted width, length, and area (in each model, age had $p<.005$ and BMI had $p<.05$). However, upon close inspection of the data, we noticed that there were three potential outliers with respect to width, length, and area. When these subjects were removed age and in most cases BMI, continued to predict the variables extracted from the Poincaré plots. However, the OSA status, whether from the OSA or control group was no longer a significant predictor in any model. This is likely due to the fact that all of these outliers occurred in the OSA group, and two of these outliers occurred in young (<30yo) subjects. It remains to be seen whether these outliers are representative of an important group of potential OSA cases or true random noise.

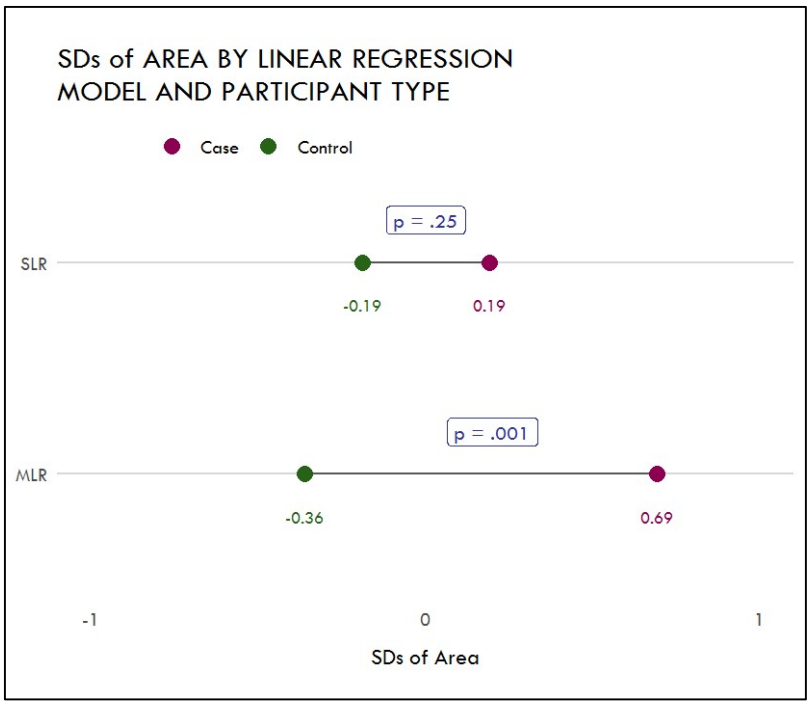


A



B

Figure 5. Poincaré plot statistical analysis. SDs of each parameter compared to total population, using single linear regression models (top lines) and multiple linear regression models (bottom lines). **A** – SDs of width. **B** – SDs of length. **C** – SDs of area.



C

Figure 5. Poincaré plot statistical analysis. SDs of each parameter compared to total population, using single linear regression models (top lines) and multiple linear regression models (bottom lines). **A** – SDs of width. **B** – SDs of length. **C** – SDs of area.

CHAPTER IV: DISCUSSION

Comparison to similar research

The significant findings in the present study were that differences in HRV between cases with moderate OSA and without OSA were detected. Participants were selected at random without matching demographics, therefore, it was important to control for confounding factors such as age, sex and BMI during the data analysis. Study findings were consistent with previous studies in that there was a detectable *change* in HRV parameters; however, it is important to note that these observations were not always consistent with other studies (Aytemir et al., 2007; Dingli et al., 2003; Lado et al., 2012; Narkiewicz et al., 1998). For example, the present study was most consistent to the study

by Kim et al. (2015) in which HRV parameters were analyzed using overnight PSG. The present study found an increase in plot width while Kim and colleagues observed an increase in RMSSD. This was the opposite of study expectations. . Width of a Poincaré plot is considered equal to RMSSD despite coming from different analytic methods. Both parameters represent PNS modulation and one would expect these values to decline since SNS overactivity is typically seen in the majority of the literature (Aytemir et al., 2007; Dingli et al., 2003; Lado et al., 2012; Narkiewicz et al., 1998; Sequeira et al., 2019).

The results of the present study were also consistent with previous work in which screening for OSA by HRV was explored (Roche et al., 1999). They evaluated HRV by using 24-hour Holter monitors and found that the difference between recorded daytime and nighttime measurements held more predictive power of OSA than did nighttime measurements alone. These measurements included RMSSD and SDNN, which were both elevated. Again, increase in these parameters is the opposite of what was anticipated. In Roche's discussion they reviewed autonomic activity changes around an apneic event. Early in an obstruction, the PNS branch is stimulated but as hypoxemia sets in, SNS activity increases and stimulates an arousal. Abrupt and large changes in heart rate and blood pressure are seen throughout this apneic cycle which the authors summarized as 'enhanced nocturnal HRV' characteristic of sleep-related breathing disorders. This provides a possible explanation for the unexpected results.

To make a more direct comparison to similar research, it is useful to explore other studies that have used Poincaré plots. As discussed previously, these plots allow for visual interpretation of the dynamic changes in the heart that can be missed in other

analytic methods. Very few peer-reviewed publications exist which feature research using Poincaré plots specifically to detect HRV changes in OSA. The earliest was conducted by Aljadeff et al. (1997) who described HRV changes in children with OSA compared to children who were primary snorers but who did not have OSA. Each group contained seven participants and the study was conducted using overnight PSG. They found that children with OSA exhibited dramatic changes in HRV induced by apneic events. Poincaré plots showed complex fan-shaped patterns where HRV was reduced at faster heart rates but increased at lower heart rates, with the latter finding being more significant. These patterns resemble some of the patterns found in the present study in the OSA group as shown in **Figure 4B.** To illustrate this phenomenon further, Aljadeff and colleagues displayed Poincaré plots around single apneic events. They found a high heart rate with limited variability during an obstruction followed by a low heart rate and enhanced HRV upon recovery from the apneic event. This sudden high HRV was a consequence of rebound bradycardia from the obstruction. This is in line with Roche's 1999 study and his conclusion of 'enhanced nocturnal HRV'. When comparing the current results to Roche's study, the behavior of the plots makes more sense.

A later study by Mermigkis et al. (2009) used Poincaré plots to assess HRV in overnight sleep studies to evaluate CPAP therapy effectiveness. Poincaré plots were recorded before and after CPAP treatment for 25 patients diagnosed with moderate to severe OSA during an overnight PSG study. They analyzed the plots during different stages of sleep by measuring the plot width, length and area. The results revealed significant decreases in plot width after CPAP treatment, as well as decreased plot area, particularly in the sleep stages N1-2. This latter finding was interpreted as indirect

evidence of a reduction in sympathetic heart activity. They felt that this was proof of treatment effectiveness and that this measurement was easy to obtain via Poincaré plot analysis. Although the present study only described HRV differences between OSA patients and controls rather than analyzing treatment, study findings could be viewed as consistent. Plot width and area were reduced in normal controls, just as Mermigkis found that these parameters were reduced in patients after receiving OSA treatment.

Lastly, Chaidas et al. (2014) evaluated autonomic imbalance in children with OSA using Poincaré plot widths and measurements of morning urine norepinephrine. They found that children with OSA and moderate-to-severe hypoxemia had significantly smaller Poincaré plot widths than controls, and that this was negatively related to AHI and positively related to SpO2, meaning the more severe the disease, the narrower the plot width. The authors considered the Poincaré plot width as representing nocturnal parasympathetic activity, therefore these reduced plots would indicate reduced PNS drive. They confirmed SNS over-reactivity by also showing higher morning urine norepinephrine levels. These findings are the opposite of what was found in the present study. A possible explanation to this is that Chaidas chose to exclude periods of wakefulness in his Poincaré plots, which would include arousals from apneic events. Because rebound bradycardia and a large swing in HRV occurs in this arousal period, it would be reasonable to expect that the Poincaré plot widths would be narrower if wakeful periods were removed.

The method for plot analysis used in the present research was modeled after the studies by Mermigkis et al. (2009) and Chaidas et al. (2014). It is important to note that there are no standardized HRV parameters in OSA populations using Poincaré plot

measurements and it is difficult to interpret results with such limited evidence from other studies. In addition, there were some differences in the present study compared to previously published studies. .

To further examine and explain the current results, a literature review was completed to explore how Poincaré plots explain diseased states. Brennan et al. (2002) described visual patterns of Poincaré plots, where a balanced ANS appeared "comet" shaped, progressively wider along the x=y axis, signifying increased short-term variability as what would be expected in health. He also described a "cigar" shaped plot, being narrow and representative of SNS activity or repressed PNS activity, which represents abnormal function. In this simplified explanation, one would expect to see OSA represented as narrow plots, knowing that SNS over-reactivity is representative of OSA. However, PNS and SNS are complicated in their interactions and are not reciprocal. A good example of this is in a study by Woo et al. (1994) who described Poincaré plot patterns in patients with advanced heart failure. They found both narrow plots as well as "complex" plots in heart failure patients. Complex plots are those that display initial narrow clustering leading into widely dispersed clusters, wider than comet-shaped plots. The point of this is to express that there is an expected "normal" pattern in ANS balance and health and that variations in both directions can be representative of ANS imbalance and poor health. In other words, as Shaffer and Ginsberg (2017) pointed out, even though some diseases will depress non-linear HRV measurements, elevated HRV does not always indicate health.

Since there does not appear to be a standardized Poincaré plot pattern for OSA, more research should be done to explore and confirm typical patterns for this group. The

findings of the present study suggest a larger, more complex pattern may be representative of OSA and could be interpreted as an ANS imbalance. In the OSA group, there were a few participants with narrow plots as well (see **Figure 4C**). This may have been due to not controlling for demographics or comorbid diseases and further studies could elucidate this matter further. Therefore, comparisons from one person to the next may or may not be useful at this time unless in large aggregate numbers, but perhaps comparisons of changes in the pattern within the same person, as in from pre- and post-treatment, may provide further insights.

Limitations

The method used in this study to measure plot parameters were subjective, largely by estimating the bulk of the plot which included artifacts. Artifacts result from technical issues such as from electrodes and ECG recording devices as well as from inadvertent physiological events. These artifacts can distort time-domain and frequency-domain measurements and, although it has been suggested that these artifacts be edited, there is no consensus on a method (Peltola, 2012). In the present study, the only editing done prior to analysis was by not including obvious outlying artifacts. A possible alternative method to editing would have been visually inspecting the raw ECG data and removing artifacts, but this was not possible within the study design presented here. According to Guzik et al. (2007), an advantage of the Poincaré plot was that 'outliers' (i.e. artifacts or ectopic beats) are easily identified visually and that they do not influence HRV to the degree seen in time- and frequency-domain analysis.

Another limitation to the present study was that it was retrospective in nature and that participant selection was done randomly with groups being un-matched in demographics with many unknown variables. For example, it was not known if patients had pre-existing disease or whether they had received previous treatment for sleep-related breathing disorders. The literature tells us that people with OSA are more likely to have comorbid conditions than their healthy counterparts. Also, all patient data was collected from a sleep center. Patients are usually referred to a sleep center for an overnight PSG when there is already a suspicion of some form of sleep-related disorder.

Future Applications

Currently, OSA treatment effectiveness is based on measuring a decrease in AHI and resolution of symptoms (e.g. decreased daytime sleepiness). An important concept to keep in mind is that AHI represents the number of apneic/hyponeic events that occur in an hour but does not describe the duration or magnitude (Sequeira et al., 2019). It is important to also measure end outcomes as well. In the treatment of OSA, a significant end outcome is reducing cardiovascular risk. Measuring blood pressure changes is one way to evaluate cardiovascular risk changes. In the study of physiology in healthy normals and in those with chronic diseases like OSA, , measuring HRV is frequently used, but the application and interpretation of HRV parameters are typically outside the area of expertise for other providers such as dentists. If treatment of OSA is meant to be multi-disciplinary, then understanding research and applying it to clinical use is a must for all providers involved. It is in this capacity that the use of Poincaré plots can be potentially helpful.

If there is an easily identifiable difference on a Poincaré plot with someone that has OSA compared to a healthy person, this might be easy to use to evaluate effectiveness of OAT. As seen in this study and with the limited evidence from other studies, there is no current standard, and with large variations seen within groups. A more feasible application may be using Poincaré plots for evaluation and follow-up of OSA treatment efficacy on an individual basis, i.e. comparing changes to one's own baseline. Further studies are needed to corroborate this application.

Currently, CPAP is regulated at home. Patients with OSA typically have one baseline PSG, and then they are sent home with a CPAP device that is able to monitor its effectiveness remotely (e.g. records pressure, hours of use and AHI). Conversely, OAT effectiveness is sometimes not monitored and when it is, it is usually by a second PSG with titration of the appliance during the sleep study. A more practical approach would be to have the patient titrate the device at home until they reach subjective comfort and improvement. From there, they could use a 24-hour Holter monitor which provides ECG recordings that can be used to analyze HRV, to include Poincaré plots as well as AHI changes.

CHAPTER V: CONCLUSIONS

The present study revealed significant differences in HRV between patients with moderate OSA and without OSA, while controlling for confounding variables such as age, sex and BMI. Previous research evaluating HRV in OSA using Poincaré plots is limited. This study adds evidence that HRV is altered in patients with moderate OSA

compared to those without OSA. It also highlights the Poincaré plot as simple and potentially valuable, particularly as a research tool in measuring treatment outcomes which could be useful in multi-disciplinary care. OSA is commonly treated with a multi-disciplinary approach, including physicians and dentists. Therefore, making evidence-based data easier to understand across fields would fortify the relationships between providers and enhance clinical practice, potentially improving patient outcomes. Further studies are needed using Poincaré plots in assessing ANS characteristics of OSA and the potential health benefits of therapy.

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