The impact of altitude on the prevalence and characteristics of Restless Legs Syndrome

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DECLARATION

I, Pariska Munian, declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at any other University.

Signature of candidate

20th day of March 2024 in Durban .

Abstract

Restless Legs Syndrome (RLS) is a neurological sensory disorder, characterised by the irresistible urge to move due to unpleasant, deep-seated paresthesias in the legs. The urge to move usually occurs in the evening, when an individual is at rest and the sensations experienced are alleviated with movement. The prevalence of RLS in a general population ranges from 2.5 to 10%, from studies across the globe. Differences in RLS prevalence have been noted between different ethnic groups, with individuals of European ancestry exhibiting greater prevalence of RLS compared to individuals of Asian and African ancestry. The aetiology of RLS is unclear; however, there is evidence of central nervous system iron dysregulation. Low partial pressure of oxygen at high altitude, which is a large distance above sea level, may exacerbate iron dysregulation which may account for the greater prevalence of RLS at high compared to low altitude, which is an area at sea level. However, the impact of altitude on the prevalence of RLS requires further investigation and is the aim of this study. To investigate the effect of altitude on the prevalence and characteristics of RLS, a questionnaire was administered to the general South African population at two altitudes: low altitude (Durban, South Africa) and higher altitude, (1753m above sea level, Johannesburg, South Africa). The survey was completed by 1291 participants (416 at low altitude and 875 at higher altitude). Using an online questionnaire, data were collected on demographic characteristics (including age, sex and ethnicity), the Cambridge-Hopkins RLS questionnaire (to assess the presence/absence of RLS), self-reported iron deficiency, subjective measures of sleep, measures of daytime sleepiness (using the Epworth Sleepiness Scale) and levels of fatigue (using the Fatigue Assessment Scale). RLS was significantly more prevalent at the higher altitude (n = 69, 7.9%) compared to low altitude (n = 20, 4.8%), which may be due to an increase in iron dysregualtion at high altitude, resulting from the low partial pressure of oxygen. Factors associated with RLS also were exacerbated at higher altitude; these include increased RLS severity ($p = 0.003$), increased daytime sleepiness ($p = 0.04$) and decreased self-reported iron levels ($p = 0.03$) in individuals with RLS at higher altitude compared with low altitude. RLS was less prevalent in individuals with African ancestry than in those with European ancestry at the higher altitude ($p = 0.0025$). However, RLS was more prevalent in individuals with African ancestry than in those with Indian ancestry at low altitude ($p = 0.0004$). My data therefore support that altitude appears relevant to the pathophysiology of

RLS, with high altitude presenting as a risk factor for RLS and exacerbating some characteristics of RLS.

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ABBREVIATIONS

CH-RLSq	Cambridge-Hopkins Restless Legs Syndrome questionnaire
CAD	Coronary Artery Disease
CNS	Central Nervous System
CVD	Cardiovascular Disease
DCSAD	Diagnostic Classification of Sleep and Arousal Disorders
ESS	Epworth Sleepiness Scale
FAS	Fatigue Assessment Scale
Hb	Hemoglobin
HTDI	Hopkins Telephone Diagnostic Interview
IRLSS	International Restless Legs Severity Scale
IRLSSG	International Restless Legs Syndrome Study Group
MPQ	McGill Pain Questionnaire
MRI	Magnetic Resonance Imaging
PLM	Periodic Leg Movements
RLS	Restless Legs Syndrome

RLS-DI	Restless Legs Syndrome – Diagnostic Index
RLS-QLI	Restless Legs Syndrome - Quality of Life Instrument
ROS	Reactive Oxygen Species
SF-36	Short Form-36 Health Survey
SIT	Suggested Immobilization Test
UVR	Ultraviolet Radiation

1 INTRODUCTION

Restless Legs Syndrome (RLS) is a "neurological sensory disorder/sleep-related movement disorder" which is often undiagnosed in individuals (Vizcarra-Escobar et al., 2015). RLS is characterised by the irresistible urge to move due to unpleasant, deep-seated paresthesia in the lower limbs, which is only relieved with movement (Allen et al., 2003). The uncomfortable feeling in the legs typically begins in the evening and progressively worsens as the night progresses (Gupta et al., 2017). The uncomfortable sensations usually begin when a person is at rest, which often results in increased difficulty falling asleep and a restless sleep at night (Gupta et al., 2017).

Periodic limb movements (PLM) are the involuntary leg movements which occur during sleep and are experienced in up to 80% of patients with RLS (Montplaisir et al., 1997). PLM is most often common in patients with RLS (Guo et al., 2018). Periodic Limb Movement Disorder (PLMD) is a rare sleep disorder distinguished by the periodic and repetitive movements of the lower limbs during sleep (Hornyak et al., 2006). PLMD is a sleep disorder, while as PLM is associated with movement and is characterised by spontaneous motor activity which cause increased arousals from sleep.

The aetiology of RLS remains largely unknown; however, the symptoms associated with RLS have been shown to originate from the central nervous system (CNS). Prevailing theories on the cause of RLS include dopaminergic dysfunction (Erichsen et al., 2010), brain iron dysregulation (Nordlander., 1953; Ekbom., 1960), and peripheral hypoxia (Salminen et al., 2014), and are discussed in detail below. At this time, there is no biological assay available for the diagnosis of RLS, and as such, RLS is diagnosed by subjective diagnostic criteria. However, this method of diagnosis may cause an apparent difference in prevalence results due to heterogeneity in screening tools. The current screening tool for RLS is the Cambridge-Hopkins diagnostic questionnaire (CH-RLSq), which assesses if a patient is positive for RLS, based on a scoring system.

The prevalence of RLS may be influenced by age, sex, and geographical location, as well as ethnicity. The prevalence of RLS may be influenced by altitude. There are two important factors

that might be responsible for the increase of RLS prevalence at high altitude, hypoxia and changes in the body iron at high altitude (Connor et al., 2011).

1.1 Diagnosis of RLS

Karl-Axel Ekbom described the first formal diagnostic criteria for RLS in 1960, where he stated, "The sensations appear only when the patient is at rest, most often in the evening and early part of the night, and produce an irresistible need to keep the legs moving. Furthermore, the sensations are not felt in the skin but deep down inside the legs" (Ekbom, 1960). Since the description by Ekbom, the clinical features of RLS have not drastically changed, however Ekbom's description of the sensations has been altered over time. Over decades that followed, the diagnostic criteria evolved to emphasize the urge to move, rather than the physical sensations felt (Allen et al., 2014). In 1979, the formal diagnostic definition of RLS was first published in the 'Diagnostic Classification of Sleep and Arousal Disorders' (DCSAD), from the 'Diagnostic Classification Committee of the American Sleep Disorders Association' (Howard., 1979). This definition was not agreed upon by experts treating RLS, who described RLS as a disorder associated with movement being initiated and maintained rather than a disorder associated with the urge to move when at rest (Allen et al., 2014). The DCSAD described the essential features of RLS as: "an individual feels extremely disagreeable deep sensations of creeping inside the calves whenever sitting or lying down. These dysesthesias cause an almost irresistible urge to move the legs, thus interfering with sleep" (Howard., 1979). Similarly, to Ekbom's description, the DCSAD stated that the sensations felt in the lower limbs were the primary cause of the urge to move. This is contrary to the findings of current studies that claim that the primary characteristic of RLS is the urge to move and can occur in the absence of the uncomfortable sensations (Allen et al., 2014). The DCSAD stated that RLS severely reduces sleep time, causing excessive daytime sleepiness. Recent studies do not entirely support this as they demonstrate that although patients with RLS have reduced total sleep time, their daytime sleepiness scores are considered as normal (Allen et al., 2014).

In 1990, the 'American Sleep Disorder Association' released a revision of the DCSAD RLS diagnostic criteria (Allen et al., 2014). This revision was done by experts in the field of sleep medicine, without the consensus of RLS experts. RLS was classified as an 'Intrinsic Sleep Disorder' and defined as 'disagreeable leg sensations', commonly experienced before sleep and

causing an irresistible urge to move the legs. This definition of RLS emphasized symptoms at the onset of sleep and at night, contradicting the current assessment of RLS which states that symptoms occur solely during waking hours, plus are common in the late afternoon and evenings (Allen et al., 2014).

As a response to the perceived inaccuracies surrounding the 1990 RLS diagnostic criteria, the 'International Restless Legs Syndrome Study Group' (IRLSSG) was founded in 1995 (Allen et al., 2014). The IRLSSG consisted of 28 founding members, who together developed the first diagnostic criteria, which was based on the consensus of global RLS experts. The IRLSSG set the "four minimal criteria" for RLS, which are still used as the basis for diagnosis today (Allen et al., 2014). Using the minimal criteria as a basis led to an accumulation of RLS research, which led to the exposure of limitations in the 1995 initial IRLSSG conceptualization of RLS diagnostic criteria. In 2003, an epidemiology workshop was held with the purpose of revising the 1995 IRLSSG criteria (Allen et al., 2014). During this workshop, the 1995 IRLSSG criteria was upgraded plus reworded to provide better dependability for RLS diagnosis by researchers (Allen et al., 2003). These diagnostic criteria included wording changes that led to the following 2003 criteria:

1. "A sudden urge to move the legs, usually occurring with an uncomfortable sensation in the legs".
2. "The impulse to move or unpleasant sensations start or worsen during sessions of rest or inactivity".
3. "The longing to move or unpleasant sensations are partially or wholly relieved by movement".
4. "The symptoms worsen in the evening or night".

In the year 2014, the RLS criteria were once again amended, with an additional criterion to differentiate RLS from RLS mimics, such as leg cramps (Allen et al., 2014). RLS is now diagnosed according to five-point diagnostic criteria, which was outlined by the IRLSSG, as follows (Allen et al., 2014):

1. “The longing to move the legs is commonly accompanied by or caused by an uncomfortable sensation in the legs; however, this is not always the case. An unpleasant sensation being felt by the individual is unnecessary for the diagnosis, an urge to move the legs can suffice”.
2. “The need to move the legs and the feeling of an uncomfortable sensation begins or is exacerbated during an elongated time of rest such as lying down”.
3. “The need to move the legs and the feeling of an unpleasant sensation can be slightly or completely relieved by movement of the legs, such as walking. The sensation will be alleviated as long as movement continues”.
4. “The need to move the legs and the feeling of an unpleasant sensation during times of rest only appear or are exacerbated in the later part of the day or the night”.
5. “The instances of the above features are not only seen as symptoms primary to another medical condition, example arthritis, leg cramps or habitual tapping of the foot”.

Prior to the development of the latest diagnostic criteria, epidemiological studies of RLS were restricted by the absence of a validated diagnostic questionnaire, which could be completed by a patient, which has a high specificity and was able to provide a positive predictive value. The majority of diagnostic questionnaires, completed by patients, have either not been validated, or exclude items separating the diagnosis of RLS from other medical conditions which have similar symptoms. Three diagnostic tools for RLS have been validated. The first instrument incorporates the five diagnostic criteria for RLS into a single question, which can be self-administered. The RLS Diagnostic Index (RLS-DI) includes both non-essential and essential criteria for RLS into an algorithm to improve diagnosis and is based upon patient interview and the collection of objective data from chart review and polysomnography (Beneš and Kohnen., 2009). The second diagnostic tool used to diagnose RLS is the ‘Cambridge-Hopkins diagnostic questionnaire for RLS’ (CH-RLSq) which was developed in 2005, and included commodities describing the primary diagnostic features of RLS and would provide a basic diagnosis (Allen et al., 2009). The final diagnostic tool used to diagnose RLS is ‘Hopkins Telephone Diagnostic Interview’ (HDTI), a diagnostic clinical interview that can be used by someone who is familiar with the basic characteristics of RLS, to make a diagnosis without any in-person contact (Allen et al., 2009). In 2008, Allen et al. conducted a large population-based prevalence study of RLS. From the study population questionnaires, a sample including 185 participants were selected for a separate clinical interview which would be administered telephonically, and RLS was diagnosed using the

validated HDTI. This telephonic interview was conducted by qualified RLS experts. All telephonic interviews conducted were not aware of the information on the diagnostic questionnaire and took place within 2–4 months after the questionnaire had been finished by the subject. The HTDI was then reviewed by the other experts, who were unaware of the previous diagnosis. The CH-RLSq had a normalized sensitivity of 87.2% and specificity of 94.4%, for patients with RLS compared to healthy controls. This finding means that the CH-RLSq has a high ability to correctly identify individuals with/without RLS. The predictive values in the sample were 85.5%, meaning that there was a high ratio of patients diagnosed as positive in comparison to all those who had positive test results. Patients with RLS are classified as having definitive RLS or indicated as RLS+, meaning they fulfill the diagnostic criteria and meet all the necessary requirements laid out by the CH-RLSq for the presence of RLS. The criterion for definitive RLS was derived on the basis of the formal clinical definition of RLS and the exclusion of "mimic conditions". Patients without RLS, or healthy controls, are indicated as RLS- as they do not meet any of the RLS diagnostic requirements. A scoring criterion was also set to determine if the individual was 'RLS probable'. RLS probable means an individual that have some of the characteristics of RLS, but are not in fulfillment of the complete diagnostic criteria (Cho et al., 2009). Therefore, the CH-RLSq can be used to provide a reasonable level of both sensitivity and specificity to screen for RLS in a population-based study (Allen et al., 2009).

1.2 Characteristics of RLS

1.2.1 Descriptors of sensations associated with RLS

The uncomfortable sensations associated with RLS are commonly described as "aching, throbbing, pulling, itching, crawling, or creeping" (O'Keeffe., 1996). Patients with RLS often have difficulty in describing the sensations they are feeling and in differentiating between sensory and motor symptoms (Benes et al., 2007). Sensory symptoms, which may include pain, have a strong association with quality of life but are not as well comprehended as motor symptoms and sleep disruptions in RLS (Winkelman et al., 2013). Researchers investigating the sensory symptoms of RLS report that most patients with RLS do not use conventional words to describe their RLS sensations, and often resort to the use of emotive/affective words as sensory descriptors (Winkelman et al., 2013). Karroum et al., (2012) conducted a study to characterise

the verbal descriptors of RLS sensations. The study included 56 participants with primary/idiopathic RLS, who were interviewed in person, and 738 participants, who were sent a questionnaire to complete via post (Karroum et al., 2012). Study participants were asked to detail their RLS sensations and the 'McGill Pain Questionnaire' (MPQ) (Melzack, 1975) was used to assess RLS sensations. The study found that all the participants from both of the groups had atypical sensations associated with the irresistible urge to move the legs, typical of RLS. The majority (95%) of patients reported spontaneous "electrical," "prickling," "burning," "tingling," and "itching" sensations. More than 25% of participants chose the sensory words "electric shocks," "irradiating," and "tingling," from the MPQ and the affective words "exhausting," "distressing," "unbearable," "irritating," and "depressing." The participants also used more heat-associated language, rather than cold descriptors to describe their sensations. Moreover, 47– 61% of patients with RLS perceived these uncomfortable sensations as painful, indicating that not all patients with RLS experience pain (Karroum et al., 2012). The results from this study were very similar to the findings of another study where researchers investigated the validity of the current diagnostic descriptors in 41 participants with RLS and found the most frequent spontaneous descriptors to be: "irritating", "painful", and "urge to move"; with prompted descriptors including: "restless", "uncomfortable", and "need to stretch" (Kerr et al., 2012). This study concluded that the most frequently used descriptors were different from the terminology used in the RLS diagnostic criteria and that including these frequently used descriptors may improve the accuracy of RLS is diagnosed (Kerr et al., 2012). In addition, the researchers found that the terminologies used to describe the sensations of RLS were changed when participants with RLS experienced pain. Patients selected more pain-related literary terms and selected more intense descriptors than the non-painful RLS group (Kerr et al., 2019). Participants experiencing painful RLS used words such as, "aching", "painful", "cramping" and "unbearable", which showed that RLS descriptors were changed when a participant experienced pain, indicating a possible aetiological difference in participants who have painful RLS compared to non-painful RLS.

A study, conducted by Picchietti et al. (2011), analysed symptom descriptors from patients with paediatric RLS, where professional interviewers ran in-person interviews with the young patients, aged 3-17 years old, who were positive for all the criteria indicating definite RLS. The study consisted of 33 patients with RLS, who were provided with 16 contrasting groups of descriptors for RLS sensations and the most common descriptors used were, ''need to

move/kick,'' ''pain/hurts,'' ''uncomfortable/cannot get comfortable,'' and ''like bugs or ants/crawling''. The "urge to move" was common amongst the majority of patients, across all adolescent ages. This "urge to move" occurred during passive activities, when the individual was physically inactive, most frequently when in a supine position. Some individuals indicated that they ''could not control'' their lower limbs or were unable to prevent them from moving while others emphasised that if they did not move when the urge arose, their lower limbs would become exceedingly uncomfortable (Picchietti et al., 2011). All the studies focusing on RLS descriptors indicate the importance of using the most precise group of descriptors for RLS in order to improve diagnostic accuracy and optimise treatment of RLS.

1.2.2 Severity

RLS can vary in severity, ranging from slightly annoying to severely disrupting sleep and quality of life (Allen et al., 2005). Symptoms may occur once or twice per week in moderately severe cases but can also appear more than this in severe cases (Allen et al., 2014). The IRLSSG developed, and validated, a ten-item scale in order to assess the severity of a patient's RLS (International Restless Legs Syndrome Study Group., 2003). This 'International Restless Legs Severity Scale' (IRLSS) is described as having a high measure of accuracy and is the main scale for assessing the severity of RLS, including the assessment of symptom severity and the impact of symptoms on quality of life (Allen et al., 2003). As such, the IRLSS is an important tool for assessing the efficacy of new treatments. Although the IRLSS is used extensively as a measure of RLS severity, the scale does not capture all aspects of severity, such as the number of limbs involved or the rapidity with which symptoms develop when a patient is at rest.

1.2.3 The effect of RLS on sleep and quality of life

The symptoms of RLS are worse at night and usually occur while lying down and therefore have a large impact on sleep (Allen et al., 2010). Patients with RLS experience an increased frequency of sleep disturbances, when compared to healthy controls (Abetz et al., 2004; Berger et al., 2004; Allen et al., 2005; Allen et al., 2010; Kushida et al., 2007). A review paper reported that 14 studies observed insomnia as a prominent clinical consequence of untreated RLS (Earley and Silber, 2010). Due to the diversity of the study populations, as well as the different diagnostic criteria and symptoms, the results obtained from these various studies differ in SF-36 scores and

sleep and wakefulness hours of the RLS participants. However, in all studies, 50-85% of RLS participants reported experiencing an inability to rest, affecting their time taken to fall asleep and the inability to stay asleep (Earley and Silber, 2010). A sleep study conducted in 2007 compared polysomnography findings in patients with idiopathic RLS to that of healthy controls (Hornyak et al., 2007). The study concluded that patients with RLS expressed prolonged sleep onset latencies, shorter amount of time spent asleep, lower quality of sleep and longer REM sleep latency compared to the healthy controls (Hornyak et al., 2007). The 'RLS-Quality of Life Instrument' (RLS-QLI) is a questionnaire which was custom-developed, in 2004, to evaluate the effect of RLS on the quality of life among patients with RLS (Atkinson et al., 2004). The RLS-QLI was used by researchers to assess the symptoms of RLS and the consequences on quality of life (Kushida et al., 2004). The results of the RLS-QLI showed that due to the increased sleep disruptions involved with RLS, patients often experience decreased level of alertness and increased amount of emotional distress throughout the day. Overall, sleep studies have confirmed sleep disturbances, as well as decreased sleep efficiency and an overall reduced total sleep time in patients with RLS, which may cause an increased amount of daytime sleepiness and reduce an individual's quality of life (Earley and Silber, 2010).

An instrument used to measure an individual's quality of life based on their health, not specific to RLS, is the 'Short Form-36 Health Survey' (SF-36) (Jenkinson et al., 1993). SF-36 is a multipurpose health questionnaire which is comprised of 36 questions, assessing the physical and mental health of an individual (Ware, 2000). Low scores on the SF-36 from patients with RLS indicate an increased impact of RLS on quality of life (Allen et al., 2005). The quality of life in patients with RLS was examined in five major studies using the SF-36 (Abetz et al., 2004; Berger et al., 2004; Allen et al., 2005; Allen et al., 2010; Kushida et al., 2007). Studies ranged from 58 to 433 participants with RLS. The overall results were consistent across all five surveys, where participants with RLS scored lower on the SF-36 compared to healthy controls (Earley and Silber, 2010). The low SF-36 scores of participants with RLS suggested that the disorder significantly decreased a person's quality of life (Abetz et al., 2004).

Depression and anxiety have both been linked to patients with RLS symptoms. Six population-based studies have investigated the correlation between depression and anxiety with RLS (Banno et al., 2000; Bassetti et al., 2001; Mosko et al., 1989; Sevim et al., 2004; Sukegawa et al., 2003;

Ulfberg et al., 2001). The results from all six studies consistently show an increase in prevalence rates or an increased risk of depressive episodes and symptoms of anxiety in patients with RLS. A separate case-controlled survey investigated the association between RLS and Major Depressive Disorder and Panic Disorder in a Baltimore community (included 982 healthy controls and 42 patients with RLS). The survey data showed a 16.7% increased risk for both depression and anxiety in the patients with RLS compared to the healthy controls (Lee et al., 2008). Similarly, a study using questionnaires found that patients with RLS were at a significantly higher risk for both anxiety and depression, as compared to the healthy controls (Winkelman et al., 2006). Therefore, there is a strong association between the symptoms of RLS and depression and anxiety, which may also further exacerbate sleep disturbances, as sleep and depression share an intricate reciprocal relationship (Lee et al., 2008). Depression negatively impacts an individual's daily functioning and well-being, resulting in a decrease in quality of life.

1.3 Aetiology of RLS

The exact aetiology of RLS is unknown but previous research suggests that abnormal regulation of dopamine, low iron levels in the brain and genetic linkages may play a vital part in the development of the symptoms RLS, and these are likely not mutually exclusive (Muth, 2017). Below is a brief overview of the factors implicated in the aetiology of RLS.

1.3.1 Dopaminergic dysfunction

The pathogenesis of RLS has been linked to dopaminergic dysfunction, as the symptoms of RLS have been shown to improve with treatment by dopamine agonists (Erichsen et al., 2010).Dopamine may modulate movement by controlling the tone and contraction in skeletal muscles influencing the postsynaptic D1 and D2 receptors located in the striatal locus, which is responsible for motor control in the muscle pairs around specific joints (Korchounov et al., 2010). Therefore, the dysregulation of dopamine may be associated with the symptoms of RLS as the hypothalamic dopaminergic A11 cell group is the solitary source of spinal dopamine, and the A11 nuclei in the diencephalon spinal dopaminergic tract is theorised to be the anatomical region of origin of dopaminergic dysfunction in RLS (Sharples et al., 2014). Dopaminergic dysfunction arises when the A11 cells cluster together (Sharples et al., 2014). The closeproximity

of the A11 nuclei to the hypothalamus' suprachiasmatic nuclei is also known to modulate cyclical dopamine levels (Sharples et al., 2014), with the lowest plasma concentrations, in the CNS, occurring in the evening, which corresponds with the circadian diagnostic criterion of RLS.

The loss of A11 dopaminergic control in the grey matter of the spinal cord may cause alterations to an individual's autonomic function (Clemens et al., 2006). Clemens et al., (2006) reported that the decreased spinal dopamine input causing autonomic inhibition, may induce atypical visceral feelings in the muscles, which are frequently described as the inability to remain still, as well as muscle restlessness, a characteristic of RLS and PLM. PLM can be described as sudden jerks of movement and RLS is characterized by feelings of discomfort, both of which can be caused by loss of supra-spinal inhibitory influence (enhanced spinal excitability) (Clemens et al., 2006). Decrease of spinal dopamine release also explains dopaminergic drug treatments used to alleviate RLS/PLM symptoms: the drug-induced increases in dopamine override decreased function in the CNS dopamine signaling, resulting in a more regulated passage of dopamine to the spinal cord. Furthermore, tyrosine hydroxylase is the rate-limiting enzyme that is required for the synthesis of dopamine, and iron is required for the activity of this rate-limiting enzyme. As such, iron or iron deficiency specifically, has also been implicated in the aetiology of RLS.

1.3.2 Brain iron dysregulation

Iron deficiency, particularly in the brain, has been regarded as a significant contributing factor of RLS (Nordlander, 1953; Ekbom, 1960). As mentioned in section 1.3.1, iron is essential for the production and regulation of dopamine (Sharpleset al.,2014). The main role of iron in the body is the transportation of oxygen to vital organs; however, iron has many other functions including the synthesis of the neurotransmitter's dopamine and noradrenalin (Hare et al., 2013; Guo et al., 2017). Additionally, to the synthesis of dopamine, iron also has a role in the conversion of tyrosine and levodopa into dopamine, as iron is a co-factor of tyrosine hydroxylase (the enzyme responsible for the conversion) (Guo et al., 2017). Therefore, decreased iron may disrupt dopamine synthesis, potentially causing the onset of RLS symptoms. A 2001 study used magnetic resonance imaging(MRI) techniques to quantify iron levels in the brain, established that patients with RLS had a lower iron concentration in the substantia nigra and in the red

nucleus, compared to healthy controls (Allen et al., 2001). When examining the effect of RLS on brain chemistry, it was observed to be similar to that of brain iron deficiency (Hare et al., 2013). Researchers observed that in patients with RLS, there appeared to be a marked decrease in the reabsorption and transmission of dopamine, as well as reduced ferritin levels in the cerebrospinal fluid (Allen et al., 2001).

Ferritin is a protein involved in the storage and release of iron, as well as provides an indirect measure of plasma iron levels (Muñoz et al., 2010). In healthy individuals, iron is released into the circulation when hepcidin binds with ferroportin, consequently causing the ferroportin to degrade which results in decreased serum iron (Weinstock et al., 2020). Patients with RLS have been seen to have an increased concentration of hepcidin, leading to a more rapid degradation of ferroportin, thereby resulting in iron deficiency (Weinstock et al., 2020). Several studies have revealed a strong relationship between serum ferritin levels and the severity of RLS symptoms; more specifically, a reduction in ferritin levels was associated with an increase in RLS severity (O'Keeffe et al., 1994; Sun et al., 1998). Overall, the data propose that the capability of the brain to transport and store iron is impaired in idiopathic RLS (Sharpleset al.,2014).

Low iron levels may also play a role in local hypoxia and ischaemic injury in patients with RLS (Wessling-Resnick, 2010). Researchers found that there is an activation of the hypoxic pathway in the brains of patients with RLS due to low iron, which increases the expression of proteins in the hypoxia pathway (Connor et al., 2017). Although there is a lack of evidence that patients with RLS are hypoxic, it has been observed that environmental hypoxic conditions may worsen RLS symptoms (Connor et al., 2017).

1.3.3 Peripheral Hypoxia

Peripheral hypoxia has been linked with the appearance of RLS symptoms. The strong correlation linking peripheral hypoxia and RLS severity indicates that a pathophysiologic link between peripheral hypoxia and the symptoms associated with RLS might exist (Salminen et al., 2014). A case-control study conducted the suggested immobilization tests (SIT) on 15 patients with RLS and 14 healthy controls, and non-invasively assessed peripheral oxygen and carbon dioxide partial pressures (Salminen et al., 2014). The study found that patients with RLS had a decreased partial pressure of oxygen in their lower limbs, but not their chest, and that those with

severe RLS had a higher oxygen gradient between their feet and chest. Carbon dioxide levels remained unchanged. Therefore, Salminen et al (2014) concluded that there are links between peripheral hypoxia and the emergence of RLS symptoms and the severity of RLS (Salminen et al., 2014).

Some researchers have hypothesised that the hypoxia inducible factor-1α (HIF-1α) pathway was activated in substantia nigra neurons and brain microvasculature in patients with RLS (Patton et al., 2011). This pathway is normally activated when the body experiences low oxygen concentrations/ hypoxia and plays a role in regulating oxygen homeostasis, as well as regulating the shift to anaerobic metabolism (Ziello et al., 2006). To test the activation of the HIF-1α pathway in patients with RLS, the substantia nigra tissue of six patients with RLS and six healthy controls were immunohistochemically analysed for HIF-1α, neuronal nitric oxide synthase (nNOS) and nitrotyrosine immunoreactivity. Microvessel lysates were acquired from the cortex tissue of the brain and immunoblot analyses were used to quantify HIF-1α, HIF-2α and vascular endothelial growth factor (VEGF) expression. Patton et al (2011) found a drastic increase in the immunoreactivity of HIF-1α in the substantia nigra neurons in five of the six patients' with RLS in comparison to the healthy controls. Additionally, an up-regulation of nNOS and nitrotyrosine expression was observed in the substantia nigra of four of the six patients with RLS when compared with the healthy controls (Patton et al., 2011). The expression of HIF-2α and VEGF was also drastically increased in the microvasculature lysates in the RLS cortical brain tissue in comparison to the healthy controls; an indication that your tissues aren't getting enough oxygen, resulting in the upregulation of VEGF resulting in new blood vessels growth to bring in more oxygen. Overall, this study demonstrates the activation of the hypoxic pathway in many types of cell in patients with RLS. An increase in nNOS and nitrotyrosine levels indicates that nitric oxide plays a role in the activation of the hypoxic pathways, and the activation of the hypoxia pathway may which may be caused due to an iron deficiency (Patton et al., 2011). As altitude increases, there is a decrease in the air pressure, reducing the atmospheric partial pressure of oxygen and causing the reduction in the oxygen saturation of haemoglobin (Hb) in the blood plasma (Muckenthaler et al., 2020). The people living at high altitudes need to increase and maintain adequate oxygenation levels; resulting in an increased rate of erythropoiesis (increased red blood cell production). Erythropoiesis requires a higher than normal amount of iron in the body

because it increases the body's capacity to transport oxygen (Muckenthaler et al., 2020). The increased erythropoiesis at high altitude may cause iron levels to be reduced in the muscle and brain, subsequently causing changes in the dopamine levels and consequently leading to the development of RLS (Connor et al,. 2011). This mechanism is further elaborated on in section 1.5.

1.3.4 Altered neurotransmitter pathways

Although the pathogenesis of RLS is often connected to dopaminergic dysfunction, as well as iron deficiency, some researchers have hypothesised that other neurotransmitters or neuromodulators, namely glutamate, gamma-hydroxybutyric acid (GABA), and adenosine might also have a role in the pathogenesis of RLS (Jiménez-Jiménez et al., 2019). Experimental animal models have used iron deficiency, which causes sensorimotor symptoms mimicking the symptoms of RLS, to initiate changes in dopaminergic, glutamatergic and adenosinergic neurotransmission, thus indicating their possible rolein the pathogenesis of this disease (Wang et al., 2004). The principle inhibitory neurotransmitter in the CNS is GABA and has been associated in the pathophysiology of RLS due to the improvement of RLS symptoms when treated by GABAergic drugs (Rizzo et al., 2002; Allen et al., 2013; Winkelman et al., 2014). Symptoms of RLS have also been improved with the treatment of alpha-2-delta calcium-channel ligands (including Gabapentin and Pregabalin), indicating a role of glutamate in the pathogenesis of RLS as well (Rizzo et al., 2002; Allen et al., 2013; Winkelman et al., 2014). Multiple proton MR spectroscopy (1 HMRS) studies have been conducted, which examined the status of excitatory amino acids (aspartate and glutamate) and/or GABA in RLS, linking these neurotransmitters to the pathophysiology RLS (Rizzo et al., 2002; Allen et al., 2013; Winkelman et al., 2014). With regards to the involvement of GABA, a study has shown a link between GABA levels and PLM indices as well as with, RLS severity (indicating a positive correlation with GABA levels in the thalamus and a negative correlation with GABA levels in the cerebellum), which indicated that the overactivity of the cerebellum contributed to the RLS symptoms through its effect on the striatum, via the thalamic nuclei (Allen et al., 2013). In a separate study, partial ablations were made to the descending glutamatergic pathways to the spinal cord in rodents which induced movements, similar to those associated with RLS, during the transition from sleep to wakefulness (Guo et al., 2018). Partial ablation to the secondary

motor cortex and somatosensory cortex had a similar effect, inducing movements similar to those associated with RLS during rapid eye movements (REM) sleep (Guo et al., 2018). Yepes et al. (2017) showed that rats that were fed an iron deficient diet had a larger release of glutamate at corticostriatal glutamatergic terminals, suggesting that the aforementioned terminals may be related to symptoms of RLS. The thalami of RLS patients suffering from severe and frequent sleep arousals contain an upregulation of glutamate (Allen et.al, 2013). Glutamate is responsible for sufficient arousals from sleep (Allen et.al, 2013).

Adenosine is another neurotransmitter/neuromodulator linked the pathophysiology of RLS (Jiménez-Jiménez et al., 2019). The deficiency of iron in the brain is associated with changes in adenosinergic transmission and the downregulation of A1 receptors (Ferre et al., 2018). Striatal adenosine A1 and A2 receptors are major modulators of adenosine signaling (Ferre et al., 2018). Iron deficiency diet in rodents resulted in downregulation of the A1 receptor in the cortex and striatum, and an A2 receptor density increase in the striatum (Ferre et al., 2018). This caused a disruption in the adenosine-dopamine-glutamate equilibrium in the striatum, and an inhibitory effect of A1 receptors on glutamate neurotransmission in the cerebral cortex (which play a role in hyperarousal and PLMS). These results suggest that adenosine neurotransmission may contribute to the pathophysiology of RLS (Ferre et al., 2018).

1.3.5 Genetics

Several global epidemiological studies have investigated the role of family history in the pathophysiology of RLS (Vogl et al., 2006; Ulfberg et al., 2007). The number of patients with RLS with a positive family history has been found to vary widely, with the lowest being 18.5% in India (Rangarajan et al., 2007) and the highest prevalence being 59.6% in South Tyrol (Vogl et al., 2006). A Swedish study conducted in 2007 reported that 28.3% of patients with RLS had a positive family history (Ulfberg et al., 2007), while a French study reported that 40.9% of individuals affected by RLS had a familial history (Tison et al., 2007). The inheritance of RLS follows a pattern of autosomal dominance in one third of familial cases, specifically in families with early onset RLS, typically before the age of 45years (Ohayon et al., 2012). This pattern of dominance was first hypothesized in 1997 after a study in France found that 63% of patients with RLS reported that at least one member in their family had similar symptoms (Montplaisir et al., 1997). Another study by Winkelmann et al. (2000) in Germany, found that in a population of 300

patients with RLS, only 11.7% of those individuals had a clear and positive family history. In contrast, other studies have claimed that the inheritance of RLS is bimodal, with autosomal dominance being present with early onset RLS, while RLS at a later stage is due to other factors such as dopaminergic dysfunction and brain iron dysregulation (Winkelmann et al., 2002). A study on the clinical correlation of RLS in monozygotic twins reported that the concordance rate of RLS between identical twins was 83.3%, however the age of disease onset, as well as the signs and symptoms of the disease varied in the twins (Ondo et al., 2000). Desai et al. (2004) found that when assessing RLS in monozygotic and dizygotic twins, the heritability of RLS was 0.6, meaning that 60% of the variability in the RLS population is due to genetic differences among people. The signs and symptoms in both familial and idiopathic RLS are similar; however, RLS can be seen at an earlier age in people with a family history (Desai et al., 2004).

Genetic factors have been linked to the causation of RLS because idiopathic RLS commonly affects patients a positive family history (Ekbom et al., 2009). Though no specific gene mutations have been found in patients with RLS (Picchietti et.al, 2017), single nucleotide polymorphisms (SNPs) such as BTB Domain Containing 9 (BTBD9), Meis Homeobox 1 (MEIS1), Mitogen-Activated Protein Kinase 5 (MAP2K5)/SKI family transcriptional corepressor 1 (SKOR1), protein tyrosine phosphatase receptor type delta (PTPRD), and TOX high mobility group box family member 3 (TOX3) have been identified in global association studies, conducted on patients with RLS (Picchietti et.al, 2017). SNPs are polymorphic and frequent in same species members. SNPs in the alleles of the PTPRD and BTBD9 genes exhibit a strong correlation with RLS. The rs1975197 SNP in the PTPRD gene is related to the likeliness of the development of PLM in RLS patients (Moore et.al, 2014), and the rs3923809A allele in the BTBD9 gene has also been linked to increase PLM in patients with RLS (Moore etal., 2014). It is important to note that a majority of genetic studies have been performed in populations with European ancestry, which may not be an accurate reflection of RLS genetics across the globe.

In conclusion, theories regarding the pathogenesis of RLS have been shown to have a strong genetic link. Symptoms are usually not different across idiopathic and secondary RLS. Though a variety of mechanisms may be involved in the pathogenesis of RLS, the similarity of the symptoms across all subsets of RLS indicates common pathways may be involved. The pathogenesis of RLS can also be linked to dopaminergic dysfunction, iron deficiency, hypoxia

and genetic linkages. The symptoms of patients with RLS have been observed to respond well to dopaminergic therapy, and multiple sources have noted the prominence of iron deficiency in patients with RLS.

1.4 Prevalence of RLS

Epidemiological studies on the prevalence of RLS in a general population have been conduceted worldwide, with a large number of these studies conducted in Europe and North America. The general prevalence estimates of RLS range from 2.5 to 10% (Garcia-Borreguero et al., 2006). However, as shown in Table 1.1, when including studies from all continents, the prevalence range increases to 0.03 – 24.2%. This variance may be the consequence of differences in study design and population sampling (Koo, 2015), differences in population dynamics and/or due to a lack of biological assays available to diagnose RLS (as described in section 1.1above). The most recent change to the RLS diagnostic criteria, made in 2014, may cause the results of all studies conducted prior to 2014 to have overestimated the prevalence of RLS due to the lack of excluding mimics of RLS.

Table 1.1: Studies conducted on the prevalence of Restless Legs Syndrome (RLS),in adult populations, listed according to geographic location

Continent	Author, Year of Publication	Location, Altitude (m)*	Age of Participants (years)	Sample Size, Sex	Diagnostic Criteria	Reported Prevalence (%) of RLS	Study Design and Methodology
Africa	Winkler et al., 2010	Tanzania, 1018	≥14	7654 ♀: 8329 ♂: 3825	IRLSSG 2003	0.03	In person interview conducted in a midsized hospital. Patients who answered one RLS question positively were questioned again,and diagnosed, byan RLS expert.
	Ferreira et al., 2013	Mozambique, 345	≥18	118 ♀: 79 ♂: 39	Unspecified IRLSSG criteria	6.77	An in-person interview was conducted on participants who experienced chronic pain. Thereafter, RLS diagnosis was made by an RLS expert.
	Burtscher et al., 2014	Tanzania, 1018	≥14	35 ♀: 27 ♂: 8	IRLSSG 2003	0.47	In person evaluation. Patients who positively answered anRLS question were questioned again, and diagnosed, by a neurologist.
	Fawale et al., 2016	Nigeria, 380	≥65	633 ♀: 494 ♂: 105	IRLSSG 2003	3.5	Participants comprised of elderly individuals attending the general outpatient clinic in State Hospital.
Asia	Kageyama et al., 2000	Japan, 438	≥20	4612 ♀: 3600	Unspecified IRLSSG	8.5	Single question asked – “Have you ever experienced sleep disturbance due to creeping sensation or hot feeling in your legs?”

				♂: 1012	criteria		
	Tan et al., 2001	Singapore, 15	≥55	157 Sex distributi on not specified	IRLSSG 1995	0.6	Individuals were examined for RLS, positive to four IRLSSG criteria
	Kim et al., 2005	South Korea, 282	40–69	9939 ♀: 5228 ♂: 4711	Unspecified IRLSSG criteria	12.1	Study conducted on the general Korean population during an in-person interview. A positive answer to a single RLS question indicted a positive RLS diagnosis.
	Mizuno et al., 2005	Japan, 483	≥65	3287 Sex distribut-ion not specified	IRLSSG 1995	1.06	Postal questionnaire was sent out and probable RLS cases (n = 150) were then interviewed in-person and clinically examined, where it was found that only 35/150 were positive for RLS.
	Rangarajan, 2007	Bangalore, India, 920	18-90	1266 ♀: 567 ♂: 699	IRLSSG 2003	2.2	In person examination. Person tested positive if all four IRLSSG criteria were met
	Cho et al., 2008	South Korea, 282	20-69	5000	IRLSSG 2003	7.5	A population-based study, conducted telephonically using the Korean version of the

				♀: 2530 ♂: 2470			'John Hopkins telephone diagnostic interview' for RLS.
	Yokoyama et al., 2008	Japan, 483	≥70	1769 ♀: 1000 ♂: 769	Unspecified IRLSSG criteria	11.4	In person interview conducted at home. Single question asked, "Is your sleep interrupted by a creeping sensation or hot flushes in your legs?"
	Nomura et al., 2008	Japan, 483	≥20	2812 ♀: 1589 ♂: 1223	IRLSSG 2003	1.8	Interview conducted over the phone. Person tested positive if all four IRLSSG criteria were met – this was followed by an assessment by a neurologist
	Tsuboi et al., 2009	Japan, 483	≥65	1251 ♀: 812 ♂: 439	IRLSSG 1995	1.0	In person survey conducted, answers were then verified in a telephone interview and an in-person interview was conducted
	Cho et al., 2009	South Korea, 282	18-64	6509 ♀: 3928 ♂: 2581	IRLSSG 2003	0.9	A population-based study, conducted telephonically using the Korean version of the 'John Hopkins telephone diagnostic interview' for RLS.
	Park et al., 2010	South Korea, 282	40-69	1274 ♀: 1000 ♂: 274	IRLSSG 2003	6.5	Researchers distributed a questionnaire to participants who agreed to participate. Positive answers for the four IRLSSG criteria.
	Kim et al.,	South Korea,	65	714	IRLSSG	8.4	In person interview conducted with physician who had a priorunderstanding of sleep

	2010	282		Sex distribution not specified	2003		disorders. Positive answers for the four IRLSSG criteria.
	Chen et al., 2010	Taiwan, 1150	15-70	4011 Sex distribution not specified	IRLSSG 2003	1.6	Participants were questioned telephonically; positive to four IRLSSG criteria.
	BaHammam et al., 2011	Saudi Arabia, 665	≥18	1303 Sex distribution not specified	IRLSSG 2003	5.2	Face-to-face interview. Participants who exhibited RLS mimics were excluded from the study.
	Kim et al., 2012	South Korea, 282	≥65	1990 ♀: 1089 ♂: 901	IRLSSG 2003	9.5	Questionnaire administered; positive to four IRLSSG criteria.
	Li et al., 2012	Wangtai China, 587	≥16	2101 ♀: 1040 ♂: 1061	IRLSSG 2003	7.2	In-person interview conducted on a random population in China. Participants were asked to complete a questionnaire in order to determine a RLS diagnosis.
	Ma et al., 2012	Shanghai, China, 4	≥50	2609 ♀: 1714	IRLSSG 2003	0.7	A survey was asked both telephonically and in-person to the general population. Questions used were based on the RLS diagnostic criteria.

				♂: 895			
	Mahmood et al., 2015	Pakistan, 900	≥18	390 Sex distribution not specified	IRLSSG 2003	23.6	Positive to four IRLSSG criteria.
	Ishaq et al., 2020	Pakistan, 900	18-26	300 Sex distribution not specified	IRLSSG 2003	8	Positive to four IRLSSG criteria.
Europe	Rothdach et al., 2000	Germany, 263	65-83	869 ♀: 673 ♂: 196	IRLSSG 1995	9.8	Two RLS-trained physicians assessed the prevalence of RLS using the four standard criteria in face-to-face interviews. They also performed a standardized neurological examination for each participant.
	Ulfberg et al., 2001	Sweden, 320	18-64	♂: 2608 ♀: 140	IRLSSG 1995	5.8	Random samples of individuals were sent a questionnaire, which included questions about their general quality of sleep and sleep habits.
	Sevim et al., 2004	Mersin, Turkey, 450	≥18	3234 ♀: 1663 ♂: 1571	IRLSSG 1995	3.2	Data obtained from a community sample of adults.
	Berger et	Germany,	20-79	4310	IRLSSG	10.5	In-person interview and a physical examination were conducted on participants, who were

	al., 2004	263		♀: 2292 ♂: 2018	1995		randomly selected.
	Happe et al., 2004	Germany, 263	25-75	1312 ♀: 694 ♂: 618	IRLSSG 2003	8.8	Health survey conducted in-person.
	Rijsman et al., 2004	Netherlands, 30	≥50	1485 ♀: 696 ♂: 789	Unspecified IRLSSG criteria	7.1	Study was performed by postal questionnaire.
	Allen et al., 2005	France, 375 Germany, 263 Italy, 538 Spain, 600 United Kingdom, 162	≥18	France - 1884 Germany – 1929 Italy – 1768 Spain- 1894 UK – 1950 Sex distributi	IRLSSG 2003	France - 10.8 Germany – 4.1 Italy – 6.7 Spain- 4.9 UK – 8.6	Interviews with participants carried out telephonically via random-digit dialing. In-person interview conducted by a trained RLS expert. Randomly selected participants from randomly selected locations were interviewed

				on not specified			
	Hogl et al., 2005	Italy, 538	50-89	701 ♀: 366 ♂: 335	IRLSSG 1995	0.6	Cross-sectional study of a random sample of a general population. The diagnosis of RLS was established via questionnaires. Participants underwent a physical examination.
	Tison et al., 2005	France, 375	≥18	10,263 ♀: 5501 ♂: 4762	IRLSSG 1995	8.5	In-person interview conducted on a random sample, using the diagnostic criteria to assess the prevalence of RLS.
	Bjorvatneta l., 2005	Denmark, 31 Norway, 500	≥18	1005 1000 Sex distributi on not specified	IRLSSG 2003	8.8 14.3	Population-based cross-sectional study; of randomly selected adults participated in a telephone interview in Norway and Denmark.
	Vogl et al., 2006	Italy, 538	≥18	530 Sex distributi on not specified	IRLSSG 2003	8.9	Three loci on chromosomes 12q, 14q and 9p, were isolated in a population. A two-step strategy was used to identify patients with RLS.
	Hadjigeorgi ou et al.,	Greece, 498	≥18	3033	IRLSSG	3.9	Population-based survey of RLS was conducted, where individuals were randomly

	2007			♀: 1884 ♂: 1419	2003		recruited.
	Ulfberg et al., 2007	Sweden, 320	18-90	1000 ♀: 510 ♂: 490	IRLSSG 2003	4.9	Cross-sectional study was conducted via telephone interviews.
	Broman et al., 2008	Uppsala, Sweden, 320	20-59	1335 ♀: 749 ♂: 586	IRLSSG 2003	18.8	A questionnaire was given out by mail, to a randomly selected sample of participants. Approximately every 50th person was chosen from the national registration records and responses.
	Westtron et al., 2008	Sweden, 320	18-64	♀: 3516	IRLSSG 2003	15.7	A random sample of women was selected from the population. They received questionnaires on RLS, as well as general health questions.
	Erer et al., 2009	Turkey, 450	40-95	1124 ♀: 574 ♂: 550	IRLSSG 2003	9.7	Two-phase study conducted on participants:phase one included an in-person interview, using the RLS diagnostic questionnaire to determine RLS status and phase two included assessment by a physician.
	Tasdemir et al., 2010	Turkey, 450	≥18	2111 ♀: 1104 ♂: 1007	IRLSSG 2003	3.4	Descriptive, cross-sectional, in-person study. A voluntary population was randomly selected for the study. All the suspected RLS participants underwent neurological examination.
	Celle et al., 2010	France, 375	≥18	667 ♀: 396 ♂: 271	IRLSSG 2003	24.2	Individuals randomly selected from the French population, underwent clinical assessment, mood evaluations and nocturnal polygraphs in order to determine RLS prevalence.
	Juuti et al.,	Finland, 164	57	995	IRLSSG	18.0	Health survey conducted in a random urban population

	2010			♀: 556 ♂: 439	2003		
	Benediktsdottir et al., 2012	Iceland, 500 Sweden, 320		601 Sex distribution not specified	IRLSSG 2003	Iceland - 18.3 Sweden - 11.5	Face-to-face interview was conducted; positive to four IRLSSG criteria
	Pekmezovic et al., 2013	Serbia, 473	≥18	2112 Sex distribution not specified	IRLSSG 2003	5.1	A community-based study consisting of an in-person interview in a randomly selected adult population.
	Ohayon and Roth., 2022	United Kingdom, 162 Germany, 263 Portugal, 372 Italy, 538 Spain, 660	15-100	18,980 ♀: 9739 ♂: 9241	International Classification of Sleep Disorders	5.5	Cross-sectional studies were performed. Subjects underwent telephone interviews.
South America	Miranda et al., 2001	Chile, 1871	≥18	100 ♀: 69 ♂: 31	IRLSSG 1995	13.0	Relatives of outpatients and uremic patients undergoing chronic hemodialysis were telephonically interviewed.

	Castillo et al., 2006	Ecuador, 1117	25-85	500 ♀: 310 ♂: 190	IRLSSG 2003	2.0	Survey conducted telephonically to assess RLS symptoms involving natives from sea level and high altitude. The process consisted of two phases: the creation of the epidemiological instrument and the telephone survey.
	Persi et al., 2009	Buenos Aires, Argentina, 25	≥18	471 ♀: 284 ♂: 187	IRLSSG 2003	20.2	Participants from high and low population density areas completed a self-assessment questionnaire.
	Eckelietal., 2011	Brazil, 320	≥18	1081 ♀: 431 ♂: 650	IRLSSG 2003	6.4	A transversal study was conducted. Participants were interviewed by a neurologist and/or sleep physician.
	Castillo et al., 2014	Ecuador, 1117	≥40	665 ♀: 386 ♂: 279	IRLSSG 2003	6.0	Details provided in Table 1.2
North America	Lavigne and Montplaisi, 1994	Canda, 487	≥18	2019 ♀: 1023 ♂: 996	Unspecified IRLSSG criteria	15.0	In person survey conducted, two questions: 1. "At bedtime, does restlessness in your legs wake up during the night", 2. "Do you feel unpleasant sensation in your leg muscles that require you to move your legs/walk to be more comfortable?"
	Phillips et al., 2000	Turkey, 1132	≥18	1803 Sex distribution not	Unspecified IRLSSG criteria	9.4	Survey conducted telephonically

				specified			
	Allen et al., 2005	United States of America (USA), 763	≥18	5964 Sex distribution not specified	IRLSSG 2003	7.6	Participants were interviewed to determine the "presence, frequency, and severity of RLS symptoms".
	Phillips et al., 2006	USA, 763	≥18	1506 ♀: 775 ♂: 731	IRLSSG 2003	9.7	Telephonic survey used to assess presence of RLS.
	Lee et al., 2006	USA, 763	≥18	1028 Sex distribution not specified	IRLSSG 2003	4.2	Subjects included adults of different ethnic backgrounds. RLS diagnosis was determined in a questionnaire and based on the presence of RLS symptoms.
	Winkelman et al., 2006	USA, 763	30-60	2821 ♀: 1486 ♂: 1335	Unspecified IRLSSG criteria	15.8	Community-based epidemiology study.
	Froese et al., 2008	Canada, 487	≥18	430 ♀: 245	IRLSSG 2003	17.7	A community-based, door-to-door, cross-sectional survey of three indigenous North American groups was conducted.

				♂: 185			
	Winkelman et al., 2008	USA, 763	≥18	3433 ♀: 1874 ♂: 1559	IRLSSG 2003	5.1	Study conducted on adults who experienced cardiovascular health as a result of sleep-disordered breathing.
	Gao et al., 2009	USA, 763	>56	65,554 23,119 Sex distribution not specified	IRLSSG 2003	4.1 6.4	This study included adults who were unaffected by diabetes, arthritis and pregnancy.
	Ram et al., 2010	USA, 763	≥16	6139 ♀: 3174 ♂: 2965	Unspecified IRLSSG criteria	0.4	In person interview by research assistant – no other details submitted.
	Allen et al., 2011	USA, 763		61,792 Sex distribution not specified	IRLSSG 2003	7.3	Participants were selected at random to answer a questionnaire, in order to make a RLS diagnosis.

Table adapted from Koo (2015), Sleep Med Clin 10 (3), from p. 191-197 &Ohanyon et.al (2012), Sleep Med Rev 16 (4), from p. 285-288.

RLS, Restless Legs Syndrome; IRLSSG, International Restless Legs Syndrome Study Group; UK, United Kingdom; USA, United States of America

♀ representative of females; ♂ representative of males.

*Altitudes not provided in the manuscripts were calculated based on the reported location

1.4.1 Ethnicity differences in RLS prevalence

The prevalence of RLS has predominantly been assessed in populations with a European Ancestry, with the prevalence reports ranging from 5-25% in European and North American countries, determined using the 2014 five-point diagnostic criteria (Picchietti etal., 2017). Many epidemiological studies do not report on the ethnic backgrounds of participants therefore some assumptions, based on expected ethnicity for the geographic location, need to be made when evaluating the available literature. A study conducted in 2012 reviewed the prevalence data available at the time and concluded that there was a larger range of prevalence (3-19%) in populations with European Ancestry, compared to other ethnicities, such as Japanese and Middle Eastern, which had a prevalence ranging from 0.1-17.5% (Ohayon et al., 2012). However, these studies were either administered prior to the addition of the fifth criterion in the diagnostic criteria that excluded RLS mimics, or researchers used less well-defined diagnostic criteria (Ohayon et al., 2012). A minimal amount of research has been conducted on RLS prevalence in ethnic groups other than those of European ancestry. When researching South American population groups, researchers found an RLS prevalence ranging from 2.0 – 20.2% in ethnic groups of mixed European and South American ancestry (Picchietti et al., 2017). The majority of the RLS prevalence studies conducted in Asia has included individuals of different ethnic backgrounds, although presumably of Asian ancestry, ranging from Han Chinese origin to Korean (Table 1.1). Asian population groups had a lower RLS prevalence than South American and European ancestry groups, with a range between 0.7 – 12.1% (Picchietti et al., 2017). A lower prevalence of RLS has also been found in the African American population (0.03 – 6.8%), in comparison to the population of European ancestry (Table 1.1). However, reports of this have been inconsistent; a 2006 study concluded that the prevalence of RLS is equal in both African ancestry (4.7%) and European ancestry individuals (3.8%) in America (Lee et al., 2006). Yet another study concluded that RLS was less prevalent in African Americans (2.7%) compared to individuals with European ancestry (23.9%) (Allen et al., 2013). Prevalence studies conducted in Africa (Table 1.1) showed lower prevalence rates in black Africans located in Mozambique, Nigeria and Tanzania (0.03 – 6.77%) compared to the global RLS prevalence (Ferreira et al., 2013; Fawale et al., 2016; Winkler et al., 2010; Burtscher et al., 2014).

The tendency towards higher prevalence rates in European ancestry compared to other population groups suggests that ethnicity may be a risk factor in the development of RLS. It is important to take ethnicity into account when conducting RLS analyses in order to determine prevalence, as the prevalence of RLS may be influenced by the genetic differences in each ethnic group; however there has not been conclusive research linking ethnic genetics and RLS prevalence. South Africa has an extremely diverse ethnicity, with the largest ethnic groups being Black African, White, Indian/Asian and Coloured. Coloured is a recognised population group by the South African government that may be defined as mixed race in other countries. Other possible proposed factors related to ethnicity may include geographic location (latitude) and UV exposure and melanin levels according to the various population groups (Koo, 2012). Although RLS does appear to be more common in population groups with European ancestry compared to other ethnic groups, it is important to note that the methodology for these studies were variable as different diagnostic criteria were used, which may impact the results. Data on differences in RLS prevalence related to ethnicity are still too fragmentary to reach definitive conclusions.

1.4.2 Sex differences in RLS prevalence

Researchers believe that sex may be a contributing risk factor for the onset of RLS. Previous studies indicate that RLS is more prevalent in females, with RLS being diagnosed in twice as many females than males (Seeman, 2020). The prominence of RLS in females may be due to pregnancy, which is a cause of secondary RLS, as well as iron-deficiency anaemia which is more common in females due to the loss of blood during menstruation (Coad & Conlon, 2011). The physiological changes, such as the increased iron requirements, incurred with pregnancy have been hypothesised to be associated with the pathophysiology of RLS (Manconi et.al, 2004). Pregnancy increases the body's iron requirements two-to three-fold as iron is needed for the synthesis of haemoglobin and for the developing foetus (Soma-Pillay et al., 2016). As previously discussed, iron deficiency has been linked to the pathogenesis of RLS, which may account for the higher prevalence of RLS in pregnant women. A study evaluating sex differences in RLS found that 53.7% of the female participants had low ferritin levels compared to the 22.2% of male participants. In addition, RLS severity scores were higher in the female participants compared to the male participants (Holzknecht et al., 2020).The results from this study indicate a possible sex difference in the phenotypical presentation of RLS, which primarily manifests as

sensory symptoms in females. However, more research is needed to understand why RLS is more common in females than males.

Not all studies have shown a female preponderance for RLS. Fawale et al. (2016) found no significant sex difference between patients with RLS and healthy controls in an elderly Nigerian population. However, the authors acknowledge that the results of this study require the validation of larger studies with a larger representation of male participants in order to properly ascertain sex distribution of RLS in an African population. The study found that in the 75–84 year age group, males have higher odds of RLS, with the male to female ratio being 2:1 (Fawale et al., 2016). These data conflict with those obtained from previous studies in Europe, North America and Asia (Ohayon et al., 2012).Other mechanisms involved in the tendency for RLS to affect females are still unknown, and further research is needed to understand how the pathogenesis of RLS differs in females versus males.

1.4.3 Age differences in RLS prevalence

Researchers support the idea that the there is an increased risk of RLS at a higher age, and that the symptoms of RLS worsen as age increases (Allen & Earley., 2000). Individuals of 60 years of age or older have double the risk of developing RLS as compared to younger individuals (Garcia-Borreguero et.al, 2006). Even so, the onset of RLS symptoms at a young age is not a rare occurrence (Guo et al., 2017), and one study reported that approximately 45% of individuals experience their first episode of RLS before 20 years of age- which would be considered early-onset RLS. Early on-set RLS is the manifestation of RLS symptoms before the age of 45 years. However, most researchers report the onset of RLS beginning at a later age (>50years) (Guo et al., 2017). A 2004 study reviewed the correlation between age and onset of RLS symptoms. Researchers found that there was 3% prevalence for 18-29 years old individuals, 10% prevalence in people aged 30-79 and 19% prevalence in those over 80 years of age (Zucconi and Ferini-Strambi, 2004). A significant bimodal distribution with a large prevalence peak occurring at 20 years of age and a smaller peak in the mid-40s was observed when investigating age-of-onset of RLS (Whittom et al., 2007). The age at which RLS onset occurred differed depending on if there was a genetic link of RLS present or not (Whittom et al., 2007). Age of onset was also clearly differentiated with early onset of RLS being exhibited in primary RLS, as compared with those with secondary RLS showing a later age of onset, concluding that early- and late-onset RLS

could be distinguished depending on familial history and aetiology of RLS (Whittom et al., 2007).

1.4.4 Latitude differences in RLS prevalence

As discussed in section 1.4 above, RLS prevalence studies have been done in 27 different European and North American countries to date, with prevalence ranges from 0.6 - 24.2% (Table 1.1), with nearly all studies using the IRLSSG diagnostic criteria, either from 1995 or 2003, depending on when the study was done. Koo (2011) speculated that the range in prevalence's may be due to latitude differences in the studies conducted. This is due to the circadian nature of RLS, as well as the correlation between RLS symptoms and endogenous melatonin levels (Koo, 2012).In particular, light or ultraviolet radiation (UVR) may be related to the expression of RLS (Koo, 2012). Koo (2012) hypothesised that as the UVR decreases with increasing distance from the equator, geography may affect the prevalence of RLS and result in an increase in prevalence with the increasing distance from the equator. Koo (2012) found that after mapping the RLS prevalence of both American and European regions, frequency of RLS was positively correlated with latitude. Countries closer to the equator ranged from 3.4 - 3.8% prevalence while those at more northern latitudes ranged from 14.3 - 18.3% (Koo, 2011). These results support that latitudinal tracking factors, such as UVR, may be associated with the expression of RLS. Based on the information in Table 1.1 the latitude theory supports the RLS prevalence of African countries, which are closer in proximity to the equator, and exhibit a relatively low RLS prevalence, ranging from 0.03 - 6.77%. This may be due to ethnicity differences in the study populations as participants from African countries are more likely to have a high amount of UV exposure which increases melanin and melatonin levels (Wright et al., 2016)). The latitude theory also supports the relatively high prevalence of RLS in European countries (0.6- 24.2%), located further from the equator. Presently, more research is needed to verify the latitude theory; another factor likely to influence UV exposure is altitude.

1.5 The effect of altitude on RLS

Living at higher altitudes, greater distance above sea level, has been proposed to increase the risk of RLS (Castillo et al., 2006). An area at high altitude is generally considered to be above 2400m

however the threshold of what is considered ‘high’ altitude is variable (Luks., 2012). Although the prevalence studies in Table 1.1 did not include altitude, the overall trendshows that most of the studies done in Asia are roughly at sea level which may account for the lower prevalence seen in these populations, amongst other factors. Interestingly, the Europeans studies in Table 1.1 were conducted at relatively low altitudes, yet exhibited a higher prevalence of RLS in comparison to other continents. The higher prevalence may be due to factors other than altitude, such as genetics and lack of melanin. South America (prevalence ranging from 2.0 – 20.2%) had a slightly higher prevalence than North America (prevalence ranging from 0.4 04 - 17.7%), which followed the altitude trend as the South American studies were conducted at higher altitudes than the North American studies. Table 1.2 details the studies conducted to investigate the effects of altitude on the prevalence of RLS.

Table 1.2: Studies specifically focusing on the effects of altitude on the prevalence and characteristics of Restless Legs Syndrome

Author, Year of Publication	Location, Altitude (m)	Age of Participants (years)	Sample Size (n)	Diagnostic Criteria	Prevalence (%) of RLS Reported	Study Design and Methodology	Conclusion
Castillo et al., (2006)	Andean city of Quito, Ecuador, South America Mountainous Region – 1286m Coastal Region –4m	25 - 85	250 from the mountainous regions and 250 from the coastal region	IRLSSG 2003	Mountainous Region – 3.2% Coastal Region – 0.80%	Cross-sectional survey conducted telephonically consisting of 550 households, performed in 2004 by a sleep-trained, neurologist.	The total prevalence of RLS in the adults who lived at a higher altitude was significantly greater than the number of adults living at sea level
Stefani et al., (2016)	Austria, Europe Simulated High Altitude – 3000m Low Altitude – 574 m	Mean age:39.7	Five untreated RLS individuals Five controls	Diagnostic criteria not stated		Two randomised nights of polysomnography: One night in asimulated high-altitude environment with normobaric hypoxia and a control night at low altitude. Both nights were performed in the same setting.	Study found that Periodic Limb Movements of Sleep (PLMS) increased, at high altitude, in both RLS participants and controls and concluded that peripheral hypoxia may play a role in increasing the prevalence of RLS at a high altitude.

Gupta et al., (2017)	Himalayan and sub-Himalayan region of India 400 m above sea level (low altitude), 1900-2100 m above sea level (mild altitude), and 3200 m above sea level (high altitude)	18 - 84	1689 participants	IRLSSG 2006	Low Altitude – 2.5% Mid Altitude – 12.2% High Altitude – 11.8%	Cross-sectional door-to-door study conducted at low and high altitudes using random sampling. Individuals were screened for RLS using the Cambridge–Hopkins RLS diagnostic questionnaire.	There was a greater prevalence in participants living at high altitude. The low prevalence of RLS in the Asian population was consistent with results from previous studies.
Düz et al., (2019)	Turkish aircrew admitted to Istanbul MedipolUniversity Hospital Neurology Department (Altitude not specified)	Not provided	301 Turkish aircrew 272 controls (non-aircrew)	IRLSSG 2006	Aircrew group - 6.7% Controls - 7.9%	Both aircrew and controls were asked to fill in the IRLSSG Questionnaire	No significant difference was found in the prevalence of RLS in the aircrew and the controls, therefore it was concluded flying at high altitude was not a risk factor for RLS.

IRLSSG, International Restless Legs Syndrome Study Group; PLMS, Periodic Limb Movements of Sleep

Altitude influenced the prevalence of RLS, with an increase in prevalence rate at higher altitudes compared to lower altitudes (Table 1.2). Castillo et al. (2006) conducted a telephonic survey in native South America, concluding that the prevalence of RLS patients living in the mountainous region at a higher altitude (2816m above sea level) was higher (3.2%) than in individuals living at sea level (0.80%). The principle mechanism of this phenomenon is still not completely understood, but ideas include abnormalities in the metabolism of iron at higher altitudes may be implicated in the development of RLS (Castillo et al., 2006). The authors suggest that the physiological and metabolic adaptations which are associated with exposure to high altitudes for an elongated amount of time may be putting these individuals at an increased risk for RLS (Castillo et al., 2006).

There are two important factors that may be responsible for the increase of RLS prevalence at high altitude, hypoxia and changes in the plasma iron levels at high altitude (Connor et al., 2011). As altitude increases, there is a decrease in air pressure which reduces the partial pressure of inspiratory oxygen in the surrounding atmospheric air, which then reduces the oxygen saturation of haemoglobin (Hb) in the blood (Muckenthaler et al., 2020). As a result of the reduced oxygen saturation at high altitude, Hb levels rise in response to decreased amount of oxygen available. Low blood oxygen levels are detected by peritubular fibroblasts in the renal cortex of the kidneys, via prolyl hydroxylases, which will then stabilize the α-subunits of the hypoxia-inducible factor-1 and -2 (HIF-1α and HIF-2α)(Muckenthaler et al., 2020). Once stabilization has occurred, HIFα subunits form heterodimers with ARNT (aryl hydrocarbon receptor nuclear translocator/HIFβ) and cause the upregulation of oxygen-dependent genes that allow cells to adapt to hypoxic conditions (Muckenthaler et al., 2020). Erythropoietin (EPO) is a renal hormone which is upregulated and secreted into the blood circulation, in order to increase the maturation of red blood cells in the bone marrow (Muckenthaler et al., 2020). EPO drives maturation from CFU-E (precursor erythroblasts) to erythroblasts. This process may take approximately 7–8 days, with extra days required for the maturation into adult erythrocytes. Therefore, the production of red blood cells responds to hypoxic conditions in the body (Muckenthaler et al., 2020).

Oxygen is attached to the iron molecules which are present in each hemoglobin subunit in the red blood cells (Muckenthaler et al., 2020). Therefore, the increased levels of erythropoiesis, which

occur at high altitude, require higherthan normal amounts of iron that needs to be supplied by dietary absorption and by mobilization from iron stores. The erythrocytes produced daily require more than 2×10^{15} iron atoms every second (Muckenthaler et al., 2020). However, in populations with an increased need for iron, such as in individuals living at high altitude, iron stores are less likely to be replenished at a fast-enough rate to accommodate the body's needs (Muckenthaler et al., 2020). Iron exists throughout the brain and is an essential cofactor of tyrosine hydroxylase, which is the rate-limiting enzyme of dopamine synthesis (Beard et al., 1994). Researchers found that an iron-deficiency results in high levels of tyrosine hydroxylase in the caudate, putamen, and ventral midbrain, as well as an increase in the extracellular dopamine levels (Beard et al., 1994). As previously stated, the dysregulation of dopamine is widely associated with the aetiology of RLS. Therefore, at high altitudes, increased erythropoiesis requires an increased amount of iron which needs to be supplemented by the diet or using up reserve iron stores (Muckenthaler et al., 2020). People living at high altitude undergo persistent exposure to low oxygen availability however, the effects this has on the metabolism of iron is not well studied. Research studies show mixed findings with some saying that ferritin levels remain within physiological range at high altitude (Beall CM et al., 1990), while other research shows that ferritin levels of women living at high altitude decreased by a third (Cook et al., 2005). Secondary RLS is prominent in pregnant women, who are most vulnerable as their oxygen requirements are greater and their iron stores are not able to be replenished fast enough and will therefore take a longer time to adapt to the low oxygen level partial pressures at high altitudes (Muckenthaler et al., 2020). As previously mentioned, RLS is associated with low iron levels and therefore patients with RLS may be more vulnerable to the increased iron demand at high altitude leading to an exacerbation of RLS symptoms, causing an increased prevalence. The partial pressure of oxygen is lower at a high altitude resulting in reduced tissue oxygenation, possibly leading to peripheral hypoxia (Salminen et al., 2014).

A pilot study investigating the effect of altitude on RLS conducted a two-night experiment, in which five patients with RLS and five healthy controls underwent polysomnography (Stefani et al., 2016). Patients with RLS and healthy controls were put into a simulated high-altitude environment of 3000m and into a control environment, which was their regular home altitude of 574m, in random order. Researchers found that PLMs increased in both participant groups at the

simulated high altitude, concluding that peripheral hypoxia could play a role in RLS severity. However, a one-night intervention was not long enough to show the cause of this phenomenon, either iron dysregulation or dopaminergic dysfunction, was beyond the scope of the study (Stefani et al., 2016).

Gupta et al. (2017) conducted a study in the Indian Himalayas; and revealed RLS was five times greater at a high altitude (1900-3200m) (11.8-12.2%) as compared to those living at a low altitude (400-700m) (2.5%). Researchers hypothesised that the increased prevalence of RLS at high altitude may include brain iron dysregulation, which may lead to dopaminergic dysfunction, as well as hypoxia, a similar hypothesis to Castillo et al (2006). The reduced iron availability at a high altitude may lead to a brain iron deficiency, which in turn affects dopamine levels (Gupta et al., 2017). The imbalance of dopamine in the body may result in the onset of RLS symptoms, thereby increasing the RLS prevalence. Gupta et al. (2017) found that the low iron levels at a high altitude may also result in hypoxia in the leg muscles, which result in feelings in the legs similar to those present in RLS.

In contrast to the previous research mentioned a study conducted in 2019 found no significant difference in the prevalence of RLS in the aircrew and the controls and concluded that flying at high altitude was not a risk factor for RLS (Düz et al., 2019). However, the study's test group consisted of flight crew, who do not experience staying at high altitudes for large enough amounts of time to be affected by the low partial pressure of oxygen at high altitude. Another factor which may influence these results is that the cabin pressure of a plane is controlled differently and would not affect the oxygen levels of the flight crew.

There have been a limited amount of studies focusing on altitude's effect on RLS. Although the studies that have been conducted tend to support that RLS symptoms are more prevalent at higher altitudes, this has not been consistently demonstrated, and requires a wider diversity of populations, at different altitudes to be studied. Studying the effect of altitude on RLS in a South African population will contribute to our understanding of the aetiology of RLS, as South Africa has a diverse, heterogenous population of European, African and Asian ancestry residing at higher altitude (Johannesburg) and at sea level (Durban), both located at a similar latitude. RLS has a massive impact on quality of life, which is why further investigation is necessary. With the

knowledge that living at a high altitude could increase an individual's risk of developing RLS, iron supplementation can be provided to people at risk living at high altitude or it could help elucidate the cause of RLS. Given that there is speculation that the characteristics of RLS may differ depending on the underlying aetiology, it is also worthwhile exploring whether these characteristics change with altitude as hypoxia and low iron is more prevalent at high altitude, than compared to low altitude (Netzer et al., 2013).

1.6 Aim

The aim of this study was to determine the impact of altitude on the prevalence and characteristics of RLS in a South African population by comparing the prevalence and characteristics of RLS at low altitude (Durban) and at higher altitude (Johannesburg).

The study objectives were to:

Determine the prevalence of RLS at low altitude (Durban) and at higher altitude (Johannesburg) in a South African population.

Determine the relationship between RLS and altitude i.e. does the severity of RLS increase at a higher altitude.

Determine the relationship between daytime sleepiness in the RLS+ cohort at higher and low altitude i.e. does daytime sleepiness increase at higher altitude.

1.7 Justification

Previous research has shown that the prevalence of RLS is greater at high altitudes, but none of this research is based in Johannesburg, South Africa which has an altitude of 1753m compared to Durban which is at low altitude (0m). One of the reasons why this phenomenon is still not fully understood is because the contribution that high altitude plays in the development of RLS has been minimally studied. The availability of oxygen in the air is lower at a high altitude which causes a low amount of oxygen to enter the cells and tissues in the body, which causes a subsequent cascade of events, such as increased erythropoesis and an increased need for iron in the body, which may cause peripheral hypoxia. The decreased availability of oxygen at high altitudes may also lead to the increased metabolism of iron, decreasing the bodies iron reserves which need to be replenished.

Anemia may result if the body is unable to keep up with the increased iron demand at high altitude, during hypoxia or if an iron deficiency occurs. High altitudes could contribute to RLS through hypoxia or an iron deficiency. Therefore, the aim of this study is to investigate the impact of altitude on the prevalence and characteristics of RLS.

2 METHODS

2.1 Ethical Approval

Ethics for this study has been granted by the Human Research Ethics Committee (HREC) at the University of the Witwatersrand, which adheres to the Declaration of Helsinki (M200213, Appendix A). The participants of the study were first provided with an information sheet that detailed the study procedures and notified them that they were free to withdraw from the study at any time. All participants gave informed and written consent prior to participation.

2.2 Study Population

The data for this cross-sectional study were collected between 2020 and 2022. Participants were recruited if their current (for at least the past year) residential location was at either 0m above sea level (low altitude, Durban/ eThekwini, South Africa) or 1753m above sea level (higher altitude, Johannesburg, South Africa). Both locations are at similar latitude (between 26.2041° S, 28.0473° E and 29.8587° S, 31.0218° E). Participants self-identified their sex (male, female or other) and their ethnicity (African ancestry, Coloured, Indian/Asian, European ancestry or other) according to pre-defined categories determined by StatisticsSA (http://www.statssa.gov.za; Population Census 2016). The inclusion criteria were to be South African residents, ≥18 years of age (legally consenting), and living at one of the two above-specified locations. It was explained to participants that by completing the survey they were consenting to taking part in the study. Participants were excluded if they had not been living at their current residential location for longer than one year, if they were determined as RLS probable using the Cambridge-Hopkins RLS diagnostic screening questionnaire, or did not complete all the necessary questions in the survey (all questions were optional).

2.3 Study Design

Figure 2.1 provides a summary of the study design. A self-administered, online survey (Appendix B) was electronically distributed to local communities in the general South African population. Participants were recruited from the two altitudes, via a snowball sampling technique; using university email systems; private emails and social media platforms.

Participants were able to complete the online questionnaire, using any electronic device that had internet access. The questionnaire consisted of customized general demographic questions, including age, sex, ethnicity, current residential location, and medical history specific to certain conditions (e.g. low iron deficiency). To examine the effect of altitude on the prevalence of RLS, as well the effect of altitude on other RLS characteristics (e.g. severity and sleepiness) and sleep habits, the following validated questionnaires were included in the survey: The Cambridge-Hopkins RLS diagnostic screening questionnaire (CH-RLSq)(Allen et.al, 2009), used to confirm the presence of RLS; the International restless legs severity scale (IRLSS)(Allen et al., 2003), used to assesses the severity of symptoms that affect RLS patients; the Epworth Sleepiness Scale (ESS) (Johns., 1991), used to measure daytime sleepiness; and the Fatigue Assessment Scale (FAS) (Michielsen et al., 2003), used to evaluate the symptoms of chronic fatigue. These questionnaires/scales are discussed in detail below. Survey responses were used only if RLS screening questionnaire questions and their area of current residential location was answered. If residential location and RLS questions were answered, but age, sex, or sleep related information was unanswered, the data from those participants were still included in prevalence analyses, but not for the characteristic analysis.Survey responses were collected using Research Electronic Data Capture (REDCap), a data management platform hosted by the University of the Witwatersrand. REDCap is a secure web browser used for data management, survey distribution, and as a collection and sorting tool for survey responses (Harvey., 2018).

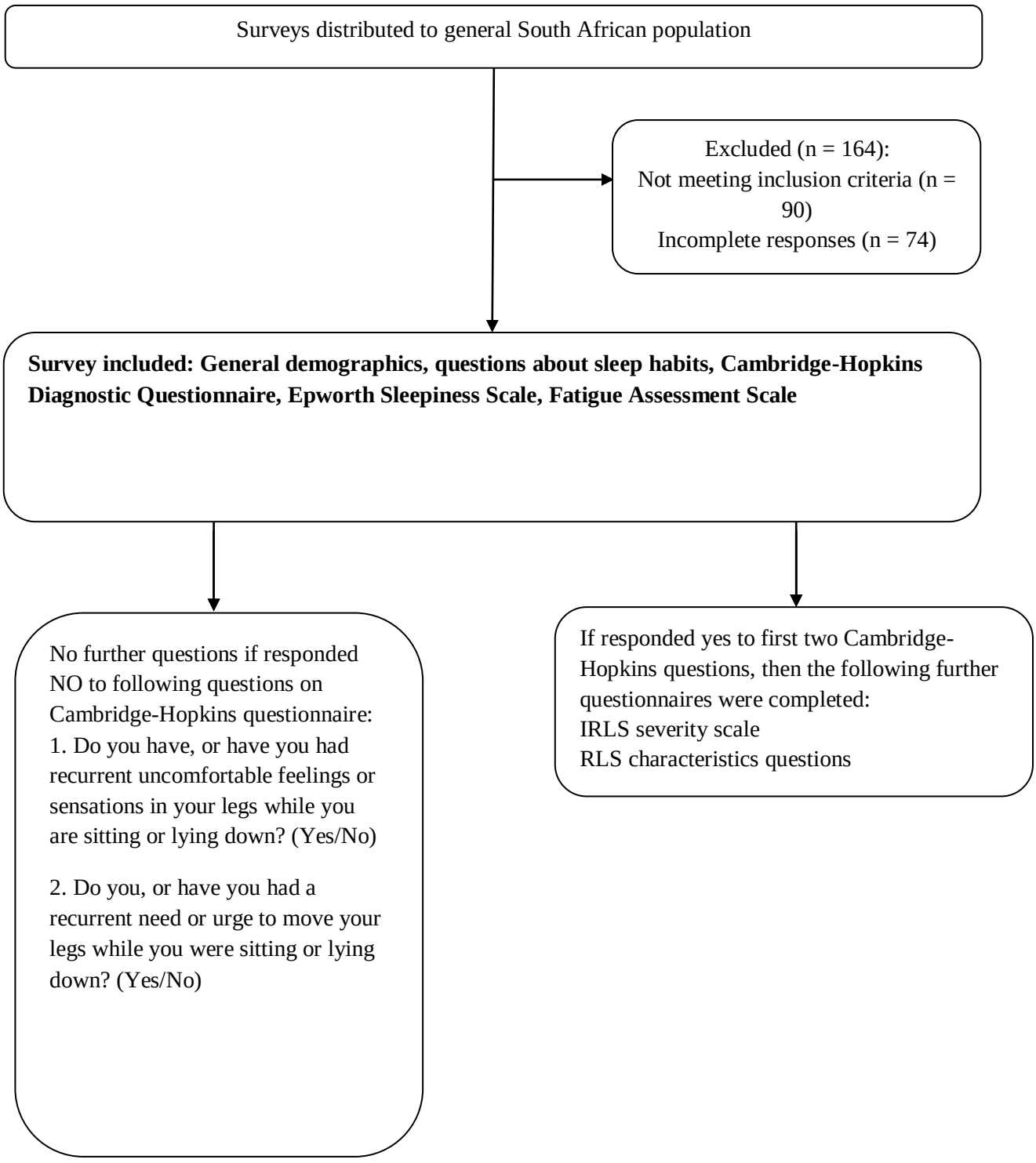

Figure 2.1: Flow diagram showing the study design of survey distribution according to responses

RLS, Restless Legs Syndrome; IRLS, International Restless Legs Syndrome

Below, I discuss the details of each questionnaire completed by the eligible participants after screening. It is important to note that not all questions included in the questionnaire were analysed, as the main focus of this study is on the effects of altitude on the prevalence of RLS and on the most prominent characteristics of RLS.

2.4 Measures: Questionnaires included in the survey

The following measures have not been validated in a South African population, however the participants of our study could all understand and speak English and the measures have been validated in English speaking populations. However, this is a limitation of the study.

The survey distributed included general customized demographic questions (eg, age and ethnicity) and questions regarding basic health status (Appendix A). The survey included a list of diseases and participants were asked to indicate if they were diagnosed with a specific disease by a doctor, as well as to indicate any medications they were currently taking. Participants were asked in the survey to indicate if they were experiencing low-iron levels or anemia. For the purpose of this study, the response to this question was reported as self-reported iron levels. Questions about the general sleep habits of participants were included. Participants were asked to indicate, the average amount of time usually spent asleep, the average number of times they wake up in the night and the average time taken to fall asleep. Participants were then asked to rate, on a scale of 1-100, the quality of their sleep and to comment on what they thought caused their sleep disturbances (if any occurred) throughout the night. The survey then went on to ask if the participant experienced any uncomfortable sensations, while lying down, in the evening and if an affirmative answer was indicated, the participant was then given a list of questions regarding the uncomfortable sensations experienced at night, which included selecting the most appropriate descriptors of the sensations being felt, from the list provided (eg. itching and burning). Participants were then asked to answer the following questionnaires described below:

2.4.1 Cambridge-Hopkins RLS diagnostic screening questionnaire

To determine RLS status amongst participants, the most recent version of the CH-RLSq (Allen et al., 2009) was used (Appendix C). The CH-RLSq is a validated, seven-item diagnostic tool used to confirm the presence of RLS. The Cambridge-Hopkins diagnostic questionnaire for RLS has

been widely used in various studies to assess and diagnose RLS. Several studies have highlighted the effectiveness of the Cambridge-Hopkins questionnaire as a diagnostic tool for RLS. For instance, Gupta et al. (2015) emphasized that the Cambridge-Hopkins questionnaire is a valuable diagnostic tool for RLS and can be utilized in epidemiological studies (Gupta et al., 2015). Similarly, Allen et al. (2009) found that the Cambridge-Hopkins RLS questionnaire provides a reasonable level of sensitivity and specificity for identifying RLS in population-based studies (Allen et al., 2009).

The CH-RLSq includes questions regarding the basic diagnostic features of RLS, in order to provide a basic differential diagnosis. The CH-RLSq consists of a set of questions, with specific options for answers (eg. Yes/No/Don't know), allowing the participants to be categorised according to the absence or presence of RLS, as well as RLS probable. If a participant fulfilled the diagnostic criteria for RLS and did not have any RLS mimic conditions, they were classified as having RLS (RLS+). Participants that did not fulfill the RLS diagnostic criteria were considered to not have RLS (RLS-). The CH-RLSq was also used to determine probable RLS cases, dependent on the answers selected; however, these cases were excluded from all statistical analyses, as they did not fulfill the diagnostic criteria of RLS however, could not be included in the RLS- group because of the presence of some RLS symptoms/RLS mimics. If an affirmative answer was provided to the first two questions of the Cambridge-Hopkins diagnostic questionnaire, participants were directed to the questionnaires listed below:

2.4.2 International restless legs severity scale

The International restless legs severity scale (Allen et al., 2003) is a validated, ten-item scale, which assesses the severity of symptoms that affected RLS participants in the previous week (Appendix D). Participants rated their symptoms, on a scale of "none" to "very severe". Severity is rated for each question as follows: 0 – no symptoms, 1–mild symptoms, 2–moderate symptoms, 3–severe symptoms, and 4–very severe symptoms. These scores are added for a total score between 0–40. Scores totaling to 0 indicate no symptoms. Scores totaling between 1-10 indicate mild RLS severity; 11 – 20 moderate RLS severity; 21-30 indicates severe RLS; and, 31-40 indicates very severe RLS.

2.4.3 Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS) (Johns., 1991) is a validated scale used as a measure of daytime sleepiness (Appendix E) and was completed by all participants. The scale consists of a list of eight situations in which the participant is asked to rate their tendency to become sleepy, during various activities, on a scale of 0 to 3. Selecting 0 indicates no chance of sleeping, 1 indicates a slight chance of sleeping, 2 means a moderate chance of sleeping and 3 characterises a high chance of sleeping. Total Scoring can be between 0 – 24. A score of 0-7 means that it is unlikely a person is abnormally sleepy, while a score of 8-9 indicates an average amount of daytime sleepiness. Scoring between 10-15 indicates excessive daytime sleepiness. A score of 16-24 means a person is excessively sleepy and should consider seeking medical attention.

2.4.4 Fatigue Assessment Scale

The Fatigue Assessment Scale (FAS) (Michielsen et al., 2003) is a 10-item validated scale which evaluates the symptoms of chronic fatigue and was completed by all participants (Appendix E). The scale includes ten statements which refer to how a person usually feels. For each statement, one of the five answers provided are selected. Answers range from "never" to "always", with 1= never, 2 = sometimes, 3 = regularly, 4= often and 5 = always. Items 4 and 10 are reverse-scored. Total scores range from 10, which indicate the lowest level of fatigue, to 50, indicating extremely severe fatigue.

2.5 Statistical Analysis

Data were collected using REDCap electronic data capture tools hosted by the University of the Witwatersrand. Data was first downloaded from REDCap into Microsoft Excel (2007), and then manually sorted and participants were divided into three categories: RLS+, RLS- and RLS probable. Following this, participants were then further divided into different ethnicity groups (European ancestry, African ancestry, Indian and Coloured) at higher and low altitude. The data was assessed for normal distribution and categorical variables are presented as frequency and percentages and continuous variables are presented as mean (standard deviation, SD) or median [interquartile range, IQR]. p values > 0.05 were considered to be significant. Based on a cited global prevalence of ~10% (Ohayon et.al, 2012), and a 95% confidence interval with a 5%

margin error, a minimum of 384 participants, from each altitude, were required for our study. All data analyses were performed using GraphPad Prism v.9.

Comparisons were made between the higher altitude and low altitude and between the RLS+ and RLS- groups at each altitude. RLS prevalence statistics were compared using a Fisher's exact test, which was corrected for multiple comparisons when comparing different ethnic groups. The coloured population (n = 67) were included in prevalence statistics, but were excluded from all ethnicity statistics due to the small sample size and to increase statistical power. Prevalence statistics at both altitudes, which included age, sex and ethnicity, were conducted on both RLS+ and RLS- participants and this was done using a Fisher's exact test. Self-reported iron levels were also examined in both RLS+ and RLS- participants, at both higher and low altitude, using the Fisher's exact test.RLS severity scores at both altitudes were compared, for the RLS+ participants only, using an unpaired t-test or Mann-Whitney U-tests. Sleepiness and fatigue (ESS and FAS scores, respectively) were analysed using one-way Analysis of Variance (ANOVA) tests, with Turkey post-hoc test for all participants. Descriptors of RLS, presence of painful RLS, family history of RLS and treatment being sought/taken were all compared in the RLS+ participants, at higher and low altitude using a Fisher's exact test. Duration of RLS and age of onset, as well as current age were compared in RLS+ participants at both higher and low altitudes using an unpaired t-tests or Mann-Whitney U-tests.

3 RESULTS

3.1 Demographics of the study cohort

The demographic characteristics of the study population (N=1272) are presented in Table 3.1 and Figure 3.1. This study included a total of 1291 participants, who were classified by the altitude of their residential location; low altitude (Durban, n = 416)or higher altitude (Johannesburg, n = 875) and further sub divided by the presence (RLS+) or absence (RLS-) of RLS or probable RLS (excluded from statistical analyses due to uncertainty of RLS diagnosis) based on the results from the CH-RLSq (Allen et.al, 2009). Figure 3.1 shows the distribution of the total number of questionnaires received, which were divided by altitude and RLS status. Participants located at the higher altitude comprised 67.8% of the surveys collected. The majority of the survey feedback were from individuals of the African ancestry population (40.5%), followed by European ancestry (28.6%), Indian/Asian (25.0%) and Coloured (5.8%).The mean (SD) age of participants living at higher altitude were 23(8)years and 31(12) years for participants living at low altitude.

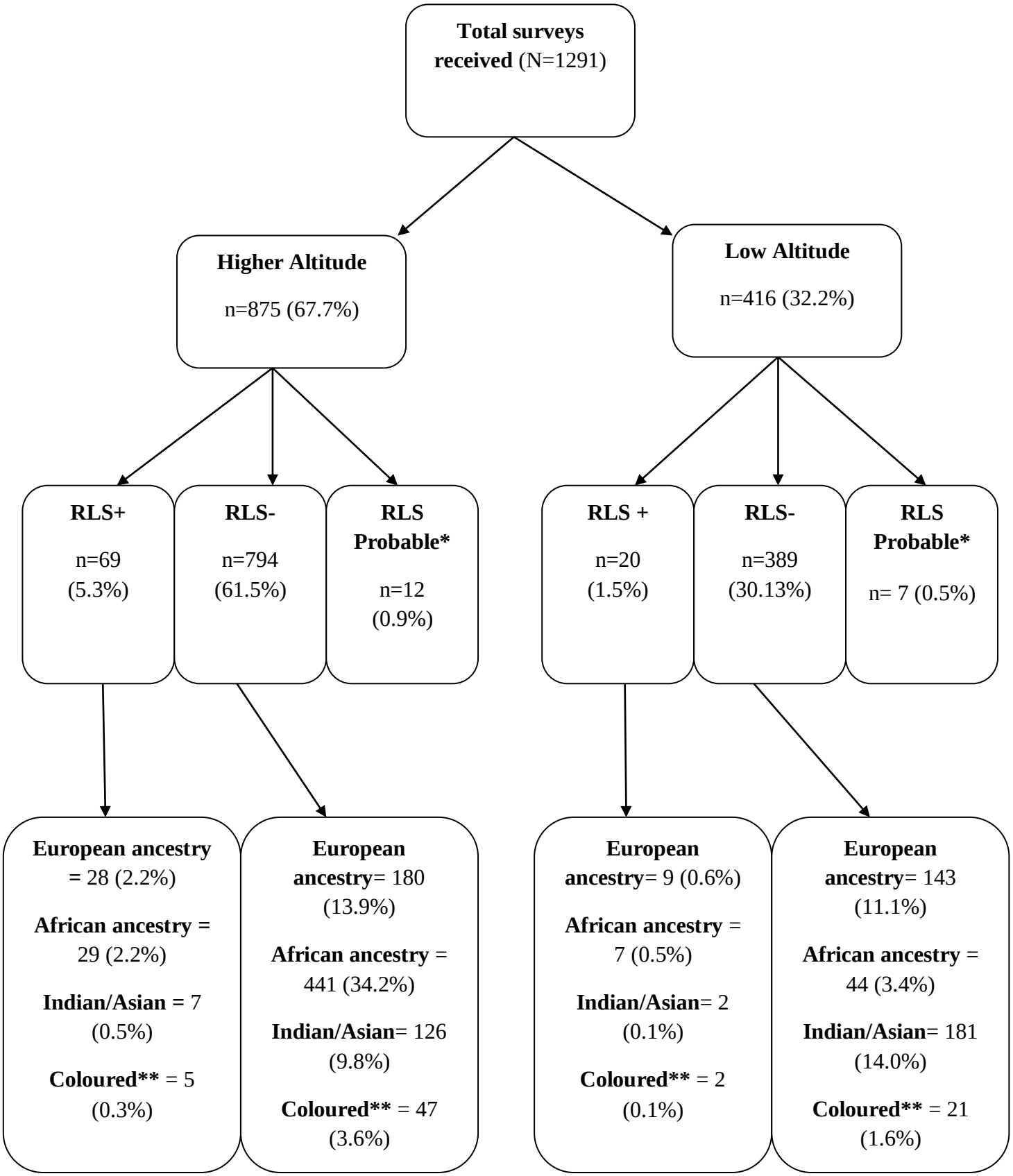
Total surveys received (N=1291)
Higher Altitude
n=875 (67.7%)
Low Altitude
n=416 (32.2%)
RLS+
n=69 (5.3%)
RLS-
n=794 (61.5%)
RLS Probable*
n=12 (0.9%)
RLS +
n=20 (1.5%)
RLS-
n=389 (30.13%)
RLS Probable*
n= 7 (0.5%)
European ancestry = 28 (2.2%)
African ancestry = 29 (2.2%)
Indian/Asian = 7 (0.5%)
Coloured** = 5 (0.3%)
European ancestry= 180 (13.9%)
African ancestry = 441 (34.2%)
Indian/Asian= 126 (9.8%)
Coloured** = 47 (3.6%)
European ancestry= 9 (0.6%)
African ancestry = 7 (0.5%)
Indian/Asian= 2 (0.1%)
Coloured** = 2 (0.1%)
European ancestry= 143 (11.1%)
African ancestry = 44 (3.4%)
Indian/Asian= 181 (14.0%)
Coloured** = 21 (1.6%)

Figure 3.1: Flow chart showing the distribution of the population, according to altitude, restless legs syndrome (RLS) status and ethnicity (European, African, Indian and Coloured ancestry).

RLS+, participants with RLS; RLS-, participants without RLS

*RLS probable individuals excluded from all statistical analysis due to uncertainty of RLS diagnosis

**Coloured population included in prevalence statistics, but excluded from all ethnicity statistics due to small sample size of colored population.

3.2 Demographic profile of Restless Legs Syndrome population

The overall RLS prevalence in our South African cohort was 6.7%, with participants living at higher altitude demonstrating a significantly higher prevalence than participants at low altitude [69 (7.9%) vs. 20 (4.8%), p =0.04], as shown in Table 3.1. The RLS+ cohort at higher altitude was significantly younger than the RLS+ cohort at low altitude [24 (8) vs. 29 (10)] years, p = 0.0041).There was no significant sex difference between the RLS+ cohorts at either altitude (p = 0.2)(Table 3.1). This study primarily consisted of participants of African ancestry, followed by those of European ancestry and Indian/Asian ancestry, with the least number of participants from Coloured ancestry. At the higher altitude, participants with European ancestry had a significantly higher prevalence of RLS compared to the African ancestry participants (p = 0.0025). At low altitude, the African ancestry population had a significantly higher RLS prevalence than the Indian/Asian population,(p= 0.0004) (Table 3.1). There were no other significant differences in prevalence rates of RLS between the ethnic groups (Table 3.1).

Table 3.1: Frequency details and comparison of demographics for individuals with and without Restless Legs Syndrome (RLS) living at higher altitude (n=875) and low altitude (n=416)

	High Altitude		Low Altitude		
	RLS +	**RLS –**	**RLS+**	**RLS-**	**p-value**
Sample Size, n (%)	69 (7.9)	794 (90.7)	20 (4.8)	389 (93.5)	**0.045**
Age (years), mean (SD)	24 (8)	24 (8)	29 (10)	31 (12)	**0.0001**
Sex					
Female, n (%)	54 (9.1)	536 (89.8)	13 (4.5)	275 (94.5)	0.24
Male, n (%)	15 (5.4)	258 (92.8)	7 (5.6)	114 (91.2)	0.24
Ethnicity					
European Ancestry, n (%)	28 (13.0) [a]	180 (83.7)	9 (5.8)	143 (92.9)	**0.022**
African Ancestry, n (%)	29 (6.2)[c]	441 (93.6)	7 (13.5)[d]	44 (84.6)	0.07
Indian/Asian,n (%)	7 (5.1) [b]	126 (91.9)	2 (1.0)	181 (97.3)	0.02

Data shown as n(%) or as mean(SD)

Test conducted to obtain p-value: RLS status, T-test; Age, T-test; Sex, Fisher's-Exact; Ethnicity, Fisher's-Exact

[a]$p<0.017$ (adjusted for multiple comparisons, Turkey HSD), European ancestry population higher altitude vs. low altitude

[b]$p<0.017$ (adjusted for multiple comparisons, Turkey HSD), Indian/Asian population higher altitude vs. low altitude

[c]$p<0.017$ (adjusted for multiple comparisons, Turkey HSD), European ancestry vs. African ancestry population at higher altitude

[d]$p<0.017$ (adjusted for multiple comparisons, Turkey HSD), African ancestry vs. Indian/Asian population at low altitude

RLS +, participants with restless legs syndrome; RLS-, participants without restless legs syndrome

3.3 Sleep - and iron related variables

The ESS scores were greater in the RLS+ cohort at higher altitude compared to in the RLS+ cohort at low altitude ($p = 0.001$), as well as compared to the RLS- cohort at higher altitude ($p = 0.0001$). However, there was no significant difference in ESS scores when comparing the RLS- cohorts from high and low altitude ($p = 0.67$) (Table 3.2).

No statistically significant difference was found in fatigue (FAS scores) between the RLS+ cohorts between altitudes ($p = 0.083$). There was also no significant difference in subjective sleep qualitybetween the RLS+ cohorts between altitudes ($p = 0.061$). There was a significant difference in the sleep onset duration, which showed that the RLS+ cohorts at higher altitude had a greater sleep onset duration than the RLS+ cohort at low altitude ($p = 0.008$) (Table 3.2).

The values, regarding self-reported iron levels, in Table 3.2 indicate the number (%) of participants who reported low or normal iron levels at both higher and low altitude. There were a greater number of participants at higher altitude who reported having low iron levels, therefore a significant difference was found when comparing the self-reported iron levels of the overall cohort between higher altitude and low altitude ($p = 0.027$) (Table 3.2). There was also a significantly greater number of participants in the RLS+ cohort at higher altitude who reported having low iron, than the number of participants in the RLS+ cohort at low altitude, ($p = 0.03$) (Table 3.2). A significant difference was also found with a larger number of participants in the RLS+ cohort reporting having low iron, than the number of participants in the RLS- cohort at higher altitude ($p = 0.0001$, Table 3.2). No significant difference was found in the iron levels between the RLS+ and RLS- cohorts at low altitude ($p = 0.09$) (Table 3.2).

Table 3.2: Sleep- and iron- related variables for individuals with and without Restless Legs Syndrome (RLS) living at higher altitude (n=875) and low altitude (n=416)

	Higher Altitude		**Low Altitude**		
	RLS+	**RLS-**	**RLS+**	**RLS-**	**p-value**
Epworth Sleepiness Scale score, mean (SD)	11.8 (4)	7.6 (5)	9.7 (3)	7.7 (4)	**0.01**
Fatigue Assessment Scale Score, mean (SD)	33.2 (1.0)	29.9 (5.2)	32.7 (2.4)	20.6 (3.8)	0.08
Sleep Quality score, mean (SD)	54.7 (5.2)	58 (2.0)	61.6 (3.5)	76 (3.9)	0.06
Self-reported Sleep Characteristics					
Sleep duration ≤ five hours, n (%)	13 (18.8)	365 (5.1)	3 (15)	126 (91)	0.24
Nighttime awakenings ≥ four times, n (%)	6 (8.7)	118 (56)	2 (10)	62 (13.5)	0.33
Sleep onset duration ≥ 20 minutes, n (%)	46 (66.6)	451 (13.4)	7 (35)	101 (22)	**0.008**
Self-Reported Iron Levels					
Low Iron, mean (SD)	54 (6.2)	310 (35.9)	11 (2.6)	135 (32.4)	0.03

Normal Iron, mean(SD)	15 (1.7)	484 (56.0)	9 (2.1)	254 (61.0)	0.16
	p-value comparing the number of participants who indicated low iron levels at higher altitude = **0.00**		p-value comparing the number of participants who indicated low iron levels at low altitude = **0.09**		

Data shown as n(%) or as mean(SD)

Test conducted to obtain p-value: ESS, T-test; FAS, T-test; Sleep Quality, ANOVA (Turkey); Self-reported sleep characteristics , Fisher's-Exact; Self-reported iron levels, Fisher's-Exact

RLS +, participants with restless legs syndrome; RLS-, participants without restless legs syndrome

3.4 Comparisons of RLS+ populations at higher altitude and low altitude

Table 3.3 exhibits the different RLS characteristics associated with the RLS+ cohort at higher altitude and low altitude. The higher altitude RLS+ cohort experienced significantly higher IRLS severity scores compared with the low altitude RLS+ cohort ($p = 0.0025$, Table 3.3). There was no significant difference found between the RLS+ cohorts, at higher versus low altitude, when comparing age of onset of RLS ($p = 0.31$), duration of RLS ($p = 0.1$) and familial history ($p = 0.92$) (Table 3.3).

There was no significant difference when comparing those who sought treatment for RLS ($p = 0.75$), and those who were taking treatment for RLS between higher altitudes and low altitude, ($p = 0.38$).There also was no significant difference when comparing the choice of RLS descriptors between the two altitudes (Table 3.3).

Table 3.3: Comparison of RLS characteristics for individuals with Restless Legs Syndrome (RLS) living at high altitude (n=69) and low altitude (n=20)

	High Altitude (n = 69)	**Low Altitude (n = 20)**	**p-value**
IRLS Severity score, mean (SD)	14.99 (7.1)	9.05 (8.5)	**0.00**
Frequency of RLS symptoms > four times per week, n (%)	26 (89.6)	7 (35)	0.20
RLS Duration (years), mean (SD)	6.5 (6.5)	9.9 (10.4)	0.10
Age of RLS Onset (years), mean (SD)	19.1 (2.4)	22.06 (7.5)	0.31
Family History of RLS, n (%)	23 (33.3)	5 (25)	0.92
Sought RLS Treatment, n (%)	8 (11.5)	3 (15)	0.75
Currently Taking RLS Treatment, n (%)	16 (23.1)	7 (35)	0.38
RLS descriptors, n (%)			
Painful	34 (49.2)	10 (50)	0.82
Itchy	10 (14.4)	4(20)	0.51
Creepy	7 (10.1)	3 (15)	0.69
Aching	34 (49.2)	6 (30)	0.20
Insects Under the Skin	5 (7.2)	0 (0)	0.59
Electric Current	20 (28.9)	5 (25)	1.00
Tugging	7 (10.1)	1 (5)	0.68
Numbness	24 (34.7)	11 (55)	0.12
Burning	5 (7.2)	1 (5)	1.00
Pins-and-Needles	22 (31.8)	5 (25)	0.78

Pulling	9 (13.0)	3 (15)	1.00
Tingling	13 (18.8)	4 (20)	1.00
Legs Falling Asleep	20 (28.9)	4 (20)	0.57
Indescribable feeling	11 (15.9)	1 (5)	0.29

Data shown as n(%) or as mean(SD)

Test conducted to obtain p-value: unpaired T-test for IRLS severity, Frequency of symptoms, RLS duration, and Age of RLS onset. Fisher's-Exact for Family history, sought treatment, Taking treatment, and RLS descriptors.

RLS +, participants with restless legs syndrome; RLS-, participants without restless legs syndrome; IRLS, International Restless Legs Syndrome

4 DISCUSSION

This cross-sectional study found the prevalence of RLS to be 6.70% in a South African population. RLS was significantly more prevalent in individuals living at a higher altitude (7.89%), than individuals living at low altitude (4.81%). RLS was more prevalent in individuals of European ancestry, compared to other ethnic groups at higher and low altitude, while the participants of African ancestry had a significantly higher prevalence of RLS than the Indian/Asian participants at sea-level. In the cohort of patients with RLS, RLS severity, daytime sleepiness and delayed sleep onset were significantly greater in those living at high altitude compared with those living at low altitude. Other characteristics of RLS (age of onset, duration of RLS, family history, RLS descriptors) were similar between higher and low altitude. A greater number of participants in the total population self-reported low iron levels at the higher altitude compared to the low altitude and in the RLS+ cohort at the higher altitude compared to the RLS+ cohort at low altitude.

4.1 RLS prevalence

4.1.1 The role of ethnicity on RLS prevalence in a South African population

The prevalence of RLS obtained in our study (6.70%) was lower than the reported global prevalence which is based mainly from European and North American data, and average of 12.5% worldwide (Koo, 2012). The South African population consists of a vast range of racial and ethnic diversities, with the highest proportion of individuals being of African ancestry. Previous studies, conducted in Africa (Table 1.1) have shown that populations of African ancestry have a lower RLS prevalence (0.03 – 6.77%), compared to the global RLS prevalence (Ferreira et al., 2013; Fawale et al., 2016; Winkler et al., 2010; Burtscher et al., 2014). Furthermore, previous RLS prevalence studies conducted in Indian/Asian populations also showed a lower prevalence, in comparison to European ancestry (Winkler et al., 2010; Rangarajan et al., 2007). Therefore, the lower prevalence rate found in our study may be due to the individuals of African ancestry and Indian/Asian ancestry having a lower incidence of RLS than individuals in European and North American populations.

Ethnicity differences in the prevalence of RLS could be attributed to physiological differences between ethnicities, as well as geographic and genetic factors. Physiologically, it has been shown that individuals of African ancestry have increased ferritin and transferritin levels, associated with higher iron levels within the body (Zacharski et al., 2000). Given that iron deficiency is associated with the pathophysiology of RLS (Earley et al., 2005), it is possible that individuals of African ancestry are less susceptible to RLS due to the increased ferritin and transferritin levels. Geographically, RLS might be influenced by factors such as altitude (Gupta et.al, 2017) and line of latitude (Koo, 2012). It is hypothesized that UV radiation may sustain RLS symptoms, as UV radiation promotes the production of reactive oxygen species (ROS), therefore causing the formation of radical hydroxyl, a conversion which requires iron (Trenam et al., 1992). UV radiation also facilitates the release of iron from ferritin stores (Aubailly et al., 1991). This may impact RLS symptoms, as iron is used for the regulation of dopamine. Melanin is higher in individuals of African ancestry due to increases in the activity of the melanogenic enzyme, tyrosinase, in melanocytes 91 (Alaluf et al., 2002). Melanin absorbs UV rays and protects the body from UV radiation damage (Alaluf et al., 2002), and since it is higher in individuals with darker skin, it may explain the decreased RLS prevalence in individuals of African ancestry. Exposure to UV rays causes an increase in serum α-melanocyte stimulating hormone (α-MSH), a hormone which creates RLS symptoms when administered to humans (Koo et al., 2008). When individuals of European ancestry are exposed to UV rays, the increase in α-MSH concentrations may induce RLS symptoms (Koo et al., 2008). However, when individuals of African ancestry are exposed to UV radiation, due to the naturally high levels of melanin, they have been shown to experience negligible increases in α-MSH (Koo, 2012). Lastly, it is possible that epigenetic influences from the environment may facilitate the interaction between RLS and genetics in different ethnic groups (Jimenez-Jimenez et al., 2018). However, to my knowledge, no research evaluating the genetics of RLS in different ethnic groups has been conducted to-date. Thus, there is potential that RLS prevalence can be influenced by genetic variability, and research focusing on this aspect of the pathophysiology is especially interesting in a South African context, due to the genetic diversity.

The low prevalence of RLS in the South African Indian/Asian population group from our study agrees with other studies done in Indian population groups. The first Indian/Asian RLS prevalence study conducted in South India reported .an RLS prevalence of 2.1% (Rangarajan et

al., 2007). These results were supported by a 2009 study conducted in India, where the prevalence of RLS was 1.5% in the Indian/Asian population compared to the general prevalence of European populations (Bhowmik et al., 2007). As discussed above, this difference can be due to physiological and geographic factors as the Indian/Asian populations have higher levels of melanin, compared to individuals of European ancestry (Wakamatsu et al., 2006). However, somewhat in contrast to this hypothesis, the Coloured population in our study had the lowest RLS prevalence. This may simply be due to the low response rate (75 of 1291 total respondents) we received from the Coloured population, however there is a lack of literature on RLS in the Coloured population, essentially made of multi-racial ethnicity, that is fairly unique to South Africa (de Wit et al., 2010).

4.1.2 The role of age on RLS prevalence in a South African population

The younger age group in our study population (24 (8) years) may be a contributing factor to the lower RLS prevalence, compared to global RLS prevalence, as RLS onset is more likely to occur in older individuals (Allen &Earley., 2000). The study group at high altitude was significantly younger (24 (8) years) than the study group at sea level (29 (10) years). Nevertheless, RLS was found to be more prevalent at higher altitude, despite the younger population. Although the prevalence of RLS increases with age (Phillips et al., 2000), it is possible that due to the higher altitude, RLS symptoms became more prominent at a younger age. The effect of altitude on RLS will be discussed in section 4.2. A 2017 study reported that approximately 45% of individuals experience their first episode of RLS before 20 years of age, however the onset of RLS beginning at a later age is more prevalent (>50years) (Guo et al., 2017). Researchers found that there was a 3% RLS prevalence for individuals aged younger than 29 years old, 10% prevalence in people aged 30-79 years old and 19% prevalence in those over 80 years of age (Zucconi&Ferini-Strambi., 2004). Expanding the age range of our population may result in different prevalence rates of RLS in the South African population, however despite the younger age of the study population, RLS still featured in the South African population and warrants further exploration. The median age of the South African population is 28 years old and our sample is close to the median age, which may indicate accuracy of the RLS prevalence found in our results.

4.1.3 The role of sex on RLS prevalence in a South African population

Sex is proposed as a contributing risk factor for RLS (Seeman., 2020). In particular, RLS is reportedly diagnosed twice as many times in females than men (Seeman., 2020). The prominence of RLS in females may be due to pregnancy, which is a cause of secondary RLS, as well as iron-deficiency anaemia which is more common in women and is also known to be associated with RLS (Coad & Conlon., 2011). In our study, although we had more females (69.03%) complete the survey than males; RLS was not found to be more common in females than males. We found no significant difference in sex between the RLS populations at higher and low altitude, or within each altitude.

It is possible that the sex difference in RLS prevalence may not be apparent yet given the younger age of participants at high altitude. The findings of our study are in conjunction with another African study by Fawale et al. (2016) who found that there were no significant sex differences between patients with RLS and healthy control participants (Fawale et al., 2016). However, previous studies in Europe, North America and Asian show a greater RLS prevalence in females (Ohayon et al., 2012). It is possible that ethnicity is the reason for the lack of significant difference in sex distribution seen in this study. However, a larger study group is needed to properly ascertain sex distribution of RLS in an African population.

4.2 Factors contributing to differences in RLS prevalence at different altitudes

We found that the RLS prevalence was greater at higher altitude (7.89%) compared to low altitude (4.89%). This is consistent with previous literature which has reported an increase in altitude to be a risk factor for RLS (Castillo et al., 2006). Castillo et al. (2006) reported the overall prevalence of RLS in adults living at a higher altitude (3.2%) was significantly higher than that of adults living at low altitude (0.80%). Similar findings were reported by Gupta et al., (2017) who found that in an Indian population at low altitude, RLS prevalence was low (2.5%), which was consistent with the tendency to see lower RLS prevalence in Indian individuals. However, the prevalence at high altitude (3200m) was higher (11.8 – 12.2%) than is usually reported in both low- and high-altitude European prevalence studies (Table 1.1). Although the difference in RLS prevalence between altitudes in the current study is not as large as that reported by Gupta et al (2017), it should be noted that the high altitude in the current study was

lower than that of the Gupta study (1753m vs 3200m above sea-level) and contained different population demographics.

The reason RLS may be more prevalent at high altitude is not fully understood, but Castillo et al. (2006), hypothesised that the increase may be due to two factors: 1) changes in iron metabolism and 2) altitude-induced hypoxia. A pilot study, detailed in Table 1.2, investigated the effect of altitude on RLS and reported greater leg movements, and by proxy RLS symptoms, at high altitude thus supporting a role for peripheral hypoxia in RLS (Stefani et al., 2016). Individuals living at high altitude undergo persistent exposure to low air pressure, which reduces the partial pressure of inspiratory oxygen in the atmosphere, causing reduced oxygen saturation of Hb in the blood (Muckenthaler et al., 2020). Low partial pressure of oxygen stimulates erythropoesis which results in the increased demand for iron. Iron deficiency is regarded as a significant contributing cause of RLS (Allen et al., 2013). Therefore, at high altitude, the increased demand for iron, in people with an iron-deficiency and thus an already iron deficient system, may increase the risk of developing RLS symptoms.

There were a greater number of participants at higher altitude compared to low altitude who reported an iron deficiency. There was also a significantly greater self-reported iron deficiency between the RLS+ cohorts at higher altitude versus low altitude, ($p = 0.03$) as well as between the RLS+ and RLS- cohorts at high altitude only ($p = 0.0001$, Table 3.2). The low iron levels and increased iron demand of individuals living at higher altitude may play a role in local hypoxia and ischaemic injury (Wessling-Resnick, 2010). Low iron in the brain of RLS patients activates the hypoxic pathway (Connor et al., 2017) and peripheral hypoxia is associated with the appearance of RLS symptoms (Salminen et al., 2014). Due to high altitude being a cause of hypoxia and hypoxia leading to the appearance of RLS symptoms, it may explain why our study, which showed an increase in self-reported low iron at higher altitude, found an increase in RLS prevalence at higher altitude.

Previous studies indicate the prevalence of RLS is about nine times greater in people with iron-deficient anemia than in the global populations (Allen et al., 2013). The uptake of iron in the CNS is regulated by the expression of transferrin receptor 1 in endothelial cells of the blood-brain barrier (Rouault et al., 2006). Iron bound to transferrin in the systemic circulation is endocytosed by the endothelial cells in the brain, and elemental iron is released to the brain

interstitial fluid by ferroportin (Rouault et al., 2006). As previously stated, patients with RLS have a higher concentration of hepcidin, subsequently causing a more rapid degradation of ferroportin, which may lead to an iron deficiency (Weinstock et al., 2020). Allen et al (2015) hypothesised that RLS was a result of an altered iron status in the CNS, indicating the low body iron may increase the prevalence of RLS (Allen et al., 2015). Researchers assessing the regulatory proteins in RLS patients found that H-ferritin, was downregulated in the brains of individuals with RLS (Allen et al., 2001). H-ferritin is the most abundant transporter of iron and is also used for iron storage in the brain. A downregulation, as seen in RLS patients, may cause iron deficiency due to lack of transport and storage of iron (Wu et al., 1999). Brain iron dysregulation in the CNS has been linked to the onset of RLS symptoms.

Our study also found that there were significantly lower iron levels in the RLS+ cohort compared to the RLS- cohort, at high altitude. In contrast, the iron levels between the RLS+ and RLS- population, at low altitude, were not significantly different. As previously discussed, living at high altitude increases the need for iron (Muckenthaler et al., 2020). Therefore, living at a high altitude is a risk factor for iron deficiency anemia, resulting in inadequate iron transport across the blood-brain barrier which may then disrupt dopamine synthesis, potentially causing the onset of RLS symptoms and subsequently RLS. Therefore, living at higher altitude may be the reason for the larger amount of self-reported iron deficiency in the RLS population at higher altitude compared to low altitude.

In 2020, a South African study that had adjusted for hemoglobin (Hb) levels at higher altitude in order to detect iron deficiency in women in Johannesburg (Silubonde et al., 2020), found a 39% anaemia prevalence (21.3% iron deficiency anemia) (Silubonde et al., 2020).Silubonde et al. (2020) showed an increase in the prevalence of iron deficiency in Johannesburg, South Africa, compared to the prevalence of iron deficiency at low altitude, which is consistent with the results found in this study. Low iron may be caused by the high altitude of the area, resulting in the increased prevalence of RLS.

As previously stated, ethnicity may also be a contributing factor in the difference in RLS prevalence at different altitudes. The vast ethnic diversity found in South Africa may differ at various geographical locations, leading to differences found in RLS prevalence. As stated in 4.1.1, previous studies, conducted in Africa (Table 1.1) show that populations of African

ancestry (Ferreira et al., 2013; Fawale et al., 2016; Winkler et al., 2010; Burtscher et al., 2014) and Indian/Asian ancestry (Winkler et al., 2010; Rangarajan et al., 2007) have a lower incidence of RLS than individuals in European and North American populations. It is possible that there is a larger population of individuals of African and Indian/Asian ancestry living at low altitude in comparison to high altitude, which may be the cause of the lower RLS prevalence rate at low altitude.

4.3 The effect of altitude on the characteristics of RLS

4.3.1 Severity

Individuals living at higher altitude exhibited a higher mean IRLSSG severity score (14.9 [2-33]) than those living at low altitude (9.05 [1-32]) indicating that RLS is significantly more severe at high altitude. The RLS+ cohort at higher altitude had moderately severe symptoms indicating moderate discomfort in the legs, 2 – 3 days a week, while the RLS+ cohort at low altitude had mild symptoms one day a week or less. There are no previous studies indicating the RLS severity measures between high and low altitudes, but studies that have focused on hypoxia, which is more prevalent at higher altitude, found a relationship between hypoxia and severity of RLS (Salminen et al., 2014). The findings of Salminen et al. (2014) suggested a pathophysiologic link between peripheral hypoxia and the severity of RLS symptoms. The study found that patients with severe RLS had a higher oxygen gradient between their feet and chest, compared to the healthy controls and concluded that hypoxia played a role in the severity of RLS (Salminen et al., 2014). Hypoxia due to high altitude may therefore have contributed to the higher RLS severity found in this study at higher altitude. However, there could be other causes for this result and more research is needed to confirm the result found in this study.

4.3.2 Sleep variables associated with RLS

A significant difference in daytime sleepiness was found between the RLS+ cohort and the RLS- cohort at higher altitude, where the RLS+ cohort exhibited greater daytime sleepiness. This was to be expected as patients with RLS experience increased frequency of sleep disturbances (Abetz et al., 2004; Berger et al., 2004; Allen et al., 2005; Allen et al., 2010; Kushida et al., 2007). Studies conducted in sleep clinics have reported that insomnia is a common consequence of RLS

(Earley and Silber, 2010). 50-85% of RLS patients reported insomnia, affecting their sleep onset and maintenance. Overall, sleep studies have confirmed sleep disturbances, as well as decreased sleep efficiency and an overall reduced total sleep time in RLS patients (Earley and Silber, 2010). The RLS+ population at high altitude had a moderate IRLSSG severity score meaning discomfort in the legs 2 – 3 days a week, which may have caused a reduced total sleep timeand may explain the higher level of daytime sleepiness between the RLS+ and RLS- participants at high altitude.

In addition, the current study found that there was a significantly higher level of daytime sleepiness in RLS+ cohort at higher altitude compared to RLS+ cohort at low altitude. Sleep at higher altitude is characterised by poor subjective quality, frequent awakenings, brief arousals, marked nighttime hypoxia, and periodic breathing (Wickramasinghe et al., 1999). Although the increase in arousals during sleep at higher altitude seems to be linked to respiratory periodicity and hypoxia, the mechanisms contributing to the sleep changes are not well understood (Weil et al, 2004).Hypoxia in conjunction with periodic breathing is the primary cause of sleep disruption at higher altitude (Wickramasinghe et al., 1999). Poor sleep at night may subsequently lead to an increase in daytime sleepiness (Stepanski et al., 2012). The increased sleep fragmentation at higher altitude is the reason for poor sleep quality and may account for some of the elevated levels of daytime sleepiness at high altitude (Wickramasinghe et al., 1999).

There was a significant difference in the duration of sleep onset between the RLS+ cohort at higher and low altitude, as the RLS+ cohort at higher altitude had a longer duration of sleep onset ($p = 0.008$). A sleep study compared polysomnography findings in a large group of patients with idiopathic RLS and healthy controls and concluded that patients with RLS expressed prolonged sleep onset latencies, shorter total sleep time, lower sleep efficiency and longer REM sleep latency compared to the healthy controls (Hornyak et al., 2007). Our study found no significant difference in the sleep duration, night-time awakenings, and measures of fatigue or in the sleep quality between RLS+ cohort at higher and low altitude. In contrast, previous literature indicates that there is an increase of fatigue and a decrease in the sleep quality in patients with RLS compared to healthy controls (Allen et al., 2005). These inconsistent findings may be due to our small study sample or that younger participants are more resilient to sleep loss (Pasula et al., 2018).It is possible that fatigue and sleep quality are related to the severity of RLS and severity

at high altitude was moderate, while as severity at low altitude was mild. Researchers comparing the differences in sleep quality, sleepiness and fatigue according to severity of symptoms of RLS found that the severity of RLS symptoms affects not only sleep quality but also multiple aspects of quality of life (Cuellar et al., 2007).

4.3.3 Presentation of RLS

This study found no significant difference in the descriptors of RLS between the RLS+ cohorts at higher and low altitude, which means that altitude was not seen to affect the sensations of RLS. RLS is notoriously difficult for individuals to describe, but consistent descriptive words include aching, throbbing, pulling, itching, crawling, or creeping (O'Keeffe, 1996). Picchietti et al., (2011) analysed symptom descriptors from patients with paediatric RLS and found the most common descriptors to be, ‘‘Need to move/kick,’’ ‘‘pain/hurts,’’ ‘‘uncomfortable/ cannot get comfortable,’’ and ‘‘like bugs or ants/crawling’’. Similarly, in our study, the RLS+ cohorts, at both higher and low altitude used words such as itching, creepy-crawly, aching, tugging, numbness and burning to describe the sensations of RLS, indicating that the descriptors of sensations of RLS do not seem to be severely altered by altitude.

Researchers have found that there is a need for more accurate descriptors in order to improve the diagnostic accuracy of RLS. Kerr et al (2012) investigated the accuracy of the current diagnostic descriptors and concluded that the most frequently used descriptors differ from the terminology used in the RLS diagnostic criteria (Kerr et al., 2012).It is possible that with more specific RLS descriptors, there may be a difference in the sensation descriptors between higher and low altitude RLS+ patients.

The results of this study showed no significant difference between RLS+ cohort who sought treatment between higher and low altitude, despite the greater severity of RLS at higher altitude. The lack of individuals seeking treatment may be due to the financial economy in South Africa, as many individuals cannot afford medical assistance (Ponsar et al., 2011). This may also account for the low overall prevalence of RLS in South Africa as individuals are more focused on burdens perceived as a greater threat to their wellbeing and survival. Durgin et al., (2015) investigated the economic burden of RLS and found that RLS patients had a higher estimated direct and indirect cost than healthy individuals. Researchers also found that the increasing

severity of RLS is associated with increased economic burden for RLS patients (Durgin et al., 2015).Socioeconomic factors play a role in the ability of people being able to seek treatment.

4.4 Limitations and future study directions

There are a few study limitations that need to be recognised. First, the demographic distribution of the sample is a limitation because the overall sample size at different altitudes, and of each race group at different altitudes, should be larger, to facilitate making any conclusive statements about ethnicity and RLS. Future studies should increase the sample size at both higher and low altitudes in order to obtain a clearer picture of the role that altitude plays in RLS. The small sample size of the mixed ancestry ethnic group (e.g Coloured population) led to them being excluded in the statistical analysis. Future studies should focus on increasing the sample size of this group as it provides an excellent crossover between populations of European and African ancestry. Also important to note is that the diagnostic surveys included in this study have not been validated in a South African population, however the participants of our study could all understand and speak English and the measures have been validated in English speaking populations. However, this is a limitation of the study. As RLS severity, age of onset and prevalence is affected by age, having a RLS cohort in a similar age range may impact the RLS prevalence results obtained. Ideally, the sample of future studies should include individuals of a wide age range. In our study iron levels were self-reported which is not ideal as not every individual may be aware that they have an iron deficiency. Future studies should consider measuring the iron of each participant in order to gain the most accurate results. It is also important to note that this study did not account for parity when surveying participants, and it is recommended that future studies assess parity. In addition, future studies should measure the partial pressure of oxygen in participants at both high and low altitude, in order to determine hypoxia and its role in RLS. In the study, questionnaires were administered electronically which excludes a large proportion of the population from lower socioeconomic backgrounds, ideally questionnaires should be administered in-person as well as electronically as to get a more diverse study group for assessment.

4.5 Conclusion

This study found that higher altitude may play a role in the prevalence of RLS in a South African population, where there is an increase in the prevalence of RLS at higher altitude. RLS prevalence in the SA population is low, compared to global RLS prevalence, but the results obtained in this study can contribute to the overall understanding of RLS, given the diversity of the population. The current study has shown that prevalence of RLS is affected by ethnicity at both higher and low altitude and RLS is more prevalent in individuals of European ancestry as compared to other ethnicities. This study found that the age of RLS onset, and the sex prevalence in RLS are not altered by an increase in altitude. Factors associated with RLS, such as RLS severity, daytime sleepiness and decreased iron levels are exacerbated by higher altitude in individuals with RLS, which may be due to hypoxic conditions. Although this study found that RLS severity increases at higher altitude, there was no difference in the descriptors used to describe RLS sensations between altitudes. Overall, this study concludes that high altitude is a risk factor for RLS and exacerbates the major characteristics of RLS.

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6 Appendices

Appendix A: Ethical Clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

2022/05/24

Dr S Kerr, et al
School of Physiology
Medical School
University

Sent by e-mail to: Samantha.Kerr@wits.ac.za

Dear Dr Kerr

Re: **Protocol Ref No:** M200213
Protocol Title: *Prevalence and phenotypic characteristics of Restless Legs Syndrome (RLS) and Periodic Limb Movements (PLM) in a South African population*
Principal Investigators: Dr S Kerr, et al

Thank you for your e-mail of 2022/05/19.

I confirm that we have noted and approve of the current investigating team on this project, which comprises:

1. Dr S Kerr (Principal Investigator)
2. Dr C Dafkin
3. Dr S Iacovides
4. Ms A Zoras (MSc student)
5. Ms P Munian (MSc student)
6. Ms J Bourne (Hons student)

Thank you for keeping us informed.

Yours Sincerely

J. Burns
.....
Mr I Burns
For the Human Research Ethics Committee (Medical)

CB Penny
.....
Dr CB Penny, Chairperson, Human Research Ethics Committee (Medical)

Appendix B: Sleep Questionnaire

Sleep in SA population

We have obtained approval for this study from the Wits Human Research Ethics Committee (M200213)

14. Please indicate any conditions, in addition to those listed on the previous page, that you are currently (within the last 6 months) taking any chronic (long-term) medications for: _____

_____

15. Please indicate the chronic medications and the dosages that you are taking: _____

_____

16. How long do you usually sleep for? ☐0 - 5 hours ☐6 - 8 hours ☐more than 9 hours

17. How many times do you wake up during your sleep at night?

☐0 to 1 times ☐2 to 3 times ☐4 to 5 times ☐more than 5 times

18. How long does it usually take you to fall asleep?

☐0 - 10 minutes ☐11 - 20 minutes ☐21 - 30 minutes ☐31 - 40 minutes ☐more than 40 minutes

19. How would you rate the quality of your sleep? On a scale from 0 to 100:_____

20. Do you suffer from any sleep disturbances? If so, please list._____

21. Please include any comments that you feel may contribute towards your sleep disturbances, if any (e.g. newborn baby feeding at night): _____

22. Have any of your close relatives experienced a sleep problem? (check all that apply)

☐Parent ☐Child ☐Sibling ☐Not applicable ☐I don't know

23. Do you have, or have you had, recurring uncomfortable feelings or sensations in your legs while you are sitting or lying down? ☐Yes ☐No

24. Do you, or have you had, a recurring need or urge to move your legs while you were sitting or lying down?

☐Yes ☐No

25. How often do you experience an urge to move your legs, accompanied by unpleasant sensations?

☐16 or more times a month ☐5-15 times a month ☐2-4 times a month ☐ Once a month or less ☐ Never

26. How would you describe the urge to move or unpleasant sensations in your legs? (check all that apply)

☐ Itching
☐ Creepy-crawly
☐ Aching
☐ Like insects crawling under the skin
☐ Like an electric current
☐ Tugging
☐ Numbness
☐ Burning
☐ Pins-and-needles
☐ Painful
☐ Pulling
☐ Tingling
☐ Like leg is falling asleep
☐ Indescribable

We have obtained approval for this study from the Wits Human Research Ethics Committee (M200213)

27. Are you more likely to have these feelings when you are resting (either sitting or lying down) or when you are physically active? ☐Resting ☐Active

28. Do these feelings usually start when you are resting (either sitting or lying down)? ☐Yes ☐No

29. If you get up or move around when you have these feelings do these feelings get any better while you actually keep moving? ☐Yes ☐No ☐I don't know

30. If yes, how often do you feel unpleasant, restless feelings in your legs that can be relieved by walking or movement?

☐16 or more times a month ☐5-15 times a month ☐2-4 times a month ☐ Once a month or less ☐ Never

31. Which times of day are these feelings in your legs MOST likely to occur? (Please indicate one or more than one)

☐Morning ☐Midday ☐Afternoon ☐Evening ☐Night ☐About equal at all times

32. Which times of day are these feelings in your legs LEAST likely to occur? (Please indicate one or more than one)

☐Morning ☐Midday ☐Afternoon ☐Evening ☐Night ☐About equal at all times

33. Will simply changing leg position by itself once without continuing to move usually relieve these feelings?

☐ Usually relieves ☐ Does not usually relieve ☐I don't know

34. Are these feelings ever due to muscle cramps? ☐Yes ☐No ☐I don't know

35. Do these feelings occur only when sitting or only when lying down or both when sitting and lying down?

☐Neither ☐Only when sitting ☐Only when lying down ☐Both when sitting and lying down

36. When you experience the urge to move or unpleasant sensations how do they affect your ability to sleep? (check all that apply) ☐Little or no effect ☐Interfere with my falling asleep ☐Awaken me after falling asleep ☐ Keep me from getting a good night's sleep ☐ Disturb my bed partner

37. In the past 12 months, how often did you experience these feelings in your legs? (Please choose one)

☐Everyday ☐4-5 days per week ☐2-3 days per week ☐1 day per week ☐2 days per month ☐1 day per month or less ☐Never

38. Approximately how old were you when you first noticed these feelings in your legs? (Please write age in years)_____

39. How does alcohol influence your urge to move or the unpleasant sensations?

☐Makes them worse ☐Makes them better ☐I don't know ☐Never drink ☐No effect

7	How severe was your mood disturbance due to your symptoms - for example being angry, depressed, sad, anxious or irritable?	○	○	○	○	○

48. Overall, how much relief from your arm or leg discomfort did you get from moving around? ☐No Relief

☐Mild Relief ☐Moderate Relief ☐Either complete or almost complete Relief ☐No symptoms to be relieved

49. How often did you get symptoms? ☐Very often (6-7 days per week) ☐Often (4-5 days per week)

☐Sometimes (2-3 days per week) ☐Occasionally (1 day per week) ☐Rarely (less than 1 day per week)

50. When you had symptoms, how severe were they on average? ☐Very severe (8 hours or more per 24 hour day)

☐Severe (3-8 hours per 24 hour day) ☐Moderate (1-3 hours per 24 hour day)

☐ Mild (less than 1 hour per 24 hour day) ☐None

The following statements refer to how you usually feel. Please choose the answer to each question that is most applicable to you. Please give an answer to each question, even if you do not have any complaints at the moment.

		Never	Sometimes (less than once a month)	Regularly (few times a month)	Often (weekly)	Always (daily)
1	I am bothered by fatigue	○	○	○	○	○
2	I get tired very quickly	○	○	○	○	○
3	I don't do much during the day	○	○	○	○	○
4	I have enough energy for everyday life	○	○	○	○	○
5	Physically, I feel exhausted	○	○	○	○	○
6	I have problems starting things	○	○	○	○	○
7	I have problems thinking clearly	○	○	○	○	○
8	I feel no desire to do anything	○	○	○	○	○
9	Mentally, I feel exhausted	○	○	○	○	○
10	When I am doing something, I can concentrate quite well	○	○	○	○	○

How likely are you to doze off or fall asleep in the following situations after you've had your usual night's sleep? (please select the most appropriate option for each question)

		Never	Rarely	Sometimes	Most nights/days	Always
1	Sitting and reading	○	○	○	○	○
2	Watching tv	○	○	○	○	○
3	Sitting inactive in a public place	○	○	○	○	○
4	Passenger in a car for an hour without a break	○	○	○	○	○
5	Lying down to rest in the afternoon	○	○	○	○	○
6	Sitting and talking to someone	○	○	○	○	○
7	Sitting quietly after lunch with no alcohol	○	○	○	○	○
8	In a car, stopped for a few minutes in traffic	○	○	○	○	○

having a satisfactory family, home, social, school or work life?

Appendix C: Cambridge-Hopkins Restless Legs Syndrome questionnaire

Answer the questions as completely as you can. Please **mark the checkbox** for the best answer to each question.

1. Do you have, or have you had, recurrent uncomfortable feelings or sensations in your legs while you are sitting or lying down?	☐ Yes ☐ No
2. Do you, or have you had, are current need or urge to move your legs while you were sitting or lying down?	☐ Yes ☐ No
If you answered YES to either question 1 or 2 continue Question 3. If you answered NO to BOTH stop here The following is about these feelings	
3a. How often do you experience an urge to move your legs, accompanied by unpleasant sensations?	☐ 16 or more times a month ☐ 5-15 times a month ☐ 2-4 times a month ☐ Once a month or less ☐ Never
3b. How would you describe the urge to move or unpleasant sensations in your legs (check all that apply)?	☐ Itching ☐ Creepy-crawly ☐ Aching ☐ Like insects crawling under the skin ☐ Like an electric current ☐ Tugging ☐ Numbness ☐ Burning ☐ Pins-and-needles ☐ Painful ☐ Pulling ☐ Tingling ☐ Like leg is falling asleep ☐ Indescribable
4. Are you more likely to have these feelings when you are resting (either sitting or lying down) or when you are physically active?	☐ Resting ☐ Active
5. Do these feelings usually start when you are resting (either sitting or lying down)?	☐ Yes ☐ No

6. If you get up or move around when you have these feelings do these feelings get any better while you actually keep moving?	☐ Yes ☐ No ☐ Don't know
6a. If yes, how often do you feel unpleasant, restless feelings in your legs that can be relieved by walking or movement?	☐16 or more times a month ☐5-15 times a month ☐2-4 times a month ☐Once a month or less ☐ Never
7. Which times of day are these feelings in your legs most likely to occur? (Please indicate one or more than one)	☐ Morning ☐ Midday ☐ Afternoon ☐ Evening ☐ Night ☐ About equal at all times
8. Which times of day are these feelings in your legs least likely to occur? (Please indicate one or more than one)	☐ Morning ☐ Midday ☐ Afternoon ☐ Evening ☐ Night ☐ About equal at all times
9. Will simply changing leg position by itself *once* without continuing to move usually relieve these feelings?	☐ Usually relieves ☐ Does *not* usually relieve ☐ Don't know
10. Are these feelings ever due to muscle cramps?	☐ Yes ☐ No ☐ Don't know
10a. If so, are they always due to muscle cramps?	☐ Yes ☐ No ☐ Don't know
11. Do these feelings occur only when sitting or only when lying down or both when sitting and lying down?	☐ Neither ☐ Only when sitting

	□ Only when lying down □ Both when sitting and lying down
12. When you actually experience the feelings in your legs, how distressing are they?	□ Not at all distressing □ A little bit □ Moderately □ Extremely distressing
13. When you experience the urge to move or unpleasant sensations how do they affect your ability to sleep (check all that apply)?	□ Little or no effect ☐Interfere with my falling asleep ☐Awaken me after falling asleep ☐Keep me from getting a good night's sleep ☐Disturb my bed partner
14. In the past 12 months, how often did you experience these feelings in your legs? (Please indicate one)	☐Everyday ☐4-5daysperweek ☐2-3daysperweek ☐1dayperweek ☐2dayspermonth ☐1daypermonthorless ☐Never
15. Approximately how old were you when you first noticed these feelings in your legs? (please write age)	Yrs
16. How does alcohol influence your urge to move or the unpleasant sensations?	□ Makes them worse ☐Makes them better ☐Don't know ☐Never drink ☐No effect
17. How does exercise influence your urge to move or the unpleasant sensations?	□ Makes them worse ☐Makes them better

	☐Don't know ☐Never exercise ☐No effect
18. Is this urge to move or unpleasant sensation made worse by over-the-counter sleeping or cold medications (e.g. Benadryl)?	☐ Makes them worse ☐Makes them better ☐Don't know ☐Never take over-the-counter sleep medications ☐No effect
19. WOMEN: Did you first notice these feelings in your legs during pregnancy?	☐ Yes ☐ No
19a. If yes have you noticed these other than related to pregnancy?	☐ Yes ☐ No
19b. How were these feelings affected by pregnancy?	☐ Makes them worse ☐Makes them better ☐Don't know ☐No effect
Makes them worse ☐Makes them better ☐Don't know ☐No effect	☐ Left ☐Right ☐Both
21. Do you perceive this urge to move or unpleasant sensation as painful?	☐ Yes ☐ No ☐Don't know
22. If you have experienced an urge to move or unpleasant sensation in your legs, have you sought medical treatment?	☐ Yes ☐ No
23. Did you ever experience, or were you ever told that you suffered from "growing pains" as a child?	☐ Yes ☐ No ☐Don't know

Appendix D: Epworth Sleepiness Scale (ESS)

		Grading Scale				
		Never	**Rarely**	**Some-times**	**Most nights/days**	**Always**
1	How likely are you to doze off or fall asleep in the following situations after you've had your usual night's sleep:					
a	Sitting and reading	1	2	3	4	5
b	Watching TV	1	2	3	4	5
c	Sitting inactive in a public place	1	2	3	4	5
d	Passenger in a car for an hour without a break	1	2	3	4	5

e	Lying down to rest in the afternoon	1	2	3	4	5
f	Sitting and talking to someone	1	2	3	4	5
g	Sitting quietly after lunch with no alcohol	1	2	3	4	5
h	In a car, stopped for a few minutes in traffic	1	2	3	4	5

Appendix E: Fatigue Assessment Scale

Fatigue Assessment Scale (FAS)

The following 10 statements refer to how you usually feel. For each statement you can choose one out of five answer categories, varying from *never* to *always*. 1 = *never*; 2 = *sometimes;* 3 = *regularly*; 4 = *often*; 5 = *always*.

	Never	Sometimes	Regularly	Often	Always
1. I am bothered by fatigue (WHOQOL)	1	2	3	4	5
2. I get tired very quickly (CIS)	1	2	3	4	5
3. I don't do much during the day (CIS)	1	2	3	4	5
4. I have enough energy for everyday life (WHOQOL)	1	2	3	4	5
5. Physically, I feel exhausted (CIS)	1	2	3	4	5
6. I have problems starting things (FS)	1	2	3	4	5
7. I have problems thinking clearly (FS)	1	2	3	4	5
8. I feel no desire to do anything (CIS)	1	2	3	4	5
9. Mentally, I feel exhausted	1	2	3	4	5
10. When I am doing something, I can concentrate quite well (CIS)	1	2	3	4	5

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Note: The abbreviations after the items indicate the scale from which the items has been abstracted. The following are the scales:
CIS - Checklist Individual Strength
WHOQOL - World Health Organization Quality of Life assessment instrument
FS - Fatigue Scale

Appendix F: Turnitin Report