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PIRITTA AUVINEN

Restless legs symptoms in primary health care patients with depression

RESTLESS LEGS SYMPTOMS IN PRIMARY HEALTH CARE PATIENTS WITH DEPRESSION

Piritta Auvinen

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ABSTRACT

The purpose of the dissertation was to investigate the prevalence of restless legs symptoms in depressed subjects in primary health care and look into its possible pathophysiology. Depression is a common mood disorder worldwide and is associated with several comorbidities, such as restless legs syndrome and pain. Restless legs symptoms cause an uncomfortable feeling in the lower limbs especially at rest.

The dissertation consists of four studies. The aim of this dissertation was to investigate (I) the prevalence of restless legs symptoms in depressive subtypes, (II) the association of depressive and restless legs symptoms in a follow-up setting, (III) the association of inflammatory markers with restless leg symptoms and depression and (IV) investigate a relationship between depression, restless legs symptoms and musculoskeletal pain.

Data were collected from the Finnish Depression and Metabolic Syndrome in Adults study. The study was conducted in the Central Finland Hospital District in 2008-2009 and included subjects over the age of 35 who had depressive symptoms and were followed up in 2015-2016 in primary health care. Depressive symptoms were screened by conducting the BDI survey for all participants. If a score was higher than 10, a M.I.N.I. interview was conducted of that subjects to assess the diagnosis and subtype of depression. A total of 706 patients were included and 426 controls were selected by random sampling. Restless legs symptoms, widespread pain, and other information were determined using a self-administered form.

The results of the study indicated that restless legs symptoms are common in primary care patients, especially in those with depression, but there is no significant difference in the prevalence between the subtypes of non-melancholic and melancholic depression (43.4% and 52.4%). In the follow-up study, moderate to high leisure time

physical activity protected subjects from restless legs symptoms, whereas a high number of depressive symptoms were associated with subjects having restless legs symptoms. Among the inflammatory markers, a higher concentration of TNF-α was associated with restless legs symptoms in subjects with depressive symptoms and clinical depression. Widespread pain and restless legs symptoms occurred in conjunction with control subjects as well as the subjects with depressive symptoms. Pain intensity was higher in the subjects with restless legs symptoms regardless of depressive symptoms or depression.

These results indicate that restless legs symptoms have a cross sectional and longitudinal association with depression being common and having an association with a TNF-α mediated inflammatory process especially in patients with depressive symptoms with or without clinical depression. This dissertation provides information on the prevalence of depression and restless legs symptoms in primary health care. The results of this study warrant future research of a causal relationship and mechanism between depression and restless legs symptoms.

National Library of Medicine Classification: QW 568, W 84.6, WL 108, WM 171.5, WM

Medical Subject Headings: Restless Legs Syndrome; Depressive Disorder; Depression; Mood Disorders; Primary Health Care; Tumor Necrosis Factor-alpha; Musculoskeletal Pain

Keywords: Restless legs syndrome; depressive disorder; depression; primary health care; TNF-alpha; pain

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TIIVISTELMÄ

Tämän tutkimuksen tavoitteena oli tarkastella perusterveydenhuollon masentuneiden potilaiden levottomat jalat oireita. Masennustila on yleinen mielialahäiriö maailmanlaajuisesti. Masennustilaan liittyy useita liitännäissairauksia. Masennustila ja levottomat jalat oireet esiintyvät usein yhdessä. Levottomat jalat oireet aiheuttavat epämiellyttävää tunnetta alaraajoihin, etenkin levossa.

Tutkimuksessa analysoitiin (I) masennustilan ja levottomat jalat oireiden esiintymistä masennuksen alatyypeissä, (II) masennustilan ja levottomat jalat oireiden esiintymistä pitkäaikaisseurannassa, (III) inflammaatiomarkkereiden yhteyttä masentuneiden potilaiden levottomat jalat oireisiin ja (IV) laaja-alaisen kivun, masennuksen ja levottomat jalat oireiden yhteyttä.

Tutkimuksessa käytettiin aineistona Finnish Depression and Metabolic Syndrome in Adults -tutkimusta. Tutkimus toteutettiin Keski-Suomen sairaanhoitopiirin alueella. Tutkimukseen otettiin mukaan yli 35-vuotiaat potilaat, joilla oli masennusoireita vuosina 2008-2009 ja seuranta toteutettiin 2015-2016. Masennusoireita selvitettiin tekemällä kaikille Beck Depression Inventory -oirekysely ja pistemäärän ollessa yli 10 jatkettiin Mini-International Neuropsychiatric Interview -haastatteluun masennusdiagnoosin ja alatyypin varmistamiseksi. Aineistossa oli 706 potilasta sekä lisäksi 426 henkilön kontrolliryhmä valittiin satunnaisotannalla. Levottomat jalat oireita, laaja-alaista kipua sekä muita tietoja selvitettiin itsetäytettävällä lomakkeella.

Tutkimuksen tuloksissa selvisi, että masennustilan yhteydessä levottomat jalat oireet ovat yleisiä perusterveydenhuollon potilailla, mutta esiintyvyydessä masennustilan alatyyppien, ei-melankolinen ja melankolinen masennustila, välillä ei ole merkittävää eroa (43.4% ja 52.4%). Seurannassa vähintään kohtalainen vapaa-ajan fyysinen aktiivisuus suojasi levottomat jalat oireilta, mutta korkea määrä masennusoireita taas altistivat potilaita levottomat jalat oireille. Inflammaatiomarkkereista

tuumorinekroositekijä alfan pitoisuus liittyi levottomat jalat oireisiin masennusoireisilla ja masennustilaa sairastavilla potilailla. Laaja-alainen kipu ja levottomat jalat oireet esiintyivät yhdessä kontrolliryhmän henkilöillä sekä masennusoireisilla, mutta yhteyttä ei ollut masennustilaa sairastavalla ryhmällä. Sen sijaan kaikissa ryhmissä levottomat jalat oireilu lisäsi kivun tuntemisen voimakkuutta.

Tutkimus osoittaa levottomat jalat oireiden liittyvän usein masennukseen poikkileikkaus- ja pitkittäisotannassa. Levottomat jalat oireilla on yhteys tuumorinekroositekijä alfa välitteiseen tulehdusprosessiin erityisesti potilailla, joilla on masennusoireita tai diagnosoitu masennustila. Tutkimukset antoivat tietoa perusterveydenhuollon masentuneiden potilaiden levottomat jalat oireiden esiintyvyydestä ja siihen liittyvistä tekijöistä. Väitöskirjan tutkimusten pohjalta voidaan lähteä jatkossa tutkimaan masennustilan ja levottomat jalat oireiden syy-seuraussuhdetta sekä niiden etiologisia tekijöitä entistä tarkemmin.

Yleinen suomalainen ontologia: levottomat jalat -oireyhtymä; masennus; mielenterveyshäiriöt; perusterveydenhuolto; tuumorinekroositekijät; kipu

Avainsanat: Levottomat jalat oireyhtymä; masennustila; masennus; perusterveydenhuolto; tuumonekroositekijä alpha; kipu

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I am fortunate to have my wonderful friends and family by my side and happily our moments together are mostly cheerful and full of laughter. Particularly my dear friend Doctor Mari Kinnunen I cannot thank you enough for your guidance over the years. I hope to inspire others as you have inspired me. I am grateful to my fiancé Teemu for

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Lahti, 31 March 2021
Piritta Auvinen

"Alone we can do so little; together we can do so much."

— Helen Keller

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications:

I Auvinen P, Mäntyselkä P, Koponen H, Kautiainen H, Korniloff K, Ahonen T, Vanhala M. Prevalence of restless legs symptoms according to depressive symptoms and depression type: a cross-sectional study. Nord J Psychiatry. 2018 Jan;72(1):51-56. doi: 10.1080/08039488.2017.1385849.

II Auvinen P, Koponen H, Kautiainen H, Korniloff K, Ahonen T, Vanhala M, Mäntyselkä P. A Longitudinal Study of Restless Legs Symptoms among Patients with Depression. Submitted manuscript, 2021.

III Auvinen P, Mäntyselkä P, Koponen H, Kautiainen H, Korniloff K, Ahonen T, Vanhala M. J. Elevation of tumor necrosis factor alpha levels is associated with restless legs symptoms in clinically depressed patients. Psychosom Res. 2018 Dec;115:1-5. doi: 10.1016/j.jpsychores.2018.09.008.

IV Auvinen P, Koponen H, Kautiainen H, Korniloff K, Ahonen T, Vanhala M, Mäntyselkä P. The influence of restless legs symptoms on musculoskeletal pain in depression. Scand J Pain. 2020 Jul 28;20(3):603-610. doi: 10.1515/sjpain-2019-0128.

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CONTENTS

ABBREVIATIONS

5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine
BDI	Beck Depression Inventory
BMI	Body mass index
CRP	C-reactive protein
DMT1	Divalent metal transporter 1
DNA	Deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4th Edition
EEG	Electroencephalography
HDL	High-density lipoprotein
HPA	Hypothalamic-pituitary-adrenal
ICD-10	10th revision of the International Statistical Classification of Diseases and Related Health Problems
ICD-11	11th revision of the International Statistical Classification of Diseases and Related Health Problems
IDO	Indoleamine 2,3-dioxygenase
IL	Interleukin
LDL	Low-density lipoprotein
MAO-A	Monoamine oxidase A
M.I.N.I.	Mini-International Neuropsychiatric Interview
PET	Positron emission tomography
SNRI	Serotonin-norepinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
TNF-α	Tumor necrosis factor alpha

1 INTRODUCTION

Depression is a psychiatric disorder characterized mainly by low mood and exceptional fatigue or an inability to experience pleasure. Depressive disorders are mental and behavioural disorders and, more specifically, mood disorders. Depression is common globally and one in ten people on earth will have it sometime during their lifetime (1). Depression can be a transient crisis of life or a life-threatening, completely debilitating condition that can take years to recover from. Nowadays the treatment options for depression have improved and there are more options in pharmacotherapy and other treatments such as psychotherapy. Although the treatment of depression has advanced, from time to time it is still a challenge for health care professionals.

Primary health care is an important part of the chain of medical treatment for depression because identifying depressive symptoms and initiating adequate treatment or – if necessary – referring early is crucial in the development of the depression. Initially, most patients seeking medical care for depression symptoms turn to primary-care physicians rather than psychiatrists directly. Inferior treatment outcomes for depression are associated with either abandonment of treatment or delayed treatment initiation (2). Some patients with depression are treated in primary health care until they are in remission without the need to continue treatment in specialized medical treatment centers.

In addition to diagnosing and treating depression, it is important to pay attention to comorbidities. Cognitive impairment, cardiovascular diseases, diabetes, obesity, sleeping problems as well as restless legs syndrome and pain are associated with depression (3-5). Sometimes the pain may be the first or the only sign of depression. Restless legs syndrome also known as Willis-Ekbom disease is a medical condition with established neuropathology, inflammatory and genetic associations. Restless legs symptoms are unpleasant sensations in the legs and an intense urge to move them especially in the evenings. Cardiometabolic risk factors such as a high body mass index (BMI) and low physical activity, have been found to be associated with restless legs syndrome as well as depression. The reason why restless legs symptoms and depression often occur together is not yet well established. This dissertation was intended to study restless legs symptoms and depression in a long-term follow-up, and their association with inflammatory markers and musculoskeletal pain.

2 REVIEW OF THE LITERATURE

2.1 DEPRESSION

Depression is a disorder consisting of several different symptoms such as depressed mood, loss of interest and fatigue. Like all other emotions, a depressive mood as a reaction to a loss or exhaustion is a normal feeling but if the symptoms cumulate and persist long enough, clinical depression may be diagnosed by a physician. Depression has a negative and widespread impact on mental and physical health. Depression is a disorder that can significantly affect a patient's life. Depressed patients become debilitated and isolated from their normal patterns of life and depression usually affects relationships, family life, and work, in addition to health. (6)

2.1.1 Prevalence

It is recognized that depression is a global health burden. Depression has a greater negative influence on the population in several countries like Finland, the Russian Federation and Algeria than e.g., Australia, Japan, People's Republic of China and United Mexican States. The negative effects of depression include inability to work, risk of suicide and several somatic diseases such as ischemic heart disease. (7) There is heterogeneity in the prevalence of depression; in particular, the lifetime prevalence of major depressive disorder in Taiwanese adults was low at 1.2% whereas it is high for example in the United States (20.6%) (8, 9). An earlier study including 30 countries determined that the one-year prevalence of depression was 7.2%, and the lifetime prevalence was 10.8% (1). The prevalence of a major depressive disorder in the Finnish population was 7.4% in a nationally representative sample of Finns aged 30 years and above, hence the consequences of depressive disorder is a public health problem. Being widowed, separated or unmarried increased the risk for depression, along with being female and younger. (10)

2.1.2 Diagnosing

The 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) is a medical classification list by the World Health Organization that specifies the symptoms of depression and the criteria for diagnosing depression along with other diseases and health problems. Depression is identified based on symptoms and the exclusion of other potential conditions causing similar

symptoms. There are no specific diagnostic procedures, such as a blood test or imaging, to confirm or diagnose depression. The main symptoms of depression in the ICD-10 are depressed mood for most of the day, loss of interest or enjoyment and unusual exhaustion or lack of energy. Other depressive symptoms are a lack of self-confidence or self-esteem, increased self-blame, recurrent thoughts about death or suicide or self-harm, lack of ability to concentrate, psychomotor changes, sleeping disorder and changes in appetite. A depression diagnosis requires at least two main symptoms and two other symptoms. The depressive symptoms persist over two weeks. (11)

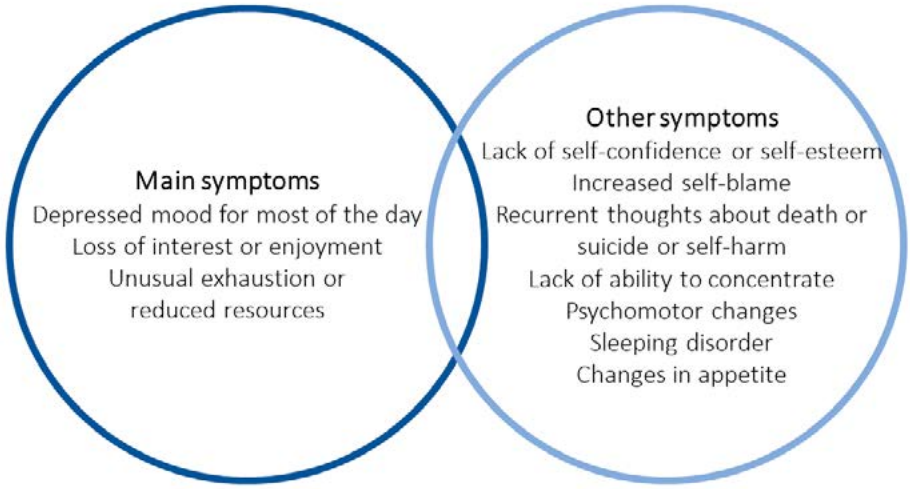

Figure 1. Diagnosis requires at least two main symptoms and two other symptoms and the depressive symptoms persist over two weeks. ICD-10 depression diagnostic criteria modified from World Health Organization (11).

The World Health Organization has published a new disease classification system, the 11th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-11). ICD-11 is due to be introduced in 2022 but the material is already officially published now for planning, translation and training. In the updated version, mood disorders are subdivided into depressive disorders that include single episode depressive disorder, recurrent depressive disorder, dysthymic disorder, mixed depressive and anxiety disorder, and bipolar disorders. Symptoms of a depressive episode include a depressed mood or diminished interest in activities almost daily, lasting for a period of at least two weeks, accompanied by difficulty in concentrating, feelings of worthlessness or excessive or inappropriate guilt, hopelessness, recurrent thoughts of death or suicide, changes in appetite or sleep, psychomotor agitation or retardation, and reduced energy or fatigue. A minimum of five of the ten symptoms is required. (12)

A diagnosis of depression can be made only by a physician based on the medical criteria. Various questionnaires can be used to help in screening and diagnosing depressive symptoms in a clinical assessment. One of the best known and widely used questionnaires is the Beck Depression Inventory (BDI), first published in 1961, which asks for self-reported symptoms and takes only a few minutes of time. The form was developed by Aaron T. Beck and included 21 question about the somatic, cognitive, emotional and motivational manifestation of depression. The BDI is used worldwide for evaluating the severity of depression symptoms in healthy and psychiatric populations. All questions measure the severity of a symptom of depression, from mild to severe and the total score is between 0 and 63. (13) The BDI is used as a part of diagnostics but the diagnosis of clinical depression is based on the symptoms fulfilling the diagnostic criteria. Structured diagnostic interviews can be used in diagnostics. For example, the Mini-International Neuropsychiatric Interview (M.I.N.I.) is a psychiatric structured diagnostic interview instrument that a professional fills out with a patient and it takes about 15 minutes. The M.I.N.I. is a reliable assessment method, with 95% sensitivity and 84% specificity (14). The M.I.N.I. was created by clinicians and psychiatrists, taking into account Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) and ICD-10 psychiatric disorders (11, 15). It includes 130 questions and screens for psychiatric disorders including one personality disorder (16). Other fully-structured psychiatric interviews used include the Composite International Diagnostic Interview (CIDI), which recognizes mental disorders (17).

2.1.3 Subtypes

There are several subtypes of depressive disorders, such as atypical, seasonal affective, psychotic, anxious and postpartum depression. This study is focused on melancholic

and non-melancholic depression (18). Melancholic depression is a relentless and severe manifestation of clinical depression. Melancholic depression involves fewer specific personality traits, such as neuroticism, impulsivity and aggressivity, than atypical depression (19). The prevalence of metabolic syndrome is decreased in melancholic depression, albeit depression generally predisposes a person to the syndrome. Patients with non-melancholic depression have a higher prevalence of metabolic syndrome than melancholic patients (20). Melancholic depression is characterized by psychomotor slowing, loss of appetite, a distinct quality of mood, social withdrawal, immobility, slowed speech, a non-reactive mood and a loss of emotion. Patients with melancholic depression have a lower working memory and speed of information processing compared to non-melancholic patients (21). The most substantial disadvantage of melancholic depression is its notable prevalence of suicide ideation (22, 23).

2.1.4 Pathophysiology

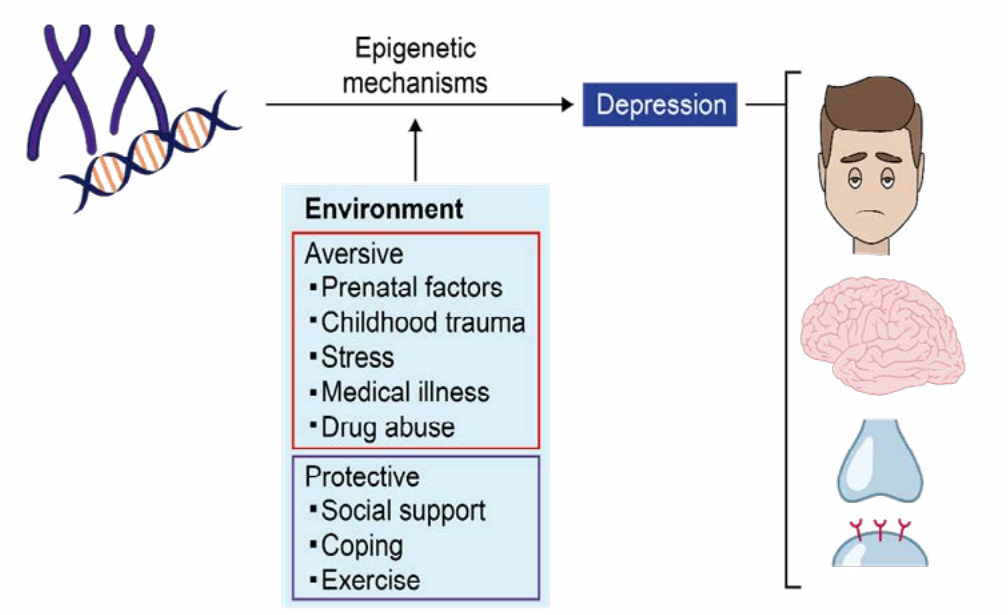

Figure 2. The emergence of depression is understood as a multifactorial process that progresses over time. Key predisposing factors include genes and acquired biological susceptibility as well as environmental factors. Depression affects behavioral, brain network and molecular processing. The molecular effects have an influence on neurotransmission, neuroplasticity, stress hormones and inflammation. Figure modified from Otte et al. (28)

The pathophysiology of depression is composed of the involvement of different pathophysiological mechanisms. Depression is located partly in one's genes and an epigenetic modification of gene expression could onset depression (24). A psychological load or times of crisis in life are risk factors for contracting depression. The importance of one's environment is substantial, as childhood maltreatment, physical neglect and sexual abuse are associated with depression repeatedly. (25) All forms of bullying, such as verbal, physical, and cyber bullying, have been associated with depression in pediatric patients (26). Moreover, childhood emotional abuse is a strong predictor of depression and has a crucial impact on the development of adult-onset depression (27).

2.1.5 Monoamine hypothesis

The monoamine hypothesis of depression is based on serotonin and noradrenaline function in the brain's neurotransmitting, where monoamines are endogenous chemicals that enable interaction between cells. Serotonin is a more prominent factor than noradrenaline but noradrenaline has an effect on e.g., memory consolidation (29). Dopamine does not have a consistent association with depression. However, it may be that in depressed patients the number of dopamine receptors has increased. (30, 31) Serotonin, also called 5-hydroxytryptamine (5-HT), is a monoamine synthesized from tryptophan (Figure 2). A serotonin transporter is a crucial factor in serotonergic signaling in the central nervous system (32). Besides its effect on serotonin metabolism, the serotonin transporter is responsible for synaptic homeostasis.

Abnormalities in the serotonin transporter function induce increased anxiety and stress-related behaviors. (33) The monoamine hypothesis is linked to the pathogenesis of depression and a notable number of antidepressants have an impact on serotonin regulation. Antidepressants like selective serotonin reuptake inhibitors (SSRIs) have an effect on serotonin, and some antidepressants, such as serotonin-norepinephrine reuptake inhibitors (SNRIs), also impact noradrenaline. (34) The interesting point is that a person's the gut microbiota has effects on the SSRIs response. The gastrointestinal tract has a bidirectional relationship with the central nervous system through the gut-brain axis. The gut-brain axis has been proposed as one pathogenesis of depression (230).

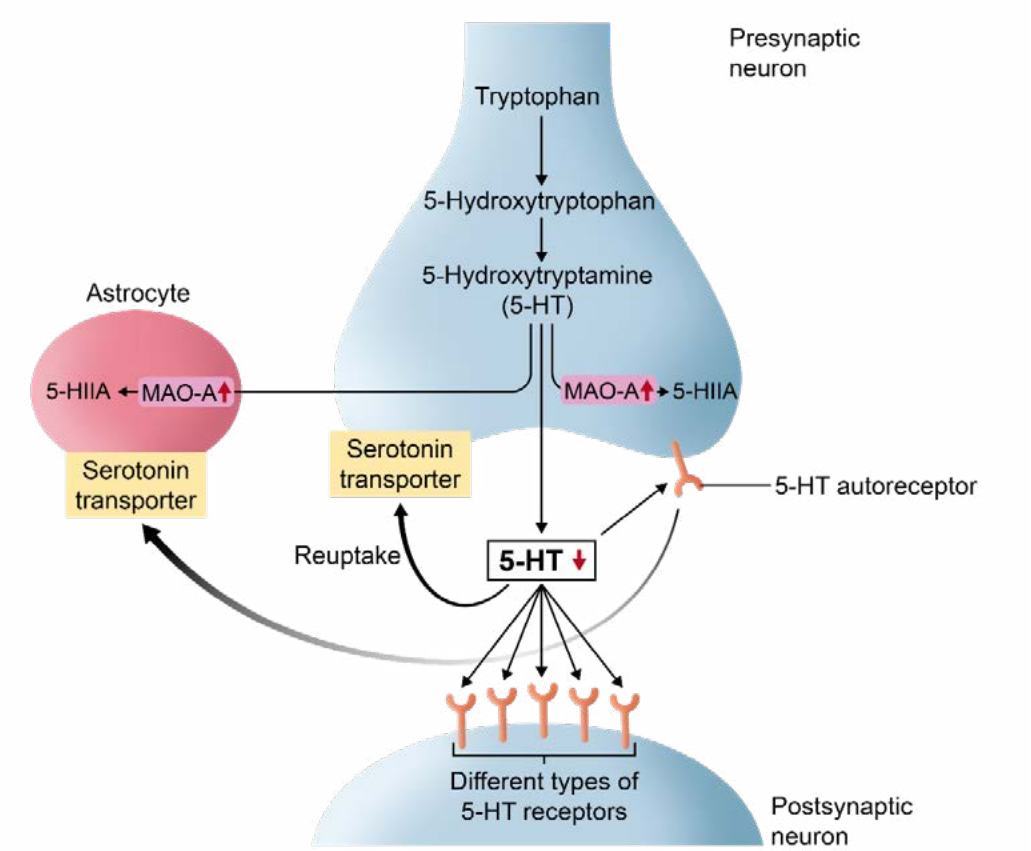

Figure 3. Based up the serotonin theory of depression, 5-HT is synthesized in the central nervous system from tryptophan. The synthesis of 5-HT is regulated by both serotonin-degrading monoamine oxidase A (MAO-A) and the serotonin transporter, which returns the neurotransmitter back to the presynaptic neuron for storage. Depression increases the catalytic activity of MAO-A, which reduces the concentration of 5-HT. Figure modified from Higuchi et al. (35)

2.1.6 Hypothalamic-pituitary-adrenal axis

The hypothalamic-pituitary-adrenal (HPA) axis is a major pathway signaling and regulating stress. Stress activates the hypothalamus, which causes a secretion of the adrenocorticotrophic hormone-releasing factor and vasopressin. The adrenocorticotrophic hormone-releasing factor inserts adrenocorticotrophic hormone from the pituitary, which has an influence on the secretion of cortisol; it has been reported that, depressed patients show hypersecretion of cortisol (36). A previous study

found that cortisol decreases electroencephalography (EEG) signal strength and perfusion in the thalamus (37). However, the explanatory or particular mechanisms that link depression and increased cortisol concentration together is not well understood.

2.1.7 Structure of the brain

The structure of the brain is altered in people with serious depression compared to healthy subjects. Depressed patients have abnormalities in the subcortical white matter and hippocampus, which interrelate with dysregulation of the HPA-axis activity (38). A change in the cortico-striatal-pallidal-thalamic circuit has been acknowledged to be related to the brain volume in the prefrontal, orbitofrontal and anterior cingulate cortices, whereas basal ganglia structures are diminished (39). The hippocampus and prefrontal cortex have atrophy caused by decreased concentrations of the brain-derived neurotrophic factor which participates in the regulation of synaptic plasticity (40). Neuroimaging analysis has found increased activity in the posterior cingulate cortex and lateral orbitofrontal cortex besides low gray matter volume in the right inferior temporal gyrus and left angular gyrus (41, 42). Additionally, depressive patients have a hyperactive amygdala and decreased functional activity in the cerebellum (43).

2.1.8 Inflammation

Depression and systemic inflammation are growing area of interest. Elevated levels of chemokines, adhesion molecules, acute phase proteins and prostaglandins are connected to the pathophysiology of depression. In particular, an increased concentration of immunologic factors, such as C-reactive protein (CRP), interleukin (IL) -1 and IL- 6, is linked to depression; however, tumor necrosis factor alpha (TNF-α) has not been reported to have a definite link to depression. (44, 45) Cytokines are presumed to have an impact on the metabolism of serotonin, activation of the HPA axis, dopamine and tryptophan concentration, which lead to a reduction in serotonin (46). TNF-α is associated with indoleamine 2,3-dioxygenase (IDO) activation because TNF-αs are the main inducers. IDO is an enzyme that is present in macrophages and other cells and it precipitates the depression inhibiting development of tryptophan to serotonin. (47)

2.1.9 Comorbidities related to depression

Depression increases the relative risk of cognitive impairment, cancers, heart disease, diabetes mellitus, obesity, disability and mortality (3, 4). In addition to the aforementioned, depression is associated with certain lifestyle factors, restless legs

symptoms, pain, other psychiatric disorders and a range of somatic diseases. An association between depression and metabolic syndrome, which includes characteristics of obesity, hyperglycemia, hypertension and dyslipidemia, is significant. (48) Non-melancholic depression is more likely to be related to metabolic syndrome than the melancholic subtype (20). Depressed people have an elevated risk of developing type 2 diabetes (49). Depression is not associated with the onset of type 1 diabetes but patients with type 1 diabetes are more likely to be depressed than controls (50). Depressed patients are more likely to develop heart disease than non-depressed subjects. The connection between coronary heart disease and depression is well-known. The connection does not go away even after taking into account the impact of various risk factors such as blood cholesterol, blood pressure, and congestive heart failure. (4) Apart from patients with heart failure or atrial fibrillation, depression has been found to be a self-sufficient prognosticator of mortality and hospitalization (51).

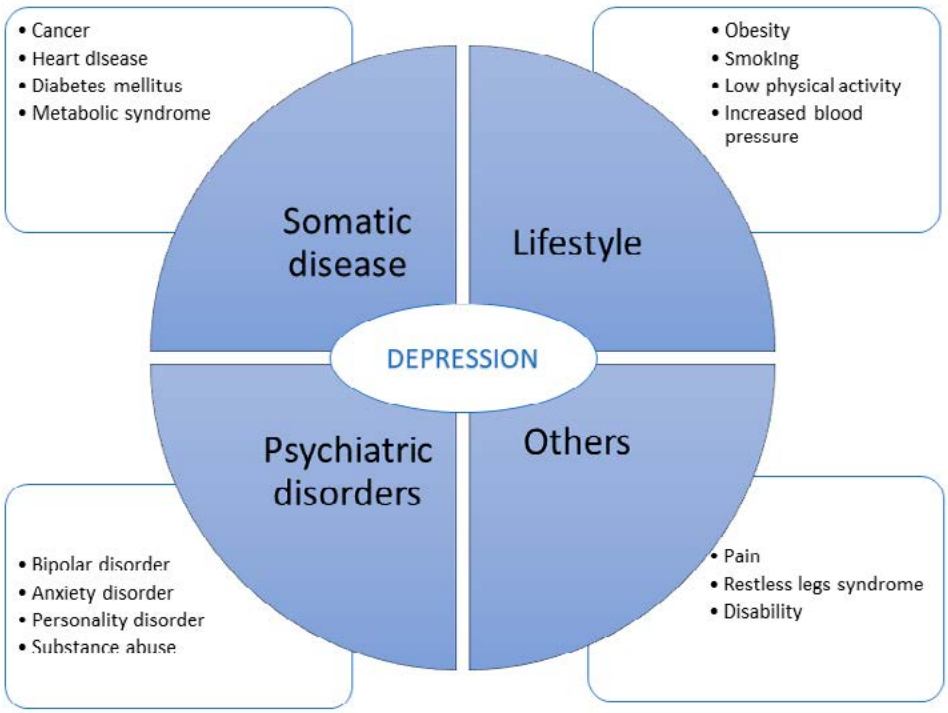

Figure 4. Comorbidities related to depression (3,4, 52-56, 58, 59, 78).

2.1.10 Lifestyle factors

In general, depression has an indirect impact on average life expectancy, compounding comorbidity and lifestyle factors, and at worst it is a fatal disorder resulting in suicide. Depression predisposes a subject to an unhealthy way of life, such as smoking, excessive alcohol consumption, low physical activity and increased blood pressure and waist circumference. (52-56) Obesity and depression are cognate subjects and the relation is bidirectional, in other words, depression aggravates obesity and vice versa. The mechanism that links depression and obesity together is a result of biological, behavioral and psychological factors, in addition to inflammation, dysfunction of the HPA-axis and changes in leptin and insulin metabolism (57). Furthermore, insomnia is associated with the risk of developing depression but insomnia is also a symptom of depression, and sleeping problems, like restless legs syndrome, are often associated with depression (58, 59). Depressive patients have a risk of various unhealthy lifestyle factors but the association with metabolic syndrome is bidirectional and fluctuating depending on, for instance, depression subtype (20, 55, 56, 60).

2.1.11 Restless legs symptoms

Insomnia in particular is associated with restless legs symptoms and depression (61). Restless legs syndrome impairs the quality of life substantially and involves a variety of comorbid health problems (62, 63). Psychiatric disorders, especially mood and anxiety disorders, and restless legs syndrome exist together regularly but the linking mechanism is not known (5, 64). The relationship between depression and restless legs syndrome is presumably bidirectional although in previous prospective studies restless legs syndrome preceded both clinical depression and a new onset of depressive symptoms (65, 66).

2.1.12 Pain

The phenomenon of pain has not changed much over time but a new definition has been proposed by the International Association for the Study of Pain: "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (67). Pain is a subjective experience influenced by biological, psychological, and social factors. Pain can be classified in several ways, and the pain-causing mechanism (Figure 5.) or temporal classification is one dimension of these. Acute pain starts abruptly, usually with a clear temporal and causal relationship to current surgery, injury or illness; thus acute pain improves as the tissue heals. Subacute pain lasts for 6-12 weeks after the injury or illness that caused it. Subacute

pain may predispose a person to the onset of chronic pain lasting longer than 3 months (68, 69). Chronic pain continues after the initial injury heals or persists past the normal healing time. Chronic pain is proposed to be divided into the following categories: chronic primary pain, chronic cancer pain, chronic post-traumatic and postsurgical pain, chronic neuropathic pain, chronic headache and orofacial pain, chronic visceral pain, and chronic musculoskeletal pain. (69)

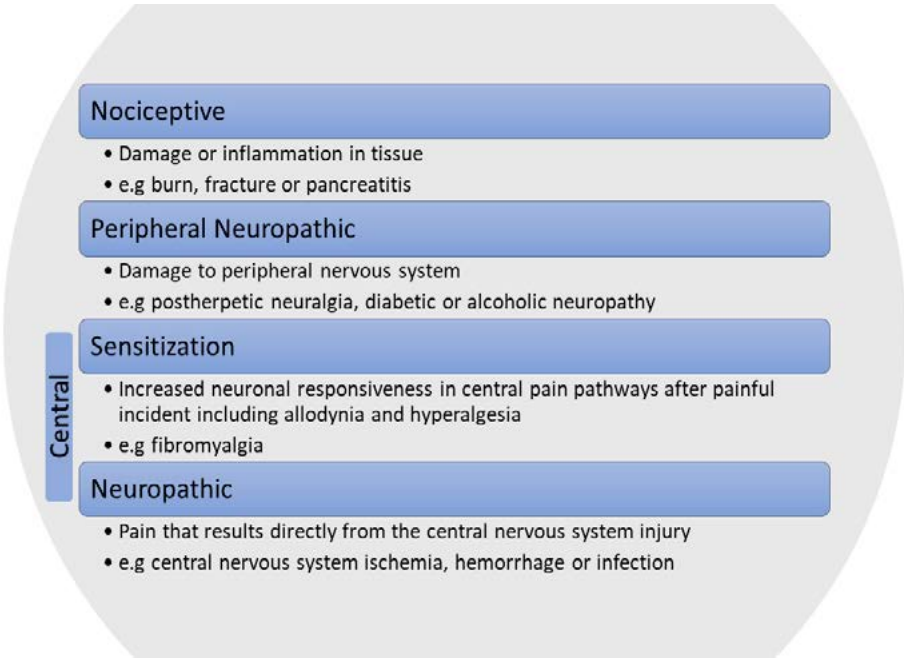

Figure 5. An example of a classification based on the mechanism of pain (70-72).

Depressed patients usually have chronic and widespread musculoskeletal pain. Somatic symptoms, such as sleep problems, mood disturbance and fatigue, are common in depression, and stress aggravates pain. (71) The relationship between depression and restless legs symptoms is well-known; furthermore they show an association with pain (58, 73). An explanatory mechanism that causes widespread pain in depressed patients is not consistent but the effects of monoamines and inflammation, such as IL-6, are alternatives (74-76). In addition to low-spirits and a frail quality of life, compared to non-depressive people, depressed subjects are more sensitive to pain (77). The mean prevalence of pain in patients with depression was 65% in a previous review study containing 56 articles. Previous studies of primary care patients have found that the typical symptoms of depression, such as anhedonia or dejection, are more challenging

for the patient to identify than pain. Occasionally the patient may experience pain as the only symptom of depression. (78) Depression is associated with several painful diseases such as chronic lower back pain and fibromyalgia (79, 80). Often chronic headache, migraine and tension-type headaches are associated with depression (81). A previous study found that patients with chronic widespread pain have personality traits, such as high harm avoidance and low self-directedness, which are also associated with depression (82, 83).

2.1.13 Psychiatric disorders

Depression is one of several mood disorders and another common one is bipolar disorder. Bipolar disorder includes periods of depression and hyperactivity, and between episodes there are no symptoms at all or they are milder than during the actual episodes. (84) Depression and anxiety disorders have several affinities in pathophysiology, risk factors and treatment, notably, the action of the serotonin-noradrenaline system is likely related to depression and anxiety disorder. Occasionally anxiety disorders occur with concomitant depressive symptoms (6). Some patients with depression also have a personality disorder and a disadvantageous personality disorder clearly appears to prolong recovery from depression (85, 86). Substance abuse, which means using alcohol, drugs, prescription medicine, and other substances in a detrimental way, is linked to depression. Having depression and substance abuse disorder at the same time can make it difficult to recover from both disorders (87).

2.1.14 Treatment

Pharmacological and non-pharmacological treatments are available for depression. There are options from psychotherapy to medication and to healthy lifestyle changes available. Antidepressant therapy, psychotherapies, brain electrotherapy, and transcranial magnetic stimulation have been strongly demonstrated in clinical trials (88-91). There are several different types of antidepressants and all marketed antidepressants are more effective than placebos in treating depression. The first-generation antidepressants are tricyclic antidepressants and irreversible monoamine oxidase inhibitors. The current antidepressants include SSRI (e.g., citalopram and fluoxetine) and SNRI medications (e.g., duloxetine and venlafaxine). There are also other antidepressants such as vortioxetine, mirtazapine and bupropion. (88)

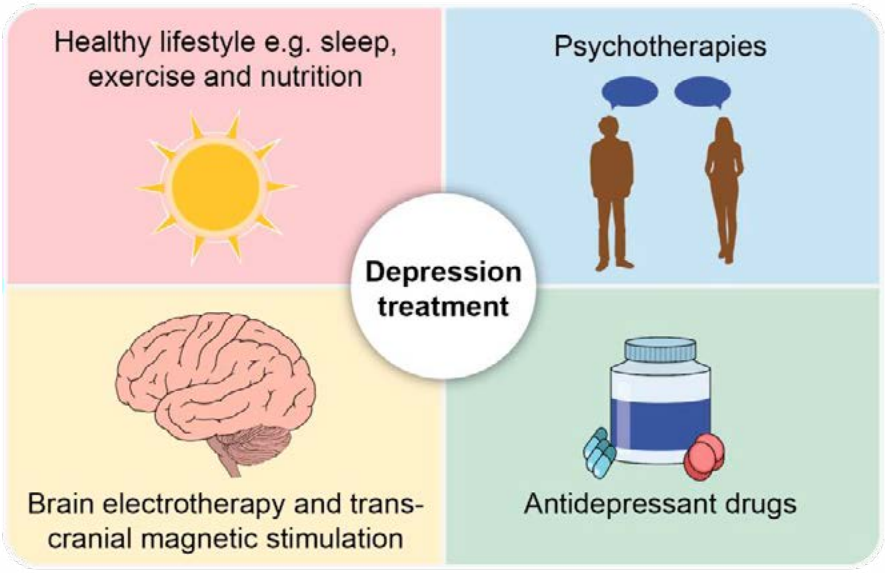

Figure 6. Pharmacological and non-pharmacological treatments for depression (88-91).

2.2 RESTLESS LEGS SYMPTOMS

Restless legs syndrome is a common medical condition that refers to a symptom that occurs in the legs exclusively during rest. It is a sensorimotor movement disorder characterized by an uncomfortable sensation that results in an urge to move one's legs. The symptoms of restless leg syndrome have a wide spectrum of severity which can cause pain or even lead to severe insomnia (92).

2.2.1 Prevalence

The prevalence of restless legs syndrome varies depending on, for example, populations and geography. The prevalence of restless legs syndrome in healthy subjects has been 12.5% in the Netherlands, and 11.5 % in Norway and Denmark (93, 94). Previously, the prevalence of restless legs syndrome in the Finnish population has been found to be 11.4-20% in women and 7.7-15% in men (95, 96). The highest prevalence has been reported in Norway 26.8%, in France 24.2 % and in Australia 18 %; a lower prevalence case has been found in the Nigerian population, for instance, at 3.5% (97-100). Primary health care patients commonly have a high prevalence of restless legs syndrome, such as 21.5 % in Italy, 19.6 % in Appalachia in the Eastern United States, and 24 % in the Northwestern United States (101-103). Patients with

depression have an elevated prevalence of restless legs syndrome 31.5 %, and geriatric patients also have a high prevalence 36.8 % (61, 104).

2.2.2 Diagnosing

International Restless Legs Syndrome Study Group delineated five criteria for diagnosing restless legs syndrome: "(1) an urge to move the legs usually but not always accompanied by, or felt to be caused by, uncomfortable and unpleasant sensations in the legs, (2) the urge to move the legs and any accompanying unpleasant sensations begin or worsen during periods of rest or inactivity such as lying down or sitting, (3) the urge to move the legs and any accompanying unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues, (4) the urge to move the legs and any accompanying unpleasant sensations during rest or inactivity only occur or are worse in the evening or night than during the day, and (5) the occurrences of the above features are not solely accounted for as symptoms primary to another medical or behavioral condition (e.g. myalgia, venous stasis, leg edema, arthritis, leg cramps, positional discomfort, habitual foot tapping)" (105).

Symptoms of restless legs syndrome were described as a need to move, crawling, tingling, restlessness, ache, cramping, creeping, pulling or pain (106). The symptoms occur especially in the evenings but daytime symptoms are not excluded (107, 108). Diagnostic criteria based on self-reported symptoms and a patient's anamnesis form the cornerstone of the diagnosis. Physicians do not have an existing laboratory procedure for diagnosing the syndrome. The line between symptoms and syndrome is not definitive. Criteria established by International Restless Legs Syndrome Study Group is a guiding principle in diagnosing restless legs syndrome but in the examination of restless legs there are several dissimilar instructions with high specificity (101).

2.2.3 Pathophysiology

Clinical experience and scientific knowledge of restless legs syndrome have increased during the last decade, but the specific pathogenesis is still unclear. Restless legs syndrome is idiopathic or develops secondary to a variety of medical conditions. However, the symptoms in idiopathic and secondary types are similar and a precise method for differential diagnostics does not exist. Notwithstanding, previous studies have proposed divergent but potential alternatives for pathophysiology, e.g., neuroinflammation, deficient dopaminergic neurotransmission, hormones, iron deficiency, genetics, a lack of folate and peripheral hypoxia. (109-111) Restless legs

syndrome has inheritable characteristics, thus the majority of patients with restless legs syndrome have a family history (112). The pathophysiological mechanisms are related partly to genetics and there have been identified risk loci related to IL-1B and IL-17A genes (113, 114).

2.2.4 Dopaminergic neurotransmission

Dopamine is a catecholaminergic neurotransmitter and has an influence on e.g., the prefrontal cortex, amygdala, thalamus, mesocortical and mesolimbic systems. Dopamine influences emotions and rewards behaviors and also regulates voluntary movement and postural control (115). Noteworthy evidence of a relation between dopamine and restless legs syndrome is the response to medical treatment, i.e., dopamine agonists used to treat restless legs symptoms. Dopamine agonists are the first-line treatment and have a more effective response than levodopa, which is the precursor to dopamine. (116) Dopaminergic hypoactivity is based on positron emission tomography (PET) imaging, which enabled the discovery of increased dopamine receptor availability in the thalamus and the anterior cingulate cortex. Increased receptor availability could be a result of either dopamine deficiency or an alteration of binding ability. (117)

2.2.5 Iron metabolism

Iron deficiency is one of the hypotheses of restless legs symptoms. The central nervous system is more involved in the impairment of iron metabolism than the peripheral parts. In serum samples, there has not been an association between restless legs syndrome and concentrations of ferritin, transferrin or iron. (118-120) Instead, decreased iron and ferritin concentrations have been discovered in cerebrospinal fluid whereas transferrin concentration is elevated (121, 122). Restless legs syndrome has been found to be associated with differences in the iron metabolism of the brain in unequal regions, the substantia nigra, thalamus, putamen, and pallidum above all (123, 124). A lack of iron in the nervous system has an influence on dopaminergic function, resulting in a disturbance of monoamine neurotransmitter synthesis. Alternatively, dopamine treatment has an effect on iron homeostasis, increasing the intracellular iron content of macrophages. (109, 125)

2.2.6 Inflammation

Knowledge of the inflammation process in restless legs symptoms is scarce. Restless legs syndrome has been linked to inflammatory diseases, such as gastrointestinal

disorders, rheumatologic diseases and pulmonary disorders. The inflammatory markers IL-6 and CRP do not have a constant relation to restless legs syndrome but among hemodialysis patients an association between restless legs syndrome and increased levels of IL-6 and CRP has been reported (126-128). Restless legs syndrome elevates the neutrophil-to-lymphocyte ratio, which is one of the markers of systemic inflammation (129).

2.2.7 Comorbidities related to restless legs

Restless legs symptoms do not inflict life-threatening consequences but increase the risk of mortality although symptoms have a persistent influence on daily life (62, 130). Several diseases, e.g., Parkinson's disease, multiple sclerosis and irritable bowel syndrome, and general poor health-related quality of life are closely linked to restless legs syndrome (131-134). Restless legs symptoms are in conjunction with an eminent feeling of impairment in daily functioning and insomnia (135). Patients with restless legs symptoms often suffer from psychosomatic symptoms, especially somatization (136). Restless legs syndrome is linked to more psychopathological symptoms, such as hypochondriasis and hysteria and a low internal locus of control, than specific personality characteristics such as higher mental arousal or low self-confidence (137-139). Physical inactivity, obesity, elevated cholesterol and smoking are associated with restless legs syndrome unlike the alcohol consumption or high blood pressure (140-142). In one study, the presence of restless legs symptoms was longitudinally associated with lower physical function (143).

In a previous study the prevalence of restless legs symptoms was 54.8 % in women with multi-site pain (144). Restless legs symptoms are defined as unpleasant feelings that may be experienced as pain without any other source of pain. Patients with restless legs syndrome have more long-term and intense pain, hyperalgesia and musculoskeletal pain than healthy individuals. (145-147) Various painful conditions such as migraine, tension-type headache and fibromyalgia occur more often in people with restless legs symptoms than in those without (148-152). Widespread pain is linked to restless legs symptoms in patients with severe bronchitis or emphysema or asthma (153). Furthermore, patients with Parkinson's disease and restless legs symptoms report a sense of pain more frequently than subjects without restless legs symptoms (154).

2.2.8 Treatment

In clinical guidelines dopaminergic drug therapies have been suggested to be the primary treatment for restless legs symptoms. Dopamine agonists alleviate dopamine deficiency by stimulating striatal dopamine receptors. Dopamine agonists (e.g.,

pramipexole and rotigotine) are effective treatments compared with a placebo (155). Pramipexole inhibits dopamine synthesis, release and circulation (156). Levodopa is a more effective treatment option than placebo but not as effective as dopamine agonists (157). Oral or parenteral iron therapy ameliorate restless legs symptoms probably better than a placebo (158). The effectiveness of α2δ ligands (gabapentin or pregabalin), opioids and benzodiazepines for restless legs symptoms treatment is unknown, and these drugs are prone to be abused (159, 160). Besides pharmacological therapies, acupuncture has been studied in patients with restless legs symptoms but there is a lack of large-scale clinical trials (161).

2.3 INFLAMMATORY MARKERS, DEPRESSION AND RESTLESS LEGS SYMPTOMS

CRP is a commonly used inflammation marker and is primarily associated with tissue injury, stress and bacterial inflammation. Additionally, it is an acute-phase plasma protein synthesised mainly by hepatocytes and works by providing immunity and eliminating foreign bodies (162). CRP has an association with the activation of cytokines, especially IL-6 and TNF-α (163). In addition to being linked to depression, CRP is associated with cardiovascular events such as myocardial infarction and death from cardiovascular causes (75, 164). However, the relation between restless legs syndrome was insignificant in a previous study (165). There are not established mechanisms explaining the relationship between CRP and depression. Antidepressants such as fluoxetine and escitalopram have a reducing effect on CRP levels (166).

Cytokines are key regulators of the body's defense reactions. The differentiation, growth, and functional regulation of cells in the immune system are under the control of cytokines. In addition to CRP levels, TNF-α concentration is also elevated in most inflammatory disorders. The activity of TNF-α is possibly connected to the invasion of bacteria into the bloodstream and several other diseases, e.g., rheumatoid arthritis and psoriasis (167-169). TNF-α is a critical factor for proinflammatory actions and various cells, e.g., the activated macrophages, monosytes, T-cells, lymphocytes and astrocytes, are able to produce TNF-α (167).

Major theories that link TNF-α and depression together are a change in the HPA axis, genetic polymorphisms and changes in serotonin and dopamine transporters; however, the relationship between TNF-α and depression is multifaceted (45). Based on previous studies, an increased concentration of TNF-α in particular does not have definite importance in the pathophysiology of depression (170-173). However, the connection between depression and TNF-α has been found to exist invariably (174-176). There is not a lucid association between the concentration of TNF-α and subtypes

of depression such as melancholic and atypical subtype (177, 178). The data on TNF- α and restless legs syndrome is limited, but one previous study found that the relation is insignificant (179).

IL-6 is a cytokine that has an impact on, inter alia, metabolic mechanisms, B-cell activation and the hepatic acute phase reaction. Depressed patients have a probabilistic higher serum IL-6 concentration than healthy controls. (75, 180) IL-1ra is an anti-inflammatory protein belonging to the IL-1 family. Among subjects aged 65 years and older, high plasma levels of IL-1ra were associated with a higher risk of developing depressive symptoms over time. However, the connection is more complicated. In the aforementioned study, depressive symptoms are linked to increased IL-1ra concentration in males but not in females (181). Unfortunately, there were no research findings on the connection between IL-6 or IL-1ra and restless legs symptoms at the time of the writing of this dissertation.

2.4 SUMMARY

Depression is a major public health problem globally. The identification and diagnosis of depression has evolved in recent years. Treatment of depression is challenging, with several comorbidities associating with it such as pain, cardiovascular diseases, sleep problems, substance abuse, anxiety and personality disorders. The focus of this thesis is on the restless legs symptoms of depressed patient. Restless legs symptoms and depression became a point of interest because they seem to be associated but scientific knowledge of this relationship is scarce.

There is a limited amount of data about the prevalence of restless legs symptoms in a primary care setting in Finland. At the time of the writing of this dissertation, the effect of the depression subtypes, melancholic and non-melancholic depression, on restless legs symptoms had not been studied. Non-melancholic depression showed a higher prevalence of metabolic syndrome but it is not known how this affects the prevalence of restless legs symptoms. There were data on the association between depression and restless legs symptoms but limited information on the change in both conditions in a follow-up.

The factor linking depression and restless legs symptoms is not known precisely but one proposed hypothesis is inflammation. Increased concentrations of CRP, IL -1 and IL- 6 are associated with depression but their influence on restless legs symptoms has been less studied. The role of neuroinflammation in the genesis of restless legs symptoms in patients with depression is assumed. The relationship between depression, restless legs symptoms and inflammation markers, including CRP and TNF-α, has not been studied before, hence elucidating the connection seems appropriate

research. In addition to being painful by themselves, restless legs symptoms may be associated with several painful conditions as well as with depression. Thus, one interest in this study was to investigate the role of depression in the relationship between restless legs symptoms and pain.

3 AIMS OF THE STUDY

The general aim of this thesis was to investigate the prevalence, prognosis, and association of restless legs symptoms with inflammatory factors and pain in depressed and non-depressive subjects in primary health care.

The specific aims were:

I. to analyze the association between restless legs and the severity of depressive symptoms and the prevalence of restless legs symptoms in subjects without depressive symptoms, with depressive symptoms without clinical depression, and in subjects with melancholic or non-melancholic depression subtypes.

II. to analyze the association between restless legs symptoms and depressive symptoms with or without clinical depression in a longitudinal setting.

III. to evaluate the association of circulating concentrations of the inflammatory markers TNF-α and CRP with restless legs symptoms among subjects without depressive symptoms, among subjects with depressive symptoms without clinical depression, and among subjects with clinical depression.

IV. to study the prevalence and intensity of pain among subjects without depressive symptoms, among subjects with depressive symptoms without clinical depression, and among subjects with clinical depression.

4 SUBJECTS AND METHODS

4.1 SETTING AND DATA COLLECTION

The Finnish Depression and Metabolic Syndrome in Adults (FDMSA) study was conducted in the Central Finland Hospital District, which includes 274,000 residents. The baseline study was conducted in the years 2008-2009 and the follow-up approximately six years after the baseline in the years 2015-2016. New subjects aged 35 years or older who went themselves or were referred by a general practitioner to a depression nurse case manager due to depressive symptoms were included in the study. Individuals with a score of least 10 in the 21-item BDI were enlisted in this study and altogether 706 subjects were involved in the catchment area. The group of controls was selected by random sampling simultaneously with the patient recruitment in 2008-2009. An age-, sex- and community-stratified random sample representing the population in the study region was taken by Statistics Finland (http://www.stat.fi). Statistics Finland is the Finnish authority established for statistics. It produces the majority of Finland's official statistics and is an independent organization under the Ministry of Finance. The subjects in the control group were persons 35 years or older and residents of the participating municipalities. They had a BDI score of less than 10 and no current psychiatric diagnosis.

4.2 SUBJECTS

A total of 1,105 subjects participated in study I and IV, of whom 67.1 % (n=742) were women and 32.9% (n=363) men. The characteristics of the study subjects are shown in Table 1. The study population included 410 controls without a psychiatric diagnosis (mean BDI score=3.2 ± 2.7) and 256 were subjects with depressive symptoms without a depression diagnosis (mean BDI score=17.8 ± 6.4). Of all the subjects, 439 received a depression diagnosis, 149 subjects (mean BDI score=20.2 ± 7.7) had non-melancholic depression and 290 subjects (mean BDI score=25 ± 8) melancholic depression. In the study groups 27 subjects did not respond to the question about restless legs symptoms.

In study II a total of 1,105 subjects, consisting of the patients and the controls, were included in the baseline study. In all, 298 (27%) participants withdrew from the study during the follow-up: 20 because of death, 244 did not respond and 34 declined to participate. The study included subjects who were involved in baseline and follow-up research. Among the subjects with diagnosed depression, 54% of men and 69% of

women participated in the follow-up (p=0.004). Among the subjects with depressive symptoms, the mean age of non-participating subjects was 49 years while among participating ones it was 55 years (p<0.001). Otherwise, there were not any significant differences between subjects who dropped out and participating subjects in terms of sex, BDI score and restless legs symptoms. At the baseline, 333 subjects were controls with no psychiatric diagnosis (mean BDI score = 3.1 ± 2.7), 192 were subjects with depressive symptoms without a depression diagnosis (mean BDI score = 17.6 ± 6.2) and 282 subjects had received a depression diagnosis (mean BDI score = 23.2 ± 7.8). The diagnosed depressive subjects had less leisure time physical activity, more smoking, a higher triglyceride concentration, heart rate and BMI than the controls. The control subjects felt more rested and had more sufficient sleep compared to both patient groups.

In study III, the data do not include subjects (N = 78) with CRP values above 30 mg/l or TNF-α values above 50 ng/l, ensuring that microbe-based contamination would not substantially affect the outcome. Under these circumstances, 1,027 subjects, consisting of patients and controls, were included in study III; men (33.0%) and 688 (67.0%) women. Of these subjects, 396 were controls without a psychiatric diagnosis, 243 were subjects with depressive symptoms without a depression diagnosis, and 388 had a depression diagnosis.

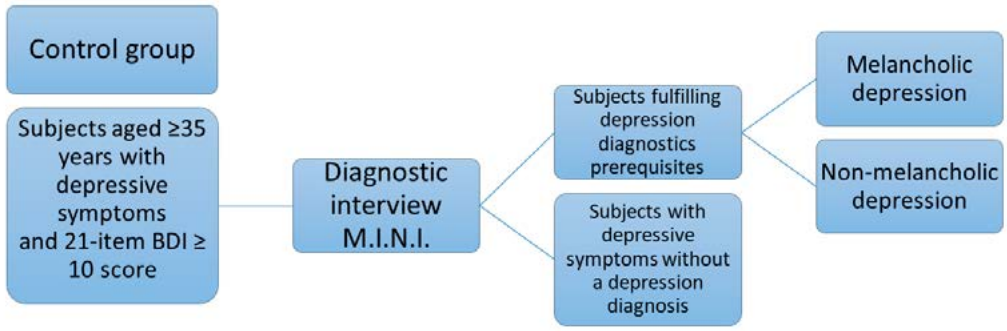

Figure 7. Schematic diagram of the division of patients into study groups alongside control subjects (I-IV).

4.3 MEASUREMENTS

In this thesis, all the participants filled out a questionnaire which contained questions about current smoking, years of education, use of alcohol (number of drinks per week), leisure time physical activity (number of 30-min exercise sessions), previously

diagnosed somatic disorders and use of medications, including antidepressants. In an outpatient setting, the study nurse measured blood pressure, weight, height and waistline of the participants, taking about 30 minutes of time.

Leisure time physical activity was classified as low (0–2 sessions per month), moderate (1–2 sessions per week) and high (three or more sessions per week). Sufficient sleep was evaluated by the question "Do you think that your sleep is sufficient (1=yes, almost always; 2=yes, often; 3=rarely or hardly ever; 4=I cannot say)". Subjects responding "almost" or "often" were regarded as having sufficient sleep. Feeling rested in the morning was evaluated by the question "How tired do you feel during the first 30 minutes after you have woken up in the morning (1=very tired; 2=quite tired; 3=quite rested; 4=I feel fresh)?" Subjects responding "quite rested" or "feeling fresh" were regarded as rested in the morning.

4.3.1 Diagnosing depression

The 21-item BDI is a 21-question multiple-choice, self-report inventory that is a widely used tool for screening, assessing and following up on depressive symptoms. In the study the severity of depressive symptoms was measured with the 21-item BDI and the psychiatric diagnosis was confirmed with a M.I.N.I diagnostic interview conducted by a trained study nurse (13, 16, 182). The M.I.N.I. takes into account psychiatric disorders from DSM-IV and ICD-10 and has accurate sensitivity and specificity (183). Among the subjects who had a BDI score of 10 or higher, a diagnosis of depression was determined with a M.I.N.I. diagnostic interview. The melancholic subtype was identified by M.I.N.I. criteria for a major depressive episode with melancholic features such as lack of appetite, feeling of intense sadness or guilty, waking up at least two hours before usual and difficulty falling asleep again (16, 184). In the follow-up years 2015-2016, the participants filled out a standard questionnaire including the same structured question of restless legs symptoms and BDI as at the baseline.

4.3.2 Restless legs symptoms

Restless legs symptoms were assessed with a structured and tested question that took into account the core characteristics of restless legs syndrome: an urge to move the legs, primarily during rest or inactivity, and partial or total relief with movement, with the presence or worsening of discomfort exclusively in the evening or at night. The question was enquired in a written form and was based on self-reported symptoms. The symptoms were determined with a Finnish version of the question: "When you try to relax in the evening or sleep at night, do you ever have unpleasant, restless feelings in your legs that can be relieved by walking or movement?" (101).

Table 1. Demographic and clinical traits of the subjects.

	Controls (A)	21-Item Beck's depression inventory score ≥10			P-value
	N=410	Without depression (B) N=256	Non-melancholic depression (C) N=149	Melancholic depression (D) N=290	
Male, n (%)	165 (40)	67 (26)	48 (32)	83 (29)	<0.001
Age, mean (SD)	53 (10)	53 (11)	51 (10)	51 (10)	0.003
Education years, mean (SD)	12.0 (3.4)	11.0 (3.3)	11.1 (3.0)	11.0 (3.1)	<0.001
Body Mass Index, kg/m^2, mean (SD)	26.8 (4.6)	27.9 (5.9)	28.0 (5.1)	28.1 (6.2)	0.005
Current smoking, n (%)	67 (16)	59 (23)	40 (27)	102 (35)	<0.001
Alcohol use, doses per week, n (%)					0.10
0	66 (16)	61 (24)	36 (24)	67 (23)	
1-9	292 (71)	168 (66)	91 (61)	189 (65)	
≥10	52 (13)	27 (11)	22 (15)	34 (12)	
Leisure time physical activity, n (%)					<0.001
Low	49 (12)	43 (17)	35 (23)	73 (25)	
Moderate	181 (44)	122 (48)	63 (42)	116 (40)	
High	180 (44)	90 (35)	51 (34)	99 (34)	
Heart rate, beats/min, mean (SD)	67 (9)	68 (9)	71 (10)	69 (9)	<0.001
BP, mmHg, mean (SD)					
Systolic	129 (16)	130 (16)	132 (15)	130 (16)	0.18
Diastolic	81 (10)	81 (10)	84 (11)	81 (10)	0.046

Plasma glucose, mmol/l, mean (SD)	5.68 (1.01)	5.63 (0.85)	5.97 (1.60)	5.81 (1.32)	0.060
Serum cholesterol, mmol/l , mean (SD)	5.04 (0.88)	5.11 (0.96)	5.05 (1.01)	5.11 (1.03)	0.69
Serum LDL cholesterol, mmol/l, mean (SD)	3.10 (0.82)	3.10 (0.84)	3.05 (0.85)	3.06 (0.97)	0.86
Serum HDL cholesterol, mmol/l, mean (SD)	1.56 (0.42)	1.57 (0.45)	1.50 (0.43)	1.61 (0.50)	0.20
Serum triglycerides, mmol/l, mean (SD)	1.20 (0.65)	1.37 (1.63)	1.41 (0.89)	1.35 (0.80)	0.012
Perceived to have sufficient sleep, N (%)	348 (85)	150 (59)	80 (54)	94 (32)	<0.001
Felt rested in the morning	355 (87)	158 (62)	88 (59)	125 (43)	<0.001

The results in the table are shown as numbers (percentages) and means (standard deviation). SD, standard deviation; BMI, body mass index; alcohol dose, 12 grams of pure alcohol; Low, 0–2 sessions per month; Moderate, 1–2 sessions per week; High, 3 or more sessions per week; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

4.3.3 Blood samples

The blood sample collection procedure was conducted in the health centers' laboratories by a trained nurse and in an outpatient setting. Lipids, fasting glucose and CRP were measured and diagnoses were based on fasting blood samples drawn between 8 and 11 o'clock a.m. after 12 h of fasting. Plasma glucose, serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were analyzed using Modular Analytics SWA (Hitachi High-Technologies Corporation, Tokyo, Japan). In addition, 3 tubes of serum, 3 tubes of plasma and a tube of whole blood were frozen at -70 Celsius and the samples were stored at -70 Celsius in locked freezing conditions at the Central Finland Hospital laboratory. TNF-α was determined from the frozen samples and the concentration was analyzed using an Immulite 1000 immunoassay analyzer (Siemens Healthcare Diagnostics Products Ltd., Gwynedd, UK).

4.3.4 Pain

Musculoskeletal pain was assessed by enquiring: "Do you have pain?" with response categories: 1) not at all; 2) I have pain rarely or temporarily; and 3) I have pain frequently or continuously in the joints, back, neck, or multisite. The prevalence of continuous widespread pain was defined according to category 3 (frequent or continuous pain in the joints, back, neck, or multisite). Pain intensity was based on three pain-related questions. Participants were asked if they have had 1) pain or stiffness in joints; 2) neck pain; or 3) back pain during the last four weeks. Answers (0, have not had; 1, have had mild pain; 2, have had difficult pain; 3, have had severe pain) were summed up into a total score (scale ranging between 0 and 9), which accounted for pain intensity (185).

4.4 STATISTICAL ANALYSES

In study I, statistical significance between groups was tested with analysis of variance, the Kruskal–Wallis test and a chi-square test. The relationship between the prevalence of restless legs symptoms and depression groups were analyzed using a crude and adjusted logistic regression models. The model was adjusted using age, smoking, BMI, leisure time physical activity and years of education. A conceivable non-linear relationship between the prevalence of restless legs symptoms and BDI score was also assessed by using a 5-knot restricted cubic spline regression adjusted for sex and age. The lengths of the distributions of the knots were located at the 5th, 27.5th, 50th, 72.5th and 95th percentiles. A visual assessment of the residuals and influence

diagnostics was used to validate the assumptions underlying the logistic regression model.

The data in study II and IV are presented as means with standard deviation and as counts with percentages. In study II, statistical comparisons between groups were made using analysis of variance for continuous variables and a chi-square test or logistic models for categorical variables. Repeated measures were analyzed using generalizing estimating equations models to measure changes in the prevalence of restless legs symptoms, with an unstructured covariance structure. Sex, age, smoking, BMI and leisure time physical activity were used as covariates in these models. Multivariate logistic regression was used to investigate the association between baseline characteristics (restless legs symptoms, sex, age, education, BMI, smoking, leisure time physical activity and BDI score) and restless legs symptoms in the follow-up. A conceivable nonlinear relationship between restless legs symptoms in the follow-up and the change in BDI score were assessed by using 3-knot-restricted cubic spline logistic models.

Statistical comparisons in study III between the groups were performed by analysis of variance, the Kruskal-Wallis test, chi-square test or Fisher-Freeman-Halton test, when applicable. When adjusted models were used, analysis of covariance was applied (the models included age, smoking, alcohol use, sex, BMI and leisure time physical activity as covariates). Statistical significance between groups in study IV was tested by analysis of variance or a chi-square test. When adjusting for confounding factors, an analysis of covariance (tested variable was continuous) or logistic regression model (tested variable was categorical) was applied; models included age, sex, smoking, use of alcohol, education years, BMI, use of antidepressants, and leisure time physical activity as covariates. In the case of violation of the assumptions (e.g., non-normality), a bootstrap-type test was used in study III and IV. The significance for pairwise comparisons were corrected for multiplicity using Hommel's multiple comparison procedure (at a significance level of 0.05) in study II and IV. The normality of the variables in the all data was tested by using the Shapiro-Wilk W test. The Stata 14.1, 15.0, 15.1 and 16.0 StataCorp LP statistical package was used for the analyses (StataCorp LP, College Station, TX).

4.5 ETHICAL ASPECTS

The study protocol was approved on the 17th of April 2007 by the Ethics Committee of Central Finland Central Hospital, Jyväskylä, Finland. Notification was based on written and oral patient information and written informed consent was obtained before any study procedures. If there were abnormalities in clinical or laboratory examinations, the

subjects were referred to their own health center. The subjects were able to withdraw from the study at any time and it did not affect their treatment and participation in the study was voluntary. The original data were stored in a locked room. The data available to the researchers did not have names, birthdates or identity numbers.

5 RESULTS

5.1 ASSOCIATION BETWEEN RESTLESS LEGS AND THE SEVERITY OF DEPRESSIVE SYMPTOMS (I)

Subjects with elevated depressive symptoms were more often current smokers and had lower levels of leisure time physical activity and had higher concentrations of triglycerides than the controls. The prevalence of restless legs symptoms was 39.2% (433) among all participants. The lowest prevalence (24.6%) was in subjects without a depression diagnosis (BDI score <10). Correspondingly, the prevalence was 43.4% in subjects with depressive symptoms without a depression diagnosis. The highest prevalence (52.4%) was in subjects with melancholic depression, and among subjects with non-melancholic depression (46.3%). The difference was not significant between non-melancholic and melancholic depression. However, the difference was significant between subjects without a psychiatric diagnosis, subjects with depressive symptoms without a depression diagnosis and diagnosed depressive subjects ($p<.001$).

Figure 8 illustrates crude and adjusted (for age, education years, smoking, BMI and leisure time physical activity) prevalence of restless legs symptoms. Figure 9A shows that the prevalence of restless legs symptoms is related to an increase in BDI scores up to a score of 30. The odds ratio for restless legs symptoms increases with increasing depressive symptoms being over sixfold compared with a zero BDI score (Figure 9B).

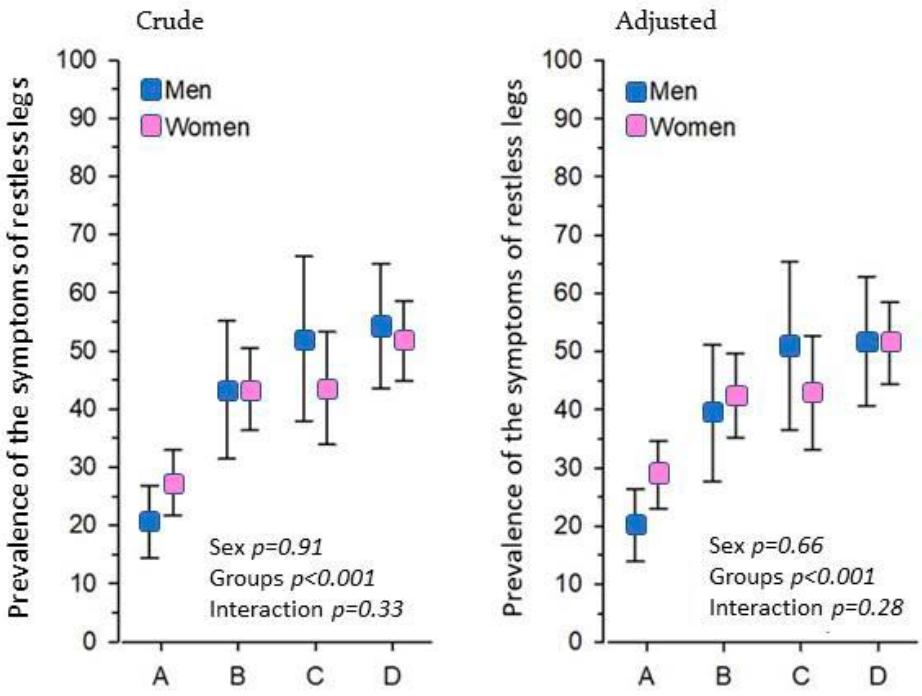

Figure 8. Prevalence of restless legs symptoms in controls (A), subjects with depressive symptoms without a depression diagnosis (B), non-melancholic depression (C) and melancholic depression (D) in men and women. Crude prevelences are shown in the left panel, and adjusted (for age, smoking, education years, body mass index and leisure time physical activity) prevalence are in the right panel. Groups show difference between controls (A), subjects with depressive symptoms without a depression diagnosis (B) and diagnosed depressive subjects (C and D).

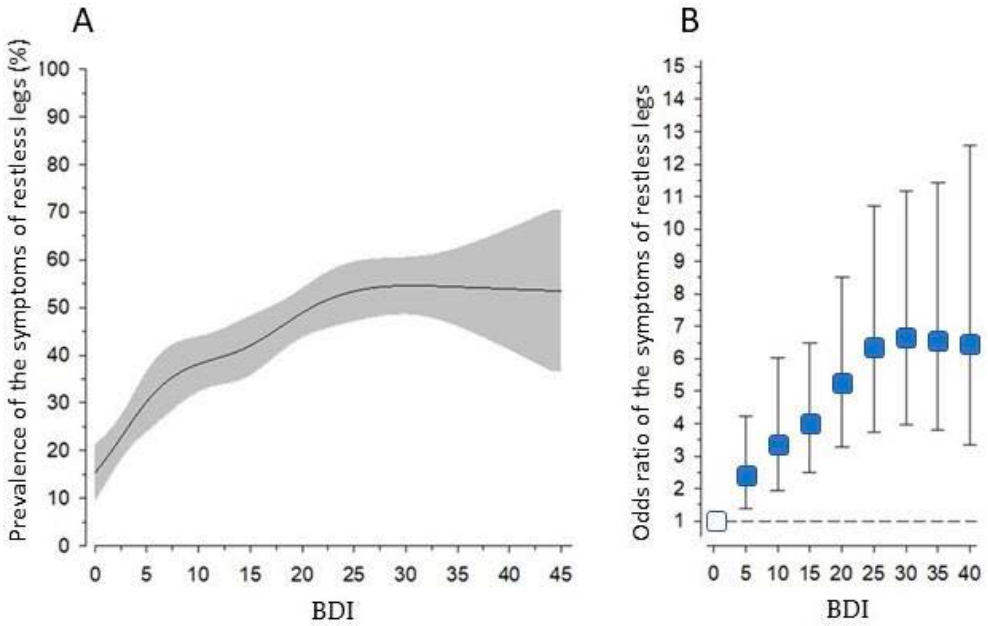

Figure 9. The relationship between BDI score and age- and sex -adjusted prevalence (A) and odds ratio (B) of restless legs symptoms. The gray area represents 95% confidence intervals.

5.2 THE EFFECT OF DEPRESSION ON THE PERSISTENCE AND DETERIORATION OF RESTLESS LEGS SYMPTOMS (II)

During the approximately 6 years of follow-up, the prevalence of restless legs symptoms declined in all groups (p=0.005; adjusted for sex, age, smoking, BMI and leisure time physical activity) (Figure 10). Group-specific changes were -2% (95% CI: -7 to 3%) in the controls, -7% (95% CI: -14 to 1%) in the subjects with depressive symptoms without diagnosed depression and -9%; (95% CI: -15 to -3%) in the subjects with diagnosed depression.

In the multivariate analysis, baseline restless leg symptoms, age and BDI score predicted the presence of restless legs symptoms at the follow-up among the subjects with diagnosed depression. Among the subjects with depressive symptoms without diagnosed depression, no predicting factors were found in addition to baseline restless leg symptoms. In the control group, the level of leisure time physical activity was inversely associated with restless legs symptoms at the follow-up.

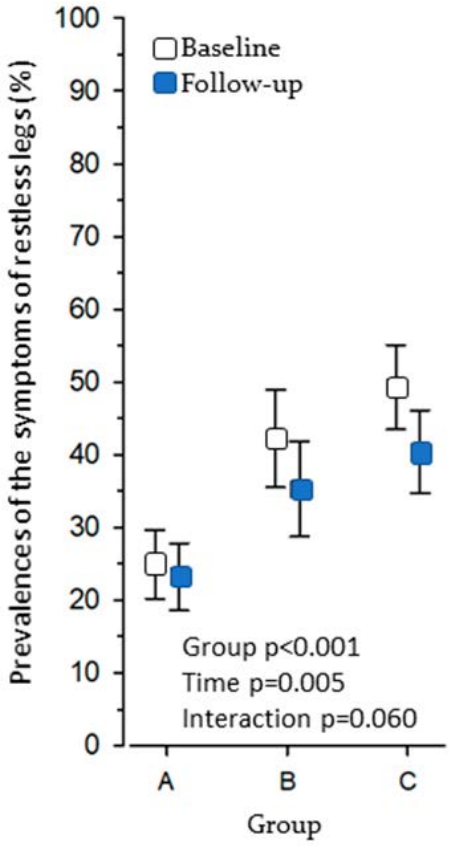

Figure 10. Prevalence of restless legs symptoms in controls (A), subjects with depressive symptoms without a depression diagnosis (B) and diagnosed depressive subjects (C) at baseline and follow-up. The prevalence are adjusted for sex, age, smoking, leisure time physical activity and BMI.

At the follow-up, the BDI score had changed significantly in all study groups ($p<0.001$). In the control subjects, the BDI score had increased (mean 1.1; 95% CI:0.7 to 1.6) whereas the subjects with depressive symptoms without a depression diagnosis (mean -7.7; 95 % CI: -9.0 to -6.5) and the diagnosed depressive subjects (mean -10.2; 95% CI: -11.4 to -9.1) had a decreased BDI score at the follow-up compared with baseline.

The change in BDI score was associated with the presence of restless legs symptoms in the control group (OR 1.11 [1.04 to 1.19]; $p=0.002$) and in the group of subjects with diagnosed depression (OR 1.05 [1.01 to 1.08]; $p=0.005$). In subjects with depressive symptoms without diagnosed depression (OR 1.03 [0.98 to 1.08]; $p=0.15$) there was not a significant association between BDI score and restless legs symptoms at the follow-up (Figure 11).

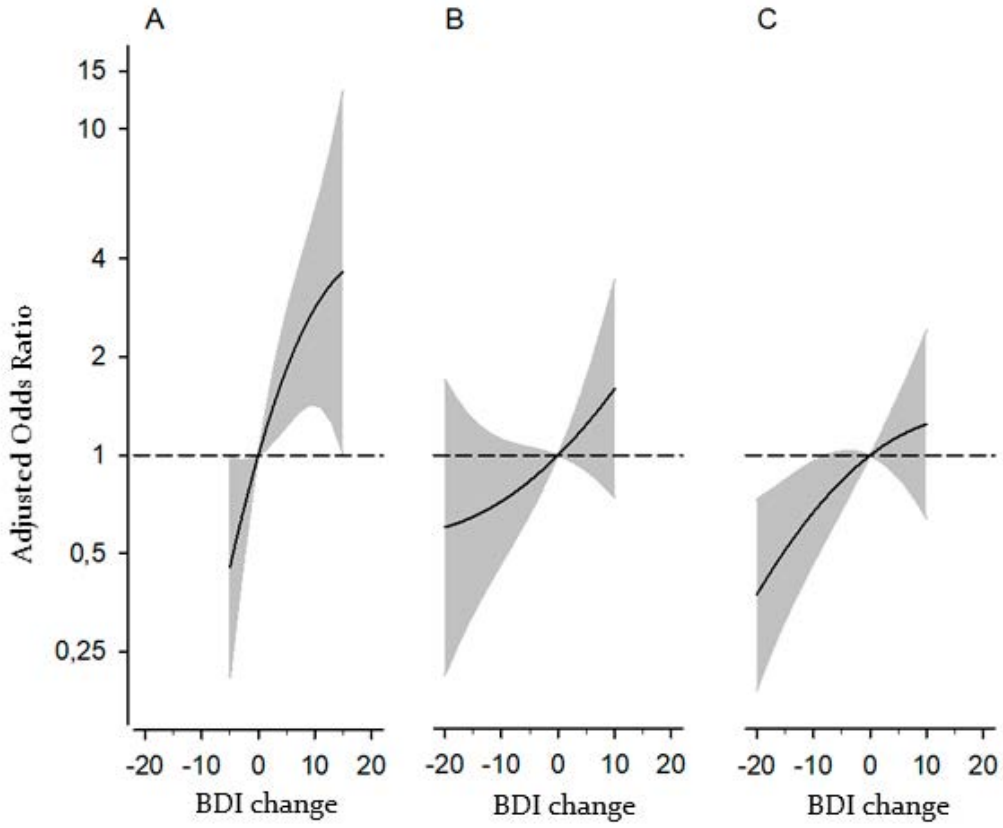

Figure 11. The change in the BDI score and odds ratios of restless legs symptoms in the follow-up in controls (A), subjects with depressive symptoms without a depression diagnosis (B) and diagnosed depressive subjects (C). The odds ratios are adjusted for sex, age, education years, smoking, body mass index, physical activity, BDI score and restless legs symptoms at baseline. The gray area represents 95% confidence intervals.

5.3 ASSOCIATION BETWEEN INFLAMMATORY MARKERS, RESTLESS LEGS SYMPTOMS AND DEPRESSION (III)

Those subjects who were diagnosed with depression had higher CRP concentrations than the controls. The difference in the levels of TNF-α between the controls and the depressive subjects was not significant. The concentration of TNF-α was significantly higher in the subjects with restless legs symptoms (7.4 ng/l ± 3.2) compared with the subjects without restless legs symptoms (6.7 ng/l ± 2.3) ($p < 0.001$ adjusted for sex, age, smoking, alcohol use, body mass index and leisure time physical activity) (Figure 12).

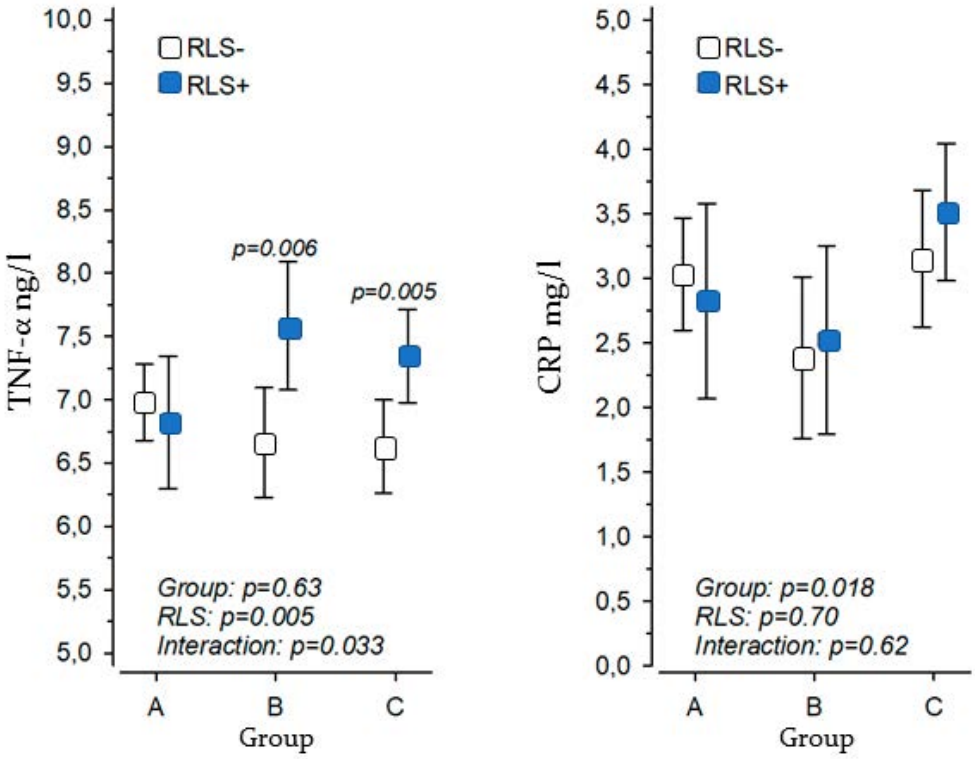

Figure 12. Relationships of TNF-α and CRP to restless legs symptoms in controls (A), subjects with depressive symptoms without a depression diagnosis (B) and diagnosed depressive subjects (C). Results are adjusted for sex, age, smoking, alcohol use, BMI and leisure time physical activity.

Among the subjects with a diagnosis of depression (p = 0.005) and the subjects with depressive symptoms without a depression diagnosis (p = 0.006) restless legs symptoms were associated with increased concentrations of TNF-α. In the control subjects the TNF-α levels were similar in those with and those without restless legs symptoms. The difference in the TNF-α levels between non-melancholic and melancholic depression subtypes was insignificant. The concentration of TNF-α between the patient groups and the controls was not significant if restless legs symptoms were not taken into account.

5.4 INFLUENCE OF RESTLESS LEGS SYMPTOMS ON MUSCULOSKELETAL PAIN IN THE DEPRESSED SUBJECTS (IV)

The adjusted (for age, sex, smoking, use of alcohol, education years, BMI, use of antidepressants, and leisure time physical activity) prevalence of continuous widespread musculoskeletal pain was examined between subjects with and without restless legs symptoms in the three groups: the controls, the subjects with symptoms of depression without a diagnosis, and the subjects with diagnosed depression (Figure 13). The prevalence was different between the three study groups: the controls 4.6% (95% CI: 2.8 to 7.1), the subjects with symptoms of depression without a diagnosis 16.0% (11.7 to 21.1), and the subjects with diagnosed depression 22.1% (18.3 to 23.3) (p=0.006; adjusted for age, sex, smoking, education years, use of alcohol, leisure time physical activity, BMI and use of antidepressants). After multiple corrections, all groups differed significantly from each other.

Compared to subjects without restless legs symptoms, subjects with restless legs symptoms had continuous widespread musculoskeletal pain more often in the control subjects (p=0.001; 2.3% vs. 10.5%) and the subjects with depressive symptoms without a depression diagnosis (p=0.024; 9.1% vs. 18.7%). In subjects with diagnosed depression, there was not a significant difference in continuous widespread musculoskeletal pain (p=0.98; 19.5% vs. 19.4%) between subjects without or with restless legs symptoms. Figure 14 shows the adjusted (for age, sex, education years, smoking, use of alcohol, leisure time physical activity, BMI and use of antidepressants) intensity of pain and restless legs symptoms between the study groups. The difference between increased intensity of pain and the study groups was negligible but restless legs symptoms had a relevant association with increased intensity of pain in all groups (p<0.001).

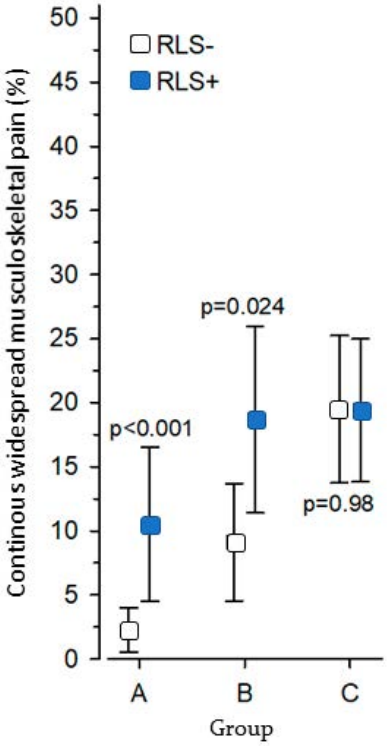

Figure 13. Prevalence of continuous widespread musculoskeletal pain according to restless legs symptoms in controls (A), subjects with depressive symptoms without a depression diagnosis (B), and subjects with diagnosed depression (C) (adjusted for age, sex, education years, smoking, use of alcohol, leisure time physical activity, BMI and use of antidepressants).

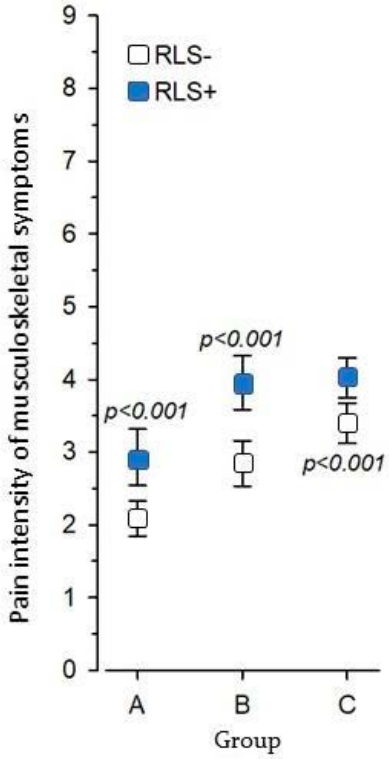

Figure 14. Intensity of pain according to restless legs symptoms in controls (A), subjects with depressive symptoms without a depression diagnosis (B), and subjects with diagnosed depression (C) adjusted for age, sex, education years, smoking, use of alcohol, leisure time physical activity, BMI and use of antidepressants.

6 DISCUSSION

The results of this thesis focused on the prevalence, prognosis, and association of restless legs symptoms with inflammatory factors and pain in depressed and non-depressed subjects in primary health care. Firstly, this study indicated that restless legs symptoms were common in the depressed primary care patients. However, there was no significant difference in the prevalence between the subtypes of depression, melancholic and non-melancholic. Secondly, in a follow-up study moderate to high leisure time physical activity seemed to provide protection against restless legs symptoms. Thirdly, among the inflammatory markers, a higher concentration of TNF-α was associated with restless legs symptoms in the depressed subjects and among the subjects with depressive symptoms without a depression diagnosis but not in the control subjects; however, CRP did not have a similar association. Fourthly, pain intensity was higher in the subjects with restless legs symptoms regardless of depressive symptoms or depression.

6.1 PREVALENCE OF RESTLESS LEGS SYMPTOMS (I)

Based on these results, there was no substantial difference in the prevalence of restless legs symptoms between non-melancholic and melancholic depression. The results of study I suggest that depression by itself rather than metabolic disturbances linked to non-melancholic depression, partially explain the association with restless legs symptoms. In addition, there was a linear association between the severity of depressive symptoms and restless legs symptoms. This result ties well with a previous study wherein subjects with restless legs symptoms received a high BDI score, especially from those items that were related to physical symptoms of depression and lower points from the items related to cognitive–affective symptoms (186, 187).

In line with previous studies, restless legs symptoms occurred more frequently with depression. In addition, a higher level of depressive symptoms was related to increased odds of having restless legs symptoms. Compared with the controls the prevalence of restless legs symptoms was twofold higher in patients with depression. The prevalence was quite high also in the controls. A similar conclusion was reached by some previous studies, which reported a high prevalence of restless legs syndrome, for instance, 21.5 and 24% (101, 102). Depression shows an association with a higher BMI, smoking and lower leisure time physical activity. There are indications that physical activity may be a

significant factor in contributing to the likelihood of depression (188, 189). Understanding the effects of physical activity on restless legs symptoms is likely to be even more multidimensional than the link between physical activity and depression. A recent study suggested that the relationship between restless legs symptoms and depression is bidirectional (190). It is probable that effective treatment of depression relieves the symptoms of restless legs syndrome. Furthermore, a previous study has indicated that proper treatment of restless legs symptoms improves mood (191).

6.2 LONGITUDINAL ASSOCIATION OF RESTLESS LEGS SYMPTOMS WITH DEPRESSION (II)

The follow-up study indicated differences in the longitudinal relationship between depression and restless legs symptoms. The baseline scores for depressive symptoms predicted restless legs symptoms only in the subjects with diagnosed depression. A change in the depressive symptoms between baseline and follow-up was associated with restless legs symptoms in the controls and in the subjects with diagnosed depression. Furthermore, in the controls, a moderate or high level of leisure time physical activity provided protection against restless legs symptoms.

The course of restless legs symptoms in the study seemed to be quite stable. This is consistent with what has been found previously in a hospital-based study and a survey of subjects with restless legs syndrome (192, 193). In the previous prospective studies, restless legs syndrome preceded both clinical depression and a new onset of depressive symptoms (65, 66). In study II, the baseline scores for depressive symptoms predicted restless legs symptoms in the follow-up with those participants who had diagnosed depression at the baseline. A higher score of depressive symptoms during the follow-up period increased the odds of having restless legs symptoms. This finding is concordant with a previous study based on subjects with restless legs syndrome which suggested that a change in a depression score is associated with a change in restless legs symptoms (192).

It is plausible that pramipexole, ropinirole and gabapentin enacarbil treatment of restless legs symptoms improves mood because they may also alleviate depressive symptoms (191, 194). In study II, a decreasing level of depressive symptoms was associated with decreasing odds of having restless legs symptoms in the follow-up. About seven out of ten diagnosed depressed subjects in the study used antidepressants at the baseline. Among them, the follow-up BDI-score was markedly lower compared with the baseline, suggesting a treatment response. There are also studies in which antidepressant use has been linked to restless legs symptoms though recent results have strongly questioned this relationship (195-198).

The subjects with diagnosed depression perceived their sleep least often sufficient and felt least often rested in the morning. Insomnia may contribute to the relationship between depression and restless legs symptoms but an increasing number of depressive symptoms after the follow-up seemed to also predispose the non-depressive people to restless legs symptoms. Only a few of the non-depressed controls reported problems with sleeping at the baseline. However, when comparing these results to previous results, it must be pointed out that restless legs syndrome with insomnia increased the severity of depression more than primary insomnia with similar insomnia severity (199). Therefore, sleep is not the only explanation in the longitudinal relationship between depressive and restless legs symptoms presented in this thesis.

Research to examine the nature of the causation between depression and restless legs symptoms and to determine possible underlying mechanisms is limited. Cardiometabolic risk factors related to lifestyle could be one link between depression and restless legs symptoms. A high BMI and low physical activity have been found to increase the risk for developing restless legs syndrome (143). The relation between a high BMI and level of depressive symptoms may not be straightforward and the association may be influenced by sex and ethnicity (200, 201). Likely there is still a long way to go to comprehend the causal relationship between depression and restless legs symptoms accurately.

These results provide evidence that in the control subjects a moderate or high level of leisure time physical activity protected them against restless legs symptoms. This finding is concordant with a previous finding indicating that the presence of restless legs symptoms is longitudinally associated with lower physical function (143). In another previous study, exercise was associated with beneficial anti-inflammatory effects e.g., inhibiting the production of tumor necrosis factor and stimulating the occurrence of anti-inflammatory cytokines (202). The finding that exercise among subjects with depression did not have the protective effect against restless legs symptoms may be considered an indication that subjects with depression probably have a more severe pathophysiology related to restless legs symptoms than the control subjects.

6.3 RESTLESS LEGS SYMPTOMS AND INFLAMMATORY MARKERS (III)

The results from study III provide evidence that depressive symptoms or clinical depression and elevated circulating TNF-α concentration was associated with restless legs symptoms. The concentration of TNF-α was similar in the control group despite restless legs symptoms. It has been known that the link between depression and TNF-α

concentration is undermined, for example, by obesity (203). An anti-TNF-α drug, infliximab, relieved depression in patients with e.g., ankylosing spondylitis and Crohn's disease but the activity of the other disease did not have an impact on the end result (204, 205). In a study with mice, infliximab relieved depression-like behavior, but no similar finding was repeated in a study of humans with treatment-resistant depression (206, 207). The anti-TNF-α treatment had problems, including infections, that would limit the usefulness of treatment for depression with severe restless legs symptoms (208). The subjects with diagnosed depression had a higher CRP concentration than the control subjects, which has also been reported in previous studies (209). These results showed that CRP did not have a similar association with restless legs symptoms in the depressive subjects as TNF-α.

TNF-α has the potency to initiate divalent metal transporter 1 (DMT1) onset when iron uptake increases in the astrocytes, microglia and neurons (210-212). The theory involves the assumption that DMT1 enables the transfer of iron ions across the endosomal membrane in the cells. In the brain DMT1 controls iron homeostasis and iron is indispensable for deoxyribonucleic acid (DNA) synthesis and protein metabolism, including serotonin and dopamine (213). Increasing TNF-α concentration is assumed to be a pathway connecting iron metabolism and the dopaminergic system together in depressive subjects. In the previous studies, subjects with restless legs syndrome had differences in iron metabolism in the different regions of the brain, such as the putamen, pallidum, thalamus and substantia nigra. (124, 214) Parkinson's disease patients with restless legs syndrome had a lower concentration of iron and ferritin but a higher level of transferrin in the cerebrospinal fluid compared to patients without restless legs syndrome. It is still unknown how decisive a pathway DMT1 is for iron metabolism in the brain system but an animal study revealed a nascent connection between the function of DMT1 and mood disorders in mice (215, 216). In addition to the aforementioned, TNF-α has an impact on monoaminergic systems with dopamine and serotonin functions. In both peripheral and brain tissues, an elevated concentration of TNF-α improves the activation of indoleamine 2,3-dioxygenase (IDO) (207). IDO precipitates the depression inhibiting development of tryptophan to serotonin and TNF-α decreases the bioavailability of serotonin and has an effect on the reuptake of serotonin (217, 218).

There is evidence that CRP is a more likely immunomarker associated with depression than TNF-α (174). The pathophysiology of restless legs symptoms may include effects of the immune system, but the effect between CRP and restless legs symptoms did not appear to be the most likely option (128). Study III suggested that particularly in depressive subjects, TNF-α may have an association in the explanation of restless legs symptoms.

6.4 RESTLESS LEGS SYMPTOMS AND PAIN (IV)

There was a significant relationship between restless legs symptoms and widespread musculoskeletal pain in the control subjects and the subjects with depressive symptoms without a depression diagnosis. Restless legs symptoms did not show a relationship with widespread musculoskeletal pain in subjects with diagnosed depression even though they reported the most pain. The subjects with restless legs symptoms and depression symptoms without diagnosed depression seemed to experience continuous widespread musculoskeletal pain as frequently as the subjects with a depression diagnosis. An interesting finding was that restless legs symptoms independently increased the intensity of pain in all three groups in a similar way. It has previously been suggested that restless legs symptoms have a bidirectional effect on chronic pain and depression. Also, it is known that restless legs symptoms were associated with dopaminergic dysfunction. (219) Depression itself seemed to increase the predisposition to widespread musculoskeletal pain but consequently restless legs symptoms did not affect pain in subjects with a depression diagnosis.

The comorbidity between pain and depression is known and explained by, for example, the action of IDO and inflammation (226). Dysregulation of the HPA axis and hypercortisolism have been found to associate with depression but patients with widespread pain or restless legs syndrome have had an insignificant affiliation (36, 227, 228). It is by now generally accepted that pain and depression affect each other. Depression predisposes a person to obesity and low physical activity; in addition to that, it predicts pain or a pain-related disability more likely than vice versa (53, 55, 220). Depression is associated with adverse metabolic features but a high triglyceride and glucose concentration have also been found to be associated with musculoskeletal pain, such as back and tendon pain (221, 222).

The previous study reported that a high concentration of IDO explained in part the association between pain and depression (223, 224). Regrettably data between restless legs symptoms and IDO did not exist; however, in one study, somatization was linked to the activation of IDO and restless legs symptoms (225). The monoamine hypothesis, including noradrenaline, dopamine and serotonin insufficiency, has linked depression with the chronic pain mechanism (76). Inflammatory markers, such as CRP and IL-6, increase in depression and have an influence on monoamines; in addition to these, IL-6 is linked to chronic widespread musculoskeletal pain (74, 75). Along with pain and depression, systemic inflammation could be a contributor in restless legs symptoms (229).

6.5 STRENGTHS AND LIMITATIONS OF THE STUDY

This study was based on representative sample of middle-aged and elderly subjects in the Finnish population. Informative data were collected from control subjects and depressed subjects and on their lifestyle factors, health status and laboratory tests, amongst other things. Determination of sample size was successful and it was sufficient for the results. A representative control group was properly used in order to compare data. One clear benefit was that the data were also used as a longitudinal study. The same subjects at the baseline and at the follow-up were able to be assessed with the same measures of depressive symptoms, depression diagnostics and restless legs symptoms.

In addition to self-reported depressive symptoms, a diagnosis of depression was based on a diagnostic interview. The BDI was a practical method for evaluating depression symptoms. In this study it was assumed that the subjects who had a BDI score below 10 did not presumably have clinical depression. This study setting did not distinguish the sources of BDI scores. Therefore, it was not possible to assess the importance of affective or cognitive symptoms of depression based on the BDI. The M.I.N.I. was not conducted for subjects who had a BDI score below 10. Nonetheless the M.I.N.I. was a precise instrument for diagnosing and subtyping depression. The study nurses were properly trained to ensure the quality of the interview.

The definition of restless legs symptoms was based on a structured questionnaire. According to a previous validation study of a group of 521 subjects in a neurology clinic, the questionnaire had 100% sensitivity and 96.8% specificity (101). One limitation regarding implementation is that the study did not include a differential diagnosis of idiopathic, secondary, or iatrogenic restless legs symptoms. The study did not collect information on how often or long the symptoms had lasted. It is possible that the method used in this study even with high sensitivity and specificity could result in false positive responses. Because of the limitations above the decision was made to investigate restless legs symptoms instead of syndrome.

This study included persons aged 35 or older, so the results cannot be generalized to younger age groups. In the present study, a quarter of the original baseline sample was lost in the follow-up. It is possible that those who did not respond represented a more severe progression of the depressive symptoms. However, the participating subjects represented, in a fairly balanced way, all the groups of the original sample indicating no evidence of serious bias.

6.6 SUMMARY AND IMPLICATIONS

The results from study I demonstrated that restless legs symptoms are common in primary care among subjects with depression, regardless of the depression type. The prevalence of restless legs symptoms did not differ between the subjects with non-melancholic depression and those with melancholic depression. Study II found that a higher number of depressive symptoms was a risk factor for restless legs symptoms in subjects with clinical depression. Also, a low level of leisure time physical activity in the control subjects was a risk factor for restless legs symptoms. In study III the main conclusion that can be drawn is TNF-α level was associated with restless legs symptoms among subjects with depressive symptoms. Study IV confirmed the findings about restless legs symptoms were related to continuous widespread musculoskeletal pain in the control subjects and subjects with depressive symptoms without a depression diagnosis. It is notable that pain intensity was higher in the subjects with restless legs symptoms regardless of depressive symptoms or depression. This may alter or improve aspects of clinical management of pain in subjects with restless legs symptoms; there should be more focus on the prevention and treatment of either condition.

The severity of depressive symptoms is related to the prevalence of restless legs symptoms. To our knowledge, this was the first study to investigate a relationship between restless legs symptoms and depression according to depression subtypes. Restless legs symptoms were most common in the subjects with diagnosed depression, and the odds of having restless legs symptoms increased with the BDI score. In this study higher level of depressive symptoms was a risk factor for restless legs symptoms in subjects with clinical depression. This might be due to the fact that effective treatment of depression probably relieves restless legs symptoms. In the future, it would be necessary to study the precise role of antidepressants in the course of restless legs symptoms based on a prospective setting.

The findings from the longitudinal setting suggest that in the prevention and treatment of restless legs symptoms among subjects with depression, the priority is the effective treatment of depression. Together, the present findings suggest that among the population without depressive symptoms, perhaps promoting physical activity and mental health should be included in a preferred strategy. The result now provides evidence that the course and prognostic factors are different in the control subjects, subjects with depressive symptoms who do not meet the diagnostic criteria of depression, and in subjects with diagnosed depression. This finding emphasizes the proper assessment of depressive symptoms and diagnostics of depression in patients

needing treatment for restless legs symptoms and provides a good starting point for discussion and further research.

Based on the results of the present study, an elevated concentration of TNF-α was associated with restless legs symptoms in subjects with depressive symptoms with and without diagnosed depression, but not in control subjects. The results at least cautiously suggest neuroinflammation plays a role in the genesis of restless legs symptoms in subjects with depressive symptoms with and without clinical depression. The study indicates a relationship between TNF-α and restless legs symptoms in depressive subjects, which has not yet been extensively studied.

These findings encourage future work and provide a potential mechanism for the pathophysiology of restless legs symptoms to demonstrate the effects of the immune system. Based on these results, it is proposed that TNF-α may have an effect in the manifestation of restless legs symptoms, particularly in depressive subjects. Importantly, the results provide evidence that restless legs symptoms have a multidimensional and considerable relation to pain.

Depression is a major health problem. This study indicates that subjects with depression very often have restless legs symptoms. Therefore, it can be recommended that treatment guidelines for depression should consider this common and important comorbid condition. These findings should be taken into account during clinical evaluations and treatment of patients who visit a physician due to restless legs or depressive symptoms. When the patient comes to a physician’s practice for restless legs symptoms, it is appropriate to enquire about their mood. The results of this study reaffirm that depressed patients should be provided with adequate screening, diagnosis, and treatment of restless leg symptoms in primary care.

7 CONCLUSIONS

Based on the research questions, these are the most significant findings from the thesis

I. Restless legs symptoms are common in the subjects with depression but melancholic or non-melancholic depression subtypes did not have an influence on prevalence.

II. In a longitudinal setting a higher amount of baseline depressive symptoms was a risk factor for restless legs symptoms in subjects with clinical depression and low levels of leisure time physical activity in the controls. Increased depressive symptoms were associated with increased prevalence of restless legs symptoms at the follow-up both in subjects with depression and non-depressed control subjects.

III. TNF-α concentration was associated with restless legs symptoms among subjects with depressive symptoms whether they had clinical depression or not; however, CRP did not have a similar association.

IV. Restless legs symptoms had an association with continuous widespread musculoskeletal pain in the control subjects and the subjects with depressive symptoms without a depression diagnosis. Intensity of pain was associated with restless legs symptoms in non-depressed controls, depressed subjects without depression diagnosis and subjects with a depression diagnosis in a similar way.

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Depression is a common mood disorder worldwide and is associated with several comorbidities, such as restless legs syndrome and pain. The general aim of this thesis was to investigate the prevalence, prognosis, and association of restless legs symptoms with inflammatory factors and pain in depressed and non-depressive subjects in primary health care. The results of the study indicated that restless legs symptoms are common in primary care patients, especially in those with depression.

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