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Sex Differences in Lyme Disease Symptomatology in the Northeast United States

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This is to certify that the doctoral dissertation by

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Walden University
2020

Abstract

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Dissertation Submitted in Partial Fulfillment

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Abstract

Guided by the gender-analysis-matrix theoretical framework, 3 key areas of research inquiries focused on the relationship between sex and Lyme disease, symptoms of Lyme disease and sex, and severity of Lyme disease symptoms on sex. A quantitative secondary data analysis was used to address the research questions. A clinician specializing in caring for individuals with Lyme disease provided the dataset, containing responses to the Horowitz Multiple Systemic Infectious Disease Syndrome Questionnaire for Lyme disease. A cross-sectional, comparative research design incorporating 2 statistical techniques for analysis—the independent samples *t* test and multivariable regression analyses—was used to examine symptom counts and the severity of symptom, scoring the severity. Study findings from 235 participants (40 males, 17%, and 195 females, 83%) indicated no sex differences in type, number, and severity of chronic Lyme disease symptoms. The top 5 Lyme disease symptoms—fatigue, disturbed sleep, stiff neck or back, neck cracks, and joint pain—ranked the same for males and females, varying little in percentages. The positive social change implications derived from the findings of this study are to improve understanding of sex differences in chronic Lyme disease. This study not only addressed clinical presentations, but also issues of sex bias, which can result in the development and implementation of sex-based medical, psychological, and social interventions leading to epidemiological interventions to reduce the prevalence of this debilitating disease.

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Dedication

This project is dedicated to my parents, Frank and Margaret Scotti. I was born with Lyme disease and my father has provided infinite support for me, especially after my mother's death from Lyme disease complications, misdiagnosed as "cancer." Prior to my mother's death, she impressed upon me the importance of continuing research in Lyme disease to provoke further the understanding of this cruel disease. I am hopeful that this research has contributed to better disclosure of this controversial indication.

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A special thank you to my father, Frank Scotti, Esq. for all his support in motivating me to continue to push through and finish the program.

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Chapter 1: Introduction to the Study

Introduction

Lyme disease, also known as *borreliosis*, is an infectious disease that can be transmitted by the *Ixodes* tick, if that tick is infected with the *Borrelia burgdorferi* (*B. burgdorferi*) bacterium (Centers for Disease Control and Prevention [CDC], 2015a). Lyme-literate professionals distinguish between Lyme disease and *chronic* Lyme disease, also known as *posttreatment Lyme disease* (PTLDS; Johns Hopkins Medicine, 2018). In contrast to Lyme disease, which has specific symptomatology, chronic Lyme disease has persistent symptoms (Donta, 2012). Most often, the symptoms of chronic Lyme disease are neurological and cardiac related, neither responding to typical antibiotic treatment nor to confirmation using serological testing (Lantos, 2015).

Lyme disease appears to affect males and females equally (Rebman, Soloski, & Aucott, 2015). Lyme disease is far more common in females than males; indeed, chronic Lyme disease affects nine females for every male (Muñoz-Grajales, González, Alarcón, & Acosta-Reyes, 2016). The cause of chronic Lyme disease and reasons for the higher prevalence of chronic Lyme disease among females remain largely unexplained. The goal of this study was to enhance understanding as to why such sex differences in the susceptibility, prevalence, and severity of chronic Lyme disease exist.

Severity of symptoms for Lyme disease varies between individuals, whether male or female. Untreated, undiagnosed, or late diagnosis of Lyme disease leads to serious health problems that can resemble other indications or conditions, leaving individuals to experience a variety of symptoms that worsen the longer they are left untreated (Muth,

2019). The severity of symptoms was scored in this study using a 4-point Likert-type scale of 0 for none, 1 for mild, 2 for moderate, and 3 for severe for each symptom listed in the Horowitz Multiple Systemic Infectious Disease Syndrome (MSIDS) Questionnaire tool, also called the Horowitz MSIDS Questionnaire (HMQ). In this study, I examined whether significant sex differences exist in reported presentations of Lyme disease, as well as sex differences in the number and severity of Lyme disease-related symptoms for males with Lyme disease versus females with Lyme disease.

An increased understanding of potential sex differences regarding chronic Lyme disease can result in the development and implementation of sex-based medical, psychological, and social interventions, potentially leading to epidemiological interventions that reduce the prevalence of this debilitating disease. Sex differences are important epidemiological factors that impact the prevalence and severity of infectious diseases (Vázquez-Martínez, García-Gómez, Camacho-Arroyo, & González-Pedrajo, 2018). Sex as a variable in infectious-disease research typically has been overlooked and the influence of sexual dimorphism (where the two sexes of the same species exhibit different characteristics beyond the differences in their sexual organs), is probably underrepresented (Ingersoll, 2017). Sex bias is a major challenge in clinical research, as major adverse effects observed in single-sex studies cannot forecast whether males and females will respond differently to a drug, vaccine, or treatment.

Hormonal, genetic and environmental factors between males and females may influence immune responses and sex-related outcomes, and both sexes should be shielded against immune-mediated and infectious diseases with the long-term goal of

individualizing therapies for males and females independently (Ruggieri, Anticoli, D'Ambrosio, Giordani, & Viora, 2016). Although confounding variables may be of interest or considered, limitations in the data set used with regard to availability of certain data elements preclude the incorporation of some potential confounders (e.g., presence of autoimmune diseases that differ in prevalence between males and females and exhibit symptoms that are similar to those present in persons with chronic Lyme disease) in this research analysis.

The purpose of Chapter 1 is to provide a comprehensive overview of this research study. In the background of the study section, I summarize pertinent empirical literature and note gaps in this body of literature. These gaps provide the rationale for this study. I elucidate the statement of the problem and the purpose of the research and present the research questions and associated null and alternative hypotheses. The chapter continues with a discussion of the guiding theoretical framework, followed by the nature of the research, pertinent definitions, research assumptions, an articulation of scope, delimitations, and limitations. Research outcomes currently available on sex-divergent responses to treatments and therapies are finite and suggest the need for additional basic biomedical research in this area, especially with Lyme disease patients (Ruggieri et al., 2016). A summary section concludes each chapter.

Background of the Study

In 2006, the Infectious Diseases Society of America (IDSA) identified specific criteria for chronic Lyme disease, identifying Lyme disease as the harbinger of chronic Lyme disease (Wormser, Dattwyler, Shapiro, Halperin, Steere, Klempner, Krause,

Bakken, Strle, Stanek, Bockenstedt, Fish, Dumler, Nadelman, 2006). Also, individuals with chronic Lyme disease must have received a diagnosis of Lyme disease with clinical findings documented first (Wormers et al., 2006). The IDSA concluded that individuals with chronic Lyme disease do not respond to oral antibiotics (which is the general course of treatment for initial Lyme disease), yet report chronic or intermittent symptoms including extreme fatigue, musculoskeletal pain, neurological and cognitive impairments persisting at least 6 months after the completion of antibiotic therapy, and other symptoms so severe that they impede daily functioning (Wormers et al., 2006; Lantos, 2015). This clinical confusion regarding chronic Lyme disease has led to controversial debates in the literature. Some clinicians and researchers posited that chronic Lyme disease is a polymicrobial disease distinctly different from Lyme disease and associated with such diseases as fibromyalgia or chronic fatigue syndrome (Rawls, 2018). Others defined Lyme disease as a psychosomatic disorder (Lantos, 2015).

Each year, state health departments report more than 300,000 new cases of Lyme disease to the CDC (2018). This has led the CDC to declare that Lyme disease is the fastest growing vector-borne infectious disease in the United States (CDC, 2018). However, factual evidence has shown that between 3 and 28% of individuals initially diagnosed with Lyme disease progress to chronic Lyme disease (Lantos, 2015). The dearth of solid data, coupled with the lack of objective clinical tests, compelled researchers to deduce that the prevalence rate of chronic Lyme disease in the general population has “become nearly impossible to discern” (Lantos, 2015, p. 326).

Empirical evidence demonstrated similar prevalence rates of Lyme disease across sex groups; yet in contrast, individuals with chronic Lyme disease are considerably more likely to be females than males, with prevalence ratios ranging from 7:1 to 9:1 (Wormser & Shapiro, 2009). Researchers have conducted several analyses to better understand sex-based differences in the epidemiology, clinical presentation, and immunologic response of chronic Lyme disease. Case in point, Rebman et al. (2015) observed sex differences in immune responses to Lyme disease that may promote the observation of higher rates of Lyme disease among females. Basic immune responses diverge between females and males, and it is evident that females have higher absolute numbers of CD4+ lymphocytes compared to males, which may contribute to their increased immune responses (Whitacre, 2001). The Whitacre and Rebman studies did not consider differences in Lyme disease symptomatology based on sex. Although sex hormones have long been recognized for their roles in reproductive functions, in the past 2 decades, scientists have observed that sex hormones are fundamental signaling modulators of the mammalian immune system (Ackerman, 2006). Additionally, sex hormones have conclusive roles in lymphocyte maturation, activation, and the synthesis of antibodies and cytokines, as these hormones contribute to the creation of autoimmunity (Ackerman, 2006).

Lyme disease impacts people in different ways and diagnosing the chronic form of the disease is challenging (Lymedisease.org, 2015). Lyme disease takes, on average, 2 years for an individual to obtain a correct diagnosis (Auwaerter, 2015; LymeDisease.org, 2015). The prevalence of Lyme disease among females may be higher than is reported, resulting from misdiagnoses (Wormer & Shapiro, 2009). Females who had been

diagnosed or misdiagnosed with such illnesses as fibromyalgia, chronic fatigue syndrome, or depression may actually have chronic Lyme disease (Wormser & Shapiro, 2009). Imperfect diagnostic tools for Lyme disease may also contribute to its under-identification and subsequent misdiagnoses, mostly for females (Aucott, Morrison, Munoz, Rowe, & Schwarzwalder, 2009). In response to the potential for misdiagnosis, Horowitz (2013) developed the Horowitz differential diagnostic approach as a road map to identify the multiple elements of the MSIDS questionnaire tool. The MSIDS questionnaire has been validated as a tool for distinguishing between individuals with confirmed Lyme disease and healthy individuals without Lyme disease (Citera, Freeman, & Horowitz, 2017) in the clinical-assessment stage. The HMQ has been deemed valid and effective as a low-cost screening tool for medical practitioners to assist in the necessary clinical assessment of individuals presenting with possible Lyme disease or related tick-borne infections (Citera et al., 2017).

In a small retrospective study with 125 participants, Schwarzwalder, Schneider, Lydecker, and Aucott (2010) found verification of sex-based differences using the Enzyme-Linked Immunosorbent Assay (ELISA) and Immunoglobulin G (IgG) serologic response to early Lyme-disease stages. Schwarzwalder et al. acknowledged that such differences could have cognizant implications on the merit of diagnosis, treatment, and disease classification. A later study further underscored a sex-based gap (Rebman et al., 2015). Although some research has been done in this area, referencing a research study with a sample size of 85 individuals (Ljøstad & Mygland, 2009), additional research is needed to appreciate the extent of the differences between the sexes and Lyme disease,

from the earliest stages of antigen exposure to the final effector stages of immunity in response to exogenous and self-antigens (Whitacre, 2001).

The identification of individuals with Lyme disease is a major health concern and a critical public health threat in the United States and Europe (Citera et al., 2017). This problem is on the brink of becoming the most prevalent spreading vector-borne epidemic worldwide, as pathogen-carrying ticks ride migratory birds throughout wide geographic areas, proliferating the infection (Citera et al., 2017). Much remains unexplored and undiscovered regarding sex and sex-based differences in the epidemiology, clinical presentation, and immunologic response to chronic Lyme disease (Rebman et al., 2015). Additionally, treatment and therapies are not sex specific of gender-sensitive medicine, and specific data on sex differences are lacking (Guerra-Silveira & Abad-Franch, 2013).

Problem Statement

Lyme disease is suspected to be a parasitic infection transmitted by the bite of a hard tick associated with several species of the genus Ixodes (Hatchette et al., 2015). Lyme-literate practitioners hypothesize that a parasitic association with Lyme-disease infection is evident due to the discovery of multiple microbes contributing to this illness and indication. The ongoing battle in this research is due to multiple strains of *Borrelia* and coinfections on top of Lyme infection relates primarily to different types of ticks in many geographic locations (Rawls, 2018). Different strains of *Borrelia* species have been confirmed as causative agents of Lyme disease with 100 known strains in the United States and 300 strains worldwide (Lymestats.org, 2018). Because Lyme disease impacts people differently, and depending on susceptibility criteria such as sex, the symptoms of

Lyme disease may not appear for many years (Columbia University Irvine Medical Center, 2018). Thus, a correct diagnosis is quite difficult. Lyme disease is often misdiagnosed as many other indications including psoriatic arthritis, joint pain, multiple sclerosis, rheumatoid arthritis, chronic fatigue syndrome, thyroid disease, amyotrophic lateral sclerosis, and the four types of lupus: systemic lupus erythematosus, discoid (cutaneous), drug-induced lupus, and neonatal lupus (CanLyme, n.d.). As symptoms manifest uniquely for each individual through several factors and variables, no standardized diagnostic test exists, though one test generally used to diagnose early Lyme disease is the western-blotting method (Mayo Clinic Staff, 2018).

Western blotting is a method and sensitive assay used for immunodetection and characterization of specific proteins by taking advantage of the specificity inherent in antigen–antibody recognition (Gallagher & Chakavarti, 2008). Tests, such as the western blot test, identify antibodies to the *Borrelia* bacteria strain and not the bacteria itself (IGeneX, 2017). These antibodies may not have been produced by the body in a quantity necessary to show a positive outcome in diagnostic results. Specificity to a diagnostic test will indicate the level of the specific antigen or antibodies found in the sample and provides a level of confidence that the individual has the disease, even if dormant at the time of drawing the blood sample negative (Lalkhen & McCluskey, 2008). The sensitivity of a diagnostic test can correctly identify those individuals with the disease, so if the highly sensitive test is negative, the individual does not have the disease, called a true negative (Lalkhen & McCluskey, 2008).

The western-blot test for Lyme disease provides a second-tier confirmation of the physician assessment of clinical symptoms (CDC, 2015b). Most treating physicians do not elucidate patients' familial history of Lyme disease or biological sex as factors for Lyme disease (vom Steeg & Klein, 2016). Individuals with chronic Lyme disease are significantly more likely to be females than individuals diagnosed with either Lyme disease or post-Lyme disease syndrome (Wormser & Shapiro, 2009). Based on this finding, individuals with chronic Lyme disease consistently diverge as a function of sex from individuals with *B. burgdorferi* infection or post-Lyme-disease syndrome. Also, illnesses such as fibromyalgia, chronic fatigue syndrome, or depression, with a female preponderance, could be misdiagnosed as chronic Lyme disease (Wormser & Shapiro, 2009).

In another study, the biological plausibility of sex effects on Lyme disease was explored with a review of charts or medical records. In 2010, researchers demonstrated no remarkable differences in clinical presentation of Lyme disease by sex (Schwarzwalder et al., 2010). However, a positive ELISA and median number of IgG bands were significantly higher among males. Sex-based differences in the magnitude of ELISA and IgG serologic response to early Lyme disease occurred (Schwarzwalder et al., 2010). As a result, such differences have consequences for the relevance of diagnosis, treatment, and perhaps disease classification (Schwarzwalder et al., 2010).

Overall, more studies are needed to evaluate a sex-based link to Lyme disease (Schwarzwalder et al., 2010). Sex is important in health, health care, and medical research, due to practitioners knowing predisposition such as females being more likely

to suffer from autoimmune diseases, have osteoporosis, depression, and anxiety, whereas males are more likely to develop Parkinson's disease and cardiovascular disease early in life, according to researchers at Stanford University (Conger, 2017). Also, researchers at Stanford University are finding increasing evidence of the influence of biological sex on health pertaining to disease, indicating researchers are just beginning to understand the magnitude of the problem (Conger, 2017).

Lyme disease has generated a great deal of controversy over a long period of time (Pettengill, 2018). Currently, diagnosis of Lyme disease is a clinical diagnosis or assessment, primarily determined by evaluating an individual's medical history, symptoms, and exposure to ticks (Lymedisease.org, 2018b). Most physicians and practitioners, excluding those who are Lyme-literate, follow the CDC recommended testing strategy, which is a two-step testing algorithm, screening with an ELISA test, and reflecting positive or equivocal results in western-blot tests (Pettengill, 2018). Lyme disease hinders an individual's immune system, so it does not react or respond to the infection. Thus, 20 to 30% of tests create false-negative antibody results (Lymedisease.org, 2018b).

As previously explained, if a diagnostic test for Lyme disease is sufficiently specific, the specificity to a diagnostic test will indicate the level of the specific antigen or antibodies found in the blood sample, thereby providing a level of confidence that the individual has the disease, even if dormant at the time of drawing the blood sample (Lalkhen & McCluskey, 2008). When the specificity is low, false negatives occur (Gallagher & Chakavarti, 2008). When the *Borrelia burgdorferi* bacterium is transmitted

to an individual, it quickly evades recognition and attack from the individual's immune system by first changing proteins on its outer cell wall, thereby effectively disguising itself and hiding in the tissues before eventually forming a slimy substance called a biofilm (Holtorf Medical Group, 2019). The biofilm becomes a very protective layer that renders the bacteria up to 1,000 times more resistant to antibiotics than other bacteria (Sapi et al., 2016).

Sex analysis is a critical element of health systems research (London School of Hygiene & Tropical Medicine, 2014). For this study, it was essential to distinguish between sex and gender. Most societies view biological sex as a binary concept, with two firmly fixed categories (male and female), based on a person's reproductive functions, (e.g., genitals, sex chromosomes, gonads, hormones, reproductive structures; Gender Spectrum, 2017). Sex refers to male and female, as gender refers to masculine and feminine (Nobelius, 2004). In general terms, *sex* refers to the biological differences between males and females, such as the genitalia and genetic differences, whereas *gender* is more difficult to define but can refer to the role of a man or woman in society, or an individual's concept of themselves (i.e., gender identity; Newman, 2016). Male and female genitalia, internal and external, are different; similarly, levels and types of hormones present in male and female bodies are different (Newman, 2016). These vital divergent factors must be considered in treating Lyme disease and associated autoimmune disease (Horowitz, 2013). As an example, females with rheumatoid arthritis experience a significant delay in referrals to an early arthritis clinic in comparison with males (Regitz-Zagrosek, 2012).

Sex analysis can be incorporated into health-systems research at any stage of the research process and includes the consideration of sex when defining the research aim, objectives, or questions in the development of the study design and data-collection tools, the process of data collection, the explication and exchange of results, and in research-uptake activities (London School of Hygiene & Tropical Medicine, 2014). Sex frameworks and tools can help researchers create research methods, inclusive of research questions, data collection, and analysis (London School of Hygiene & Tropical Medicine, 2014).

Several studies addressed human male–female differences in overall mortality, susceptibility to allergic and autoimmune diseases, or individual infectious disease risk; yet surprisingly, a comprehensive test of the major hypotheses outlined above is currently unavailable (Guerra-Silveira & Abad-Franch, 2013), to the best of my knowledge. The use of sex analysis is now affording beneficial new insights into prevention and management of chronic diseases such as Lyme disease in all stages of infection (Canadian Women's Health Network [CWHN], 2012).

Despite growing appreciation of the gravity of sex-driven elements, progress toward sex assimilation as standard practice has been gradual and inconsistent in health research and medical practice (Day, Mason, Logusky, & Rochon, 2016). It is often assumed that males and females have the same symptoms for infectious diseases. Biological differences between males and females should be acknowledged and contemplated in emerging disease programs (World Health Organization [WHO], 2011). Approximately 79% of people worldwide (as of 2012), diagnosed with an autoimmune

disease, are females (CWHN, 2012). These diseases also often occur divergently in males and females, with various ages of onset and an assortment of symptoms (CWHN, 2012).

Purpose of the Study

This study was designed to investigate the epidemiologic consequence of sex-based differences of chronic Lyme disease. Sex differences in the pathogenesis of infectious diseases may reflect variations with immune responses during infection (vom Steeg & Klein, 2016). The lack of expertise about the relationship of sex to infectious diseases has been shown in a variety of disciplines including epidemiology, medical and biological sciences, social sciences, and demography (WHO, 2007). If infectious diseases are considered in the context of biological sex, it could be hypothesized that sex results in physiological differences (e.g., hormonal regulation of immune responses) in the control and clearance of a pathogen, as well as in anatomical differences that may influence exposure and transmission of a pathogen (vom Steeg & Klein, 2016). Important sex differences in the brain seem to arise from biology; an example is gonadal sex steroids or genes found on sex chromosomes that influence sex differences in neuroanatomy, neurochemistry and neuronal structure, and connectivity (Zagni, Simoni, & Colombo, 2016). The sexes differ in the intensity, prevalence, and pathogenesis of infections caused by viruses, bacteria, parasites, and fungi (vom Steeg & Klein, 2016).

This study entailed a comparative social inquiry to determine if a statistically significant difference between the sexes would emerge in chronic Lyme symptomatology and severity, which has not been defined in the outcome analyses of Lyme disease by sex. Sex significantly contributes to shape immune responses, contributing to variation in

the pathogenesis of infectious disease in males and females, and the prevalence of what is called autoimmune diseases (Ruggieri et al, 2016). Males and females differ in their innate immune responses, which is one part of the immune system that responds when activated by the presence of antigens and their chemical properties, suggesting that some sex differences are germ line-encoded (vom Steeg & Klein, 2016). The sexes provide various genetic backgrounds, anatomic niches, immunological profiles, and hormonal situations that can be directly affected by pathogens such as viruses, bacteria, parasites, and fungi, as well as the chronic development of diseases following infection (vom Steeg & Klein, 2016). Hormones, genes, and behaviors contribute greatly to sex differences in the culmination of infection (vom Steeg & Klein, 2016). Lyme disease is considered a polymicrobial infection, thereby inferring that Lyme disease is not a single or stand-alone infection (Rawls, 2018). Key variables investigated for a comparative assessment were biological sex (male or female) and chronic Lyme condition symptomatology.

The evidence is fast becoming clear that chronic disease affects males and females differently, but this is relatively new knowledge. Until now, most research on chronic disease did not consider biological sex (CWHN, 2012). The U.S. National Institute of Mental Health has recommended the incorporation of sex as a variable in experimental and clinical studies to address sexual dimorphisms influencing sex differences when treating chronic disease indications, such as Lyme disease (Zagni et al., 2016). In most cases, the exact mechanism interposing the dimorphism in infectious disease pathogenesis is unknown, partly because sex has not been considered a biological variable for the analysis of outcome data (vom Steeg & Klein, 2016).

Increasing evidence confirms that sexual dimorphism in bacterial infections has been mainly attributed to the differential levels of sex hormones between males and females and sex-bias also depends on the effects of sex hormones on specific bacterial species (Vázquez-Martínez et al., 2018). Additionally, sex differences are important epidemiological factors that affect the severity of infectious diseases, with current research showing males as more susceptible to gastrointestinal and respiratory bacterial diseases and sepsis, and females more susceptible to genitourinary tract bacterial infections, highlighting the role of specific hormone receptors involved in the sex-bias of bacterial infections (Vázquez-Martínez et al., 2018). In this study, I examined if critical symptoms and severity are delineated by sex; these factors were not defined specifically in previous outcome analyses of Lyme disease subjects by sex.

Research Questions

The study focused on investigating the research gap, based on biological sex and Lyme disease, by exploring the following research questions:

RQ1: What is the relationship between biological sex and the frequency of symptoms (unexplained fevers, sweats, chills, or flushing; unexplained weight change [loss or gain]; fatigue, tiredness; unexplained hair loss, swollen glands; sore throat; testicular pain/pelvic pain; unexplained menstrual irregularity; unexplained breast milk production, breast pain; irritable bladder or bladder dysfunction; sexual dysfunction/loss of libido; upset stomach; change in bowel function [constipation or diarrhea]; chest pain or rib soreness; shortness of breath/cough; heart palpitations, pulse skips, heart block; history of heart murmur or valve prolapse; joint pain or swelling; stiffness of the neck or

back; muscle pain or cramps; twitching of the face or other muscles; headaches; neck cracks or neck stiffness; tingling, numbness, burning or stabbing sensations; facial paralysis [Bell's Palsy]; eyes/vision—double, blurry; ears/hearing—buzzing, ringing, ear pain; increased motion sickness, vertigo; lightheadedness, poor balance, difficulty walking; tremors; confusion, difficulty thinking; difficulty with concentration or reading; forgetfulness, poor short term memory; disorientation; getting lost, going to wrong places; difficulty with speech or writing; mood swings, irritability, depression; disturbed sleep—too much, too little, early awake; exaggerated symptoms or worse hangover from alcohol, (Citera et al., 2017) of individuals with confirmed chronic Lyme disease?

$H_0$1: No statistically significant relationship exists between biological sex and the symptoms of individuals with confirmed chronic Lyme disease.

H_a1: A statistically significant relationship exists between biological sex and the symptoms of individuals with confirmed chronic Lyme disease.

RQ2: Are there differences in the number of symptoms (unexplained fevers, sweats, chills, or flushing; unexplained weight change [loss or gain]; fatigue, tiredness; unexplained hair loss, swollen glands; sore throat; testicular pain/pelvic pain; unexplained menstrual irregularity; unexplained breast milk production, breast pain; irritable bladder or bladder dysfunction; sexual dysfunction/loss of libido; upset stomach; change in bowel function [constipation or diarrhea]; chest pain or rib soreness; shortness of breath/cough; heart palpitations, pulse skips, heart block; history of heart murmur or valve prolapse; joint pain or swelling; stiffness of the neck or back; muscle pain or cramps; twitching of the face or other muscles; headaches; neck cracks or neck stiffness;

tingling, numbness, burning or stabbing sensations; facial paralysis [Bell's Palsy]; eyes/vision—double, blurry; ears/hearing—buzzing, ringing, ear pain; increased motion sickness, vertigo; lightheadedness, poor balance, difficulty walking; tremors; confusion, difficulty thinking; difficulty with concentration or reading; forgetfulness, poor short term memory; disorientation; getting lost, going to wrong places; difficulty with speech or writing; mood swings, irritability, depression; disturbed sleep—too much, too little, early awake; exaggerated symptoms or worse hangover from alcohol; Citera et al., 2017) associated with Lyme disease between adult females compared with adult males diagnosed with Lyme disease?

$H_0$2: No statistically significant differences exist in the number of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

H_a2: Statistically significant differences exist in the number of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

RQ3: Are there significant differences in the severity of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease?

$H_0$3: No statistically significant differences exist in the severity of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

H_a3: Statistically significant differences exist in the severity of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

Theoretical Foundation

The WHO gender-analysis-matrix (GAM) for emerging infectious diseases provided a meaningful theoretical framework for this study. I selected the GAM as the theoretical foundation because the model contains relevant constructs on infectious disease and on the exploration of effects of sex on disease transmissions and outcomes (WHO, 2011). The WHO GAM is an analytical tool that uses a participatory methodology to simplify the definition and analysis of sex issues by communities that are affected by them, and specifically in connection to chronic Lyme disease compared to chronic illnesses (Global Development Research Center, 2016). The participatory methodology of the GAM matrix was described using a simple 38 x 2 table format, modified to include the 38 symptoms on the x-axis and males and females on the y-axis. The participatory method includes the males and females of the community (sample) in two groups to estimate the associated symptoms per group. The GAM matrix comprised an x-axis that represents Levels of Analysis (symptoms) and a y-axis for the Categories of Analysis (sex—male/female) confirmed chronic Lyme disease (Global Development Research Center, 2016).

An example of the table and modified matrix appear in Table 1.

Table 1

List of Symptoms Derived From HMQ

List of symptoms	Males	Females
Unexplained fevers, sweats, chills, or flushing		
Unexplained weight change (Loss or Gain)		
Fatigue, tiredness		
Unexplained hair loss		
Swollen glands		
Sore throat		
Testicular pain/Pelvic Pain		
Unexplained menstrual irregularity		
Unexplained breast milk production, breast pain		
Irritable bladder or bladder dysfunction		
Sexual dysfunction / loss of libido		
Upset stomach		
Change in bowel function (Constipation or Diarrhea)		
Chest pain or Rib soreness		
Shortness of Breath / Cough		
Heart palpitations, pulse skips, heart block		
History of Heart Murmur or Valve Prolapse		
Joint pain or Swelling		
Stiffness of the neck or back		
Muscle pain or cramps		
Twitching of the face or other muscles		
Headaches		
Neck cracks or Neck Stiffness		
Tingling, numbness, burning or stabbing sensations		
Facial Paralysis (Bell's Palsy)		
Eyes/Vision – Double, Blurry		
Ears/Hearing – Buzzing, Ringing, Ear Pain		
Increased motion sickness, vertigo		

(table continues)

List of symptoms	Males	Females
Lightheadedness, poor balance, difficulty walking		
Tremors		
Confusion, difficulty thinking		
Difficulty with concentration or reading		
Forgetfulness, poor short-term memory		
Disorientation; getting lost, going to wrong places		
Difficulty with speech or writing		
Mood swings, irritability, depression		
Disturbed sleep—Too Much, Too Little, Early Awake		
Exaggerated symptoms or worse hangover from alcohol		

Source: "Empirical Validation of the Horowitz Multiple Systemic Infectious Disease Syndrome Questionnaire for Suspected Lyme Disease, by M. Citera, P. Freeman, & R. Horowitz, 2017, *International Journal of General Medicine, 2017*(10), 249–273.

Conceptual Framework

The conceptual framework builds on aspects that influence health outcomes using health-related considerations such as sex-specific treatments and therapies, given that incidence of infectious disease is often male biased (Guerra-Silveira & Abad-Franch, 2013). Researchers proposed two main hypotheses to explain this observation: the physiological hypothesis emphasizes differences in sex hormones and genetic architecture, and the behavioral hypothesis stresses sex-related differences in exposure (Guerra-Silveira & Abad-Franch, 2013).

Gender medicine must consider the needs of both sexes. For instance, more data on males are necessary on osteoporosis and depression, while more data on females are urgently called for in the cardiovascular area (Regitz-Zagrosek, 2012) and for infectious diseases. The new conceptualization of evidence-based sex-based medicine, which includes primary variations of biology and behavior between males and females, should

enhance health care for both sexes (Regitz-Zagrosek, 2012). Aggregated data sets can mask differences between the sexes, leading to assumptions that all individuals share the same experiences; this bias can impact the validity and reliability of research in nullifying ways (London School of Hygiene & Tropical Medicine, 2014).

Biological sex influences a wide range of physiological functions and influences a wide assortment of diseases including those of the cardiovascular, pulmonary, and autoimmune systems, as well as diseases encompassing gastroenterology, hepatology, nephrology, endocrinology, hematology, and neurology (Oertelt-Prigione & Regitz-Zagrosek, 2012). The scientific literature reflects sex distinctions with over 10,000 articles addressing sex differences in clinical medicine, epidemiology, pathophysiology, clinical manifestations, outcomes, and management (Regitz-Zagrosek, 2012). A new curiosity in understanding the biology of this disparity, as well as funding opportunities, has directed attention to research priorities on sex differences (Whitacre, 2001).

Nature of the Study

This study employed a cross-sectional comparative-research design with a quantitative method. Secondary data were used in the study analysis. Information obtained from psychometrically validated surveys was used to examine the sex of patients to see if sex links to the severity of Lyme disease and symptoms (Citera et al., 2017). The sex of individuals with Lyme disease was the independent variable and the number of symptoms and severity of symptoms were the dependent variables in this study. Information on participants' sex, the primary independent variable of the study, was assessed while accounting any covariates and confounders that may have influenced

the severity of the outcome under investigation. The data collection and participant selection were from a sample of adults in the target population in the United States. Patients were positively diagnosed with chronic Lyme disease.

The quantitative-method design used in this study was best described as a cross-sectional comparative research design. A cross-sectional comparative quantitative-survey research design was used to compare females to males on several parameters. Participants' survey information was obtained from the HMQ for Lyme disease. Horowitz provided a data set for this study, and those data were used for the analyses.

Definitions

The terms and concepts listed below were defined in the framework of an epidemiologic study.

Autoimmune disease: The term autoimmune disease refers to a varied group of illnesses that involve almost every human organ system such as diseases of the nervous, gastrointestinal, and endocrine systems, as well as skin and other connective tissues, eyes, blood and blood vessels (American Academy of Allergy, Asthma, & Immunology, 2018).

Autoimmunity: Autoimmunity is the underlying problem in autoimmune diseases because the body's immune system becomes misdirected and attacks the organs it was designed to protect (American Autoimmune Related Diseases Association, 2017).

Borrelia burgdorferi: *Borrelia burgdorferi* (*B. burgdorferi*) is a spirochete tick-borne obligate parasite whose normal reservoir is a variety of small mammals in the United States (Burgdorfer et al., 1982). Whereas infection of these natural hosts does not

lead to disease, infection of humans can result in Lyme disease, as a consequence of the human immunopathological response to *B. burgdorferi* (Wooten & Weis, 2001).

Gender: Gender refers to the role of a man or woman in society (gender role), or an individual's concept of themselves (gender identity; Newman, 2016).

Lyme disease: Lyme disease is a multisystem disorder that may involve dermatological, musculoskeletal, nervous system, or cardiac manifestations (Artsob, 1993).

Sex: Sex refers to male and female, as gender refers to masculine and feminine (Nobelius, 2004).

Assumptions

This research was based on assumptions. With regard to data collection and analysis in this study, the first assumption was that all participants consented to complete the questionnaire as part of the validation study from which the secondary data set was derived. Horowitz granted access to a data set from the validation study published in December 2017.

The second assumption was that the sample population in the data set of 236 participants was sufficient for the analysis of this research. The sample size was determined to be acceptable to establish at least an 80% statistical power. As the data set was known to include 236 subjects, the power was determined based on the data set provided by Horowitz.

Scope and Delimitations

The delimitations defining the boundaries of this research were based on the data set authorized for use by Horowitz from the 2017 validation study for the HMQ. The data set provided did not provide personal demographics of participants such as residence, date of first infection, date of diagnosis, or any treatment received for Lyme disease. For this research, I used the data set to compare the specific sample of participant data between males and females and the symptoms and severity identified through the anonymous completion of the questionnaire.

Limitations

The limitations for the design and methodology for this research were influenced by the data set provided by Horowitz's (2017) validation study. The first limitation for this cross-sectional study design related to sample bias. The data set provided for this research consisted of a subsample of a larger sample of individuals with confirmed Lyme disease, recruited from three medical practices involved in the data-collection study. The data accrued through recruitment in social media and the questionnaire survey was provided by e-mail invitation, directing potential participants to click on a link explaining the purpose of the survey and informed-consent information (Citera et al., 2017). The cross-sectional study design reflected bias, identifying prevalent cases rather than incident cases.

The second limitation to the study was recall bias. Cross-sectional studies may also exhibit recall bias, because disease or assessment of disease may influence participants' responses to questionnaires (Thelle & Laake, 2015). The study may be

limited on practical importance because the purpose of this study was not tailored to the practical aspects of Lyme-disease intervention. That is, no treatments or interventions were included in the data collection. All survey responses were anonymous with no identifying information requested from participants; the time, location, or known transmission of the infection were not included in the data set used for this research (Citera et al., 2017). For a quantitative method, its application was not used to or effectively measure subjective experiences or emotional states of individuals affected by an outcome or those who are exposed to a risk factor (DiClemente, Salazar, & Crosby, 2013; Frankfort-Nachmias & Nachmias, 2008).

Significance

The relevance of this current study as it relates to the intent to address the identified gap was that the impact of sex-based differences on chronic Lyme-disease infections abridge the substantial lapses in misdiagnosis and treatment of the disease. Despite nearly 4 decades of scientific inquiry into transmission dynamics, immunopathology, and treatment outcomes of Lyme disease, much remains unknown, with a general lack of research examining potential sex-based differences in this infectious-disease setting (Klein & Roberts, 2015). The abridged gap would promote effective health measures on practical intervention approaches for chronic Lyme disease.

Hence, understanding differences between sex influences on the issues of the pathophysiology of Lyme neuroborreliosis, PTLDS or chronic Lyme disease, may allow for better knowledge of underlying differences in the immune response between males and females following infection, which could affect pathogen clearance, development of

autoimmune-like responses, and seroconversion on two-tier antibody tests (Rebman et al., 2015). The sex differences in autoimmune diseases underscore the necessity for sex analysis (CWHN, 2012). These diseases also often occur differently in males and females, with different ages of onset and different kinds of symptoms (CWHN, 2012).

The improvement of medical, public health, epidemiological, and social practices on sex-driven approaches to addressing chronic Lyme disease may substantially reduce the incidence and prevalence of Lyme disease in a target population. Such unique contributions would advance research, health-promotion measures, advocacy awareness, and informed decision-making processes in individual, organizational, and social settings. Current research has the potential to inform future research of new initiatives for a therapeutic approach that can alter disease pathogenesis, rather than targeting disease sequelae (Ackerman, 2006). Therefore, the positive social-change implications derived from the findings of this study could substantially improve understanding of epidemiologic sex-based factors and considerations for diagnosis, testing, and choice of treatment options on personal, sex-specific, and social well-being.

If any correlation exists based on sex-based link to the number of symptoms and the severity of symptoms, then positive social-change implications would not be limited only to advancement of current testing approaches, but potentially could encourage better Lyme-disease education and understanding, awareness, and choice of health care treatment options. In practice, any connection between sex to the number and severity of symptoms could allow practitioners or epidemiologists the opportunity to effectively measure the incidence, prevalence, risk of Lyme disease, and subsequent parameters

based on sex-specific criteria or assays. An example of subsequent parameters is the clinical-practice guidelines that insurance companies follow for coverage of the treatment for Lyme disease. These parameters must be met and followed by general practitioners for insurance coverage to be used for treatment of this disease. That is, the type of antibiotic treatment and therapies and duration of these treatments are parameters outlined by insurance companies, respectively. The theoretical application of the GAM in this study supports population-based benefits and perceptions of sex-driven differences, not as a weakness of individual integrity, but as a strength in medical practices, public health programs, epidemiological studies, and social consciousness of the population.

Summary

In summary, hormones, genes, and behaviors contribute significantly to biological sex differences in the outcome of infection (Klein & Roberts, 2015). Lyme disease affects males and females differently, as does the different age of onset, and different kinds of symptoms (CWHN, 2012). Researchers at the Lyme Disease Research Foundation in Lutherville, MD, evidenced preliminary findings indicating females with Lyme disease display more clinical symptoms than do males with Lyme disease and are also less likely to seroconvert on antibody tests for serodiagnosis of Lyme disease following treatment (Worcester, 2012).

Chapter 2: Literature Review

Introduction

The incidence of Lyme disease increased from over 3.5 cases per 100,000 people in 1991 to 8.1 cases per 100,000 in 2016 in the United States (CDC, 2017). Lyme disease affects individuals differently, and it can take almost 2 years to have an accurate diagnosis of Lyme disease, due to the variability of the symptoms or lack thereof (Auwaerter, 2015; Lymedisease.org, 2015). Individuals diagnosed with Lyme disease, chronic Lyme disease, or post-Lyme disease syndrome are more likely to be females than males (Wormser & Shapiro, 2009). Yet, uncertainties persist concerning the influence of sex on symptoms, severity of symptoms, and health-related quality of life between males and females diagnosed with Lyme disease. These uncertainties suggest the need for further epidemiological studies to explore this gap in the literature. Rebman et al. (2015) emphasized that sex-based uncertainties still exist in the exploration of Lyme disease severity and symptomatology. These authors noted that sex-based differences of the host's immune system for Lyme disease response should be explored at the cellular level as well as after early and late manifestations of Lyme disease (Rebman et al., 2015). These researchers concluded that sex-based differences in the epidemiology, clinical presentation, and immunologic response of Lyme disease infections remain unspecified at this time (Rebman et al., 2015), thereby leaving a gap in the literature on this topic.

This chapter provides a review of literature describing the justification for further Lyme disease research inquiry, based on prior publications on this topic. I describe and summarize the conclusions of several published studies relating to the topic under

investigation and the relevance of these studies to the problem statement and purpose of this study.

Literature Search Strategy

The literature reviews for this research topic were accessed through various databases including ProQuest, Google, Google Scholar, PubMed, Medscape, Journal Watch, and Clinicaltrials.gov. The literature review for this study included peer-reviewed journals and non-peer-reviewed articles. Nonfiction books and publications written by Lyme-literate medical practitioners, patients, and private Lyme organizations were also reviewed. Considering that sex and gender are often mistakenly used as interchangeable terms, distinct searches were performed using the terms sex and gender with Lyme disease, and infectious diseases. The search terms used for online publications included *Lyme disease, Lyme disease diagnosis and treatment, Lyme disease and family history, Lyme disease and sex/gender disparities, susceptibility to Lyme disease, Lyme Encephalopathy, Lyme disease and health-related quality of life, Lyme disease and sex/gender differences, Lyme disease and IGeneX testing, Western Blot testing for Lyme disease, state reporting guidelines for Lyme disease cases, sex and gender differences in health, sex gap and autoimmune diseases, sex and gender differences with infectious diseases,* and *sex and autoimmune diseases.*

As a paradigm of this search pattern, I performed the literature search using PubMed for Lyme-disease conditions with the key term *Lyme disease.* Keywords generated a total of 42 articles published from 1984 to 2017. Searches performed with the keywords *sex/gender differences of Lyme disease* generated only one article in the

American Journal of Epidemiology database. Similarly, I searched using the term *sex differences of Lyme disease*, and from this search three articles emerged. In contrast, a literature search in the *American Journal of Epidemiology* using the term *gender differences of Lyme disease* generated two articles. Comparable literature searches were performed using the databases specified above.

Theoretical Foundation

The WHO's GAM (2011) for emerging infectious diseases was used as the theoretical framework for this study. I selected the GAM as the framing foundation because the model contains relevant constructs on infectious disease and on the exploration of the effects of sex and gender on disease transmissions and outcomes (WHO, 2011). I considered the WHO GAM as the foundation for sex and gender, engaged as a tool used to analyze the impact of male–female differences on emerging infectious diseases (WHO, 2011).

Another interesting use of the GAM was the WHO Gen*der Mainstreaming Manual for Health Managers* which also addresses issues with malaria, such as in the United Republic of Tanzania where some evidence indicates that malaria treatment practices differ by the sex of the clinician (WHO, 2011). Aside from pregnancy, possibly complicating the course of malaria with risks for females and children, regardless of the child's sex, the use of the GAM framework uncovered the possibility that when one or several family members contracted malaria, females become overburdened by the responsibility of caring for sick people (WHO, 2011).

Sex analysis is an important component of health-systems research (London School of Hygiene & Tropical Medicine, 2014). For this study, it was important to distinguish between sex and gender. Most societies view sex as a binary concept, with two rigidly fixed categories (male and female) based on an individual's reproductive functions, (i.e., genitals, gender chromosomes, gonads, hormones, reproductive structures; Gender Spectrum, 2017; Newman, 2016). Sex refers to male or female, but gender refers to masculine or feminine (Nobelius, 2004). In general terms, sex refers to the biological differences between males and females, but gender is more difficult to define. Gender can refer to the role of a man or woman in society (i.e., gender role), or it can refer to individuals' concepts of themselves (i.e., a gender identity; Newman, 2016).

Male and female genitalia, internal and external, are different; similarly, the levels and types of hormones present in male and female bodies are also different (Newman, 2016). These factors must be considered when treating for Lyme disease and associated autoimmune diseases (Horowitz, 2013). As an illustration, the referral of female individuals with rheumatoid arthritis to an early arthritis clinic was considerably delayed in comparison with male individuals (Regitz-Zagrosek, 2012).

Sex analysis can be incorporated into health-systems research at any stage of the research process. Consideration of biological sex differences must be made when defining the research aim, objectives, or questions during the development of the study design and data-collection tools, the process of data collection, the interpretation and communication of results, and the research-uptake activities (London School of Hygiene & Tropical Medicine, 2014). Sex frameworks and tools can help researchers develop

their research methods, including research questions, data collection, and analysis (London School of Hygiene & Tropical Medicine, 2014). Several studies addressed human male–female differences in overall mortality, susceptibility to allergic and autoimmune diseases, or individual infectious-disease risk. Yet, a critical and comprehensive test of the major hypotheses outlined above is currently unavailable (Guerra-Silveira & Abad-Franch, 2013).

Conceptual Framework

Conceptual frameworks are particularly common when research involves testing; however, in this research (Swaen, 2018), I used the framework to review my hypotheses in the differences between male and female and symptoms and the severity of symptoms with chronic Lyme disease. According to findings from a prospective cohort study of 77 patients in a 2012 study, females with Lyme disease displayed more clinical symptoms than males and were less likely to seroconvert following treatment (Worcester, 2012). These findings suggest to us that there may be a difference between how males and females respond to infection with Lyme disease (Crowder, 2012). Also noted by Crowder, findings emphasized the need for additional research on sex-based differences in the effects of Lyme disease, which have not been thoroughly explored in Lyme disease or in other infectious diseases. Figure 1 outlines the framework for this research below.

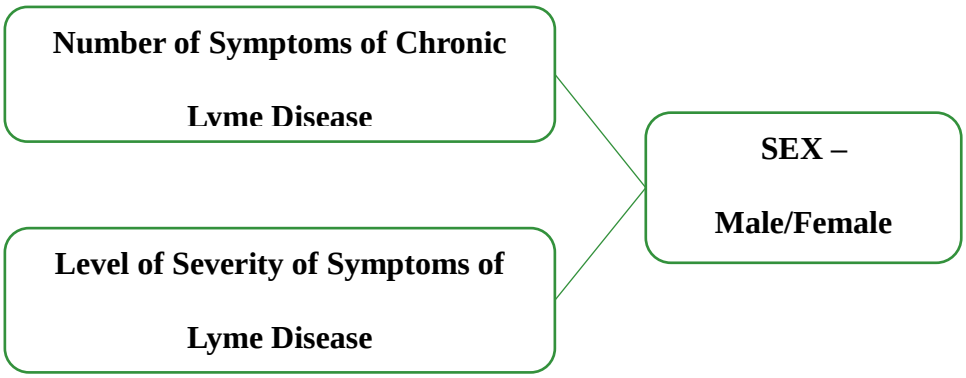


Figure 1. Example of GAM model.

Overview of Literature Review

Lyme disease is a vector-borne infectious disease. The name Lyme disease is more specific to findings based on a group of children infected in Lyme, CT, in the late 1970s (Dimeo-Ediger, 2017). I briefly review the history of Lyme disease to aid in understanding an expanding epidemic in the world today. I also discuss published literature on how chronic Lyme disease has been shown to be present disproportionately in females more than males. Similarly, I explore literature covering sex bias in medicine in this section of the chapter, as sex biases arise when diagnosis rests on sameness in symptoms, severity, and treatment between males and females.

I explain the confusion between sex and gender when infectious disease affects each stage of the human life cycle, as in age groups. I discuss the literature that explored issues relating to family history of Lyme disease and health-related quality of life in chronically ill individuals (male and female) diagnostically positive for Lyme disease. Finally, the literature-based evaluation of chronic illnesses, as the result of infectious

diseases, I describe its effect on social and economic burdens, along with differences between sexes over their lifecycle.

History of the Lyme Disease Epidemic

Lyme disease is the leading vector-borne infectious disease in the world (Langhoff, 2011). The disease is transmitted to the host by a group of corkscrew-shaped, gram-negative spirochetes called *Borrelia burgdorferi* "sensu lato" (or "s.l.", meaning broad sense and abbreviated as *Bb.*; Langhoff, 2011). *Borrelia burgdorferi* is a genospecies that contains four groups consisting of several strains (Langhoff, 2011). Lyme disease has been a known disease for several decades, but in the past 8 years, Lyme has emerged as an issue of cultural and medical relevance widely discussed by practitioners and nonpractitioners (Dimeo-Ediger, 2017). Lyme disease is now the fastest growing vector-borne disease in the world, described by many researchers as a distant cousin of the syphilis spirochete (Langhoff, 2012). The disease may have spread from Europe to the United States in the early 1900s, but health experts only recently recognized Lyme disease as a distinct illness (News-Medical.Net, 2009). Reported cases seem to have surfaced beginning in Europe as early as the late 1800s, followed by U.S. cases from the late 1940s to the early 1950s, with a cluster of arthritis cases emerging in Connecticut during the late 1960s (Langhoff, 2011). In the late 1800s in Europe, Lyme disease was referenced as a rash of the hands (Horowitz, 2017). Some medical practitioners described Lyme disease as a skin lesion (Liotta, 2014). Furthermore, reports from Europe and the United States indicated the same lesions as part of a condition called Bannwarth syndrome, including radiculitis, Bell's Palsy, and meningitis (Halperin, 2015).

In 1909, Afzelius described the disease as an expanding ring-like rash, and 10 years later linked the presence of a rash to joint problems caused by a tick's bite (Horowitz, 2017). In 1922, the disease was found to align with neurological problems, and in 1930, with psychiatric disturbances with arthritis symptoms (Horowitz, 2017). By 1965, Robbin described how expanding circular rashes seemed to respond to penicillin treatment (Horowitz, 2017). Five years later, Wisconsin dermatologist Scrimenti published the first report of an erythema chronicum migrans rash in the United States (Horowitz, 2017). Yet, by 1977 in rural Connecticut, all these details and symptoms were not connected with individuals who were ill. Steere, a Yale rheumatologist, reported symptoms including fever, headache, and migratory joint pains, as well as multiple cardiovascular and neurological abnormalities (Horowitz, 2017). In the 30 years since Lyme disease was identified, in addition to the bacterial infection that would be known as Lyme disease, other species of *Borrelia* were identified and along with *B. burgdorferi sensu stricto*, were collectively classified as belonging to the *Borrelia burgdorferi sensu lato* complex (Smith, 2017).

Lyme disease is a complicated illness linked to larger groups of the species of *Borrelia* bacteria (Langhoff, 2012). Yet, many species of *Borrelia* exist throughout the world (Langhoff, 2012). The etiologic agent for Lyme disease in the northeast United States is a bacterium that belongs to a group of spirochetes, identified by Burgdorfer from the National Institute of Health, while studying ticks on eastern Long Island (Horowitz, 2017), leading to the species of *Borrelia* subsequently being named *Borrelia burgdorferi* (Horowitz, 2017). Worldwide, more than 20 *Borrelia* species have been identified, all

associated with Lyme or Lyme-like disease in humans (K. Smith, 2017). *Borrelia burgdorferi sensu stricto* is the predominant group of Lyme-disease strains detected in individuals from the United States and Canada, with *Borrelia garinii* and *Borrelia afzelii*, in addition to *Borrelia burgdorferi* sensu stricto strains, detectable in individuals from other countries (Langhoff, 2012).

Documented Lyme Disease Symptoms

Lyme disease is transmitted by the bite or blood-sucking action of an insect, and ticks are commonly mentioned for Lyme-disease transmission (Langhoff, 2012). Langhoff (2012) indicated that many other insects can transmit the *Bb* spirochete. Lyme disease has three recognized stages: the first, the Early Lyme disease stage (Nordqvist, 2016) can last for several days, and sometimes is present with an erythema migrans (or bull's eye) rash (Nordqvist, 2016). The second stage, Early Disseminated Lyme, may last for several weeks to months after initial infection. Symptoms in this stage include facial paralysis/Bell's palsy, meningitis, painful headaches, a stiff neck, swelling of large joints such as the knees, numbness, shooting pains in the arms and legs, or palpitations or abnormal heartbeat (Nordqvist, 2016). The underlying denominator for most chronic illnesses, such as Lyme disease, is inflammatory symptoms, and these symptoms all have an element of inflammation (Horowitz, 2017). The third stage, Late Disseminated Lyme, can last for weeks, months, and even years after initial infection. A symptomatic episode during this stage may include arthritis, difficulty concentrating, unrestful sleep, memory loss or cognitive impairment, and tingling or numbness in the hands or feet (Nordqvist, 2016).

Some individuals may have an initial manifestation of the Lyme disease infection at the chronic or late stage, possibly due to inaccurate testing or misdiagnosis (Nordqvist, 2016). Horowitz (2017) developed a more comprehensive list of all possible symptoms of Lyme disease, especially in individuals who failed classical treatments. This list built on treatment of Lyme disease, associated coinfections, and overlapping causes that can lead to MSIDS. Lyme disease symptoms include unexplained fevers, sweats, chills or flushing, unexplained weight change (loss or gain), fatigue or tiredness, unexplained hair loss, swollen glands, sore throat, testicular pain in males, and pelvic pain in females (Horowitz, 2017). Irritable bladder or bladder dysfunction, general dysfunction or loss of libido, upset stomach, change in bowel function (constipation or diarrhea), chest pain or rib soreness, shortness of breath, cough, heart palpitations, pulse skips, heart block, any history of heart murmur or valve prolapse, joint pain or swelling, stiffness of the joints, neck, or back, and muscle pain or cramps are also listed as common symptoms of Lyme disease (Horowitz, 2017). Twitching of the face or other muscles, headaches, neck cracks, neck stiffness, tingling numbness, burning or stabbing sensations, facial paralysis (Bell's palsy), eyes/vision issues (double, blurry, and floaters), ears/hearing problems (buzzing, ringing, and ear pain), increased motion sickness, vertigo, lightheadedness, wooziness, poor balance, difficult walking, tremors, confusion, difficulty thinking, difficulty with concentration or reading, forgetfulness, poor short-term memory, disorientation (getting lost and going to wrong places), difficulty with speech or writing, mood swings, irritability, depression, disturbed sleep (too much or too little or early awakening), and exaggerated symptoms or worse than normal symptoms that appear

similar to a hangover from alcohol are also part of the common symptoms observed among individuals with Lyme disease (Horowitz, 2017). A number of these symptoms occur in conditions other than Lyme disease, so they should not be considered hallmark symptoms of Lyme disease (Citera et al., 2017). Many conditions can yield symptoms that might be mistaken for Lyme disease (Citera et al., 2017).

Borrelia bacteria attack specific areas or organs of the body (Horowitz, 2017). The eye, brain tissue and glial cells, the heart, collagen, skeletal muscle fibers, and the synovial membrane that surrounds the joints are known organs or body tissues that are vulnerable to *Borrelia* attack upon infection (Horowitz, 2017). Early-stage Lyme disease is typically characterized by *erythema migrans*, a bull's eye rash that appears 3 to 14 days after a tick bit at the site when an individual has been bitten (CDC, 2015a; Grisanti, 2015; E. Shapiro, 2014). In a study of more than 6,000 individuals diagnosed with Lyme disease, researchers found that only 17% of participants recalled having *erythema migrans* (Lymedisease.org, 2015). Individuals will not often know they have been bitten by an infected tick, and most individuals do not understand that Lyme disease can also be transmitted by contact with body fluids such as urine, tears, semen, contaminated blood and breast milk, or even mites, spiders, mosquitoes, fleas, and biting flies (Doyle, 2011). Most people believe they might have Lyme disease when they present with symptoms. Other symptoms of early stage Lyme disease include fatigue, chills, fever, headache, joint, paint, and swollen lymph nodes (Auwaerter, 2015; CDC, 2015a). The overwhelming realization of Lyme-disease symptoms makes the diagnosis of the disease very difficult, complex, and problematic (Auwaerter, 2015; Lymedisease.org, 2015).

Lyme disease is often misdiagnosed (Grisanti, 2015; Lymedisease.org, 2015). Results from the Lymedisease.org (2015) study showed that Lyme disease was initially misdiagnosed as a mood disorder in 59% of participants. Misdiagnosis as chronic fatigue syndrome was in 55% of cases and 49% of cases as fibromyalgia (Lymedisease.org, 2015). Lyme disease is commonly misdiagnosed as psoriatic arthritis, rheumatoid arthritis, motor neuron disease, multiple sclerosis, Hashimoto's thyroiditis, amyotrophic lateral sclerosis, systemic lupus erythematosus, discoid (cutaneous) lupus, drug-induced lupus, and neonatal lupus (Auwaerter, 2015; Lymedisease.org, 2015).

Diagnostics Testing for Lyme Disease

In October 1994, the Dearborn Conference, the *Second National Conference on Serologic Diagnosis of Lyme Disease*, was sponsored by the CDC, the Association of State and Territorial Public Health Laboratory Directors, the Michigan Department of Health, US Food and Drug Administration, the National Institute of Health, the Council of State and Territorial Epidemiologists, and the National Committee for Clinical Laboratory Standards (Langhoff, 2012). The purpose of the conference was to establish surveillance-case definitions and criteria for reporting emerging Lyme-disease cases to the CDC, as well as to standardize laboratory diagnostic tests to detect Lyme infections. Accuracy of diagnostic testing depends heavily on the sensitivity and specificity of the individual test, which varies substantially by manufacturers' approach (Langhoff, 2012).

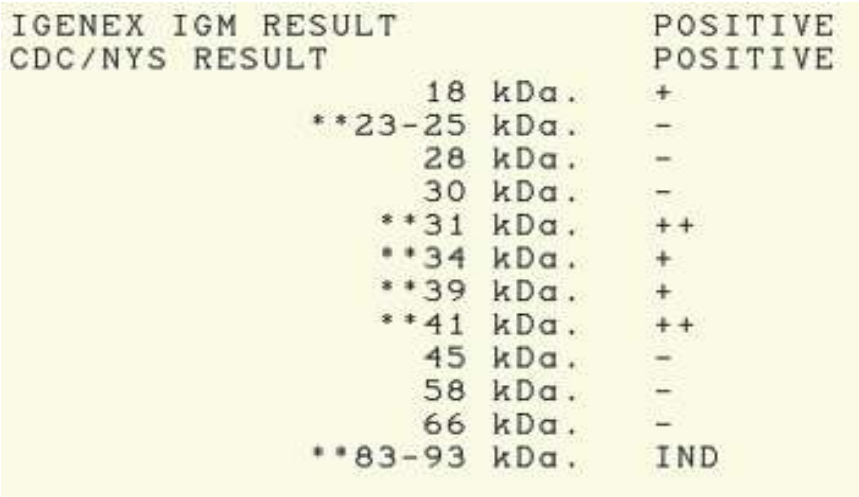
Strains from other areas of the United States and the rest of world, including those which cause neurologic, skin, cardiac, or other manifestations of Lyme disease, were excluded from the Dearborn's standardization criteria (Langhoff, 2011). The CDC

recommended a two-tier system of diagnostic testing that included the ELISA diagnostic test followed by the western-blot test for Lyme disease. Yet individuals are unlikely to have their Lyme-disease infection detected by these tests if their infection strain was excluded from the test probes (Horowitz, 2017). Thus, the recommended two-tier system is still problematic and may not produce positive test results, especially given that some strains are excluded from the test-probe standardization (Horowitz, 2017).

As symptoms present differently for each individual resulting from several different factors, a standardized diagnostic test has not been conclusively developed (Auwaerter, 2015; CDC, 2015a). If there is no case definition or definitive laboratory test to identify individuals with a diagnosis of chronic Lyme disease, systematic clinical evaluation is difficult. Diagnosis often rests entirely on the clinical judgment of a Lyme-literate physician (Wormser & Shapiro, 2009). That said, the western-blot banding pattern for antibody proteins in the serologic test has been found to be of some utility. This test is measured in kilodaltons (kDa), (Langhoff, 2012). The bands are separated and recorded by molecular weight and expressed in kDa. The banding pattern of 31kDa represents the organism's outer surface protein A or 34kDA of the outer surface protein B.

Another test that has become the standard to diagnose Lyme disease is the IGeneX western-blotting method (Mayo Clinic Staff, 2018). Figure 2 illustrates an example of positive results of an IGeneX western-blot test for Lyme disease. Using the CDC/New York State criteria shown in Figure 2, the IgM western blot is reported as positive if two of the following bands are present: 23–25, 39, and 41 kDa (Kaplan, 2004).

In contrast, according to the IGeneX criteria, the IgM western blot test is reported as positive if two of the following bands are present; 23–25, 31, 34, 41, and 45 kDa. IgG is a sign of a past exposure to or past infection by the organism (Kaplan, 2004).



IGENEX IGM RESULT	POSITIVE
CDC/NYS RESULT	POSITIVE
18 kDa.	+
**23-25 kDa.	-
28 kDa.	-
30 kDa.	-
**31 kDa.	++
**34 kDa.	+
**39 kDa.	+
**41 kDa.	++
45 kDa.	-
58 kDa.	-
66 kDa.	-
**83-93 kDa.	IND

Figure 2. IGeneX IgM result band markings.
Note. From *What You Should Know About Lyme Disease*, by IGeneX, 2017, http://igenex.com/lyme_disease.htm

When reporting bands, the reporting laboratory marks each band with the following indicators of intensity, shown in Figure 3.

-	Not present
+	Low
++	Medium
+++	High
+/-	Equivocal = indeterminate (there, but not as intense as Low)

Figure 3. Sample IGeneX results.
Note. From *Interpreting the IgG & IgM Western Blot for Lyme Disease*, by M. Kaplan, 2004, http://www.anapsid.org/lyme/wb.html

Sex Differences and Infection

Despite the potential importance of differences in biological sex for the transmission, course, and outcome of some infectious diseases, no clear understanding exists of the implications of the effects of sex on the surveillance of and response to outbreaks, especially for diseases that are not generally transmitted (WHO, 2007). Sex (biological) and gender (sociocultural) factors are important predictors or determinants of health outcomes (Day et al., 2016). Infectious diseases rarely affect males and female equally, despite demographic sex ratios (Guerra-Silveira & Abad-Franch, 2013). Thus, it is critical to integrate sex and gender considerations throughout the research process to produce the best possible health outreach, facilitate optimal health-promotion measures, and improve the target population's quality of life (Day et al., 2016).

Most autoimmune diseases are more frequent in females than in males (Regitz-Zagrosek, 2012). For instance, systemic lupus erythematosus is more frequent in females of reproductive age, as serum estrogen concentration affects its severity (Regitz-Zagrosek, 2012). Sjögren syndrome is also more frequent in females, again, involving sex hormones in the pathophysiology. Fibromyalgia, a poorly understood disease, is more frequent in females than in males, as is rheumatoid arthritis. Most interestingly, females experience a significant diagnostic delay for rheumatic diseases in comparison to males (Reitz-Zagrosek, 2012).

Wormser and Shapiro (2009) investigated the implications of sex on chronic Lyme disease. The researchers compiled data on sex in this cross-sectional study, based on a systematic review of published studies of antibiotic treatment in U.S. individuals

with post-Lyme-disease syndrome (*n* = 184) or chronic Lyme disease (*n* = 490), and on cases of adults with Lyme disease reported to the CDC from 2003 to 2005 (*n* = 43,282). Study results showed that individuals with chronic Lyme disease were significantly more likely to be females than were individuals diagnosed with either Lyme disease or with post-Lyme disease syndrome (Wormers & Shapiro, 2009). Medical personnel must therefore consider the needs of both sexes, as sex influences health outcomes.

Sex can also modify behavior as a function of certain hormones, such as testosterone, which causes aggressive behavior associated with risk-seeking and neglecting personal health or behaviors (Denson, O'Dean, Blake, & Beames, 2018). Sex differences may lead to genomic and epigenetic modifications (Reitz-Zagrosek, 2012), and these modifications and their physiological effects are different in males and females. Also, sex hormones modify DNA repair and epigenetic mechanisms (Reitz-Zagrosek, 2012). The role of sex must be incorporated in research and health care practices, due to the biological significance of sex with infectious diseases (Reitz-Zagrosek, 2012).

The murine model on the *Borrelia hermsii* infection pattern shows that males have a significantly higher initial peak level of *spirochetemia* than females (Strle et al., 2013). Another example of a single-center population-based study of children with Lyme neuroborreliosis showed that facial nerve palsy was a more common symptom in girls than boys, whereas headaches or neck stiffness was more common in boys than girls (Tveitnes & Oymar, 2015). The proportion of children with headache and neck stiffness did not differ significantly between sexes, but headache or neck stiffness as the only

symptom of *Borrelia hermsii* infection was statistically significant in boys but not in girls (Tveitnes & Oymar, 2015).

Autoimmune diseases are more prevalent in females than males (Whitacre, 2001). Sex-based differences, if present in the host immune response upon infection of Lyme disease, may be revealed with in-depth analysis of the innate and adaptive cellular elements mobilized during the early and late manifestations of Lyme disease (Rebman et al., 2015). Many autoimmune diseases show a female bias (Rebman et al., 2015). Indeed, basic immune responses differ between females and males and it is notable that females have higher absolute numbers of CD4+ lymphocytes relative to males, which likely contributes to the increased responses observed (Whitacre, 2001).

With Lyme disease, reinfection in females is common (McClelland & Smith, 2011). A study of postmenopausal females who experienced recurrent *Borrelia burgdorferi* infection revealed increased numbers of cells spontaneously secreting interferon-c, interleukin-4, and interleukin-10 (Jarefors et al., 2006). These ratios and responses are opposite to the T-helper-1 cell dominant response necessary for successful clearance of the *B. burgdorferi* infection (Jarefors et al., 2006). Sex steroids also have indirect effects that must be considered in Lyme infection (Jarefors et al., 2006). Sex hormones modulate the hypothalamic-pituitary adrenal axis, which in turn modulates the stress response, as oophorectomy results in decreased corticosterone concentrations, when orchiectomy enhances the corticosterone response (Whitacre, 2001). Female species in the animal kingdom, including humans, have higher corticosterone-cortisol concentrations than males (Whitacre, 2001). In addition, glucocorticoids suppress the

production of sex hormones and the corresponding actions of these hormones in tissues (Bereshchenko, Bruscoli, & Riccardi, 2018). The sharp spike of corticotropin-releasing hormone and cortisol at parturition, or childbirth, undoubtedly contributes to the decline of estrogen postpartum. The discovery of an estrogen-response factor in the promoter region of the gene-encoding corticotropin-releasing hormone suggests that these two hormone systems are interregulated. Therefore, interactions between the sex hormones, hypothalamic-pituitary adrenal axis, and immune-system responses are complex and intricate, and must be considered when Lyme disease is suspected. Based on this biological network and interactions, all known intrinsic factors associated with this interactive relationship must be considered when evaluating the effects of sex differences in autoimmunity (Whitacre, 2001). Lyme disease is still a complex infection that is slowly becoming more identifiable than before, and so much about the responses from infection, inflammation, and immune dysfunction is still unknown (Rebman et al., 2015).

Although Lyme disease is an autoimmune condition that causes an autoimmune response in the body, it can also mimic, and therefore "cause" other autoimmune diseases (Schneider, 2015). Many autoimmune diseases differ in their clinical presentation between males and females (Whitacre, Reingold, & Looney, 1999). The predominance of autoimmune diseases among females suggests that biological sex hormones may modulate susceptibility (Whitacre et al., 1999). Although sex differences in autoimmune disease are well recognized, sex dimorphism in the immune response and the importance of sex hormones in promoting differences between males and females needs further study (Whitacre et al., 1999).

Rebman et al. (2015) also explored the effects of sex on the pathology, diagnosis, and treatment of Lyme disease. The researchers presented a detailed outline of the prevalence of early Lyme disease for sexes with late Lyme disease, discerning that objective neurologic or rheumotologic conditions were reported more often in males than in females (Rebman et al., 2015). In contrast, the syndromes of more tenuous and complex origins, known as chronic Lyme disease or posttreatment Lyme disease syndrome, appear more commonly in females than in males (Rebman et al., 2015).

Females with chronic manifestations of Lyme disease may suffer instead from a severe immune response brought on by the illness because they are often told they suffer from a variety of other illnesses including depression, rheumatoid arthritis, fibromyalgia, and chronic fatigue syndrome, or unexplained medical symptoms (Cameron, 2016). Researchers from Johns Hopkins University School of Medicine suggested that ongoing symptoms may be blamed, in part, on an immune response (Aucott et al., 2016). They also speculated that high CCL19 chemokine (a signaling protein secreted by cells to simulate the attraction of white blood cells to the place of infection) elevations have been reported in immune illnesses, and possibly a reflection of ongoing, immune-driven reactions suggesting that persistent bacteria and/or spirochetal antigens after antibiotic therapy may advance the disease (Aucott et al., 2016).

Severity of Lyme Disease Symptoms

Researchers compared the severity of chronic Lyme disease with other chronic conditions qualitatively, using an online survey of 3,090 subjects suffering from chronic Lyme disease and clinically diagnosed with symptoms persisting 6 months following

antibiotic treatment (Johnson, Wilcox, Mankoff, & Stricker, 2014). Individuals with confirmed chronic Lyme disease showed a significantly impaired health-related quality of life and used healthcare services more compared to the general population and individuals with other chronic illness (Johnson et al., 2014). Researchers scored severity using the CDC Health-Related Quality of Life 9-item metric, which includes a 4-item Healthy Days Core Module and a 5-item Healthy Days Symptoms Module, shown in Figure 4 (Johnson et al., 2014).

Variable
4-item Healthy Days Core Module
General health rating (Excellent = 1, Poor = 5)
Physical health not good (# days out of 30)
Mental health not good (# days out of 30)
Physical or Mental health limited usual activities (# days out of 30)
5-item Healthy Days Symptoms Module
Pain limited activities (# days out of 30)
Sad, blue or depressed (# days out of 30)
Worried, tense or anxious (# days out of 30)
Not enough rest (# days out of 30)
Very healthy/full of energy (# days out of 30)

Figure 4. Healthy days symptom module.
Note. From Severity of Chronic Lyme Disease Compared to Other Chronic Conditions: A Quality of Life Survey, by L. Johnson, S. Wilcox, J. Mankoff, & R. Stricker, 2014, https://doi.org/10.7717/peerj.322

The overall comparative health status of individuals with long-term or chronic Lyme disease showed that greater time to diagnosis and greater time since infection significantly correlated with poorer self-reported health status (Johnson et al., 2014).

Chronic illnesses account for 84% of healthcare costs, and those with chronic illnesses are the greatest users of healthcare services (G. Anderson, 2010). Furthermore, the costs for individuals with an activity limitation are roughly double those of individuals without an activity limitation (G. Anderson, 2010). Compared with the general population, individuals with chronic Lyme disease were five times more likely to visit doctors and health care professionals and more than twice as likely to be seen in an emergency department. In addition, they were almost twice as likely to stay overnight in a hospital and roughly six times more likely to receive or pay for home care visits (Johnson et al., 2014).

In an ongoing chronic-Lyme-disease quality of life study, MyLymeData2018, which continuously gathers data on symptom severity for individuals with diagnosed chronic Lyme disease. MyLymeData compares symptoms to the severity and quality of life of individuals suffering from other chronic diseases (Lymedisease.org, 2018a). Of chronic Lyme disease individuals, 72% reported fair or poor health status compared to 16% of the general population. This frequency significantly exceeds that of other chronic diseases, with congestive heart failure (62%) and fibromyalgia (59%) being the closest ((Johnson et al., 2014). Individuals with chronic Lyme disease reported an average of three severe or very severe symptoms, with 13% reporting at least one symptom and 63% reporting two or more symptoms as severe or very severe (Johnson et al., 2014). This finding is at odds with IDSA guidelines, which view these symptoms as no more than the "aches and pains of daily living" and therefore, the poor health status conforms with the severity of symptoms reported (Johnson et al., 2014).

Another numeric approach to score the severity of symptoms of patients with Lyme disease is the Nutech functional score. This 43-point positional (every symptom is subgraded and each alternative gets some points according to its position) and directional (moves in direction bad to good) scoring system was developed by Nutech Mediworld (Shroff & Hopf-Seidel, 2018). The Nutech Functional Score grades each symptom from 1 to 5 in the direction from BAD → GOOD (Shroff & Hopf-Seidel, 2018). Originally developed in 2004 as a numeric scale to score symptoms of cerebral palsy, the scale was modified for Lyme-disease symptoms in 2017 (Shroff & Bharthakur, 2015).

Summary

The reviewed literature underscored how sex hormones play a role in the genesis of autoimmunity (Ackerman, 2006). The distinct differences between males and females in the incidence of infections, the severity of disease, and the likely outcome are a consequence of sex-related differences in immune-cell composition and activation following exposure to a pathogen (Galligan & Fish, 2015). Future research may provide a therapeutic approach that can alter disease pathogenesis, rather than targeting disease sequelae (Ackerman, 2006). Alternatively, sex-based differences in the host immune response initiated in Lyme disease may emerge with more in-depth analysis of the innate and adaptive cellular elements that are mobilized during early and late manifestations of Lyme disease (Rebman et al., 2015). Many autoimmune diseases show a female bias and several lines of evidence show that autoimmune processes are a component of Lyme disease (Rebman et al., 2015). The awareness that males and females differ in their

response to specific pathogens and to treatments for infectious diseases may yield sex-specific personalized treatments (Klein & Roberts, 2015).

In this study, I presented an analysis of sex differences using the tools of the GAM for emerging infectious diseases. This analysis showed the relationship between the sex of the individuals and the severity of their Lyme-disease infections, as a theoretical foundation. The matrix comprised an x-axis signifying Levels of Analysis (confirmed chronic Lyme disease) and a y-axis for Categories of Analysis (sex—male/female). Limitations in the data set precluded the incorporation of some potential confounders in this research analyses that are unknown in the data set used for this research.

These two hypotheses showed that current treatments for infectious diseases are not sex-based, thus forming the basis of the conceptual framework. This conceptual framework provides evidence of a new-concept of sex-based medicine that considers the needs of appropriate treatment and therapies for males and females. Researchers at Johns Hopkins University (2018) performed a study that showed evidence of severe and lingering symptoms in some individuals following treatment of Lyme disease. PTLDS causes severe symptoms in the absence of a clinically detectable infection. Findings from this research show the need for more accurate identification of these individuals, possibly due to delayed diagnosis and exposure to inappropriate antibiotic and steroid treatment prior to receiving appropriate treatments (Johns Hopkins Medicine, 2018).

The known etiological agent of Lyme disease is accompanied by one or more bacterial, viral, parasitic, or fungal infection (Horowitz, 2017). Horowitz defined chronic

Lyme disease, treated or not, as an MSIDS because symptoms can linger after initial infection, further explaining how Lyme disease is ultimately a clinical diagnosis. The lingering symptoms described by Aucott et al. (Johns Hopkins Medicine, 2018) need to be identified and addressed in subsets of individuals for more accurate identification. The research approach for this project provided types of subsets as sex, defined as male and female at birth. The HMQ, created by Horowitz, validated and published with Citera et al. in 2017, was the basis for this study, describing the gap in the identification of Lyme disease between males and females, regardless of age. I describe the HMQ further in the section describing the method used for data collection.

Chapter 3: Research Method

Introduction

As noted in Chapter 1, I designed this study to investigate the epidemiologic impact of sex-based differences of chronic Lyme disease. Specifically, this study entailed a comparative inquiry to explore differences between males and females on their Lyme-disease symptomatology. The key variables investigated for the comparative assessment were biological sex (male or female) and chronic Lyme condition symptomatology. I describe the methodology used to meet the goals of this current study in this chapter. This chapter includes the research design, research method, sampling method, data-collection strategies, and statistical approach.

Research Design and Rationale

The research design selected for this study was a comparative cross-sectional study design. In a cross-sectional comparative study design, selected participants in two different groups are compared on one or more variables of interest from data gathered at one point in time (Jadhav, 2016). In cross-sectional studies, researchers evaluate the relationship between variables using statistical analyses; however, cause and effect relationships cannot be definitively determined given that temporality (timing or duration) is not always clear (Grand Canyon University, 2018; Thiese, 2014). For this study, the key variables under investigation are sex (male and female), Lyme-disease symptoms, and the severity of those symptoms. Biological sex served as the predictor variable, and Lyme-disease symptom, and severity of symptoms were the dependent or criterion variables.

RQ1 was *What is the relationship between biological sex and the frequency of symptoms of individuals with confirmed chronic Lyme disease?* To address RQ2, the difference in the symptoms of Lyme disease were assessed based on sex to determine whether the symptoms are sex-based. Additionally, RQ3 included in the study the severity of symptoms identified for classification to appraise whether the severity of symptoms is sex-specific by analyzing the significance of the severity of Lyme symptoms between males and females.

Lyme disease is an infectious disease investigated in this study's scope to provide better understanding of how to effectively address sex-driven pathological differences and manage the disease. The decision to use a cross-sectional comparative quantitative-method design was based on the objective to measure associations among sex differences with Lyme-disease infection. For that purpose, the secondary data source was a questionnaire approach used with a cross-sectional design and quantitative method, which provided the data used in this study analysis. The secondary data consisted of responses to the HMQ, based on the secondary data's dictionary codebook, obtained from the data source. The HMQ, designed and validated by Horowitz and colleagues, is intended for use in the clinical assessment and care of individuals with Lyme disease to provide information about an individual's Lyme-disease diagnosis and symptom assessment (Citera et al., 2017).

The full list of the 38 symptoms from the HMQ were considered in this study. As noted in that section, a count of the number of symptoms was calculated by summing the number of symptoms presented (i.e., possible scores could range from 0–38 symptoms).

Severity of each symptom was measured on a 4-point Likert-type scale ranging from 0 (absence of symptom) through 3 (severe); further details are provided in the symptom index section.

According to the CDC, as of 2017, Lyme disease sickens approximately 300,000 Americans per year, making the disease more common than the West Nile virus or any other illness transmitted by insects or arachnids (Ginsberg et al., 2017). Lyme disease can have a long latency period between exposure and disease manifestation or diagnosis, regardless of the sex of those infected and their location of residence (LaMorte & Sullivan, 2016). Patients' current residence may not be the point of acquired infection and may not be a collected data point, as the diagnosis and confirmation of their Lyme infection can happen long after initial infection. Therefore, the onset or duration of infection is not a variable for consideration nor is it a confounding variable, as it is not relevant to the differences experienced between males and females.

Methodology

Population

The data set used for this investigation were gathered by Horowitz through the HMQ for Lyme disease. The data set contains questionnaire data on 82 variables from a subset of Horowitz's total population of 1,190 participants. Horowitz created the MSIDS, which was validated for use as a screening tool in 2017 (Citera et al., 2017). Horowitz is medical director of the Hudson Valley Healing Arts Center in Hyde Park, New York (Bay Area Lyme Foundation, 2018). This location is an integrative medical center that combines classical and complementary approaches in the treatment of Lyme disease and

other tick-borne disorders. Horowitz has treated more than 12,000 individuals with chronic Lyme disease in the last 29 years, with patients coming from all over the United States, Canada, and Europe (Bay Area Lyme Foundation, 2018).

I requested the Hudson Valley Healing Arts Center, in Hyde Park, New York, make available to me a data set drawn from a population of individuals with chronic Lyme disease. I contacted Dr. Freeman, head of the Horowitz research group, as the lead investigator for the 2017 empirical validation of the MSIDS questionnaire study, and Citera for a data set that fits the parameters and variables of the research in this study. I obtained a data set from Horowitz for use in this project, gathered through the HMQ for Lyme disease. Horowitz provided the deidentified data for this study, and the data set was used for analyses in this current study. This data set was used as a secondary data source and confidentiality agreements were signed with the academic research group at the State University of New York (SUNY)-New Paltz, NY.

Sampling and Sampling Procedures

In the secondary data source, data accrued using the MSIDS questionnaire. The inclusion criteria were that respondents must be either male or female, age 18 or older, who have been confirmed with a diagnosis of Lyme disease. The demographic characteristics in the data set include individuals living in the United States who were previously treated or are currently being treated for Lyme disease. The specific variables examined from the data set were the sex of a respondent (male or female) and the number of 38 different symptoms and severity of those 38 symptoms of Lyme disease. Data accrued from medical records, specific questionnaires, confirmation of Lyme disease,

age, residence, and sex of the individual listed in the data set. As the data used in this project were previously collected for research publication by Citera, Freeman, and Horowitz 5 years prior to this current study, Horowitz approved the use of the data set and Citera provided the confidentiality agreement for signature before I accessed the data set. The data set included 1,190 patient records of whom 236 have documented evidence of Lyme disease and met the criteria for a clinical diagnosis, 568 healthy individuals with no confirmed diagnosis of Lyme disease, and 386 individuals with missing or incomplete data (Citera et al., 2017). The data set of the 236 participants with confirmed Lyme disease was the data set approved for my use in this research. Institutional Review Board (IRB) approval was granted June 2, 2014 and data collection took place between 2014 and 2016 for the 2017 publication of the validation study (Citera et al., 2017).

Instrumentation and Operationalization of Constructs

The source of the secondary data set used in this study was the HMQ data collected from the 2017 validated MSIDS questionnaire, fielded by Horowitz. This data set consisted of four sections, with each section providing clinical diagnostic information about an individual's likelihood of having Lyme disease or other tick-borne illnesses (Citera et al., 2017). The variables from their data set that corresponded for use in my research are biological *sex* (male and female), the *MSIDS 38 symptom checklist*, rated for frequency of symptoms as 0 (never), 1 (sometimes), 2 (most of the time), and 3 (all of the time), and the severity of symptoms rated 0 (never), 1 (sometimes), 2 (most of the time), and 3 (all of the time; Citera et al., 2017). It is important to note that the variable *sex* is

defined as male or female at birth. All participants in the data set self-identified their biological sex as either male or female.

The 38 symptoms from the checklist follow: unexplained fevers, sweats, chills, or flushing; unexplained weight change (loss or gain); fatigue, tiredness; unexplained hair loss; swollen glands; sore throat; testicular pain/pelvic pain; unexplained menstrual irregularity; unexplained breast milk production, breast pain; irritable bladder or bladder dysfunction; sexual dysfunction/loss of libido; upset stomach; change in bowel function (constipation or diarrhea); chest pain or rib soreness; shortness of breath/cough; heart palpitations, pulse skips, heart block; history of heart murmur or valve prolapse; joint pain or swelling; stiffness of the neck or back; muscle pain or cramps; twitching of the face or other muscles; headaches; neck cracks or neck stiffness; tingling, numbness, burning or stabbing sensations; facial paralysis (Bell's palsy); eyes/vision—double, blurry; ears/hearing—buzzing, ringing, ear pain; increased motion sickness, vertigo; lightheadedness, poor balance, difficulty walking; tremors; confusion, difficulty thinking; difficulty with concentration or reading; forgetfulness, poor short term memory; disorientation; getting lost, going to wrong places; difficulty with speech or writing; mood swings, irritability, depression; disturbed sleep—too much, too little, early awake; exaggerated symptoms or worse hangover from alcohol (Horowitz, 2013).

Sample Size and Power Analysis

The need for clarity on having sufficient data on individuals with Lyme-disease confirmed by Lyme-literate practitioners is important in maintaining statistical power to minimize the possibility of a Type I (false positive) or Type II error (false negative).

$$t = \frac{\overline{X}_1 - \overline{X}_2}{S_{\overline{X}_1 - \overline{X}_2}}$$

The statistical technique used for this analysis was a multivariable analysis for the symptoms by sex and then analyzing severity specifically using negative binomial regression. G*Power was used to test whether the actual sample of 236 was large enough to have adequate power to detect differences between the sex groups. To achieve 95% statistical power, and assuming a medium effect size of 0.15, an alpha level of 5%, and five independent variables (i.e., sex, age, race/ethnicity, employment status, and education), a sample size of 138 individuals would be needed. The formula for multiple linear regression is as follows:

$$\mu = \exp(\ln(t_i) + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5$$

where μ = the dependent variable, t_i is the exposure to a particular observation, b = the amount that an independent variable is modified by the regression equation (i.e., the slope of a regression line), and X is a given independent variable. The G*Power 3.1 program was used for the sample-size calculation presented in this section of the dissertation. In the Horowitz data set, 236 individuals met the criteria for inclusion in the study, which is more than the 210-sample size required by G*Power for a regression and a *t* test, respectively.

Data-Analysis Plan

This study required descriptive and inferential statistics to be calculated to address the dissertation requirements. To do so, the Statistical Package for Social Sciences (SPSS) was used for statistical analysis. Specifically, descriptive statistics were computed

for all data. Means and standard deviations were calculated for continuous variables and percentages and frequencies were computed for categorical variables. Two statistical techniques were used: an independent samples *t* test and negative binomial regression. The *t* test served as a descriptive analysis to compare the crude differences between males and females for RQ1 (symptom count), whereas inferential analyses were performed with the negative binomial model for RQ2 and RQ3. I describe each later in this chapter. As noted earlier, I used a secondary data source for this study. In many cases, the purpose for which the secondary data are collected may not be similar or the same as the primary purpose intended by the secondary users of such data. For this study, the biological sex of the individual with Lyme disease was the primary independent variable of interest and the number of symptoms and severity of symptoms were the dependent variables in this study. I coded the data to ensure these forms of the variables could be achieved. The following research questions were addressed in this current study.

RQ1: What is the relationship between biological sex and the frequency of symptoms (see the list of 38 symptoms) of individuals with confirmed chronic Lyme disease?

$H_0$1: No statistically significant relationship exists between biological sex and the symptoms of individuals with confirmed chronic Lyme disease.

H_a1: A statistically significant relationship exists between biological sex and the symptoms of individuals with confirmed chronic Lyme disease.

RQ1 is an overarching guide to this project and was analyzed using a *t* test to describe the difference in symptoms across the sexes. The goal of this question was to

describe the dependent variable (a continuous measure), representing the frequency (never to always) of symptoms. The independent variable is biological sex, a dichotomous variable. The independent sample *t* test fits for this analysis method because it can be used to compare two means across two independent groups. The independent samples *t* test is appropriate when the expectation is to see if the mean score of a continuous dependent variable varies as a function of a dichotomous independent variable (Ritchey, 2008).

I also checked Levene's test for homogeneity of variance as part of this test to determine whether the two groups have roughly the same variance (i.e., homogeneity of variance) or have different variances (i.e., heterogeneity of variance; Statistics Solutions, 2020). I checked the dependent variable to determine if it is normally distributed, using the Shapiro–Wilk test. If the normality assumption was in question, a nonparametric test (such as the Mann–Whitney U test) was used in place of the independent samples *t* test (Statistics Solutions, 2018a).

RQ2: Are there differences in the number of symptoms associated with Lyme disease between adult females compared with adult males diagnosed with Lyme disease?

$H_0$2: No statistically significant differences exist in the number of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

H_a2: Statistically significant differences exist in the number of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

A multiple linear regression analysis, in this case a negative binomial regression, was used to investigate RQ2. Negative binomial regression is appropriate when the dependent variable is a count variable and there is a mixture of continuous and categorical independent variables (Hilbe, 2011). In the negative binomial regression equation, the outcome variable (or *y*) is a count variable that adds together the number of symptoms of Lyme disease a respondent has (0 to 38), and b_1X_1 is the estimated regression coefficient that quantifies the association between being either male or female and the outcome variable, adjusting for the impact of several confounding variables in the regression equation (LaMorte, 2016).

Multivariable analysis is necessary when analyzing infectious diseases that are influenced by a number of factors that impact an exposed individual's immunologic response to the causative agent/pathogen (Katz, 2011). For this analysis, these factors that were to be considered in the multivariable modeling were age, race/ethnicity, employment status, and education of each respondent but were not available for analysis in the data set.

RQ3: Are there significant differences in the severity of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease?

$H_0$3: No statistically significant differences exist in the severity of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

H_a3: Statistically significant differences exist in the severity of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

RQ3 aimed to examine the differences between males and females in symptom severity. Similar to RQ2, a count variable was constructed to look at the relationship between sex and the severity of each symptom when controlling for other factors, as listed in RQ2. The justification for using this method is the same as with RQ2, which is "Are there differences in the number of symptoms associated with Lyme disease between adult females compared with adult males diagnosed with Lyme disease?"

Threats to Validity

A quantitative cross-sectional survey research requires that all variables be measured simultaneously (Public Health Action Support Team, 2017). Horowitz (2017) validated the MSIDS questionnaire at the time of Lyme disease testing or confirmed such testing was performed for each participant. Thus, Horowitz provided the survey and assessment tools to the individuals simultaneously, which met the requirements for a quantitative survey research study design.

A limitation in the validity of this study is that no causal inference can be made for individuals with Lyme disease based on sex and the number and severity of symptoms. Only a correlational inference can be made, as the data are not experimental in nature. Correlation is not the same as causality (Creswell, 2009; Glanz, Rimer, & Viswanath, 2008; Rudestam & Newton, 2015; Szklo & Nieto, 2014).

Another primary limitation of validity in this study is one shared by all quantitative survey research, that validity can be a problem with surveys or questionnaires. Questionnaires tend to be standardized, which can create difficulty in asking questions other than general ones targeted to a broad range of people. Construct validity was used to test the reliability of the HMQ. The researchers used this type of testing to determine correlations with the use of the questionnaire, proving it accurately differentiated individuals with Lyme disease from those without Lyme disease. Citera et al. (2017) concluded that the questionnaire measured acceptable levels of internal reliability using Cronbach's coefficient alpha and exhibited evidence of convergent and divergent validity upon the conclusion of the validation study for MSIDS questionnaire (Citera, et al., 2017). In other words, convergent and divergent validity are both subtypes of construct validity. Construct validity is a test (i.e., questionnaire) designed to measure a particular construct (i.e., difference between symptoms and severity of individuals with Lyme and those without). Convergent validity takes two measures that are supposed to be measuring the same construct and shows that they are related, whereas conversely, divergent validity shows that two measures that are not supposed to be related are in fact, unrelated (Lund Research, 2012).

A cross-sectional design is applied in a study to evaluate the prevalent rather than incident outcomes and thus excludes people who develop the outcome but die before the conclusion of the study (Carlson & Morrison, 2009). As explained in Chapter 1, it is sample bias reflected in the cross-sectional study design that identifies prevalent cases rather than incident cases. In other words, sample bias is a possible limitation, potentially

affecting validity due to the size of the sample and without follow-up on the survey data. Recall bias can be a factor in the collection of the data collection due to self-reporting on symptoms and their relative severity, for which the researcher cannot adjust. The measured association in a cross-sectional study is between exposure and having the outcome rather than to exposure and developing the outcome (Carlson & Morrison, 2009).

One limitation addresses external validity, insofar as no generalization to the entire U.S. population can be made in this study beyond the specified target population—individuals with confirmed Lyme disease—because the current study is not an extensive multisite study. In other words, the residence of participants was not a data point collected and many survey questionnaires were completed through electronic data capture through a database website. This study is therefore somewhat limited in generalizability primarily, based on how useful the results of a study are for a broader group of people. Additionally, study outcomes are not based on any treatment or interventional study, as no participants were treated with any specific medication or therapy. The purpose of this study is not tailored to Lyme-disease intervention because data used were from secondary sources following Lyme treatment and follow-up with the participants through a questionnaire survey. The application of a quantitative method was not used to effectively measure subjective experiences or emotional states of individuals affected by an outcome or those exposed to a risk factor (DiClemente et al., 2013; Frankfort-Nachmias & Nachmias, 2008).

Ethical Procedures

Horowitz granted access to a data set from the 2017 validation study led by Citera, a professor in the Department of Psychology at the State University of New York at New Paltz, New Paltz, NY. Freeman, a researcher at Hudson Valley Healing Arts Center in Hyde Park, NY working with Horowitz, contacted Citera for approval to provide a data set from their research. The Human Research Ethics Board (the IRB) of the SUNY-New Paltz provided a confidentiality agreement for my signature and after all documents were in place, the data set was approved for my use in this research.

The survey component for the 2017 validation study of the HMQ was certified as exempt on June 2, 2014 (Citera et al., 2017). The application for approval was filed with the Walden IRB for acceptance of the use of secondary data in the analysis for this research. Upon approval from the Walden University IRB, the data set was released to me for use and analyses by the Human Research Ethics Board [the IRB] of SUNY-New Paltz.

Summary

In this research study, I measured all variables simultaneously. In other words, the number and severity of Lyme disease symptoms in adult males with confirmed Lyme disease were compared to adult females with confirmed Lyme disease. In the data set retrieved from Horowitz's and Citera's research databases, all data were made available for the analyses supporting this research. The goal was to determine whether the null hypothesis for each posed research question should be rejected, based on the *p*-value

level of significance. The results of this study and the conclusions drawn from these findings are discussed in detail in Chapters 4 and 5, respectively.

Chapter 4: Results

Introduction

Lyme disease is an illness comprised of multiple infections in addition to the primary parasitic spirochete infection, *Borrelia burgdorferi* (Ross, 2018). These other infections are known as coinfections and can be viral, bacterial, fungal, and parasitic infections, such as *Bartonella, Babesia, Anaplasma, Ehrlichia,* and *Clostridium difficile* (Berghoff, 2012). Combinations of these chronic coinfections create MSIDS, a term coined by Horowitz, (lymeactionnetwork.org, 2017). Along with these coinfections are other factors such as allergies, environmental toxins, and a compromised immune system, and consequently, a chronic Lyme-disease infection is a multifaceted and complex illness making the diagnoses and treatment an extremely difficult process (Lyme Action Network, 2017). Lyme disease is definitively an illness that causes direct and indirect dysfunction to most body organs and systems, yet is distinct for each individual (Ross, 2018).

I designed this study to investigate the epidemiologic consequence of sex-based differences of chronic Lyme disease. Sex differences in the pathogenesis of infectious diseases may reflect variations with immune responses during infection (vom Steeg & Klein, 2016). This study entailed a comparative inquiry to determine if a statistically significant difference exists between males and females in chronic Lyme symptomatology and severity, an area that has received limited attention in previous research. Sex—being female or male—influences one's immune responses, contributing

to variation in the pathogenesis of infectious disease in males and females and the prevalence of autoimmune diseases (Ruggieri et al., 2016).

The focus of this study was to investigate the research gap based on biological sex and Lyme disease by exploring the following research questions:

RQ1: What is the relationship between biological sex and the frequency of symptoms of individuals with confirmed chronic Lyme disease?

$H_0$1: No statistically significant relationship exists between biological sex and the symptoms of individuals with confirmed chronic Lyme disease.

H_a1: A statistically significant relationship exists between biological sex and the symptoms of individuals with confirmed chronic Lyme disease.

RQ2: Are there differences in the number of symptoms associated with Lyme disease between females compared with males diagnosed with Lyme disease?

$H_0$2: No statistically significant differences exist in the number of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

H_a2: Statistically significant differences exist in the number of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

RQ3: Are there significant differences in the severity of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease?

$H_0$3: No statistically significant differences exist in the severity of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

H_a3: Statistically significant differences exist in the severity of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease.

In this chapter, I describe the secondary data-collection process. review any modifications of the data collected, provide the methods used for statistical analysis, and present the results from this research.

Data Collection

The data used for this study were secondary data provided by a clinician specializing in caring for individuals with Lyme disease. The data include responses to the HMQ for Lyme disease. The HMQ is a questionnaire used by health care practitioners as part of the clinical assessment for diagnosing Lyme disease. Citera et al. (2017) provided a data set from the valuation of the HMQ for Lyme disease. The dataset included 236 persons with a confirmed Lyme-disease diagnosis from 2014 through 2016.

Of the 236 persons with a confirmed Lyme disease diagnosis, one person had missing information for one of the symptoms (bowel function), which comprises the outcome variable for the inferential analyses. Therefore, I excluded this individual from the analysis, bringing the total sample for this study to 235 individuals who had complete data for all 38 symptoms.

Sex and age were the only demographics provided in the final data set. The data set did have programmed columns for race, employment status, and education, but these data were not collected to ensure the anonymity of participants, as originally approved by the SUNY IRB. Although these variables were originally thought to be included in the data set because the questions were listed in the codebook, once I received the data, I realized these variables were not collected and thus could not be used for this project. These variables were not included in any of the research results collected for the HMQ validation study.

Results

Study Population

In Table 2, the percentages and frequencies for the primary independent variable, sex, are presented.

Table 2

Sex Frequency of Participants with Confirmed Lyme Disease

	Frequency	Percent
Female	195	83.0
Male	40	17.0

Note: $N = 235$.

The mean age of participants was approximately 48 years old ($SD = 13.71$). The mean age was 46–50 and the range of ages was under 20 to over 80. Table 3 provides the distribution of the sample by age.

Table 4 provides univariate statistics for the count of symptoms and severity of symptoms. The mean of the severity of symptoms score was calculated by adding all

scores in the *Section 1: Symptom Frequency Score* of the HMQ (Horowitz, 2014). If the score was between 21 and 45, the probability of a tick-borne disorder was high and indicated the individual should see a health care provider for further evaluation (Horowitz, 2014). When the results of Section 1 of the HMQ show a high frequency of symptoms scored, those with a score of 3 are then listed in *Section 2: Most Common Lyme Symptoms* of the HMQ. These are those symptoms characterized as high probability of having Lyme-MSIDS (Horowitz, 2014). The severity score is the calculated total of those symptoms with a score of 3 per symptom from *Section 1: Symptom Frequency Score*. The following section, *Section 2: Most Common Lyme Symptoms Score* is where the top common symptoms of Lyme disease are listed. Again, these are those with a high-frequency score, characteristic of those with a high probability of having Lyme-MSIDS (Horowitz, 2014). Table 4 shows the main dependent variables used in the inferential analysis to test the research questions.

Table 3

Age Distribution/Ranges of MSIDS Data Set

Age range	Frequency	Percent
< 20	10	4.7
21–25	9	3.9
26–30	11	4.6
31–35	15	6.5
36–40	14	6.0
41–45	20	8.0
46–50	60	25.6
51–55	32	13.0
56–60	23	9.8
61–65	24	9.8
66–70	9	3.8
71–75	5	2.0
76–80	2	0.8
> 80	2	0.8
	235	100.0

Note: *N* = 235.

Table 4

Means and Standard Deviations, Scale Variables

Variable	Mean	*SD*	Median	Range
Count of symptoms	21	7.88	21	0–37
Severity of symptoms	35	17.80	33	0–94

Note: N = 235.

Hypothesis Testing

To test the hypothesis, a series of interferential analyses were conducted along with the proper assumptions for each test. Hypothesis testing is organized by research question.

Research Question 1

The results for RQ1, "What is the relationship between biological sex and the frequency of symptoms of individuals with confirmed chronic Lyme disease?" are given below. To first understand the symptomology of Lyme patients, I compared males and females for each of the 38 symptoms. Fatigue was the most common symptom for members of both sexes, as seen in Table 5, listed by the most common symptoms among females.

I used the Shapiro–Wilk statistic to assess whether the data for frequency of symptoms was normally distributed. The Shapiro–Wilk obtained was 0.985 with $df = 235$ and a p-value of .017), suggesting evidence of deviation from normality. In addition, I produced a Normal Q-Q plot to assess normality. For a normal distribution, the points must be about the same distance from the line in the Normal Q-Q plot shown in Figure 5 (Glen, 2019). I ran parametric independent samples t tests and Mann–Whitney U tests for the count of symptoms variable.

Table 5

Frequencies and Percentages for Symptoms by Sex

	Males		Females	
	Frequency	Percent	Frequency	Percent
Fatigue	36	90	183	93
Disturbed sleep	33	82	172	88
Stiff neck or back	32	80	169	86
Neck cracks	32	80	158	81
Joint pain	31	77	156	80
Mood swings	30	75	156	80
Concentration/reading	26	65	157	80
Forgetfulness	24	60	154	79
Muscle pain	30	75	152	77
Headaches	30	75	149	76
Fevers	22	55	146	74
Confusion	27	67	144	73
Upset stomach	20	50	132	67
Light-headedness	23	57	130	66
Bowel function	19	47	123	63
Tingling, numbness	27	67	120	61
Speech/writing	19	47	120	61
Shortness of breath	24	60	114	58
Ears/hearing	24	60	110	56
Sexual dysfunction	20	50	109	55
Heart palpitations	19	47	103	52
Twitching	24	60	100	51
Motion sickness/vertigo	19	47	100	51
Weight change	15	37	94	48.
Eyes/vision	20	50	94	48
Chest pain	21	52	92	47
Disorientation, getting lost	13	32	89	45
Bladder	12	30	86	44
Sore throat	20	50	85	43
Hair loss	9	22	82	42
Swollen glands	16	40	83	42
Tremors	18	45	63	32
Worse hangover	12	30	57	29
Heart murmur	5	12	50	25
Menstrual irregularity	0	0	41	21
Testicular/pelvic pain	12	30	38	19
Breast pain	1	2	33	16
Facial paralysis	8	20	26	13

Note: N = 235; 195 Female participants/40 Male participants.

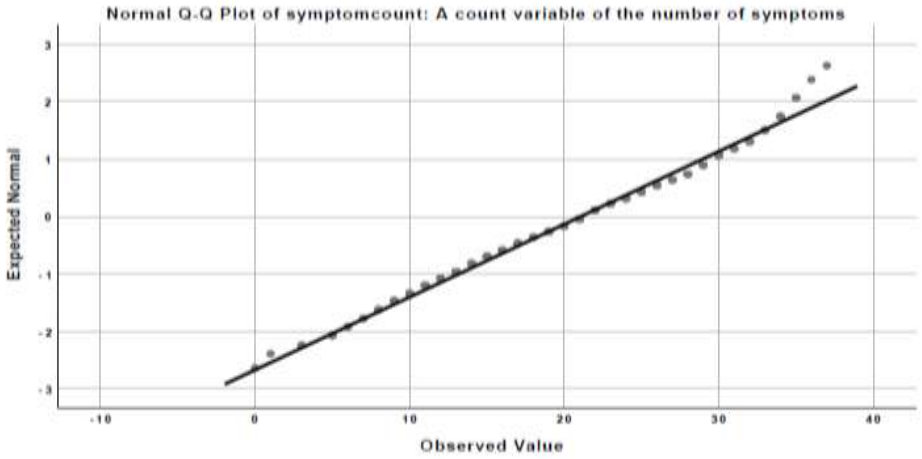


Figure 5. Q-Q plot for count of symptoms.

Table 6 lists the results of the *t* test. The mean symptom count for females was 21 with a standard deviation of 7.66, whereas the mean symptom count for males was 19 with a standard deviation of 8.81. Results of the independent samples *t* test were not statistically significant ($t = 1.51$; $df = 233$; $p = .133$), suggesting no difference between males and females in the frequency of symptoms of Lyme disease. The Levene's test of homogeneity of variance was statistically nonsignificant ($F = 1.230$; $p = .268$), suggesting the data are homoscedastic, meaning "having the same scatter."

Table 6

T Test for Frequency of Symptoms by Sex

	N	Mean	*SD*	*t*	*df*	Sig
Female	195	21	7.66		233	.133
Male	40	19	8.81			

Note. $N = 235$.

The results of the Mann–Whitney U test were also statistically nonsignificant (U = 3373.5; p = .178), which suggests no difference emerged between males and females in the frequency of symptoms of Lyme disease (see Table 7).

Table 7

Mann-Whitney U Test of Frequency of Symptoms by Sex

	N	Mean rank	MWU	Sig
Female	195	120	337350	.178
Male	40	104		

Note: N = 235, MWU = Mann–Whitney U test.

Research Question 2

The results for RQ2, "Are there differences in the number of symptoms associated with Lyme disease between adult females compared with adult males diagnosed with Lyme disease?" were determined using a negative binomial regression model with the outcome of frequency of symptoms and independent predictors of age and sex. The Omnibus X^2 test, summarized in Table 8, was not statistically significant (X^2 = 0.352, df = 2; p = .839), suggesting that the neither of the independent variables of age and sex have any effect on the number of symptoms per participant, respectively.

Table 8

Negative Binomial Regression of Number of Symptoms onto the Predictors

Variable	*B*	*SE(B)*	*p*
Intercept	3.004	0.283	0.000
Sex of Respondent (Female)	0.104	0.179	0.560
Sex of Respondent (Male, referent group)	0.000	—	—
Age of Respondent (years)	-0.001	0.005	0.853
Omnibus X^2	0.352		0.839

Note. $N = 235$.

Research Question 3

The results for RQ3, "Are there significant differences in the severity of symptoms associated with chronic Lyme disease between adult females and adult males diagnosed with chronic Lyme disease?" appear in Table 9. Table 9 shows descriptive statistics for males and females in symptom severity. Females have a higher mean severity score ($M = 36.2$) than males (30.1).

Table 9

Severity of Symptoms by Sex

Variable	*M*	*SD*	Range
Severity of symptoms (Females)	36	17.5	2–94
Severity of symptoms (Males)	30	18.4	0–83

Note. $N = 235$.

A negative binomial regression model was fit with the outcome of severity of symptoms and independent variables of age and sex. The Omnibus X^2 test, shown in Table 8, was not statistically significant ($X^2 = 1.060$, $df = 2$; $p = .589$), suggesting that age and sex have no impact on the severity of symptoms (see Table 10).

Table 10

Negative Binomial Regression of Severity of Symptoms Onto the Predictors

Variable	*B*	*SE(B)*	*p*
Intercept	3.413	0.282	0.000
Sex of Respondent (Female)	0.185	0.177	0.295
Sex of Respondent (Male)	0.000	—	—
Age of Respondent	0.000	0.005	0.974
Omnibus X^2	1.060		0.589

Note. $N = 235$.

Assumption Tests for Negative Binomial Regressions

Several assumptions that must be met in multiple linear regression: linearity, homoscedasticity, independence of errors, normality of errors, and multicollinearity (Allisonn, 1999). The first assumption, linearity, proposes that the relationships of the variables under investigation are linear in nature. The way to investigate whether this assumption holds is to check the plot of the regression standardized residuals, or the Normal P-P plot. As long as a linear trend is evident in the plot, the assumption of linearity is met (Mertler & Vannatta, 2010). The Normal P-P plot for number of symptoms as a dependent variable and severity of symptoms as a dependent variable shows that this assumption was met (see Figures 6 and 7).

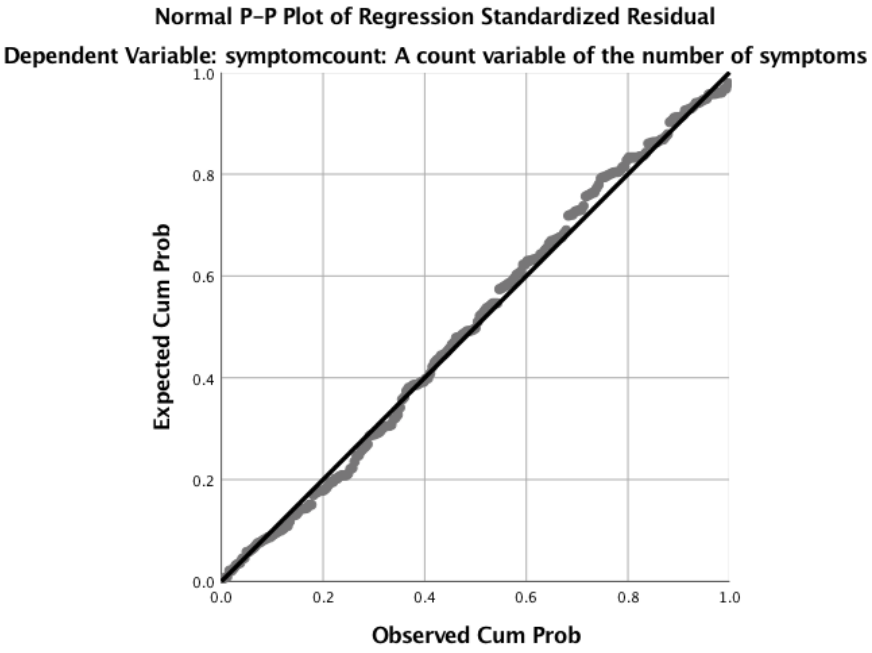


Figure 6. Normal P-P plot of regression standardized residual dependent variable: symptom count—A count variable of the number of symptoms.

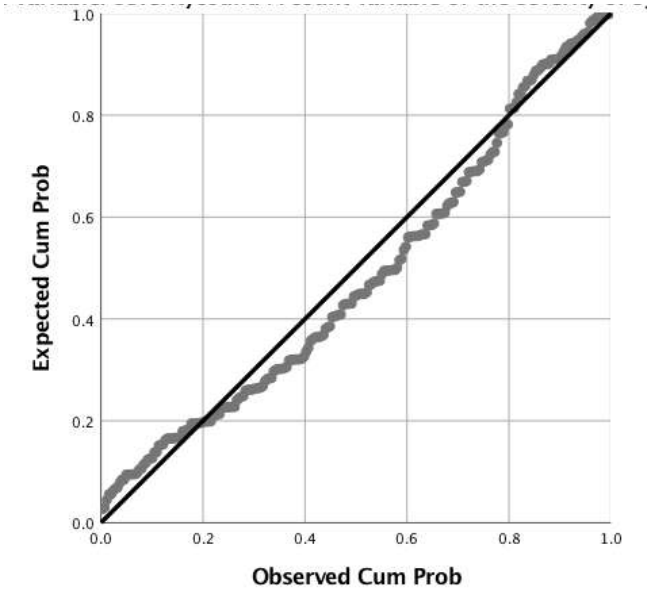


Figure 7. Normal P-P plot of regression standardized residual dependent variable: severity count—A count variable of the severity of symptoms.

The second assumption, homoscedasticity, confirms that the degree of random noise (or error) in the regression equation remains relatively constant or homoscedastic (Allison, 1999). The Breusch–Pagan Test (Breusch & Pagan, 1979) is essentially a chi-square test for heteroscedasticity. If the value of chi-square is statistically significant, the data are considered heteroscedastic and corrective measures are required. The Breusch–Pagan test was statistically non-significant for the severity of symptoms ($c^2 = 0.308$, $df = 2$, $p = 0.857$) and the number of symptoms ($c^2 = 2.483$, $df = 2$, $p = 0.289$). This assumption was met.

The third assumption, independence of errors, confirms that the disturbance terms in the regression equation are uncorrelated. This assumption is checked with the Durbin–Watson statistic. The Durbin–Watson statistic ranges from 0 to 4, with a midrange value of 2. As a general rule, values of the Durbin–Watson statistic closer to 2 indicate independence of errors; values below 1 and above 3 suggest correlation of errors (Gujarati, 2003). The Durbin–Watson statistic for the severity of symptoms was 1.878, and for the number of symptoms was 2.055. This assumption was met.

The fourth assumption, normality of errors, is predicated on the understanding that all errors are normally distributed in a regression equation. As long as all other assumptions are met, the violation of this assumption can be discounted (Allison, 1999). The Shapiro–Wilk Test of the standardized residuals is the test used to check this assumption (S. Shapiro & Wilk 1965). The value of the test is statistically significant for the severity of symptoms (0.964, $df = 235$, $p < .001$) and the number of symptoms (0.985,

$df = 235$, $p < .05$). This assumption was not met; however, given that all other assumptions were met, corrective action is unnecessary at this time.

Multicollinearity is not a violation of the assumptions of regression per se; however, multicollinearity does make it difficult to find statistically significant coefficients in a regression model (Allison, 1999). Multicollinearity is typically checked by calculating variance-inflation factors (VIFs). A VIF of 10 or greater typically indicates potential multicollinearity (D. Anderson, Sweeney, & Williams 2002). All VIFs in all modes for both dependent variables were under 2.0. This assumption was met.

Summary

This chapter included a summary of the results of this study. No difference between the frequency of Lyme-disease symptoms, the number of symptoms, or the severity of symptoms between males and females in this data set. The sex or age of the participant does not contribute to the outcomes of the participant. Chapter 5 includes the interpretation of the research findings.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

Historically, sex as a variable in infectious-disease research has been overlooked (Ingersoll, 2017). Recently however, the biological pathways responsible for sex-based differences in the manifestations of infectious diseases have begun to be unveiled (van Lunzen & Altfeld, 2014). The purpose of the present study was to investigate the sex-based differences for chronic Lyme disease. According to the CDC, Lyme disease is the most prominent of vector-borne disease cases reported each year in the United States, with a larger number of cases than many other diseases more familiar to the public, including breast cancer, colon cancer, and human-immunodeficiency-virus infection (Vector Disease Control International, 2019). Late-stage or chronic Lyme disease can be the result of failing to properly diagnose and treat the infection early, leading individuals to experience symptoms weeks, months, or years after the presumed initial infection date (Vector Disease Control International, 2019).

I performed analyses for this research study using secondary data of symptoms and related severity of those symptoms between the sexes. The data accrued using the MSIDS questionnaire developed and validated by Horowitz (Citera et al., 2017). The study population for this research consisted of 40 males who comprised 17% of the participant participation, and 195 females, comprising the remaining 83%. All participants included in the data set were confirmed to have chronic Lyme disease. The data collected from these participants were anonymous through a web-portal database.

Therefore, no identifiers such as race, ethnicity, or residence were collected using this questionnaire.

The purpose of this study was to observe if any differences emerged between the sexes from this data set of people with confirmed late-stage Lyme disease (chronic Lyme disease). No statistically significant differences emerged between males and females in the number or severity of symptoms. In this chapter, I present further interpretation of these findings and the implications of this research pertaining to the advancement of knowledge of Lyme-disease research. In addition, I discuss the limitations of this study and offer recommendations for future research. Finally, I conclude this chapter with a summary that highlights the key essence of the study.

Interpretation of the Findings

I designed the present research to examine the relationship between the frequency and severity of Lyme-disease symptoms with patient sex. I discuss the findings in answer to these research questions and my interpretation of these findings. To the best of my knowledge, based on a thorough review of the literature, this study is the first to examine sex differences regarding the type, number, and severity of chronic-Lyme-disease symptoms, not including diagnostic findings. Therefore, it is not possible to make any direct comparisons between this study's results and those of previous studies found in the literature. Instead, I discuss the study findings in the context of the current knowledge of chronic Lyme disease in the scientific community.

For this study, I used HMQ data collected from the 2017 validated MSIDS questionnaire fielded by Horowitz (Citera et al., 2017). The data set comprised data from

235 participants who met study criteria (i.e., males and females of 18 years of age or older who had a confirmed diagnosis of Lyme disease). Of the 235 participants, 195 (83%) were females and 40 (17%) were males. The research sample, derived from the chronic Lyme disease population, is comparable to samples of other ongoing research in the chronic-Lyme-disease population in age and sex.

Using the three research questions, I examined sex differences in type, number, and severity of chronic-Lyme-disease symptoms, respectively. Results from analyses (i.e., independent samples *t* test, Mann–Whitney U test, and negative binomial regression) conducted to address the research questions were not significant. Research performed in search of differences between males and females focusing on chronic Lyme disease has been developed using statistical observations and public CDC statistics, highlighting the important difference between clinical practice data collection (as was used for this study) and statistical data collection and CDC-reported cases, which are both inherently skewed toward diagnosis, based on laboratory tests (Lee-Lewandrowski, Chen, Branda, Baron, & Kaufman, 2019).

Relationship Between Sex of Individual and Symptoms of Lyme Disease (RQ1)

The first research question asked about the relationship between biological sex and the frequency of symptoms in individuals with confirmed Lyme disease. The frequencies and percentages for the 38 symptoms by sex were comparable. Indeed, the five most prevalent Lyme-disease symptoms—fatigue, disturbed sleep, stiff neck or back, neck cracks, and joint pain—ranked the same for males and females, varying little in percentages. *Fatigue* was experienced by 90% of males and 93% of females; *disturbed*

sleep was experienced by 82% of males and 88% of females; *stiff neck or back* was experienced by 80% of males and 86% of females; *neck cracks* were experienced by 80% of males and 81% of females; and *joint pain* was experienced by 77% of males and 80% of females, reported for the individual participants in this study. Furthermore, findings indicated that sex has no effect on the number or severity of symptoms, after controlling for age.

Relationship with Number of Symptoms of Lyme Disease and Sex of Individual (RQ2)

The second research inquiry was to determine what, if any, differences emerged in the number of symptoms associated with Lyme disease between sexes of participants with confirmed Lyme disease. This is the first study to assess sex differences in type, number, and severity of Lyme-disease symptoms, thereby nullifying the ability to compare results with prior work. The minimal research on chronic-Lyme-disease sex differences has used public CDC laboratory test data rather than clinical data. For example, Wormser and Shapiro (2009), using CDC data, found that individuals with *chronic Lyme disease* were considerably more likely to be female than male, with prevalence ratios ranging from 7:1 to 9:1. These findings differ from CDC data reports showing higher prevalence rates of *Lyme disease* in males than females. Scholars have voiced concerns about the use of laboratory data.

Females may be at higher risk of contracting chronic Lyme due to diagnostic delays if laboratory diagnostic testing is more effective in males, as has been suggested. The empirical focus on the diagnosis of chronic Lyme disease using CDC lab data obfuscates

the importance of understanding potential sex differences regarding chronic Lyme disease symptoms regardless of sex differences in prevalence rates. (Johnson, Shapiro, & Mankoff, 2018, p. 143).

Study findings can be examined in relation to what is known in the scientific community. In this study, the most common symptoms reported by participants—male and female—were fatigue, disturbed sleep, stiff neck or back, neck cracks, and joint pain. Results from the study by Rebman et al. (2017) showed that patients with chronic Lyme disease reported significantly higher levels of fatigue, sleep problems, and pain, compared to healthy control patients. In a study examining the effects of dapsone as a treatment for chronic Lyme disease, Horowitz and Freeman (2016) found that chronic-Lyme-disease-positive/*Babesia*-negative patients did not evince reductions in the symptoms of disturbed sleep and head pain posttreatment. Results from this study in relation to those found in the Rebman et al. (2017) and Horowitz and Freeman's (2016) studies suggest that fatigue, disturbed sleep, and pain may be common among chronic-Lyme-disease patients and may be more severe in these patients than in healthy individuals.

Relationship with Severity of Symptoms of Lyme Disease and Sex of the Individual (RQ3)

The third research inquiry was to determine if any significant differences emerged in the severity of symptoms associated with chronic Lyme disease and sex. It has been 4 decades since the acknowledgment of chronic Lyme disease, and still much remains unknown regarding sex-based differences in the clinical presentation of this infection

(Rebman et al, 2015; 2017). Indeed, sex differences in Lyme disease have not been comprehensively examined in the literature. The only study that has examined sex differences focused on childhood Lyme disease (Tveitnes & Oymar, 2015). The results from Tveitnes and Oymar's (2015) study showed that a significantly higher percentage of girls (86%) than boys (62%) reported facial nerve palsy whereas a significantly higher percentage of boys (30%) than girls (10%) reported headache or stiffness in the neck. Tveitnes and Oymar's results cannot, however, be compared to findings in this study, conducted with adults diagnosed with chronic Lyme disease, due to the two different types of Lyme disease, as well as age differences in hormonal production, associated comorbidities, and duration of illness. However, Tveitnes and Oymar did emphasize the importance of studying sex differences with regard to chronic Lyme disease and related infections.

Limitations of the Study

As with any empirical work, this study had some limitations. The use of a secondary data set and sex as an independent variable (that could not be manipulated) precluded the ability to use a true experimental design and instead required the use of a cross-sectional comparative design. The true experimental design, which has experimental and control conditions and involves the manipulation of the independent variable, is the only design that can determine cause and effect (Imai, Tingley, & Yamamoto, 2013). As this study did not use a true experimental design, causality could not be determined (i.e., that sex caused or did not cause the number or severity or chronic-Lyme-disease symptoms).

The original study recruitment and data-collection procedures may have introduced certain biases, and thus limitations, into this study. The secondary data set used for this research came from a subsample of individuals with confirmed Lyme disease who were recruited by three practices involved in the original study. The data accrued with recruitment through social media and the survey was provided by e-mail invitation, directing potential participants to click on a link explaining the purpose of the survey and informed-consent information (Citera et al., 2017). The open recruitment may have resulted in a self-selection bias. That is, patients who had a higher number of chronic-Lyme-disease symptoms and a higher degree of severity of symptoms may have been more inclined to participate in the study in comparison to patients whose symptoms were minimal or mild.

A related bias that potentially influenced study findings was the Neyman bias, or prevalence-incidence bias, which happens as a result of a significant amount of time passing between exposure to a disease and the investigation and reporting of the disease and its symptoms (Tripepi, Jager, Dekker, & Zoccali, 2010). The Neyman bias is a problem in cross-sectional research (Yu & Tse, 2002). The data set contained information from prevalence cases, which are typically more ill patients, rather than incidence cases, or newly diagnosed patients. Thus, the original open-recruitment process may have excluded patients with few disease symptoms and low severity of symptoms but included patients with a high number or more severe symptoms. The symptoms reported by participants may have been influenced by the length of time since infection/reinfection and diagnosis: participants who were infected for a longer period of time or reinfected

may have more symptoms or more severe symptoms. Moreover, the time of infection and time of diagnosis were potential confounding variables that could not be addressed in this study.

Additional limitations included that the data set did not include information on comorbidities associated with chronic Lyme disease that may have exacerbated symptoms. Individuals can have a number of symptoms that fall under the broad spectrum of more than one disease, due to a multisystem immunological breakdown over years of multiple health issues (Crystal, 2019). Another concern was that the existing ELISA serologic test for Lyme disease may have resulted in the initial exclusion of patients who actually did have Lyme disease. A positive ELISA test result only proves exposure to the *Borrelia* infection and does not indicate a current infection. Thus, the test can indicate a historical or late-stage (chronic-Lyme-disease) infection. In contrast, false negative results are typical, especially if the seroconversion postinfection has not occurred. Seroconversion can take up to 8 weeks before true positivity is proven (AMBOSS, 2020).

Recommendations

This study can prompt the development of future empirical work. The outcomes from this research show no statistically significant differences between males and females with respect to the type, frequency and severity of symptoms for chronic Lyme disease. It is important to note, however, that this is the only study to date that has examined potential sex differences regarding the type, number, and severity of chronic Lyme disease symptoms. There remains a need for additional studies. The analysis of this

research highlighted the unintentional imbalance in study participants with more females than males. Replication studies, especially those utilizing sex-equivalent sample groups, are needed to affirm or contradict the non-significant findings noted in this study. Cross-sectional studies that control for or include as additional independent variables pertinent factors such as ethnicity, geographical residence, time since infection or diagnosis, and additional diagnoses are needed, as they may parse out significant effects and/or interactions that could not be assessed in this study. Studies utilizing different observational designs, such as cohort studies (i.e., comprised of a sample of exposed and non-exposed patients) and case control studies (i.e., comprised of a sample of patients with and without Lyme disease) would be beneficial. Indeed, the case-control approach has become the primary design used in chronic disease epidemiological research (Giesecke, 2017).

Knowing the age of the individual is important for assessment of the individual's status of immunity. Future research should consider that an individual's immune system changes with aging and then the immune system becomes slower to respond; the body may heal more slowly; changes in hormone production; the immune system's ability to detect and correct cell defects declines; and autoimmune disorders may develop which all effects of aging (Martin, Zieve, & Conaway, 2018). There is a strong argument for considering age-related per sex clinical assessments and diagnostic testing requirements in future research to eliminate overlooking Lyme disease infections.

The study findings were dependent upon the appropriate diagnosis of Lyme disease and chronic Lyme disease. There remains, however, uncertainty among

physicians regarding the cause, origin, and specific diagnostic criteria of chronic Lyme disease (Greenberg, 2017). Clinical assessment by non-Lyme-literate practitioners can lead to misdiagnoses and affect proper reporting of chronic Lyme disease symptoms with related frequency of those symptoms. There is a need for continuous dialogue among practitioners regarding diagnostic and treatment concerns for chronic Lyme disease. The difficulty that physicians have diagnosing chronic Lyme disease is due in part to the lack of use of a standardized assessment tool, such as the MSIDS (Horowitz, 2013). Not only is there a need for additional studies that use the MSIDS, there is a need to train practitioners on the use of this and other assessments. Moreover, as treatment response may differ between males and females, there is a need for practical assessment and empirical examination of potential sex differences with regard to treatment modalities.

Implications

This secondary data set does not identify pertinent research differences in chronic Lyme disease between females and males. These findings were unremarkable between sexes seen in this research. However, current research in 2018, performed in both rural and urban areas, shows more medical claim lines with Lyme disease diagnoses were submitted for females than males (FAIR Health, 2019; Leland, 2019). Additionally, Lyme disease diagnoses by sex in rural areas (56% females & 44% males) was slightly less than in urban areas (61% females & 39% males) (FAIR Health, 2019). The question regarding the knowledge of the practitioner performing the clinical assessment and whether or not the practitioner understands that the symptoms can reflect late stage or chronic Lyme disease. According to Rebman et al. (2015), comparison to other infectious

and chronic diseases where sex differences are more pronounced, differences in the number of CDC-reported cases of Lyme disease by sex are unremarkable. That question that one then asks is "Why?"

A better data set for researching the differences in the symptoms and severity of symptoms with chronic Lyme disease should begin with a managed recruitment with equal cohorts for analysis to ensure a potentially more appropriate outcome. As previously stated, annually CDC-reported number of confirmed cases of Lyme disease in the U.S. from 2001 to 2017, are listed by age and sex, and the outcome just from that report identifies more cases reported for males (214,885) compared to females (168,961) across all age groups (Elflein, 2019). With any other indication, age is a condition considered when diagnosis is being determined and the age of individual specific to their sex identifier, is medical common sense for consideration. Unfortunately, a large portion of the medical community does not believe that chronic Lyme disease is a problem, nor indeed, that it exists (Cox, 2019).

It is important that practitioners understand the risks associated with chronic Lyme disease. A misdiagnosis of chronic Lyme disease can lead to further misdiagnoses, especially among sexes. For example, individuals with chronic Lyme disease may not realize the associated cancer risk they have (Envita Medical Center, 2019). The Envita Medical Center (2019) reported having a surprisingly high number of patients with late-stage cancer who tested positive for Lyme disease. The organization also reported an association between Lyme disease and tumor development (in both males and females) (Envita Medical Center, 2019). The need for diagnosing Lyme disease, whether acute or

chronic, in males or females, is critical in not only proper diagnosis of Lyme disease but to further prevent the development of associated chronic or terminal diseases.

Conclusion

Lyme disease is known as the *great imitator* as its symptoms mimic or imitate up to 350 different diseases (Mott, 2019). Lyme disease is quickly becoming the untreated epidemic of the 21st century, as patients around the world struggle to find a "Lyme-literate" doctor who can help them regain their health, often without success (Holtorf, 2020). Many physicians have only a general textbook understanding of Lyme disease and its symptomatology, and if they do have a deeper understanding of the disease, they often do not understand the complexity in the presentations of Lyme disease symptoms (IGeneX, 2020). Moreover, the symptoms of Lyme disease may differ and/or be more or less pronounced among individuals, depending on susceptibility criteria such as sex (Columbia University Irvine Medical Center, 2018).

Many individuals with Lyme disease often continue to experience symptomatology after treatment and may be diagnosed as having chronic Lyme disease. Multiple symptoms can present at various times for an individual with chronic Lyme disease, which can be migratory, resulting in an ebb and flow in the number and severity of symptoms. Chronic Lyme disease remains a controversial diagnosis, with some physicians disagreeing as to its actual existence (Horowitz, 2013). Moreover, the non-specific symptoms associated with chronic Lyme disease makes clinical assessments difficult to diagnose and treat properly (Holtorf, 2020). Controversies surrounding its actual existence coupled with the lack of valid and reliable diagnostic assessments for

chronic Lyme disease likely contributed to the lack of empirical examination of potential sex differences with regard to the type, number, and severity of symptoms. This is a concern, as sex analysis is a critical element of health systems research (London School of Hygiene & Tropical Medicine, 2014).

The findings from this study indicated no sex differences with regard to type, number, and severity of chronic Lyme disease symptoms. This, however, was just one study, and certain study limitations and biases (discussed previously) may have contributed to the non-significance of findings. There remains a crucial need for additional studies that examine sex differences with regard to symptoms for both Lyme disease and chronic Lyme disease. Understanding sex-based differences, such as sex-based antibodies to diseases, is becoming more obviously important as the issue of sex bias is much deeper than clinical presentation (Lymedisease.org, 2020). Going forward, it is imperative that individuals research and seek out referrals to Lyme-literate practitioners before spending precious time and money on diagnostics that may be inconclusive and treatments that may do more harm than good.

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