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Endometriosis: an investigation into persistent pelvic pain

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ENDOMETRIOSIS:

AN INVESTIGATION INTO PERSISTENT PELVIC PAIN

by

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ENDOMETRIOSIS: AN INVESTIGATION INTO PERSISTENT PELVIC PAIN

ELENA MARTEL

ABSTRACT

Objectives: This study aims to explore the relationship between central pain amplification and persistent pelvic pain. No previous studies have utilized bimanual pelvic examination findings, in addition to pressure-pain analysis, in an effort to investigate the mechanisms contributing to Chronic Pelvic Pain (CPP) in women with endometriosis.

Methods: Participants included 144 women aged 18-50 years old, diagnosed with CPP and/or surgically-confirmed endometriosis compared to healthy controls. Participants were categorized into four groups, including pain-free endometriosis (Endo Ø Pain), painful endometriosis (Endo + Pain), Chronic Pelvic Pain without endometriosis (CPP Ø Endo), and healthy controls. Pressure-pain Quantitative Sensory Testing (QST) was conducted on all participants to determine levels of pain thresholds. External systemic tenderpoints were assessed utilizing standardized fibromyalgia tenderpoint criteria. A pelvic examination was performed on participants in the gynecological sample in order to assess internal tenderness. One-way ANOVA, chi-square analyses were conducted to assess descriptive variables. Correlation calculations were performed to assess the relationship between pressure-pain thresholds and tenderpoints. Generalized Linear Models (GLM) were conducted to analyze the differences on pressure-pain thresholds and pelvic exam tenderpoints between groups, while controlling for age and number of comorbid chronic pain syndromes (CPS).

Results: Endo Ø Pain had a significantly higher stage of endometriosis and were significantly older than the Endo + Pain group. Healthy controls had less external systemic tenderpoints than all patient subgroups. CPP Ø Endo and Endo + Pain had more CPS than healthy controls and Endo Ø Pain. Pelvic exam tenderpoints ($r= -0.31$, $p < 0.01$) and systemic external tenderpoints ($r = -0.35$, $p <0.01$) were negatively correlated with low pressure-pain threshold. Systemic external tenderpoints were negatively correlated with the high pressure staircase ($r= -0.41$, $p < 0.01$), but pelvic exam tenderpoints were not. The GLMs conducted revealed that Endo Ø Pain had significantly higher low pressure-pain threshold compared to both Endo + Pain (difference = 0.57, $p < 0.01$, CI= 0.12, 1.02) and healthy controls (difference = 0.55, $p < 0.05$, CI= 0.08, 1.02). The CPP Ø Endo group had significantly lower high pressure-pain threshold scores compared to healthy controls (difference = 0.38, $p < 0.05$, CI= 0.05, 0.71). The Endo + Pain group also had significantly lower high-pain thresholds as compared to healthy controls (difference= 0.31, $p < 0.05$, CI= 0.04, 0.58). CPP Ø Endo group had significantly more pelvic tenderpoints compared to Endo Ø Pain (difference = 2.81, $p < 0.001$, CI = 1.36, 4.27); Endo + Pain group also had significantly more pelvic exam tenderpoints than the Endo Ø Pain (difference= 2.10, $p < 0.001$, CI = 0.96, 3.24).

Conclusions: The findings of this study suggest that pain thresholds, as measured by QST pressure-pain testing, are associated with persistent pelvic pain. CPP Ø Endo and Endo + Pain experienced more systemic tenderness, and more CPS than the Endo Ø Pain group and healthy controls. This indicates that chronic pain, not endometrial lesions, are likely responsible for the development of centralized pain amplification. Although the

etiology of endometriosis and CPP is poorly understood, the findings of this study contribute to the idea that central sensitization is associated with the shared underlying pain mechanism in chronic pain syndromes.

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LIST OF ABBREVIATIONS

CEC: Circulating Endometrial Cell

CI: Confidence Interval

CMSI: Complex Medical Symptom Inventory

CNS: Central Nervous System

CPP: Chronic Pelvic Pain

CPS: Chronic Pain Syndrome

CS: Central Sensitization

DIE: Deeply Infiltrating Endometriosis

GBS: Gracely Box Scale

GLM: Generalized Linear Model

IBS: Irritable Bowel Syndrome

MBM: Michigan Body Map

NSAID: Non-Steroidal Anti-Inflammatory Drug

OCP: Oral Contraceptive Pills

QST: Quantitative Sensory Testing

SD: Standard Deviation

SI Joint: Sacroiliac Joint

INTRODUCTION

Endometriosis Background:

Endometriosis is a chronic inflammatory condition affecting 6-10% of the female population globally (Bulletti, Coccia, Battistoni, & Borini, 2010) . Defined as the existence of endometrial glands and stroma outside of the uterus, the diagnosis is accompanied by various side effects, including dysmenorrhea, infertility, dyspareunia, and pelvic pain. The etiology of the disease is unknown, although it is believed to be a multifactorial illness with both epigenetic and environmental influences (Johnson, Hummelshoj, & World Endometriosis Society Montpellier Consortium, 2013). Evidence has shown risk factors related to gynecologic history, family history, nutrition, alcohol use, embryonic factors and race (Riazi et al., 2015). Endometriosis may increase a woman's risk of cancer or autoimmune disorders (Surrey et al., 2017).

In cases of endometriosis, ectopic endometrial tissue can appear throughout the body, causing inflammatory reactions and subsequent pain. The discovery of Circulating Endometrial Cells (CECs) in the peripheral blood of endometrial patients sheds light on the origin of these distant metastases. The invasiveness of these cells leads to recurrence, widespread circulation, and lesion presence in organs such as the lungs (Chen et al., 2017). While endometrial lesions are most common in pelvic organs and the ovaries, extrapelvic lesions have also been reported. More commonly, the gastrointestinal and genitourinary systems are involved, as well as the lungs, diaphragm and breast. In rare cases, endometrial lesions have been discovered in the brain. A 2004 case report by Sarma et al. described the pathological diagnosis of cerebellar endometriosis, and the

accompanying side effects of the central nervous system involvement (Sarma et al., 2004). On MRI, the endometrioma in the brain phenotypically resembled that found in the pelvis in the more common endometriosis patient.

Although the disease typically presents in adolescence, diagnosis often occurs after years of persistent pelvic pain. Endometriosis is typically identified in women of reproductive age, however, cases of pre-menarcheal diagnoses have been reported (Marc R. Laufer, Sanfilippo, & Rose, 2003). Even so, the age of menarche is a variable influencing the onset of the disease. Treloar et al. reported that menarche prior to age 14 strongly correlates with endometriosis. There is an increased risk of developing endometriosis in those with both an earlier age of menarche and onset of dysmenorrhea (Treloar, Bell, Nagle, Purdie, & Green, 2010). Endometriosis is laparoscopically diagnosed in 67% of adolescent girls reporting Chronic Pelvic Pain (CPP), but resistant to standard clinical treatments such as non-steroidal anti-inflammatory drugs (NSAIDs) or oral contraceptive pills (OCPs) (M. R. Laufer, Goitein, Bush, Cramer, & Emans, 1997). The majority of adult patients experience cyclic pelvic pain, while adolescents with endometriosis report acyclic CPP (Dessole, Melis, & Angioni, 2012).

During surgical confirmation of endometriosis, the disease state is staged according to standards set by the American Society of Reproductive Medicine. Peritoneal and ovarian lesions are categorized as red (including red, pink, and clear lesions), white (including white and yellow/brown lesions), and black (including black and blue lesions). In addition to lesion color, depth of tissue implantation (centimeters) is also assessed. Adhesions are categorized as filmy or dense in addition to other pathological findings.

Stage I of the disease is considered “minimal”, Stage II is “mild”, Stage III is “moderate”, Stage IV and V are “severe”. As the severity of the stages increases, so does the implantation depth. Staging is determined based on a weighted point system as the pelvic region is assessed for number, size, and location of endometrial lesions. The presence of disease in other regions outside of the pelvis, including the bowel and urinary tract, are also considered (“Revised American Society for Reproductive Medicine classification of endometriosis,” 1997).

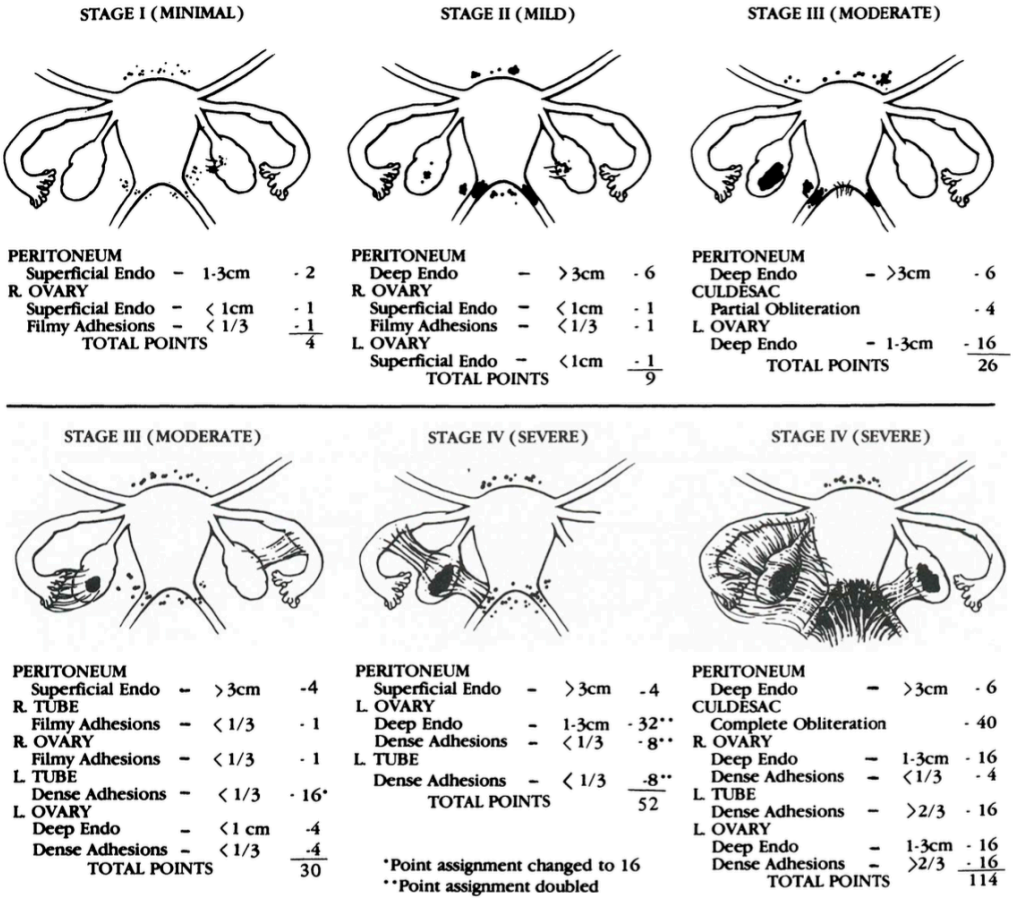

Figure 1: Guidelines for Operative Endometriosis Staging
Revised American Society for Reproductive Medicine classification of endometriosis: 1996 - Fertility and Sterility. (n.d.).

Endometriosis can be classified into three generalized categories based on physical presentation. Deeply infiltrating endometriosis (DIE), the most aggressive presentation of the disease, can be distinguished from peritoneal endometriosis and endometriotic ovarian cysts (Laganà et al., 2017). The lesions must infiltrate >5mm of tissue in order to meet clinical criteria for DIE categorization. An operative report by Chopin et al. found that surgical excision of DIE lesions successfully alleviated pain symptoms post-operatively (Chopin et al., 2005).

The symptoms of endometriosis, including pain, fatigue, infertility, and gastrointestinal problems, vary with disease severity. As previously reported with patients, the severity of symptoms does not positively correlate with advanced stages. A patient with Stage I endometriosis could experience higher pain levels and increased symptoms compared to those with more advanced stages (Laganà et al., 2017). It has been reported that red and clear lesions are the most painful, although they are typically identified in adolescent patients with lower stages of endometriosis (Marc R. Laufer et al., 2003). This finding substantiates the claim that staging has little relationship with symptom burden.

Pain associated with endometriosis can be categorized as dysmenorrhea or CPP. The distinction between the two categories is based on painful days per month, with dysmenorrhea pain lasting 5-13 days monthly, and CPP pain occurring at least 14 days every month (As-Sanie et al., 2013). Approximately 20-25% of the endometriosis population is clinically asymptomatic, experiencing no pain as a result of the disease

(Bulletti et al., 2010). This “pain-free” group of endometriosis patients may still experience symptoms aside from pain, including infertility and abnormal menstruation.

Endometriosis commonly affects reproductive capability in women. Approximately 30-50% of women diagnosed with endometriosis are infertile (Bulletti et al., 2010). Of the infertile women of reproductive age, 50% are also diagnosed with endometriosis (Laganà et al., 2017). Although the underlying mechanism responsible for the significant correlation between an endometriosis diagnosis and infertility is unknown, abnormal pelvic anatomy is a likely cause (Bulletti et al., 2010). Endometrial lesions lead to fibrosis and adhesions that distort the tissue, causing inefficient ovum release (Mounsey, Wilgus, & Slawson, 2006). IVF can be considered an appropriate method of assisted reproduction on a case-dependent basis.

An endometriosis diagnosis introduces various psychological stressors on the patient. Surgical intervention, hospitalizations, medication regimens and delayed diagnoses lead to increased costs. As a result, many patients are left with the financial burden of their disease. On average, it takes 8-11 years of persistent symptomology before an endometriosis diagnosis is made (Riazi et al., 2015)**.** This delay induces both emotional and psychological stress, in addition to financial hardship. Recent literature has reported depression and anxiety as the most common disorders comorbidly diagnosed with endometriosis (Laganà et al., 2017).

Chronic Pelvic Pain Background:

Endometriosis can be differentiated from Chronic Pelvic Pain (CPP), which is defined as persistent, noncyclic pain perceived to be in pelvic structures, with a pain duration of at least six months (Speer, Mushkbar, & Erbele, 2016). Although CPP affects one in seven women, the underlying pathophysiology is generally unknown. The pain cannot be directly attributed to a sole identifiable cause, such as menstruation, gastrointestinal discomfort, or sexual activity. Endometriosis is often primary to CPP, although many cases of consistent pelvic pain are reported without evidence of endometrial lesions. Of the population of women with CPP, only 25-38% are diagnosed with endometriosis (Kontoravdis et al., 1999). In addition to endometriosis, pelvic pain can be secondary fibroids, irritable bowel syndrome, interstitial cystitis, and various other disease states affecting the gastrointestinal and genitourinary systems.

CPP affects 15% of women, and is often accompanied by various comorbidities, including irritable bowel syndrome, abdominal wall pain, and endometriosis (Allaire et al., 2017). Although the etiology of the disease is unknown, sensitization of the central nervous system (CNS) may be the underlying mechanism responsible for the chronicity of the pain. CPP has the potential to cause pain in the gastrointestinal, urologic, gynecologic, and musculoskeletal systems. As a result of the multifactorial nature of the disease, it can be suggested that CNS pain processing, as well as psychologic factors, are to blame. Prior studies have provided evidence of the presence of central sensitization in patients with CPP, but it is currently unknown if this centralized pain amplification is the cause or effect of chronic pelvic pain (Kaya, Hermans, Willems, Roussel, & Meeus,

2013). The literature on pelvic pain has indicated a fundamental role of the CNS in the pathogenesis of CPP. It is unknown if central sensitization is secondary to CPP, or if it is the cause of the chronic pain. Brain morphology abnormalities, overactive nociceptors, and autonomic dysregulation have been reported in studies investigating central sensitization and CPP (Kaya et al., 2013).

Similar to women diagnosed with endometriosis, CPP patients report high levels of anxiety and depression. A study by Lagana et al. determined that depression was present in 86% of CPP patients, but only 38% of women with pain-free endometriosis. Comorbid mood and anxiety disorders further amplify the experience of pelvic pain for endometriosis and PP patients (Laganà et al., 2017). Further studies focused on the effect of psychological factors on the development of CPP could provide greater insight into those at-risk for developing persistent pelvic pain. Women with CPP have a poorer quality of life than pain-free women, but the addition of endometriosis does not affect psychological well-being (Souza et al., 2011).

Diagnosis and Treatment:

Endometriosis Diagnosis:

Misdiagnosis is a common occurrence, due to the nonspecific symptoms of the illness. Shared symptomology with other genitourinary and gastrointestinal illnesses provide various differential diagnoses. A British study by Zondervan et al. reported that the majority of participants with endometriosis were primarily diagnosed with irritable

bowel syndrome (IBS). A third of these women had symptoms of pain for over two years prior to an accurate diagnosis (Zondervan et al., 1999).

Table 1: Differential Diagnoses of Endometriosis

Symptom	Differential Diagnosis
Dysmenorrhea	· Primary · Secondary (e.g. myomas, infection, adenomyosis, cervical stenosis)
Dyspareunia	· Diminished lubrication or vaginal expansion due to insufficient arousal · Gastrointestinal abnormalities (e.g. constipation, IBS) · Infection · Musculoskeletal abnormalities (e.g. pelvic relaxation, levator spasm) · Pelvic Vascular Congestion · Urinary abnormalities (e.g. urethral syndrome, interstitial cystitis)
Generalized Pelvic Pain	· Endometritis · Neoplasms, benign or malignant · Nongynecologic causes · Ovarian torsion · Pelvic adhesions · Pelvic inflammatory disease · Sexual or physical abuse
Infertility	· Anovulation · Cervical Factors · Luteal phase deficiency · Male factor infertility · Tubal disease or infection

Adapted from: Mounsey, A., Wilgus, A., & Slawson, D. C. (2006). Diagnosis and Management of Endometriosis. *American Family Physician*, *74*(4), 594–600.

The diagnosis of endometriosis is an imperfect process. Ultrasound, MRI, clinical evaluation, and medical history are utilized throughout the diagnostic process. Although

laparoscopy is the golden standard of disease state confirmation, less invasive methods should be utilized prior to surgery. MRI and ultrasound imaging techniques can help rule out other causes of pelvic pain, and assist in the diagnosis (Dessole et al., 2012). Biopsy of endometrial tissue has also been introduced as a potentially less-invasive method of diagnosis. Pelvic biopsy has a predictive value between 91% (positive predictive value) and 96% (negative predictive value). Endometrial density has also been found to be fourteen times denser in women with endometriosis . The diagnostic process is the same in both adolescents and adult women with suspected endometriosis.

To date, laparoscopic procedures are performed to visually assess these lesions in an effort to both diagnose and stage endometriosis. Due to comorbid pain syndromes, laparoscopic exploration is often the only method that can reliably eliminate other potential chronic pain illnesses. Staging is based on size, depth, and range of endometrial foci, via direct visualization upon laparoscopy. Histological examination of biopsied lesions is necessary to confirm an endometriosis diagnosis (Laganà et al., 2017).

Prior to laparoscopy, medical history and physical examinations are conducted on patients with persistent pelvic pain. Clinical signs of the disease include musculoskeletal symptoms, fatigue, and menstrual cycle irregularities, including pain. In addition, phenotypic physical exam findings such as masses, decreased uterine mobility, adnexal tenderness, and nodularity can indicate increased risk of endometriosis (Riazi et al., 2015). Typically, the report of pelvic pain and dysmenorrhea leads to the suspicion of endometriosis, requiring further investigation. Patients suffering from dysmenorrhea are 2.5 times more likely to have endometriosis than patients without pelvic pain (Treloar et

al., 2010). Clinical diagnosis of endometriosis, prior to operative confirmation, is more likely with advanced stages of the disease (Riazi et al., 2015). Currently, there is no standardized, fully-validated questionnaire that allows the screening of reproductive-aged women for endometriosis. Clinicians heavily rely on the gathered medical history to rule out low-risk patients, and to refer high-risk endometriosis patients for laparoscopy.

A genetic biomarker of endometriosis has yet to be discovered. As a result, the diagnosis and staging the disease relies on radiology, as well as minimally invasive laparoscopic procedures (Galazis & Miskry, 2018). Currently, researchers are investigating the diagnostic potential of above-average circulating endometrial cells (CECs) quantities in patients with surgically confirmed endometriosis. Unlike chemokines CXCL8, CCL5, and CCL2, which have been analyzed as potential biomarkers for the diagnosis of endometriosis, CEC levels are unaffected by hormonal changes during the menstrual cycle (Chen et al., 2017). This potential biomarker could serve as a more reliable, noninvasive screening and diagnostic tool.

Endometriosis Treatment:

Current treatment is aimed at alleviating the symptoms, specifically pain, associated with endometriosis. Endometriosis is an estrogen-dependent disorder, and evidence has shown some resistance to progesterone (Johnson et al., 2013). The role of estrogen in endometriosis is further highlighted by the cessation of symptoms in post-menopausal women, with the exception of those who utilize hormone therapy (Stratton & Berkley, 2011). The treatment agents include oral contraceptives, androgens, aromatase

inhibitors, selective progesterone receptor modulators, and levonorgestrel-releasing intrauterine system. Some conventional treatments also decrease the disease state itself, although they are not considered curative methods. These treatments include GnRH agonists and antagonists, as well as progesterone antagonists (Bulletti et al., 2010).

When pharmacological methods are ineffective, endometriosis can be treated surgically. The effectiveness of surgery at minimizing pain is unrelated to disease stage at the time of treatment (Abbott et al., 2004). Ablation of lesions can improve fertility in cases of minimal or mild endometriosis (Bulletti et al., 2010). Although surgery does alleviate symptoms for a majority of women, approximately 20% of patients experience no post-operative pain relief (Abbott et al., 2004).

The primary treatment plan for adolescents with endometriosis is targeted at pain management. These methods include analgesics such as NSAIDs and hormonal management, including OCPs, but are often ineffective. GnRH analogue treatments are not indicated for patients under 18 years of age (Dessole et al., 2012). Although previous studies have reported that the use of GnRH analogues in adolescent patients can negatively impact bone mass, more recent research has determined the potential for combined therapy. Divasta et al. discovered GnRH analogues, used in conjunction with norethindrone acetate, negate issues regarding bone mass (Divasta, Laufer, & Gordon, 2007).

A successful treatment plan for the population of adolescents resistant to treatment by OCPs and NSAIDs includes ablation of endometrial tissue via laparoscopy. It is important to note that adolescent endometrial lesions can phenotypically vary from

lesions in adult women with endometriosis. It has been reported that red lesions are predominantly found in teenagers, while blue or brown lesions are found in older patients (Roman, 2010). After laparoscopic ablation is conducted in adolescents, pharmacological treatment should be continued in order to target any microscopic remnants of the disease (Dessole et al., 2012) . Younger patients are more likely to have post-operative lesion recurrence, specifically when conservative surgical methods are used, further validating the need for continued medication adherence (Fedele, Bianchi, Zanconato, Bettoni, & Gotsch, 2004).

Chronic Pelvic Pain Diagnosis and Treatment:

The diagnosis of CPP is also based on both the physical exam and the patient's medical history. Oftentimes, patients undergo laparoscopy, only to rule out an endometriosis diagnosis. CPP can persist, despite targeted gynecologic treatments of endometriosis. Oftentimes, the patients with continued discomfort also suffer from various comorbid pain syndromes, including central sensitization (Allaire et al., 2017). Due to the various comorbidities associated with CPP, treatment plans are often targeted at specific symptoms. Curative treatments of CPP are currently unknown, and the disease is often refractory to current methods. By identifying specific underlying causes, physicians can potentially ameliorate symptomology.

A study by Laufer et al. on 32 adolescents below the age of 22 years investigated the etiology of persistent pelvic pain in those resistant to conventional treatments (M. R. Laufer et al., 1997). Only 69.6% of these patients were found to have endometriosis,

indicating that one third had no known cause of their pain. Considering the side effects of invasive surgery, advances in the clinical diagnosis of CPP are needed to avoid unnecessary operations.

The current use of laparoscopy to both diagnose endometriosis and rule out endometriosis in CPP patients, comes with various well-known side effects. Surgery should be avoided when possible, but other diagnostic measures of endometriosis are limited. Non-operative methods, that can be used clinically, are vital to improving the standard of care of pelvic pain patients. Standardized clinical methods could both expedite diagnosis time and ameliorate the diagnostic process overall. There is no self-reported questionnaire that exists to allow for the symptom-based screening of endometriosis. A validated questionnaire, in addition to a physical pelvic examination, would provide a non-invasive, reliable method of diagnosis. Those patients most at risk based on this assessment could then undergo laparoscopy for staging and confirmation of the disease state. This method would eliminate the need for surgery of those suffering from chronic pelvic pain, who are unlikely to have endometriosis.

Central Sensitization

Central Sensitization (CS) is the hyperactivity of the central nervous system in response to stimuli (Kaya et al., 2013). As defined by Fleming et al., the enhanced sensitivity is secondary to neuronal plasticity that increases sensitivity to future stimuli (Fleming & Volcheck, 2015). The two components of central sensitization are allodynia, when innocuous stimuli cause pain, and hyperalgesia, when mildly painful stimuli cause

heightened pain levels. Allodynia, a painful sensation caused by light touch, has been indicated in patients with advanced stages of endometriosis. The mechanism behind allodynia is poorly understood, although research has implicated both nervous system mechanisms, including phenotypic changes in peripheral and central neurons, as well as immune mechanisms (Lolignier, Eijkelkamp, & Wood, 2015). Evidence has shown that the hypersensitivity is amplified by the spinal dorsal horn, limbic system, and cortical structures (Simis et al., 2015).

There is significant comorbidity between chronic pain conditions, including fibromyalgia and CPP. Overlap not only exists in the diagnosis of these diseases, but also in their symptomology, including abdominal bloating, chronic pelvic pain and anxiety (Fleming & Volcheck, 2015). The commonalities between these chronic pain conditions suggest a shared underlying mechanism, such as CS. Several studies have reported a decreased dermal and muscular pain threshold to various stimuli in CPP patients (Giamberardino, Tana, & Costantini, 2014). Using fMRI, brain activity changes resulting from CS can be visualized, and therefore targeted. Proposed treatments of central sensitization include non-invasive brain stimulation and techniques aimed towards the CNS.

Quantitative Sensory Testing

Quantitative Sensory Testing (QST) allows the delineation of somatosensory functioning, including peripheral nerve fibers and CNS mechanisms (Blankenburg et al., 2010). QST methods utilize the use of thermal, mechanical, electrical and chemical

stimuli to assess detection and pain thresholds. Detection thresholds require the participant to report the first recognition of the applied stimuli. This could include the detection of a von Frey hair (a series of increasingly thick nylon filament), or the detection of a temperature change on thermal testing. Pain thresholds ask the participant to report the maximum application of stimuli that they are able to sustain on their skin. Typical pain thresholds include thermal application, both hot and cold, or pressure using a pressure algometer or a pressure cuff. QST is a validated measure of somatosensory testing in both children (Blankenburg et al., 2010) and adults (Moloney, Hall, & Doody, 2012)

QST has been implicated as a necessary medium when assessing the prevalence of central sensitization in patients with CPP (Giamberardino et al., 2014). A recent study by Antolak & Antolak (2018) utilized QST protocols in men diagnosed with CPP. Abnormal pain thresholds were discovered in the majority of participants, using warm detection thresholds (88%) and pinprick sensation detection (92%) (Antolak & Antolak, 2018). Abdominal pain and detection QST protocols have been utilized in women, without significant findings (Whitaker et al., 2016). To date, there is a substantial gap in the literature regarding the relationship between central sensitization, as measured by QST pain thresholds, and persistent pelvic pain.

Specific Aims and Objectives

This study aims to explore the relationship between central pain amplification and persistent pelvic pain. No previous studies have utilized bimanual examination findings, in addition to pressure-pain analysis, in an effort to investigate central sensitization. Evidence has shown a positive relationship between chronic pain syndromes and centralized pain. The following hypotheses are suggested based on the categorization of CPP as a chronic pain syndrome:

Aim I:

To determine which participant groups (Endo Ø Pain, Endo + Pain, CPP Ø Endo, and Healthy Controls) have the lowest pressure-pain thresholds, as measured by QST testing on the non-dominant thumbnail.

Hypothesis I:

Patients with CPP Ø Endo will therefore report the lowest pressure pain threshold on QST testing. Endometriosis patients with pain will report the next lowest pressure pain threshold. Significant differences between the healthy controls and the Endo Ø Pain groups are not anticipated.

Aim II:

To determine which groups report the highest levels of tenderness during the pelvic bimanual examination.

Hypothesis II:

Patients in the CPP Ø group, as well as the Endo + Pain group, will report the highest number of pelvic exam tenderpoints. Patients in the Endo Ø Pain group will not significantly differ from Healthy Controls on tenderness during the bimanual pelvic exam.

Aim III:

To determine the relationship between pain reported during the pelvic examination and thumbnail pressure pain.

Hypothesis III:

Pressure-pain thresholds will negatively correlate with pain levels reported during the pelvic examination. Patients experiencing high levels of tenderness during the pelvic exam will also report greater sensitivity during the thumbnail pressure assessment.

Aim IV:

To determine the relationship between reported pelvic tenderness, pressure-pain thresholds, and the number of comorbid chronic pain syndromes.

Hypothesis IV:

Patients with a greater number of comorbid chronic pain syndromes will report lower pressure-pain thresholds on QST, as well as increased external and pelvic exam tenderpoints.

METHODS

This study was completed as a collaborative project between the Biobehavioral Pain Laboratory in the Department of Psychiatry and Anesthesia at Boston Children's Hospital (BCH) and the Michigan Medicine Von Voigtlander Women's Clinic at Von Voigtlander Women's Hospital. The Institutional Review Board at the University of Michigan approved this study as a means of collecting data from human participants in an effort to study endometriosis. Potentially eligible participants were approached during their clinical visits at Michigan Medicine. This study utilized obtained surgical notes and imaging, self-reported pain questionnaires, quantitative sensory testing, and a bimanual pelvic examination. All participants provided informed consent prior to the onset of their study visit.

Participants:

Women with CPP and/or endometriosis, aged 18-50 years were recruited from local advertisements and a pelvic pain referral center. Age-matched healthy controls were recruited from local advertisements. All study participants received $40 compensation.

Phone interviews were conducted to screen potential participants. Requirements for the pain subgroups included a surgically confirmed history of endometriosis or chronic pelvic pain, as well as a performed pelvic surgery within the past five years. Pain-free women with no history of pelvic pain, endometriosis, or chronic pain syndrome were recruited as healthy controls. Potential participants were screened with the M.I.N.I.

International Neuropsychiatric Interview, a diagnostic interview used to assess psychiatric illness (Sheehan et al., 1998). Potential healthy controls with psychotic, mood, or anxiety disorders were excluded. Studies have shown that up to 70% of patients diagnosed with a chronic pain syndrome also suffer from comorbid depression and anxiety (de Heer et al., 2014). As a result, endometriosis and chronic pelvic pain participants with comorbid mood or anxiety disorder were included in this study, but those meeting criteria for psychotic illness were excluded. A history of suicide attempt or substance abuse within two years of the study were additional exclusionary criteria for this study. Participants with severe physical impairment were also excluded.

Various measures were taken to minimize the influence of hormonal variability in all participants. Included participants had no history of prior hysterectomy or oophorectomy. Pregnant, lactating, or menopausal women were also excluded from participation. For this study's purposes, menopause was defined cessation of menses for 12 consecutive menses, unrelated to exogenous hormonal influences. Additionally, all QST was performed on participants between days 2 and 10 of the menstrual cycle, in an effort to decrease the effects of hormone fluctuation. Participants taking hormonal contraceptives were able to complete study visits at any point in their menstrual cycle. Participants were asked to abstain from opioid analgesic use within 48 hours of the study visit.

Participants were categorized into groups based on self-reported questionnaires, medical history, and diagnosis at surgery. Classifications included endometriosis with pain (Endo + Pain), pain-free endometriosis (Endo Ø Pain), and chronic pelvic pain

without endometriosis (CPP Ø Endo). Endometriosis patients suffering from both dysmenorrhea and CPP were grouped into the Endo + Pain group. Although prior studies have further separated these endometriosis patients into two pain subgroups (endometriosis with CPP and endometriosis with dysmenorrhea), no significant differences were found between the groups (As-Sanie et al., 2013). As previously defined by this group, CPP is defined as moderate to severe pelvic pain that is ≥ 4 (on a 0-10 pain rating scale where 10 is the most painful), lasting at least 6 months, and occurring at least 14 days monthly. Dysmenorrhea is defined as moderate to severe pelvic pain that is ≥ 4 (on a 0-10 pain rating score), lasting at least 6 months, but only occurring 5-13 days each month. Dysmenorrhea pain typically occurred around the time of menstrual bleeding, whereas CPP is typically unrelated to menses. Participants categorized in the Endo Ø Pain group reported fewer than 4 days of moderate or severe pelvic pain per month.

The majority of patients were previously diagnosed, prior to the study, at a different medical institution. When available, surgical biopsy and pathology documentation was utilized to corroborate diagnoses. Operative case reports were completed, depicting the physical characteristics of the endometriosis, including location, severity and pelvic adhesive disease. Surgical biopsy and pathology were not required, but were utilized when available. Any presence of endometriosis was staged in accordance with the revised American Fertility Society endometriosis scoring system ("Revised American Society for Reproductive Medicine classification of endometriosis,"

1997) For the purposes of this study, operative reports were reviewed by an endometriosis expert, blind to study results.

All participants completed both demographic and medical history questionnaires. Medical questionnaires assessed surgical history, medication use, and menstrual pattern. The Complex Medical Symptom Inventory (CMSI) was administered to evaluate participants for the existence of chronic cormorbid pain syndromes (CPS), including fibromyalgia, chronic fatigue syndrome, interstitial cystitis, irritable bowel syndrome, chronic lower back pain, chronic migraine headache, temporomandibular disorder, and vulvodynia (Williams & Schilling, 2009). Evidence has shown that the above listed chronic pain syndromes are associated with CNS pain amplification (Phillips & Clauw, 2011). As a result, many chronic pain syndromes have a shared underlying mechanism. Participants diagnosed with endometriosis and/or CPP, as well as a CPS, were included in this study, due to the high prevalence of comorbid chronic pain syndromes. Potential healthy controls were excluded if they met the criteria for any pain syndrome, as assessed by the CMSI.

Participants in the three pelvic pain groups were asked to complete additional questionnaires aimed at assessing their history of pelvic pain, including severity, pattern, symptom characteristics and prior treatment plans. Participants numerically rated the severity of their pelvic pain and physical discomfort on a scale of 0-10 (0 as no pain, 10 as the worst pain). They also reported the average number of days per month in which they suffered from moderate to severe pelvic pain. Pain patients provided written consent allowing the release of their most recent operative reports, which were then reviewed.

The reviewer of the operative reports was blinded to all other study data, and had a significant expertise in surgical evaluation of gynecological disorders, including endometriosis.

Quantitative Sensory Testing:

In order to determine individual sensory and pain thresholds, Quantitative Sensory Testing (QST) was performed on all participants. As defined by Cruz-Almeida and Fillingim (2018), "QST refers to a group of procedures that assess perceptual responses to systematically applied and quantifiable sensory stimuli for the purpose of characterizing somatosensory function or dysfunction." For the purposes of this study, a research coordinator administering the QST testing was blinded to all other study data.

Prior research provided a specific QST staircase method that allowed the assessment of pressure-pain in fibromyalgia patients (Harris et al., 2006). Previously published studies described the adaptation of this QST staircase method for chronic pelvic pain patients (As-Sanie et al., 2013). The QST was conducted by administering ascending pressure stimuli to the non-dominant thumbnail of all participants in an effort to assess centralized pain amplification. The application of pressure allowed the quantification of self-reported pressure-pain sensitivity, using the Gracely Box Scale (GBS), a 0-20 numerical descriptor scale (Gracely, Petzke, Wolf, & Clauw, 2002). The pressure apparatus uses calibrated weights, applied to a hydraulic piston that produces controlled pressure using water-filled tubing to a second piston with a 1 cm^2 rubber probe. As an ascending staircase, the applied pressure was applied in an increasing

manner, from 0.25 kg/cm^2 up to a maximum pain tolerance, or to 10 kg/cm^2. Ascending pressure was applied in increments of 0.25-0.5 kg/cm^2. The GBS was used to record perceived pain intensity at each pressure along the ascending pressure series. These results were used to determine the starting point for the individualized staircase pressure administration.

The response-dependent multiple random staircase pressure application was then applied to the non-dominant thumbnail. The 36 stimuli delivered were applied at 20 second intervals to the thumb. Based on the participant's self-reported pain rating, the intensity (kg/cm^2) needed to elicit a specific response was determined. A computer program utilized these pain ratings to adjust the stimulus intensity, randomly applying the pressure needed to elicit "faint", "mild", and "slightly intense" pain. According to the GBS, faint pain measures as a 0.5, mild pain as a 7.5, and slightly intense pain as a 13.5 on the 0-20 descriptor scale. Currently, no standardized norms for pressure-pain analysis in endometriosis and chronic pelvic pain patients exist. A prior fibromyalgia study utilizing QST provided evidence that a 1.5 kg/m^2 difference in pressure (SD= 2.0) will induce pain in chronic pain patients, as compared to healthy controls (Giesecke et al., 2003). Accordingly, this study utilized this value when determining the pressure increments (kg) for the ascending staircase.

Physical Exam:

In order to guarantee consistency throughout the study, every physical exam was performed by Sawsan As-Sanie, MD, a board-certified obstetrician-gynecologist. A

standardized case report used to assess location and summary of internal and external tenderness was completed throughout the physical examination. Prior to the exam, participants were given a figure of an anatomical figure (shown below), and asked to mark an 'X' on the area of maximal abdominal tenderness.

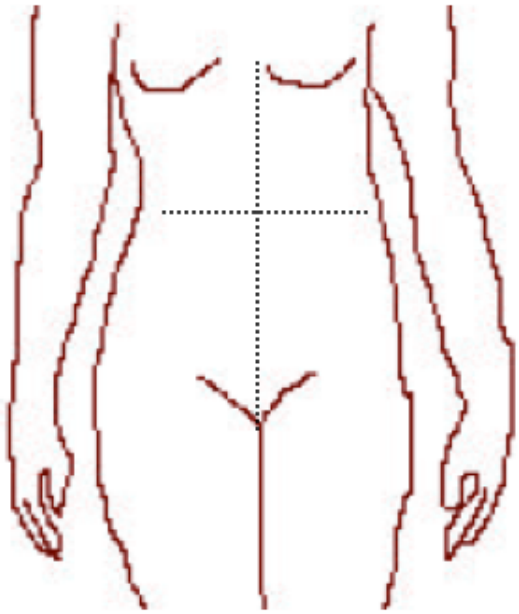

Figure 2: Abdominal Quadrants

External systemic tenderpoints were assessed by the standardized criteria for fibromyalgia diagnoses, in an effort to assess centralized pain. Due to a lack of imaging or lab test for the diagnosis of fibromyalgia, a physical exam consisting of 18 potential tenderpoints is widely used. For the purposes of this study, the fibromyalgia tenderness exam was used to evaluate centralized pain mechanisms in the patient population, as

compared to healthy controls. These sum of the individual's tenderpoints were then compared to the pressure-pain score the received using the staircase QST methodology.

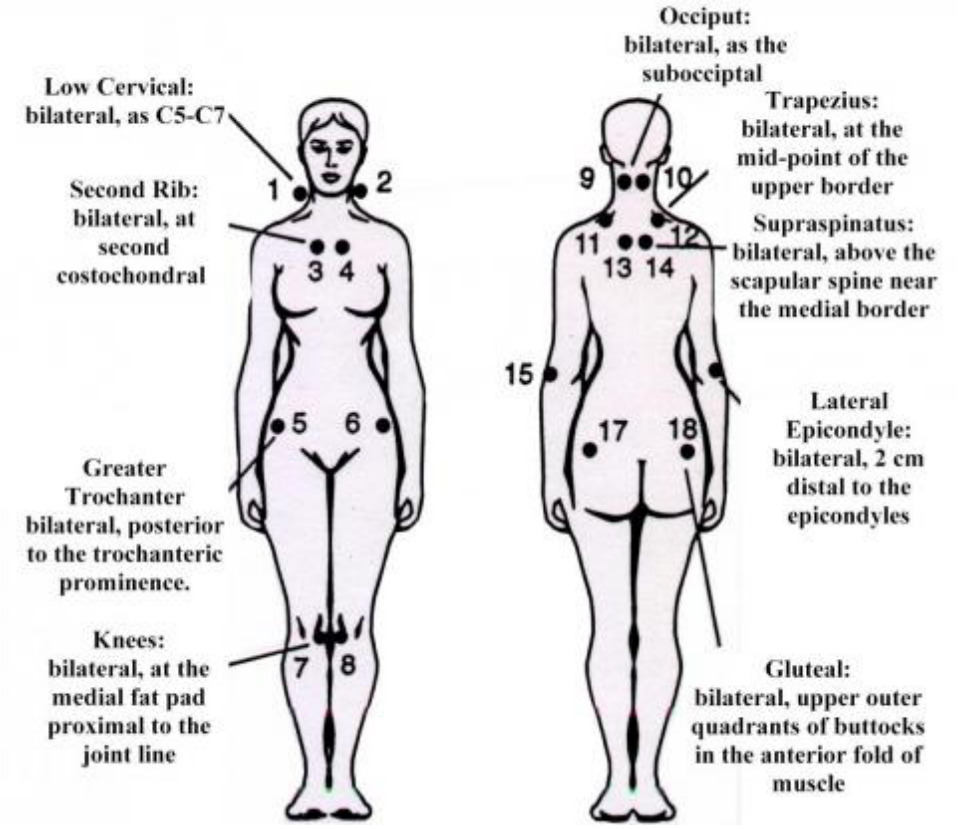

Figure 3: Fibromyalgia Tenderpoints
Leskowitz, E. (2008). Energy-Based Therapies for Chronic Pain (pp. 225–241). https://doi.org/10.1007/978-1-59745-344-8_11

External Tenderness:

An external physical exam was performed on the gynecological participant sample to determine areas of tenderness and discomfort. This

participant sample included those diagnosed with chronic pelvic pain and/or endometriosis, but did not include healthy controls. To assess abdominal tenderness, the four quadrants of the abdomen were palpated manually as the patient was asked to report any tenderness experienced. Using a standardized physical examination protocol, areas of potentially reported abdominal tenderness included right upper quadrant (RUQ), left upper quadrant (LUG), right lower quadrant (RLQ), left lower quadrant (LLQ), suprapubic, and periumbilical regions.

During the study visit, allodynia was defined as painful sensation experienced during the light touch across the abdominal wall. The pattern of abdominal tenderness was also assessed upon external exam, and was categorized as diffuse, focal, not localized, or no-pain. “Diffuse” tenderness was defined as crossing into more than one quadrant. “Focal” tenderness was defined as being reproducible in only one small area (<1 inch in diameter). “Not localized” tenderness was defined as non-reproducible pain on abdominal exam. The number of trigger points was also determined during external physical exam. The focal point of tenderness was assessed, as well as reproduction of 50% of the chief complaint. It was also determined if these trigger points worsened with abdominal flexion. Pubic symphysis tenderness and sacroiliac (SI) joint tenderness was also evaluated.

Internal Tenderness:

Participants in the gynecological sample (CPP Ø Endo, Endo + Pain, Endo Ø Pain) underwent a single digit pelvic examination, as well as a bimanual exam. The single digit exam was used to identify vulvar or vestibular tenderness, pain near the ischial spine, bladder/urethral tenderness, as well as pelvic floor tenderness. Using a pressure algometer, pelvic floor sensitivity was assessed, targeting the levator ani, obturator internus, and piriformis muscles. Approximately 2kg of pressure was applied to specific internal regions, and participants were asked to give a score from 0-10, where 0 indicated no pain whatsoever, and 10 indicated the worst imaginable pain. Currently, there is no standardized protocol that can be utilized to examine these values.

The standard bimanual exam was also performed in order to identify any potential abnormal findings. Central uterine tenderness was defined as pain with moving of the cervix, uterosacral ligament, and/or the uterus. Uterine size (normal versus enlarged) and mobility (freely limited versus limited mobility) were assessed. Uterosacral nodularity, adnexal mass and tenderness, and rectovaginal masses were also investigated via internal palpation. The pattern of identified pelvic tenderness was categorized as “diffuse”, “focal”, “not localized”, or “no pain” on the standardized exam report. Location of the ‘worst’ pain was determined as well as a brief summary of the discomfort experienced by the patient in that region. “Worst” pain locations included abdominal wall, pelvic

floor, vagina, vulva, uterus/cervix, suprapubic, and adnexa. These six regions were displayed in a figure presented on the standardized case report (Figure 4).

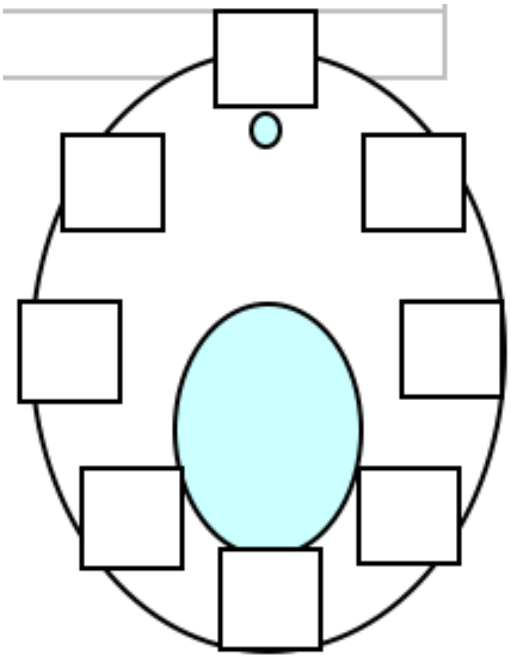

Figure 4: Areas of Pelvic Exam Tenderness for Case Report

Statistical Analyses:

All analyses were performed using SPSS, version 23 (IBM Corp., Armonk, NY). ANOVA and Chi-square analysis was performed to see how participant subgroups varied for both demographics and specific medical variables. Continuous variables between groups were analyzed using one-way ANOVA tests. Categorical data, such as race, education, and occupation was analyzed using the Chi-squared test.

Correlations

Two correlational analyses were conducted in order to explore significant differences between participant subgroups, when looking at the association between the pressure staircase and reported tenderpoints (internal and external). A primary score for pelvic exam tenderpoints was calculated as the sum of the number of areas identified as tender during the pelvic exam. Correlations were run between the total external tenderpoints and all (low, medium, and high) pressure-pain QST. Correlations were also run between the pressure pain staircases and total internal tenderpoints, as measured upon physical exam.

Generalized Linear Models

Generalized Linear Models (GLM) were used to further explore the physical exam findings on low and high pressure-pain thresholds. Two separate generalized linear models were performed, one using the individualized lowest staircase QST pressure (used to indicate faint discomfort), and another using the individualized highest pressure (indicating "slightly intense" pain). Both pressure pain variables were natural-log transformed to create two versions of the pain rating score. Both age and number of comorbid chronic pain syndromes were controlled throughout all GLM analyses. A GLM was also conducted to examine variation across the gynecological groups (CPP Ø Endo, Endo Ø Pain, Endo + Pain) on pelvic exam tenderpoints. The total pelvic tenderpoints

score was natural log transformed prior to GLM analysis. The healthy controls did not undergo a pelvic exam, and were therefore excluded from this GLM.

Analyses were conducted using Version 23 of SPSS for Windows. All p-values were 2-sided and considered statistically significant if less than 0.05.

RESULTS

Participant Sample:

A total of 144 pre-menopausal women participated in this study. The participants included 30 healthy controls, and 122 women diagnosed with CPP or endometriosis. Due to missing data, 8 women in the gynecological sample were excluded from data analysis, leaving 30 healthy controls and 114 patient participants. Based on clinical diagnoses, relevant surgical evidence, and medical history, participants were categorized into 3 subgroups. Of the gynecological sample, 35 women were categorized in the endometriosis without pelvic pain group (Endo Ø Pain), 57 women were categorized as endometriosis with pelvic pain, including dysmenorrhea and CPP (Endo + Pain), and 22 were categorized as Chronic Pelvic Pain without endometriosis (CPP Ø Endo).

The sample was predominantly caucasian (83.8%). The women in this sample were aged 18-50 years (mean= 31 years, SD= 7.99). One-way ANOVAs looking at groups differences in age were conducted; $F(3, 140) = 5.53$, $p = 0.001$). Post-Hoc Scheffe tests were conducted, showing significant mean age differences. A significant age difference was found between Endo Ø Pain (n=35, mean= 35.45 years) and Endo + Pain (n=57, mean = 28.75 years) subgroups, where patients without pain were significantly older (difference=6.79, $p < 0.05$). No other between-group age comparisons were found to be statistically significant.

When comparing patient subgroups, there were no significant differences in number of prior pregnancies, abdominal surgeries, or pelvic pain treatment surgeries. No

differences between groups were found regarding the current use of hormonal contraception, and of these women, all were taking either estrogen-progestin or progestin-only contraceptives (As-Sanie et al., 2013). There were no significant differences in body mass mass index (BMI) between participant subgroups (n= 149, mean = 26.2, range= 18.61- 42.92). Chi-square analysis were used to assess categorical variables. No significant differences were found in occupation, education, or race between groups.

Table 2. Participant Characteristics

Variable	***Frequency***
Age (years)(range, M) N= 14, [SD]	18-50, 31.1 [8.17]
Race (N= 142)	
White	83.8
Asian or Asian American	3.5
Black or African American	4.9
Native American	0.7
Other	7.0
BMI (range, M) N=149	18.6-42.9, 26.2
Pain Duration (years) (range, M, [SD]) N= 109	5.2-7.6, 6.39 [6.25]
Time Since Last Surgery (years) (range, M, [SD]) N= 111	1.3-1.9, 1.55 [1.61]

NOTE: Values represent percentages unless otherwise noted.

Medical Variables:

A One-way ANOVA analysis was conducted to examine years since last surgery between Endo Ø Pain, Endo + Pain, and CPP Ø Endo subgroups; ($F(2, 108)= 3.691$, $p= 0.028$). Post-Hoc Scheffe tests determined that Endo + Pain (n=54, mean= 1.80) had significantly more time (years) since last surgery than the CPP Ø Endo (n=22, mean= 0.74) subgroup (difference = 1.06, $p < 0.05$). CPP Ø Endo subgroup had surgical intervention more recently than the Endo + Pain subgroup. No other significant differences were found between subgroups when considering years since surgical intervention. Healthy controls were not included in this analysis.

No significant differences in pain duration (years) were found between groups. Post-Hoc Scheffe tests were conducted to compare endometriosis stage between the two endometriosis patient subgroups. It was discovered that the Endo Ø Pain group (n= 35, mean= 2.74) had a significantly higher stage of endometriosis than the Endo + Pain (n= 55, mean= 2.09) group at the time of the initial study visit (difference= 0.652, $p <0.05$).

Using the fibromyalgia criteria, external systemic tenderpoints (range= 0-18) were tested throughout the body. One-way ANOVA was conducted to determine differences between participant subgroups ($F(3,104)=10.786$, $p = 0.000$). Healthy controls had significantly less tenderpoints (n= 30, mean= 1.47) than all patient subgroups, including Endo Ø Pain (n= 29, mean= 4.83), Endo + Pain (n= 42, mean= 6.93), and CPP Ø Endo (n= 7, mean= 9.00). Healthy controls had significantly less tenderpoints than Endo Ø Pain (difference= -3.361, $p <0.05$), Endo + Pain (difference = -5.462, $p < 0.01$), and CPP

Ø Endo (difference = -7.533, $p < 0.05$). No significant differences were found between the patient subgroups.

Chronic comorbid pain syndromes (CPS) were also analyzed using one-way ANOVA analyses. Significant differences between healthy controls (mean= 0.07) and Endo + Pain (mean= 1.73) were found. Participants with CPP Ø Endo (mean= 2.29) also had significantly more CPS than healthy controls. No significant differences were found between controls and the Endo Ø Pain (mean= 0.63) groups. Significant differences were found between the Endo Ø Pain and Endo + Pain subgroups, as well as the Endo Ø pain and CPP Ø Endo subgroups. Participants with CPP were found to have greater comorbid chronic pain syndromes than both healthy controls and participants with endometriosis, but no pain. During the physical exam, the periumbilical and suprapubic regions were also assessed, although no participants, in any subgroup, reported tenderness in these areas. Abdominal skin allodynia was also unreported by all participants.

Table 3: Medical Variables Across Groups

Variable	Endo Ø Pain Mean (*SD*) [N]	Endo + Pain Mean (*SD*) [N]	CPP Ø Endo Mean (*SD*) [N]	Healthy Controls Mean (*SD*) [N]	*F* Ratio
Age	35.54 (*7.98*) [35][b]	28.75 (*7.40*) [57][a]	30.77 (*7.35*) [22]	30.77 (*8.64*) [30]	5.529***
Pain duration	4.47 (*6.31*) [34]	7.71 (*6.24*) [56]	5.96 (*5.67*) [19]		3.006
Endo stage	2.74 (*1.09*) [35][b,c]	2.09 (*1.30*) [55][a,c]	0 (*0*) [22][a,b]		44.129***
External systemic tenderpoints	4.83 (*4.56*) [29][d]	6.93 (*5.34*) [42][d]	9.00 (*5.57*) [7][d]	1.47 (*2.11)* [30][a,b,c]	10.786***
Years since last surgery	1.67 (*1.64*) [35]	1.80 (*1.63*) [54][c]	0.74 (*1.32*) [22][b]		3.691*
Comorbid pain syndromes	0.63 (*0.88*) [35][b,c]	1.73 (*1.78)* [55][a,d]	2.29 (*2.28)* [21][a,d]	0.07 (*0.25)* [30][b,c]	13.917***

*Note. Baseline variables that differ significantly at *p<.05, **p<.01, ***p<0.001 across groups are indicated with the superscript of the differing group.*
a= Endo with no pain, b= Endo with pain, c= CPP with no endo, d= healthy controls

Correlations

Pearson correlations were conducted in order to analyze the correlational relationship between total pelvic exam tenderpoints, systemic external tenderpoints, and pressure- pain staircases (low and high; kg). Significant correlations were found between various continuous variables.

Pelvic exam tenderpoints were negatively correlated with low pressure-pain threshold ($r= -0.31$, $p < 0.01$). Systemic external tenderpoints were also negatively correlated with low pressure pain threshold ($r = -0.35$, $p <0.01$). These negative correlations signify that the lower the threshold for low pressure-pain upon QST testing

moderately correlates with a higher number of both systemic and pelvic tenderpoints. The high pressure staircase was found to be negatively correlated with the total systemic external tenderpoints ($r= -0.41$, $p < 0.01$). For the high pressure staircase, there was no significant correlation to pelvic examination tenderpoints. The total number of systemic external tenderpoints was positively correlated ($r= 0.27$, $p < 0.05$) with pelvic exam tenderpoints. The high pressure staircase stimulus was positively correlated with the low pressure staircase (0.53, $p < 0.01$).

Table 4: Correlation between Tenderpoints and Pressure-Pain

		Pelvic Exam Tenderpoints	Low Staircase (kg)	High Staircase (kg)	External Systemic Tenderpoints
Pelvic Exam Tenderpoints	Pearson Correlation	1	-.314**	-.163	.267*
	N	95	89	89	69
Low Staircase (kg)	Pearson Correlation	-.314**	1	.533**	-.354**
	N	89	147	146	110
High Staircase (kg)	Pearson Correlation	-.163	.533**	1	-.414**
	N	89	146	146	110
External Systemic Tenderpoints	Pearson Correlation	.267*	-.354**	-.414**	1
	N	69	110	110	118

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Generalized Linear Models

Generalized Linear Models (GLM) were conducted to explore significant differences between groups on pressure-pain thresholds (low and high), while controlling for age and number of comorbid chronic pain syndromes (CPS). In this model, there are factors, which are categorical variables, as well as covariates, which are continuous variables. In the current model, covariates included age and CPS. The low pressure-pain staircase and high pressure-pain staircase was natural log transformed in order to normalize the data set, prior to conducting GLMs with the dependent variables. The GLM was conducted three separate times, with the dependent variables including low pressure-pain threshold, high pressure-pain threshold, and total pelvic exam tenderpoints.

Low-Pressure Threshold:

The GLM was conducted to examine low pressure-pain threshold as a dependent variable across the four participant groups with age and CPS as covariates. The mean low pressure-pain threshold was 1.03 kg, SD= 0.91. Significant differences emerged between the groups on this model, holding age and CPS constant. Bonferroni post-hoc analyses revealed the following significant differences in pairwise comparisons: Endo Ø Pain had significantly higher low pressure-pain threshold compared to both Endo + Pain (difference = 0.57, $p < 0.01$, CI= 0.12, 1.02) and healthy controls (difference = 0.55, $p < 0.05$, CI= 0.08, 1.02). The omnibus test, used to compare the fitted model against the intercept-only model, determined that the model is significant ($p < 0.01$).

High-Pressure Threshold:

The GLM was conducted to examine high pressure-pain threshold as a dependent variable across the four participant groups with age and CPS as covariates. The mean high pressure-pain threshold was 4.10 kg, SD= 1.96. Significant differences emerged between the groups on this model, holding age and CPS constant. Bonferroni post-hoc analyses revealed the following significant differences in pairwise comparisons: the CPP Ø Endo group had significantly lower high pressure-pain threshold scores compared to healthy controls (difference = 0.38, $p < 0.05$, CI= 0.05, 0.71). The Endo + Pain group also had significantly lower high-pain thresholds as compared to healthy controls (difference= 0.31, $p < 0.05$, CI= 0.04, 0.58). The omnibus test determined that the model is significant ($p < 0.05$).

Pelvic Exam Tenderpoints:

A GLM was conducted to explore the differences between the gynecological participant groups (CPP Ø Endo (n= 16), Endo Ø Pain (n=28), and Endo + Pain (n=41)) on pelvic exam tenderpoints, once again controlling for age and CPS. Bimanual pelvic examinations were not performed on healthy controls in this study, therefore healthy controls were excluded from this generalized linear model. The mean total pelvic exam tenderpoints for the sample was 2.54 (range= 0-9, SD= 2.25). Bonferroni post-hoc analyses revealed the following significant differences in pairwise comparisons: the CPP Ø Endo group had significantly more pelvic tenderpoints compared to Endo Ø Pain

(difference = 2.81, $p < 0.001$, CI = 1.36, 4.27); Endo + Pain group also had significantly more pelvic exam tenderpoints than the Endo Ø Pain (difference= 2.10, $p < 0.001$, CI = 0.96, 3.24). The omnibus test determined that the model is significant ($p < 0.001$).

DISCUSSION

The high prevalence of persistent pelvic pain among women of reproductive age creates a demand for improved understanding of endometriosis and CPP. In the general population, the incidence of endometriosis in women of reproductive age is estimated to be 10% (Mounsey et al., 2006). Studies have shown that CPP in women aged 18-50 directly accounts for $881.5 million per year in medical costs (Mathias, Kuppermann, Liberman, Lipschutz, & Steege, 1996). Between associated pain, delayed diagnosis, and treatment aimed at symptoms, clinical advances are needed.

This study aimed to explore the relationship between centralized pain and pelvic pain, in women diagnosed with endometriosis and/or CPP. By using pressure-based quantitative sensory testing (QST), pressure-pain thresholds were determined for all participants. Physical examinations, both internal and external, allowed tenderness to be assessed clinically across the gynecological sample. Similar to endometriosis, the pathogenesis of CPP is not fully understood. Research has indicated that central sensitization has a role in the underlying mechanism of the disease, specifically in the chronic nature of the pain (Kaya et al., 2013).

The standardized protocol used for the pelvic (single digit and bimanual) exams allowed for consistency throughout this study. Additionally, all physical exams were conducted by the same physician, further standardizing the process. The researcher that administered the QST to the participants was blinded to all other study results, eliminating experimenter bias when determining pressure-pain sensitivity. Another strength of this study is the accuracy of participant categorization into the three patient

groups. The use of medical history, surgical diagnoses, operative case reports, and relevant pathology allowed confidence in group classification by limiting the potential for misdiagnosis. The QST protocol was performed by a single researcher, blinded to all other study results, providing consistency and eliminating bias. Additionally, a single physician conducted the bimanual pelvic examinations, adding an additional layer of consistency throughout all patient visits.

Endo Ø Pain patients were found to be significantly older than Endo + Pain patients. There are various possible explanations for this finding, including poorly age-matched participant recruitment, age at diagnosis, and delayed diagnosis. Previous studies have found that the probability of diagnosis increases with age, as does the severity of symptoms (Bulletti et al., 2010). Future research should continue to look age age as a risk factor for pain associated with endometriosis. As reported by The Endometriosis Association, two thirds of women with endometriosis, regardless of age at diagnosis, report the onset of pelvic pain prior to 20 years of age (Dessole et al., 2012). Future research focusing on adolescents most at risk for endometriosis and CPP could provide further insight into the disease.

It was determined that CPP Ø Endo group had surgery more recently than the Endo + Pain group. Pain duration (in years) between these groups did not differ, indicating that the CPP Ø Endo group had a greater delay in diagnosis than the Endo + Pain group. Both patient groups were experiencing pain as a major symptom of their illness, but those with endometriosis received an operatively confirmed diagnosis earlier. This finding suggests that women with Chronic Pelvic Pain, but without endometriosis

lesions, have an even harder time receiving accurate clinical diagnoses. Prior studies have determined that the probability of clinical diagnosis increases with endometriosis stage (Riazi et al., 2015), further delaying the diagnostic process for women with CPP without secondary endometriosis.

Pain-free endometriosis participants were found to have a higher stage of the disease than the endometriosis group with CPP or dysmenorrhea. Several studies have found no relationship between endometriosis stage and symptom burden (Laganà et al., 2017). Similarly, a recent study on post-operative pain in spinal patients found that pain was unrelated to severity of disease (Sieberg et al., 2013).

External systemic tenderpoints were assessed on the physical exam, as a method of determining centralized pain. As expected, healthy controls had significantly fewer external tenderpoints than all patients in the gynecological sample. No differences were found between patient groups, suggesting that all diagnoses of pelvic pain shared an underlying centralized pain mechanism. Many patient participants also reported comorbid chronic pain syndromes (CPS), which could also be related to the development of central sensitization. Research has shown a high prevalence of central sensitization in patients with CPS, including fibromyalgia (FM), IBS, and interstitial cystitis (Phillips & Clauw, 2011).

Analysis of CPS discovered that Endo + Pain and CPP Ø Endo had significantly more comorbid illnesses than healthy controls. Participants of these two groups share a common symptom of pain, indicating a similar centralized pain amplification process. The Endo Ø Pain did not differ significantly from healthy controls, suggesting that the

associated pain, not the endometriosis diagnosis, is responsible for the shared underlying mechanism of CPS. This finding suggests that the pain, not the endometrial lesions, are responsible for the central pain amplification secondary to persistent pelvic pain. Women suffering with painful endometriosis, whether experiencing dysmenorrhea or chronic pelvic pain, are more likely to have a comorbid secondary CPS than pain-free endometriosis patients.

Pelvic examinations, including abdominal palpation and internal tenderness assessment, were performed on patients in the gynecological sample. External tenderpoints for the pelvic exam included the right upper quadrant, left upper quadrant, right lower quadrant and left lower quadrant abdominal regions. Internal tenderpoints were assessed by single-digit and bimanual exams. The sum of pelvic exam tenderpoints was significantly correlated with the total number of systemic external tenderpoints, used to assess centralized pain. This finding suggests that internal tenderness is another identifier of central sensitization. Future studies on patients with chronic pain could utilize an internal tenderpoint assessment, via pelvic exam, in conjunction with the current external tenderpoint identification protocol. Generalized linear models analyzing total pelvic exam tenderpoints between groups found that both the CPP group without endometriosis and the painful endometriosis group had more tenderpoints than the pain-free endometriosis sample. As a result, internal tenderpoints can be a useful measure of identifying both chronic pelvic pain and central sensitization.

Pain thresholds, as measured by QST pressure-pain testing, were found to be significantly correlated with both pelvic exam tenderpoints and external systemic

tenderpoints. It was determined that a higher number of tenderpoints, identified both externally and internally, correlated with lower the pressure-pain threshold. This finding suggests that patients experiencing clinical tenderness also have lower thresholds for pressure in non-painful areas, such as the thumbnail. Similar findings were identified for systemic external tenderpoints on the higher pressure-pain thresholds. For the high pressure staircase, no relationship was found with pelvic exam tenderpoints.

When pressure-pain was further delineated between groups, it was found that pain-free endometriosis patients had higher low-pain thresholds than painful endometriosis participants. This finding suggests that CPP and dysmenorrhea associated with endometriosis could lead to the development of central sensitization, not the endometriosis diagnosis itself. When analyzing the high-pressure pain threshold between groups, the CPP without endometriosis and the painful endometriosis groups had lower pain thresholds than healthy controls. This further confirms that the addition of endometriosis does not affect amplified pain measured by QST, but pelvic pain does. These differences cannot be attributed to other significant differences between groups, including age and CPS.

No participants reported pain in the periumbilical or suprapubic regions during the physical exam, regardless of their group categorization. This finding may suggest that these regions are not affected by centralized pain amplification. Additionally, no participants reported skin allodynia during the examination. Previous studies have found a positive correlation between reduced pressure-pain threshold and abdominal and perineal allodynia in women with CPP (Jarrell, Malekzadeh, Yang, & Arendt-Nielsen,

2015). This study did not test for perineal allodynia, therefore it is unknown if the participants had hypersensitivity to light touch in this region. Bedside testing of central sensitization by assessing allodynia has been validated, and should be continued with pelvic pain patients to further understand this symptomology (Jarrell et al., 2015). Prior research has discovered that phenotypic changes in both neurons and immune cells are implicated in the etiology of allodynia, a discovery that further connects the CNS and centralized pain (Lolignier et al., 2015).

The delay in diagnosis is a source of major frustration for women suffering from endometriosis. Early diagnosis will not only lessen the financial burden of unnecessary testing, but also years of suffering without adequate treatment. Non-invasive clinical diagnosis is currently difficult, but a reliable, efficient method would deliver more accurate and earlier initial diagnoses. Currently, there is no standardized approach to the physical exam that allows identification of pelvic pain patients most at-risk for endometriosis. Examination of both external genitalia as well phenotypic findings on the internal exam are focused on nodularity, visible lesions, or masses, not tenderness (Riazi et al., 2015). By focusing future research on pain, instead of lesions, the underlying mechanism responsible for chronic pelvic pain and endometriosis could be identified.

Limitations

Due to the state demographics of Michigan, the participant population was predominantly caucasian. Prior studies have provided conflicting evidence regarding the effects of race and ethnicity on the prevalence of endometriosis, so a more diverse sample

size would provide more evidence on the matter. It has been reported that endometriosis does not vary based on ethnicity (Bulletti et al., 2010). Other studies have reported that the risk of the disease is increased in patients of Asian descent (Riazi et al., 2015). A larger sample size would have allowed more sophisticated models of statistical analysis. For example, Generalized Linear Models allows the analysis of interaction effects and predictors, but a larger participant population would be needed to utilize these analyses. Missing data on the physical exam, predominantly for the CPP Ø Endo group, contributed to the smaller sample size. Participant group sizes were slightly unbalanced based on exclusionary criteria during the recruitment process.

Another limitation of this study is the potential inclusion of asymptomatic endometriosis patients in the "healthy-control" participant group. Healthy controls were recruited based on age and lack of pain, but did not undergo surgery to rule-out the possibility of pain-free endometriosis. Although it is unlikely that any of the controls consisted of this small asymptomatic population, at most this would have affected 2 controls (2-9%) (As-Sanie et al., 2013).

The absence of a validated protocol for assessing internal tenderpoints creates another study limitation. Although a thorough physical examination, including an internal bimanual exam and external assessment, was completed in order to identify these regions, it was the first of its kind with no previous clinical foundation. For the purposes of this study, a standardized case report was developed. All pelvic exams were performed by a single physician, providing as much standardization and consistency as possible throughout the study. Without a previously validated case report, the reliability of the

physical exam methodology was based on the expertise of the experimenter. Additionally, pelvic examinations were not performed on healthy controls. Although the protocol of this study allowed for comparison between the gynecological sample, none of the physical exam findings were compared to non-patient scores. Future research including all participant groups in the physical exam component of the study would provide stronger evidence of abnormal pelvic exam findings in the endometriosis and CPP populations.

The method of QST used in this study did not allow specific identification of the underlying CNS mechanism responsible for the central sensitization found in chronic pain patients. Brain imaging was not utilized in this study, but previous research has provided evidence that women with pain-free endometriosis have increased gray matter in the antinociceptive pain regulatory system. This volume increase in the periaqueductual gray was not identified in women with chronic pelvic pain (As-Sanie et al., 2012).

Future Directions

As the first study to integrate a bimanual pelvic exam with quantitative sensory testing, there are various applications, both clinical and research-based, that can be applied to the future of gynecology. Recently, the Michigan Body Map (MBM) has been developed as a standardized self-report measure used to assess areas of chronic pain (Brummett et al., 2016). Patients are given a body map figure (Figure 5) and asked to check off regions of the body in which they have experienced persistent or recurrent

chronic pain (> 3 months). Those without chronic pain indicate “No chronic pain” on the MBM. While this measure has often been utilized for diagnosing fibromyalgia, the MBM can also assess centralized pain resulting from other etiologies. Developed by the University of Michigan Medical School, this protocol is reliable, valid, and accurate; P= 0.013 (Brummett et al., 2016). The findings of this study suggest that internal tenderness is another identifier of centralized pain. The introduction of an internal tenderpoint to the MBM could create a more comprehensive assessment of central pain amplification.

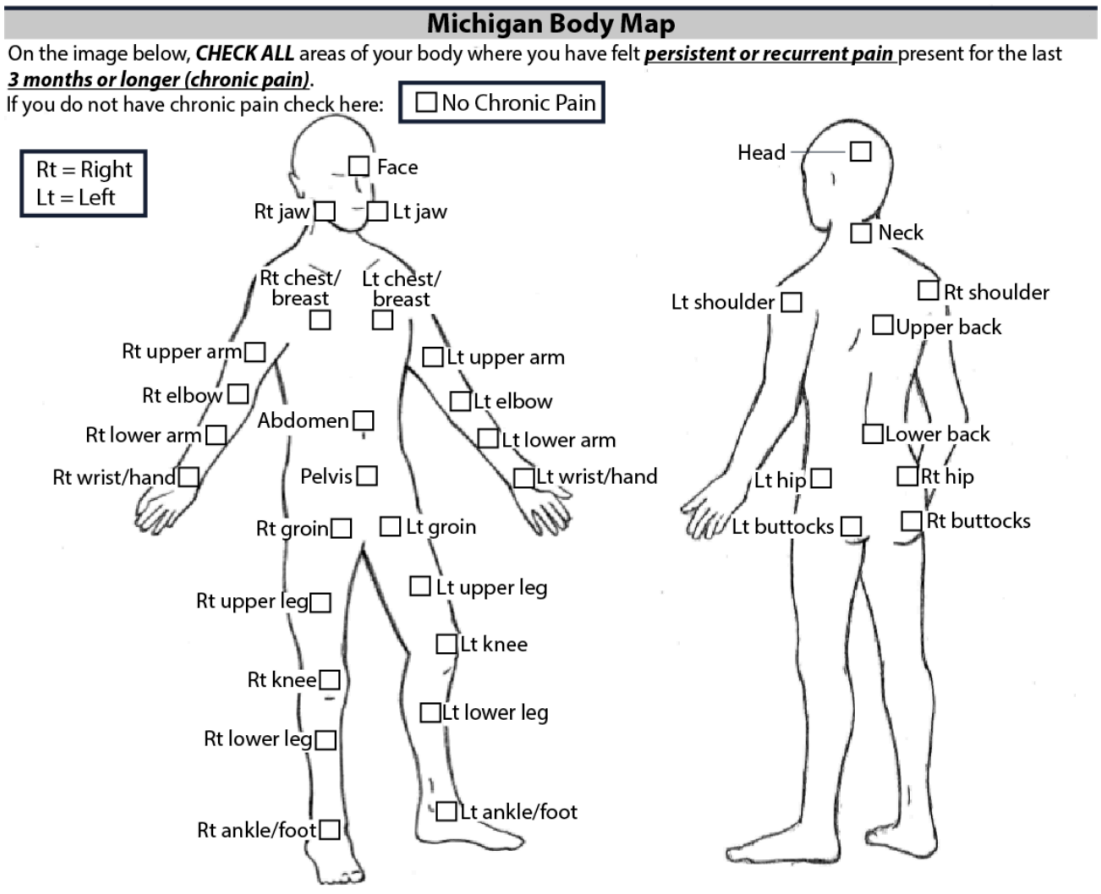

Figure 5: Michigan Body Map
Brummett, C. M., Bakshi, R. R., Goesling, J., Leung, D., Moser, S. E., Zollars, J. W., … Hassett, A. L. (2016). Preliminary validation of the Michigan Body Map. *Pain*, *157*(6), 1205–1212.

This study utilized pressure-pain QST protocols to assess central sensitization. Our results suggest that patients diagnosed with chronic pain conditions have lower pressure-pain thresholds, indicating greater amplification of the central nervous system. It would be beneficial to continue QST assessments on patients with chronic pain, incorporating more methods of testing, in addition to the use of a pressure algometer. QST methods include thermal and mechanical detection and pain thresholds (Cornelissen et al., 2014). Other modalities of QST, aside from pressure pain thresholds, could be implemented in future work, such as staircase thermal testing.

Currently, most endometriosis research is aimed at the presence and severity of endometrial lesions outside of the uterus. It has been well-established that chronic pain is associated with the disease, although the underlying mechanism responsible for this pain is poorly understood. Future research focused on the role of the CNS in endometriosis and CPP could advance our understanding of chronic pain syndromes. Neuroimaging and genetic biomarker exploration could provide tangible evidence for a shared underlying pain mechanism among CPS. Sensory neurons are key players in wound healing, a component of chronic illness that could explain the centralized aspect of pain amplification (Lolignier et al., 2015).

Future research should also be aimed at minimizing the invasive nature of endometriosis diagnosis. The potential use of circulating endometrial cells as a biomarker for endometriosis, in addition to a thorough pelvic exam, could reduce unnecessary laparoscopic surgeries. Furthermore, the addition of an internal tenderpoint to the MBM could further identify high-risk patients. As a result, only those patients most at risk for

endometriosis would need to sustain exploratory surgical diagnosis. Although laparoscopic investigation is currently the golden standard of diagnosis, endometriosis patients would benefit from less invasive methods with fewer side effects.

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