Assessing brain-derived neurotrophic factor as a novel clinical marker of endometriosis

Jocelyn M. Wessels, Ph.D.,[a] Vanessa R. Kay, B.Sc.,[a] Nicholas A. Leyland, M.D.,[a] Sanjay K. Agarwal, M.D.,[b] and Warren G. Foster, Ph.D.[a]

[a] Department of Obstetrics and Gynecology, McMaster University, Hamilton, Ontario, Canada; and [b] Department of Reproductive Medicine, University of California, San Diego, La Jolla, California

Objective: To evaluate novel clinical markers of endometriosis including the neurotrophins brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophin 4/5 (NT4/5) and compare them to others previously reported in the literature including cancer antigen 125 (CA-125) and C-reactive protein (CRP).
Design: Prospective study.
Setting: University hospital.
Patient(s): One hundred thirty-eight women were prospectively and consecutively recruited (April 2011–April 2015; cases: undergoing endometriosis surgery, n = 96; controls: benign gynecological surgery, n = 24 combined with healthy women, no history of pelvic pain, not undergoing surgery, n = 18).
Intervention(s): Collection of peripheral blood, gynecological and demographic information, eutopic biopsy in women undergoing laparoscopy.
Main Outcome Measure(s): Circulating BDNF, NGF, NT4/5, CA-125, and CRP were quantified by ELISA.
Result(s): Plasma concentrations of BDNF were significantly greater in women with endometriosis (1,091.9 pg/mL [640.4–1,683.1]; n = 68, untreated) than in controls (731.4 pg/mL [352.1–1,176.2]; n = 36), whereas circulating NGF, NT4/5, CA-125, and CRP were not different. When assessed for their ability to differentiate between women with revised Classification of the American Society of Reproductive Medicine stage 1 and 2 or stage 3 and 4 disease and controls, BDNF was the only putative marker able to identify stage 1 and 2 disease, with a sensitivity and specificity of 91.7% and 69.4%, respectively, using an arbitrary cutoff value of 1,000 pg/mL. We also demonstrated that circulating BDNF in women with endometriosis who were receiving ovarian suppression for disease was equivalent to that in the control group. This suggests that BDNF may also offer the opportunity to monitor patient response to treatment.
Conclusion(s): Plasma BDNF is a potentially useful clinical marker of endometriosis that is superior to NGF, NT4/5, CA-125, and CRP.
(Fertil Steril® 2016;105:119–28. ©2016 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Key Words: Biomarker, CA-125, C-reactive protein, endometriosis, neurotrophin

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/wesselsj-bdnf-clinical-marker-endometriosis/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Endometriosis is a chronic gynecological disease of unknown etiology characterized by the presence of endometrial fragments at ectopic locations (1, 2). It affects approximately 10% of women of reproductive age from all ethnicities and is a major cause of severe pelvic pain, suffering, infertility, and hysterectomy (2–5). In the absence of a suitable diagnostic marker, the interval between onset of symptoms of endometriosis and confirmed diagnosis by laparoscopy is 11.7 years in the United States (6). Lost time from work, costly medical interventions, and surgical procedures all contribute to endometriosis being one of the largest health care expenditures, with the annual cost of treatment and patient care reaching approximately $22 billion in the United States (7–9) and

Received June 15, 2015; revised September 1, 2015; accepted September 2, 2015; published online September 25, 2015.
J.M.W. has nothing to disclose. V.R.K. has nothing to disclose. N.A.L. has nothing to disclose. S.K.A. has nothing to disclose. W.G.F. has nothing to disclose.
Supported by the Canadian Institutes of Health Research (CIHR; grant MOP142230; to W.G.F.), and the Vanier Canada Graduate Scholarship-CIHR (to J.M.W.). J.M.W. is a member of the CIHR-sponsored Reproduction and Early Development in Health training program. The funding bodies had no role in study design, collection, analysis, or interpretation of data.
Reprint requests: Warren G. Foster, Ph.D., Department of Obstetrics and Gynecology, HSC-3N52D McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada (E-mail: fosterw@mcmaster.ca).

Fertility and Sterility® Vol. 105, No. 1, January 2016 0015-0282
Copyright ©2016 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
http://dx.doi.org/10.1016/j.fertnstert.2015.09.003

$1.8 billion in Canada (10). Significantly more resources are spent on endometriosis than on other chronic conditions (migraines, asthma, and Crohn's disease) (8), and thus identification of a clinical marker of disease remains a top priority.

Emerging evidence suggests an important role for the neurotrophins, a family of growth factors including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin 3, (NT-3), and neurotrophin 4/5 (NT4/5), in uterine physiology (11, 12) and endometrial pathology (13–17). Results of a small study suggest that women with endometriosis have elevated circulating BDNF concentrations compared with healthy controls, which decreased after surgical removal of lesions (18). However, this study was limited to patients with stage 1 and 2 disease only, and controls were healthy women alone. Moreover, the prior study was limited to BDNF, and thus it is unknown how well BDNF would compare to other clinical markers in this population. Subsequently, protein expression for BDNF and its high affinity receptor were found to be greater in the uterus of women with endometriosis compared with in disease-free controls (13, 16). Therefore, the objectives of this prospective case-control study were to assess the suitability of circulating concentrations of neurotrophins including BDNF, NGF, and NT4/5 as independent clinical markers of endometriosis and to contrast our results with other putative clinical markers of endometriosis including cancer antigen 125 (CA-125) and C-reactive protein (CRP) in the same population of women. Herein we present the results of our interim analysis of the study data.

MATERIALS AND METHODS

Study Participants

One hundred thirty-eight women were recruited and screened for inclusion in the study (Supplemental Fig. 1). One hundred twenty women undergoing gynecological laparoscopy between April 2011 and April 2015 for pelvic pain thought to be due to endometriosis were prospectively and consecutively recruited. Of these, 96 were found to have endometriosis (cases, n = 96) and 24 were diagnosed with other benign gynecological conditions (symptomatic controls, n = 24). Eighteen women with no history of pelvic pain and not undergoing surgery were also recruited (asymptomatic controls, n = 18). The study exclusion criteria were individuals unable to provide consent, age under 18, pregnancy, or a diagnosis of adenomyosis in the control group (three of 138). Adenomyosis was diagnosed by the gynecological surgeon using pelvic ultrasound and surgical evidence of disease. Women receiving hormone therapies for endometriosis in the 3 months before study enrollment were excluded from the untreated group of cases but were included in the treated group of cases (Supplemental Fig. 1) to determine the effect of endometriosis treatment on circulating clinical markers. All participants completed demographics and gynecologic questionnaires from which menstrual cycle length, date of last menstruation, and pelvic pain were determined. Pelvic pain was assessed using a nonstandardized pelvic pain test consisting of four separate 5-point questions on a visual analog scale and totaled out of 20. Menstrual cycle stage was determined by uterine biopsy for women undergoing surgery and using the date of last menstruation for those not undergoing surgery. During laparoscopic surgery women were categorized as a case or symptomatic control by a gynecological surgeon, and the diagnoses were confirmed by pathology reports. The stage of endometriosis was determined by the surgeon during surgery according to the revised Classification of the American Society of Reproductive Medicine (rAFS) (19). This study was approved by the Research Ethics Board, McMaster University (Institutional Review Board no. 06-064, 14-066-T), and all participants provided written informed consent before surgery.

Peripheral blood was collected from participants into plasma and serum separator tubes (BD Canada) by a nurse at McMaster University Medical Centre. As our initial primary markers/endpoints are found in plasma, serum was not collected from most asymptomatic controls (n = 16) nor from a few other cases (n = 11). Blood was placed on ice, transferred to the laboratory, and centrifuged at 3,000 rpm, and approximately 200 μL of plasma or serum was aliquoted into 1.8 mL cryovials (Sarstedt) and frozen at $-80°$C.

BDNF Assay

Plasma samples were thawed at room temperature, and BDNF concentrations were quantified in triplicate using the BDNF Emax immunoassay ELISA (Promega) following the manufacturer's protocol. Briefly, 96-well NUNC maxisorp plates (Fisher Scientific) were coated with antihuman BDNF antibody overnight. Freshly thawed plasma samples were diluted 1:10 with the provided sample buffer. After incubation, the absorbance was read at 450 nm within 30 minutes using the Biotek Synergy spectrophotometer (Fisher Scientific). The kit sensitivity was 15.6 pg/mL.

NGF and NT4/5 Assays

Serum samples were thawed at room temperature, and circulating NGF was quantified in duplicate in neat serum using the human β-NGF Mini ELISA Development Kit (Peprotech) following the manufacturer's protocol. Incubations for the sample and detection antibody were lengthened to 3 and 2.5 hours, respectively. The kit has a sensitivity of 16 pg/mL. NT4/5 was quantified in duplicate using the Human NT-4 ELISA (RayBiotech), which has a sensitivity of 2 pg/mL. The plates were incubated with neat serum overnight at 4°C and according to the manufacturer's protocol. ELISAs were read as above.

CA-125 and CRP Assays

Circulating CA-125 and CRP were quantified in duplicate using the Human CA-125/MUC16 Quantikine ELISA Kit (R&D Systems) and Human CRP ELISA (Life Technologies), following the manufacturers' protocols. Plasma samples were thawed at room temperature and diluted 1:3 (CA-125) or 1:4,000 (CRP) with the diluent provided. The sensitivity of the CA-125 and CRP assays is 0.035 U/mL and 10 pg/mL, respectively. ELISAs were read as above.

Download English Version:

https://daneshyari.com/en/article/6178573

Download Persian Version:

https://daneshyari.com/article/6178573

Daneshyari.com