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Short Communication

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Brain Derived Neurotrophic Factor as a Non-invasive Biomarker for Detection of Endometriosis

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Abstract

Background: Endometriosis is an estrogen-dependent chronic progressive gynecological disease that affects around 10% of women of reproductive age. A recent study shows that brain-derived neurotrophic factor (BDNF) has the potential as a clinical marker in the diagnosis of endometriosis. We aimed to determine whether BDNF levels are correlated with pain scores associated with endometriosis.

Methods: Fifty women who underwent laparoscopy surgery at Dr. Soetomo General Hospital and Dr. Ramelan Navy Hospital were prospectively recruited from October 2017 until August 2018. A blood sample was obtained before surgery and BDNF was measured using the Human BDNF Quantakine® kit. The relationship of BDNF levels in serum with the diseases's level of pain and stages was compared between cases and controls. BDNF validity as an endometriosis diagnosis biomarker was assessed using receiver operating characteristic (ROC) analysis.

Results: Serum concentrations of BDNF were significantly greater in women with endometriosis (30.42±7.41 *pg/ml*), compared to controls (25.66±3.30 *pg/ml*). Serum concentrations of BDNF were moderately correlated with the patient's reported pain scores (r=0.44, p=0.01). Receiver operating characteristic curve analysis confirmed the potential of BDNF in the diagnosis of endometriosis. Using a cut-off value of 27.06 *pg/ml*, the sensitivity and specificity were reported to be 66.7% and 64.3%, respectively.

Conclusion: BDNF serum levels in endometriosis women are significantly higher than in women without the disorder. BDNF serum level seems to have low accuracy and predictive value as a diagnostic marker for endometriosis. However, there was a moderate relationship between BDNF serum level and the degree of pain.

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Introduction

Endometriosis is an estrogen-dependent chronic progressive gynecological disease affecting around 10% of reproductive-age women (1). About 50% of female adolescents complain of chronic pelvic pain, infertility, or dysmenorrhea as a result of endometriosis (2). In addition to pain and infertility, endometriosis also brings detrimental consequences upon women's social functioning, vitality, and well-being. Clinicians still find it difficult to diagnose endometriosis based only on history, symptoms, and imaging. The gold standard for diagnosis is direct visual inspection of the pelvis through surgery, usually by laparoscopic surgery, which has many consequences for both the patient and the medical provider. It includes potential com-plications of the procedure, financial costs, and resource limitation in most developing countries. This causes delays in the diagnosis and treatment, causing rapid development of the disease over time. In the United States and the United Kingdom, the interval between initial symptoms and diagnosis was 11.7 years and 8 years, respec-tively. The discovery of a reliable and non- invasive diagnostic technique for endometriosis should remain a prime concern. Scientists have made many efforts to find a novel

marker for endometriosis diagnosis, ranging from biochemical properties of blood, peritoneal fluid, and endometrial tissue. A recent study suggests that brain derived neurotrophic factor (BDNF) has a potential as a clinical marker for endometriosis diagnosis. BDNF protein is a member of the neurotrophin that was first identified in the nervous system and synthesized by the endometrium (3). BDNF concentrations in serum are known not to be affected by age and weight gain and tend to be more stable in comparison to plasma level (4). Previous studies have shown higher BDNF levels in eutopic endometrium and plasma in women with endometriosis compared to healthy cases (5, 6). Therefore, the purpose of this prospective study was to measure serum levels of brain derived neurotrophic factor (BDNF) in women with endometriosis and the disease-free controls; furthermore, an attempt was made to assess whether BDNF is a reliable non-invasive clinical biomarker for endometriosis diagnosis or not.

Previous studies have shown higher BDNF levels in eutopic endometrium and plasma in women with endometriosis compared to healthy cases (5, 6). Therefore, the purpose of this prospective study was to measure serum levels of Brain Derived Neurotropic Factor (BDNF) in women with endometriosis and the disease-free controls; furthermore, an attempt was made to assess whether BDNF is a reliable non-invasive clinical biomarker for endometriosis diagnosis, and to find the correlation between serum BDNF and the severity of pain.

Methods

Patient selection: This study was conducted at Dr. Soetomo General Hospital Surabaya and Dr. Ramelan Navy Hospital an affiliated hospital. All enrolled subjects were given informed consent and willing to take part in this study. The ethical clearance number was 610/Panke.KKE/X/2017. The samples of the study were obtained from populations that met inclusion-exclusion criteria, comprising 50 cases. The sample size was calculated by binomial test. Women of reproductive age who underwent laparoscopic gynecological surgery were all chosen sequentially from October 2017 to August 2018. Subjects with endometrial cysts, pain, infertility, and endometriosis detected through laparoscopic surgery were included in the endometriosis group. Laparoscopic tubal ligation was the criterion to select the control group members.

The diagnosis and surgical procedures were carried out by an obstetrician-gynecologist consultant of reproductive endocrinology and infertility. In addition, the degree of endometriosis was classified into 4 grades using the American Society for Reproductive Medicine (ASRM) classification. The level of pain was determined using visual analog scale (VAS), from scale 0 (no pain) to 10 (worst pain). Subjects with pregnancy, obesity, a history of active smoking, consumption of psychoactive drugs, and certain physical diseases such as psychiatric disorders, neurological disorders, or mood and behavior disorders were not included. All subjects did not consume alcohol and had not received hormonal therapy in the previous three months.

Serum BDNF measurement: Blood samples were obtained before laparoscopy surgery during the luteal phase (4-7 days before the preceding menstruation). Patients were asked to fast for at least eight *hr*, and then 5 *ml* of venous blood sample was drawn. Serum preparation was done by laboratory personnel. The blood samples underwent serum preparation, then the serum samples are immediately stored at -20 degree Celcius until analyzed. Serum BDNF levels in patients and controls were measured using the enzyme-linked immunosorbent assay (ELISA) with Human BDNF Quantakine® kit. BDNF was measured according to the manufacturer's instructions. All results were expressed in *pg/ml*.

Statistical analysis: Differences in serum BDNF between the endometriosis and the control group were examined by baseline variables, including body mass index (BMI), menstrual age, day of sampling, and parity. The relationship between BDNF serum level and the degree of endometriosis, level of pain, dysmenorrhea, infertility, and laparoscopic findings was assessed by the Spearman rank correlation. Moreover, the relationship of BDNF levels in serum with level of pain was analyzed using Pearson correlation. To evaluate the validity of BDNF as a biomarker of endometriosis, receiver operating characteristic (ROC) analysis was done by calculating the area under the ROC (AUC) curve, sensitivity, and specificity. The specified BDNF cut-off value was then calculated in a cross-table to determine the positive predictive value (PPV) and negative predictive value (NPV) of the specified cut-off level. The level of statistical significance was set at $p< 0.05$. All statistical calculations and data analyses

were performed using SPSS version 17.0 (IBM, USA).

Results

There were a total of fifty women who underwent laparoscopic surgery from October 2017 to August 2018. Thirty-six women were found to have endometriosis by laparoscopic surgery and were assigned to the endometriosis group. Fourteen women who underwent laparoscopic tubal ligation went to the control group. The mean age of the control group was higher compared to the endometriosis group (38.14±4.4 *vs*. 31.47±6.5, p= 0.03). A statistically significant difference was also found in the number of parities (2.5±0.85 *vs*. 0.17±0.38, p<0.01). The length of the menstrual cycle and sampling days in the two groups did not differ significantly.

The largest proportion in the endometriosis group in this study had symptoms of dysmenorrhea (94.4%), followed by infertility (86.1%). Intense pain with a visual analog scale value (VAS) >7 was found in 50% of women. The findings on laparoscopic examination and anatomical pathology demonstrated ovarian endometriosis in 88.9% of cases and peritoneal endometriosis was detected in 11.1% while the rate of stage III and IV endometriosis was reported to be 30.6% and 58.3%, respectively (Table 1).

The endometriosis group showed a significantly higher serum BDNF compared to the control group. Correlation analyses between endometriosis stages with mean BDNF revealed no significant difference. A moderate relationship was found between BDNF levels in serum and the degree of pain experienced by subjects (r=0.44 with p=0.01). The ROC curve is shown in figure 1, and the area under the curve for BDNF in women with endometriosis compared to individuals without endometriosis was 0.713 (p=0.02). Using a cut-off value of 27.06, the sensitivity and specificity values were 66.7% and 64.3%, respectively. The capability of BDNF serum at respective values to detect endometriosis is shown in table 2 with PPV of 82.8%, NPV of 53.57%, positive likelihood ratio of 1.86, and negative likelihood ratio of 0.52.

Table 1. Sample characteristics of women with endometriosis

Characteristics	Endometriosis (%) (n=36)
Chief complaint	
Dysmenorrhea	34 (94.4)
Infertility	31 (86.1)
Visual analogue scale (VAS)	
0-3	2 (5.6)
4-7	16 (44.4)
>7	18 (50)
Laparoscopic results and pathological anatomy findings	
Ovarian endometriosis	32 (88.9)
Peritoneal endometriosis	4 (11.1)
Staging of endometriosis	
Gr. I	3 (8.3)
Gr. II	1 (2.8)
Gr. III	11 (30.6)
Gr. IV	21 (58.3)

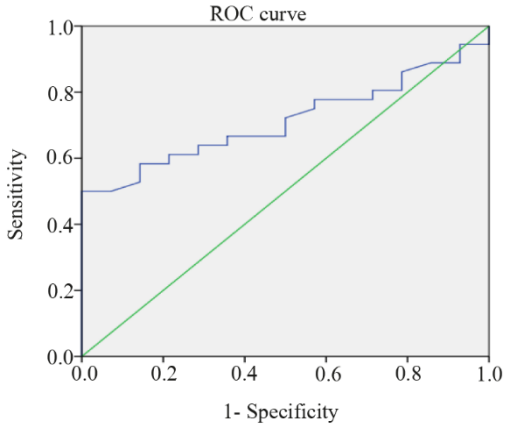

Figure 1. Area under the curve (AUC) of BDNF serum levels in cases with endometriosis

Table 2. BDNF serum diagnostic test in cases with endometriosis

		Endometriosis		
		(+)	(-)	Total
BDNF	≥27.06	24	5	29
Serum	<27.06	12	9	21
		36	14	50

Discussion

Brain derived neurotrophic factor (BDNF) is one of the neurotrophins found in the central and peripheral nervous system and is also expressed in several peripheral tissues, including tissues in the reproductive system (7, 8). Synthesis of BDNF and its receptor, TrkB, in the normal ovary was known to play a role in the oocyte maturation process (9, 10). Besides, endometrial cells are one of the sources that produce BDNF (3). BDNF

concentrations in plasma and serum in the luteal phase are known to be higher than follicular phase (11, 12) in women without endometriosis; therefore, blood sampling in this study was carried out during the luteal phase. The luteal phase was selected based on the history of the menstrual period, and it became one of the limitations of our study.

Serum concentrations of BDNF were significantly greater in women with endometriosis (30.42± 7.41 *pg/ml)*, compared to controls (25.66±3.29 *pg/ml*). This result is consistent with previous studies which demonstrated that plasma BDNF (6, 13, 14) and serum BDNF levels (11) were higher in the endo-metriosis group compared to the control group. An increase in estrogen is known to cause an increase in BDNF expression in plasma (12) through estrogen response element (ERE) that is found in the BDNF gene (15). Other studies revealed molecular mechanisms that occur in endometriosis lesions. Through this mechanism BDNF increase is caused by the interaction of inflammatory factors [Interleukin-1β (IL-1β)] and estradiol (E2) with their receptors, causing an increase in expression of extracellular signal-regulated kinase 1/2 (ERK1/2). Through phosphorylation of transcription factor, cAMP response element binding protein (CREB) causes BDNF synthesis in endometriosis lesions (11). BDNF protein synthesized in subsequent endometriosis lesions is secreted into the peritoneal fluid (11). BDNF is also able to reach peripheral circulation (plasma and serum) through capillary blood vessels formed around endometriosis tissue (6, 11, 13, 14).

The expression of the TrkB receptor is known to increase in women with endometriosis (11, 16). Binding between BDNF with TrkB receptors in endometriosis lesions causes endometriosis cell proliferation through ERK/STAT3 (signal transducer and activation of transcription 3) signal pathway. Moreover, the activation of ERK/STAT3 pathways could be induced by E2 (17). Although BDNF plays a role in endometriosis cell proliferation, based on this study, there was no association between BDNF serum and the severity of endometriosis. The results of this study indicate an association between pain and BDNF levels. Other studies have demonstrated that there is an association between BDNF in plasma (13, 14), and serum (11) with pain in endometriosis, causing BDNF to have an effect on induction of endometriosis pain.

As a chronic gynecological disease with a variety of clinical symptoms, endometriosis is shown to be implicated in deteriorating the patient's quality of life. The majority of patients have a prolonged complaint of pain and infertility before a diagnosis is established, and therapy is given (18). Delay in diagnosis is thought to be the main reason for late detection among patients with endometriosis (18).

Dysmenorrhea is one of the pain symptoms often found in women with endometriosis. Patient's complaint of dysmenorrhoea in this study was considerably high, yet several findings also second this finding with reported rates of 60-90% (14, 19). The pathogenesis of endometriosis pain can be explained by several mechanisms including nociceptive, inflammatory, and neuropathic pain. Moreover, the presence of active peritoneal lesions and peritoneal adhesion to surrounding organs also triggers the release of pain mediators (20, 21) that subsequently causes nociceptive pain.

A study by Tokushige et al. showed the presence of sensory nerve fibers type Aδ and C near the location of endometrial glands and stroma (22); also, nerve growth factor (NGF) and its receptor, TrkA, are highly expressed in endometrial glands and stroma (23, 24). NGF is one of the neurotrophins that has been known to play an essential role as a mediator in neurons, causing pain sensations in the peripheral area. In endometriosis, NGF causes nociceptor growth that increases the number of sensory neurons, especially small fibers of sensory and sympathetic ganglion neurons which participate in mediation of pain sensations and contribute to persistent inflammatory and neuropathic pain in endometriosis (20).

The increase of NGF in inflamed tissue (endometriosis) will be followed by retrograde transport of NGF in the target tissue to the dorsal ganglion. Then, it will cause an increase in preprotachykinin (PPT) production, calcitonin gene-related peptide (CGRP), and BDNF expression in small and medium-sized dorsal ganglion (25). In addition, BDNF is known to play a role in axon branching in sensory neurons (26). Research in mouse models of migraine shows the increase of BDNF in neurons followed by an increase in BDNF serum levels (27), suggesting that BDNF levels in the brain or neurons can reach peripheral circulation, due to the ability of BDNF to cross the blood-brain barrier (28).

Calculation of diagnostic accuracy shows that

level of BDNF in cannot be used as a useful biomarker for endometriosis; this is due to the sensitivity, specificity, NPP and NPV <90%, and also the difference in positive and negative like-lihood ratios <10. However, the use of marker combinations or the use of a scoring system is expected to increase the value of endometriosis diagnostic tests.

Conclusion

BDNF serum levels in women with endometriosis are significantly higher than women without endometriosis. However, no association was found between BDNF serum levels and endometriosis grading. Therefore, the examination of BDNF level in serum has a low accuracy and low predictive value as a diagnostic marker for endometriosis diagnosis. However there was a moderate relationship between BDNF level in serum and the degree of pain.

Conflict of Interest

The authors declare no conflict of interest.

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