RESEARCH ARTICLE

Simple Immunocytochemistry from Menstrual Blood in Diagnosis of Endometriosis

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Abstract

Introduction: The menstrual reflux containing viable endometrial cells precede the development of endometriosis lesion. Based on that, we can assume that we can detect the presence of endometriosis by utilizing the endometrial cells in the menstrual blood sloughing. The purpose of this study was to prove that simple immunocytochemistry technique on a sample of menstrual blood may show endometrial cells.

Materials and methods: A case–control study involving 60 women was performed, from February 2007 to February 2008. Screening for suspected endometriosis was performed by history taking, physical diagnosis and additional examination. Diagnostic laparoscopy or laparotomy and biopsy were performed afterward. Endometriosis histopathologically defined as the presence of epithelial cells of endometrial glands and stroma in the tissue being examined. Menstrual blood sampling is performed by taking 20 drops of blood into 20 mL preservative solution. In this study, the cells/tissues staining by immunohistochemistry using diaminobenzene (DAB), and for comparison hematoxylin–eosin staining (HE) was used so that the stromal cells which express caspase-3, caspase-9 and MMP-9 are stained brown with a blue background.

Results: The endometrial cells were successfully isolated using a preservative solution, and all samples from endometriosis subjects could be analyzed for the expression of caspase-3, caspase-9, and MMP-9.

Discussion: The results of this study lead to a conclusion that immunocytochemistry analysis of the menstrual blood endometrial cells can be applied as a noninvasive method for establishing the diagnosis of endometriosis in daily practice.

Keywords: Caspase-3, Caspase-9, Endometriosis, Menstrual blood, Noninvasive.

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Introduction

Endometriosis is a progressive gynecological disease, which is characterized by the presence of tissue resembling endometrial glands and stroma in the pelvic peritoneum, and other extrauterine tissue. Endometriosis can stimulate chronic inflammatory reaction and endometriosis lesions will proliferate and implant into the surrounding tissues, causing fibrosis, adhesions and organ distortion.[1–3]

Epidemiologically endometriosis is often experienced by women of reproductive age, of all ethnic and social groups, suffering from chronic pelvic pain, infertility, and disorders of both the intra-abdominal and intrapelvic organs. Internal organ disorders occur because of adhesions, suppression, and obstruction of the gastrointestinal tract, urinary tract and reproductive organs. This disease shows a high recurrence (75%) after treatment is discontinued, it becomes progressive, interferes with daily activities, lowers productivity, causing heavy stress, which ends up with decreased quality of life of the sufferers.[1–3]

The prevalence of the disease is not precisely known due to constraint of having definite diagnosis of endometriosis where surgical procedure is required and be followed by histopathological verification. The estimated prevalence of endometriosis around the world is 10%, which means that approximately 70 million women worldwide suffer from endometriosis. The disease was first suspected more often in women of reproductive age (25–29 years old), although generally confirmed diagnosis is often too late because women came after infertility complaints. Most endometriosis (3–4.1%) was found by chance when laparoscopy for sterilization in women without complaints and symptoms and higher (around 20%, range 2–78%) during laparoscopy in women with infertility complaints and even higher (24%, range 4–78%) in women with pelvic pain.[1–3]

On the other hand, the incidence of endometriosis increases from year to year. In the developed countries, endometriosis is a health problem that is quite prominent. In the United States, the disease is found in more than 7 million women, including teenagers. Furthermore, it turns out that endometriosis is the third order of gynecological diseases are often an indication for major surgery (laparotomy) with or without hysterectomy, which takes care for a long time and spends a high cost. The annual fee for the management of this disease in 1995 is 2–6 million US dollars and continued to increase to reach 22 million US dollars a year in 2002.[2] Thus it appears that endometriosis is a disease with many problems, ranging from etiopatogenesisnya (allegedly multifactorial), clinical manifestations, to how diagnostic and therapeutic invasive and

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risky.[4] Therefore, in the present and the future endometriosis will also weigh on the economy of developing countries such as Indonesia.

In Indonesia, endometriosis has also been a matter of reproductive health and show a similar pattern of findings, i.e., especially in women of reproductive age and premenopausal (range 18–49 years), with a chief complaint of dysmenorrhea and infertility. Of the 33 million women of childbearing age turned out 17 million of whom are teenage patients of whom 3.3 million (10%) and an estimated one-third of infertility associated with endometriosis. The incidence of infertility groups according to the three major hospitals in the three big cities in Indonesia ranges from 13.6% to 65.5%. When the prevalence of endometriosis in the world extrapolated to the amount of 10%, then the number of endometriosis sufferers in Indonesia until now estimated at more than 11 million people (with an estimated population of Indonesia at 220 million people).[4]

It is known that endometriosis showing symptoms vary and are not always typical, from asymptomatic, minimal to severe. The results of physical examination, visual inspection when the operating and histopathological findings are often not appropriate. Thus, the way the diagnosis is not always easy, but getting an early diagnosis can be established sooner treatment begins so that the severity of the disease can be reduced.[5,6] Until now, clinical examination, laparoscopy, ultrasonography (USG), computerized tomography scan (CT scan), magnetic resonance imaging (MRI) and examination carcinoantigen-125 (CA 125) is a way to diagnose the disease. Followed laparoscopic biopsy lesions have been used to get to know and look for lesions and perform staging which provides accuracy of the results of histopathology of 50–67%.[7,8]

Although laparoscopy is regarded as a diagnostic method commonly used up to now, but this way is not always easy to do and not always accepted by the female suspect suffered from endometriosis since it is an invasive action. As a result, there is often a delay in diagnosis, and even let loose detection of disease.[1,6,9] Investigations such as ultrasound, computed tomography (CT) scan, MRI, and CA-125 is a diagnostic method that is noninvasive, but these methods only useful for diagnosing severe abnormalities.[1,6–9]

Aetiopathogenesis has been proposed for a long time, but the theory of implantation of Sampson remains the main theory used to underlie the pathogenesis concept at this time. Immunological factors, genetic, hormonal, and inflammation have also extensively covered the incidence of the disease through the theory.[2,3,5,6]

Implantation of endometrial cells was separated when menstruation is thought to occur because the immune system defects,[2,5,6] and the intrinsic molecular aberrations due to dysfunction of endometrial,[3] so that endometrial tissue can implant on the network that is not supposed to. Thought that the endometrial cells attached to the peritoneum must be the cells are viable longer; that is to say, the cells are impaired in the process of apoptosis.[10,11] Apoptosis is a series of molecular events that started with a couple of different ways and all ended activate caspases. An enzyme caspase protein essential as implementing the regulation of cell death program. Deviation display caspase lead to failure of the apoptosis process, so that presents viable endometrial cells, as occurs in the development of neoplasms.[12,13]

Implantation of cells occurs due to local degradation of extracellular matrix tissue (ME) by proteolytic enzymes when endometrial cells attached to the ME. Two groups proteolytic enzyme thought to play a role, namely fibronectin, and laminin (a serine protease), and matrix metalloproteinases (MMPs). Matrix metalloproteinases are influenced by several factors, such as the specific tissue inhibitor of metalloproteinases (TIMPs) which act as mediators of inflammation and hormonal processes. Suspected estrogen plays a role in the maintenance of MMP production, while progesterone acts opposite to lower production of MMP. In this disease there is a change of humoral immunity; peritoneal fluid of endometriosis patients experienced an increase in the number of immune cells. This situation is believed to further facilitate the development of the disease rather than prevent it. Nevertheless, the abnormal immunological response is not yet clearly revealed.[6] On the other hand, the pattern of clinical manifestations is influenced by environmental factors and genes. Environmental factors will affect the genotype led to different phenotypes. The influence of genetic factors on the pathogenesis of endometriosis is seen in the fact that this disease occurs six to nine times more frequently in female first-generation derivative.[14] Clinical manifestations of endometriosis are very diverse, and how invasive diagnostic laparoscopy, as well as making handling difficult thought to look for ways diagnostic other options that are not invasive, early, safe, accurate, and affordable cost. Thus thought also that disease progression to a more severe following concomitant impact will be prevented (Table 1).

Based on the pathogenesis of endometriosis is caused by regurgitation of menstrual blood, it is unthinkable that the way other diagnostic noninvasive can be done by predicting proteins from endometrial cells is able to live and exist in the menstrual blood. These proteins are similar to those found in the cells of endometriosis. Of the various types of aberrant proteins turned out that already found in the endometrial tissue is caspase proteins and MMP. Caspases have been known to play a role in apoptosis, whereas MMP plays a role in the process of implantation of cells into tissues of the body.[14,15] Both play an important role at the beginning of the formation of endometriosis lesions. As already known, the process of cell apoptosis takes place following the pattern of the complicated cascade, involving caspase-9, and the end of all of this by activating caspase-3. This compound is a doubt-there cell death by destroying the DNA. On the other hand, from the air turns every kind MMP is MMP-9 protein at the transcription stage can be triggered on a large scale by many compounds in the body cells and is only shown by trophoblast cells, osteoclasts, leukocytes, and their precursors. Any deviation protein display, including caspase-3 and MMP-9, is closely related to genetic variations, or polymorphisms of

Table 1: Detection rate of caspase-3, caspase-9, and MMP-9 in immunocytochemistry of menstrual blood sample of women with and without endometriosis

	Endometriosis						*Without endometriosis*					
	Caspase-3		*Caspase-9*		*MMP-9*		*Caspase-3*		*Caspase-9*		*MMP-9*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Unreadable	0		0		0		0		0		0	
Readable	30	100	30	100	30	100	30	100	30	100	30	100

the gene. This variation is different in each ethnic.[12,13,15] The MMP-9 gene polymorphism in the promoter region are involved in the transcription process.[15–17]

Given that these proteins play an important role in the onset of disease, it is thought that the analysis of caspase-3, caspase-9, and MMP-9 in menstrual blood can be used as markers to diagnose endometriosis. Until now generally, immunocytochemistry technique is still difficult, especially for the isolation of endometrial cells in menstrual blood.[18] If the endometrial cells in menstrual blood can be isolated and abnormal protein can be detected by immunocytochemistry technique then this method can clarify the pathogenesis of the disease and are also able to diagnose the disease is noninvasive. It is expected to be developed another option diagnosis means that noninvasive. This method can connect the clinical manifestations of endometriosis with a view caspase-3, caspase-9, and MMP-9 in menstrual blood. These findings on the future thought may provide opportunities to develop ways of early diagnosis, management, and prevention of disease in addition to clarify the pathogenesis.

Materials and Methods

Subjects

The subjects were female patients suspected of suffering from endometriosis who came to the Polyclinic-Fertility Reproductive Endocrinology, Gynecology Clinic FKUP/RSHS, and the hospital network of RSHS. All patients with suspected endometriosis women who came went to the Polyclinic of Fertility Reproductive Endocrinology, Gynecology Clinic FKUP/RSHS and hospital networks RSHS much as 150–200 patients for a period of one year. Women with external endometriosis, endometriomas been diagnosed with endometriosis or externally by histopathology results. Controls were women with histopathological results not endometriosis.

Exclusion criteria were pregnant women. Being in the treatment of hormone, for at least three months, unless GnRH after nine months from the last administration. Another gynecologic disease, such as infection, malignancy, and other serious illnesses. Suffering from other diseases that complicate research, like other systemic diseases. The number of study participants who collected is sixty in total.

Clinical Examinations

Anamnesis, examination, and physical ultrasonography performed by researchers in an attempt to make a diagnosis. In the surgery is performed classification, then taken of the sample tissue. In patients who had surgery, surgery of data collection to determine the degree of the lesion.

Sample Processing Procedures of Menstruation Blood

Menstrual blood sampling is performed by dripping 20 drops of blood into 20 mL preservative.

Immunocytochemistry

Endometriosis by histopathologic criteria is the presence of epithelial cells of endometrial glands and stroma in the endometrial tissue examined. In this study, staining cells/tissues in immunohistochemistry using diaminobenzene (DAB), and the comparison is used hematoxylin–eosin staining (HE) so that the stromal cells which express MMP-9 and caspase-3 and caspase-9 will be stained brown with a blue background.

Data Analysis

The data analysis research conducted descriptive and analytic. Age grouping is intended to eliminate confounding incidence, and sort out cases that occur during adolescence (under 20 years), young adults (20–24 years), the peak age first diagnosed (25–35), and in perimenopause. The descriptive calculation is made by calculating the amount of the number and percentage, whereas analytical calculations were made with the statistical test adapted to the hypothesis to be tested.

Results

The study lasted for a year (February 2007–February 2008) in Reproductive Endocrinology Clinic-Fertility and Gynecology FKUP/RSHS and Hospital Network RSHS. Through history, physical examination and other investigations carried out screening of patients with endometriosis, then performed a diagnostic laparoscopy or laparotomy, which ended with a biopsy of the lesion. Biomolecular examination caspase-3, caspase-9, and MMP-9. Based on the results of microscopic histopathology as ascertainment, assessed the relationship of clinical manifestations of endometriosis cases and not endometriosis with menstrual blood biomolecular markers. Found 63 (42.28%) cases of endometriosis, while 86 (57.72%) classified as not endometriosis.

Each immunocytochemistry result is then interpreted according to the color criteria which shows the strength and weakness of the protein by endometrial cells (histoscore), as follows:

- Intensity+: light brown in color
- Intensity++: brown
- Intensity+++: dark brown in color

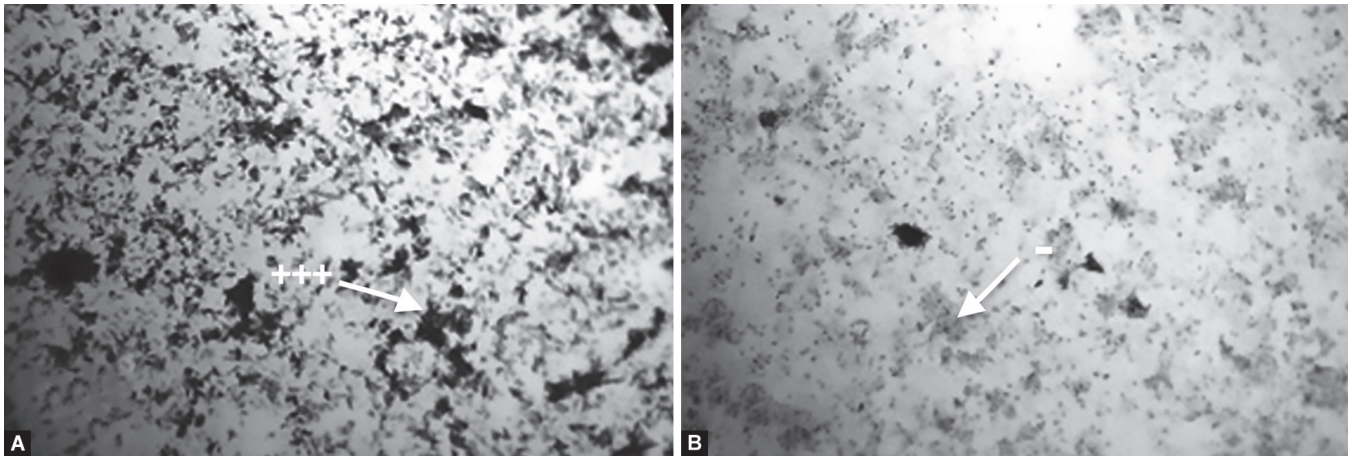
We found the immunocytochemistry of caspase-3 obtained from first day of menstrual blood of women with endometriosis showed higher expression compared to that obtained from non-endometriosis women (Fig. 1).

We tried to observe the expression of caspase-3 obtained from the third day of menstrual blood, and we found fewer cells stained with caspase-3. Yet comparison between women with endometriosis still showed higher expression of caspase-3 compared to that of nonendometriosis women (Fig. 2).

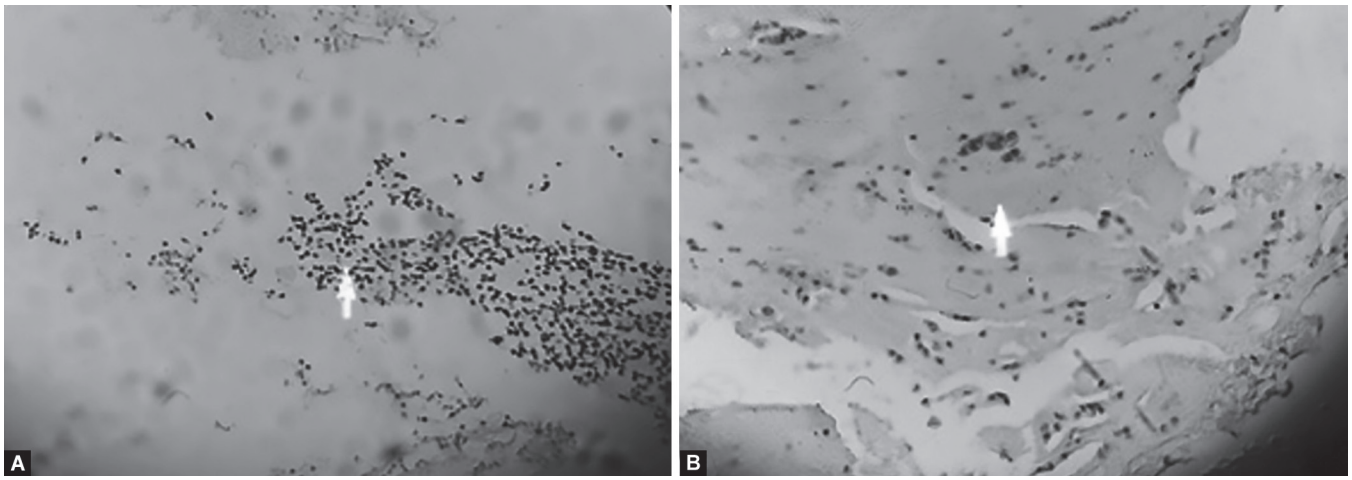
In our study, we also showed the expression ratio caspase-3/caspase-9 was higher in patients with endometriosis compared to that of nonendometriosis (Fig. 3).

Discussion

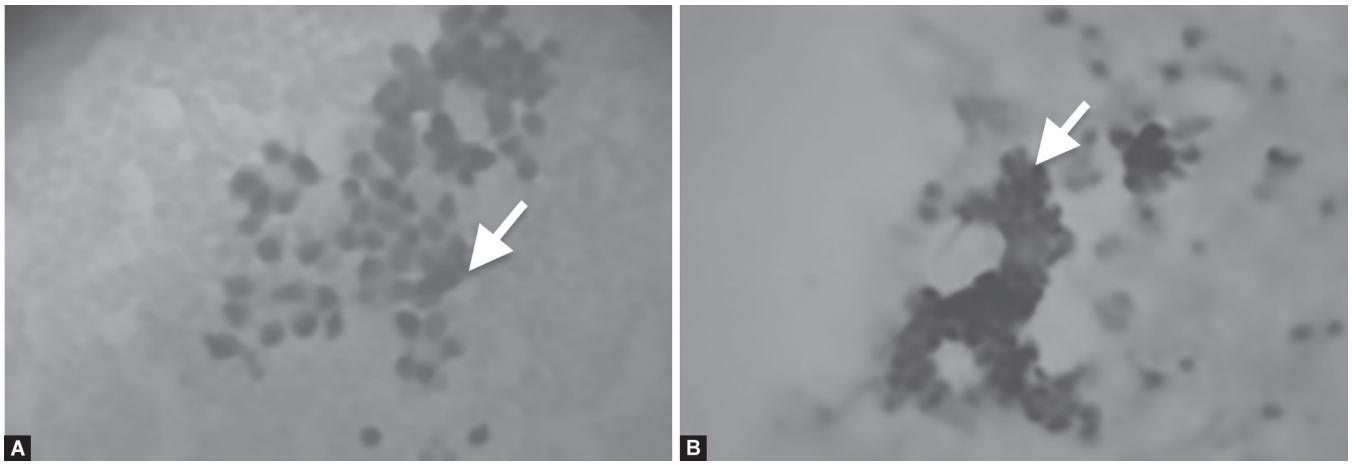
Physiologically apoptosis was detected in endometrial glandular epithelium which is at the end of the secretion phase and menstrual phase, while little apoptosis was detected during the proliferative phase or initial phase of secretion. Eutopic endometrium of endometriosis patients changes like ectopic tissue. The changes are not found in eutopic endometrium of women without endometriosis. This fact has led to the view that there is a primary defect in endometriosis in eutopic endometrium. The elements of cells and tissues derived from the change eutopic endometrium and overflow into the peritoneal cavity allegedly high potential for implantation and grow on the surface of the peritoneum, developing into endometriosis. The reduced apoptosis in cells suspected endometriosis is an important factor in the development of this disease.[6,12,13] In this study, patients with endometrial cells of endometriosis based disorders endometrial response to progesterone expected found intact and alive because



Figs 1A and B: (A) Immunocytochemistry of endometrial cells from the first day of menstrual blood from women with endometriosis; (B) Without endometriosis



Figs 2A and B: (A) Immunocytochemistry of endometrial cells from the third day of menstrual blood from women with endometriosis; (B) Without endometriosis



Figs 3A and B: The expression of caspase-3/caspase-9 in endometrial cells from menstrual blood; (A) Endometrial cells from menstrual blood without expressions of caspase-3/caspase-9 (magnification 200×); (B) Endometrial cells from menstrual blood with expressions of caspase-3/caspase-9 (magnification 400×)

of impaired apoptosis, so that they can live longer. As a regulator of apoptotic caspase enzymes in endometriosis experiencing irregularities, thus weakening apoptosis and caspase shown decreases in endometrial cells from menstrual blood.

Results of this study confirm what previous researchers claim that endometrial cells can be found either in the network endometriosis (ectopic endometrium), an abundance of menstrual blood and peritoneal fluid.[13,19] It has been thought that the analysis

of cells through menstrual blood cannot be done because it is already necrosis and only a few cells are found so a lot of research endometriosis use of scrapings/endometrial biopsy or make menstrual blood culture.

Results showed that there was no difference in appearance rating protein caspase-3, caspase-9 and MMP-9 in the endometriosis group than non-endometriosis. The preparation cannot be analyzed is the preparation that does not contain endometrial stromal cells, while other cells there looked good (it can be analyzed) although the amount is too little. This condition is thought that menstrual blood is taken after the expiration of the first days of menstruation because it is important ascertained menstrual blood storage time is right, namely the first or second day of menstruation. Thus, this study managed to alienate menstrual blood and endometrial cells also displayed satisfactorily managed and can be analyzed properly, so the chances to diagnose and assess prognosis noninvasive endometriosis can be further developed.

Results showed negative expression of caspase-3 and caspase-9 tend to be greater in patients with endometriosis compared to normal women. The differences of each examination for the two groups were not significant. Odds ratios for caspase-3 is 1.43. This means that women with endometriosis have a display alleged caspase-3 were negative, and 1.43 times likely to have endometriosis compared to without endometriosis. Similarly, when the caspase-9 negative views on women with suspected endometriosis, then the chances of suffering from endometriosis is 5.46 times compared to women without endometriosis.

MMP-9 is a protein that plays a role in the destruction of the extracellular matrix, due to the properties of these cells were able to implant once attached to the peritoneum. Expression of MMP-9 (+3) and the expression of MMP-9 (+2) in both groups the difference was not significant. Have believed that strong apoptosis disorders prevalent in endometriosis.[20,21] In this study, the percentage of expression of MMP-9 (+2) more precisely, while all cases of endometriosis showed expression of caspase (+3) and (+2). Ten cases (29%) endometriosis that normal visual assessment showed the expression of MMP-9 (+2). An odd ratio value of 1.72 states that in this study patients with endometriosis to display its MMP-9 in endometrial cells (+2) and (+3), likely to have endometriosis by 1.72 times higher than normal women. As the results of the immunocytochemistry for caspase-3 and caspase-9, increased expression of MMP-9 in endometriosis tend to be higher than non-endometriosis (Fig. 3).

The expression of caspase-3, caspase-9 and MMP-9 menstrual blood endometrial cells in this study shows that clarify the relationship display the pathogenesis of endometriosis is decrease apoptosis and increasing destruction (degradation) of the extracellular matrix. A similar figure has been widely studied in eutopic endometrial tissue.[20,22,23] These results, although not significant but showed a tendency that cannot be ignored. This phenomenon is evidence that must be taken into account, namely the possibility of subclinical disorder endometriosis can already ditasah first before clinical disorders arise. If this is so then finding molecular markers before the disorder becomes clinically important.[24]

In a previous study, it has been found a consistent relationship between histopathologic diagnosis of endometriosis by symptoms, physical examination, expression of MMP-9 (++) and (+++), the expression of caspase-3 and caspase-9 were detected through endometrial cells. Judging from the degree of endometriosis, it appears that the symptoms and physical signs associated with a high degree of disorder endometriosis (stage III and IV), among other menstrual disorders. This situation can occur due to ovarian enlargement will disrupt the follicles producing steroid hormones, thus giving rise to menstrual disorders. There appear to be differences may not be significant with non-cystic ovarian endometriosis is accompanied by abnormal uterine bleeding. The findings in endometriosis occur due to the excessive destruction of the extracellular matrix which is not shared by other types of cysts.

In endometriosis minimal degree or degrees I found no symptoms and physical disorders, but there is a consistent relationship between the appearance of MMP-9, caspase and CC genotypes. Of the 34 cases of endometriosis (54%) found the number of cases of degree I, II and normal by 12 (35%) cases. The expression of caspase-3 was decreased by 30 (88.23%) cases, while for the decreased expression of caspase-9 was found in 100% of cases Expression of MMP-9 were increased (++) amounted to 18 (53%) cases, while (+++) by 16 (47%) cases. CT allele expression of MMP-9 was increased was 8 (34%) cases, while the CC genotype expression of 26 (76.55%) cases. Seeing the results of the suitability of the results of histopathologic examination with endometriosis are quite high (the expression of caspase-3 and caspase-9), the caspase-3 and caspase-9 can be used to detect mild cases of endometriosis (minimal lesions).

The above discussion shows a different relationship with the clinical manifestations of biomolecular markers were significant in the group of endometriosis, although the relationship is not consistent. The results of this study cannot be extrapolated to the general population, because the difference between biomolecular markers (caspase-3, caspase-9, MMP-9) in endometriosis and non-endometriosis is not significant.

In practical terms, these findings can be used as a support for the noninvasive diagnosis, especially in cases of endometriosis with clinical manifestations which cannot be determined by a noninvasive method that has been commonly used (ultrasound, MRI, CT-scan, and CA-125). These cases usually require proving diagnostic laparoscopy. With that relationship was found to have the value of this diagnostic laparoscopy for patients who refuse a noninvasive diagnostic method is advantageous. Besides this significant association can be used an excuse to start hormonal therapy, which until now has not had a basis/rationale.

For women with symptoms of endometriosis that supports the allegations, but without obvious physical examination abnormalities or simply suffer from endometriosis grade I and II, the examination can also be encouraged to strengthen clinicians in starting a noninvasive treatment. One of the benefits of early detection is to prevent the disease in women with endometriosis do not continue with complications. Prevention can be done by eliminating and minimizing the risk of leading to the development of the disease, among others, avoidance of exposure to toxic substances by improving lifestyle, such factors cigarettes, alcohol, hormones, dioxins, or excessive grease. The use of antioxidants (free radical) can avoid developing the disease.

Sampling on days 1, 2, and 3 menstruation, some samples still showed endometrial cells with more scattered groups of cells (Fig. 1) (such as the results of immunocytochemistry on the 3rd day) (Fig. 2). Endometrial cells are still visible depending on when decay occurs and also depending on the menstrual blood that can be collected during the sampling. Some patients first day of menstruation the endometrium shed in large quantities, then the picture of UTI was

seen on the first day and then the next day is becoming rarer but still visible on day 2 and 3. For patients whose decay endometrium much going on day 2, then the blood sample of 0.5 cc (10 drops) cannot be collected on the first day, and the exposure of endometrial cells can still be seen on the 3rd and 4th with clumps of cells are more dispersed. Depending on the day to how the decay of the endometrium is greatest (day 1 or 2), the endometrial cells are still exposed either to be read to day to 3 for which decays largest on the 1st or 4th day of menstruation in menstrual decays highest on the day 2 menstruation.

Conclusion

The results of this study lead to a conclusion that immunocytochemistry analysis of the menstrual blood endometrial cells can be applied as a noninvasive method for establishing the diagnosis of endometriosis in daily practice. The selection of protein markers that are more specific and sensitive still needed.

References

1. Xu M, Vincent K, Kennedy S. Diagnosis of endometriosis. In: Rombauts L, Tsaltas J, Maher P, et al., ed. Endometriosis. Melbourne: Blackwell Publishing; 2008. pp. 133–148.
2. D'Hooghe T, Vodolazkaia A, Kyama C, et al. Health economics of endometriosis. In: Rombauts L, Tsaltas J, Maher P, et al., ed. Endometriosis. Melbourne: Blackwell Publishing; 2008. pp. 3–16.
3. Stacey A, Missmer, Anshu P, et al. The etiology of endometriosis: environtment, In: Rombauts L, Tsaltas J, Maher P, et al., ed. Endometriosis. Melbourne: Blackwell Publishing; 2008. pp. 49–67.
4. Adyono WD. The Impact of intraoperative administration of GnRH analogue toward clinical menifestation, immune system and quality of life subjects with endometriosis, Dissertation in Bahasa Indonesia. University if Indonesia; 2003.
5. D'Hooghe TM, Hill J. Endometriosis. In: Berek JS, ed. Berek and Novak's Gynecology. Philadelphia: Lippincott Wiliams and Wilkins; 2007. pp. 1137–1184.
6. Speroff L, Fritz MA. Endometriosis. Clinical Gynecologic Endocrinology and Infertility. Washington: Lippincott William and Wilkins; 2005. pp. 1104–1133.
7. Marchino GL, Gennarelli G, Enria R, et al. Diagnosis of pelvic endometriosis with use of macroscopic versus histologic findings. Fertil Steril 2005;84(1):12–15. DOI: 10.1016/j.fertnstert.2004.09.042.
8. Marchino GL, Gennarelli G, Enria R, et al. Laparoscopic visualization with histologic confirmation represents the best available option to date in the diagnosis of endometriosis. Fertil Steril 2005;84(1):42–49. DOI: 10.1016/j.fertnstert.2005.03.025.
9. Ballard K, Lowton K, Wright J. What's the delay? A qualitative study of women's experiences of reaching a diagnosis of endometriosis. Fertil Steril 2006;86(5):1296–1301. DOI: 10.1016/j.fertnstert.2006.04.054.
10. Matsuzaki S, Canis M, Pouly J, et al. Endometrial dysfunction in endometriosis: biochemical aspects. In: Rombauts L, Tsaltas J, Maher P, et al. Endometriosis. Melbourne: Blackwell Publishing; 2008. pp. 89–100.
11. Braun DP, Ding J, Shaheen F, et al. Quantitative expression of apoptosis-regulating genes in endometrium from women with and without endometriosis. Fertil Steril 2006;20:1–6.
12. Kumar V, Abbas AK, Fausto N. Cellular adaptations, cell injury, and cell death. In: Robbins and Cotran Pathologic basis of disease. Philadelphia: Elsevier Saunders; 2005. pp. 26–31.
13. Harada T, Kaponis A, Iwabe T, et al. Apoptosis in human endometrium and endometriosis. Hum Reprod Update 2004;10(1):29–38. DOI: 10.1093/humupd/dmh007.
14. Bischoff F, Simpson JL. Genetics of endometriosis: heritability and candidat genes. Best Pract and Res Clin Obstet Gynecol 2004;18(2):219–232.
15. Montgomery GW, Treloar SA, Kennedy SH, et al. Genetic variation and endometriosis risk. In: Rombauts L, Tsaltas J, Maher P, et al., ed. Endometriosis. Melbourne: Blackwell Publishing; 2008. pp. 37–48.
16. Han YJ, Kim HN, Yoon JK, et al. Haplotype analysis of the matrix metalloproteinase-9 gene associated with advanced-stage endometriosis. Fertil Steril 2008;3:47.
17. Shan K, Fu ZL, Hui D, et al. Polymorphisms in the promoter regions of the matrix metalloproteinase-7,9 and the risk of endometriosis and adenomyosis in China. Mol Human Reprod 2006;12(1):35–39.
18. Dabbs DJ. Diagnostic immunochemistry. Philadelphia: Elsevier Inc.; 2006.
19. Sharpe-Timms KL. Endometrial anomalies in women with endometriosis. Ann NY Acad Sci 2001;943:131–147. DOI: 10.1111/j.1749-6632.2001.tb03797.x.
20. Leyendecker G, Herbertz M, Kunz G, et al. Endomeriosis result from the dislocation of basal endometrium. Hum Reprod 2002;17(10):2725–2736. DOI: 10.1093/humrep/17.10.2725.
21. Matarese G. Endometriosis as an autoimmune disorders: the possible leptin link? In: Rombauts L, Tsaltas J, Maher P, et al. ed. Endometriosis. Melbourne: Blackwell Publishing; 2008. pp. 80–88.
22. Garcia-Velasco JA, Arici A. Apoptosis and the pathogenesis of endometriosis. Semin Reprod 2003;21(2):165–172. DOI: 10.1055/s-2003-41323.
23. Osteen KG, Yeaman GR, Brunertran KL. Matrix metalloproteinases and endometriosis. Semin Reprod Med 2003;21(2):155–163.
24. Vigano P, Parazzini F, Somigliana E, et al. Endometriosis: epidemiology and etiological factors. Best Pract Res Clin Obstet Gynecol 2004;18:177–200. DOI: 10.1016/j.bpobgyn.2004.01.007.