

Expression of natural killer cell activity with CD107a on ectopic endometrium in woman with endometriosis compared with non-endometriosis

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Abstract. Some factors have an important role in endometriosis pathogenesis; there is an immune cell that plays an important role in endometrial cells that have reflux. Woman with endometriosis experienced the cellular immune disorder. It is suspected that decrease of NK cell in the peritoneal fluid caused by its qualitative defect with CD107a expression as the best marker. The aim of this study was to compare expression of NK Cell activity with CD107a between awoman with endometriosis and non-endometriosis. A case-control study from March until July 2015 in Haji Adam Malik General Hospital. The case group was ectopic endometrial tissue block paraffin and control group was normal endometrial tissue block paraffin. This study included 23 patients in endometriosis group and control group respectively. A majority proportion of CD107a expression in endometriosis group was +1 (16 patients (69.6%)), while the control group was +3 (9 patients (39.1%)). Expression of NK cell activity with CD107a in patients with endometriosis was lower than the control group ($p < 0.05$). It suggested that cellular immune factors may play a role in the pathogenesis of endometriosis.

1. Introduction

Endometriosis is a gynecological disease marked by the presence of glands and ectopic endometrial stroma or outside of the uterine cavity. This is associated with pelvic pain and infertility.[1,2] Until today, the pathogenesis of endometriosis is still unclear, but the most popular hypothesis and fundamental of the mechanism of endometriosis occurrence is retrograde menstruation and immunological defects in the dispose of the debris.[3] The immune system seems to have an important role regarding acceptance and rejection of endometrial cells that experience reflux. Also, immune cells also contribute to the progression of the disease by secreting various cytokines that regulate cell proliferation, inflammation, and angiogenesis.[4,5] Natural Killer (NK) cell itself is one of the main immune cells in the pathogenesis of endometriosis.[6,7,8,9,10] Some studies found that there was a decline in activity and cytotoxicity of NK cells in the peritoneum fluid.[2,11,12] Dmowski *et al.* found that a failure in the system of collecting and cleaning residual menstrual blood (retrograde menstrual debris) is mainly due to the decreased function of NK cells in endometriosis.[13] The reduced activity took place mainly in the follicular phase, in which those endometrial cells were lysed by a cell that should be retrograded by NK cells.[4,5] However, it is still controversial in which based on Djaganata's study, there was no significant association of Natural Killer cells (CD56) expression based on Allred scores and the incidence of endometriosis, and found no significant differences between the mean of the Natural Killer expression cells in endometriosis and non-endometriosis.[14]



According to Ahn (2014), a decreased in NK cell cytotoxicity occurs not as a reduction in quantity but as a functional defect because the number of NK cells did not appear to differ between patients with endometriosis and non-endometriosis.[15] Therefore, it is important to assess the activity assessment compared with the number of NK cells. Recently, the expression of CD107a is shown as markers because they are directly involved in exocytose activity by NK cell cytotoxic granulation NK.[2,10] Alter G (2004) showed that CD107a in blood serum experience increased the regulation of NK cells after stimulation. Induction of CD107a is expressed simultaneously with the secretion of cytokines, lysis of target cells and is closely linked to the extent of target cell lysis process which takes place by NK cells. This study also showed that this marker is more sensitive for NK cell activity compared to intracellular cytokine examination. In endometriosis, a decrease in CD107a will describe a decrease in NK cell activity.[10] The aim of this study is to compare the expression of NK cell activity with CD107a between a woman with endometriosis and non-endometriosis.

2. Material and Methods

This was a case-control design conducted in Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital Medan from March until July 2015. We included endometriosis as a case group and non-endometriosis as a control group. Endometriosis group was 23 paraffin blocks of ectopic endometrial tissue of endometriosis patients obtained from laparotomy and laparoscopy with the inclusion criteria of 20-45-year-old women, endometriosis confirmed by laparoscopy (laparoscopy was performed by some doctors), regular menstrual history, no other gynecology disorders, not using hormonal medication during last 3 months, had no history of neurological disease and no history of previous pelvic surgery. The control group was 23 paraffin blocks of normal endometrial tissue obtained from biopsy tissue of healthy women with infertility with the inclusion criteria have normal menstrual cycles, not using contraception during the last six months, and there is no leukocytosis. Damaged paraffin blocks or failed immunohistochemical staining that cannot be read were excluded in the study. Expression activity of NK cells with CD107a examined by immunohistochemical staining methods based on the quantitative score of proportion. Score 0 if no cells are stained (0%); +1 if 1-25% of cells stained with a weak intensity; +2 when 26-50% of cells stained with moderate intensity; +3 when 51-75% of cells stained with strong intensity; and +4 when > 76% of cells stained with very strong intensity. Interpretation of the results was performed by two double-blind clinical pathologists with the conformity assessment results with the Kappa test. Results were analyzed with SPSS 17. To analyze the differences in the value of the expression activity of NK cells with CD107a of the endometriosis and normal endometrium groups Fisher Exact test with 95% confidence interval. P-value <0.05 was considered statistically significant.

3. Result

We performed 23 paraffin blocks of endometriosis tissue as the case group and 23 paraffin blocks on normal endometrial tissue as the control group. In this study, the majority of endometriosis patients were under 30 years (43.5%) and nullipara (100%) (Table 1).

Table 1. Distribution of research subject characteristic based on age and parity.

Characteristics	Group			
	Endometriosis		Normal Endometrium	
	n	%	n	%
Age (year)				
<30	10	43.5	11	47.8
30-40	9	39.1	12	52.2
>40	4	17.4	0	0
Total	23	100	23	100
Parity				

Nullipara	23	100	7	30.4
Primipara	0	0	7	30.4
Multipara	0	0	9	39.2
Total	23	100	23	100

Table 2. Distribution of endometriosis case based on the stadium.

Stadium	Endometriosis	
	n	%
1 (minimum)	0	0
2 (low)	2	8.7
3 (moderate)	10	43.5
4 (severe/high)	11	47.8
Total	23	100

From table 2, we found the majority of patients (47.8%) including the stage 4 (severe) and no one is at stage 1 (0%).

Table 3. Difference of natural killer cell activity (CD107a) on endometriosis tissue and normal endometrium tissue.

Expression Proportion CD107a	Study Group		Total (n,%)	p-value ^a
	Normal Endometrium (n,%)	Endometriosis (n,%)		
Negative	0 (0)	1 (4.3)	1 (2.2)	0.001
+1	6 (26.1)	16 (69.6)	22 (47.8)	
+2	8 (34.8)	6 (26.1)	14 (30.4)	
+3	9 (39.1)	0 (0)	9 (19.6)	
+4	0 (0)	0 (0)	0 (0)	
Total	23 (100)	23 (100)	46 (100)	

^aFischer exact test

Table 3 showed that the majority proportion of CD107a expression in endometriosis group was +1 (16 patients (69.6%)), while the control group was +3 (9 patients (39.1%)). and there was asignificant differential expression of CD107a in endometriosis compared with normal endometrium (p=0.001).

4. Discussion

Some factors expected to have an important role in endometriosis pathogenesis; there is an immune cell that plays an important role in endometrial cells that have reflux. In this study, we found that the expression of NK cell activity by CD107a in woman with endometriosis was lower than non-endometriosis. The results are consistent with those reported by Oosterlynck et al. which states that there is a relationship between NK cell activity and endometriosis.[11] Ahn et al. (2014) showed that the number of NK cells not decreased but its function that decreased.[15] Nevertheless, it is still controversial where Weed et al. found that there are no defects quantitatively from the decline of NK cell activity in peritoneal fluid or the peripheral blood. NK cells encountered by the number of relatively large cytolysis granules containing perforin and granzyme with different types. After contact with the target, NK cell granules will run into the contact zone with suspected target cells (known as the immunological synapse) and the contents removed to perform lysis.[17] Cytolysis granule membrane is coated by LAMP-1 or CD107a. This family member of LAMP-1 is a membrane protein that is experiencing high glycosylation and represents about 50% of the lysosome membrane protein. Parts that have high glycosylation of molecules on the luminal side of the carrier also has been alleged to be involved in protecting cellular membranes from the lyses enzymes attack in the granules, and further protect the extracellular membrane of effectors cells after degranulation takes place. However, the exact function remains unclear.[18] Perforin is important for lyses activity of cytotoxic

lymphocytes, and is required for the delivery and release of granzyme-B into the target cell cytoplasm. Decreased expression of perforin even in small amounts correlated with a significant decrease in cytotoxicity. NK cells do not release its granules all at once, and only secrete one peliosis granules subgroup after activation. Also, not all fusion events will result in the complete opening of the pores and release the contents of granules. Furthermore, perforin processed in lysosomes to give the mature form of the protein; one interesting possibility is that perforin delivery was disturbed to lysosomes can also affect the level of maturation. Thus, decrease number of perforin on peliosis granules or incompatible maturation can interfere the NK cell cytotoxicity. Therefore, the damage LAMP-1 expression can affect the other NK cell and lead to conduction disorders of important peliosis granule proteins such as perforin and slower peliosis granules movements. This will result in the inability of NK cells to deliver granzyme-B to the target cells and inhibition of NK cell cytotoxicity. All indicate that the LAMP-1 (CD107a) is not only a marker of NK cell degranulation but also an important component in NK cell cytotoxicity.[19] Later known that menstruation also expresses HLA-G (MHC class-I) on the endometrial tissue to enter the peritoneal cavity. Because peritoneal NK cells play an important role in this system, the interruption of toxicity via MHC class-I can cause endometrial cells survive and implantation into the peritoneal fluid. Therefore, it was concluded that in women with endometriosis, there is the expression of MHC class-I in the peritoneal fluid of those women, thereby disrupting the ability of the functional toxicity of NK cells, resulting in endometrial tissue into the peritoneal fluid can survive and precipitate endometriosis.[20] Ahn SH et al. (2015), in a review article about pathophysiology and immune dysfunctions in endometriosis, stated that the immune cell behavior in women with endometriosis helps the survival of endometriotic lesions via upregulation of inflammatory pathways and loss of the toxicity of NK cells in the peritoneal cavity has been proven.[15] Somigliana et al. reported that the existence of immunosuppressant both in normal endometrial stromal cells and endometriosis. This suggests that the normal endometrium have innate immunosuppressive ability against the cytotoxic activity of NK cells so that the pregnant state allows the implantation of the embryo. In women with endometriosis, immunosuppression effect on NK cell cytotoxicity are greater, so that the area of the peritoneum allows endometrium fragment to develop into lesion.[21] Further study is required with larger samples to prove that cellular immune play a role in endometriosis.

5. Conclusion

The expression of NK cell activity with CD107a in a woman with endometriosis was lower than non-endometriosis, and there was significant differential expression of CD107a between endometriosis and normal endometrium. It suggested that cellular immune factors may play a role in the pathogenesis of endometriosis.

References

- [1] Eskenzi B and Warmer M L 1997 Epidemiology of endometriosis *Obstet. Gynecol. Clin. North Am.* **24** 235-58
- [2] Jeung I H, Chung Y J, Chae B, et al. 2015 Effect of helixor A on natural killer cell activity in endometriosis *Int. J. Med. Sci.* **12(1)** 42-7
- [3] Siregar H S 2014 *L-selectin P213S polymorphism and macrophage profiles in endometriosis; anew insight into the pathophysiology of endometriosis* (Berlin: Lambert Academic Publishing, Schaltungsdients Lange o.H.G.) pp 9-63
- [4] Simoens S, Dunselman G, Dirksen C, et al. 2012 The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres *Human Reprod.* **27** 1292–9
- [5] Bedaiwy M A and Falcone T 2003 Peritoneal fluid environment in endometriosis, clinicopatological implication *Minerva Gynecol.* **55** 1-13
- [6] Sampson J A 1997 Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity *Am. J. Obstet. Gynecol.* **14** 422–69

- [7] Wu M Y, Yang J H, Ching Y U, *et al.* 2000 Increase in the expression of killer cell inhibitory receptors on peritoneal natural killer cells society for reproductive medicine *Elsevier Sci. Inc.* **74(6)**
- [8] IL-2 signaling and its primary biological effect in different immune cell Available from: <http://www.rndsystems.com/Pathway.aspx?p=15455&r=15436>
- [9] Gaffen S L and Liu K D 2004 Overview of interleukin-2 function, production and clinical applications *Elsevier Ltd. Cytokine* **28** 109-23
- [10] Alter G, Malenfant J M and Altfeld M 2004 CD107a as a functional marker for the identification of natural killer cell activity *J. Immunol. Methods* **294** 15-22
- [11] Oosterlynck D J, Meuleman C, Waer M, *et al.* 1993 Immunosuppressive activity of peritoneal fluid in women with endometriosis *Obstet. Gynecol.* **82** 206-12
- [12] Gagne D, Rivard M, Page M, *et al.* 2003 Blood leukocyte subset are modulated in patients with endometriosis *Fertil. Steril.* **80(1)**
- [13] Dmowski W P, Ding J, Shen J, *et al.* 2001 Apoptosis in endometrial glandular and stromal cells in women with and without endometriosis *Human Repro.* **16** 1802-8
- [14] Djaganata S P 2015 Ekspresi imunohistokimia sel natural killer pada endometrium ektopik penderita endometriosis [Tesis] (Medan: FK USU)
- [15] Ahn S H, Monsanto S P, Miller C, Singh S S, Thomas R and Tayade C 2015 Review article pathophysiology and immune dysfunction in endometriosis *Bio. Med. Res. Int.* 1-12
- [16] Agarwal A 2006 Role of oxidative stress in endometriosis *Reprod. Bio. Med. Online* **13(1)** 126-34
- [17] Meiling H, Lingya P L, Baozhen W, *et al.* 1994 A case-control epidemiologic study of endometriosis *Chin. Med. Sci. J.* **9** 114-8
- [18] Orange J S and Ballas Z K 2006 Natural killer cells in human health and disease *Clin. Immunol.* **118** 1-10
- [19] Loza M L, Zamai L, Azzoni L, *et al.* 2002 Expression of type 1 (interferon gamma) and type 2 (interleukin-13, interleukin-5) cytokine at distinct stages of natural killer cell differentiation from progenitor cells *Blood* **99** 1273-81
- [20] Robertson M J 2002 Role of chemokines in the biology of natural killer cells *J. Leukoc. Biol.* **71** 173-83
- [21] Peters P J, Borst J, Oorschot V, *et al.* 1991 Cytotoxic T lymphocyte granules are secretory lysosomes, containing both perforin and granzymes *J. Exp. Med.* **173** 1099-109