Research Report

Distribution of Regulatory T-Cell ($Cd4^+$, $Cd25^+$) in the Peritoneal Fluid of Endometriosis Patients

(*Sebaran Kadar Sel T Regulator ($Cd4^+$, $Cd25^+$) Cairan Peritoneum Pasien Endometriosis*)

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Abstract

Objectives: To observe the distribution of Regulatory T-cell ($CD4^+$, $CD25^+$) in the peritoneal fluid of endometriosis patients, as related to the local immune system of the peritoneum.

Method: A cross-sectional descriptive research Perioneal fluid was taken from endometriosis patients and non-endometriosis patients who underwent laparoscopic or laparotomy surgery at Raden Saleh Reproductive Health Clinic and Central Operating Theatre between June 2008 to March 2009. Total leukocyte and its differential count was counted manually. Regulatory T-cell was labelled with monoclonal antibodies for CD45 (PerCP-347464), CD4 (SIPC-340133) dan CD25 (PE-341009) and counted by Flowcytometry (Facscalibur) using cellquest pro software. Data were collected and analyzed with SPSS ver 11 software.

Result: Thirty-three patients were participated in this study, 12 non-endometriosis and 21 endometriosis patients were analyzed. 10 endometriosis patients were classified as minimal-mild, while 11 others were in moderate-severe stage. Higher median level of Regulatory T-cells were found in endometriosis patients (0.52%) compared to non-endometriosis cases (0.12%), even it was not significantly different (p 0.403). The median level of Regulatory T-cell in minimal-mild endometriosis (0.46%) was not significantly different with moderate severe endometriosis (0.52%) (p 0.981).

Conclusion: The percentage and total number of Regulatory T-cell in the peritoneal fluid did not show significant difference between endometriosis and non-endometriosis patients. Endometriosis patients shows tendency of higher percentage and total number of Regulatory T-cell than non-endometriosis group. The stage of endometriosis apparently does not show any significant difference in terms of Regulatory T-cell in peritoneal fluid.

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Keywords: Regulatory T-cells, CD4 CD 25, peritoneal fluid, endometriosis

Abstrak

***Tujuan**: Untuk mengetahui sebaran kadar sel T Regulator (CD4+, CD25+) cairan peritoneum pada kasus endometriosis terkait dengan sistem imun lokal di peritoneum.*

***Metode**: Penelitian ini bersifat deskriptif potong lintang. Kasus endometriosis yang datang ke klinik Kesehatan Reproduksi Raden Saleh RSUPNCM dan dilakukan laparoskopi Juni 2008 sampai dengan Maret 2009 dimasukkan sebagai sampel sebagai kontrol diambil kasus non endometriosis yang dilakukan laparoskopi maupun laparotomi di Klinik kesehatan Reproduksi Raden Saleh dan OK sentral RSUPNCM.*

***Hasil**: Didapatkan 33 sediaan yang terdiri dari 12 kasus non endometriosis dan 21 kasus endometriosis. Didapatkan kecenderungan lebih tingginya kadar T_{reg} maupun jumlah total T_{reg} pada kasus endometriosis dibandingkan non endometriosis. Hal ini sesuai dengan asumsi bahwa peningkatan sel T regulator dapat menginhibisi sifat sitotoksik dari limfosit T sitotoksik dan sel NK melalui mekanisme langsung dari sel ke sel. Meski demikian secara statistik tidak didapatkan perbedaan yang bermakna pada kedua kelompok. Derajat endometriosis ternyata tidak menunjukkan perbedaan yang bermakna terhadap pola sebaran T_{reg}.*

***Kesimpulan**: Sebaran kadar sel T regulator dalam cairan peritoneum penderita endometriosis dan non endometriosis tidak menunjukkan perbedaan bermakna secara statistik, namun pada kelompok kasus menunjukkan kecenderungan nilai yang lebih tinggi dibandingkan pada kasus nonendometriosis.*

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***Kata kunci**: sel T regulator, CD4 CD 25, cairan peritoneum, endometriosis*

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INTRODUCTION

Endometriosis is a gynecologic disorder commonly affecting females during their reproductive age. The prevalence is estimated as high as 4% of all females in reproductive age. Endometriosis could also be found in 20-40% of females who came for infertility problems.[1-4] Even though endometriosis was already founded in the early of 17th century, but until now what is the actual etiology and pathogenesis of endometriosis still become a big enigma.[5,6]

Endometriosis is a gynecologic disorder characterized by the growth of endometrial tissue outside of the uterus (ectopic endometrium).[7] In 1927, Sampson introduced an idea about the possible origin of endometriosis lesion probably coming from endometrial sheding inside uterine cavity during menstruation. Menstrual blood consists of endometrial tissue could flow backward to the abdominal cavity, instead of flow out normally through the vaginal canal (retrograde menstruation).[8] Cells that contains inside the menstrual blood shows good capability to survive inside a peritoneal cavity.[9,10] 76% of females with patent tubes under laparoscopic observation shows regurgitation of menstrual blood to peritoneal cavity.[11] However, not all female with menstrual regurgitation will suffer pelvic endometriosis.[12] There should be

contributions from other local factor that finally could trigger endometriosis condition. One factor that considered has a potential role in the pathogenesis of endometriosis is the characteristics of immune system in the peritoneal cavity which normally should prevent the implantation and growth of the endometrial tissue.[13]

Previous studies have discovered the role of T helper 1/T helper 2 (Th1/Th2) in endometriosis.[14] Th1 and Th2 cells are a subset of T helper lymphocyte and has an ability to release certain cytokines. Th1 cytokines release will induce cellular immunity, while Th2 on the contrary will induce humoral immunity. The immune system responses are mainly regulated by the presence of another T lymphocyte subset called regulatory T cells (T_{reg}). The important role of T_{reg} is to maintain the balance between Th1 and Th2 to assure adequate and proper immune response. Currently, there are many researches are trying to study the role of T_{reg} cells to prevent the incidence of transplantation tissue rejection, autoimmune reaction, and immune responses in general.[15,16] Since endometriosis tissue survival in peritoneal cavity is very obvious, the question regarding to the role of local immune system in the pelvic cavity become more interesting. T_{reg} has been known to have abilities in regulating immune response by either through direct cell to cell contact or by releasing certain kind of cytokines. Therefore, the idea of the role of T_{reg} in endometriosis development could become a possibility. There were many hypothesis and studies also trying to describe how the local immune system in the pelvic cavity reacts to the presence of endometriosis lessions. However, there are two possibilities that could explain why the local immune system do not react properly to endometriosis lessions. Low immune response in pelvic cavity could be induced by substances released from the endometriosis tissue, or the local immune system itself could not react properly.

This study basically would like to observe the role of T_{reg} in regulating the local immune system. First basic thought about the possible role of T_{reg} on this circumstances is a high amount of T_{reg} cell possibly could inhibit the cytotoxic activities of $CD8^+$ and NK cells. There was a study showing the changes of Natural Killer (NK) cell.[17] On the contrary, if the number of T_{reg} is low it might also contribute to the process of tissue destruction by the inability to regulate proper immune response of T lymphocyte. Uncontrolled T T helper lymphocyte could induce particular cytokines release that could be used as supporting factors for endometriosis growth and development.

METHODOLOGY

The design of this research is an observational study consisting of several cross sectional studies. A cross sectional study held on February 2008 until March 2009 in the IBS operating chamber and the Raden Saleh Reproductive Health Clinic and Makmal Endokrinologi FKUI-RSUPNCM, Jakarta. This is an observational study. Data and specimen were taken in cross sectional fashion.

The study was done from February 2008 to March 2009. Patients who underwent laparotomy or laparoscopy for endometriosis or other gynecology problems at the Central Operating Theatre (COT) or Raden Saleh Clinic at Dr Cipto Mangunkusumo General Hospital, Jakarta and match with the inclusion criteria were asked to participate in the study. Peritoneal fluid was taken from each patient and sent to Makmal Laboratory Faculty of medicine University of Indonesia for further analysis. Number of T_{reg} cell was counted using Facscalibur machine using CD45 (PerCP-347464), CD4 (SIPC-340133) and CD25 (PE-341009) markers. T_{reg} cells was counted using cellquest pro software. Data were collected and analyzed with SPSS ver. 11 software. Each quantitative variable was calculated for mean and standard deviation. Correlation between two quantitative variables was measured with Pearson method for normal distributed data. On the other hand if data was not normally distributed the correlation between two variables was analyzed by Spearman method. Correlations between qualitative or quantitative variables were analyzed by t-test or chi square for normally distributed data and Mann Whitney or Kruskal Wallis if data was not normally distributed.

Table 1. Distribution of regulatory T cells

Variable	Non endometriosis (n=12)			Endometriosis (n=22)			p
	Mean	SD	Median	Mean	SD	Median	
Content T_{reg}	1.864	4.903	0.115	5.429	14.016	0.520	0.403
Total T_{reg}	2.114	6.427	0.000	415.698	1618.480	3.864	0.386

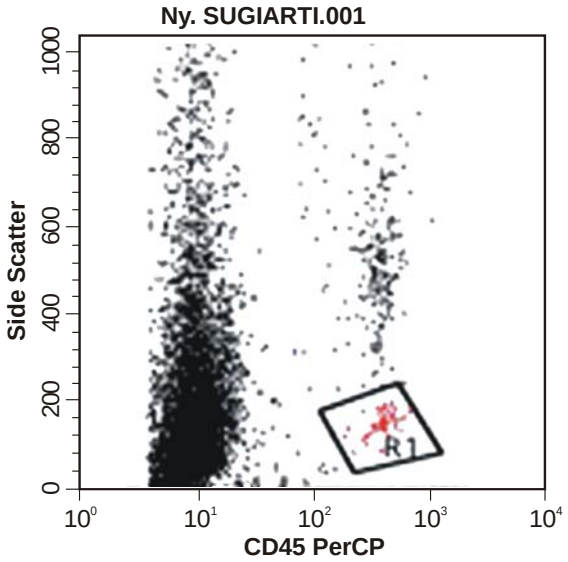
Info: p is considered significant if <0,05

To observe whether is there any difference in terms of T_{reg} level based on the severity of endometriosis, endometriosis group was divided into minimal-mild endometriosis and moderate-severe endometriosis according to the criteria from American Fertility Society (AFS). The median for T_{reg} precentages in minimal-mild endometriosis group (0.455) and in moderate-severe endometriosis group (0.520), do not show significant difference (p 0.981). Moreover, The median for total number of Treg in minimal-mild endometriosis group (1.987), and in moderate-severe endometriosis group (11.448), also do not show significant difference (p 0.291).

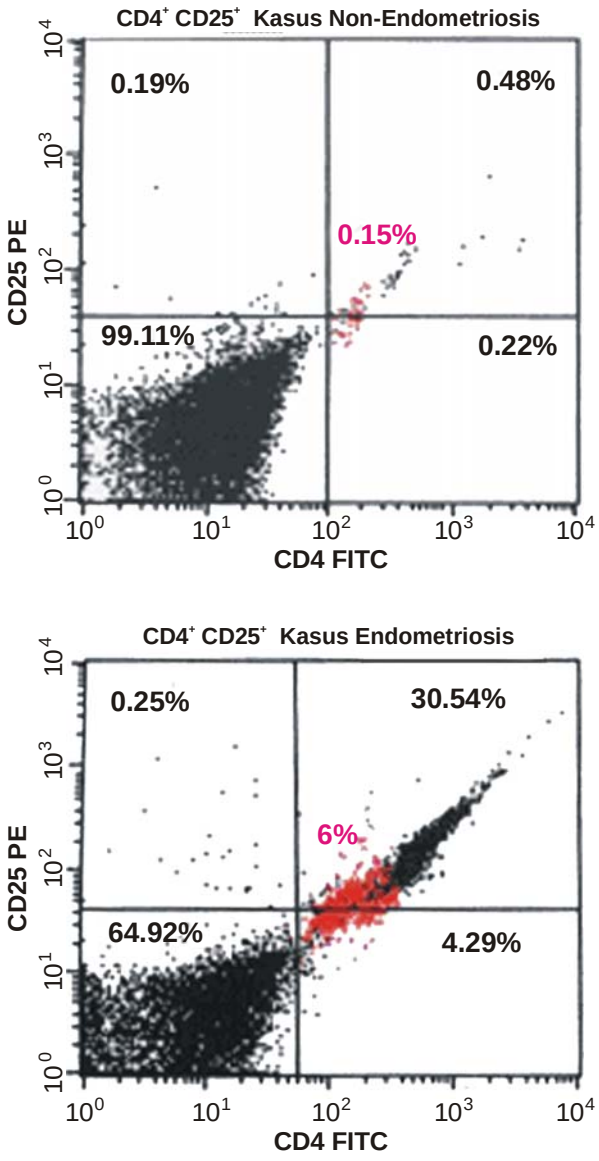
Table 2. Distribution of Regulatory T cells in endometriosis group

Variable	Mild-moderate endometriosis (n=10)			Severe Endometriosis (n=11)			p
	Mean	SD	Median	Mean	SD	Median	
Content T_{reg}	5.35	13.828	0.455	5.50	14.85	0.520	0.981
Total T_{reg}	14.966	32.714	1.987	779.85	2220.706	11.448	0.291

Information: p is considered significant if <0,05



Picture 1. Lymphocyte gating from the leukocyte population distribution based on side scatter and CD45



Picture 2. Gating of T_{reg} $CD4^+$ $CD25^+$ content in non-endometriosis and endometriosis cases

In the initial phase of the study, gating was done to take lymphocyte cells from the acquired leukocyte population (side scatter and CD45), while gating from the flowcytometry result shows that there is still no uniformity in deciding the limit of T_{reg} content. Uniformity in gating decision is achieved after several gating attempts.

DISCUSSIONS

From thirty three patients who is participating in this study 63.6% of them were diagnosed as endometriosis. These finding is consistent with previous studies that state endometriosis is involved in as much as 68% cases of infertility.[18]

There are several factors involved in the pathogenesis of endometriosis, including hormonal factors, environmental factors, excessive retrograde menstruation because of obstruction, and local immune system disruption in the pelvic cavity.

Sampson argued that endometriosis is derived from the implantation of cells found in menstrual fluid that spilled into the abdominal cavity. The viability of cells contains in the menstrual blood and their ability to implant and grow in the peritoneal cavity has been showed in previous studies.[9,10] 76% of women with patent fallopian tubes proved by laparoscopic observation shows menstrual blood registration that could induce pelvic endometriosis.[11] However, approximately only 10% of women who experience menstrual blood regurgitation might suffer endometriosis.[12] That means, some factors could play essential roles in the pathogenesis of pelvic endometriosis. Some hypothesis were saying about the possible role of the significant amount of menstrual blood regurgitated into the abdominal cavity. Moreover, there were also studies which try to describe the possible immune system disruption in the pelvic cavity, thus the cleaning mechanisms cannot working properly. The inability of pelvic immune system to clean the regurgitated menstrual blood loss could trigger the possibility of endometrial tissue implantation and growth in the pelvic cavity. Recent studies also shows some unsatisfactory result from either surgical or medical treatment in some particular endometriosis cases. This is often showed by the incidence of recurrency in particular endometriosis cases.[19] Therefore, the current alternative treatment for endometriosis that has been strongly considered so far is to modulate the immune response to endometriosis. Initial study has shown the changes in terms of quantity and activity of the leukocyte population in endometriosis patients. Moreover, the dysfunction of killing activity of the Natural Killer (NK) cell and macrophages in endometriosis patients also has been detected already.

These findings has been considered as an indication of immune response failure to endometriosis in the pelvic cavity.[10,25] the failure of immune system to give adequate response to particular agents could reflect a possible disruption in immune regulation. Human immune system has been designed to react adequately and properly to each agents in order to prevent the human body from harmful agents. On the other hand the human immune system should also regulated not to induce harmful immune reactions to

its' body. The regulation of human immune system is complex and involving particular immune cells and its' substances. One of the prominent immune regulator cell in human immune system is a subpopulation of T Lymphocyte called Regulatory T cell (T_{reg}). Previous studies shows this cell carry a specific markers on its' surface ($CD4^+CD25^+$) and plays an important role in peripheral tolerance, immune regulation and transplantation reaction. Since regurgitated menstrual blood coud be considered as a transplant tissue that is trying to reach the peritoneal layer, thus the immune response reaction from the pelvic cavity could decide the final result from this reaction. Based on this thought, T_{reg} possibly play an essential role in the implantation and growth of the endometriosis tissue in the pelvic cavity by regulating local immune response to regurgitated endometrial tissue. One study shows the ability of T_{reg} in preventing the occurrence of graft rejection during transplantation by suppressing T Lymphocyte response.(26)

This study shows either the percentage and the total number of T_{reg} is higher in endometriosis group, eventhough the difference is not statistically significant. However, through this finding we might expect the presence of T_{reg} in relative higher number in endometriosis patients could be associated with the possibility of its' role in inhibiting killing or cytotoxic activities from the Cytotoxic T Lymphocyte (CTL) and NK cell. Thus, defect in the killing activity of local CTL and NK cell in pelvic cavity could facilitate the implantation and subsequent growth of the endometriosis tissues.

This study was also trying to observe the correlation between the presence of T_{reg} and the severity of endometriosis. There is a tendency of higher T_{reg} percentage and total number in moderate severe endometriosis compared to minimal-mild endometriosis. However, the difference between two groups unfortunately is not statistically significant. This finding also shows early indication regarding the hypothesis of possible immune defects in the pelvic cavity.

However, previous study that has been conducted by Tariverdian etal in 2009 shows an opposite results. He found a lower T_{reg} in endometriosis group. Therefore, there is some conflicting results compared to the previous study. In order to answer that issue, a more detail study to observe the real interaction between immune cells in the pelvic cavity is required. This study could only shown the tendency in terms of percentage and total number of T_{reg}. How T_{reg} could influence the other local immune cells in pelvic cavity still become a question. It was realized since the beginning that studying a local immune response is more difficult since it could not be representated by the peripheral cells or substances. Therefore, the procedure to collect the local specimen could become a factor that might influence the result.

CONCLUSION

There is no difference in terms of T_{reg} percentage or total number in pelvic cavity between endometriosis and non-endometriosis patients. There is no difference according to T_{reg} between minimal-mild endometriosis and moderate severe endometriosis.

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