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Expressions of CD 56 + CD 16 + peripheral natural killer (pNK) cells in early idiopathic recurrent miscarriage

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SUMMARY: **Expressions of CD 56 + CD 16 + peripheral natural killer (pNK) cells in early idiopathic recurrent miscarriage.**

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Objective. *To determine: 1) Is CD56 + CD16 pNK cells higher in early idiopathic recurrent miscarriages compared to normal patients? 2) Are CD56 + CD16 + pNK cells lower in early idiopathic recurrent miscarriages compared to normal patients? 3) Are there any associations between CD56 + CD16 + pNK cells with early idiopathic recurrent miscarriage?*

Methods. *This study was an observational analytic study with cross sectional design comparing CD56+ CD16+ CD3+ in patients with recurrent early idiopathic miscarriage (case group) with patients who had offspring (control group). This study was conducted at the Department of Obstetrics and Gynecology H. Adam Malik General Hospital Medan and several hospitals network. The study was conducted by taking blood samples in the luteal phase, and CD56 +, CD16 +, and were examined. Data analysis using SPSS computer program. This study was conducted on the approval of the patient and has been approved by the Ethics Committee of University of Sumatera Utara Medical Faculty.*

Results. *The mean score of CD56 + CD16 +% lymph of the early idiopathic recurrent miscarriage group was lower than the control groups' mean score, but the statistical test results with the Mann-Whitney test showed no significant difference in the CD56 + CD16 +% lymph score of the early idiopathic recurrent miscarriage group with the control group. The mean score of CD56 + CD16 + Abs Count of the recurrent idiopathic early recurrent miscarriage group was lower than the mean score of CD56 + CD16 + Abs Count of the control group, and the statistical test results with the Mann-Whitney test showed no significant difference in CD56 + CD16 + Abs Count values of recurrent idiopathic early recurrence with the control group. The ROC value of serum CD56 + CD16 +% lymph level was 0.63. Cut off point is 7.50 with sensitivity of 82% and specificity of 46%. The ROC value of serum CD16 + CD56 + Abs Count was 0.73. The cut off point was 197.50 with a sensitivity of 82% and a specificity of 64.*

Conclusion. *The score of CD56 + CD16 + cell pNK values were lower in the group of patients with early idiopathic recurrent miscarriage than the control group. CD56 + CD16 + pNK cells have been shown to be associated with early idiopathic recurrent miscarriage.*

KEY WORDS: CD56 + CD16 + - pNK cell - Recurrent miscarriage.

Introduction

Recurrent miscarriage or habitual abortion is a condition in which two or more miscarriages occur consecutively at less than 20 weeks' gestation and/or fetal weight of less than 500 grams (1, 2). Recurrent miscarriage rate is 1 in 300 pregnancies, and epidemiologic studies found a 1 to 2% rate of recurrent miscarriage incidence (3). The risk of recurrent miscarriage in women who have had a miscarriage 2 times consecutively is 30%, 3 times is 33% (3, 4). The risk will decrease after the mother succeeds giving birth to a live baby, therefore early monitoring and prevention is necessary for high-risk mothers.

The immunologic and endocrine mechanisms of the system have been known to play a role in this problem. Approximately 30% of embryos fail at preimplantation stage, 30% also fail after implantation embryo in the uterus but this is detected

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in only a few cases after serum human chorionic gonadotrophin (hCG) and 10% clinical examination with miscarriage (5). Recurrent miscarriage in preimplantation and post implantation diagnosed infertile or subfertile. The reaction of the maternal immune system to the fetus that can lead to recurrent miscarriage can be classified as an autoimmune reaction when the maternal immune system attacks the tissues and organs themselves, or the aloimmune reaction in which the maternal immune system is abnormal and negatively affects the fetus or placental antigen, including antibodies maternal cytotoxic, inadequate maternal antibodies and impaired function and distribution of natural killer (NK) cells (6, 7).

Abnormal chromosomes in the embryo cause implantation potential and preimplantation of miscarriage. The presence of these autoantibodies is associated with recurrent miscarriage, infertility and Recurrent Implantation Failure (RIF) as well as NK cells and T helper type 1 (Th 1) cytokines produce lymphocytes (8). NK cells are part of the innate immune system and are found in blood vessels peripheral 10-15% and endometrium. Although both the natural killer peripheral (pNK) and the natural killer uterus (uNK) are expressed on the surface of the CD56 + antigen, pNK cells are phenotypically different and function with uNK cells and less than 10% of pNK cells resemble uNK cells (9). More than 90% of pNK cells are CD56dim and CD16 + where 80% of uNK is CD56bright and CD16 + (10-12). Both pNK and uNK cells are known to be associated with reproductive failure. PNK hyperactivity is closely associated with increased NK cell mass and increased cytotoxic migration of NK cells in the uterus in reproductive failure where this will increase the rate of miscarriage (13, 14). High concentrations of NK cells of the CD56 + CD16 + type have been found in the uterus of the mother undergoing miscarriage, suggesting cytotoxic activity is likely to occur at the site of implantation (12, 15). Increasing the amount and activity of NK cells can predict a tendency for subsequent miscarriages to occur and be considered a causative and prognostic factor for sterility, infertility and miscarriage (16-18).

PNK cells are an important part of recurrent immunologic disorders found in recurrent miscarriage (14). In general ≥10% of pNK cells increase, where >12% NK cells 5 days after miscarriage in cases of recurrent miscarriage are associated with immunologic factors, further more of 18% of NK cells, describing miscarriage can not be avoided (13).

The examination method for NK cell activation has been re-evaluated, showing that incubation of peripheral mononuclear blood cells (PBMC) with different targets leads to activation and increased CD69 + expression in NK cell lymphocytes (8, 19, 20). Incubation of new PBMC blood cell isolates or whole blood with K 562 cells stimulates NK cells and causes a significant increase in CD69 + expression in NK cells, but CD56 + only slightly increases in NK cells (21). CD69 + expression, and more significantly up regulation of CD56 + CD69 + CD3 subset - correlates with NK cell cytoxicity and is significant in NK cell activation (22).

During pregnancy, CD56 + CD16 + accumulates in decidua and becomes the dominant leukocyte population (23). The association between increased pNK cell and reproductive failure is one of the subjects of controversy in reproductive therapy (24). In Hadinedoushan (25), Karami et al. (26), and Atia (27) research, it was found that CD56 + pNK increased significantly in recurrent miscarriage. In contrast Souza et al. (28), Kolarz (29), and Katano et al. (30), indicated that there was no difference between recurrent and normal abortions. But in Fukui et al. (31) and King et al. (32) research, it was found that recurrent miscarriage was associated with decreased CD56 + pNK. There was no agreement on the levels of CD56 + CD16 + pNK cells from recurrent idiopathic recurrences so it is worth re-examining to be able to know: 1) Is CD56 + CD16 + pNK cells higher in early idiopathic recurrent miscarriages compared to normal patients? 2) Are CD56 + CD16 + pNK cells lower in early idiopathic recurrent miscarriages compared to normal patients? 3) Are there any associations between CD56 + CD16 + pNK cells with early idiopathic recurrent miscarriage?

Methods

This research is an analytic observational type study with cross sectional design that is comparing CD56 + CD16 + mother with recurrent early diopathic miscarriage (case) with mother who have offspring (control), aimed to assess the relationship between variables analyzed by bivariate and multivariate analysis method.

This research was conducted in the Department of Obstetrics and Gynecology H. Adam Malik General Hospital Medan and some of its network hospitals, namely: Dr. Pirngadi Hospital Medan, Rumkit Tk.II Putri Hijau Kesdam / Bukit Barisan, Haji Hospital Medan, Sundari Hospital, and IVF Laboratory HFC Division Endocrinology Reproduction and Infertility Department of Obstetrics and Gynecology of University of Sumatera Utara Medical Faculty and Clinical Pathology Laboratory H. Adam Malik General Hospital Medan.

Participants from this study were divided into two groups: the case group that were mothers with recurrent early idiopathic recurrences, and control group who were mothers who had offspring within the last 12 months, with a sample size per group of 20 individuals each. The study was conducted by taking blood samples in the luteal phase, and CD56 +, CD16 + were examined. Data analysis using SPSS program.

This study was conducted on the approval of the patient and has been approved by the Ethics Committee of University of Sumatera Utara Medical Faculty.

Results and discussions

From the characteristics of research subjects we found that there was a significant difference in mean age between recurrent miscarriage group and control group (p = 0.037). Significant associations were found between age group with occurrence of recurrent miscarriage cases and control group (p = 0.043) (Table 1).

Tribal classification showed that in the recurrent miscarriage group, there were more cases of recurrent miscarriage (54.50%). In the control group, Javanese tribe is the most widely found that is 45.50%, while the Batak tribe found only 31.80%.

Based on maternal level of education indicates that the study group of recurrent idiopathic early maternity miscarriage mostly with undergraduate education as many as 12 (54.5%) and lowest junior high education as many as 1 (4.5%). While in the control group it was found that most of the cases with undergraduate education were 11 (50%) and the lowest of junior high education was 1 (4.5%). Result of statistical test with Fisher exact test got p value 0.912 which showed no significant difference of education level of case group and control group.

TABLE 1 - THE MEAN. DISTRIBUTION AND RELATIONSHIP OF RESEARCH SUBJECTS BASED ON PATIENT CHARACTERISTICS IN EARLY IDIOPATHIC RECURRENT MISCARRIAGE GROUP WITH CONTROL GROUP.

Patients Characteristics		**Early Idiopathic Recurrent Miscarriage (n=22)**		**Control (n=22)**		**p value**
Mean Age (years)		30.23 ± 4.11		27.86 ± 3.11		0.037***
Age Group (n%)	20 - 25	2	(9.1%)	5	(22.7%)	
	26 - 30	8	(36.4%)	13	(59.1%)	0.043**
	31 - 35	12	(54.5%)	4	(18.2%)	
Total		**22**	**(100%)**	**22**	**(100%)**	
BMI (kg/m^2)		22.70 (20.5-23.2)		22.55 (20.3-23.4)		0.557****
BMI	Underweight	0		0		
	Normal	16	(72.7%)	17	(77.3%)	1.0*
	Overweight					
	Obese 1					
	Obese 2	6	(27.3%)	5	(22.7%)	

Information: normally distributed data is presented in the form of mean ± standard intersection. abnormally distributed data is presented in median (minimum / maximum), p = significance (p <0.05). B = significant; ** = Fisher exact test test; *** = t-test test * Chi square test **** Mann-Whitney test

Work as an housewife was found to be a trend with a history of recurrent miscarriage compared to other occupations, while employment as a private employee was found to be 59.1% in the control group compared with the case group. Work is grouped into two groups of occupations, namely indoors / outdoors / field, which aims to see if there is a relationship between the types of work with recurrent miscarriage. From the results of the analysis with Fisher exact test obtained p value > 0.075 which showed no significant difference in the work between early repeat idiopathic recurrence group and control group. In the early idiopathic recurrent miscarriage group found 50% working in the room, the remaining 50% are also working in the field. Different results were found in the control group, as many as 40.9% worked in the room, the remaining 59.1% worked in the field.

On anthropometric examination, mean BMI showed no significant difference in early and idiopathic recurrent miscarriage group and control (p = 0.557 using Mann-Whitney test). The average BMI span in the recurrent miscarriage group was almost identical to that of the control group, whereas in the hereditary group it was found to be between 20.3 kg/m^2 and 23.4 kg/m^2. In the spread of BMI categories, recurrent idiopathic recurrent miscarriage groups found a higher percentage in the normal category than obese were nearly three times that of obese (72.7% and 27.3%). The result of statistical test with Fisher exact test was obtained p > 0.05 which showed no significant difference of BMI between groups.

In this study, it was found that the mean CD56 + CD16 +% lymph early recurrent idiopathic miscarriage group was 11.86 ± 7.32 lower than the mean maternal group who had the offspring of 15.09 ± 7.00. However, statistical test results with Mann-Whitney test obtained p >0.05 which showed no significant difference of CD56 + CD16 +% lymph value of recurrent idiopathic early miscarriage group with control group. Hence the hypothesis that the CD56 + CD16 +% lymph value of recurrent idiopathic early recurrence is different from the control group (Table 2).

The results of this analysis showed that CD56 + CD16 +% lymph value had no effect on the incidence of recurrent early idiopathic recurrence.

The analysis result with ROC graph showed that Area Under Curve was equal to 0.63. This illustrates the ability of the test by measuring the serum CD56 + CD16 +% lymph level to distinguish between mothers who had an early idiopathic recurrent miscarriage with a control of 63%, statistically classified as less good.

Cut off point for control based on CD56 + CD16 +% lymph level according to ROC chart is 7.50 with sensitivity of 82% and 46% specificity. This value explains that women who have CD56 + CD16 +% lymph levels are equal to or less than 7.50, will generally have recurrent miscarriages, while mothers with CD56 + CD16 +% lymph values greater than 7.50 will not have recurrent miscarriages.

Table 3 shows that the mean CD56 + CD16 + Abs count rate of early recurrent idiopathic recurrent miscarriage group was 261.59 ± 248.12 lower than the mean CD56 + CD16 + Abs Count control group of 436.68 ± 212.39. Statistical test results with Mann-Whitney test obtained p value <0.05 which indicates there is a significant difference CD56 + CD16 + Abs Count value of early recurrent idiopathic miscarriage group with control group. Hence the hypothesis that there is a difference CD56 + CD16 + Abs Count between early recurrent idiopathic recurrent miscarriage group with the control group is accepted.

TABLE 2 - MEAN DISTRIBUTION AND RELATION OF RESEARCH SUBJECTS BASED ON CD56 + CD16 +% LYMPH VALUE ON EARLY IDIOPATHIC RECURRENT MISCARRIAGE WITH CONTROL GROUP.

$CD56^+$ $CD16^+$% lymph	**N**	**Mean**	**Std. Deviation**	**p value**
Early Idiopathic Recurrent Miscarriage	22	11.86	7.318	0.129**
Control	22	15.09	6.996	

Information: normally distributed data is presented in the mean ± standard deviation, p = significance limit (p <0.05). ** Mann-Whitney test

TABLE 3 - DIFFERENCES OF CD VALUES OF 56+ 16+ ABS COUNT OF STUDY GROUPS.

$CD56^+ CD16^+$ Abs Count	N	Mean	Std. Deviation	p value
Early Idiopathic Recurrent Miscarriage	22	261.59	248.124	0.009**
Control	22	436.68	212.389	

In the endometrium premenarche and post menoupause uNK cells become few (33, 34). The number of CD56 + cells increases in the proliferation phase and reaches the highest levels in the final phase of secretion, this increase that supports the occurrence of pregnancy (11, 35, 36).

This change in the number of uNK cells has a correlation with hormonally induced decidualization and correlates also with fluctuations in chemokine expression rather than embryonic implantation correlations.

The uNK cells overexerate in the uterus at the time of implantation and this corresponds to the trophoblast, and has a role in the process of implantation, the formation of the placenta. The presence of NK cells on the maternal-fetal surface during implantation indicates that the trophoblast is the target cell for uNK. Differentiation of the endometrial epithelium in humans is a dynamic state of the menstrual cycle and early pregnancy in which uNK cells play a role in this process (37-39).

The analysis result with ROC graph showed that Area Under Curve equal to 0,73. This illustrates the ability of the test through the measurement of serum CD56 + CD16 + Abs Count levels to distinguish between mothers with recurrent early idiopathic miscarriage with a control of 73%. Statistically quite good.

Cut off point for control mother based on CD56 + CD16 + Abs Count according to ROC chart is 197,50 with sensitivity of 82% and specificity of 64%. This value explains that mothers with CD56 + CD16 + Abs Count counts equal to or less than 197.50 will generally have early repetitive idiopathic recurrences, whereas mothers with CD56 + CD16 + Abs Count scores greater than 197.50 will not have a miscarriage.

A small number of CD56 bright and CD16 + cells circulating in the peripheral blood phenotypically show similarities to uNK cells, but there is a difference between CD56 bright cells in the blood and uterus. All CD56 bright cells are a temporary agranular which is in the decidua of some agranular 56 bright CD cells but mostly shows a typical feature of large granular lymphocytes (11).

The number of CD56 + cells increases in the proliferative phase and reaches the highest levels in the final phase of secretion, an increase that supports the occurrence of pregnancy (11, 35, 36).

The number of uNK cells increases during the secretion phase and if there is no pregnancy process, it will die on premenstrual, and this will reduce the production of solubel factors which maintain vascular integrity and this will trigger menstrual disorders. UV cells are important at the time of renewal, diffraction and disturbance of the endometrium during the menstrual cycle, expression of RNA to vascular endothelial growth factor-C (VEGF-C), placental growth factor (PGF), angiopoietin-2 (Ang-2) in secretion phase (40).

Table 4 shows that most (77.8%) who have CD56 + CD16 + Abs Count values below the cut off point (<197,5) have recurrent early idiopathic recurrences. Statistically with Chi-square test showed a significant relationship (p <0,005).

Odd Ratio of recurrent idiopathic recurrent miscarriage was 7.87 times in the group with CD56 + CD16 + Abs Count under cut off point (<197.5) compared to the CD56 + CD16 + Abs Count group above the cut off point (≥197, 5).

Table 5 shows that most (71.4%) who have CD56 + CD16 +% lymph values below the cut off point (<7.5) have early recurrent idiopathic recurrences. Statistically with Chi-square test showed no significant relationship (p <0.005).

Odd Ratio of recurrent idiopathic recurrent miscarriage was 3.75 times in the group with CD56 + CD16 +% lymph score below cut off point (<7.5) compared to the group having CD56 + CD16 +% lymph values above the cut off point (≥7.5).

TABLE 4 - THE ASSOCIATION OF RECURRENT MISCARRIAGE WITH CD56 + CD16 + ABS COUNT = 197.5.

$CD16^+56^+$ Abs Count	Miscarriage			p value	OR
	Recurrent miscarriage	None	Total		
< 197.5	14 (77.8%)	4 (22.2%)	18 (100%)		
≥197.5	8 (30.8%)	18 (69.2%)	26 (100%)	0.002	7.87
Total	22 (50.0%)	22 (50.0%)	44 (100%)		

TABLE 5 - THE ASSOCIATION OF RECURRENT MISCARRIAGE WITH CD56 + CD16 +% LYMPH CUT OFF POINT = 5.5.

$CD56^+ CD16^+$ % lymph	Miscarriage			p value	OR
	Recurrent miscarriage	None	Total		
< 7.5	10 (71.4%)	4 (28.6%)	14 (100%)		
≥7.5	12 (40%)	18 (60%)	30 (100%)	0.052	3.75
Total	22 (50.0%)	22 (50.0%)	44 (100%)		

Conclusion

From the results of this study it can be concluded:

1. Mean CD56 + CD16 +% lymph values of the early idiopathic recurrent miscarriage group were lower than the control group's mean, but the statistical test results with the Mann-Whitney test showed no significant difference in CD56 + CD16 +% lymph score of early idiopathic recurrent miscarriage group with control group.
2. Mean CD56 + CD16 + Abs Count of recurrent idiopathic early miscarriage group lower than CD56 + CD16 + Abs Count control group, and statistical test results with Mann-Whitney test showed no significant differences in CD56 + CD16 + Abs Count of recurrent miscarriage group early idiopathy with the control group.
3. ROC value of serum CD56 + CD16 +% lymph was 0.63. Cut off point is 7.50 with sensitivity of 82% and specificity of 46%.
4. The ROC value of serum CD16 + CD56 + Abs Count is 0.73. The cut off point was 197.50 with a sensitivity of 82% and a specificity of 64%.

Suggestions

1. Further research is undertaken in the form of multicentre research in Indonesia so that it can be proven the role of CD56 + CD16 +, pNK, Enk recurring early idiopathic miscarriage in various ethnic in Indonesia.
2. Further studies of CD56 + CD16 +, pNK, eNK in early idiopathic recurrent miscarriages such as CD4 +, CD25 +, peripheral blood cell, also play an important role in the implantation process and the success of a pregnancy, in order to make a marker of pNK to recurrent early miscarriage in Indonesia.

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