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Evaluation of the role of CD56 cells in unexplained recurrent spontaneous abortion

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***Abstract*---**Objective: To determine whether peripheral natural killer (NK) cells play a role in recurrent miscarriage by evaluating the percentage of these cells using flow cytometry and comparing it with the percentages in normal multiparous women and women who experienced miscarriage only once. Methods: This case-control study was carried out on a total of 108 women. The study participants consisted of two groups of cases: unexplained recurrent miscarriage cases (n=36) and explained recurrent miscarriage cases (n=36). Additionally, a control group of normal multiparous women or women with only one previous miscarriage (n=36) was included. Detailed clinical assessments, laboratory investigations, and flow cytometry analysis were conducted to collect relevant data. Flow cytometry was used to determine the percentage of CD56+ NK cells in peripheral blood samples. Results: The mean age of the participants ranged from 26.72 to 28.33 years, with no significant difference among the three groups. Parity showed a statistically significant difference, with a higher proportion of nulliparous women in the unexplained abortion group. The number of previous abortions did not differ significantly between the unexplained and explained groups. Among the causes of abortion, endocrine and immunological factors were prominent. NK% CD56 levels were significantly higher in both the unexplained and explained abortion groups compared to the control group. Conclusions: Peripheral NK cells may play a role in recurrent early

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miscarriage. The elevated NK% CD56 levels observed in the unexplained and explained abortion groups indicate their potential involvement in the pathogenesis of recurrent miscarriage.

***Keywords*---**Peripheral Natural Killer Cells, Unexplained Recurrent Miscarriage, CD56+ NK Cells, Flow Cytometry.

Introduction

Recurrent miscarriage, also known as habitual abortion or recurrent pregnancy loss (RPL), is defined as the loss of three or more consecutive pregnancies with the same partner [1]. However, according to the American Society of Reproductive Medicine (ASRM), recurrent miscarriage is defined as the loss of two or more consecutive pregnancies with the same partner [2]. Early miscarriage refers to the loss of a pregnancy during the first trimester, either based on histological confirmation or ultrasonographic findings [3].

Miscarriage, the most common complication of pregnancy, refers to the spontaneous loss of a pregnancy before the fetus reaches viability. The definition varies across regions, encompassing all pregnancy losses from conception until 24 weeks of gestation in the UK, while in other parts of the world it may be defined as up to 20 weeks of gestation [4]. The etiology of early miscarriage is diverse and often subject to controversy. Multiple factors are frequently involved. The leading causes of recurrent miscarriage include genetic factors, immunologic factors, anatomical abnormalities, infections, environmental factors, endocrine dysregulation, and hematologic disorders [2].

Recent research has revealed that natural killer (NK) cell activity plays a significant role in miscarriage and impaired implantation. Increased NK cell activity can result from high levels of stress or autoimmune diseases [5]. NK cells, belonging to the group of innate lymphoid cells, are large granular lymphocytes and represent a distinct subset of cells derived from common lymphoid progenitors, alongside B and T lymphocytes. They undergo differentiation and maturation within various organs, such as the bone marrow, lymph nodes, spleen, tonsils, and thymus, before entering the circulation [6].

Among peripheral blood lymphocytes (PBLs), NK cells constitute a significant proportion, ranging from 5% to 15%. They serve as a first-line defence against intracellular pathogens and tumors [7]. NK cells can be further categorized into functionally and phenotypically distinct subsets: $CD56^{bright}$ and $CD56^{dim}$ cells. These subsets exhibit enhanced cytotoxicity compared to other NK subsets, even when in a resting state. The increased proportions and cytotoxic activity of $CD56^{dim}$CD16+ cells in the blood, as well as their presence in the endometrium of pregnant women, have been associated with pregnancy loss [8].

As the majority of pregnancies involve two parents who are not tissue-matched, successful pregnancy requires suppression of the mother's immune system. NK cells are believed to play a crucial role in this process. Uterine NK cells (uNK cells), which differ from peripheral NK cells, belong to the $CD56^{bright}$ NK cell

subset. They possess potent cytokine secretion abilities but exhibit lower cytotoxicity. Uterine NK cells share some similarities with peripheral $CD56^{dim}$ NK cells, although their receptor profiles differ slightly [9]. It is worth noting that no evidence has been found to suggest that uterine NK cells have a detrimental effect on the embryo or developing baby [10].

Therefore, this study aimed to determine whether peripheral natural killer (NK) cells play a role in recurrent miscarriage by evaluating the percentage of these cells using flow cytometry and comparing it with the percentages in normal multiparous women and women who experienced miscarriage only once.

Patients and Methods

This case-control study was conducted at the Gynecology Clinic of Benha University Hospital. The study protocol was approved by the Human Research Ethics Committee of Banha Faculty of Medicine, and written informed consent was obtained from all individual patients prior to their participation. The study was done over a period of one year, from January 2020 to January 2021.

The study included a total of 108 women who presented to the Gynecology Clinic. The study population consisted of two groups of cases: unexplained recurrent miscarriage cases (n=36) and explained recurrent miscarriage cases (n=36). Additionally, a control group of normal multiparous women or women with only one previous miscarriage (n=36) was included.

Inclusion criteria for study cases

The inclusion criteria for the study cases were as follows: females in the childbearing period, not currently pregnant, body mass index less than 35, and a history of recurrent miscarriage, defined as two or more instances of early miscarriage (excluding ectopic, molar, or elective abortion). The cause of recurrent miscarriage was unexplained in the first group and explained in the second group.

Exclusion criteria for unexplained miscarriage cases

The following exclusion criteria were applied to the cases with unexplained recurrent miscarriage: chromosomal abnormalities detected by karyotyping, structural causes such as Mullerian duct abnormalities, uterine fibroids, cervical incompetence, or previous pelvic adhesiolysis. Endocrine causes such as polycystic ovarian syndrome, uncontrolled diabetes mellitus (DM), hyperprolactinemia, or luteal phase defect (LPD) were also excluded. In addition, infectious causes (e.g., bacterial vaginosis, pelvic inflammatory diseases), immunological diseases (e.g., antiphospholipid syndrome, lupus anticoagulant), endometriosis, and inherited thrombophilia were excluded.

All cases underwent a comprehensive evaluation that included various procedures. A full history was taken from each participant, and a complete physical examination was conducted. Laboratory investigations were performed, including complete blood count, blood sugar, renal, liver, and adrenal function

tests. For diabetic cases, glycated hemoglobin levels were measured. Prolactin levels were also assessed using a standard assay with a normal range of 5-20 nl/ml or 400 IU/ml.

Ultrasound examinations were conducted using two- or three-dimensional imaging techniques, including sonohysterography. Both partners of the study cases underwent karyotyping to assess chromosomal abnormalities. Hysterosalpingography was performed to evaluate the uterine and tubal anatomy. Endometrial curettage (biopsy) was conducted, and hysteroscopy or laparoscopy was performed as needed. The nature and purpose of the study were explained to all participants, and verbal consent was obtained. Data from previous investigations were collected from patient files.

Flow cytometry analysis was conducted using two milliliters of peripheral blood collected in tubes containing K3EDTA. Fresh blood samples were used, and anti-Human CD56 antibodies labeled with Phycoerythrin (PE) were employed for the detection of NK cells. CD56+ cell analysis was performed within the lymphocyte cell range.

Statistical analysis

The statistical analysis was performed using SPSS v25 (IBM©, Armonk, NY, USA). For quantitative parametric data, the mean and standard deviation (SD) were used to present the results. Qualitative variables were described using frequency and percentage (%), and the Chi-square test was utilized for their analysis. A two-tailed P value of less than 0.05 was considered statistically significant, indicating a significant difference or association between variables.

Results

The age of the unexplained abortion group ranged from 19 to 36 years with a mean of 26.72 years, while the explained abortion group ranged from 19 to 36 years with a mean of 27.78 years. The control group age range was 19 to 37 years with a mean of 28.33 years. There was no statistically significant difference in age among the three groups (P-value = 0.309).

In the unexplained abortion group, 44.5% were nulliparous, 47.2% had one child, and 8.3% had two children. In the explained abortion group, 38.9% were nulliparous, 36.1% had one child, and 25% had two children. Regarding the control group, 47.3% had two children, 44.4% had three children, and 8.3% had four children. There was a highly significant difference in parity among the three groups. Table 1

Table 1: Parity of the three studied groups

Variable	Unexplained abortion (*n*=36)		Explained abortion (*n*=36)		Control (n=36)		x^2	P
	No	%	No	%	No	%		
Parity:								
P0	16	44.5	14	38.9	0	0		
P1	17	47.2	13	36.1	0	0		
P2	3	8.3	9	25	17	47.3	**79.21**	**<0.001****
P3	0	0	0	0	16	44.4		
P4	0	0	0	0	3	8.3		

Data were presented as frequency (%), x2: chi-square test, *: significant as P-value < 0.05.

Among the unexplained abortion group, 33.3% had experienced abortion twice before, 38.9% had it three times, 16.7% had it four times, 8.3% had it five times, and only 2.8% had it six times. In the explained abortion group, 41.7% had experienced abortion twice before, 25% had it three times, 13.9% had it four times, 11.1% had it five times, and only 8.3% had it six times. There were no statistically significant differences between the unexplained and explained groups in the number of abortions. Table 2

Table 2: Frequency of abortions of the two cases groups

Variable	Unexplained abortion (*n*=36)		Explained abortion (*n*=36)		x^2	P
	No	%	No	%		
Abortion:						
2 times	12	33.3	15	41.7		
3 times	14	38.9	9	25	2.65	0.626
4 times	6	16.7	5	13.9		
5 times	3	8.3	4	11.1		
6 times	1	2.8	3	8.3		
Mean ± SD	3.08 ± 1.05		3.19 ± 1.32		MW 0.02	0.99

Data were presented as mean ± standard deviation (SD) and frequency (%), x2: chi-square test, MW: Mann-Whitney U test.

Of the study participants, 27.8% had abortion due to endocrine causes, with polycystic ovarian syndrome (PCO) being the most common. Anatomical causes, especially cervical incompetence and submucous fibroids, accounted for 27.8% of the cases. One case had genetic causes due to paternal chromosomal abnormalities, while 41.6% had immunological causes, such as antiphospholipid syndrome (APS) and systemic lupus erythematosus (SLE). Table 3

Table 3: Causes of abortion among the explained abortion group

Causes of Abortion:		Cases (n=36)	
		No	%
Endocrine 10 (27.8%)	DM	1	2.8
	Hyperprolactinemia	3	8.3
	LPD	1	2.8
	PCO	5	13.9
Anatomical 10 (27.8%)	Septate uterus	2	5.6
	sub mucous fibroid	3	8.3
	Asherman syndrome	1	2.8
	Hypo-plastic uterus	1	2.8
	Cervical incompetence	3	8.3
Genetic 1 (2.8%)	(Paternal chromosomal abnormalities)	1	2.8
Immunologic 15 (41.6%)	APS	9	25
	SLE	6	16.7

Data were presented as frequency (%), DM: Diabetes Mellitus, LPD: Luteal Phase Defect, PCO: Polycystic Ovaries, APS: Antiphospholipid Syndrome, SLE: Systemic Lupus Erythematosus.

The CD56 levels in the unexplained abortion group ranged from 5.24% to 19.3% with a mean of 12.51%. In the explained abortion group, the range was from 3.38% to 22.13% with a mean of 9.67%. The control group had CD56 levels ranging from 5.08% to 14.06% with a mean of 8.73%. There was a highly significant difference in NK% (CD56) among the three groups. Additionally, there were statistically significant differences in the number of cases with NK% above 15% between the unexplained, explained, and control groups. Table 4

Table 4: CD56 of the studied groups

Variable	Unexplained abortion (n=36)		Explained abortion (n=36)		Control (n=36)		F	p
CD 56: Mean ± SD Range	12.51 ± 4.29 5.24 – 19.30		9.67 ± 4.62 3.38 – 22.13		8.73 ± 2.59 5.08 – 14.06		**9.02**	**<0.001***
	No	%	No	%	No	%	**x^2**	**P**
Classification: NK% (CD 56) ≤ 15	24	66.7	30	83.3	36	100	**14.4**	**0.001***
NK% (CD 56) > 15	12	33.3	6	16.7	0	0		

Data were presented as mean ± standard deviation (SD), range, and frequency (%), x2: chi-square test, CD 56: Cluster of Differentiation 56, NK: Natural Killer, F: F-statistic, *: significant as P-value < 0.05.

There was a statistically significant relationship between the cause of abortion and NK% (CD56) level, with higher levels observed in unexplained and immunologic cases compared to other causes. Table 5

Table 5: Relation between Causes of abortion among the cases group and NK % (CD56)

Variable	NK % (CD56)		F	P
	N	Mean ± SD		
Causes of Abortion:				
Unexplained	36	12.51 ± 4.29	**2.52**	**0.041***
Endocrine	10	8.59 ± 1.76		
Anatomical	10	8.7± 3.29		
Genetic	1	8.3 ± 0		
Immunologic	15	11.14 ± 6.35		

Data were presented as mean ± standard deviation (SD), CD 56: Cluster of Differentiation 56, NK: Natural Killer, F: F-statistic, *: significant as P-value < 0.05.

Regarding the relationship between the causes of abortion in the explained recurrent miscarriage group and the percentage of NK cells (CD56). In terms of endocrine causes, there were no statistically significant differences in the mean NK% (CD56) among the different variables (F = 0.89, p = 0.504). Similarly, for anatomical causes, there were no statistically significant differences observed (F = 1.41, p = 0.35). However, concerning immunologic causes, there was a statistically significant difference in the mean NK% (CD56) between APS and SLE cases (t = 6.45, p < 0.001*). The mean NK% (CD56) was higher in APS cases compared to SLE cases. Table 6

Table 6: Relation between causes of abortion among the cases of explained recurrent miscarriage group and NK % (CD56)

Variable	NK % (CD56)			Test	P
	N	mean	Sd		
Endocrine:				F 0.89	0.504
DM	1	9.42	0		
Hyperprolactinemia	3	9.69	1.88		
LPD	1	8.95	0		
PCO	5	7.68	1.74		
Anatomical:				F 1.41	0.35
Sepetate uterus	2	8.78	0.50		
sub mucous fibroid	3	7.78	1.31		
Asherman syndrome	1	5.2	0		
Hypo-plastic uterus	1	5.65	0		
Cervical incompetence	3	11.73	4.58		
Immunologic:				**t 6.45**	**<0.001***
APS	9	15.51	4.06		
SLE	6	4.58	0.76		

Data were presented as mean ± SD (standard deviation), range, and frequency (%). x2 refers to the chi-square test, CD 56 represents Cluster of Differentiation

56, NK stands for Natural Killer, F denotes the F-statistic, DM stands for Diabetes Mellitus, LPD represents Luteal Phase Defect, PCO refers to Polycystic Ovaries, APS represents Antiphospholipid Syndrome, SLE denotes Systemic Lupus Erythematosus, and * indicates statistical significance with a P-value < 0.05. The t-test was used to compare the mean NK% (CD56) between APS and SLE cases.

There were no statistically significant differences in NK% (CD56) levels among different endocrinal or anatomical causes. However, there were statistically significant differences in NK% levels between cases of APS and SLE, with a marked increase among APS cases. There was a statistically significant relationship between the cause of abortion and NK% (CD56) levels, with a higher number of immunologic cases showing levels above 15% compared to other causes. Table 7

Table 7: Relation between Causes of abortion among the explained group and NK % (CD56) level classification

Variable	NK% ≤ 15 (n=30)		NK% > 15 (n=6)		x^2	P
	No	%	No	%		
Endocrine	10	33.3	0	0	**5.52**	**0.047***
Anatomical	9	30	1	16.7		
Genetic	1	3.3	0	0		
Immunologic	10	33.3	5	83.3		

Data were presented as frequency (%), NK: Natural Killer, x2: chi-square test, *: significant as P-value < 0.05.

There were no statistically significant differences in NK% (CD56) levels among different endocrinal or anatomical causes. However, there were statistically significant differences in NK% levels between cases of APS and SLE, with a marked increase in APS cases with levels above 15%. Table 8

Table 8: Relation between Causes of abortion among the explained group and NK % (CD56) level classification

Variable	NK% ≤ 15		NK% > 15		x^2	P
	No	%	No	%		
Endocrine:	(n=10)		(n=0)		---	----
DM	1	10	0	0		
Hyper-prolactenemia	3	30	0	0		
LPD	1	10	0	0		
PCO	5	50	0	0		
Anatomical:	(n=9)		(n=1)		2.59	0.638
Sepetate uterus	2	22.2	0	0		
sub mucous fibroid	3	33.3	0	0		
Asherman syndrome	1	11.1	0	0		
Hypo-plastic uterus	1	11.1	0	0		
Cervical incompetence	2	22.2	1	100		
Immunologic:	(n=10)		(n=5)		**5**	**0.036***
APS	4	40	5	100		
SLE	6	60	0	0		

Data were presented as frequency (%), NK%: Natural Killer Cell Percentage, CD56: Cluster of Differentiation 56, DM: Diabetes Mellitus, LPD: Luteal Phase Defect, PCO: Polycystic Ovaries, APS: Antiphospholipid Syndrome, SLE: Systemic Lupus Erythematosus, x2: Chi-square test, *: Significance as P-value < 0.05.

Discussion

It is now accepted that in order for a normal pregnancy to occur, the maternal immune system must develop greater tolerance towards the semiallogeneic fetus. Natural killer (NK) cells can be found in both peripheral blood and the uterine mucosa, but there are phenotypic and functional differences between peripheral blood NK cells and uterine NK (uNK) cells [11].

Peripheral blood NK cells and uterine NK cells share the expression of certain cell surface markers. They are typically CD3 negative but express CD16 and CD56. NK cells can be further divided based on the intensity of CD56 expression, with a CD16+CD56bright subset and a CD16+CD56dim subset. Peripheral blood NK (pNK) cells are mostly CD16+CD56dim, while uNK cells are predominantly CD16+CD56bright. Importantly, the factors that regulate uterine and peripheral blood NK cells are likely to be similar. Therefore, assessing the level or activation of peripheral blood NK cells can provide information about the state of uterine cells [8].

The aim of this study is to investigate the role of peripheral natural killer cells (NK) in recurrent early miscarriage by estimating their percentage in the lymphocyte zone using flow cytometry. A case is considered positive if the percentage of NK cells is equal to or greater than 15%. A total of 108 women were included in this study, with 72 cases and 36 controls. The cases were further subdivided into two groups: 36 cases of explained recurrent miscarriage and 36 cases of unexplained recurrent miscarriage. The control group consisted of normal multiparous women and those who experienced miscarriage only once.

Collecting cases of unexplained miscarriage was easier than collecting cases of explained miscarriage, as more than 60% of all cases of recurrent miscarriage remain unexplained. The age range for the unexplained miscarriage group in our study was 19 to 36 years, with a mean age of 26.72 years. The age range for the explained miscarriage group was also 19 to 36 years, with a mean age of 27.78 years. The control group had an age range of 19 to 37 years, with a mean age of 28.33 years. There were no statistically significant differences in age among the three groups.

In another study, a cohort of 353 miscarriages was successfully karyotyped at the same center between 2002 and 2011. The women were grouped based on the number of miscarriages and maternal age. Among the 353 women, 153 were below 35 years (73 with sporadic, 48 with two, and 32 with recurrent miscarriage), while 200 were 35 years or older (81 with sporadic, 55 with two, and 64 with recurrent miscarriage) [12]. The study compared the rate and spectrum of chromosomal anomalies between sporadic and recurrent miscarriage within the two maternal age groups, using the chi-square test and Bonferroni correction for all p-values. The risk of chromosomal anomalies was estimated for maternal age,

number of miscarriages, and previous live births using multivariate binary logistic regression analysis. The results showed that sporadic and recurrent miscarriage did not significantly differ in terms of chromosomal anomaly rates (68% versus 60%), and maternal age was the only statistically significant predictor of the chromosomal anomaly risk identified. Some trends were observed in the chromosomal anomaly spectrum when comparing sporadic and recurrent miscarriage. Recurrent miscarriage exhibited a decrease in viable trisomies (37% versus 11%) and an increase in non-viable trisomies (38% versus 57%) in women over 35 years, along with an increase in unbalanced structural anomalies (4.9% versus 29%) in younger women.

The oldest participant in our study was 36 years old, limiting the direct implications of the findings. Regarding parity, significant differences were observed among the groups, with 44.5% of the unexplained abortion group being nulliparous, while 47.2% had one child and 8.3% had two children. In the explained group, 38.9% were nulliparous, 36.1% had one child, and 25% had two children. The control group had 47.3% with two children, 44.4% with three children, and 8.3% with four children. No statistically significant differences were found between the unexplained and explained groups in the number of abortions. The causes of miscarriage included endocrine (27.8%), anatomical (27.8%), genetic (1 case), and immunological (41.6%). Notably, there were significant differences in NK% (CD56) among the groups, with the unexplained group ranging from 5.24% to 19.3%, the explained group ranging from 3.38% to 22.13%, and the control group ranging from 5.08% to 14.06%.

These findings are consistent with a study conducted by Mehri Ghafourian et al. [13]. In that case-control study, they examined 25 non-pregnant women with at least three unexplained abortions and 25 non-pregnant women with a living child and no history of previous abortion. Using monoclonal antibodies anti-CD16, CD56, and CD20, and the flow cytometry method, they determined the percentage of cells with these markers. Their results showed that NK (CD16+/CD56+) cells significantly increased in women with recurrent miscarriage compared to the control group ($p \leq 0.05$). However, there were no significant differences in the percentage of CD20+ B cells between the experimental and control groups ($p > 0.05$). According to their study, an increased percentage of NK cells may be considered a risk factor for recurrent miscarriage. On the other hand, no studies have claimed that there is clear evidence linking altered peripheral blood NK cells to recurrent miscarriage.

According to the review by Wold and Arici [14], natural killer cells are abundant in the uterus and seem to play an important role in early pregnancy. Women with reproductive failure have been shown to have higher levels of natural killer cell numbers and activity in peripheral blood. However, the evidence regarding uterine natural killer cells is contradictory. While earlier studies suggested an increase in uterine natural killer cells in women with recurrent pregnancy loss, more recent investigations have not confirmed this trend. Uterine natural killer cell number or activity appears to be higher in spontaneous abortions with normal chromosomes compared to chromosomally abnormal pregnancies. Furthermore, an increase in the cytotoxic CD56 (dim) CD16+ natural killer cell subset has been observed in

the follicular fluid of patients with idiopathic infertility, and it is postulated that this increase may decrease fertilization rates in this group.

In our study, there was a statistically significant relationship between the cause of abortion and NK% (CD56), with higher levels observed in cases of unexplained and immunologic causes compared to other causes. There were no statistically significant differences in NK% (CD56) between different endocrine or anatomical causes. However, there were statistically significant differences in NK% (CD56) between cases of APS and SLE, with a marked increase among APS cases. Furthermore, there was a statistically significant relationship between the cause of abortion and NK% (CD56) levels, with a higher number of cases showing levels above 15% in immunologic causes compared to other causes.

These results are consistent with a study conducted at Oxford University by Perricone et al. [15]. In that study, NK-cell levels in the peripheral blood of APS patients without recurrent spontaneous abortion (RSA) and in APS-RSA patients were evaluated using flow cytometry. NK-cell levels were also evaluated in RSA cases without antiphospholipid antibodies (aPL) associated with endocrine, anatomical, or idiopathic conditions, as well as in healthy women. The results showed that high NK levels were found in 14 out of 25 (56%) APS-RSA patients. NK mean levels were significantly higher in APS-RSA patients than in all other conditions studied, including healthy subjects, except for idiopathic RSA.

A study by Bingham stated that maternal exposure to excessive alcohol has been associated with an increased risk of miscarriage. In our study, all cases denied being smokers or consumers of alcohol. This could be attributed to the fact that the study was conducted in a rural area and influenced by religious beliefs that prohibit these harmful habits [16].

The causes of recurrent miscarriage are extremely diverse and complex, and many events and mechanisms are still unknown. Therefore, it remains unclear whether altered levels of NK cells are a result of recurrent miscarriage or are involved in the pathogenesis of recurrent miscarriage in certain cases.

In this study, no altered levels of NK% were detected in cases of recurrent miscarriage with anatomical, hormonal, or genetic causes. This finding supports the hypothesis that altered NK% is a cause rather than a result of recurrent miscarriage.

This study had some limitations: Firstly, it was a single center study with a relativley sample size was. Additionally, the study focused primarily on the percentage of peripheral natural killer (NK) cells (CD56+) and did not explore other potential immune markers or profiles that could contribute to recurrent miscarriage. Furthermore, the study did not extensively assess confounding factors or incorporate long-term follow-up to evaluate the outcomes of subsequent pregnancies or the impact of interventions. Therefore, larger, prospective studies with comprehensive assessments and long-term follow-up are needed to provide a more comprehensive understanding of the role of NK cells in recurrent miscarriage.

Conclusions

The percentage of peripheral natural killer cells (NK%) (CD56) plays a significant role in recurrent early miscarriage. The findings demonstrate that NK% (CD56) is notably elevated in cases of unexplained recurrent miscarriage and in cases associated with immunological diseases compared to other cases, such as those attributed to genetic, hormonal, or anatomical causes.

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References

1. Egerup P, Kolte AM, Larsen EC, et al. Recurrent pregnancy loss: what is the impact of consecutive versus non-consecutive losses? Hum Reprod 2016;31(11):2428-2434, doi:10.1093/humrep/dew169
2. El Hachem H, Crepaux V, May-Panloup P, et al. Recurrent pregnancy loss: current perspectives. Int J Womens Health 2017;9(331-345, doi:10.2147/ijwh.S100817
3. Novais Nogueira Cardoso RMA, Nogueira Cardoso PLN, Azevedo AP, et al. First-trimester miscarriage: A histopathological classification proposal. Heliyon 2021;7(3):e06359, doi:10.1016/j.heliyon.2021.e06359
4. Quenby S, Gallos ID, Dhillon-Smith RK, et al. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. Lancet 2021;397(10285):1658-1667, doi:10.1016/s0140-6736(21)00682-6
5. Sharma S. Natural killer cells and regulatory T cells in early pregnancy loss. Int J Dev Biol 2014;58(2-4):219-29, doi:10.1387/ijdb.140109ss
6. Abel AM, Yang C, Thakar MS, et al. Natural Killer Cells: Development, Maturation, and Clinical Utilization. Front Immunol 2018;9(1869, doi:10.3389/fimmu.2018.01869
7. Merkt W, Sturm P, Lasitschka F, et al. Peripheral blood natural killer cell percentages in granulomatosis with polyangiitis correlate with disease inactivity and stage. Arthritis Res Ther 2015;17(337, doi:10.1186/s13075-015-0851-7
8. Poli A, Michel T, Thérésine M, et al. CD56bright natural killer (NK) cells: an important NK cell subset. Immunology 2009;126(4):458-65, doi:10.1111/j.1365-2567.2008.03027.x
9. Melsen JE, Lugthart G, Lankester AC, et al. Human Circulating and Tissue-Resident CD56(bright) Natural Killer Cell Populations. Front Immunol 2016;7(262, doi:10.3389/fimmu.2016.00262
10. Toth B, Vomstein K, Togawa R, et al. The impact of previous live births on peripheral and uterine natural killer cells in patients with recurrent miscarriage. Reprod Biol Endocrinol 2019;17(1):72, doi:10.1186/s12958-019-0514-7
11. Moffett A, Shreeve N. First do no harm: uterine natural killer (NK) cells in assisted reproduction. Hum Reprod 2015;30(7):1519-25, doi:10.1093/humrep/dev098

12. Grande M, Borrell A, Garcia-Posada R, et al. The effect of maternal age on chromosomal anomaly rate and spectrum in recurrent miscarriage. Hum Reprod 2012;27(10):3109-17, doi:10.1093/humrep/des251
13. Ghafourian M, Band NA, Pour AF, et al. The role of CD16+, CD56+, NK (CD16+/CD56+) and B CD20+ cells in the outcome of pregnancy in women with recurrent spontaneous abortion. Int J Womens Health Reprod Sci 2015;3(61-66
14. Wold AS, Arici A. Natural killer cells and reproductive failure. Curr Opin Obstet Gynecol 2005;17(3):237-41, doi:10.1097/01.gco.0000169099.98589.5b
15. Perricone R, De Carolis C, Kröegler B, et al. Intravenous immunoglobulin therapy in pregnant patients affected with systemic lupus erythematosus and recurrent spontaneous abortion. Rheumatology (Oxford) 2008;47(5):646-51, doi:10.1093/rheumatology/ken046
16. Bingham RJ. Latest Evidence on Alcohol and Pregnancy. Nurs Womens Health 2015;19(4):338-44, doi:10.1111/1751-486x.12219