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A COMPARATIVE STUDY OF CD 56+ NK (NATURAL KILLER) CELLS IN ENDOMETRIAL TISSUE OF NORMAL AND INFERTILE PATIENTS.

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Abstract

Introduction: The endometrium is the special epithelial lining of the uterine cavity which plays important roles in different phases of menstrual cycle as well as in implantation process. The natural killer (NK) cells are a type of lymphocytes circulating in peripheral blood, named because of their effector functions in killing target cells. Immune cells that share similar phenotypic characteristic but are poor killers which populate the uterine lining at implantation and during early pregnancy when the placenta is established and are identified by the surface marker CD56+.

Aims and Objectives: The aim of the study is to evaluate the mean cell count of CD56+ uterine natural killer (uNK) cells in endometrial tissue specimens and determine the association of the histopathological findings with CD56+ mean cell count.

Materials and Methods: In this cross sectional study, 50 endometrial tissue samples (biopsy and dilatation and curettage specimen) received in the department of Pathology, Regional Institute of Medical Sciences (RIMS), Imphal during the period between September, 2016 and August, 2018 were selected and included in the study. Twenty-nine cases with history of primary and secondary infertility were included in the test group and 21 cases with no history of abortions and miscarriages constituted the control group. CD56+ cells were stained using mouse monoclonal CD56+ antibody and counted manually under ten high power fields (40X) and mean cell count was calculated and statistically analysed.

Results: The age of the study group ranged from 18 to 45 years (Mean ± SE; 35.88 ± 1.13). The mean number of CD56+ NK cells was higher in control groups 56.62 ± 5.683 (Mean ± SEM; N=21) as compared to the mean number of CD 56+ NK cells in the test group 38.20 ±3.958 (Mean ± SEM; N=29). The difference was statistically significant (difference of 1.195 with 95% confidence interval of 0.536 to 3.294, p = 0.008).

Conclusion: Mean number of CD 56+ uNK cells, show significant correlation with primary infertility and repeated miscarriages (p=0.022). CD56+ NK cells may have a role in the maintenance of normal pregnancy. It might be a useful marker for predicting risk of miscarriages and infertility.

Keywords: CD 56+, Natural killer (NK) cells, Endometrial tissue, Primary and secondary infertility.

Introduction

The endometrium is the special epithelial lining of the uterine cavity consisting of a surface epithelium, glands and stroma. The endometrium plays important roles in different phases of menstrual cycle as well as in implantation process. The decidual cells during pregnancy are derived from stroma cells and are surrounded by lymphocytes.[1] Natural killer (NK) cells are a type of lymphocyte circulating in peripheral blood named because of their effector functions in killing target cells. Immune cells that share similar phenotypic characteristics but are poor killers populate the uterine lining at implantation and during early pregnancy when the placenta is established and are identified by the surface marker CD56.[2] The functions of uterine natural killer (uNK) cells are essentially unknown but available data point to a role in regulating placentation in concern with other elements of the decidua and invading trophoblastic cells. They are believed to play a role in implantation, angiogenesis and maintenance of pregnancy. Of central importance is that uterine NK cells are phenotypically and functionally different from NK cells in peripheral blood and should be regarded as a separate lymphoid subset. NK cells are the dominant immune cell type in the endometrium and play a major role in determining pregnancy outcome.[3]

The relationship between uterine natural killer cells and unexplained repeated miscarriage (RM) showed that 73.75% of the studied women with a past history of early

miscarriage had CD56+ CD16+ uNK cells in their decidua specimens and 66.25% of studied women with a past history of late miscarriage had CD56+, CD16+ uNK cells in their decidua specimens confirming that the association between early and late miscarriage and CD56+ CD16+ uNK cells in decidua specimens was significant.[4] The characterization NK cell profiles in patients with endometriosis in endometrial tissue and peripheral blood showed that populations of endometrial CD34 hematopoietic stem cells were higher ($p<0.0004$) and co-expression of NK cell marker CD56 was increased ($p<0.034$) compared with patients who had failed implantation while the levels of blood NK progenitors were similar in both groups.[5] Contradictory to the above findings, the overall expression of CD69, CD94, CD161 were also increased significantly on CD56+ NK cells in both patient groups compared to control groups ($p<0.001$).[6]

What is really happening with the CD56+ NK cells in the endometrial tissue is still vague. Based on the critical review of literatures, it is still unclear and leaves a platform for discussion on the said topic. Here we tried to make a comparative study of CD56+ NK cells in endometrial tissue of normal to those of infertile patients by evaluating the association of histopathological findings with CD56+ uNK cells mean count. This approach would narrow down the gap between the different researchers in the related field.

Materials and Methods:

The study was carried out in the department of Pathology in collaboration with the Department of Obstetrics and Gynecology, Regional Institute of Medical Sciences (RIMS), Imphal for a period of two years starting from September, 2016 to August, 2018. The study included women in the age group of 18 to 45 years. After the inclusion and exclusion criteria, a total of 50 endometrial tissue samples were included, out of which 29 samples with a history of infertility and/or miscarriage were clubbed together as Group A while the remaining 21 parous women with no history of miscarriage constituted Group B. Demographic variables such as age, number and type of miscarriage, parity, previous obstetric history, etc were taken into consideration. Brief clinical information in respect of age, marital history, parity etc of the patient and clinical data received from the case files of the patient were recoded. The samples were processed for histopathological examination(HPE), stained by haematoxylin and eosin (H/E) and Immunohistochemical (IHC) stain with Monoclonal Mouse Antihuman CD56, clone 123C3.

CD56+ uNK cells were counted manually under ten high power fields (HPF) and mean cell count was calculated. Images were taken at 10 random fields at high power (40X) magnification. Data were checked for completeness and consistency and the data entry and analysis were done using SPSS (IBM) version 21.0 software. Summarization of data like age, parity, etc. was done by using descriptive statistics like mean, standard deviation (SD) and percentage. Analytical statistics like t-test and Cramer's V were used to test the statistical significance and find the association between relevant variables, p value <0.05 was considered as statistically significant.

Ethical clearance:

The approval of the Research Ethics Board, RIMS was taken prior to the study.

Results:

The age of the study group ranged from 18-45 years (Mean ± SE; 35.88 ± 1.13). These individuals were sub-grouped into 4 age ranges upto 20 years, 21-30 years, 31-40 years and 41 years and above. More number of cases (38.0%) were in the 41years and above age group, while only 6.0% were in the upto 20 years age group. Out of the 50 case, 29 (58.0%) had miscarriage and 21 (42.0%) did not have miscarriage (Fig1). Twenty-six (52.0%) cases had early miscarriage (< 13 weeks gestation) and 3 (6.0%) had late (> 13 weeks gestation). Nineteen (38.0%) cases had miscarriage only once, 8 (16.0%) had twice and 2 (4.0%) had miscarriage thrice or more.

CD56+ NK cells were cytoplasmic stained and observed as brown color in IHC staining. The mean number of CD56 per 10 HPF was found to range between 10 to 110. Hence it was categorized into groups of 10 intervals. The maximum number of CD56 count ranged between 31-40 and 41-50 with 12 individual each comprising a total of 24 individuals of the total study population. CD56 count ranging between 51-60 was found in only one individual. The histopathological findings of the present study revealed that product of conception constituted the maximum percentage of individuals (34.0%) followed by proliferative phase endometrium constituting 30.0% of the total study population. Early secretory phase endometrium and disordered proliferative endometrium constituted 18.0% and 10.0% respectively (Fig 3, 4). Two percent showed late secretory phase endometrium and another 2.0% showed features of hydatidiform mole. Another 2 cases (4.0%) constituted the cases which had no definite histopathological diagnosis, out of which 1 case had endometrium with marked predecidualisation of stroma and another case had decidua with few glands showing Arias-Stella reaction. Figure 2 illustrates the mean number of endometrial CD56+ cells in Group A individuals was 38.20 ± 3.958 (Mean ± SEM; N=29) and group B was 56.62 ± 5.683 (Mean ± SEM; N=21). Group B individuals had a significantly increased mean number of CD56+ cells per 10 HPF as compared with Group A individuals (p value 0.0084). Upon establishing the relationship between the primary infertility and repeated miscarriage with CD56 density, our result showed that among the total

population, 10 individuals had primary infertility and 9 individuals had repeated miscarriage. In primary infertility, the CD56 density was found to be 1.20 ± 0.133 (Mean ± SEM) and that of repeated miscarriage was 1.44 ± 0.176 (Mean ± SEM). There was no significant relationship between the primary infertility and repeated miscarriage with reference to CD56 density.

Table1 illustrates the mean count of CD56+ cells per 10 HPF is significantly correlated ($p<0.05$) with primary infertility and repeated miscarriage. The mean count of CD56 per 10 HPF in primary infertility (Mean ± SD, 2.10 ± 1.28) was found to be much lesser (50%) than that of repeated miscarriage (Mean + SD, 4.33 ± 2.44).

The correlations between mean number of CD56 per 10HPF with different histopathological findings in 50 individuals show that there exists a correlation with significant level ($P<0.05$) between the two (Table 2). Among the total study individuals, 15 cases were found to be in proliferative phase endometrium (4.33 ± 0.607; Mean ± SEM) and 9 cases were in early secretory phase (6.560.884; Mean ± SEM) (p value 0.044). These two histopathological findings had a significance value of $p<0.05$ (Table 3). Upon analysing the level of association using Cramer's V co-efficient, it was found that the mean count of CD56 per 10 HPF had a very strong association (0.35 to 0.40). (Table 4)

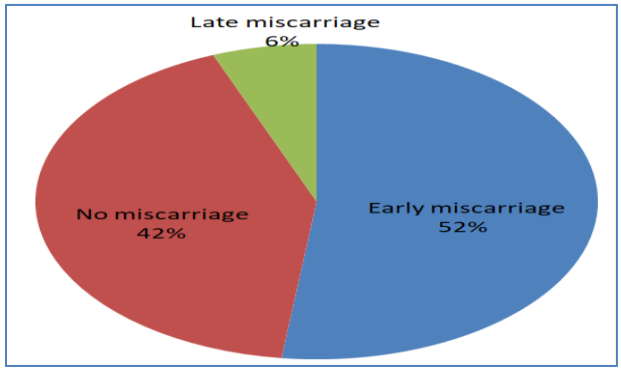

Figure 1: Distribution of miscarriage in 50 cases.

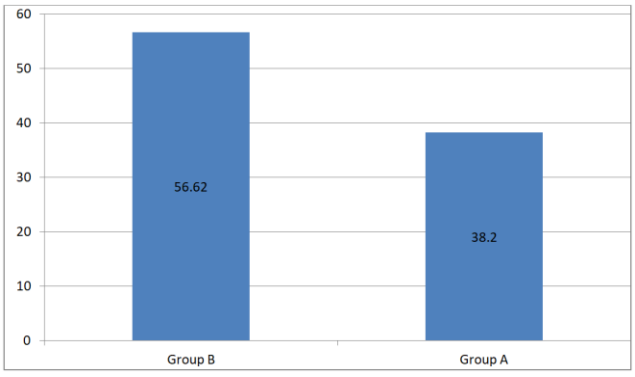

Figure 2: Bar chart of mean CD 56+ cells per 10 HPF in Group A and Group B cases.

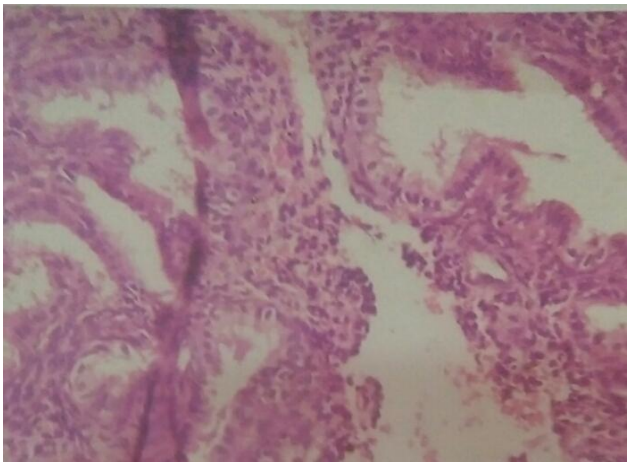

Figure 3: Photomicrograph of early secretory endometrium (40X, H&E stain)

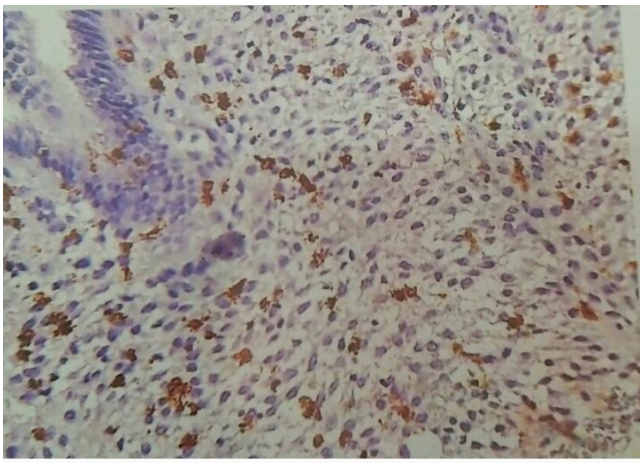

Figure 4: Photomicrograph of early secretory endometrium (40X,IHC stain, CD56)

Table 1: Relationship between mean CD 56+ count/10 HPF of primary infertility and repeated miscarriage.

	Primary infertility and repeated miscarriage	n	Mean	Standard deviation	Standard error mean	P value
Mean CD56+ count/10 HPF	Primary infertility	10	2.10	1.287	0.407	0.022
	Repeated miscarriage	9	4.33	2.449	0.816	

Table 2: Correlation between mean number of CD56+ count and histopathological findings.

		Mean number of CD 56+ per 10 HPF	Histopathological findings
Mean number of CD 56+ count	Significant (2-tailed)		0.038
	N	50	50
Histopathological findings	Significant (2-tailed)	0.038	
	N	50	50

Table 3: Relationship between histopathological findings (proliferative and early secretory phase of endometrium) and mean count of CD56+ per 10HPF

	Histopathological findings	N	Mean	Standard deviation	Standard error mean	p value
Mean count of CD56+ per 10 HPF	Proliferatve endometrium	15	4.33	2.350	0.607	0.044
	Early secretory endometrium	9	6.56	2.651	0.884	

Table 4: Level of association between mean CD56+ count per10 HPF with histopathological findings.

Co- efficient	Value	Inference
Cramer,s V	0.364	Very strong association

Value of 0.35 to 0.40 indicates very strong association.

Discussion:

The study was based on 50 individuals, consisting of 29 women with history of primary and secondary infertility which constituted the Group A and 21 women with no history of miscarriage or abortions which formed the Group B. The age of the individuals ranged from 18-45years (Mean ± SE, 35.88 ± 1.13) which is in similar range with other reports (Mean ± SD 33.6 ±5.3) of Michou VI et al 2003.[7]

In a study of Glover LE et al, populations of endometrial CD34 hematopoietic stem cells were higher and coexpression of NK cell marker CD56 was increased in successful implantation as compared with patients who had failed implantation showing that uterine NK progenitor cell populations are markedly different in patients with endometriosis who proceed to successful or failed embryo implantation.[5] This result is consistent with our present findings. However in Liu B et al and some other studies, it had been shown that uNK cell count on its own did not have a significant correlation to the pregnancy outcome but a retarded endometrium is significantly associated with a higher miscarriage rate than normally developed endometrium in women with reproductive failure.[8]

Eskicioglu F et al reported that CD56 expression of uterine NK cells may be an indicator of a healthy pregnancy (HP). The highest protein expression of CD56 was found in the HP compared to Non pregnant and RM.[9] However, it was not statistically significant; the increased expression of CD16, CD8 and also significantly increased expression of TNF may be associated with the predominant cytotoxic activity in the maternal immune system in patients with RM. A similar study by Mamedalieva NM et al showed that early miscarriages occurs as activation of cytotoxic natural killer (NK) cells with CD16+ phenotype and a pronounced suppression level of CD56+ cells endometrial type and late miscarriages occurs as cell deficit, followed by reduction of all CD8+ cytotoxic lymphocytes and CD56+ CD16+ NK cells.[10] Our present findings are consistent with those studies. Our findings are also supported by the findings of Kofod L et al who had shown that increased CD56+ uNK cells served as predictor for successful pregnancy outcome.[11]

Hachisuga T et al observed CD56+ NK cells are occasionally scattered in the endometrial stroma, although these cells exhibit several markers indicating different behaviours throughout the endometrium.[12] Our result show similar phenotype as reported earlier. There was higher mean number of CD56+ NK cells in the Group B (56.62 ± 5.683; Mean ± SEM; N=21) as compared to the mean number of CD56+ NK cells in the test group (38.20 ± 3.958; Mean ± SEM; N=29). Bulmer JN et al reported the number of uNK cells rapidly increased in the early secretory phase of the menstrual cycle which is similar with our findings.[13] The investigation regarding the mean count of uNK cells in our present study population were less than those of other reports of Tuckerman E et al.[14] These differences may be the result of ethnic or racial variance in endometrial immunological profiling as observed in the study of Kitaya K et al 2012.[15] Certain other authors observed no difference in the NK cell number or percentage in endometrial biopsies as reported in the study of Michimata T et al 2002 or in placental tissue from spontaneous miscarriages mentioned in the study of Chumbley G et al.[16,17] This may be related to the differences in the tissues studied by frozen or paraffin section and the technique used by flow cytometry or immunohistochemistry. Our result is based on paraffin sections followed by immunohistochemistry.

In the reports of Ghafourian M et al, the overall expression of CD69 and CD161 were significantly increased on CD56+ uNK cells which could be considered as immunological risk markers in recurrent spontaneous abortion and IVF failure which is contradictory with our present findings.[6] The higher concentrations of CD56 NK cells in normal conditions as reported in our result could be attributed to different reasons as stated by Michou VI et al, and also reported that the relationship between the peripheral blood concentration of CD56 cells that colonizes the endometrium with the fertility or infertility status found a significantly higher concentration of endometrial type NK cells in fertile groups than in sporadic aborters or infertile groups.[7] In their study, the augmentation of the endometrial type NK cell concentration observed in consecutive aborters was of no statistical importance when compared with the control group. This can be attributed to the fact that abortion is not only related to abnormal NK numbers, but to many other factors such as infections, autoantibodies, chromosomal abnormalities, and so on as reported in the study of Yamamoto T et al.[18]

The role of autoimmune disorders in reproductive failure, including recurrent miscarriage and recurrent implantation failure after in vitro fertilisation (IVF) did not find any significant difference in CD56 uNK cell numbers between women with unexplained RM who tested negative and those who tested positive for autoantibodies. Similarly, there was no significant difference in uNK cell numbers between women with unexplained RIF who tested negative and those who tested positive for autoantibodies. The presence of autoantibodies does not appear to affect the numbers of uNK cells in the endometrium around the time of implantation was the observation of Mariee NG et al.[19] Muller M et al reported that, while enumerating the concentrations of CD56+ NK cells, it was also found that the mean number of CD56+ NK cells in both the study and control groups did not differ significantly. However, for the late secretory phase, the mean number of CD56+ NK cells was significantly higher than that of the early secretory phase. Their findings could not identify a statistically significant correlation between the number of CD56+ NK cells and infertility.[20]

The present study shows that the number of CD56+ uNK cells is more in fertile women as compared to infertile women. This gives an insight that CD56+ uNK cells may have a role in the maintenance of normal pregnancy however it needs to be confirmed with larger sample size and other available tools. It would be more accurate to conclude if two independent investigators would have counted the number of CD56+uNK cells.

Conclusion:

Mean number of CD 56+ uNK cells, show significant correlation with primary infertility and repeated miscarriages (p= 0.022). CD56+ NK cells may have a role in the maintenance of normal pregnancy. It might be a useful marker for predicting risk of miscarriages and infertility.

References:

1. Shaw W, Bourne GL, Howkins J. Normal histology In: Padubidri VG, Daftary SN, editors. Shaw's textbook of Gynecology. 15th ed. New Delhi: Elsevier;2011. p 32-55
2. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol 2001;22(11):633-40.
3. Moffett A, Regan L, Braude P. Natural killer cells, miscarriage, and infertility. BMJ 2004;329 (7477): 1283-5.
4. Farghali MM, El-kholy AG, Swidan KH, Abdelazim IA, Rashed AR, El-Sobky E, et al. Relationship between uterine natural killer cells and unexplained repeated miscarriage. J Turk Ger Gynecol Assoc 2015;16(4): 214-8.
5. Glover LE, Crosby D, Thiruchelvam U, Harmon C, Chorcora CN, Wingfield MB, et al. Uterine natural killer cell progenitor populations predict successful implantation in women with endometriosis-associated infertility. Am J Reprod Immunol 2018;79 (3).
6. Ghafourian M, Karami N, Khodadadi A, Nikbakhat R. Increase of CD69, CD161 and CD94 on NK Cells in women with recurrent spontaneous abortion and *in vitro* fertilization failure. Iran J Immunol 2014:11(2): 84-96.
7. Michou Vl, Kanavaros P, Athanassiou V, Chronis GB, Stabamas S, Tsilivakos V. Fraction of the peripheral blood concentration of CD56+/CD16-/CD3-cells in total natural killer cells as an indication of fertility and infertility. Fertil Steril 2003;80(2):691-7.
8. Liu B, Mariee N, Laird S, Smith J, Li J, Li TC. The prognostic value of uNK cell count and histological dating in the mid-luteal phase of women with reproductive failure. Eur J Obst Gynecol Reprod Biol 2014:181: 171-5.
9. Eskicioglu F, Ozdemir AT, Ozdemir RB, Turan GA, Akan Z, Hasdemir SP. The association of HLA-G and immune markers in recurrent miscarriages. J Mat Fetal Neonat Med 2016;29(18):3056-60.
10. Mamedalieva NM, Kurmanova AM, Moshkalova GN, Kim V. Local immunity status in patients with miscarriages and herpetic infection. Gynecol Endocrin 2016;32(2):45-6.
11. Kofod L, Lindhard A, Bzorek M, Eriksen JO, Larsen LG, Hviid TVF. Endometrial immune markers are potential predictors of normal fertility and pregnancy after in vitro fertilization. Am J Reprod Immunol 2017:78(3.
12. Hachisuga T, Fukuda K, Nakamura S, Iwasaka T, Sugimori H. Local immune response in endometrial carcinomas. Br J Obstet Gynaecol 1997;104(1)110-4.
13. Bulmer JN. Cellular constituents of human endometrium in the menstrual cycle and early pregnancy. In: Bronson RA, Alexander NJ, Anderson D, Branch DW, Kutteh WH, editors. Reproductive Immunology. Oxford: Blackwell Science;1996.p.212-39.
14. Tuckerman E, Laird SM, Prakash A, Li TC. Prognostic value of the measurement of uterine natural killer cells in the endometrium of women with recurrent miscarriage. Hum Reprod 2007;22(8):2208-13.
15. Kitaya K, Tada Y, Taguchi S, Funabiki M, Hayashi T, Nakamura Y. Local mononuclear cell infiltrates in infertile patients with endometrial macropolyps versus micropolyps. Hum Reprod 2012;27(12):3474-80.
16. Michimata T, OgasawaraMS, Tsuda H, Suzumori K, Aoki K, et al. Distribution of endometrial NK cells, B cells,T cells andTh2/Tc2 cells fail to predict pregnancy

outcome following recurrent abortion. Am J reprod Immunol 2002;47(4): 196-202.

17. Chumbley G, King A, Gardner L, Howlett S, Holmes N, Loke YW. Generatioon of an antibody to HLA-G in transgenic mice and demonstration of the tissue reactivity of this antibody .J Reprod Immunol 1994;27 (3):173-86.

18. Yamamoto T, Takahashi Y, Kase N, Mori H. Role of decidual natural killer cells in patients with missed abortion: difference between cases with normal and abnormal chromosome. Clin Exp Immunol 1999:116(3)449-52.

19. Mariee NG, Tuckerman E, Laird S, Li TC. The correlation of autoantibodies and uNK cells in women with reproductive failure. J Reprod Immunol 2012;95 (1-2): 59-66.

20. Muller M, Kalmeier G, Eyal P, de Bruin A, Pool R, du Rant C, et al. An endometrial histomorphometric study of CD 56+ natural killer cells in women with unexplained infertility .S Afr J Obstet Gyanaecol 2017; 23(2):51-5.