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## Evaluation of peripheral natural killer cell as a risk factor for women with spontaneous abortion

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### Abstract

**Background:** Spontaneous abortion (SA) is one of the major pregnancy disorders and poses a serious risk to both the mother and the fetus. A large number of evidence confirms that there may be an immune background to SA. The major possibility of apparently unexplained SA is thought to be caused by an imbalance of immunological tolerance, a condition in which Natural killer NK cells may play an important role. Increased peripheral Natural Killer Cell (pNK) activity may cause SA. The aim of the current study is to find out whether natural killer cells have a role in abortion in women by measuring their concentration in the blood using the ELISA technique.

**Materials and methods:** The current study includes 50 samples from spontaneous abortion SA women and 30 samples from healthy pregnancy outcomes (control). Where it was collected from: Babylon Teaching Hospital for Maternity and Children and AL-Imam AL-Sadiq Teaching Hospital in Hilla City during the period from October 2022 to December 2022. When an ultrasound scan confirmed the death of the fetus and the signs of SA appeared on the patient, 3 ml of venous blood from patients and healthy controls were collected by the physician, and the samples were used for immunological detection of pNK cell levels by the ELISA method.

**Results:** Depending on the data that appeared in the work, there is a discrepancy in the concentrations between patients and controls, but it doesn't reach the degree of significance. The statistical analysis showed that the concentration of NK cells in patients was not significant compared with the controls' means (107.33 and 80.229) respectively and the standard deviation (46.64 and 23.73).

**Conclusion:** Results from this study suggest that the NK cells themselves may not cause SA, but rather that this rise may be due to another cause. This is because the concentration of natural killer cells is

slightly elevated in the peripheral blood of aborted women compared to healthy women, and it is only associated with cases of recurrent spontaneous abortion.

**Key words** Spontaneous abortion, natural killer cell, peripheral natural killer cell, decidual natural killer

### **Introduction**

For some women of pregnant age, spontaneous abortion (SA), commonly referred to as pregnancy loss, poses a serious risk. One of the major pregnancy conditions is spontaneous abortion (SA), which is highly hazardous for both the mother's health and the fetus. Numerous pieces of data suggest that SA may have an immunological history (Fu, *etal* 2021) and (Fan, *etal* 2020).

The ability of a healthy woman with a fully functioning immune system to successfully carry a baby to term without experiencing immunological rejection is one of the most amazing elements of reproductive biology. Local and systemic immune responses involving immunoglobulin, cytokines, hormones, and other endometrial variables have an impact on this development. These elements must work harmoniously together for successful implantation and subsequent pregnancy (Fu, *etal* 2021).

Important cytotoxic immune cells known as natural killer cells play a role in innate as well as adaptive immunity. Two significant NK subsets (Cluster of Differentiation) are CD56 bright CD16 and CD56 dim CD16. These two subsets are very functionally distinct from each other. Instead of participating in cytotoxic activities, CD56bright NK cells regulate the immune response strategy by the release of cytokines, whereas CD56dim cells release substantially more perforin and granzyme, causing strong cytolysis and expression of Killer-Cell Immunoglobulin-Like Receptor KIRs, (Fan, *etal* 2020), (Rougang, *etal* 2023) and (Salazar, *etal* 2023).

Natural killer (NK) cells play crucial roles throughout pregnancy and are attracted and stimulated by ovarian hormones. NK cells produce up to 50–70% of decidual lymphocytes during the first trimester, (El-Badawy, *etal* 2020) and (Zhang & Wei 2021). Decidual natural killer (DNK) cells differ from peripheral-blood NK cells in that they are less cytolytic and release cytokines and chemokines that promote trophoblast attack, tissue remodeling, fetal growth, and placentation, Fu, *etal* [2021], (El-Badawy, *etal* 2020) and (Zhang & Wei 2021). Uterine natural killer (UNK) cells, which are part of the innate immune system and comprise around 70% of all lymphocytes during pregnancy, are essential for the initial stage and continuation of pregnancy (Salazar, *etal* 2023) and (El-Badawy, *etal* 2020).

At the same time, NK cells predominate over the immune cells found in the maternal-fetal interface. These cells are known to regulate localized immunological responses, alter circulation, remodel the spiral artery, and facilitate trophoblast recruitment, (Wang, *etal* 2018).

Pregnancy-related NK cells are frequently CD56-bright. NK cells are well recognized for releasing a variety of cytokines with minimal cytolysis, (Zhang & Wei 2021) and (Wang, *etal* 2018). In addition to pNK cells, "tissue-resident NK" (trNK) cells are also present in peripheral tissues in humans, including the liver, lungs, skin, and uterus. A subset of CD56-bright NK cells forms the majority of trNK cells. When compared to pNK cells and trNK cells, decidual NK (dNK) cells are a specialized subset of trNK cells present in endometrial decidual tissue. These cells exhibit many distinctive morphological and functional traits, (Zhang & Wei 2021).

It has also been highlighted that dNK cells play an active role in SA remodeling in human illness. Patients with pre-eclampsia and intra-uterine growth restriction (IUGR), which are linked to insufficient remodeling of SAs and restricted trophoblast invasion in the decidua, have been shown to have fewer dNK cells. Additionally, NK can help the fetus grow and develop, and a lack of growth factors causes growth restriction in the progeny by causing incorrect bone development, (Wang, *etal* 2018), (Donskoi, *etal* 2022) and (Rougang, *etal* 2023).

When exposed to pathogens during pregnancy in the uterus, NK cells can also change their identity to become cytotoxic and perform immunological defense. Premature NK cell activation during late gestation can result in preterm labor, recurrent spontaneous abortion, uterine cancer, and recurrent implantation failure by breaking down the maternal-fetal interface's tolerance, (Zhang & Wei 2021) and (Wang, *etal* 2018). However, it is believed that a deficiency of immunological tolerance, which is a state in which NK cells may play a significant role, is the primary cause of seemingly unexplainable SA (Rougang, *etal* 2023) and (Guerrero, *etal* 2020).

Currently, peripheral NK cells are employed as an indicator for determining the initiation of therapy for individuals with infertility, (Toth, *etal* 2019) and (Dons' koi, *etal* 2022). This study sought to determine whether higher peripheral natural killer cell concentrations are a risk factor for spontaneous abortion in females...

## **Material and method**

### ***1-Study design and patient***

This study was planned in the college of science for women at the University of Babylon, and the places where samples were collected were the Babylon Teaching Hospital for Maternity and Children and the AL-Imam AL-Sadiq Teaching Hospital in Hilla City during the period from October 2022 to December 2022. The population of the study consisted of 80 subjects: 30 healthy pregnant outcomes (the control group), while the other group consisted of 50 specimens from women with spontaneous abortions (the patient group). The patients and controls were all between the ages of 16 and 45. The study population was divided into three age groups, which are 16–25, 26–35, and 36–45 years old. Women who had an induced abortion or an ectopic pregnancy were excluded from this study.

### ***2-Specimens collection and methods***

When an ultrasound scan has confirmed the death of the fetus, 5 ml of venous blood is collected in a gel tube from patients and controls. Allow the specimens to clot for 10–20 minutes at room temperature, and then place them in a centrifuge for 20 minutes at 2000–3000 RPM. Then collect the supernatant without sediment to obtain the serum layer, which was used to assess the immune status by evaluating the peripheral blood level of natural killer cells by using a quantitative sandwich ELISA kit (Bioassay Technology Laboratory (BT LAB), Shanghai, China). The procedure was done according to the guidelines provided by the manufactured scientific institute. The NK concentration in serum was determined by plotting the absorbance of each sample against a standard curve of typical concentrations supplied by the kit.

### 3-typical data

To determine the concentration of NK cells in serum need to standard curve the optical density at 450 nm (the primary wavelength) of the prepared typical concentration of NK as provided by the supplied company..

Table (1): the absorbance of standard concentrations at 450 nm

Absorbance	Concentration (nmol/ l)
0.014	0
0.0850	15
0.219	30
0.494	60
0.973	120
1.584	240
2.38	480

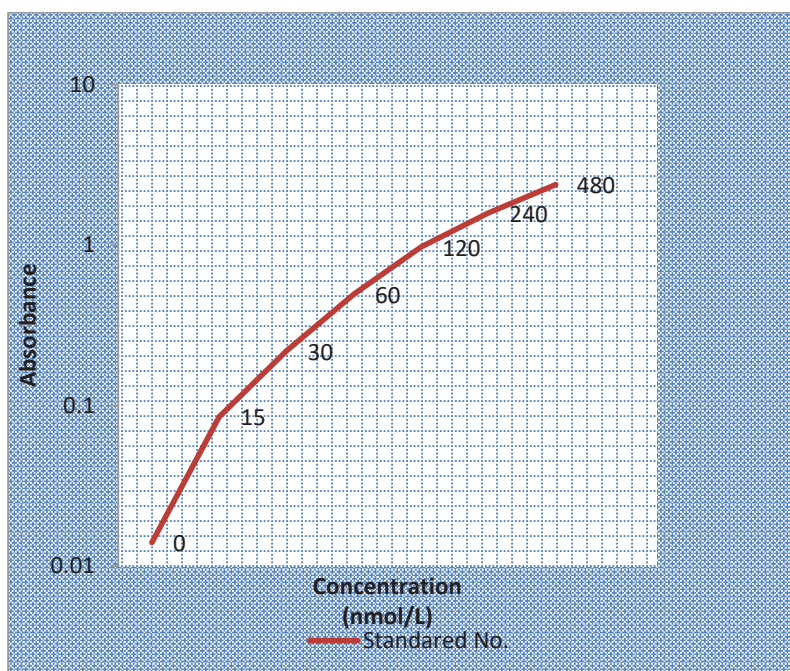


Figure 1. The standard concentration curve of NK in kit

### Results

The statistical analysis of the data indicated that there is a discrepancy in the concentrations between age groups and between patients and controls, but it doesn't reach the degree of significance. The

concentration of NK cells in patients was not significant compared with the control respectively means (107.33 and 80.229) and the standard deviation (46.64 and 23.73), as shown in Table 2. The statistical analysis for estimating the difference between the concentration in patients and controls according to age group was not significant.  $P > 0.05$

**Table (2): The concentration of Natural killer in patients according to age group**

Age group	Patient Mean ± Std. Deviation	Control Mean ± Std. Deviation	P value	
			Patient	control
16-25	96.3427 ± 48.8677	86.868 ± 20.8924	0.608 <sup>NS</sup>	0.532 <sup>NS</sup>
26-35	117.4709 ± 52.6364	74.9269 ± 28.99466		
36-45	101.6683 ± 36.1114	81.9856 ± 17.5037		
	Mean (total) 107.339 ± 46.647	Mean (total) 80.229 ± 23.737		
<b>NS: Non-significant difference under <math>p \leq 0.05</math> by One way – ANOVA</b>				

### Discussion

The results of the work showed that there is a difference between the concentration of patients and control, but it did not reach the degree of significance, perhaps because this study included samples of primary spontaneous abortion and not recurrent abortion, as well as studies that confirmed the increase of NK cells in the endometrium, so perhaps if the sample was a biopsy of the endometrium, we would have seen that, as well as some of the patients whose samples we obtained a week after the SA, and the concentration of NK cells may be in a state of decline.

Some researchers think NK cells may also be in control of a woman's body when terminating a pregnancy because of increased NK cell levels in women with recurrent miscarriages. Researchers found that women who experience recurrent spontaneous abortion have abnormal pNK subsets, which could indicate immune abnormalities at the maternal-fetal interface and may be a good indicator for the prediction of pregnancy loss, (Hou *et al* 2022). There are other potential causes for the enhanced NK cells, too. Recent research showed that, compared to controls, women with recurrent pregnancy loss (RPL) had considerably higher rates and numbers of pNK cells. However, biological activity is not always taken seriously when counting pNK or uNK cells. In comparison to NK cell counts, Natural Killer cytotoxicity (NKC) may be a more complete metric indicating NK cell properties and biological activities. Over the past two decades, the NKC test by flow cytometry has been developed into a helpful tool in clinical medicine for women with infertility. It is reported to be reproducible and suitable for both clinical and research uses (Rougang, *et al* 2023). Researchers (Zhang & Wei, 2021) confirmed that NKCs were greatly higher in non-pregnant women with a history of recurrent pregnancy loss RPL and recurrent implantation failure (RIF) compared to normal controls. Pre-eclampsia, RPL, unexplained infertility, RIF, and other reproductive diseases have all been linked to irregularities in NK cell number and cytotoxicity. The pNK cells are a reflection of the alterations in dNK cells that occur during miscarriage and can serve

as a helpful non-invasive marker in cases of reproductive failure. For use in treating women with dysregulated NK-cell immunity, additional research is required,( Bai, *etal* 2021).

### **Conclusion**

Results from this study suggest that the NK cells themselves may not cause SA, but rather that this rise may be due to another cause. This is because the concentration of Natural Killer cells is slightly elevated in the peripheral blood of aborted women compared to healthy women, and it is only associated with cases of recurrent spontaneous abortion.

### **Recommendations**

The recommendation is to expand the study population as well as take a biopsy of the endometrial layer of patients with spontaneous abortions instead of blood samples to observe uterine Natural Killer cells.

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