DOI: https://doi.org/10.30841/2708-8731.3.2026.364526

Investigation of the role of CD56, IL-2, and IL-7 in Iraqi women with spontaneous miscarriage

H. E. Mohammed[1], S. A. H. AL-Sharqi[1], B. H. Hameed[2]
[1]Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq
[2]Department of Obstetrics and Gynecology, College of Medicine, Mustansiriyah University, Baghdad, Iraq

Several articles have demonstrated an association between spontaneous miscarriage and elevated counts of cluster of differentiation CD56 natural killer cells, as well as elevated levels of interleukin-2 (IL-2), IL-7, and hematological disorders.
The objective: **to evaluate the role of CD56, IL-2, and IL-7 changes in women with spontaneous miscarriage who do not suffer from any chronic diseases.**
Materials and methods**. The research was performed in Al-Yarmouk Teaching Hospital and Al-Elwiya Teaching Hospital from October 2024 to June 2025. The study included 30 women with spontaneous miscarriage: group 1 (G1) – 16 women who had a spontaneous miscarriage between 6 and 9 weeks, group 2 (G2) – 14 women who had a spontaneous miscarriage between 10 and 14 weeks. The control group (CG) involved 20 healthy women who had term birth. All the women had no any chronic diseases, thyroid problems, blood clotting, TORCH-infections (Toxoplasmosis, Other Infections, Rubella, Cytomegalovirus, Herpes Simplex Virus) or other diseases. Tissue samples from women who had miscarriages and the placental tissue from the CG were preserved in 10% formalin for immunohistochemical staining. Additionally, serum samples were analyzed to detect IL-2 and IL-7 using enzyme-linked immunosorbent assay (ELISA). The Sysmex XP-300 hematology analyzer performed hematological analyses.**
Results**. The results showed an increase in CD56 expression in miscarriage tissue in women in G1 compared to G2. Additionally, we found no significant differences between G1 and G2 in IL-2 levels. However, IL-7 showed substantial differences between the two groups. On the other hand, we found significant difference on IL-2 and IL-7 concentrations between the study groups and the CG (term birth). White blood cell counts, lymphocyte counts, and neutrophil counts showed no significant differences between G1 and G2. However, significant difference was observed between the study groups and the CG in white blood cells, neutrophil, and platelet counts, but no significant difference was found for lymphocytes. In contrast, platelet counts revealed significant differences between the G1 and G2.**
Conclusions**. The data obtained in our study may indicate a relation between spontaneous miscarriage and immunological disturbance. A positive relation was also found between spontaneous miscarriage and inflammation.**
Keywords: *spontaneous miscarriage, interleukin-2, interleukin-7, CD56.*

Дослідження значення CD56, ІЛ-2 та ІЛ-7 в іракських жінок зі спонтанним викиднем
H. E. Mohammed, S. A. H. AL-Sharqi, B. H. Hameed

Результати кількох досліджень продемонстрували зв'язок між спонтанним викиднем і підвищеною кількістю природних клітин-кілерів кластера диференціації CD56, а також підвищеним рівнем інтерлейкіну-2 (ІЛ-2), ІЛ-7 і гематологічними розладами.
Мета дослідження: оцінити роль CD56, ІЛ-2 та ІЛ-7 у жінок зі спонтанним викиднем, які не мають будь-яких хронічних захворювань.
Матеріали та методи. Дослідження проведено в навчальних лікарнях Аль-Ярмук та Аль-Ельвія з жовтня 2024 по червень 2025 року. У дослідження увійшло 30 жінок зі спонтанним викиднем: до групи 1 (G1) – 16 учасниць, в яких відбувся самовільний викидень у терміні від 6 до 9 тиж., до групи 2 (G2) – 14 жінок, в яких відбувся самовільний викидень у терміні від 10 до 14 тиж. Контрольну групу (КГ) становили 20 соматично здорових жінок, які народили вчасно. У всіх жінок не зафіксовано хронічних захворювань, патологій щитоподібної залози, системи згортання крові, TORCH-інфекцій (токсоплазмоз, інші інфекції, вірус краснухи, цитомегаловірус, вірус простого герпесу) та інших захворювань. Зразки тканин викиднів у жінок із самовільними викиднями та зразки тканин плаценти жінок КГ зберігали у 10% формаліні для подальшого імуногістохімічного дослідження. Рівні ІЛ-2, ІЛ-7 визначали в усіх жінок у сироватці крові за допомогою імуноферментного аналізу. Аналіз крові виконували на гематологічному аналізаторі Sysmex XP-300.
Результати. Результати показали збільшення експресії CD56 у тканинах викидня у жінок G1 порівняно з G2. Не виявлено значних відмінностей у рівнях ІЛ-2 між G1 та G2. Однак рівень ІЛ-7 показав суттєві відмінності між двома групами. З іншого боку, встановлено значні відмінності вмісту ІЛ-2 та ІЛ-7 між досліджуваними групами та КГ. Не встановлено статистичної різниці у кількості лейкоцитів, лімфоцитів і нейтрофілів між G1 та G2. Проте спостерігалися значні відмінності між досліджуваними групами та КГ за кількістю лейкоцитів, нейтрофілів і тромбоцитів, але відмінностей у кількості лімфоцитів не було. Водночас кількість тромбоцитів також відрізнялася між G1 та G2.
Висновки. Отримані дані нашого дослідження можуть свідчити про зв'язок між спонтанним викиднем та імунологічними порушеннями. Також виявлено позитивний зв'язок між спонтанним викиднем та запаленням.
Ключові слова: *мимовільний викидень, інтерлейкін-2, інтерлейкін-7, CD56.*

ISSN 2708-8723 (print)
ISSN 2708-8731 (online)

The spontaneous death of a fetus before 20 weeks of gestation is known as a miscarriage or spontaneous abortion [1]. The recent studies inform that the rate of miscarriage is decreasing in women from the older age to the younger age, their early 20s [2]. Miscarriages can be caused by a variety of factors, including immunological dysregulation, smoking, obesity, thyroid pathology, and abnormalities in chromosome number [3]. Chromosomal abnormalities during the initial trimester led to increases risk of miscarriage in women [3, 4]. Spontaneous miscarriage is divided into early miscarriages, which involve missing, incomplete, and blighted ovum, occurring through less than or equal to 14 weeks of pregnancy, and late miscarriages occur after 14 weeks of pregnancy [5]. When there was no heart activity visible in the embryo, it was considered a missed abortion [6]. The presence of an empty gestational sac without an embryo is indicated of a blighted ovum [7]. An incomplete abortion occurs when some of the gestational tissue is expelled, leaving fragments in the uterus [8]. Rubella can be transmitted to fetuses by infected pregnant women and is a known cause of miscarriage [9].

In the early stages of pregnancy, natural killer (NK) cells play a crucial role in regulating essential immune processes that support successful implantation, aid in trophoblast invasion, spiral artery remodeling, and placental formation, and maintain maternal-fetal immunological tolerance. However, pregnancy problems such as recurrent spontaneous abortion and recurrent implantation failure have been linked to dysregulation of NK cells [10].

Uterine NK (uNK) cells are the most essential lymphocytes in the decidua during the first and second trimesters of gestation. They account for approximately 70% of the total lymphocytes in the first and second trimesters, and approximately 50% in the third trimester of pregnancy [11]. So, numerous cytokines, which are produced by uNK cells, contribute to a successful pregnancy. The wrong cytokine produced by uNK cells causes numerous pregnancy difficulties, such as recurrent miscarriage, preeclampsia, and intrauterine fetal growth restriction [12]. In the first trimester, approximately 90% of uNK cells are composed of the $CD56^{+}$ $CD16^{-}$ decidual NK cell subset, and the number of these cells [13].

Cytokines are classified as pro-inflammatory T helper type 1 cells (Th1) or anti-inflammatory Th2 based on their functions. Pro-inflammatory cytokines induce a cell-mediated cytotoxic reaction against intracellular pathogens, whereas Th2 immunity promotes humoral immunity [14]. The main pro-inflammatory cytokines include interferon gamma (IFN-γ), tumor necrosis factor beta (TNF-β), and interleukins (IL) – IL-2, IL-7. The main anti-inflammatory cytokines include IL-4, IL-5, and IL-13. IL-2 harms pregnancy because it is a pro-inflammatory cytokine that induces numerous cytotoxic and inflammatory responses by cell-mediated immunity, so it is known to cause miscarriage [15].

The objective: to evaluate the immunohistochemical (CD56), immunological (IL-2, IL-7), and hematological (white blood cells (WBCs), neutrophil, platelet counts) changes in women with spontaneous miscarriage who do not suffer from any known causes of miscarriage.

MATERIALS AND METHODS

The study has been conducted in Al-Yarmouk Teaching Hospital and Al-Elwiya Teaching Hospital from October 2024 to June 2025, which included 30 women with a case of spontaneous miscarriage (study group). Their age ranges from 18 to 40 years, and the gestational age of the study group is 6 to 14 weeks. Furthermore, this study included 20 women as a control group (CG) (term birth) who did not suffer from any previous miscarriage or chronic disease. The questionnaire sheet was completed by the patients participating in our study and included information on their age, medical and genetic history, family history of miscarriage, and smoking status.

The study group was divided into two: G1 (n = 16) – women who had miscarriage in the term from 6 till 9 weeks of gestation and the G2 (n = 14) – women who had miscarriage in the term from 10 till 14 weeks. The comparison between these two groups was also compared with the CG.

The miscarriage tissue samples were obtained during the curettage procedure for women who miscarried (study group), and the placental tissue of the CG was taken immediately after the placenta came out (CG). Miscarriage tissue samples of the study group and placental tissue from the CG were kept in the fixative solution (formalin 10%). They were processed after fixation by being embedded in paraffin, and a 5-μm thick section was prepared for immunohistochemical staining according to D. M. Al-Muathen et al. [16].

The staining process for monoclonal mouse anti-human CD56 (Dako, Denmark) was performed on miscarriage tissue samples and placental tissues that were fixed, paraffin-embedded, cleaned, rehydrated, and treated for antigen retrieval in a Tris-Ethylenediamine Tetraacetic acid (EDTA) solution. Sections were blocked with 3% peroxidase, incubated sequentially with primary and secondary antibodies, developed with chromogen 3,3'-diaminobenzidin (DAB), counterstained with hematoxylin, dehydrated, and finally mounted. The CD56-positive staining intensity was scored as follows: negative (< 10%), +1 mild (10–25%), +2 moderate (25–50%), and +3 severe (> 50%), according to A. I. Darka et al. [17].

About 5 mL of venous blood was collected from all participants, including cases of spontaneous miscarriage and term deliveries as controls. Blood was collected during the miscarriage from women who have experienced a miscarriage. But, for women who had term births, blood samples were taken during the delivery process.

The blood sample was divided into two parts: 2 mL were placed in an EDTA tube for hematological analysis using the Sysmex XP-300 hematology analyzer, and 3 mL were placed in a gel tube for serum separation by centrifugation at 3000 rpm for 5 minutes. All samples were screened for TORCH infections (Toxoplasmosis, Other Infections, Rubella, Cytomegalovirus, Herpes Simplex Virus), and any positive samples were excluded. The remaining serum was stored at –20 °C for IL-2 and IL-7 assays.

Commercial ELISA kits from Elabscience Biotechnology, China, were used to detect IL-2 and IL-7 in serum, following the manufacturer's guidelines. The sandwich ELISA (Huma Reader, HR, Germany) was used in the kit's operation. This kit includes a micro-ELISA plate that has been pre-coated with a human IL antibody. Standards, samples,

ISSN 2708-8723 (print)
ISSN 2708-8731 (online)

and controls were added to the micro-ELISA plate wells and combined with the specific antibody. Then, a biotinylated detection antibody specific for human IL and avidin-horseradish peroxidase conjugate were added successively to each microplate well and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain human IL, biotinylated detection antibody, and avidin-horseradish peroxidase conjugate will appear blue. The addition of the stop solution terminates the enzyme-substrate reaction, and the color turns yellow. Reagents for sample: 10 × EDTA anticoagulant (Cat. No. EEL SR003), phenylmethylsulfonyl fluoride (PMSF) protease inhibitor (Cat. No. EEL SR002), 0.25% trypsin solution (Cat. No. EEL SR001).

The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of human IL-2 and IL-7. You can calculate the concentration of human IL-2 and IL-7 in the samples by comparing the OD of the samples to the standard curve.

Exclusion criteria from the study samples:

1) Positive history for hypertension, diabetes, genetic diseases, thyroid pathology, and autoimmune diseases.
2) The specimen did not show the presence of chorionic villi after the pathology report.
3) Positive results for the TORCH test.
4) Some types of miscarriage, such as blighted ovum and septic abortion.
5) Couple with an incompatible blood group.

All participants agreed to provide the investigator with the specimens. The ethics committee of the College of Science, Mustansiriyah University, approved this work. Informed consent, as outlined in the Declaration of Helsinki, was obtained from all participants (Ref.: BCSMU/0924/0053Z).

The ANOVA test was used to analyze the repeated measures between the tested groups, with data expressed as mean (M) ± standard deviation (SD), and other descriptive statistics. Test values of $p \geq 0.05$ were considered statistically non-significant, while $p \leq 0.05$ were considered significantly different. The correlation coefficient between the various parameters in this study was performed. The statistical analysis was performed using SPSS (version 20).

RESULTS AND DISCUSSION

The questionnaire sheet was completed by the patients participating in our study and included information on their age, medical and genetic history, family history of miscarriage, and smoking status. The statistical analysis shows there was a significant difference in the age of miscarriage women ($p \leq 0.05$) between G1 and G2. In G1, the age of miscarriage women with miscarriages is older than in G2, as indicated in Table 1.

The percentage of women experiencing miscarriages is higher among related couples from G1 compared to those from G2, depending on whether the couple is related or not. The percentage of women who experienced miscarriage and had a family history of miscarriage was higher in the G1 compared to that of couples in the G2. The smoking percentage in our study is too low, as indicated in Table 2.

In the G1, there was 1 (6.25%) *primigravida* woman, and 15 (93.75%) women were *multigravidas*, whereas in the G2 there were 2 (14.28%) *primigravida* women and 12 (85.71%) women were *multigravida*. In addition, in the CG, 4 (20.00%) the women were *primiparas*, while 16 (80.00%) were *multiparas*.

The analysis of immunological data for IL-2 and IL-7 in serum samples obtained from women who miscarried indicated no significant difference ($p \geq 0.05$) in IL-2 levels between G1 and G2. The results also showed a highly significant difference ($p \leq 0.001$) between women with miscarriages (study group) and the CG. On the other hand, there was a significant difference ($p \leq 0.05$) in the serum IL-7 level of women with miscarriage between G1 and G2. The results also showed a highly significant difference ($p < 0.001$) between women with miscarriage in the study group and the CG (Table 3).

Table 1

The age of pregnant women with spontaneous miscarriage

Parameter	G1 (n = 16)	G2 (n = 14)	CG (n = 20)	p-value
Age, years	31.38 ± 6.74	28.91 ± 7.19	24.30 ± 5.41	0.05

Table 2

Comparison between G1 and G2 (according to the couple, family history, and smoking)

Tested groups	G1 (n = 16)	G2 (n = 14)
	Yes, n (%)	Yes, n (%)
A first-cousin couple relative	8 (50.0)	6 (42.8)
Family history of miscarriage	5 (31.2)	4 (28.6)
Smoking	2 (12.5)	0 (0.0)

Table 3

IL-2 and IL-7 levels in G1 and G2 with spontaneous miscarriage and the CG

IL	G1 (n = 16)	G2 (n = 14)	CG (n = 20)	p-value
IL-2, pg/mL	212.56 ± 34.05	195.56 ± 70.00	118.39 ± 41.95	0.001 when compared with the CG
IL-7, pg/mL	189.43 ± 37.34	172.02 ± 18.70	79.60 ± 7.45	0.05

ISSN 2708-8723 (print)
ISSN 2708-8731 (online)

Table 4

WBCs and lymphocyte count in G1 and G2 and the CG

Blood cells	G1 (n = 16)	G2 (n = 14)	CG (n = 20)	p-value
WBCs, cell/mL	9.40 ± 5.90	9.80 ± 2.93	8.40 ± 1.83	0.05 when compared with the CG
Lymphocyte, cell/mL	1.91 ± 0.42	2.01 ± 0.66	1.87 ± 0.85	not significant

Note: WBCs – white blood cells.

Table 5

Neutrophil and platelet count in G1 and G2 and the CG

Blood cells	G1 (n = 16)	G2 (n = 14)	CG (n = 20)	p-value
Neutrophil, cell/mL	6.81 ± 5.89	7.36 ± 2.97	3.26 ± 1.00	0.003 when compared with the CG
Platelet, cell/mL	313.93 ± 94.17	268.91 ± 76.69	225.90 ± 47.54	0.02

Table 6

Immunohistochemistry results for CD56 in the miscarriage tissue of women with spontaneous miscarriage according to scoring and intensity, abs. (%)

CD56 Score (intensity)		G1 (n = 16)	G2 (n = 14)	CG (n = 20)
0 (Zero)	Low expression groups	4 (25.00)	3 (21.42)	18 (90.00)
+1 (Mild)		2 (12.50)	6 (42.85)	2 (10.00)
+2 (Medium)	High expression groups	7 (43.75)	3 (21.42)	0 (0)
+3 (Severe)		3 (18.75)	2 (14.28)	0 (0)

Hematological analysis of venous blood from the women who experienced a miscarriage indicated no significant difference ($p > 0.05$) in the WBC and lymphocyte counts within the study group. However, the results show a significant difference in the number of WBCs counted between the study group ($p \leq 0.05$) and the CG (term birth), as indicated in Table 4. On the other hand, Table 5 shows there was no significant difference ($p > 0.05$) in the number of neutrophil counts in the study group. But there was a significant difference in the number of neutrophil counts of the study group ($p \leq 0.05$) and the CG. Likewise, there was a significant difference in the number of platelets ($p \leq 0.05$) between G1 and G2. The results also showed significant differences in the number of platelet counts between the study group and the CG.

Fig. 1 shows cell lung cancer (positive control) and placental tissue (negative control).

The staining intensity for spontaneous miscarriage tissue showed severe immunohistochemical expression of G1 in three biopsies (18.75%), which had a high expression score. While seven biopsies (43.75%) also showed high expression (+2), scoring. Meanwhile, two biopsies (12.50%) showed a low expression group (+1), and four biopsies (25.00%) scored 0. In G2, the staining intensity was severe in two biopsies (14.28%), which exhibited high expression, while three biopsies (21.42%) also showed high expression (+2). In addition, six biopsies (42.85%) showed low expression (+1), and three biopsies (21.42%) were scored. These results are presented in Table 6 and Fig. 2.

The correlations among the parameters of the study group indicate that in the miscarriage patient through G1, the correlation between the number of previous abortions and lymphocyte count is strongly positive. The correlation between WBCs and neutrophils was strongly positive, and the correlation between WBCs and platelets was moderately to strongly positive. The correlation between platelets and neutrophils was moderately to strongly positive. In G2, the correlation between neutrophils and WBCs was strongly positive. The correlation between WBCs and the number of born children was moderately to strongly negative, and a moderately strong negative correlation was observed between IL-7 and neutrophils, as indicated in Table 7.

Very early pregnancy loss (6–9 weeks) increases with the increase of maternal age, which is probably because of reduced egg quality leading to chromosomal abnormalities [18]. Also increased number of parents with consanguineous marriages because have a strong genetic bond, making their offspring vulnerable to the same autosomal recessive disorders [19]. Furthermore, according to earlier research, miscarriages in women are related to a family history of miscarriages. There may be a genetic predisposition to miscarriage that is passed down from mother to daughter, as well as environmental factors such as food habits, occupational habits, and air pollution that may put women at risk for miscarriage [20, 21].

Research has shown that in women who have miscarried, there's a significant increase in the serum levels of TNF alpha (TNF-α), IFN-γ, and IL-2 [22]. The chief action of increasing miscarriage by IL-2 is to increase the contractility of the uterine muscles; therefore, pregnancy fails and ends with miscarriage [23]. Also, increased serum IL-2 level was found in women with threatened miscarriage compared to those in women with normal pregnancy, and the pro-inflammatory cytokine profiles were related to a greater risk of threatened miscarriage [24].

In contrast to our study, it was reported that there was a decreased IL-2 concentration in women with miscarriage compared with healthy pregnancies. The reduction

ISSN 2708-8723 (print)
ISSN 2708-8731 (online)

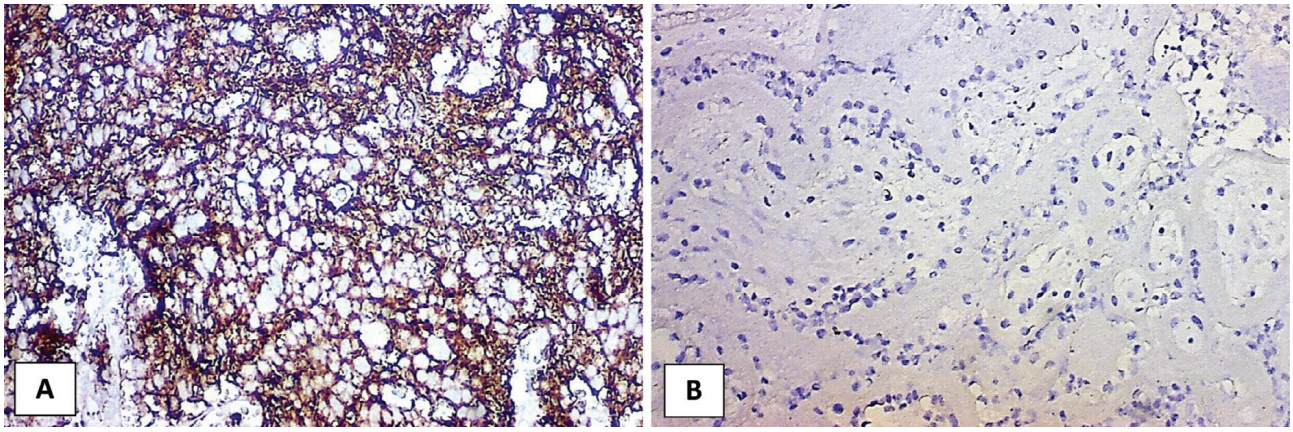

Fig. 1. Immunohistochemical staining method detection of CD56: A – in a case with small cell lung cancer (positive control); B – placenta tissue from a healthy woman's term birth (negative control) (× 10)

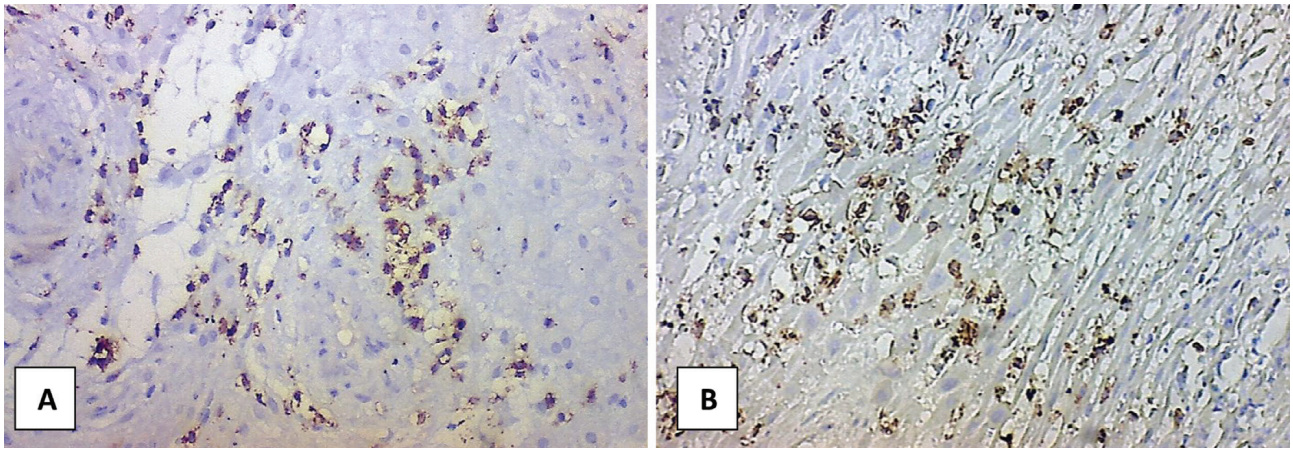

Fig. 2. Immunohistochemistry staining method detection of CD56 showed positive cytoplasmic expression: A – score 2 and medium intensity in women with 10-week gestation miscarriage; B – score 3 and severe intensity in women with 6-week gestation miscarriage (× 10)

in the levels of these cytokines may indicate a disorder in immune tolerance, which is essential for maintaining pregnancy [25]. IL-2 plays a crucial role in T-cell proliferation and function, suggesting that the immune system may be ineffectively caring for the fetus. The decrease in IL-2 expression might recommend a reduction in the defensive immune response in patients with miscarriage. Also, increasing IL-7 concentration make an inflammatory environment, disrupt fetal-maternal message and lead to implantation defects [26]. IL-7 is selected as an essential cytokine regulating the proliferation of Th17 cells. Th17 cells, the inflammatory cells, may disrupt implantation, leading to an increased level of IL-7, which can cause an imbalance favoring Th17 dominance, a condition associa-

Table 7

Correlation between study groups

G1 (n = 16)		Pearson's correlation	G2 (n = 14)		Pearson's correlation
Number of missed abortions	Lymphocyte	0.904**	Age	Number of born children	0.621*
			Neutrophil	WBCs	0.977**
			Negative correlation		
			WBCs	Number of born children	–0.635*
WBCs	Neutrophil	0.996**	IL-7	Neutrophil	–0.707*
WBCs	Platelet	0.684*			
Platelet	Neutrophil	0.679**			

Notes: * – $p \leq 0.05$; ** – $p \leq 0.01$; WBCs – white blood cells.

ISSN 2708-8723 (print)
ISSN 2708-8731 (online)

ted with miscarriage [27]. Elevated levels of IL-17 increase Th17 cell populations, which are associated with heightened miscarriage rates due to their role in promoting the secretion of inflammatory cytokines [28].

In the normal state, the uNK converts from a mainly pro-angiogenic growth factor tendency at 8–10 weeks (e.g., vascular endothelial growth factor-C, angiopetin-1, angiopoietin-2) to a cytokine secretory function at 12–14 weeks (e.g., IFN-γ, IL-1β, IL-6); thus, an elevated uNK level could result in more angiogenic factors being produced, which would raise peri-implantation blood flow and put trophoblast cells under too much oxidative stress. Additionally, according to other data, uNKs are more likely to release pro-inflammatory cytokines similar to Th1 cytokines, while suppressing anti-inflammatory Th2 cytokines, which are crucial for a healthy pregnancy [29].

Women with unexplained recurrent miscarriage exhibit an elevated expression profile of angiogenic factors in their isolated $CD56^+$ uNK cells, specifically a markedly elevated expression of basic fibroblast growth factor, vascular endothelial growth factor-A, and angiogenin [30].

On the other hand, the uNK cells can generate a variety of cytokines that help to ensure a successful pregnancy. So that preeclampsia, intrauterine fetal growth restriction, and recurrent miscarriage are among the pregnancy problems linked to the altered cytokine production profile of uNK cells [11]. So, what is seen in patients with recurrent miscarriages is a greater level of pro-inflammatory cytokines due to a lack of inhibition of uNK cells [31]. Because pro-inflammatory cytokines cause vascular endothelial cells to produce pro-coagulant substances, which in turn lead to inflammation and thrombus formation at the maternal-fetal interface, this consequently hinders the passage of blood to the placenta. Furthermore, Th1 cytokines can induce apoptosis and break down the trophoblast barrier that separates the mother's immune system from a semi-autogenous baby, which might result in miscarriage [14].

In comparison to women who underwent intentional termination, women who experienced spontaneous early pregnancy loss exhibited a larger mean number of $CD56^+$ NK cells in their decidual tissue [32].

Our result showed an increase in WBC, neutrophil, and platelet counts in women with miscarriage. It is considered the first indicator of pregnancy complications, especially during the first trimester, which leads to miscarriage and also causes preterm birth and premature membrane rupture of the uterus [33].

Increased levels of WBCs and neutrophils during the first trimester can lead to insufficient placental development and remodeling, which are associated with pregnancy complications, including an enhanced maternal immune response to fetal structures. The cause of increased neutrophils and WBCs in maternal blood may be due to maternal inflammation, which leads to endothelial dysfunction in preeclampsia [34]. The results show increased WBC and neutrophil counts in the first-trimester miscarriage group when compared to pregnancy outcomes [35]. These results agreed with our results.

Our study showed an increase in platelet count in the study group. Platelets are commonly identified as an indicator of inflammation and thrombosis. So, persistent and uncontrolled inflammatory responses can destroy placental development and cause miscarriage [36].

It has been identified that neutrophils, monocytes, lymphocytes, and platelets in the blood play a vital role in systemic inflammation [37]. Elevated levels of decidual or systemic inflammation through pregnancy may be one of the reasons for miscarriage [35]. Platelet counts in the early fetal death group were significantly higher than those in healthy pregnancies [38].

According to the results of the correlation, when the WBC count increases, neutrophil counts also increase because neutrophils are produced rapidly in response to infection and may appear in significantly greater numbers in the peripheral blood. So many neutrophils reach the inflammatory place quickly to engulf and destroy organisms [39].

On the other hand, when neutrophil levels increase, it causes a decrease in IL-7 level because neutrophils produce protease enzymes, such as metalloproteinase and elastase, which cleave IL-7. These protease enzymes cause cleavages to specific regions of IL-7, such as the loop between the α-helices [40].

CONCLUSIONS

We conclude that elevated levels of CD56 during very early pregnancy increase the risk of miscarriage. This conclusion is based on a comparison of the percentage of CD56 markers (NK cells) in women who experienced a miscarriage at 6–9 weeks of gestation versus those at 10–14 weeks of gestation. Also, elevated serum levels of IL-2 and IL-7 increase the risk of miscarriage. Furthermore, elevated level of WBCs and platelet counts may also increase the risk of miscarriage. Maternal age plays a role in spontaneous miscarriage, increasing the risk of very early miscarriage with the increase of maternal age. Also, family history of miscarriage and couples who are relatives do not have an effect on very early spontaneous miscarriage.

Conflict of interest. The authors declare no conflicts of interest.

Information about the authors

Mohammed Haneen Essa – Master's Student, Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq. *E-mail: haneenessa93@uomustansiriyah.edu.iq*
ORCID: 0009-0000-3836-9098

Al-Sharqi Sahar Abdul Hadi – Professor, Dr. Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq. *E-mail: saharalsharqi@uomustansiriyah.edu.iq*
ORCID: 0000-0002-4727-6413

Hameed Ban Hadi – Professor, Dr. Department of Obstetrics and Gynecology, College of Medicine, Mustansiriyah University, Baghdad, Iraq. *E-mail: banhadi@uomustansiriyah.edu.iq*
ORCID: 0000-0001-8451-652X

REFERENCES

1. Bettamer N, Elghazal Z, Benkia S, Fadel AA, Eltboli F. Outcomes of pregnancies in mothers with previous miscarriage at Benghazi medical center: A retrospective study. Asian J. Preg. Childb. 2024;7(1):72-92.
2. Madu AE. Miscarriage: A concise analysis of a changing trend. J Gynecol Oncol. 2020;3(4):1040.
3. Laisk T, Soares AL, Ferreira T, Painter JN, Censin JC, Laber S, et al. The genetic architecture of sporadic and multiple consecutive miscarriages. Nature Communications. 2020;11(1):5980. doi: 10.1038/s41467-020-19742-5.
4. Perkhulyn O, Pakharenko L. Cervical elastograhy in patients with cervical insufficiency and a history of anovulatory infertility. Reprod Health Woman. 2021;(3):34-6. doi: 10.30841/2708-8731.3.2021.234240.
5. Ghosh J, Papadopoulou A, Devall AJ, Jeffery HC, Beeson LE, Do V, et al. Methods for managing miscarriage: a network meta-analysis. Cochrane Database Syst Rev. 2021;6(6):CD012602. doi: 10.1002/14651858.CD012602.pub2.
6. Jiang WZ, Yang XL, Luo JR. Risk factors for missed abortion: retrospective analysis of a single institution's experience. Reprod Biol Endocrinol. 2022;20(1):115. doi: 10.1186/s12958-022-00987-2.
7. Helle N, Niinimäki M, Linnakaari R, But A, Gissler M, Heikinheimo O, et al. National register data are of value in studies on miscarriage – Validation of the healthcare register data in Finland. Acta Obstet Gynecol Scand. 2022;101(11):1245-52. doi: 10.1111/aogs.14445.
8. Kurnia E, Aisyah S, Puspitasari E. Relationship of age, parity, and history of abortion with the incidence of incomplete abortion at RSUD Dr. HM Rabain Muara Enim 2021. Sci Midwifery. 2022;10(2):842-9.
9. Jassim T, Al-Fendi A, Hashim S, Saleh T, Al-Rubaii B. Molecular detection of Rubella virus (1E genotype) in clinical sample of pregnant women, and it's related to abortion. Reprod Health Woman. 2025;(3):113-8. doi: 10.30841/2708-8731.3.2025.331563.
10. Guan D, Chen Z, Zhang Y, Sun W, Li L, Huang X. Dual Role of Natural Killer Cells in Early Pregnancy: Immunopathological Implications and Therapeutic Potential in Recurrent Spontaneous Abortion and Recurrent Implantation Failure. Cell Prolif. 2025:e70037. doi: 10.1111/cpr.70037.
11. Xie M, Li Y, Meng YZ, Xu P, Yang YG, Dong S, et al. Uterine natural killer cells: A rising star in human pregnancy regulation. Front Immunol. 2022;13:918550. doi: 10.3389/fimmu.2022.918550.
12. De Mendonça VR, Meagher A, Crespo ÂC, Kshirsagar SK, Iyer V, Norwitz ER, et al. Human term pregnancy decidual NK cells generate distinct cytotoxic responses. J Immunol. 2020;204(12):3149-59. doi: 10.4049/jimmunol.1901435.
13. Zhang J, Lye SJ. The immune potential of decidua-resident CD16+CD56+ NK cells in human pregnancy. Hum Immunol. 2021;82(5):332-9. doi: 10.1016/j.humimm.2021.01.014.
14. Yang X, Tian Y, Zheng L, Luu T, Kwak-Kim J. The update immune-regulatory role of pro- and anti-inflammatory cytokines in recurrent pregnancy losses. Int J Mol Sci. 2022;24(1):132. doi: 10.3390/ijms24010132.
15. Lawal HA, Obajimi GO, Bello YO, Onifade AF, Jimoh MA, Arowojolu AO, et al. Estimation of inflammatory cytokines in maternal serum for assessing the outcome of threatened miscarriage. J West Afr Coll Surg. 2024;14(3):280-8. doi: 10.4103/jwas.jwas_136_23.
16. Al-Muathen DM, Al-Sharqi SA, Brakhas SA, Taha AA. Possible etiological role of IL-10, IL-17 Levels, CD4+ and CD68 in the pathogenesis of herniated disc in Iraqi patients. Opera Medica Physiol. 2024;11(2):40-6. doi: 10.24412/2500-2295-2024-2-40-46.
17. Darka AI, Sel G, Barut F, Baser Acikgoz R, Balci S, Ozmen U, et al. Investigation of CD56, ADAM17 and FGF21 expressions in the placentas of preeclampsia cases. Medicina (Kaunas). 2023;59(6):1145. doi: 10.3390/medicina59061145.
18. Du Fossé NA, van der Hoorn MP, van Lith JMM, le Cessie S, Lashley EELO. Advanced paternal age is associated with an increased risk of spontaneous miscarriage: a systematic review and meta-analysis. Hum Reprod Update. 2020;26(5):650-69. doi: 10.1093/humupd/dmaa010.
19. Khayat AM, Alshareef BG, Alharbi SF, AlZahrani MM, Alshangity BA, Tashkandi NF. Consanguineous marriage and its association with genetic disorders in Saudi Arabia: A review. Cureus. 2024;16(2):e53888. doi: 10.7759/cureus.53888.
20. Woolner AMF, Nagdeve P, Raja EA, Bhattacharya S, Bhattacharya S. Family history and risk of miscarriage: A systematic review and meta-analysis of observational studies. Acta Obstet Gynecol Scand. 2020;99(12):1584-94. doi: 10.1111/aogs.13940.
21. Perkhulyn OM, Pakharenko LV, Sukhin VS, Saltovskiy OV, Kovalchuk VM, Hranovska HI, et al. Evaluation of hormonal function in women with cervical insufficiency and infertility in the history. Wiad Lek. 2021;74(10 pt 1):2412-16.
22. Lin M, Xu H, Qiu J. Inflammation in recurrent miscarriage – a comprehensive perspective from uterine microenvironment and immune cell imbalance to therapeutic strategies. Ginekol Pol. 2024;95(4):266-75. doi: 10.5603/gpl.97320.
23. Gatti KJ, Hasson SF, Alsadoon AK. Study on the role of interleukin-2 and human cytomegalovirus in cases of recurrent spontaneous abortion of women in Wasit province. Indian J Forensic Med Toxicol. 2020;14(1):828-32.
24. Hakeem LA, Bello YO, Obajimi GO, Onifade AFA, Ahmed B, Arowojolu AO, et al. Inflammatory cytokines in maternal serum between women with threatened miscarriage and normal pregnancy. Int J Gynaecol Obstet. 2024;164(1):255-61. doi: 10.1002/ijgo.15009.
25. Ozbey G, Tanriverdi ES, Cakir A, Yilmaz E. Investigation of the relationship between IL-17, IL-27, IL-2 blood levels in spontaneous abortion and healthy pregnant women. Life (Basel). 2025;15(3):326. doi: 10.3390/life15030326.
26. Demir MB, Cam SD. Hydrosalpingeal fluid leads to subfertility via endometrial TNF-α and IL-7 and NF-κB signaling pathways. Eur Rev Med Pharmacol Sci. 2023;27(7):2980-86. doi: 10.26355/eurrev_202304_31930.
27. Vilsmaier T, Amann N, Löb S, Schmoeckel E, Kuhn C, Zati ZA, et al. The decidual expression of Interleukin-7 is upregulated in early pregnancy loss. Am J Reprod Immunol. 2021;86(3):e13437. doi: 10.1111/aji.13437.
28. Najeeb N, Mohsen S, Omear H. Measuring gene expression and immune level of FOXP3 and its relationship with regulatory T cells in maintaining immune tolerance in both women pregnant and recurrent miscarriage, who suffer from autoimmune thyroid diseases. Reprod Health Woman. 2025;(1):33-40. doi: 10.30841/2708-8731.1.2025.323708.
29. Von Woon E, Greer O, Shah N, Nikolaou D, Johnson M, Male V. Number and function of uterine natural killer cells in recurrent miscarriage and implantation failure: A systematic review and meta-analysis. Hum Reprod Update. 2022;28(4):548-82. doi: 10.1093/humupd/dmac006.
30. Chen X, Liu Y, Cheung WC, Zhao Y, Huang J, Chung JPW, et al. Increased expression of angiogenic cytokines in CD56+ uterine natural killer cells from women with recurrent miscarriage. Cytokine. 2018;110:272-6. doi: 10.1016/j.cyto.2018.01.013.
31. Guerrero B, Hassouneh F, Delgado E, Casado JG, Tarazona R. Natural killer cells in recurrent miscarriage: An overview. J Reprod Immunol. 2020;142:103209. doi: 10.1016/j.jri.2020.103209.
32. Senthilnayagam B, Karthikeyan S, Sukumaran J, Srivalsan A, Rao R, Subbiah V. Decidual CD56+ natural killer cells in spontaneous early pregnancy loss- an immunohistochemical study. J Clin Diagn Res. 2016;10(10):EC27-9. doi: 10.7860/JCDR/2016/22902.8740.
33. Al-Husban N, Al-Atrash H, Alhayek N, Al-Soud K, Alhusban M. Platelet and White Blood Cell (WBC) Counts in the First Trimester and Pregnancy Outcome: Prospective Controlled Study. J Fetal Med. 2019;(2):89-94. doi: 10.1007/s40556-019-00202-0.
34. Örgül G, Aydın Haklı D, Özten G, Fadiloğlu E, Tanacan A, Beksaç MS. First trimester complete blood cell indices in early and late onset preeclampsia. Turk J Obstet Gynecol. 2019;16(2):112-7. doi: 10.4274/tjod.galenos.2019.93708.
35. Kale İ, Helvacıoğlu Ç, Muğurtay TE. Evaluation of complete blood count parameters in the first trimester: an early indicator of miscarriage. J Clin Invest Surg. 2021;6(1):48-52. doi: 10.25083/2559.5555/6.1.9.
36. Makled AH, Abuelghar W, Mostafa R. The association between platelet indices and unexplained recurrent miscarriage. Evid Based Women Health J. 2021;11(4):312-8.
37. Jassim OA, Al-Sharqi SAH, Wahab MS. Immunohistochemical expression and enumeration of mast cells in synovial tissue from Iraqi patients with Rheumatoid arthritis. Indian J Forensic Med Toxicol. 2019;13(4):672-7.
38. Shao X, Wang D, Xu Y, Guo L, Yang H, Zhou J, et al. The high platelet counts as predictor for early foetal demise. Ann Med. 2021;53(1):1502-08. doi: 10.1080/07853890.2021.1968027.
39. Chmielewski PP, Strzelec B. Elevated leukocyte count as a harbinger of systemic inflammation, disease progression, and poor prognosis: A review. Folia Morphol (Warsz). 2018;77(2):171-8. doi: 10.5603/FM.a2017.0101.
40. Vandooren J, Pereira RVS, Ugarte-Berzal E, Rybakin V, Noppen S, Stas MR, et al. Internal disulfide bonding and glycosylation of Interleukin-7 protect against proteolytic inactivation by neutrophil metalloproteinases and serine proteases. Front Immunol. 2021;12:701739. doi: 10.3389/fimmu.2021.701739.

Стаття надійшла до редакції 07.07.2025. – Дата першого рішення 11.07.2025. – Стаття подана до друку 13.08.2025

ISSN 2708-8723 (print)
ISSN 2708-8731 (online)