

A comparative clinical study of IL-18, IL-23, CD68, and VEGF in miscarriage versus normal ongoing pregnancy

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ABSTRACT

Miscarriage is a common complication of pregnancy, with various immunological and inflammatory factors contributing to its pathophysiology. The majority of its underlying causes are unknown. This study aimed to investigate the role of interleukin (IL)-18, IL-23, cluster of differentiation 68 (CD68), and vascular endothelial growth factor (VEGF) in women with miscarriage versus those with successful pregnancies. The study included 90 participants, 30 women with missed miscarriages, 30 women with incomplete miscarriages, and 30 control women with successful pregnancies. Blood levels of IL-18, VEGF, CD68, and IL-23 were determined. The results demonstrated significantly elevated levels ($p < 0.05$) of IL-18, VEGF, and CD68 in the missed miscarriage group with values of 675.82 ± 130.71 , 542.81 ± 59.96 , and 3.37 ± 0.23 ng/ml, respectively. These were followed by the incomplete miscarriage group, with levels of 160.75 ± 58.61 pg/ml for IL-18, 319.99 ± 24.08 pg/ml for VEGF, and 1.97 ± 0.26 ng/ml for CD68. The control group showed the lowest levels: 154.57 ± 50.93 pg/ml for IL-18, 260.74 ± 43.80 pg/ml for VEGF, and 1.72 ± 0.12 ng/ml for CD68. All differences were statistically significant ($p = 0.00001$). Conversely, IL-23 levels were markedly elevated in the incomplete miscarriage group (397.78 ± 56.94 pg/ml), showing a highly significant difference compared to the other groups ($p < 0.0001$). Significant moderate correlations among biomarkers

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were found in control and incomplete miscarriage groups, while in missed miscarriage there were significant positive correlations of IL-18 with IL-23, and IL-18 with CD68 with a negative significant correlation between IL-18 and VEGF. Differential levels of IL-18, VEGF, and IL-23, and CD68 indicate potential roles in miscarriage pathophysiology and suggest targeted therapeutic options.

Keywords: ILs, CD68, VEGF, cytokine, miscarriage, pregnancy

INTRODUCTION

Pregnancy loss occurring spontaneously before the twentieth week of gestation is a common health issue. It may be brought about by chromosomal abnormalities, abnormalities in the uterus, infections, and autoimmune diseases. Environmental influences, such as radiation exposure or the use of certain drugs, can also contribute to the risk of miscarriage. Furthermore, the risk of miscarriage is increased by advanced maternal age and chronic diseases like thyroid disorders and diabetes²⁻⁴. Miscarriages are common in the first trimester. Vaginal bleeding, fluid, tissue, and blood clot passing are the main symptoms of miscarriage^{5,6}. An incomplete miscarriage may be caused by abnormalities in the uterus or placental issues. Abdominal pain and severe vaginal bleeding are common side effects. Aside from discovering residual tissue in the uterus, the cervix is open^{1,5}. A missed miscarriage occurs when the foetus dies without being expelled from the mother's body. It can be occasioned by chromosomal abnormalities, maternal health conditions or placental complications^{1,5}.

Cytokines are small signaling proteins produced and released by various cells, particularly those of the immune system. They function to regulate numerous biological processes, maintain immune balance, and respond to immunological challenges. Interleukin-18 (IL-18) is a pleiotropic cytokine and is of the IL-1 family. It is an important cytokine that enhances several innate and adaptive immune processes related to physiological and pathological processes, such as infection, inflammation, and autoimmunity⁷⁻⁹. It plays a significant role in controlling immune reactions in the course of pregnancy including the regulation of immunological tolerance towards the foetus in the mother⁸. The IL-18 level imbalance in any way can be the reason for pregnancy problems, such as preeclampsia and miscarriage¹⁰.

Interleukin-23 (IL-23) is another cytokine that is released from myeloid cells, which is mainly responsible for the control of immune inflammatory response. It stimulates the development and growth of natural T helper 17 (Th-17)

cells with an important impact on pregnancy, as it governs the activity of the immune system. It induces T cell differentiation and survival and creates an immunological bridge between the mother and the foetus, thereby preventing the foetus' rejection. Disrupted IL-23 regulation results in pregnancy problems, including spontaneous miscarriage¹¹.

Cluster of differentiation 68 (CD68) is a glycoprotein mainly present on the membranes of macrophages, monocytes and other immune cells¹², which is obligatory for immune response. It is thought to be a part of the scavengers' family. CD68 is involved in the lysosomal and endosomal pathways to promote phagocytosis by attaching to different ligands that are the products of apoptotic cell debris¹³. Soluble CD68 is an essential marker of a variety of diseases, and it shows the response of phagocytic cells to inflammation or damage^{14,15}. Macrophages play a crucial role in the maternal immune system during pregnancy in the process of the remodelling of the uterine environment. They enhance the process of implantation and placentation to ensure that there is a balance between tolerance to the foetus and protection against infection. Any imbalance in this fine balance may be a cause of chronic inflammation in the uterine environment, and this can result in miscarriage¹⁶.

The vascular endothelial growth factor (VEGF) is a protein signal involved in the formation of new blood vessels (angiogenesis). It participates in both normal and disease processes and is considered cytoprotective and anti-apoptotic for endothelial cells^{17,18}. This is a consideration during the maturation of blood vessels in the placenta that guarantees sufficient blood circulation to the developing foetus via delivery of oxygen and nutrients. It also alters the immune environment of the placenta to shield the foetus against the immune response of the mother. However, poor blood vessel formation caused by abnormal signalling from this factor can lead to pregnancy complications, such as miscarriage. Elevated levels of this factor can also increase the risk of miscarriage by disrupting the delicate balance required for proper placental growth^{16,19}.

Therefore, this research aimed to compare the levels of IL-18, IL-23, VEGF, and CD68 in women who experienced a miscarriage versus those with successful pregnancies and to explore the correlation of these markers across the different groups.

METHODOLOGY

Study design and population

The study was a case-control study that took place in the Baghdad area at Fatima Al Zahra Hospital and included 90 women in the first trimester of their

pregnancy, between June and September 2023–2024. The women who took part in the research provided informed written consent to participate. During the enrolment, their medical histories were reviewed, and clinical checkups were performed. The subjects were then allocated to the case or the control group. There were 60 women in the case group who had spontaneous abortions. These women were again subdivided into 2 subgroups: 30 miscarriages that had been missed and 30 that had not been completed. The control group was composed of 30 pregnant women whose pregnancies were considered healthy and continuing, and who were selected with great care when they were compared with the women in the case group on such factors as age, gestational age and body mass index (BMI).

Inclusion and exclusion criteria

A study group of 60 women with missed abortion or incomplete abortion was the inclusion criteria. The control group comprised 30 women whose first-trimester pregnancies were normal. The age of all the participants ranged from 18 to 40 years. The investigative population encompassed women who had vaginal bleeding during the first trimester of pregnancy and had an unknown cause and an Rh-positive blood group. Medical diseases and drug use, alcohol use, smoking, and morbid obesity, severe anaemia, and Rhesus incompatibilities were used to exclude the participants.

Diagnosis of miscarriage

This was considered a missed miscarriage when the women reported termination of the pregnancy symptoms or when they had a routine antenatal check-up and the ultrasound showed no foetal heartbeat, especially when they had a crown-rump length (CRL) more than 6 mm. Conversely, unfinished abortion occurred in those women who reported with acute stomach and pelvic pain, and experienced excessive vaginal bleeding. An open cervix on clinical grounds with the passage of tissue and an ultrasound of the remaining tissue in the uterus confirmed this clinically⁶.

Sample size calculation

A level of confidence of 95 and a margin of error of 5 were applied with a standard deviation of 0.25. Then the following formula was used to estimate the sample size:

$$(1.96)^2 \times 0.25(0.25) / (0.05)^2 = 90 \text{ enrolled women [Eq 1]}$$

Sample collection and preparation

Some blood samples were taken and inserted into a gel tube to isolate the serum. Centrifugation was done at 1600 rpm at room temperature of 10 minutes. The separated serum was further kept in 20°C. It was stored in such conditions until it was subjected to serological examination.

Serological examination

The concentrations of IL18, IL23, CD68, and VEGF in the serum were detected using a commercial enzyme-linked immunosorbent assay (ELISA) kit. One of the cytokine antibodies (IL-18, IL-23, CD68 or VEGF) was applied onto the multi-well plate surface and serves as a capture antibody to fix the antigen. The conjugated antibody that was the other one aids in detecting the antigen.

Statistical analysis

Independent measures of analysis of variance (ANOVA) were used. The probability level (p-value) of less than 0.05 was deemed to be significant. The results were presented in arithmetic mean with the standard deviation (mean ± SD). Tukey HSD test was also used to compare two groups separately and significant differences were found. The estimation of the Pearson correlation coefficient between the variables among the various groups in this study was done using the Statistical Package for the social Sciences (SPSS) program (2019)^{20,21}.

RESULTS and DISCUSSION

The results in the current research were presented as mean ± SD and Matched groups were used to minimize the biased effect of these variables on the studied biomarkers. For that reason, the demographic results demonstrated in Table 1 show no statistical differences in age and BMI.

Table 1. The demographics of the study groups

Parameter	Control (mean ± SD)	Missed Miscarriage (mean ± SD)	Incomplete Miscarriage (mean ± SD)	p-value
Mother's age (years)	27.87 ± 6.85	27.13 ± 5.48	27.83 ± 6.50	0.789
Body mass index (kg/m ²)	23.80 ± 2.23	22.77 ± 4.80	24.25 ± 3.26	0.100

Non-significant at p<0.05

Results illustrated in Table 2 also showed that there were non-significant differences in the demographics, number of pregnancies, parity, previous miscarriage, or gestational weeks among the groups.

Table 2. Number of pregnancies, parity, previous miscarriages, and gestational weeks among women participating in the study

Parameter		Control (n=30)		Missed Miscarriage (n=30)		Incomplete Miscarriage (n=30)	
		Frequency	%	Frequency	%	Frequency	%
Number of pregnancies	1-3	16	53.3	15	50	11	36.6
	4-6	14	46.6	14	46.6	11	36.6
	7-9	0		1	3.3	6	20
	10-12	0		0		1	3.3
	13-15	0		0		1	3.3
Parity	0-3	24	80.0	25	83.3	17	56.6
	4-7	6	20.0	5	16.6	11	36.6
	8-11	0	-	0	-	2	6.6
Previous miscarriage	0	20	66.6	18	60	19	63.3
	1	6	20.0	7	23.3	5	16.6
	2	4	13.3	4	13.3	3	10
	3	-	-	1	3.3	3	10
Gestational weeks	8-9	12	40.0	15	50	11	36.6
	10-11	6	20.0	6	20	3	10
	12-13	12	40.0	7	23.3	12	40
	14	-	-	2	6.6	5	16.6

The results illustrated in Table 3 revealed marked variations in IL-18 levels across the study groups. Mean IL-18 concentration in the control group was 154.57 ± 50.93 pg/ml, while the missed miscarriage group exhibited a substantially higher level of 675.82 ± 130.71 pg/ml. In contrast, the incomplete miscarriage group recorded a mean IL-18 level of 160.75 ± 58.61 pg/ml. Overall, there was a statistically significant difference among the groups, with a p-value of 0.01, as shown in Table 3. The missed miscarriage group showed a markedly higher IL-18 level compared to both the control and incomplete miscarriage groups. This suggests a possible association between IL-18 elevation and missed miscarriage. As shown in Table 3, The control group (T0) recorded a mean level of 351.50 ± 92.93 pg/ml, while the missed miscarriage group (T1) had a lower mean value of 319.19 ± 33.34 pg/ml. In contrast, the incomplete miscarriage group (T2) exhibited a higher mean IL-23 level of 397.78 ± 56.94 pg/ml. Statistical analysis (ANOVA test) was performed to compare the three groups, and the results showed a highly significant difference ($p=0.00006$).

This result is attributable to the elevated IL-23 levels observed in the incomplete miscarriage group, which showed a statistically significant difference when compared separately with both the control group ($p=0.02$) and the missed miscarriage group ($p=0.000$). In contrast, no statistically significant difference was observed between the control and missed miscarriage groups ($p=0.10$).

The results of soluble CD68 levels in the serum of the three groups are shown in Table 3. There was a clear statistically significant difference among the three groups ($p<0.00001$). Statistically significant differences were also observed when each pair of groups was compared separately ($p=0.0000$). The missed miscarriage group showed the highest serum CD68 concentration (3.37 ± 0.23 ng/ml). This was followed by the incomplete miscarriage group (1.97 ± 0.26 ng/ml). The control group showed the lowest concentration (1.72 ± 0.12 ng/ml). These serological results indicate increased phagocytic activity and inflammation in the maternal circulation, especially in missed miscarriage, which contributes to pregnancy loss. Meanwhile, the incomplete miscarriage group also showed a significant increase in serum CD68 compared to the control group, which was lower than in the missed miscarriage group.

Table 3 shows serum VEGF levels across the different study groups, expressed as mean \pm standard deviation, with corresponding probability values for each comparison. The highest VEGF concentration was observed in the missed abortion group (542.81 ± 59.96 pg/ml). This was followed by the incomplete abortion group (319.99 ± 24.08 pg/ml). In contrast, the control group showed the lowest concentration (260.74 ± 43.8 pg/ml). Comparison of the three groups using the ANOVA test showed a highly significant difference. Further pairwise comparisons using Tukey's HSD test also revealed statistically significant differences between each group.

Table 3. The serum levels of the immunological biomarkers in the study groups

Biomarker	Study Group	No.	Mean \pm SD (pg/ml)	p-value	
				T0	T1
IL-18	Control group (T0)	30	154.57 \pm 50.93		
	Missed miscarriage group (T1)	30	675.82 \pm 130.71	0.023*	
	Incomplete miscarriage group (T2)	30	160.75 \pm 58.61	0.999	0.025*
p<0.01					
IL-23	Control group (T0)	30	351.48 \pm 92.93		
	Missed miscarriage group (T1)	30	319.19 \pm 33.34	0.1	
	Incomplete miscarriage group (T2)	30	397.78 \pm 56.94	0.02*	0.000**
p<0.0000					
VEGF	Control group (T0)	30	260.74 \pm 43.80		
	Missed miscarriage group (T1)	30	542.81 \pm 59.96	0.000**	
	Incomplete miscarriage group (T2)	30	319.99 \pm 24.08	0.00001**	0.000**
p<0.00001					
CD68	Control group (T0)	30	1.72 \pm 0.12		
	Missed miscarriage group (T1)	30	3.37 \pm 0.23	0.000**	
	Incomplete miscarriage group (T2)	30	1.97 \pm 0.26	0.00005**	0.000**
p>0.00001					

*: significant at $p < 0.05$; **: highly significant at $p < 0.01$

The correlation coefficients for the parameters examined are presented in Table 4. The results showed a significant correlation between IL-18 and IL-23 in the control group ($r = 0.48$, $p = 0.0073$), as well as between IL-18 and CD68 ($r = 0.34$, $p = 0.0481$). There was no significant correlation between VEGF and IL-18 ($r = -0.029$, $p = 0.1121$). In contrast, this pattern differed in the missed miscarriage group. No significant correlation was observed between IL-23 and CD68 ($r = -0.12$, $p = 0.5149$). However, a significant moderate correlation was noted between IL-23 and VEGF ($r = 0.33$, $p = 0.0495$). VEGF and CD68 exhibited a strong negative correlation ($r = -0.54$, $p = 0.0019$). Additionally, a strong negative correlation was observed between IL-18 and VEGF ($r = -0.42$, $p = 0.0207$), while significant positive correlations existed between IL-18 and

IL-23 ($r = 0.40, p=0.0299$) and between IL-18 and CD68 ($r = 0.37, p=0.0387$) in patients with missed miscarriage. In contrast, the correlation between IL-23 and CD68 was not significant ($r = 0.25, p=0.1779$), nor was the correlation between IL-23 and VEGF ($r = 0.24, p=0.2075$). Furthermore, the overall analysis revealed no significant relationship between IL-23 levels and CD68 ($r = 0.06, p=0.9211$). In the incomplete abortion group, weak and non-significant correlations were observed between IL-18 and IL-23 ($r = 0.16, p=0.4076$) and between IL-18 and CD68 ($r = -0.02, p=0.9065$). However, VEGF showed a significant negative correlation with IL-18 ($r = -0.38, p=0.0395$). The research revealed a strong significant negative correlation between IL-23 and VEGF ($r = -0.43, p=0.0109$) and a strong positive correlation between IL-23 and CD68 ($r = 0.80, p=0.001$). There was no correlation between CD68 and VEGF ($r = -0.03, p=0.8833$).

Table 4. Correlation coefficients among different variables in the study groups

Group	Parameter		Correlation Coefficient	p-value
Control	IL-18	IL-23	0.48 **	0.0073
		VEGF	-0.29 NS	0.1121
		CD68	0.34 *	0.0481
	IL-23	VEGF	0.33 *	0.0495
		CD68	-0.12 NS	0.5149
	VEGF	CD68	-0.54 **	0.0019
Missed miscarriage	IL-18	IL-23	0.40 *	0.0299
		VEGF	-0.42 *	0.0207
		CD68	0.37 *	0.0387
	IL-23	VEGF	0.24 NS	0.2075
		CD68	0.25 NS	0.1779
	VEGF	CD68	0.06 NS	0.9211
Incomplete miscarriage	IL-18	IL-23	0.16 NS	0.4076
		VEGF	-0.38 *	0.0395
		CD68	-0.02 NS	0.9065
	IL-23	VEGF	-0.43 **	0.0109
		CD68	0.80 **	0.001
	VEGF	CD68	-0.03 NS	0.8833

*: $p \leq 0.05$; **: $p \leq 0.01$; NS: not significant; positive indicates a direct correlation, negative indicates an inverse correlation.

Miscarriage is a common health issue affecting maternal health and community resources. It has a significant impact on social services. Miscarriage has diverse underlying pathologies, and the etiology remains unknown in the majority of cases. This uncertainty makes the topic a strong motivation for many researchers to investigate its causes. The current study evaluates biomarkers involved in immune response and angiogenesis. These are major pathways in pregnancy establishment and progression. An imbalance in these pathways may result in pregnancy failure. The demographic variables were matched to control for age and BMI variations. This was done to ensure that these factors would not affect our results, given their influence on miscarriage rates. The relevant data are presented in Tables 2 and 3.

Understanding the role of IL-18 in miscarriage can shed light on the mechanisms of pregnancy loss. Elevated Th1 cytokines like IL-18 may trigger an excessive inflammatory response, leading to immune-mediated fetal rejection and disruption of normal maternal and fetal functions, ultimately resulting in miscarriage²². Our study found elevated IL-18 levels in women experiencing missed miscarriage, where the deceased fetus remains in the uterus without physiological symptoms. In contrast, incomplete miscarriage involves the expulsion of fetal tissues, triggering physiological symptoms. The retained fetal tissues in missed miscarriage may act as foreign antigens, eliciting an immune response and increasing IL-18 levels. Following tissue expulsion in incomplete miscarriage, the immune system stabilizes, and IL-18 levels decrease toward those observed in healthy pregnancies. The findings of an earlier study highlighted that serum IL18 levels were significantly higher in pregnancies that ended in miscarriage, especially in women with a history of recurrent miscarriage²³. Löb and his colleagues (2021) noted that the role of IL-18 in miscarriage is not well understood. Their study found that the gene expression of IL-18 was 4.9 times higher in patients with recurrent miscarriage. In contrast, no significant changes in IL-18 gene expression were observed in spontaneous miscarriage samples when compared to healthy controls¹⁰.

Interleukin 23 is a pro-inflammatory cytokine that plays a critical role in regulating the immune response during pregnancy²⁴⁻²⁶. The maternal immune system must strike a delicate balance between tolerating the fetal allograft and mounting an appropriate immune response to protect the mother and fetus²⁷. Disruption of this balance, including changes in IL-23 levels, has been associated with various pregnancy complications, including miscarriage. Previous study has shown that women with a history of recurrent miscarriage had significantly higher serum levels of IL-23 than healthy pregnant women²⁸. High levels of this interleukin indicate an active immune response during missed and incomplete

miscarriage, and its levels reflect the physiological state and potential outcomes in each group²⁹. Saifi et al. (2014) reported that elevated IL-23 levels can disrupt the balance between T-helper 17 (Th17) cells and regulatory T (Treg) cells, increasing Th17 cell percentages and potentially leading to fetal rejection³⁰.

A study found that pro-inflammatory M1 macrophages dominate in miscarriage, while successful pregnancies show decreased M1 polarization. Elevated soluble CD68 in miscarriage cases highlights macrophages' role in early pregnancy termination³¹. VEGF plays a fundamental role in the growth of blood vessels and the function of endothelial cells, especially during pregnancy, where it participates in the formation of the placenta and the adaptation of blood flow in the uterus³². The high level of VEGF in women who suffer from both types of miscarriage (missed and incomplete miscarriage) indicates the role of this factor in pregnancy loss. This aligns with the findings of Ozturk et al. (2017) but contrasts with other studies, which reported decreased levels of this factor during miscarriage³³⁻³⁵. The increase in IL-18 levels in missed miscarriages compared to normal pregnancies may be due to a compensatory response of the placenta that fails to function effectively. This failure can lead to decreased blood supply, oxygen deficiency, and pregnancy loss. High levels of IL-18 may reflect the body's inability to restore proper placental function.

Direct studies comparing the correlations among IL-18, IL-23, VEGF, and CD68 in control, missed miscarriage, and incomplete miscarriage groups are limited. However, existing research provides insights into the individual roles of these factors. A study reported that women who experienced miscarriage exhibited higher levels of Th1-associated cytokines, including IL-18, compared to women with normal pregnancies, indicating that elevated IL-18 may play a critical role in early pregnancy loss. Studies have demonstrated that IL-23 suppresses trophoblast proliferation, migration, and epithelial-mesenchymal transition (EMT) through activation of the p38 MAPK signalling pathway, potentially leading to recurrent spontaneous abortion due to impaired trophoblast function²³. Additionally, the HIF-1 α /VEGF pathway is essential for villous angiogenesis during pregnancy. Downregulation of HIF-1 α and VEGF has been observed in missed miscarriage, highlighting their role in maintaining normal angiogenesis³⁶.

The strong correlation between IL-18 and IL-23 in the control group as well as the missed miscarriage group is a pointer to inflammatory cytokine interaction in normal and pathological pregnancy. However, in the incomplete miscarriage group, this correlation was weak and nonsignificant, possibly due to dysregulation of immune response. In both the missed miscarriage

and incomplete miscarriage groups, VEGF showed a negative correlation with IL-18, suggesting that elevated IL-18 may inhibit angiogenic activity and contribute to pregnancy complications. In contrast, no significant correlation was observed in the control group, indicating normal regulation of angiogenesis and inflammatory processes. In the incomplete abortion group, IL-23 was inversely correlated with VEGF, and the correlation was highly significant. This might suggest that IL-23 is responsible for inhibiting pathological pregnancy angiogenesis. In the missed miscarriage and control groups, the correlation was not significant. A strong positive correlation was observed in the incomplete miscarriage group, indicating that IL-23 may drive macrophage activation in cases of incomplete miscarriage. The correlation was weaker in the missed miscarriage and control groups, suggesting a progressive role of IL-23 in macrophage recruitment in severe cases. The findings of this research agree with Kwiatek et al. (2021), who measured a number of cytokines in the sera of patients with missed miscarriage compared to patients with normal pregnancy³⁷.

In missed miscarriage, retained fetal tissues act as foreign antigens, triggering immune responses, whereas tissue expulsion in incomplete miscarriage restores immune stability, lowering IL-18. Elevated IL-23 reflects active immunity in both types, and increased soluble CD68 indicates macrophage involvement in early pregnancy termination risk^{38,39}. The high level of VEGF in women who suffer from both types of miscarriage (missed and incomplete miscarriage) indicates the role of this factor in pregnancy loss. The evidence presented here suggests that inflammatory cytokines (IL-18, IL-23) and macrophage activation (CD68) are strongly correlated with pregnancy complications, particularly with incomplete miscarriage. The inverse correlation between VEGF and pro-inflammatory cytokines suggests an anti-angiogenic role in pregnancy complications⁴⁰.

In conclusion, this study reveals that elevated levels of IL-18, IL-23, CD68, and VEGF are associated with miscarriage, with specific patterns observed in missed and incomplete miscarriages. Elevated IL-18 and VEGF levels, particularly in missed miscarriage, may indicate a disrupted immune response leading to pregnancy loss, while IL-23 is more significantly elevated in incomplete miscarriage. These findings underscore the potential for targeted therapeutic strategies based on cytokine modulation to improve pregnancy outcomes and prevent miscarriage.

STATEMENT OF ETHICS

The study was approved by the Scientific Committee of Baghdad University, College of Science, under approval number (7902/22) on December 21, 2023.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Every author, including GJS, MKH, and BHH, helped with the results design and implementation as well as the manuscript's writing.

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