



# The association of matrix metalloproteinase-9 and fetal fibronectin in the first trimester threatened miscarriage

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## Abstract

**Introduction:** Vaginal bleeding during the first trimester of pregnancy, accompanied by a positive fetal heart rate and a closed cervix, is medically referred to as a threatened miscarriage. This condition is considered the most common complication within the first 12 weeks of pregnancy. It is important to note that, this condition is a potential indication of danger to the fetus, and therefore; requires immediate medical attention and monitoring.

**Objectives:** To evaluate the association of putative biomarkers matrix metalloproteinase-9 (MMP-9) and fetal fibronectin (fFN) in threatened miscarriage.

**Patients and Methods:** A case-control study on threatened miscarriage was conducted at Alkarama hospital and Ibn al Balady Hospital in Baghdad. Serum levels of fFN and MMP-9 were analyzed in 91 pregnant women with matching gestational ages, divided into two groups of threatened miscarriage (patient: n=30) and healthy pregnant women (control: n=61). The differences in serum concentration of fFN and MMP-9 between the two groups, as well as their correlation with clinical outcomes, were statistically evaluated.

**Results:** There were variations in serum concentrations of matrix metalloproteinase9 between the threatened miscarriage group (49.34±1.08 pg/mL) and the control group (12.19±4.90 pg/mL). Statistical analysis showed a p-value of 0.009. FFN levels were found to be higher in the threatened miscarriage patient group (467.85±6.22 pg/mL) compared to the control group (230.66±37.44 pg/mL), with a P-value of 0.003. A positive correlation was observed between MMP-9 and fFN ( $r=0.877$ ,  $P=0.001$ ). The 95% confidence interval for fFN was (0.9-0.992) and for MMP-9 was (0.78-0.96).

**Conclusion:** Increased levels of MMP-9 and fFN have been associated with a higher risk of fetal demise.

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## Introduction

Vaginal bleeding during the first trimester of pregnancy, accompanied by a positive fetal heart and a closed cervix, is medically referred to as threatened miscarriage. This condition is considered the most prevalent complication within the first 12 weeks of pregnancy. It is important to note that this condition is a potential indication of fetal hazard, and therefore, requires immediate medical attention and monitoring (1,2).

The majority of cases of threatened miscarriage have an idiopathic underlying cause. However, in other cases, genetic factors; causes such as endocrine disorders, hemophilia, lifestyle, environmental conditions (3,4), can be attributed to about 50% of cases. It is imperative to identify the root cause of the condition to determine the most effective course of treatment. Therefore, further research is required to gain a better understanding of the etiology of this condition

## Key point

Matrix metalloproteinase 9 and fetal fibronectin were evaluated in the serum of first-trimester pregnant women, increased levels of matrix metalloproteinase and fetal fibronectin were found in the pregnant women who complained vaginal bleeding, which may be the cause of threatened miscarriage.

and to develop effective treatment strategies.

The endometrium, which is the environment for embryo implantation, undergoes through continuous and dynamic changes. It prepares to receive an embryo and adjusts for its development (5). In recent years, significant advancements have been made in understanding implantation and the interactions between the mother and the embryo. This has provided new insights into the possible causes of conditions related to miscarriage and has opened up new research avenues into the association of maternal-fetal interface defects with miscarriage (6,7).

Throughout pregnancy, the human placenta plays a crucial role in ensuring a consistent flow of blood to the developing fetus. This is achieved by the placenta infiltrating the maternal tissues and vasculature. However, complications arising from abnormal placental development can have profound effects on both the mother and the fetus. Therefore, it is vital to closely monitor placental development to mitigate any potential risks and promote optimal health for both mother and fetus (8).

The invasion of the placental trophoblast requires the regulation of cellular adhesion as well as the remodeling and degradation of the extracellular matrix (ECM) (9). Matrix metalloproteinase-9 (MMP-9) is a 92 kDa type IV collagenase, 92 kDa gelatinase, or gelatinase B (GELB)-type enzyme. It is an enzyme that breaks down the ECM. The major structure of MMP-9 (gelatinase B) (EC3.4.24.25) includes a signal peptide, propeptide, catalytic domain, three tandem repeats of fibronectin type II inserts within the catalytic domain, a proline-rich and extensively O-glycosylated linker, and a hemopexin-like domain (10). MMP-9 plays a significant role in the penetration of human cytotrophoblast cells into the endometrium, as well as in embryo implantation and development (11). MMP-9 is a key protease in this process, and its activity is tightly regulated for proper implantation and embryonic development (12).

MMP-9 is an enzyme that requires zinc to function (11). It plays a crucial role in breaking down the maternal basement membrane, which is primarily composed of type IV collagen (11). The concentration of MMP-9 in the placental bed is low during the sixth and seventh weeks of pregnancy and gradually increases after the eighth week; by the eleventh week trophoblast cells release MMP-9 continuously (13).

Early miscarriage which occurs before the 12-week gestation mark, is caused by the overexpression of MMP-9. This overexpression is associated with trophoblast invasion and angiogenesis (13).

Throughout pregnancy, the ECM is essential for uterine structural integrity, embryo attachment, and placental invasion, serving as glue with adhesive glycoproteins and adhesion receptors (14).

Fetal fibronectin (fFN) is a glycoprotein that is an important component of the ECM. It is primarily found at the interfaces between the uterus and placenta, as well as between the chorion and decidua, where it plays a crucial role in facilitating adhesion between the mother and fetus. Fibronectin is essential for various embryonic processes, including embryogenesis, cell spreading, migration, proliferation, and apoptosis. If fibronectin is degraded, it can result in early embryonic lethality (15).

Various retrospective studies using enzyme-linked immunosorbent assay measurements of fFN have shown a closer relationship between the concentration of fFN in cervicovaginal fluid and the risk of spontaneous miscarriage. These studies have found that higher

concentrations of fFN are linked to an increased risk of spontaneous miscarriage (16). The current study aims to investigate the effects of these factors, to provide valuable insights into the underlying mechanisms of threatened miscarriage and potentially identify new targets for therapeutic interventions.

### Objectives

Evaluate MMP-9 and fFN levels in the serum of first-trimester pregnant women to determine if these potential biomarkers have an impact on women at risk of threatened miscarriage.

### Patients and Methods

#### Study design

The study involved 91 pregnant women aged 20-35 from two hospitals Baghdad al Karama Hospital and Ibn al Balady Hospital. They attended an obstetrics and gynecology unit from November 2022 to February 2023 and were divided into two groups (patient and control). All participants were in the first trimester of pregnancy between 6 to 12 weeks of gestation with body mass index (BMI) between 18 and 25 kg/m<sup>2</sup>.

#### Inclusion criteria

This study specifically includes patients who are pregnant and experiencing vaginal bleeding between 6 and 12 weeks of pregnancy. These patients must have a single-tone fetus with a positive heartbeat.

#### Exclusion criteria

- Patients with uncertain gestational age.
- Obesity.
- Smokers.
- Pregnant women with chronic diseases such as hypertension, diabetes, thyroid dysfunction, and hemophilia.

#### Sample collection

Blood samples (10 mL) were collected via venipuncture into vacuum collection tubes from each participant included in the study. The vacuum tube was then subjected to centrifugation at 4000 rpm for 15 minutes to obtain serum, which was stored at -20 °C after aliquoting into multiple Eppendorf tubes for analysis.

The concentration of fFN and MMP-9 were analyzed using an enzyme-linked immunosorbent assay.

### Methods

The Sunlong sandwich-ELISA (enzyme-linked immunosorbent assay) kit from China utilizes a pre-coated micro-ELISA strip plate with an antibody specific to MMP-9. Horseradish peroxidase-conjugated antibody is then added to standards or samples. After incubation, TMB substrate solution is added, and only wells with MMP-9 and HRP-conjugated antibodies turn blue.

MMP-9 concentration is determined by measuring optical density (OD) at 450 nm.

Similarly, the fFN kit also utilizes the Sunlong sandwich-ELISA kit from China which includes a pre-coated micro-ELISA strip plate with an antibody specific to fFN. Standards or samples are added, followed by the addition of horseradish peroxidase-conjugated antibody. After incubation, TMB (5,5'-tetramethylbenzidine) substrate solution is added, and the OD is measured at 450 nm. The concentration of fFN in samples is then calculated by comparing the OD to the standard curve.

### Statistical analysis

The collected data were entered, double-checked, and analyzed using IBM SPSS software version 26. Descriptive statistics were used to qualitatively summarize the characteristics of the collected data in this study. The independent sample t test was utilized to determine the significance among the study's groups. A *P* value of less than 0.05 was suggested to indicate differences among the study's groups. Pearson's correlation coefficient was conducted to analyze correlations. The receiver operating characteristic (ROC) curve was employed to find cutoff values and evaluate some parameters as diagnostic markers. Data were considered when; not significant  $P > 0.05$  and significant  $P \leq 0.05$ .

### Results

A total of 91 samples from both groups were investigated. The age of each study sample was normally distributed and ranged from 18 to 35 years with a mean age of  $26.16 \pm 4.25$  years for the control group and 20 to 35 years, with a mean age of  $26.96 \pm 4.06$  years for the patient group respectively. There were no significant differences between them ( $P = 0.176$ ; Table 1).

The percentages of previous miscarriages in both groups were 24.59% (15) cases and 83.34% (25) cases in the healthy and unhealthy groups respectively.

The results of MMP-9 in this study revealed a significant association between first-trimester threatened miscarriage and the protein MMP-9. The statistical analysis showed a *P* value of 0.009, indicating a strong correlation between the two variables. The patient group, consisting of women who experienced a threatened miscarriage, had a mean level of MMP-9 of  $49.34 \pm 1.08$  pg/mL, which was significantly higher than the control group's mean level of  $12.19 \pm 4.90$  pg/mL as shown in

The levels of serum fFN were measured in both the control group of the threatened miscarriage patients and in the patient group. The results showed a statistically significant difference between the two groups ( $P = 0.003$ ). Specifically, the mean level of serum fFN in the control group was  $230.66 \pm 37.44$  pg/mL, while the patient group had a significantly higher mean level of  $467.85 \pm 6.22$  pg/mL. These findings suggest a potential correlation between serum fFN levels and certain medical conditions during pregnancy.

The findings indicate a significant correlation between fFN and MMP-9 with a *P* value of 0.001 (Table 2).

### Specificity and sensitivity test for MMP-9 and fFN

Fetal fibronectin and MMP-9 were evaluated for their ability to distinguish between cases of threatened miscarriage and healthy control. The area under the receiver operating characteristic curve (AUC) serves as a measure of a marker's ability to discriminate between cases and controls. For the most severe cases of bleeding during pregnancy, the ROC area is closer to 1, while for those least affected, the ROC area is closer to 0.5 indicating a reliable test.

The analysis of the ROC curve was used to test the ability of fFN levels in serum to diagnose threatened miscarriage. The results indicate that high levels of fFN in serum for threatened miscarriage patients can indicate the presence of risk, making it a useful diagnostic marker, as shown in Figure 1.

**Table 1.** Comprehensive between the mean  $\pm$ SD of the two groups

| Measure                   | Control (n=61)<br>Mean $\pm$ SD | Patient (n=30)<br>Mean $\pm$ SD | <i>P</i> value |
|---------------------------|---------------------------------|---------------------------------|----------------|
| Age (y)                   | 26.16 $\pm$ 4.25                | 26.96 $\pm$ 4.06                | 0.176          |
| MMP-9 (pg/mL)             | 12.19 $\pm$ 4.90                | 49.34 $\pm$ 1.08                | 0.009          |
| Fetal fibronectin (pg/mL) | 230.66 $\pm$ 37.44              | 467.85 $\pm$ 6.22               | 0.003          |

**Table 2.** Correlation between fFN and MMP-9

| Correlation between | Threatened miscarriages group, <i>r</i> value | <i>P</i> value | Significance       |
|---------------------|---|----------------|--------------------|
| fFN and MMP         | 0.916   | 0.001          | Highly significant |

**Table 3.** Specificity and sensitivity test for MMP-9 and fFN

| Parameter | AUC   | Standard Error | Sensitivity | Specificity | 95%CI     | Cut-off | <i>P</i> value |
|-----------|-------|----------------|-------------|-------------|-----------|---------|----------------|
| fFN       | 0.946 | 0.024          | 90.2%       | 86.7%       | 0.9-0.992 | 276.53  | 0.001          |
| MMP       | 0.872 | 0.045          | 83.6%       | 80%         | 0.78-0.96 | 15.67   | 0.001          |

The ability of MMP-9 levels in serum to diagnose threatened miscarriage was tested by analyzing the ROC curve (Table 3). The results indicate that high levels of MMP-9 in serum for patients with threatened miscarriage can indicate the presence of the risk, making it a useful diagnostic marker, as shown in Figure 2.

## Discussion

Interactions between immune cells and trophoblasts are crucial for maintaining a healthy maternal-fetal interface during pregnancy. However, disruption in these interactions can lead to various pregnancy complications, such as spontaneous miscarriage (17).

Embryo implantation, placenta development, and other physiological and clinical pregnancy processes are all tightly tied to matrix metalloproteinases (MMPs) and tissue inhibitors of MMP-9 (18).

The increased concentration of MMP-9 observed in the current study, may be a result of infection which causes an increase in tumor necrosis factor (TNF), an inflammatory cytokine, that induces a rise in MMP-9. Moreover, a retrospective nested cohort case-control study by Castruita-De la Rosa et al, on 34 pregnant women found that elevated serum concentrations of MMP-9 were associated with and preceded the appearance of spontaneous interruption of pregnancy (19).

Research has shown that elevated levels of serum MMP-9 may be a factor in spontaneous pregnancy loss, as demonstrated in a study of 129 women in their first trimester (13).

A previous prospective study aimed to investigate the expression of MMP-9 in endometrial curettage from 135 women with recurrent spontaneous miscarriages and 120 healthy pregnant women. The study found that imbalanced MMP-9 led to excessive endometrial matrix degradation ultimately affecting pregnancy outcomes and resulting in spontaneous miscarriage in women with

recurrent infections (20).

Consistent with the findings of the current study, a case study of 30 spontaneous miscarriages and 20 requested miscarriages revealed that the mRNA expression level of MMP-9 in the spontaneous miscarriage group was higher than that in the requested miscarriage group (21).

In a previous study, conducted by researchers investigating the role of MMP-9 in recurrent pregnancy loss, specimens from the trophoblast, decidua basalis, and decidua parietalis of 40 first-trimester pregnant women were analyzed. The women were divided into two groups; those who experienced unexplained miscarriage, and those who had electively terminated pregnancies. Using immunohistochemical methods, the study found that MMP-9 levels were significantly higher in cases of recurrent pregnancy loss in both trophoblastic and decidual specimens ( $P < 0.001$ ) (22).

The current study results revealed that fFN levels were higher in patients experiencing threatened miscarriage compared to those with health-pregnancy. Elevated fFN levels may indicate inflammation, infection, placental issues, or a direct cause of miscarriage.

Infection led to an increase in several cytokines such as interleukin-1 beta (IL-1 $\beta$ ), tumor TNF alpha, and interleukin-6 (IL-6) which in turn enhance the production and expression of MMPs. This can cause degradation of the ECM and damage the uterine lining. Additionally, an insufficient placenta can weaken the attachment of the fetal sacs to the uterine wall, increasing the risk of miscarriage (23).

A study compared cervical mucus collected from 36 first-trimester pregnant women with and without bacterial vaginosis (BV) who were attending a family planning unit for first-trimester abortion. The study found that levels of fFN were significantly higher in women with bacterial vaginosis compared to those without bacterial vaginosis ( $P < 0.05$ ) (24).

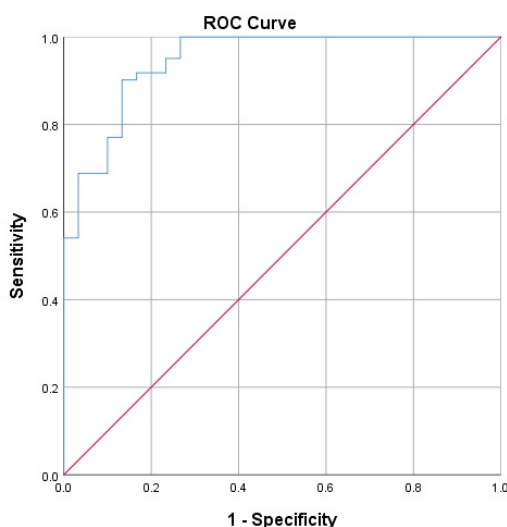


Figure 1. ROC curve for fFN parameter in the study group.

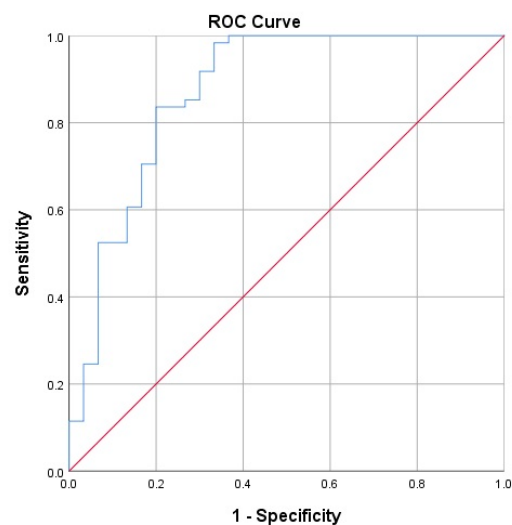


Figure 2. ROC curve for MMP-9 parameter in the study group.



In a study, fFN levels were measured in both cervicovaginal secretions and plasma of 25 healthy pregnant women in the first or second trimester as well as 28 women who were scheduled to have an abortion. The result showed that patients who were about to have an abortion had significantly higher levels of fFN in both their serum and cervicovaginal secretions compared to healthy pregnant women (25). According to previous research, fFN was positive in 17 of 49 pregnant women with recurrent miscarriages and 11 of 35 successful pregnancies, with sensitivity and specificity of 43% and 69%, respectively (16).

The prognostic significance of fFN in early pregnancy embryonic loss in patients with recurrent spontaneous abortion. The study included 84 spontaneous miscarriage patients and 31 healthy women. There was a substantial difference in the incidence of spontaneous miscarriage between the test and control groups. In fFN (+) patients, the sensitivity, specificity, and positive predictive values for predicting abortion were 82.35%, 49.25%, 29.17%, and 91.67%, respectively (26). Vaginal hemorrhage and/or trophoblast were linked to a substantial rise in fibronectin levels in the Threatened miscarriage group which confirms the current study.

### Conclusion

Our study found that increased levels of MMP-9 and fFN were linked with a higher risk of Threatened Miscarriage, which was often preceded by spontaneous pregnancy loss. Our findings imply that alterations in MMP and fFN control may play a role in Spontaneous miscarriage development.

### Limitations of the study

The study encountered challenges in gathering samples, which required a considerable amount of time, as the study samples were particular to the stage of pregnancy and the population under investigation, as these cases were pregnant women experiencing problems at an ambiguous point in their pregnancy.

### Authors' contribution

**Conceptualization:** Maha Saad Maki, Mohammed Shamil Ali, Hala Zghair Rawi.

**Data curation:** Maha Saad Maki, Mohammed Shamil Ali, Hala Zghair Rawi.

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**Visualization:** Maha Saad Maki, Mohammed Shamil Ali, Hala Zghair Rawi.

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**Writing—review & editing:** Maha Saad Maki, Mohammed Shamil Ali, Hala Zghair Rawi.

### Conflicts of interest

The authors declare that they have no competing interests.

### Ethical issues

The research conducted in this study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of the University of Technology-Iraq Bioethics Committee in scientific research (Ethical code#BCSR6). Prior to any intervention, all participants provided written informed consent. The authors have fully complied with ethical issues, such as plagiarism, data fabrication, and double publication.

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