Original Investigation

Serum osteopontin as a diagnostic marker for missed abortion: evidence from a prospective study

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Abstract

Objective: Osteopontin (OPN) is a multifunctional molecule involved in embryo implantation and blastocyst adhesion. Given its role at the maternal–fetal interface, OPN has been proposed as a potential biomarker for early pregnancy outcomes. The aim of this study was to evaluate the association between serum OPN levels and missed abortion in women presenting with early pregnancy bleeding.

Material and Methods: In this prospective study, primigravid women between 6 and 11 weeks of gestation with confirmed fetal cardiac activity were enrolled. Participants were classified into three groups according to clinical presentation: normal pregnancy, threatened abortion, and missed abortion. Blood samples were obtained at admission, and serum OPN levels were measured using an enzyme-linked immunosorbent assay. Inflammatory markers including white blood cell count, neutrophil-to-lymphocyte ratio, C-reactive protein levels, and body mass index were also recorded.

Results: The study cohort numbered 198 women, aged 18-42 years, with 38, 80 and 80 women in the normal pregnancy, threatened and missed abortion groups, respectively. OPN levels showed a significant and progressive increase with the lowest levels observed in normal pregnancies, higher levels in threatened abortion, and the highest levels in missed abortion ($p<0.001$). Receiver operating characteristic curve analysis demonstrated strong discriminative capacity of OPN for pregnancy loss (area under the curve =0.846, $p<0.001$). A cut-off value of 1.15 ng/mL was associated with 100% sensitivity, whereas a cut-off value of 2.15 ng/mL was associated with 100% specificity.

Conclusion: Elevated serum OPN levels are associated with early pregnancy loss and may serve as a potential biomarker in missed abortion. However, these findings should be interpreted with caution given the exploratory nature of the analysis.

Keywords: Diagnostic marker, early pregnancy bleeding, inflammatory biomarkers, missed abortion, osteopontin

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Introduction

Osteopontin (OPN) is an extracellular matrix glycoprotein involved in a wide range of physiological and pathological processes, including inflammation, immune regulation, cell adhesion, migration, and tissue remodeling (1). In the context of reproductive biology, OPN is expressed in the endometrium and plays a pivotal role in decidualization and embryo implantation (1,2). The expression of OPN increases during the early- and mid-secretory phases of the menstrual cycle, supporting blastocyst adhesion and trophoblast invasion

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through interactions with integrin subunits and fibronectin (3,4). Experimental studies in animal models have demonstrated that OPN deficiency leads to reduced implantation and increased embryonic resorption, suggesting a potential major role in mammalian early pregnancy maintenance (5-7).

OPN also contributes to angiogenesis in the endometrium and placenta, helping to maintain a synchronized maternal–embryonic interface and supporting blastocyst viability (8,9). OPN expression is hormonally regulated, particularly by estrogen, which stimulates OPN secretion from endometrial glands. Estrogen-induced OPN promotes blastocyst adhesion competence by enhancing integrin complex formation on the trophectoderm (8,9).

Threatened abortion, clinically defined as vaginal bleeding in early pregnancy in the presence of fetal cardiac activity, is associated with an increased risk of miscarriage, preterm birth, and fetal growth restriction (10,11). Recent evidence suggests that this condition is linked to altered inflammatory pathways, including disturbances in fibrinogen metabolism and systemic inflammatory responses, which may contribute to adverse outcomes (12). Embryo implantation is a complex process dependent on tightly regulated immune activity within the maternal uterine environment. While a balanced immune milieu is essential for successful implantation and early gestation, dysregulated or excessive inflammation may lead to implantation failure or early pregnancy loss. Uterine "CD56 bright" natural killer cells, which produce cytokines such as interferon gamma, play a pivotal role in modulating trophoblast invasion and maintaining immune tolerance at the maternal–fetal interface (13).

Although the precise mechanisms remain unclear, several studies have suggested a link between altered inflammatory responses and early pregnancy complications. These include elevated oxidative stress, dysregulated fibrinogen metabolism, and imbalances in proinflammatory cytokine production (2,14,15). Notably, patients with recurrent pregnancy loss have demonstrated increased secretion of tumor necrosis factor alpha and interleukin-12 from peripheral blood mononuclear cells, suggesting a systemic proinflammatory state (14,16). Given this inflammatory background, OPN has emerged as a promising biomarker due to its dual role in immune modulation and trophoblast interaction during early pregnancy.

Given its established roles in implantation, decidualization, immune regulation, and inflammation, OPN is a strong candidate biomarker for early pregnancy complications, such as abortus imminens and missed abortion. We hypothesized that elevated serum OPN levels may reflect underlying immunological dysregulation and could serve as a diagnostic and prognostic marker in early pregnancy loss. Therefore, the study objective was to investigate serum OPN levels in women presenting with bleeding in early pregnancy and to evaluate the association between serum OPN levels and missed abortion in these women.

Material and Methods

Study design and participants

This prospective study included pregnant women aged between 18 and 42 years, all of whom were followed at a tertiary referral hospital over the defined study period. Participants were categorized into three groups based on clinical presentation: (1) the normal pregnancy group included women without any history of vaginal bleeding; (2) the threatened abortion group included women who experienced vaginal bleeding but continued to have viable pregnancies within four weeks of admission; and (3) the missed abortion group comprised women whose pregnancies ended in miscarriage or missed abortion within the same follow-up period. All women in the missed abortion group had confirmed fetal cardiac activity at admission, and pregnancy loss was diagnosed during the four-week follow-up period.

Inclusion criteria were: a healthy eating index (HEI) score >80 which was chosen to minimize the potential confounding effects of dietary quality on systemic inflammatory markers. Since nutritional status has been shown to influence inflammatory pathways and immune mediators, this criterion allowed selection of a more homogeneous cohort for evaluation of the association between serum OPN levels and early pregnancy loss.

In addition, restricting hospital admission to within three hours of the onset of vaginal bleeding aimed to reduce temporal variability in inflammatory marker levels. This approach ensured that OPN measurements reflected the early biological response to pregnancy disturbance rather than secondary systemic inflammatory changes. Only primigravid women with no history of miscarriage, no medication use other than multivitamins, and normal gynecological and ultrasound findings were included. In addition, all participants had conceived within one year of attempting pregnancy. For the threatened abortion and missed abortion groups, only women who presented to the hospital within three hours of the onset of vaginal bleeding were eligible. The presence of any active infectious disease constituted an exclusion criterion.

Ethical considerations

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was granted by the Institutional Ethics Committee of Batman Research and Training Hospital (approval number: 2020-8, date: 02.12.2020). Written informed consent was obtained from all participants prior to inclusion in the study.

Data collection and laboratory analysis

Demographic data including age, gestational week, and body mass index (BMI) were recorded at baseline. Blood samples were collected from each participant upon admission. Serum OPN levels were measured using an enzyme-linked immunosorbent assay kit (BT LAB, Bioassay Technology Laboratory, China), according to the manufacturer's protocol. Additionally, hematologic and biochemical markers including white blood cell count (WBC), C-reactive protein (CRP) level and neutrophil-to-lymphocyte ratio (NLR) were measured as part of routine laboratory evaluation.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Shapiro–Wilk test. Variables with a normal distribution are expressed as mean ± standard deviation, while non-normally distributed variables are presented as median and interquartile range. Comparisons between three or more groups were performed using one-way analysis of variance for normally distributed data and the Kruskal–Wallis test for non-normally distributed data. Post-hoc analyses were conducted using Tukey's HSD or the Dunn–Bonferroni test, as appropriate. Differences between categorical variables were assessed using the chi-square test or Fisher's exact test.

Receiver operating characteristic (ROC) curve analysis was performed to assess the discriminative performance of serum OPN levels for missed abortion. The area under the curve (AUC), 95% confidence intervals, and optimal cut-off values were calculated. A p-value of <0.05 was considered statistically significant.

Results

A total of 198 pregnant women were included in the study. The participants' ages ranged from 18 to 42 years, with a mean age of 29.07±5.29 years. The mean gestational age was 7.82±1.14 weeks, and the mean BMI was 21.04±1.16 kg/m^2.

In terms of hematological and biochemical parameters, the mean WBC was 10.10±2.09 $\times 10^3/\mu$L, the mean CRP level was 7.20±1.43 mg/L, the mean serum OPN level was 2.56±0.99 ng/mL, and the NLR was 2.92±0.83. Descriptive statistics of the entire study population are presented in Table 1.

Participants were divided into three groups according to clinical presentation: normal pregnancy (n=38); threatened abortion (n=80); and missed abortion (n=80). Group-specific comparisons of clinical and inflammatory parameters are presented in Table 2.

When the three groups were compared, no statistically significant difference was found in terms of age (p=0.506). However, WBC, CRP, serum OPN, NLR, and BMI levels showed significant differences between the groups (p<0.001). Post-hoc analysis demonstrated that the missed abortion group had significantly higher serum OPN levels compared to both the normal pregnancy and threatened abortion groups (p<0.001).

Table 1. Descriptive statistics of the entire study population

Parameter	n	Minimum	Maximum	Mean ± SD
Age	198	18.0	42.0	29.07±5.29
Gestational week	198	6.0	11.0	7.82±1.14
WBC (x10$^3/\mu$L)	198	5.0	14.0	10.10±2.09
CRP (mg/L)	198	5.0	11.0	7.20±1.43
BMI (kg/m^2)	198	19.0	24.0	21.04±1.16
Osteopontin (ng/mL)	198	1.1	4.89	2.56±1.00
SD: Standard deviation, BMI: Body mass index, WBC: White blood cell, CRP: C-reactive protein				

Table 2. Comparison of clinical and inflammatory parameters between groups

Parameter	Normal pregnancy (n=38)	Threatened abortion (n = 80)	Missed abortion (n=80)	p-value
Age	29.74±4.90	29.15±5.60	28.68±5.18	0.506
Gestational week	8.05±1.56	7.83±1.05	7.70±0.99	NS
WBC (x10$^3/\mu$L)	6.42±1.15	10.73±0.93	11.23±1.11	<0.001
CRP (mg/L)	6.21±1.07	7.10±1.12	7.78±1.58	<0.001
BMI (kg/m^2)	21.79±1.02	20.95±1.05	20.78±1.18	<0.001
Osteopontin (ng/mL)	1.51±0.30	1.98±0.34	3.65±0.49	<0.001
NLR	1.54±0.31	2.81±0.21	3.69±0.31	<0.001
Values are presented as mean ± standard deviation. NS: Non-significant, BMI: Body mass index, WBC: White blood cell, CRP: C-reactive protein				

Moreover, the threatened abortion group had significantly higher OPN levels than the normal pregnancy group ($p<0.01$). Similarly, the NLR was significantly elevated in the missed abortion group compared to both the normal pregnancy and threatened abortion groups ($p<0.001$), and the threatened abortion group also showed significantly higher NLR values compared to the normal pregnancy group ($p<0.001$).

ROC curve analysis revealed a strong discriminative association between elevated serum OPN levels and missed abortion, with an AUC of 0.846 ($p<0.001$). The 95 percent confidence interval ranged from 0.774 to 0.918, supporting the reliability of OPNs clinical discriminative capacity. A cut-off value of 1.15 ng/mL yielded 100% sensitivity, while a cut-off value of 2.15 ng/mL provided 100% specificity. Although some overlap was observed between certain cases, this did not meaningfully affect the overall discriminative performance. The ROC curves are shown in Figure 1. Figure 1a shows the ROC curve for distinguishing healthy pregnancies from abortus imminens, indicating moderate discriminative capacity. Figure 1b shows the ROC curve for differentiating healthy pregnancies from missed abortion, demonstrating strong discriminative performance.

Upon examining the ROC curve coordinates, varying sensitivity and specificity combinations were observed for different cut-off values. At an OPN threshold of 1.15 ng/mL, sensitivity reached 100 percent, while specificity remained very low at only 5.3 percent. While cases below this threshold had an extremely low likelihood of pregnancy loss, the majority of those above it were false positives. Therefore, although this threshold may be suitable for screening purposes where high sensitivity is essential, it should not be interpreted as providing definitive diagnostic certainty. In contrast, when a cut-off of 2.15 ng/mL was applied, specificity reached 100 percent, eliminating the risk of false positives. OPN levels above this threshold encompassed the majority of pregnancy loss cases, highlighting its potential value as a highly specific marker for clinical scenarios where stronger rule in discrimination is desired. In summary, OPN levels below 1.15 ng/mL were rarely observed among pregnancy loss cases, whereas levels above 2.15 ng/mL were strongly associated with adverse pregnancy outcomes.

Discussion

This study demonstrated a significant increase in serum OPN levels in women with threatened abortion and missed abortion compared to those with normal pregnancies. The progressive rise in OPN across the clinical spectrum suggests that OPN elevation reflects the severity of early pregnancy disturbance. These findings support the hypothesis that elevated OPN levels may be associated with early pregnancy loss, particularly in the presence of vaginal bleeding.

The current results are consistent with previous reports indicating that dysregulated immune responses contribute

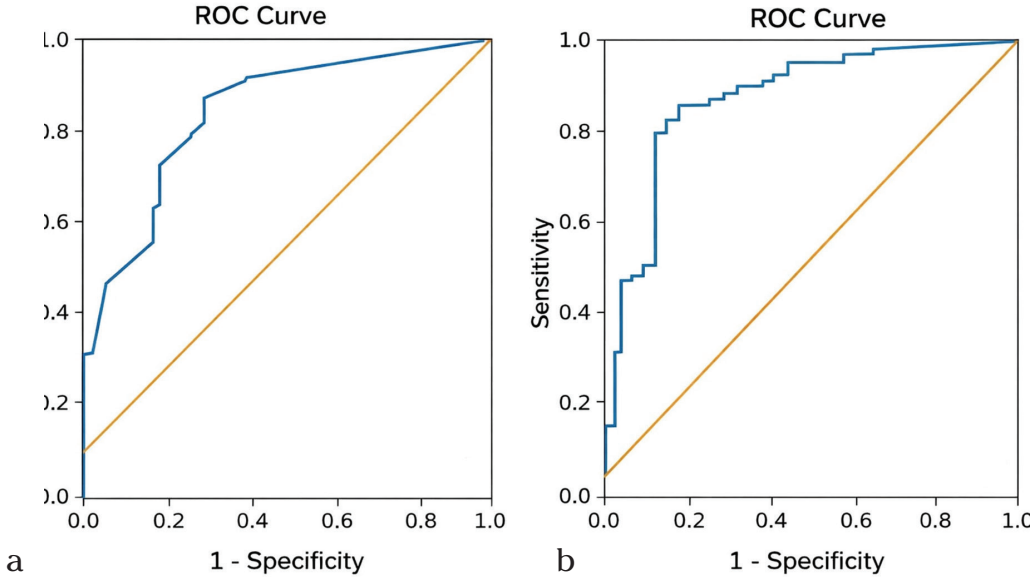

Figure 1. (a) Receiver operating characteristic (ROC) curve illustrating the association between serum osteopontin levels and threatened abortion compared with healthy pregnancies. The area under the curve (AUC) was 0.846. A cut-off value of 1.15 ng/mL provided high sensitivity, indicating potential utility for risk stratification rather than definitive diagnosis. (b) ROC curve illustrating the association between serum osteopontin levels and missed abortion compared with healthy pregnancies. The AUC was 0.846. A cut-off value of 2.15 ng/mL demonstrated high specificity, suggesting potential value in confirming adverse pregnancy outcomes in selected clinical settings

to early pregnancy complications. In line with the literature, elevated OPN levels were accompanied by increases in conventional inflammatory markers such as NLR, supporting the assumption that OPN mirrors systemic immune activation during early pregnancy (13,16). Earlier studies have shown that OPN is involved in implantation, decidualization, angiogenesis, and immune regulation at the maternal–fetal interface, and these biological functions may explain its rise during pathological implantation processes (1,5,6). Hormonal regulation by estrogen and its role in promoting blastocyst adhesion competence further reinforce the central position of OPN in early pregnancy physiology (8,9). Taken together, our findings extend previous mechanistic studies by demonstrating a clinical association between elevated OPN levels and early pregnancy loss.

The ROC curve analysis in our study revealed high diagnostic performance, with cut-off values indicating potential roles for both screening and diagnostic confirmation. A threshold of 1.15 ng/mL, showing 100 percent sensitivity, may be helpful in early identification of pregnancies at risk, whereas a level above 2.15 ng/mL, with 100% specificity, may help confirm adverse outcomes in selected clinical situations. These findings suggest that OPN could aid risk stratification and clinical decision-making, particularly in emergency settings or outpatient triage, where rapid assessment is crucial.

Another strength of this study was the inclusion of a well-defined and homogeneous population, as well as the parallel evaluation of several inflammatory parameters. Robust statistical analyses, including post-hoc and ROC methods, further enhance the reliability of the results. The availability of clinically meaningful cut-off values also increased the translational relevance of our findings and may guide future clinical protocols.

Study limitations

The study population was intentionally selected using strict inclusion criteria, including primigravid status, a narrow BMI range, a high HEI score, and the absence of overt inflammatory or infectious conditions. While this approach enhanced internal validity by minimizing potential confounding factors, it resulted in a highly homogeneous cohort and therefore limits the external generalizability of the findings. Therefore, the results should be interpreted as a proof-of-concept demonstrating an association between serum OPN levels and early pregnancy loss rather than as a broadly generalizable screening or diagnostic model.

In addition, serum OPN levels were only measured at a single time point, which limits assessment of temporal changes and precludes conclusions regarding its longitudinal or prognostic behavior during early pregnancy**.**

Other limitations should also be considered. The observational design prevents establishing a causal relationship between OPN elevation and pregnancy loss. Finally, factors such as genetic predisposition or subclinical infections were beyond the scope of this study and may have influenced inflammatory status.

Despite these limitations, this study contributes to growing evidence that inflammatory mediators may play a key role in early pregnancy failure. Longitudinal monitoring of OPN levels, as well as isoform-specific and molecular studies, may provide deeper insights into its pathophysiological role. Future research should also evaluate the clinical utility of OPN in combination with other inflammatory or hormonal markers to improve predictive accuracy in early pregnancy loss. Therefore, the present findings should not be interpreted as establishing OPN as an independent predictor of pregnancy loss.

Conclusion

Elevated serum OPN levels were associated with early pregnancy loss. Furthermore, the progressive increase from normal pregnancy to threatened abortion and missed abortion highlighted the potential clinical value of measuring OPN. ROC analysis confirmed a strong diagnostic performance, suggesting applicability in both screening and confirmation settings using different cut-off values. OPN may serve as a useful biomarker for early risk stratification in pregnant women presenting with vaginal bleeding, helping to identify cases that require closer monitoring or intervention. Larger prospective and longitudinal studies are warranted to validate these findings and to clarify the mechanistic role of OPN in early pregnancy outcomes.

Ethics

Ethics Committee Approval: *This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was granted by the Institutional Ethics Committee of Batman Research and Training Hospital (approval number: 2020-8, date: 02.12.2020).*

Informed Consent: *Written informed consent was obtained from all participants prior to inclusion in the study.*

Footnotes

Author Contributions: *Concept: D.B., C.A., Design: Y.B., C.A., Data Collection or Processing: E.O., E.G., Z.B., Analysis or Interpretation: Y.B., C.A., Literature Search: E.G., Z.B., Writing: C.A.*

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