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ELEVATED SERUM OSTEOPONTIN IN WOMEN WITH A HISTORY OF RECURRENT MISCARRIAGE: AN ANALYTICAL CASE–CONTROL STUDY

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ABSTRACT

Background: Recurrent miscarriage (RM) affects 2–6% of couples attempting to conceive and remains unexplained in nearly half of affected women despite extensive investigation. Osteopontin (OPN), a multifunctional secreted glycoprotein required for endometrial decidualization and angiogenesis, has been proposed as a peripheral marker of the maternal fetal interface pathology underlying RM, but its diagnostic value has not been fully established.

Objective: To measure and compare serum osteopontin levels between women with a history of recurrent miscarriage and healthy fertile controls, and to explore the association between serum OPN and a history of RM.

Methodology: An analytical case–control study was conducted on 44 women with a history of ≥2 pregnancy losses before 20–24 weeks of gestation (RM group) and 42 healthy fertile women with no history of miscarriage (control group), recruited at the Maternity and Children Hospital, Basra, Iraq (October 2025–February 2026). Serum OPN was measured by enzyme-linked immunosorbent assay (ELISA). Receiver operating characteristic (ROC) curve analysis was used descriptively to quantify the separation between the two predefined groups.

Results: The two groups were well matched for age ($p = 0.271$) and body mass index ($p = 0.941$). Serum OPN was significantly higher in the RM group (mean 4.57 ± 3.66 ng/mL; median 3.26 ng/mL; range 0.69–16.35 ng/mL) than in controls (mean 1.71 ± 2.07 ng/mL; median 0.91 ng/mL; range 0.02–10.74 ng/mL; $p < 0.001$). ROC analysis showed an area under the curve (AUC) of 0.82; at the exploratory Youden-derived cutoff of 1.083 ng/mL, 97.7% of RM participants and 61.9% of controls were correctly classified within this same sample. OPN showed no significant correlation with BMI.

Conclusion: Serum OPN is significantly and substantially elevated in non-pregnant women with a history of recurrent miscarriage relative to healthy fertile controls. Together with its mechanistic role in decidual and vascular remodeling, these findings identify OPN as a biologically plausible candidate for further investigation. The present case–control

design cannot establish diagnostic, predictive, or screening utility, and prospective validation in larger, longitudinal cohorts is required.

Keywords: Osteopontin; Secreted Phosphoprotein; Recurrent Miscarriage; Recurrent Pregnancy Loss; Biomarker; ELISA

1 INTRODUCTION

Recurrent miscarriage (RM) remains one of the most challenging clinical problems in reproductive medicine, with no universal consensus on its definition, aetiology, or evidence-based management. The American Society for Reproductive Medicine (ASRM) defines RM as the loss of two or more pregnancies before 24 completed weeks of gestation [1], a definition broadly echoed by the European Society for Human Reproduction and Embryology, while other guidelines require three or more losses before 20 weeks of gestation [2,3].

RM affects approximately 2–6% of couples trying to conceive and imposes substantial physical and psychological burden [4,5]. Spontaneous abortion accounts for 10–15% of clinically recognized pregnancies and up to 30% of all pregnancies, making it one of the most frequent complications of pregnancy [3]. Despite thorough clinical work-up, almost half of RM cases remain unexplained [6], which has driven active research into candidate biomarkers reflecting the underlying pathophysiology of pregnancy loss, including disturbances of the extracellular matrix and of endometrial and placental vascular remodeling.

Osteopontin (OPN), also known as secreted phosphoprotein 1 (SPP1), is a secreted, highly phosphorylated multifunctional glycoprotein that is widely expressed across tissues and is increasingly studied as a circulating biomarker across a range of physiological and disease states [7-9].

In the endometrium, OPN production is upregulated during the secretory phase of the menstrual cycle and is further enhanced during decidualization of endometrial stromal cells, indicating a role in preparing the endometrium for implantation; experimental inhibition of OPN impairs both decidualization and angiogenesis [10]. Beyond reproduction, dysregulated OPN activity is well documented in chronic inflammatory disease and tumor biology, and circulating OPN is increasingly investigated as a diagnostic and prognostic biomarker across a range of conditions [7,9].

Because OPN operates at the intersection of decidualization, angiogenesis, and local immune regulation — three processes central to successful implantation and placentation — it is biologically plausible that its dysregulation contributes to, or reflects, recurrent pregnancy loss.

Clinical data on OPN in pregnancy loss are nevertheless scarce. Earlier work has largely examined OPN in decidual or villous tissue, or in serum obtained during an ongoing or failing pregnancy, when circulating levels are already influenced by pregnancy itself [15,17]. Far less is known about serum OPN between pregnancies, when any persistent alteration linked to recurrent miscarriage would be easier to separate from normal pregnancy physiology. We therefore measured serum OPN in non-pregnant women with recurrent miscarriage and in healthy fertile controls, and examined how far the two groups differed.

Objectives

- To measure and compare serum osteopontin levels between women with a history of recurrent miscarriage and healthy women with no history of pregnancy loss.
- To describe, in an exploratory analysis, the extent to which serum OPN levels separate these two predefined groups using ROC curve analysis.

2 MATERIALS AND METHODS

2.1 Study design and setting

This was an analytical case–control study comparing women with a history of recurrent miscarriage against healthy fertile controls. The study was approved by the Research Committee of the Basra Health Department (approval no. 576, dated 11 September 2025). Participants were recruited at the Maternity and Children Hospital, Basra, Iraq, between October 2025 and February 2026. Data were collected through direct interview, and all participants provided informed consent for anonymous use of their data for research purposes.

2.2 Study participants

The RM group comprised 44 adult women with a history of two or more consecutive or non-consecutive pregnancy losses before 20–24 weeks of gestation, in accordance with ASRM criteria, with the same partner and no live birth since the most recent loss. Exclusion criteria were: molar or ectopic pregnancy; chronic systemic disease that could independently explain the losses; uterine anomalies; use of assisted reproductive technology; age <18 or >45 years; chromosomal abnormality in either partner; previous stillbirth or preterm premature rupture of membranes; and chronic infections affecting pregnancy.

The control group comprised 42 healthy, age-matched women (18–45 years) with normal fertility, at least one prior live birth, and no history of pregnancy loss, no major uncontrolled medical conditions, regular menstrual cycles with no history of PCOS, and no history of smoking or alcohol use.

2.3 Sample collection

Approximately 5 mL of peripheral venous blood was collected from each participant. Three millilitres were collected into gel-separator tubes, allowed to clot at room temperature, and centrifuged at 3,000 rpm for 10 minutes; the resulting serum was aliquoted and stored at −35°C until further analysis.

2.4 Measurement of serum osteopontin

Serum OPN concentration was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone, China). Standards, blank, and serum samples (100 µL each) were added to antibody-precoated wells and incubated at 37°C for approximately 1 hour. After discarding the well contents, Detection Reagent A was added and incubated for a further 1 hour at 37°C. Following three wash cycles, Detection Reagent B was added and incubated for 30 minutes at 37°C, followed by five further washes. Substrate solution was then added and the plate incubated in the dark at 37°C for 10–20 minutes until blue color developed; the reaction was stopped with Stop Solution, producing a color change from blue to yellow, and absorbance was read immediately at 450 nm on a microplate ELISA reader.

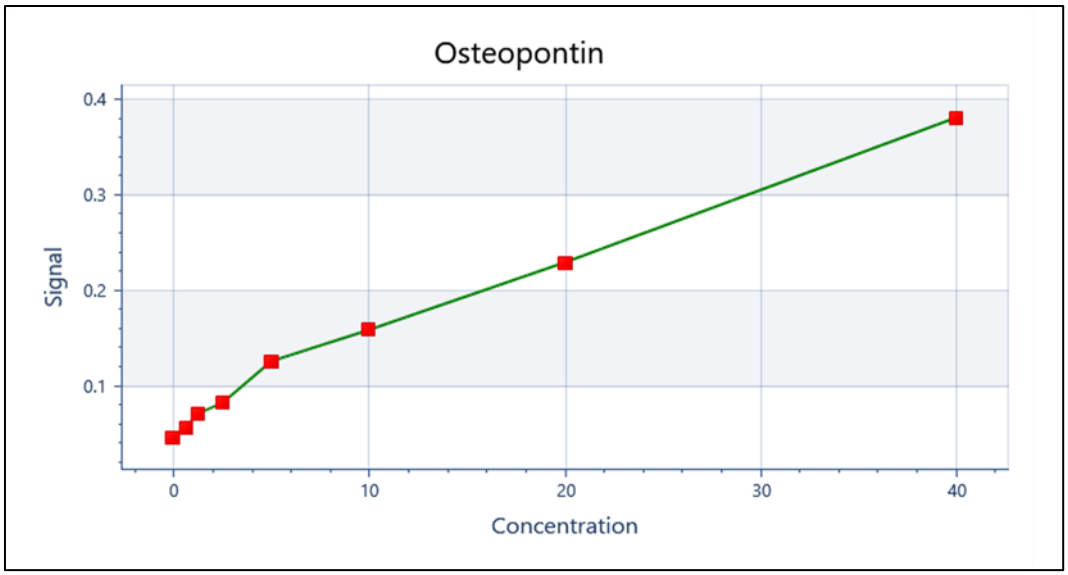

Figure 1: Osteopontin (OPN) ELISA quantitation curve.

2.5 Statistical analysis

Data were analyzed using IBM SPSS Statistics version 26. Categorical variables were expressed as frequencies and percentages and compared using Fisher's exact test. Continuous variables, which were non-normally distributed, were summarized as mean ± standard deviation, median, and range, and compared between groups using the Mann–Whitney U test. Receiver operating characteristic (ROC) curve analysis was used descriptively to quantify the separation in serum OPN (area under the curve, AUC) between the RM and control groups; the AUC is reported with its 95% confidence interval, and the exploratory cutoff was identified by maximizing Youden's index. No prospective or external validation cohort was available. Associations between continuous variables were assessed using Spearman's rank correlation coefficient. A p-value < 0.05 was considered statistically significant.

3 RESULTS

3.1 Demographic and clinical comparability

A total of 86 women were enrolled: 44 in the RM group and 42 in the control group. The two groups did not differ significantly in age distribution (p = 0.271, Fisher's exact test) or in BMI (RM: 25.04 ± 2.90 kg/m^2 vs control: 24.9 ± 2.31 kg/m^2; p = 0.941, Mann–Whitney U test), indicating that the groups were well matched and that subsequent biomarker differences are unlikely to be explained by age or body composition.

Table 1: Demographic and clinical characteristics of the study participants

Variable	RM group (n = 44)	Control group (n = 42)	p-value
Age 18–20 y, n (%)	4 (9.1%)	0 (0.0%)	0.271[a]
Age 21–25 y, n (%)	6 (13.6%)	4 (9.5%)	
Age 26–30 y, n (%)	17 (38.6%)	23 (54.8%)	
Age 31–35 y, n (%)	6 (13.6%)	5 (11.9%)	
Age 36–40 y, n (%)	11 (25.0%)	10 (23.8%)	
BMI (kg/m^2), mean ± SD	25.04 ± 2.90	24.9 ± 2.31	0.941[b]
Previous miscarriages, mean ± SD (range)	3.07 ± 1.09 (2–6)	0	< 0.001[b]

[a] Fisher's Exact Test; [b] Mann–Whitney U test. BMI = body mass index; RM = recurrent miscarriage.

3.2 Serum osteopontin levels: RM versus control

Serum OPN was significantly higher in the RM group than in the control group (p < 0.001, Mann–Whitney U test).

Table 2: Serum osteopontin levels in the RM and control groups

Parameter	RM group (n = 44)	Control group (n = 42)
Mean ± SD (ng/mL)	4.57 ± 3.66	1.71 ± 2.07
Median (ng/mL)	3.26	0.91
Range (ng/mL)	0.69–16.35	0.02–10.74
p-value	< 0.001	

Receiver operating characteristic (ROC) curve analysis was performed to describe the extent of separation in serum OPN between the RM and control groups. Serum OPN yielded an AUC of 0.82 (95% CI: [insert]), indicating moderate-to-good separation between these two predefined groups. At the cutoff value maximizing Youden's index (1.083 ng/mL), 97.7% of RM participants and 61.9% of controls were correctly classified. Because this threshold was both derived and evaluated in the same sample, these figures are optimistic and require validation in an independent cohort.

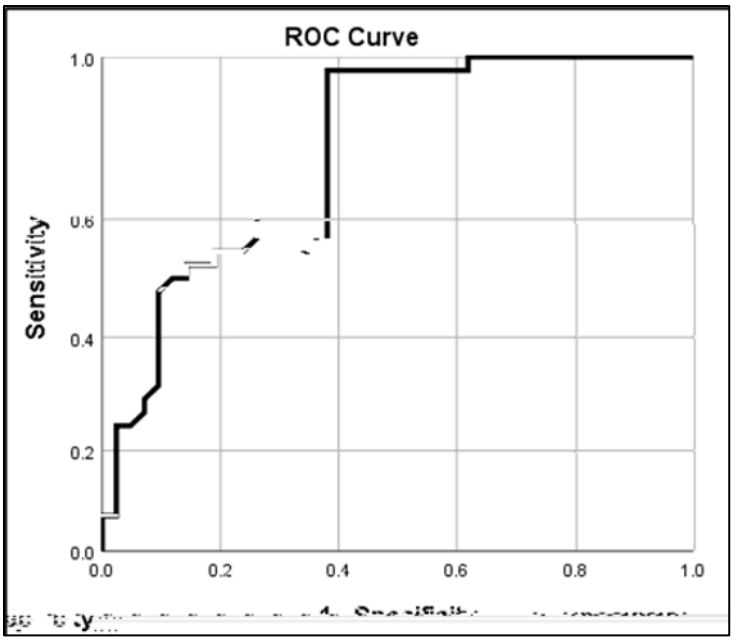

Figure 2: ROC curve analysis of serum osteopontin (OPN) for discriminating the RM group from the control group (AUC = 0.82).

4 DISCUSSION

The similarity between the RM and control groups in age ($p = 0.271$) and BMI ($p = 0.941$) indicates that the groups were broadly comparable on these two variables. Nonsignificant between-group comparisons do not, however, establish that the OPN difference is independent of age or BMI, which would require a multivariable model; this is consistent with prior reports that BMI is not consistently established as an independent predictor of recurrent pregnancy loss and is typically used to control for confounding rather than as a causal factor [11,12].

Serum osteopontin was significantly and substantially elevated in the RM group (mean 4.57 ± 3.66 ng/mL; median 3.26 ng/mL) compared with controls (mean 1.71 ± 2.07 ng/mL; median 0.91 ng/mL; $p < 0.001$), and yielded an AUC of 0.82, indicating moderate-to-good separation between the two study groups. At the exploratory cutoff of 1.083 ng/mL, 97.7% of RM participants and 61.9% of controls were correctly classified within this same sample. OPN is known to be released systemically in response to tissue stress, inflammation, and vascular injury in other conditions [7], which is consistent with the elevated serum concentrations observed here, although this study did not measure any marker of inflammation or vascular injury and therefore cannot attribute the elevation specifically to maternal–fetal interface pathology.

The pattern of high sensitivity with modest specificity at this threshold should not be interpreted as evidence that OPN functions as a screening or rule-out test. Sensitivity and specificity estimated in a case–control sample of women with an already-established diagnosis do not correspond to the performance the marker would show in an unselected clinical population, in which the prevalence of recurrent miscarriage is far lower and the predictive values would differ accordingly. These figures are reported here only to characterize the degree of separation observed between the two study groups.

This is consistent with prior clinical evidence: Bayramoğlu et al. [13] reported significantly elevated serum OPN in women with missed abortion compared with normal and threatened-abortion pregnancies, with good discriminatory performance (AUC = 0.846), supporting its potential as a non-invasive biomarker of pregnancy failure. Paravati et al. [14] found OPN significantly higher in infertile than fertile women with PCOS, proposing possible interference with embryo–endometrial interaction and implantation. Wen et al. [15], studying decidual and villus tissue rather than serum, demonstrated altered OPN expression in women with unexplained recurrent spontaneous abortion, indicating that OPN dysregulation may contribute to impaired implantation and abnormal placental development. Collectively, these findings support the involvement of OPN in the pathogenesis of recurrent miscarriage across different biological compartments.

Mechanistic evidence further underscores the essential role of decidual osteopontin in supporting normal pregnancy. Qu et al. [16] reported that first-trimester decidual OPN expression is associated with decidual natural killer (dNK) cell recruitment and increases with gestational progression, whereas in women with recurrent pregnancy loss both decidual OPN expression and dNK recruitment were reduced — a pattern that appears to contrast with the elevated

circulating OPN observed in the present study. This apparent discrepancy raises the hypothesis that local (decidual) deficiency and systemic elevation of OPN may coexist. Neither a compensatory response nor impaired tissue utilization of OPN was investigated in the present study; both remain speculative pending direct assessment of decidual and circulating OPN in the same participants.

Circulating OPN is also known to rise physiologically during pregnancy [17], so pregnancy status is an important consideration when interpreting OPN as a biomarker. Because all participants in the present study were non-pregnant at the time of sampling, the elevated OPN observed is unlikely to reflect normal pregnancy-related physiology and more plausibly represents a persistent biological alteration associated with a history of recurrent miscarriage.

Osteopontin is well established as a ligand for cell-surface integrin receptors on endothelial and trophoblast cells, thereby regulating cell adhesion, migration, and survival [7]; through these interactions it can plausibly influence angiogenesis and vascular remodeling processes required for normal placental development, although this pathway was not directly assessed in the present study. The absence of a correlation with BMI suggests that the elevation is not simply a reflection of general metabolic status, but osteopontin is a multifunctional protein influenced by inflammatory, vascular, and metabolic processes, none of which were measured here; the specificity of the observed association therefore cannot be determined from these data.

Taken together, these data establish an association between serum OPN and a history of recurrent miscarriage. Whether OPN has any diagnostic, predictive, or screening value cannot be determined from this design and would require prospective evaluation in an unselected clinical population, with independent validation of any proposed threshold.

Limitations

Several limitations should be considered when interpreting these findings. First, the study was conducted at a single center with a modest sample size (44 RM patients and 42 controls), which may limit the generalizability of the results and warrants confirmation in larger, multi-center cohorts. Second, the case–control design with a single blood draw in non-pregnant women provides only a cross-sectional snapshot of serum OPN and cannot capture how OPN levels evolve across a subsequent pregnancy or in the period immediately surrounding a pregnancy loss; longitudinal sampling would be needed to establish whether elevated OPN precedes, accompanies, or follows pregnancy loss. Third, although women with known confounding causes of pregnancy loss (chromosomal abnormalities, uterine anomalies, antiphospholipid syndrome, and others) were excluded, the study did not statistically adjust for the full range of potential RM risk factors (e.g., subclinical thyroid dysfunction, thrombophilia, or lifestyle factors), so residual confounding cannot be entirely excluded. Finally, because circulating OPN is known to rise physiologically during pregnancy, these findings apply specifically to the non-pregnant, inter-pregnancy state and should not be extrapolated to OPN behavior during an ongoing pregnancy without direct study.

5 CONCLUSION

Serum osteopontin is significantly elevated in non-pregnant women with a history of recurrent miscarriage compared with healthy fertile controls, and separates the two study groups with an AUC of 0.82. Because the groups were defined by an already-established history of pregnancy loss and the threshold of 1.083 ng/mL was both derived and evaluated in the same small sample, these findings demonstrate an association only; they do not establish diagnostic, predictive, or screening performance, and the reported sensitivity and specificity should not be applied to an unselected clinical population. Given the biological role of osteopontin in decidual and vascular remodeling at the maternal–fetal interface, serum OPN merits further investigation in larger, prospective, longitudinal studies with multivariable adjustment and independent validation of any candidate threshold.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

This study involved human subjects and was approved by the Research Committee of the Basra Health Department (approval no. 576, dated 11 September 2025).

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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