Searching for a Cheap Marker for Placenta Accreta Spectrum in a Low Resource Country: A Prospective Cohort Study

Ahmed Sherif Abdel Hamid * 1, Hazem El Zeneiny 1, Amira El Nahas 1
1-Department of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
Authors Affiliations:
Ahmed Sherif Abdel Hamid * 1: Assistant professor of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University Cairo, Egypt
Hazem El Zeneiny 1: Professor of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
Amira El Nahas 1: Lecturer of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Abstract

Objective: To assess maternal serum amyloid A (SAA) levels among women with Placenta Accreta spectrum.

Methods: We performed a prospective observational cohort study among women with Placenta Accreta Spectrum at Ain Shams University Maternity Hospital, Cairo, Egypt, between March 2017 and August 2017. The study group included women with a previous CS and diagnosed with placenta previa or accreta (20 patients in each group), while the control group included twenty women with a normally situated placenta. We collected Serum samples to measure SAA levels at the time of admission. The primary outcome was the association between SAA and the Placenta Accreta Spectrum. SAA levels were compared using the ANOVA test. We also performed Receiver Operating Characteristics characteristic analysis for previa/accrete versus normal and previa versus accreta.

Results : Each group contained 20 participants with comparable baseline characteristics. The Maternal serum amyloid-A level was 19.9±5.0 μg/mL, 18.3±5.5 μg/mL in the previa and accreta groups versus 11.4±2.1 μg/mL in the control group ($P<0.001$). The ROC analysis was significantly lowest among the normal group with no significant difference between the previa and accrete groups. Maternal serum amyloid-A had significantly high diagnostic performance in differentiating previa and/or accreta groups from the normal group and low non-significant diagnostic performance in determining previa from accreta groups.

Conclusion: Maternal SAA levels are increased in women with Placenta Accreta Spectrum.

Keywords: Serum amyloid A, Placenta Accreta Spectrum, Morbid adherent placenta, biochemical marker.

Corresponding author:
Amira Al Nahas*
Lecturer of Obstetrics & Gynecology, Faculty of Medicine, Ain Shams University Cairo, Egypt
Address: Abbaseyya Square, Cairo, Egypt.
e-mail: Egypt@2022phone/fax: +20 1117008040
ORCID number: 0009-0006-5268-9200

Introduction

Placenta accreta is defined as abnormal trophoblastic invasion of the placenta (either partly or totally) into the myometrium of the pregnant uterus. The Placenta accreta

spectrum was known as morbidly adherent placenta, which refers to the pathologic adherence of the placenta, including placenta accreta (invading the decidual surface of the myometrium), placenta increta (invading more deeply within the myometrium), and placenta percreta (penetrating the uterine serosa and invading the surrounding organs such as the bladder).[1]

Placenta accreta spectrum (PAS) disorders are life-threatening obstetrical problems with a mortality rate of approximately 7.0% and several maternal morbidities, which include obstetric hemorrhage, massive blood transfusion, urinary tract injury, and hysterectomy. The incidence of placenta accreta spectrum disorders is steadily rising in Egypt; it was recorded at 0.91% in a tertiary university hospital in Minya from 2017 to 2018. 2 In 2022 and 2023, it reached 2% in Ain Shams university hospital. This is driving our high rate of CS of 56%. [3]

Several fetal and placental hormones have been found to have different concentrations in the serum of patients with placenta previa accreta compared with those with non-accreta women. At 11–12 weeks of pregnancy, the pregnancy-associated plasma protein A (PAPP-A) is higher in the maternal serum of women with PAS disorders. At 14–22 weeks, serum β-hCG and alpha-fetoprotein (AFP) are above 2.5 multiples of the median higher in the maternal serum of women with PAS disorders. Biomarkers could be used with ultrasound imaging to screen for PAS disorders prenatally. [4]

Serum amyloid A (SAA) is an immunoregulatory protein in the acute-phase reaction.[3] Its functions include immunomodulation, cell differentiation, cell proliferation, migration, and invasion. SAA is synthesized mainly in the liver, other sources, including trophoblasts, have been described. SAA exerts immunoregulation and plays an important role in the trophoblasts' migration, invasion, and differentiation. SAA at low concentrations regulates both trophoblast invasion and metalloprotease activity within the microenvironment of the placenta, which is important for placentation and placental homeostasis. In the presence of high levels of SAA, this sequence is markedly disturbed. 5

The present study aimed to examine the hypothesis that Placenta Accreta Spectrum (PAS) might be associated with high maternal SAA.

Methods

Study Design: We performed a prospective observational cohort study. This study was done on 60 pregnant women attending the outpatient obstetric clinic or who came to the emergency ward and were all admitted to the inpatient ward as high-risk pregnancies with a diagnosis of Morbid adherent Placenta (MAP); The study group included women with a previous CS and diagnosed with placenta previa or accreta (20 patients in each group), while the control group included twenty women with a normally situated placenta. They were recruited through the period of March 2017 and August 2017. The original study is a master thesis that took the approval of the scientific committee of the Obstetrics and Gynecology Department of Ain Shams University Hospital and gained the ethical committee approval of Ain Shams University, Faculty of Medicine (Research Ethics Committee) under the number FMASU MS 26/2017.

Eligibility criteria: We studied women diagnosed with placenta previa or accreta with a history of previous CS. Women had to be more than 18 years, had a history of previous CS, and had a gestational age > 28 weeks. The placenta previa/accreta diagnosis was established by ultrasonography, Doppler, and occasionally by MRI. Women with rupture of membranes, chorioamnionitis, multiple pregnancies, or any medical disorder were excluded from the study. We excluded women with infection and medical

disorders, which could affect serum amyloid assessment 5. The control group was women with a normal placenta and no specific complaint.

The women were allocated consecutively and alienated into three groups by their clinical fate. Women with placenta previa/accreta were included in the cases group (first and second), while women with normal placenta were included in the third group.

All participants had general examinations, including blood pressure, pulse, and respiratory rate. An abdominal examination was performed to assess contractions and electronic fetal monitoring. Pelvic examinations were not done except if the patient was contracting, in which speculum examination was done.

Ultrasound and Doppler were done, and the diagnosis of placenta accreta was based on the loss of the retroplacental sonolucent zone, presence of irregular retroplacental sonolucent zone, thinning or disruption of hyperechoic serosa – bladder interface, presence of focal exophytic masses invading the urinary bladder (presence of focal exophytic mass with the same echogenicity as the placenta beyond the uterine serosa) and abnormal placental lacunae: An irregular vascular space in the placental parenchyma.

After diagnosing Morbid adherent placenta, women were admitted either for conservation or termination if they were presented at term or with significate attacks of antepartum hge. Complete laboratory investigations were performed for all participants, including CBC, coagulation profile, and liver and kidney function tests. Cross-matching of packed RBCs and plasma was done.

A sample of venous blood was taken from each patient participating in the study under aseptic conditions to assess the level of serum aa. Blood samples were centrifuged at 2500 g for 15 minutes at 4°C, separated into serum aliquots, and stored at −80°C until used for the SAA assay. Levels of SAA were assayed simultaneously for all groups using the same microtiter plates provided with the human SAA solid phases and which enzyme-linked immuno- sorbent assay kit (BioSource Europe, Nivelles, Belgium), according to the manufacturer's protocol. The inter-assay and intra-assay coefficients of variation were 7.4% and 6.1%, respectively. The sensitivity had been <0.004 μg/mL.

Participants were either admitted to the emergency room for termination or admitted to high- risk inpatient ward after being assessed by the consultant on duty, who gave the management's final decision.

Sample size justification: At the time of the study, no study used serum amyloid A in its association with the morbidly adherent placenta. So, we assigned 20 patients in each group according to the number of available kits.

Statistical Analysis: The data were analyzed using SPSS version 21.0 (IBM, Armonk, NY, USA). Numerical data were tested for normal distribution using the Shapiro–Wilk test. Normally, distributed data were presented as a mean and standard deviation; differences were assessed using the ANOVA test between groups. Categorical data were presented as numbers and percentages, and differences were compared using Fisher's exact test (for nominal data) or the chi-squared test for trend (for ordinal data). Correlations were tested using the Spearman rank correlation. ROC curve analysis was used to examine the predictive value of SAA. P values < .05 were considered statistically significant.

Results

Sixty women were included in the study, equally distributed among 3 groups (placenta previa (N=20), placenta accrete (N=20), and a normal control group (N=20). There is no statistically significant difference between the three groups regarding the demographic criteria (Age, BMI, GA, and, Hysterotomy/ CS) (Table 1).

The **Maternal serum amyloid-A level** was significantly lowest among the normal group than the previa and accreta groups (P<0.001), with no significant difference between previa and accrete groups (P = 0.351). The correlation between the maternal SAA and demographic data among the studied groups revealed no statistically significant difference (P>0.05), as shown in Table 2.

Maternal serum amyloid-A had significantly high diagnostic performance in differentiating previa and/or accreta groups from the normal group and low non-significant diagnostic performance in differentiating previa from accreta groups. Maternal serum amyloid-A level ≥15.3 (µg/mL) had high specificity& PPV and moderate sensitivity& NPV in differentiating the previa group from the control group. Maternal serum amyloid-A level ≥15.3 (µg/mL) had high specificity& PPV and low sensitivity& NPV in differentiating the accreta group from the control group. Maternal serum amyloid-A. A level ≥15.3 (µg/mL) had high specificity& PPV and low sensitivity& NPV in differentiating previa/accreta groups from the control group. (Table 3 and Fig 1a-d) .

Table 1 Clinical characteristics of studied groups

Variables	Measures	Previa (N=20)	Accreta (N=20)	Normal (N=20)	P
Age (years)	Mean±SD	27.9±3.0	28.8±2.6	28.4±2.4	**0.605 ***
	Range	23.0–33.0	25.0–33.0	22.0–32.0	
BMI (kg/m²)	Mean±SD	29.5±1.8	29.1±1.6	29.6±1.4	**0.552***
	Range	26.3–33.0	26.4–31.6	27.4–31.9	
Hystroto my/CS	Mean±SD	2.3±0.6	2.4±0.7	2.6±0.8	**0.383***
	Range	2.0–4.0	2.0–4.0	2.0–4.0	
Gestational Age (GA in weeks)	Mean±SD	32.1±2.2	32.5±2.6	32.3±2.5	**0.873***
	Range	28.0–36.0	27.0–38.0	27.0–37.0	

* ANOVA test - BMI: Body Mass Index

Table (2): Correlation between maternal serum amyloid-A levels in 3 groups

Maternal Serum Amyloid A (µg/mL)	Mean±SD	19.9±5.0	18.3±5.5	11.4±2.1	**<0.001** HS**
	Range	10.3–30.3	9.2–29.3	8.4–16.2	
	HG	**a**	**a**	**b**	

**ANOVA with post hoc test HG: Homogenous groups

Table (3): Correlation between maternal serum amyloid-A level and demographic characteristics among the studied groups

Variable	Previa (N=20)		Accreta (N=20)		Normal (N=20)	
	r	P	r	P	R	P
Age	0.137	0.565	-0.009	0.970	0.129	0.588
BMI	0.277	0.237	-0.403	0.078	0.018	0.940
Hystrotomy	0.093	0.697	-0.222	0.346	-0.075	0.753
GA	-0.280	0.233	-0.180	0.446	-0.018	0.941

Pearson correlation

Table (4): Diagnostic performance of maternal serum amyloid-A in differentiating the studied groups.

	Previa from normal	Accreta from normal	Previa/ Accreta from normal	Previa from Accreta
AUC	0.941	0.869	0.905	0.586
SE	0.038	0.059	0.038	0.091
95% CI	0.500–1.000	0.753–0.985	0.831–0.979	0.500–0.765
p-value	<0.001*	<0.001*	<0.001*	0.351
Cut off	≥15.3	≥15.3	≥15.3	≥18.3
Sensitivity	85.0% (62.1%–96.8%)	70.0% (45.7%–88.1%)	77.5% (61.5%–89.2%)	56.58% (44.90%-65.12%)
Specificity	95.0% (75.1%–99.9%)	95.0% (75.1%–99.9%)	95.0% (75.1%–99.9%)	69.35% (54.82%-72.93%)

AUC: Area under the curve, SE: Standard error, CI: Confidence interval, *Significant

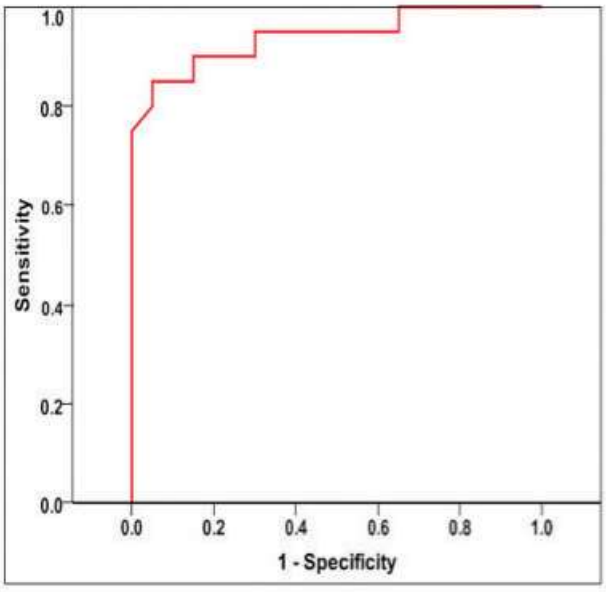

Figure (1a): ROC curve for maternal serum amyloid-A in differentiating the previa group from the normal group.

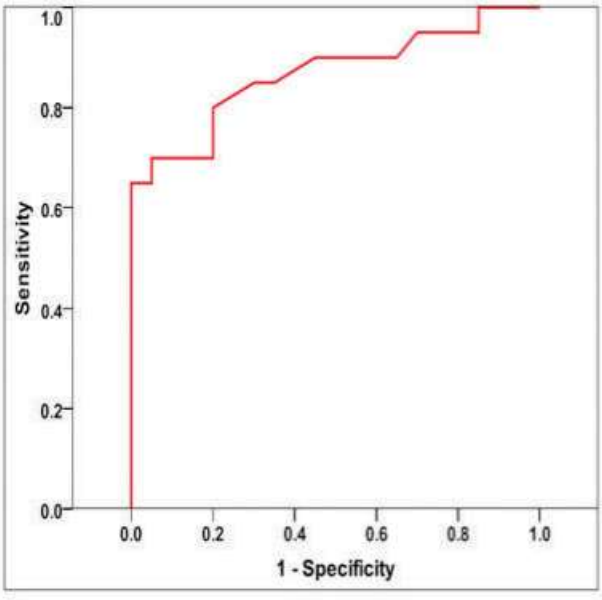

Figure (1b): ROC curve for maternal serum amyloid-A in differentiating the accreta group from the normal group

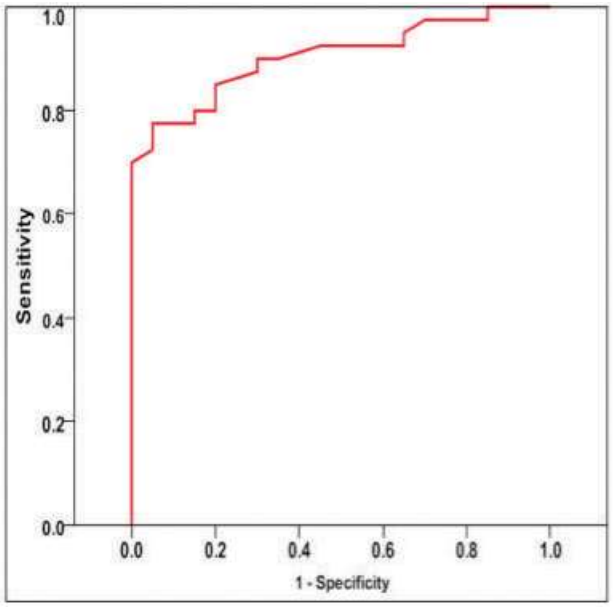

Figure (1c): ROC curve for maternal serum amyloid-A in differentiating the previa/accreta groups from the normal group

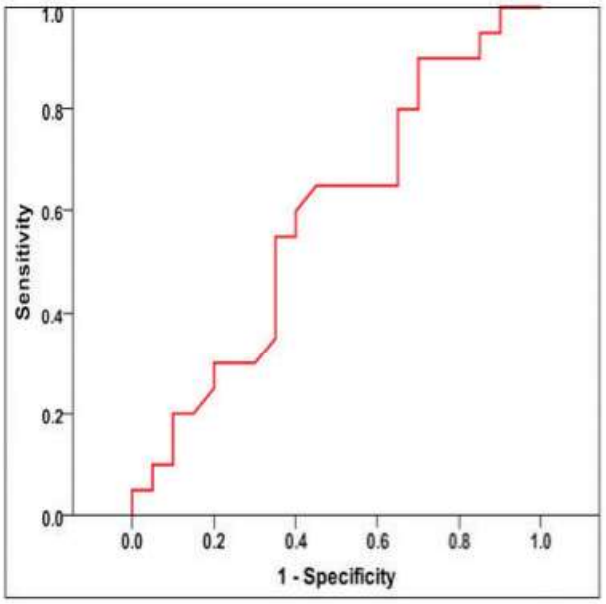

Figure (1d): ROC curve for maternal serum amyloid-A in differentiating the previa group from the accreta group

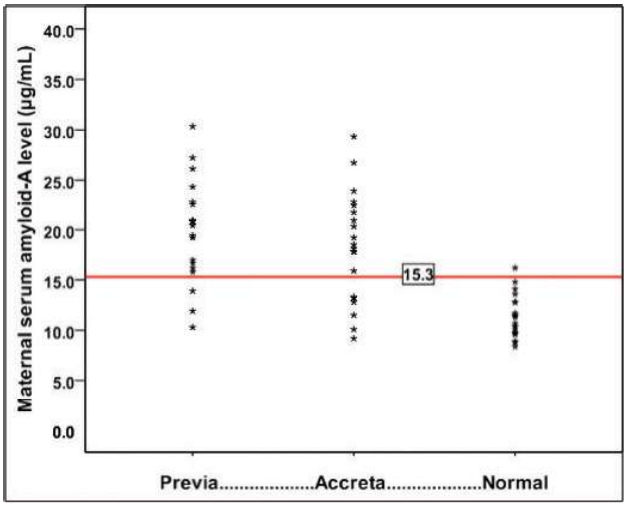

Figure (2): Diagnostic characteristics of maternal serum amyloid-A level ≥15.3 (µg/mL) in differentiating previa and/or accreta groups from the control group.

Discussion

The present study showed that maternal serum amyloid A is significantly elevated in placenta previa and placenta accreta over the normal control group. There was no correlation between levels of m SAA and the demographic criteria of selected patients. Maternal serum amyloid-A had significantly high diagnostic performance in differentiating previa and/or accreta groups from the normal group and low non-significant diagnostic performance in differentiating previa from accreta groups.

Comparison of our results to related studies

Investigators tried to analyze the association between SAA levels and the normal pregnancy and labor process, as well as abnormal pregnancy. Maternal SAA levels were measured during and after pregnancy in healthy pregnant women. SAA levels remained unchanged during normal pregnancy and were elevated only during concurrent infections. Maternal SAA concentrations were normal when measured one day before delivery. On the contrary, labor caused a marked increase in maternal

SAA levels that increased several hundred folds. [6]

SAA regulates trophoblastic invasion into the decidua at physiological levels by activating toll-like receptor 4 (TLR-4). By contrast, the increased levels of SAA are associated with increased trophoblastic invasion and syncytialization. [7]

At physiological levels, SAA regulates trophoblastic invasion into the decidua through the activation of toll-like receptor 4 (TLR-4). By contrast, the increased levels of SAA are associated with increased trophoblastic invasion and syncytialization. [7]

In the study of Sandri et al., they evaluated the role of SAA in placental invasion. SAA was expressed in the syncytiotrophoblasts, extravillous cytotrophoblasts, and decidual cells. They used the extravillous cytotrophoblasts (ECT) and a trophoblast-like human cell line to evaluate the influence of exogenous SAA on trophoblastic migration, invasion, and differentiation. They concluded that, at concentrations of 1 and 10 μg/mL, SAA doubled the invasion of ECT by stimulating the TLR-4 receptor. Also, SAA stimulated the invasion of the trophoblast-like cell line and induced both the gene expression and enzyme activity of metalloprotease (MMP-2 and MMP-9), which are proteases involved in the process of invasiveness of the EVT cells. [8]

SAA has been shown to aid in tumor cell invasion and metastasis by enhancing the Extra-cellular Matrix degradation through the induction of MMP-9 and 11 proteases activity [(9-11)].

SAA levels were also increased in some cases of gynecological cancers. In the study of Kovacevi et al. and Rossmann et al., they showed that first-trimester trophoblasts and malignant trophoblast-like choriocarcinoma cells (JAR and Jeg-3 cells) express SAA transcripts. [12-13]

Cocco et al.'s study reported that SAA gene and protein expression levels were highly expressed in Uterine serous papillary carcinoma. Interestingly, they have shown that high serum SAA levels predict different stages of the disease and could help in staging patients preoperatively. [14-15]

In contrast to the results of our study are the studies concerning increased levels of SAA in Preeclampsia, eclampsia, and Recurrent early pregnancy loss. It has been shown that there was a shallow invasion of the EVT into the endometrium in cases of Preeclampsia and that maternal SAA levels were significantly increased in preeclamptic patients over the controls. Maternal SAA levels were also increased in eclampsia and HELLP syndrome. This was explained by the insufficient blood flow to the placenta due to the shallow invasion of extravillous trophoblasts (EVT) into the endometrium.[15-16]

In the study of Ibrahim et al., serum amyloid levels were significantly higher in patients with recurrent early pregnancy loss than among their controls. They explained that SAA (At physiological levels) modulates the trophoblastic invasion into the decidua via activation of toll-like receptor 4, and maintains a functional balance between the pro-inflammatory and anti-inflammatory cytokines. Their study's increased SAA levels were associated with impaired trophoblastic invasion and syncytialization.

As SAA levels were elevated in different situations of both shallow and excessive invasiveness of Extra Villous cytotrophoblasts, it is assumed that the alteration of SAA levels in maternal blood in pre-eclampsia and Placenta accreta spectrum are the non-specific reflections of the inflammatory and injury states of the diseases. [17]

Clinical implication of our study

This study, among other studies, utilizes different biomarkers that could be used with ultrasound imaging to screen for PAS

disorders prenatally which aids in early referral to higher centers.

Strengths and limitations of the present study

The strength of our present study is that, to our knowledge, it was the first to address the association of the placenta accreta spectrum with the SAA levels. The limitations of the present study included the absence of sample-size justification due to the need for published data (at the time of the study) on SAA levels among women with the placenta accreta spectrum. Indeed, the present study suggested a possible association between SAA level and Placenta accreta spectrum, whether the exact mechanism is to be determined.

Recommendations for further studies

We recommend studying the levels of SAA in patients with the low-lying placenta in the early second trimester and following up to assess the outcome of the occurrence of the placenta accreta spectrum.

Conclusion

Maternal SAA levels are increased in Placenta Accreta Spectrum, and the exact pathophysiology is yet to be determined.

LIST OF ABBREVIATIONS

PAS ……..Placenta Accreta Spectrum

MAP……..Morbid Adherent Placenta

EVT……..Extra-villous cyto-trophoblast

Ethical approval and consent to participate

In accordance with local regulations, the protocol gained Ethical and Research approval from the council of the OB/ GYN Department, Ain-Shams University. Furthermore, the study protocol was approved by the Ethics Research Committee, Faculty of Medicine, Ain-Shams University (number: FMASU MS 26 / 2017). Written informed consent was obtained from every candidate after explaining the procedure before enrollment. WE Confirm that All methods were performed in accordance with the relevant guidelines and regulations according to the Declaration of Helsinki.

Consent for publication

NOT APPLICABLE

Availability of data and materials

All Data and ethical committee documents are available from the corresponding author on reasonable request

Competing interests

The authors report there are no competing interests to declare

Funding

This study received no financial support.

Authors' contributions

Ahmed Sherif Abdel Hamid 1, Hazem El Zeneiny 1, Amira El Nahas 1

All authors jointly contributed to the conception and design of the study.

Ahmed Sherif Abdel Hamid: Design of the study, helped in the review of literature, revision of results and data analysis, writing the manuscript,

Hazem El Zeneiny; design of the study, revision of review of literature, and revision of the manuscript

Amira El Nahas: Candidate of the master thesis, design of the study, obtaining ethical committee approval, reviewing the literature, sharing in the collection of Data, revision of results and data analysis, and contributing to writing the manuscript

Acknowledgments

Not applicable.

References

1. American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine. Obstetric Care Consensus No. 7: Placenta Accreta Spectrum. Obstet Gynecol. 2018;132(6):e259-e275.
2. El Gelany S, Mosbeh MH, Ibrahim EM, Mohammed M, Khalifa EM, Abdelhakium AK. Placenta Accreta Spectrum (PAS) disorders: incidence, risk factors and outcomes of different management strategies in a tertiary referral hospital in Minia, Egypt: a prospective study. BMC Pregnancy Childbirth 2019; 19(1): 313.
3. Abdel Hamid AS, El Zeneiny H, Fathy A, Nawara M. A pilot study to compare propranolol and misoprostol versus misoprostol and placebo for induction of labor in primigravidae; a randomized, single-blinded, placebo-controlled trial. BMC Pregnancy Childbirth. 2023 4;23(1):226.
4. Jauniaux E, Bhide A, Kennedy A, Woodward P, Hubinont C, Collins S. FIGO Placenta Accreta Diagnosis and Management Expert Consensus Panel. FIGO consensus guidelines on placenta accreta spectrum disorders: Prenatal diagnosis and screening. Int J Gynaecol Obstet. 2018;140(3):274-280.
5. Ibrahim MI, Ramy AR, Abdelhamid AS, Ellaithy MI, Omar A, Harara RM, Fet al. Maternal serum amyloid A level as a novel marker of primary unexplained recurrent early pregnancy loss. Int J Gynaecol Obstet. 2017;136(3):298-303.
6. Ibrahim MI, Ellaithy MI, Hussein AM, Nematallah MM, Allam HA, Abdelhamid AS et al. Measurement of maternal serum amyloid A as a novel marker of preterm birth. J Matern Fetal Neonatal Med. 2021;34(15):2467-2472.
7. Knebel FH, Albuquerque RC, Massaro RR, Maria-Engler SS, CampaA. Dual effect of serum amyloid A on the invasiveness of glioma cells. Mediators Inflamm. 2013;(1); 503-505
8. Sandri S, Urban BA, Fernandes I, de Oliveira EM, Knebel FH, Ruano R, et al. Serum amyloid A in the placenta and its role in trophoblast invasion. PLoS One 2014 ;, 9(3): e90881.
9. Hansen MT, Forst B, Cremers N, Quagliata L, Ambartsumian N, Grum-Schwensen B, et al. A link between inflammation and metastasis: serum amyloid A1 and A3 induce metastasis, and are targets of metastasis-inducing S100A4. Oncogene 2015; 34(4):424–35.
10. Lee HY, Kim MK, Park KS, Bae YH, Yun J, Park JI, et al.. Serum amyloid a stimulates matrix-metalloproteinase-9 upregulation via formyl peptide receptor like-1-mediated signaling in human monocytic cells. Biochem Biophys Res Commun 2005; 330(3):989–98.
11. Paret C, Schon Z, Szponar A, Kovacs G. Inflammatory protein serum amyloid A1 marks a subset of conventional renal cell carcinomas with fatal outcome. Eur Urol 2010; 57(5):859–66.
12. Kovacevic A, Hammer A, Sundl M, Pfister B, Hrzenjak A, Expression of serum amyloid A transcripts in human trophoblast and fetal-derived trophoblast-like choriocarcinoma cells. FEBS Lett 2006; 580: 161–167.
13. Rossmann C, Hammer A, Koyani CN, Kovacevic A, Siwetz M, Desoye G, et al. Expression of serum amyloid A4 in human trophoblast-like choriocarcinoma cell lines and human first trimester/term trophoblast cells. Placenta 2014; 35(8):661–4.
14. Cocco E, Bellone S, El-Sahwi K, Cargnelutti M, Casagrande F, Buza N, et al. Serum amyloid A (SAA): a novel biomarker for uterine serous papillary cancer. Br J Cancer. 2009 ; 21;101(2):335-41.
15. Engin-Ustun Y, Ustun Y, Karabulut AB, Ozkaplan E, Meydanli MM, Kafkasli A. Serum amyloid a levels are increased in pre-eclampsia. Gynecol Obstet Invest 2007; 64(2), 117–120.

16. Heitner JC, Koy C, Kreutzer M, Gerber B, Reimer T, Glocker MO. Differentiation of HELLP patients from healthy pregnant women by proteome analysis–on the way towards a clinical marker set. J Chromatogr B Analyt Technol BioMed Life Sci 2006; 840(1):10–9.
17. Lin YK, Zhu P, Wang WS, Sun K. Serum amyloid A, a host-derived DAMP in pregnancy? Front Immunol. 2022 ;5 (13):978929.