ORIGINAL ARTICLE

The role of serum and placental vascular cell adhesion molecule-1 levels in placenta accreta

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ABSTRACT

Keywords:
Vascular cell adhesion molecule-1 (VCAM-1), Serum, Invasion, Pregnancy, Placenta accreta.

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Background: Vascular cell adhesion molecule-1 (VCAM-1) is one of the cell adhesion molecules which is expressed in endothelial cells. In pregnancy, VCAM-1 is involved in placentation by promotion of angiogenesis and trophoblastic invasion. Placenta accreta (PA) is a term that refers to abnormal adherence of the placenta to the uterine myometrium. The incidence of PA rises as the number of elective cesarean sections and pregnancies with placenta previa increases. **Objectives:** This study discusses the role of VCAM-1 in normal pregnancy, the pathogenesis of PA, and its predictive value for PA occurrence. **Methods:** Our longitudinal study included 62 pregnant women. Then they were divided into *Group N:* 31 pregnant women with normal placenta and *Group P:* 31 pregnant women with placenta accreta. **Result:** Serum VCAM-1 levels were higher in case of PA than those of normal pregnancy and it had a significant predictive value for PA with markedly high sensitivity and specificity. **Conclusion:** Detection of a high serum level of VCAM-1 in the 2nd trimester can predict the occurrence of PA in healthy women. Moreover, placental VCAM-1 may be implicated in the pathogenesis of PA through enhancing trophoblastic invasion.

INTRODUCTION

Vascular cell adhesion molecule-1 (VCAM-1) is one of the cell adhesion molecules, which is predominantly expressed in endothelial cells, tissue macrophages, and placental trophoblastic cells[1]. It is implicated in cell migration and adhesion thus its role is apparent in the pathogenesis of inflammation, and tumor metastasis[2]. The prime inducing factors for VCAM-1 production are the pro-inflammatory cytokines; tumor necrosing factor-α (TNF-α) and interleukin-1 (IL-1)[3]. In pregnancy, VCAM-1 is involved in placentation by promotion of angiogenesis[4], and trophoblastic invasion [5]. Placenta accreta (PA) is an abnormal adherence of the placenta to the myometrium [6]. Decidual maldevelopment and excessive trophoblastic invasion are considered the main pathogenic mechanisms for PA[7]. It is considered a serious pregnancy-related complication that leads to catastrophic life-threatening intrapartum and postpartum hemorrhage[8]. The ultrasonography with doppler examination are the gold standard tools for prediction and diagnosis of PA in the 3rd trimester[9].

Our study aimed to highlight the potential predictive value of serum VCAM-1 levels in the 2nd trimester as a biochemical marker for the occurrence of PA.

SUBJECTS AND METHODS

From the 1st of October 2020 to the 1st of October 2021, a double-center longitudinal study was conducted by recruitment of 62 pregnant women with normal placenta and placenta accreta (PA) from Assiut Woman's Health Hospital and Aswan University Hospital, Egypt. Before recruitment, ethical approval was obtained from the ethics committee board of the Faculty of Medicine, Aswan University, **approval NO: saw/477/9/20,** and Informed consent from all eligible participants was obtained after explaining the nature of the study. The sample size was assigned according to the study's primary outcome and its power (80%) using G-power software 3.1.9.7.

Eligible participants were enrolled according to the following inclusion criteria; gestational age between 24-36 weeks, Age between 20-40 years, Singleton pregnancy, high probability of PA by two-dimensional (2D) grayscale imaging, and color Doppler flow mapping [8]. Meanwhile, women known to have metabolic syndrome, hypertension, bleeding disorders, cardiac, renal, endocrinal, autoimmune disease, and patients on anticoagulant therapy were excluded from the study. Then, the study participants were divided into two groups (n=31); ***Group N:*** pregnant women with normal placenta and ***Group P:*** pregnant women with placenta accreta. Both groups were further subdivided according to the time of blood sampling into $\mathbf{N_{T2}}$**:** normal pregnancy during 2nd trimester (24-28 W), $\mathbf{N_{T3}}$**:** normal pregnancy during 3rd trimester (32-36 W), $\mathbf{P_{T2}}$**:** PA during 2nd trimester (24-28 W), and $\mathbf{P_{T3}}$**:** PA during 3rd trimester (32-36 W).

Venous blood samples were collected from all participants in the 2nd and 3rd trimester, then centrifuged and the clear non-hemolyzed supernatant sera were analyzed for VCAM-1 level using corresponding ELISA kits purchased from Shanghai Korain Biotech Co., Ltd **catalog No**: E0203Hu according to the manufacturer's protocol.

At the time of delivery, placental tissue samples were collected in the form of two rectangular sections measuring (1.5 – 3.5 cm) in size from the decidual surface of the placenta of each participant. Half of the collected sections were fixed in 10% formalin for 24 h, then they were processed for histopathological examination[10], and the other half of placental sections were suspended in cold 1x Phosphate buffer saline (PBS), PH 7.4 and homogenized using the homogenizer, general laboratory homogenizer (GLH 650) Roto stator, then the homogenate was centrifuged, and the supernatant was analyzed for VCAM-1 levels using corresponding ELISA kits according to manufacturer's protocol. The tissue supernatant results were recalculated and expressed per milligram (mg) protein of the tissue homogenate in each sample.

All statistical analyses were carried out with SPSS software version 20 (SPSS Inc., Chicago, IL, USA). Based upon the results of normality test, Analysis was performed between groups using Kruskal Wallis H followed by the Mann-Whitney U test. Linear regression analysis was carried out. Possible predictive value of VCAM-1 was assessed by ROC test. A value of $P \leq 0.05$ was considered statistically significant. Results were expressed as means ± standard error of the mean (SEM).

RESULTS

Serum VCAM-1 levels exhibited significant higher levels in both; group P_{T2} and group P_{T3} in comparison to serum VCAM-1 levels of group N_{T2} and group N_{T3}; respectively. Moreover, serum VCAM-1 levels of 3rd trimester groups; N_{T3} and P_{T3} had significant lower values in comparison to 2nd trimester groups; N_{T2} and P_{T2} as shown in **table (1)** and **figure (1)**. Additively, in comparison to group N, placental tissue VCAM-1 level of group P showed significant higher values as shown in **table (2)**.

Interestingly, in pregnant women with normal placenta versus pregnant women with placenta accreta, serum VCAM-1 level of the 2nd trimester groups was a significant predictor (P-value < 0.001) for the occurrence of placenta accreta, at cutoff value ≥ 79.89 ng/ml, with a sensitivity of

93.5% and a specificity of 96.8%, negative predictive value of 93.7, and positive predictor value of 96.7 as shown in **table (3)** and **figure (2)**.

By hematoxylin and eosin, microscopic examination of placental sections of group N showed normally structured decidua separating basal plate of chorionic villi from the uterine muscle **(figure 3 A&B)**. Placental sections of group P **(figure 3 C&D)** showed chorionic villi lie on fibrin layer separating it from uterine muscle without intervening decidua.

Simple Linear Regression analysis was carried out to predict the level of placental expression of VCAM-1 based upon the circulating VCAM-1 level and it showed a significant regression equation ($F= (1,60) = 149.016$, $P <0.000$) with an R^2 of 0.713. Placental expression of VCAM-1 increased by 0.389 ng/mg for each 0.032 ng/ml increase of serum VCAM-1 level.

DISCUSSION

Placenta accreta (PA) is abnormal adherence of the placenta to the myometrium and is associated with life-threatening blood loss. Enhanced angiogenesis and massive trophoblastic invasion are the underlying pathological mechanisms for PA. Our study hypothesized that serum VCAM-1 may act as a predictor for the occurrence of PA and may be involved in the pathogenesis of PA through increasing trophoblastic invasion and angiogenesis. Group P exhibited substantially higher serum and placental VCAM-1 levels than group N. our result conicoid with the results of **Korkmazer et al**[5] who showed that the placental expression of VCAM-1 was significantly high in case of PA compared to normal pregnancy[5].

Pregnancy-associated expression of circulating VCAM-1 is attributed to direct production from placental trophoblastic cells[11] and endothelial production under the influence of the increased pro-inflammatory cytokines such as Tumor necrosis factor-α (TNF-α) and Interleukin-1β (IL-1β) [12,13]. The rise of VCAM-1 in the PA group could be clarified by the amplified inflammatory condition associated with PA, in the form of increased activation of macrophages that in turn increase the production of both local and circulating VCAM-1 [14,5]. In pregnancy, VCAM-1 plays a vital role in maternal immune tolerance that hinders fetal immunological rejection[15]. Moreover, VCAM-1 plays a crucial role in placentation through the promotion of trophoblastic invasion by its pro-angiogenic and cellular adhesion effects. Furthermore, VCAM-1/α4 integrin pathway plays an important role in inflammatory stimuli-induced angiogenesis, which is responsible for placental blood flow during implantation[16].

Distinctly, serum VCAM-1 level during the 2nd trimester had significant higher levels than those of the 3rd trimester. Our results are supported by the results of **Raynor and Parthasarathy**[11] and **Daniel et al**[17] who showed that serum levels of VCAM-1 were inversely proportional to gestational age[11,17]. Although, **Austgulen et al**[18] suggested that serum VCAM-1 increases with gestational age in preeclamptic patients, due to the associated vascular endothelium dysfunction[18]. Our results of decreasing serum VCAM-1 level along the progress of pregnancy is explained by down-regulation of its production from placental trophoblast at term. This decrement of VCAM-1 production helps trophoblastic separation to achieve delivery[19,20].

Remarkably, this study investigated the predictive value of 2nd trimester serum VCAM-1 levels, and the results showed that a high VCAM-1 level ≥ 79.89 ng/ml can predict the occurrence of PA with a sensitivity of 93.5% and a specificity of 96.8%.

CONCLUSION

Conclusively, the results of VCAM-1 analysis suggested that serum VCAM-1 levels were higher in case of PA than those of normal pregnancy and it had a significant predictive value for PA with markedly high sensitivity and specificity. Therefore, we suggest that detection of its high level in the 2nd trimester can predict the occurrence of PA in healthy women. Moreover, placental VCAM-1 may be implicated in the pathogenesis of PA by enhancing trophoblastic invasion through the promotion of angiogenesis.

REFERENCE

1. Harjunpää H, Asens ML, Guenther C, Fagerholm SC. Cell adhesion molecules and their roles and regulation in the immune and tumor microenvironment. Front Immunol. 2019;10(MAY).
2. Preedy VR. Adhesion molecules. Adhes Mol. 2016;5(1):1–524.
3. Qin XW, Ni XT, Mao XY, Ying H, Du QL. Cholestatic pregnancy is associated with reduced VCAM1 expression in vascular endothelial cell of placenta. Reprod Toxicol [Internet]. 2017;74:23–31. Available from: http://dx.doi.org/10.1016/j.reprotox.2017.08.002
4. Kong DH, Kim YK, Kim MR, Jang JH, Lee S. Emerging roles of vascular cell adhesion molecule-1 (VCAM-1) in immunological disorders and cancer. Int J Mol Sci. 2018;19(4):13–7.
5. Korkmazer E, Nizam R, Arslan E, Akkurt Ö. Relationship between intercellular adhesion molecule-1 and morbidly adherent placenta. J Perinat Med [Internet]. 2018;47(1):45–9. Available from: https://www.degruyter.com/view/journals/jpme/47/1/article-p45.xml
6. Jauniaux E, Grønbeck L, Bunce C, Langhoff-Roos J, Collins SL. Epidemiology of placenta previa accreta: A systematic review and meta-analysis. BMJ Open. 2019;9(11):1–9.
7. Morlando M, Collins S. Placenta accreta spectrum disorders: Challenges, risks, and management strategies. Int J Womens Health. 2020;12:1033–45.
8. Boroomand Fard M, Kasraeian M, Vafaei H, Jahromi MA, Arasteh P, Shahraki HR, et al. Introducing an efficient model for the prediction of placenta accreta spectrum using the MCP regression approach based on sonography indexes: How efficient is sonography in diagnosing accreta? BMC Pregnancy Childbirth. 2020;20(1):1–10.
9. Del Negro V, Aleksa N, Galli C, Ciminello E, Derme M, Vena F, et al. Ultrasonographic diagnosis of placenta accreta spectrum (PAS) disorder: Ideation of an ultrasonographic score and correlation with surgical and neonatal outcomes. Diagnostics. 2020;11(1):1–10.
10. Fischer A, Jacobson K, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc. 2008;2008:pdb.prot4986.
11. Raynor BD, Parthasarathy S. Maternal serum vascular cell adhesion molecule concentration during pregnancy. J Soc Gynecol Investig. 1997;4(2):78–80.
12. Farzadnia M, Ayatollahi H, Hasan-zade M, Rahimi HR. A comparative study of serum level of vascular cell adhesion molecule-1 (sVCAM-1), intercellular adhesion molecule-1(ICAM-1) and high sensitive C - Reactive protein (hs-CRP) in normal and pre-eclamptic pregnancies. Iran J Basic Med Sci. 2013;16(5):689–93.
13. Derosa G, Maffioli P. Vascular Cell Adhesion Molecule-1 (VCAM-1) Expression in Liver Disease. In: Patel VB, Preedy VR, editors. Biomarkers in Liver Disease [Internet].

Dordrecht: Springer Netherlands; 2017. p. 707–17. Available from: https://doi.org/10.1007/978-94-007-7675-3_24

14. Hecht JL, Karumanchi SA, Shainker SA. Immune cell infiltrate at the utero-placental interface is altered in placenta accreta spectrum disorders. Arch Gynecol Obstet [Internet]. 2020;301(2):499–507. Available from: https://doi.org/10.1007/s00404-020-05453-1

15. Zhang HG, Guo W, Gu HF, Chen SB, Wang JQ, Qiao ZX, et al. Correlation of VCAM-1 expression in serum, cord blood, and placental tissue with gestational hypertension associated with fetal growth restriction in women from Xingtai Hebei, China. Genet Mol Res. 2016;15(3).

16. Du W, Li X, Chi Y, Ma F, Li Z, Yang S, et al. VCAM-1+ placenta chorionic villi-derived mesenchymal stem cells display potent pro-angiogenic activity. Stem Cell Res Ther [Internet]. 2016;7(1):1–13. Available from: http://dx.doi.org/10.1186/s13287-016-0297-0

17. DANIEL Y, GAMZU R, LESSING JB, BAR-AM A, GEVA E, AMIT A, et al. Vascular Cell Adhesion Molecule-1 in Normal, Failed, and Ectopic Pregnancy. Am J Reprod Immunol. 2000;43(2):92–7.

18. Austgulen R, Lien E, Tm GV, Redman CWG. Increased maternal plasma levels of soluble adhesion molecules. Eur J Obstet Gynecol Reprod Biol. 1997;71:53–8.

19. Rajashekhar G, Loganath A, Roy AC, Wong YC. Expression and secretion of the vascular cell adhesion molecule-1 in human placenta and its decrease in fetal growth restriction. J Soc Gynecol Investig. 2003 Sep;10(6):352–60.

20. Liu B, Liu L, Cui S, Qi Y, Wang T. Expression and significance of microRNA-126 and VCAM-1 in placental tissues of women with early-onset preeclampsia. J Obstet Gynaecol Res. 2021;47(6):2042–50.

Table (1): Mean values of serum levels of VCAM-1 of all study groups (n = 31):

Data were expressed as means ± standard error. N_{T2}: Pregnant women with normal placenta in 2nd trimester, N_{T3}: pregnant women with normal placenta in 3rd trimester, P_{T2} pregnant women with PA in 2nd trimester, P_{T3} pregnant women with PA in 3rd trimester.
*: statistically significant difference (P-value ≤ 0.05).

Outcomes	Group N			Group P			Inter-groups comparison P-Value	
	2nd trimester N_{T2}	3rd trimester N_{T3}	P-Value	2nd trimester P_{T2}	3rd trimester P_{T3}	P-Value	N_{T2} & P_{T2}	N_{T3} & P_{T3}
VCAM-1	31.211 ± 3.814 ng/ml	10.983 ± 0.147 ng/ml	0.000*	105.617 ± 5.407 ng/ml	68.159 ± 3.068 ng/ml	0.000*	0.000*	0.000*

Table (2): Mean values of placental tissue VCAM-1 levels of both normal pregnancy group and placenta accreta (PA) group (n = 31):

Outcomes	Group N	Group P	P- value
VCAM-1	2.901 ± 0.096 ng/mg protein	41.911 ± 1.885 ng/mg protein	0.000*

Data were expressed as means ± standard error.
*: statistically significant difference (P-value ≤ 0.05).

Table (3): Receiver operator characteristic (ROC) curve showed the area under the curve (AUC) and the predictive value of 2nd trimester serum VCAM-1 levels:

Variable	Sensitivity	Specificity	AUC, 95%CI	P-value	+PV	-PV	Cutoff point
Normal placenta versus placenta accreta	93.5%	96.8%	0.925	< 0.001	96.7	93.7	79.89 ng/ml

AUC: area under the curve, CI: confidence interval, +PV: positive predictive value, -PV: negative predictive value.
P-value ≤ 0.05 is considered statistically significant.

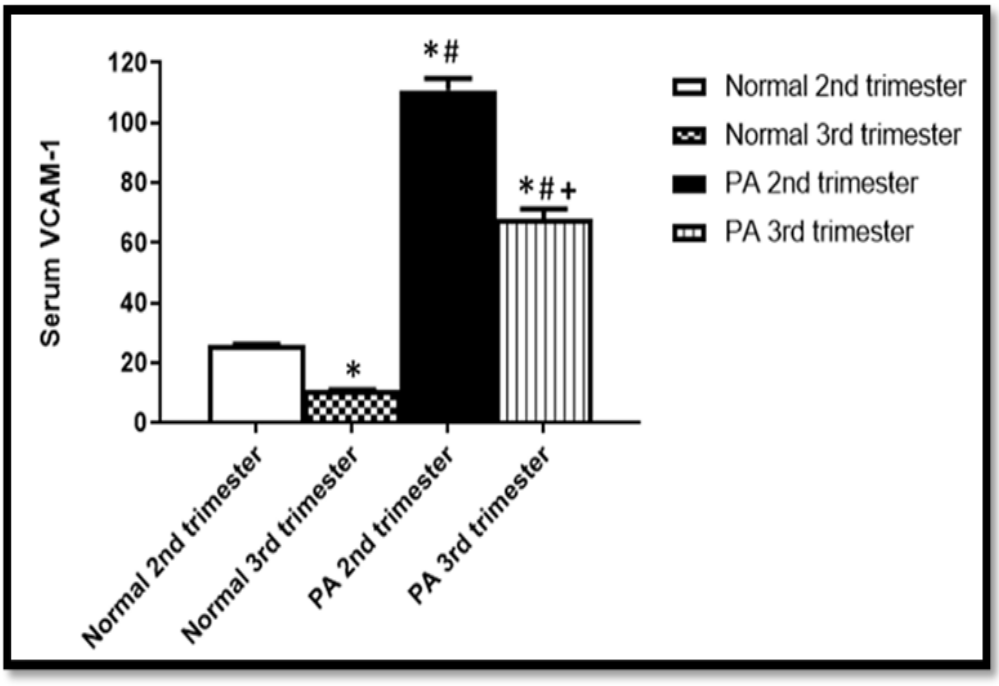

Figure (1): Serum levels of VCAM-1 of both normal pregnancy groups (2nd trimester and 3rd trimester) and PA groups (2nd trimester and 3rd trimester).

P-value < 0.05 is considered statistically significant.
*: statistically significant difference compared to normal 2nd trimester group.
#: statistically significant difference compared to normal 3rd trimester group.
+: statistically significant difference compared to PA 2nd trimester group.

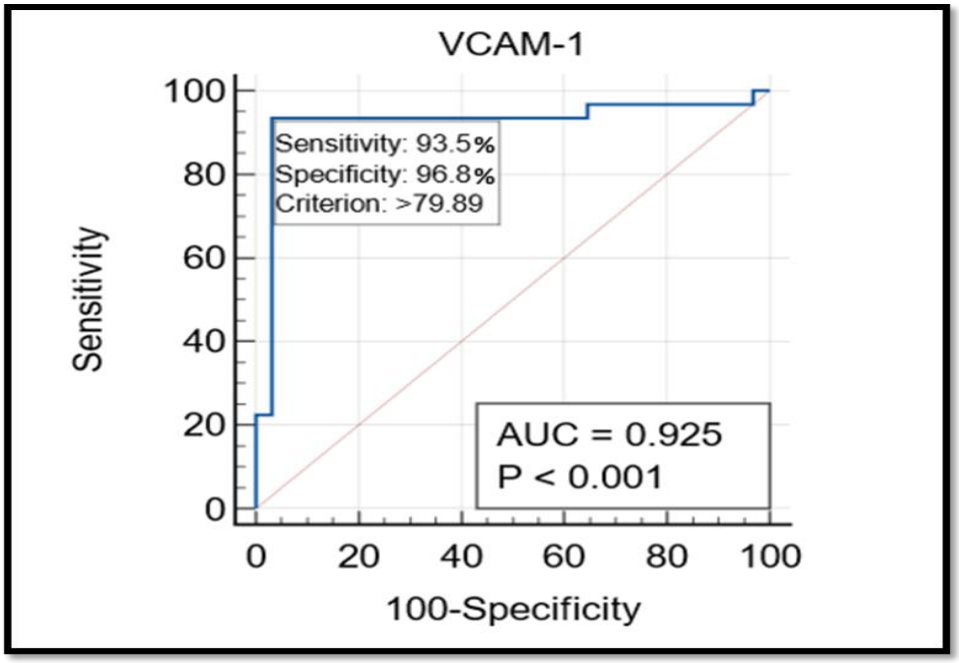

Figure (2): A receiver operator characteristic (ROC) curve for 2nd trimester serum VCAM-1 levels.

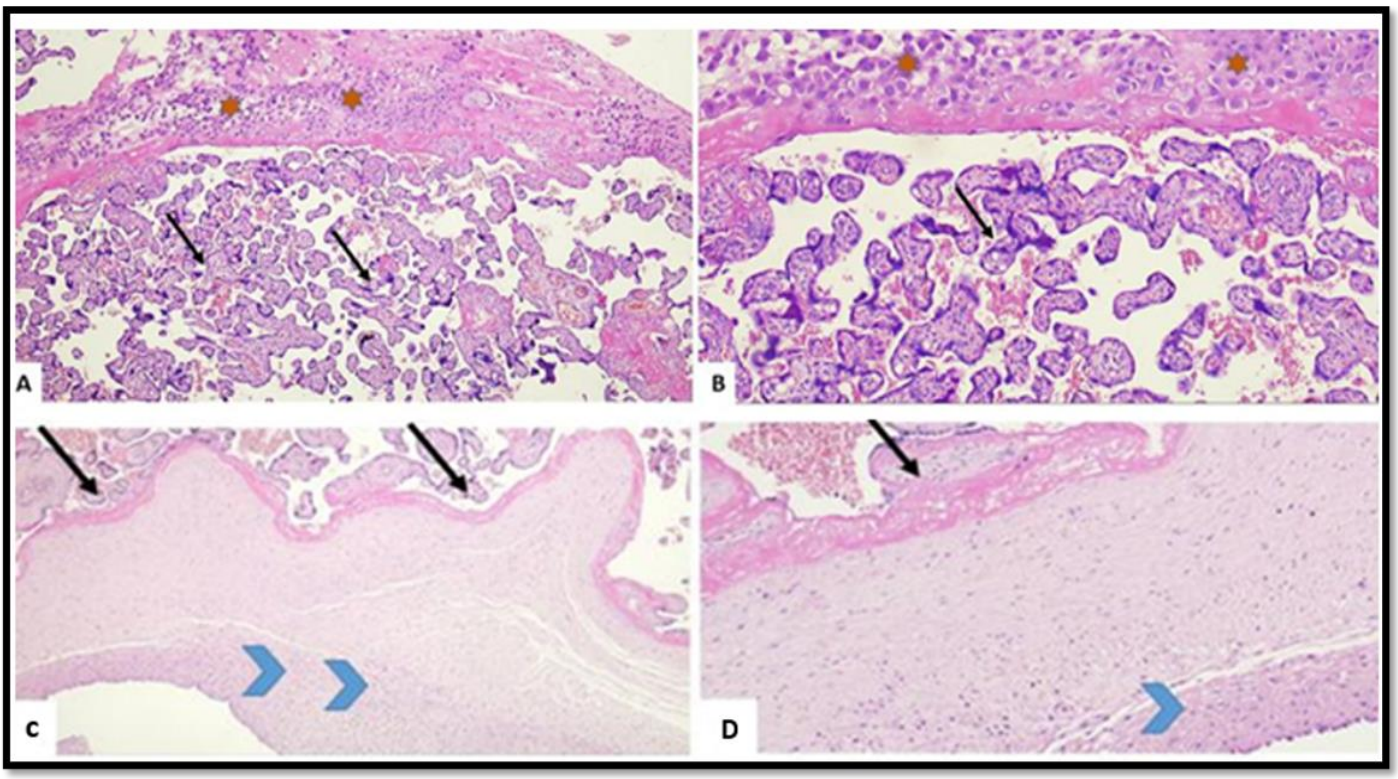

Figure (3): (A&B): **Normal placenta:** Hematoxylin and eosin-stained sections showing the decidua (brown asterisk) is attached to the basal plate of chorionic villi (black arrows) (40x, 100x respectively). **(C&D): Placenta accreta:** Hematoxylin and eosin-stained sections showing chorionic villi (black arrow) lie on the fibrin layer, that separates it from the muscle layer (blue arrowhead) without intervening decidua (40x, 100x respectively).