ISSN Print: 2706-5456
ISSN Online: 2706-5464
IJRGO 2022; 4(1): 01-05
www.gynecologyjournal.in
Received: 28-12-2021
Accepted: 06-02-2022

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Comparative study of Cripto-1 in placenta accreta and normal placenta

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DOI: https://doi.org/10.33545/27065456.2022.v4.i1a.14

Abstract
Background: Placenta accreta (PA) is a life-threatening condition that endangers both the mother and infant. PA can result in postpartum haemorrhage, coagulation dysfunction, and puerperal infection. Cripto-1 is a glycosylphosphatidylinositol-anchored small molecular signalling protein and a member of epidermal growth factors EGF-CFC family. The purpose of this research was to study Cripto-1expression in placenta accreta and normal placenta, compare intensity of staining in both placenta accrete and normal placenta and throw light on possible role of cripto-1in invasion of the trophoblast through the myometrium.
Methods: This study included 50 pregnant females with a singleton pregnancy. By ultrasound patients were classified into 2 equal groups: PA group diagnosed by (Loss of normal hypoechoic retroplacental zone, Retroplacental myometrial thickness of 1 mm, Blood vessels or placental tissue bridging uterine-placental margin, myometrial-bladder interface, or crossing uterine serosa, Multiple vascular lacunae within placenta, giving “Swisscheese” appearance) and normal Placenta (NP) group. All patients were subjected to doppler U/S, specimen collection, immunohistochemistry and Western blot.
Results: There was a significant association between accreta and control groups as regard Cripto-1 levels (P= 0.001). There was a significant relationship between degree of invasion and cripto-1 levels in accreta group (P-value = 0.001). The incidence of complications was insignificantly different in comparison to Cripto-1 levels in accreta group.
Conclusions: The increase in cripto-1 is related to the increase in the degree of invasion of the placenta, yet our study recorded that the severity of complications was insignificantly different among the different Cripto-1 levels in accreta groups. Therefore, we can correlate the risk of complications to the degree of invasion.

Keywords: Cripto-1, placenta accreta, normal placenta, trophoblast

Introduction
Placenta accrete (PA) is a clinicopathological syndrome in which the placenta unable to totally or partially detach from the uterine wall. It was first documented over 80 years ago. Many theories have been advanced to explain why and how this happens [1].
PA is a severe complication which endangers both mother and child's lives. PA can result in postpartum bleeding, coagulation malfunction, and infection. When PA is severe, the uterus must be removed. Prediction, diagnosis, and intervention could be done in early stages of PA by analysing the pathological features and pathogenesis of placenta implantation, thereby decreasing the frequency of complications related to placenta implantation and significant reduction of maternal morbidity, mortality, and perinatal adverse consequences [2].
Pathologists classified PA into three categories: PA as the chorionic villi easily attach to the myometrium, placenta increta as the villi invade the myometrium, and placenta percreta as the villi penetrate the all thickness of the myometrium [3].
Cripto-1 is a glycosylphosphatidylinositol-anchored tiny molecular signalling protein and member of the EGF-CFC family of epidermal growth factors. Cripto-1 plays a crucial function in germ layer differentiation and organ development during early embryonic development. It contributes to the activation of various signalling pathways that regulate the proliferation, differentiation, and migration of tumour cells. Cripto-1 could also regulate the migration and invasion of placental trophoblast cells and tumour cells, which have comparable biological properties.

Therefore, the impact of placental trophoblast cells on cell morphology and their capability to induce placenta implantation may play a significant role in the pathophysiology of placenta increta. A higher level of Cripto-1 expression can result in an increase in cell proliferation, migration, and invasion, aberrant placenta angiogenesis, an increase in the invasive potential of placental trophoblast cells, and placental invasion [4].

The purpose of this research was to study Cripto-1expression in placenta accreta and normal placenta, compare intensity of staining in both placenta accrete and normal placenta and throw light on possible role of cripto-1in invasion of the trophoblast through the myometrium.

Material and Methods

This research was carried out on 50 pregnant females with a singleton pregnancy, previous cesarean scar, normal course of pregnancy without any complications and underwent elective cesarean section after 37 weeks. This study conducted at the Gynecology and Obstetrics Department of Tanta University Hospital starting from February 2020 to February 2022.

The work was performed following approval from the Ethical Committee Tanta University Hospitals. An informed written consent was taken from the case or relatives of the cases.

Exclusion criteria were patients with systemic or local infection, any attack of bleeding at time of labour, and premature rupture of membranes.

By ultrasound patients were allocated into 2 equal groups: PA group diagnosed by (Loss of Retroplacental myometrial thickness of 1 mm, normal hypoechoic retroplacental zone, placental tissue bridging uterine-placental margin or Blood vessels, myometrial-bladder interface, or crossing uterine serosa, Multiple vascular lacunae in placenta, giving “Swisscheese” presence) and normal Placenta (NP) group.

All cases were undergone to: History taking, general and local examination, doppler U/S, specimen collection, immunohistochemistry and Western blot

Specimen collection

Directly following CS, the placental tissues were removed aseptically from the placenta maternal surface by the following steps: samples were taken from an accreta area and a none-accreta area for the PA group. For the normal group, Only placental tissue from the centre was taken. Tissue samples were incised under sanitary environment. Sites with hemorrhage, necrosis, and calcification were prevented. Following washing with cold saline, placental samples were instantly fixed in neutral buffered formol saline. Samples were routinely processed in ascending grades of alcohol, then cleared in Xyline, and paraffin embedded tissue blocks were prepared. Serial tissue sections (3–5-micron thickness) were prepared on positive charged slides for Routine hematoxylin & eosin staining for histopathological examination, and Cripto-1 immunostaining.

Immunohistochemistry

Following dehydration and paraffin inserting, tissues were divided into 3m thick slices and affixed on hold slides for 10 minutes of hot repair at 88°C. The slides were stripped of wax, hydrated by graded ethanol and put in sodium citrate solution in high-temperature for 1–2 min for antigen repair. Following cool down, the slides were cleaned using Tris-buffered saline (TBS). The endogenous peroxidase was inhibited with a 30% hydrogen peroxide solution for 10 minutes, followed by incubation with sheep serum at room temperature for 30 minutes and binding with the nonspecific antibody. At a dilution of 1:100, monoclonal mouse anti-human Cripto-1 antibodies (Abcam, USA) were applied to the slides and incubated overnight at 4 °C. Biotinylated rabbit anti-mouse antibody (Abcam, USA) was utilized at a dilution of 1:100 for 30 min at room temperature. After being washed with TBS, the specimens were stained with diaminobenzidine and hematoxylin before being cover slipped for microscopic examination. As a negative control, phosphate buffer solution was substituted for the main antibody.

Western blot

After three washes with PBS, tissue samples were rinsed with lymphocyte lysis buffer. By adding the extraction buffer, proteins were purified. BCA test was used to determine protein content. In accordance with the protein quantification results, the corresponding volume of total protein and 5x protein gel electrophoresis buffer were combined, and the protein was denatured at 95 °C for 10 minutes. Until the leading edge of the bromophenol blue reached the separation gel, the gel was pre-electrophoresed for 10 minutes at a constant 80V. Adjusting the voltage to maintain 120V until the dye reached the bottom of the separating gel. The protein was transferred at 110 V for one hour. Two hours at room temperature were spent blocking membranes in Tris-buffered saline-Tween-20 (TBS-T) containing 10 percent skim milk. The membranes were then treated overnight at 4°C with the primary antibody (rabbit monoclonal antibody against Cripto-1, Abcam Company, UK) diluted in TBS-T containing 3% BSA. Membranes were then incubated with secondary antibodies (an anti-rabbit IgG antibody, Beijing Dingguo Changsheng Biotechnology Company, China) for one hour at room temperature after washing with TBS-T. blots were washed three times with TBS-T, and the BM Chemiluminescence system was used for detection. Following exposure, membranes were dyed with Amido Black Staining Solution in order to determine protein content changes among samples. Software called Basic Quantifier was used to perform a densitometric study of band intensities. -actin was utilised as a control for loading. The grey values of the Cripto-1 and -actin protein bands were compared.

The primary outcome was Role of Cripto-1 in diagnosis of occurrence of Adhesive Placental Disorders. The secondary outcomes were comparison of Cripto-1 with success of conservative management, comparison of occurrence of complications and comparison with need of blood transfusion.

Statistical analysis

It was done by SPSS V25 of IBM, USA. Numerical data were represented as mean and standard deviation (SD) and were compared using Student's t-test for paired samples for the same group and unpaired student t-test for the two groups. Categorical data were represented as frequency and percent using Chi-square (x^2). A two-tailed P value < 0.05 was deemed significant.

Results

Demographic data (age, gravidity, parity, abortion, BMI, number of CS and gestational age) were insignificantly different between both groups. Incidence of intrapartum haemorrhage, bladder injury and ICU admission was significant increase in accreta group compared to controls (P= 0.007, 0.021, 0.001 respectively). Incidence of sepsis and postpartum haemorrhage was insignificantly different between accreta group and control group. The need for blood transfusion was significant increase in accreta group compared to controls (P = 0.001). Table 1

Table 1: Demographic data, Incidence of complications of studied groups

	Accreta	Control	p. value
Age	32.56±5.44	30.72 ±5.41	0.236
Gravidity	4.88±1.20	4.44±0.87	0.145
Parity	3.28±0.94	3.56±1.26	0.377
Abortion	0.72±0.84	0.40±0.58	0.124
BMI (kg/m^2)	30.00±5.78	29.44±4.42	0.702
Number of CS	3.08±0.95	2.88±0.88	0.445
Gestational age	37.44±0.51	37.72±0.68	0.105
Incidence of complications			
Intrapartum hemorrhage	13(52%)	4(16%)	0.007*
Bladder injury	7(28%)	1(4%)	0.021*
ICU	16(64%)	0(0%)	0.001*
Sepsis	11(44%)	5(20%)	0.069
Postpartum hemorrhage	4(16%)	8(32%)	0.185
No complications	2(8%)	11(44%)	0.004*
Blood transfusion			
+ve	23(92%)	2(8%)	0.001*

Data are presented as mean ± SD or frequency (%). BMI: Body mass index, CS: Caesarian section, +ve: positive,
*: significant P value

There was a significant relationship between accreta and control groups as regard Cripto-1 levels (P = 0.001). Table 2.

Table 2: Comparison of cripto-1 levels among studied groups

Cripto-1	Accreta	Control	P-value
No	0(0.0%)	20(80.0%)	0.001*
Low	14(56.0%)	5(20.0%)	
Moderate	8(32.0%)	0(0.0%)	
High	3(12.0%)	0(0.0%)	

Data are presented as frequency (%), *: significant P value

Table 3 shows Degree of invasion and success of conservative management in accreta group.

Table 3: Degree of invasion, Success of conservative management in accreta group

Degree of invasion	
Percreta	6(24%)
Increta	8(32%)
Accreta	11(44%)
Success of conservative	
Hysterectomy	15(60%)
Conservative management	10(40%)

Data are presented as frequency (%).

There was a significant relationship between degree of invasion and cripto-1 levels in accreta group (P = 0.001). The incidence of complications was insignificantly different in comparison to Cripto-1 levels in accreta group. Table 4

Table 4: Comparison between degree of invasion and cripto-1 levels and between the incidence of complications and cripto-1 levels in accreta group and Success of conservative of accreta group

Degree of invasion	Cripto-1				P-value
	Low	Mild	Moderate	Severe	
Percreta	0(0.0%)	0(0.0%)	3(37.5%)	3(100.0%)	0.001*
Increta	0(0.0%)	3(21.4%)	5(62.5%)	0(0.0%)	
Accreta	0(0.0%)	11(78.6%)	0(0.0%)	0(0.0%)	
Complications	**No**	**Low**	**Moderate**	**High**	
+ve	0(0.0%)	11(78.6%)	7(87.5%)	3(100.0%)	0.621
Success of conservative					
Hysterectomy	0(0.0%)	5(35.7%)	7(87.5%)	3(100.0%)	0.019*
Conservative	0(0.0%)	9(64.3%)	1(12.5%)	0(0.0%)	

Data are represented as mean ± SD or frequency (%)., +ve: positive, *: significant P value

Figure 1 shows Section of normal placenta showing negative staining for crypto-1 and weak positivity staining for crypto-1.

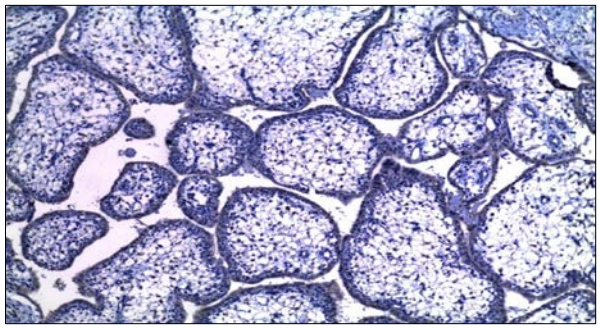

Fig 1: Section of normal placenta showing (A) negative staining for crypto-1 and (B)

Figure 2 shows Section of placenta accrete showing weak positivity staining for crypto-1, mild positivity (+) staining for crypto-1, moderate positivity (++) staining for crypto-1, and high positivity staining (+++) for crypto-1.

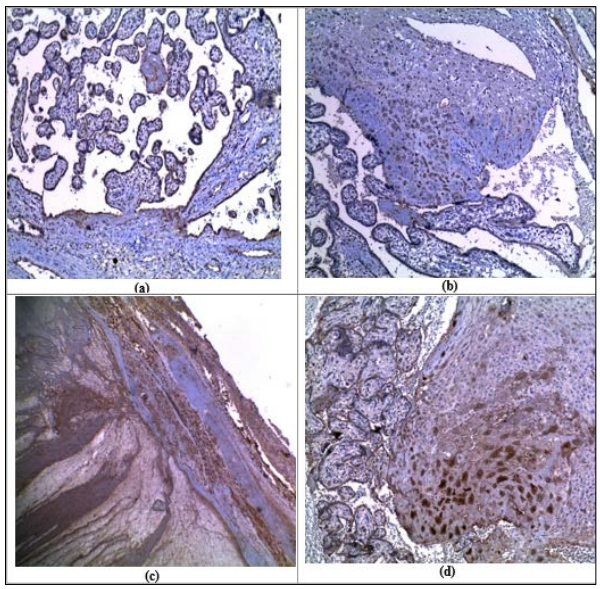

Fig 2: Section of placenta accrete showing (a) weak positivity staining for crypto-1, (b) mild positivity (+) staining for crypto-1

(c) moderate positivity (++) staining for crypto-1, (d) high positivity staining (+++) for crypto-1

Discussion

PAS points to an aberrant invasion of placental villi in the myometrium caused by a lack of basal decidua. Its pathophysiology is yet not fully understood. The most convincing theory is that previous trauma of the area (uterine surgery, cesarean delivery, etc.) enables the placenta's anchoring villi to connect to and infiltrate the myometrium [5].

In this study, there was a significant relationship between accreta and control groups as regard Cripot-1 levels.

In consistent with our results, Jiang *et al*., [4] observed that the related content of Cripto-1 protein in the PA group was significantly increased than compared to those of the Controls. Also, there is the potential that the upregulation of Cripto-1 expression is just one of the factors that contribute to the improvement of trophoblast invasion. Other essential elements that may cause excessive trophoblast invasion to require more investigation.

This study found that the incidence of intrapartum hemorrhage, bladder injury and ICU admission was significant increase in accreta group than controls. Incidence of sepsis and postpartum hemorrhage was insignificantly different between accreta group and control group.

In the same line with our findings, Matsuzaki *et al*., [6] conducted a study on 2,727,477 cases that performed caesarean delivery. They reported a significantly increased incidence of hemorrhage and urinary tract injury in accreta patients compared to none accreta participants.

In the present study, the need for blood transfusion was significant increase in accreta group than controls.

In their study, Flores-Mendoza *et al*., [7] studied 125 females of antenatal suspicions and pathologically verified PA spectrum disorders reported a significantly high estimated blood loss which was related to increase rates of blood transfusion in accreta group.

Regarding degree of invasion there were 6 (24%) patients with percreta, 8 (32%) patients with increta and 11 (44%) patients with accreta.

Similar findings were recorded by Abd ElKhabeer *et al*., [8]. They concluded that placenta accreta was the most common pathology than placenta increta and percreta, represented 50.8%, 34.4%, and 14.8% respectively.

In this study we noted that there was a significant relationship between degree of invasion and cripto-1 levels in accreta group where the levels of cripto-1 increased with the increase in the invasiveness of the placenta. We believe that it can act as a valuable prognostic biomarker in the prediction and diagnosis of the degree of invasion of placenta accreta.

Jiang *et al*., [4] supported our results by mentioned that the PA group showed increased Cripto-1 expression levels. Furthermore, expression levels differed between anatomic areas; Cripto-1 expression is significantly higher in the central region than in the non-accreta region, indicating enhanced trophoblast invasion and excessive placental invasion. They concluded that Cripto-1 participates in the control of placental tissue invasion.

One of the possible mechanisms that explain the role of Cripto-1 in invasion severity is that Cripto-1 stimulates the Wnt/-catenin signal pathway, decreases the amount of free -catenin in the cytoplasm, affects the interaction between -catenin and E-cadherin in addition the number and stability of cadherin-catenin complexes, a connecting structure of cell adhesion, and rises cell migration and invasion [4].

Caesarean hysterectomy is the conventional therapy for placenta percreta; Nevertheless, some surgeons choose for a cautious approach to avoid intraoperative problems. [9, 10]. According to our findings, The achievement of conservative administration was significantly different among Cripto-1 levels in accreta group. We previously mentioned that the cripto-1 levels were a possible indicator of the degree of the invasion and in addition, many surgeons preferred conservative management in mild cases to avoid hysterectomy potential intraoperative complications. Hence, we hypothesized, a similar trend was reflected in the form of an increase in the number of cases who underwent conservative management with mild and moderate levels of cripto-1 as it showed a higher success rate.

Conclusions

To our knowledge, no studies discussed the relation of cryito-1 to the success of conservative management or complications. On the other hand, It was already established that the increase in cripto-1 is related to increase in the degree of invasion of the placenta, yet our study recorded that the severity of complications was insignificantly different among the different Cripto-1 levels in accreta groups. Therefore, we can correlate the risk of complications to the degree of invasion.

Financial support and sponsorship: Nil

Conflict of Interest: Nil

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