International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Insulin Like Growth Factor Binding Protein 4 (IGFBP4) In Placenta Accreta Spectrum (PAS)

Noha Abdalla[1], Wafaey Gomaa[2], Mostafa Mohammed[3], Doaa Shaaban Mohamed[4], Abdel Ghaffar Ahmed[5], KamalAbdalla[6], Hisham Hussein Imam[6], Amr E. Ahmed[1]

[1]*Biotechnology and Life Sciences Department, Faculty of Post Graduate Studies for Advanced Sciences, Beni-Suef University, Egypt.*

[2]*Department of Pathology, Faculty of Medicine, Minia University, Egypt.*

[3] *Department of Biochemistry, Faculty of Medicine, Minia University, Egypt.*

[4]*Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine Beni-Suef University, Egypt*

[5]*Department of Gynaecology and Obstetrics, Faculty of Medicine, Assiut University, Egypt.*

[6]*Department of Gynaecology and Obstetrics, Faculty of Medicine, Minia University, Egypt.*

Abstract

The objective of the current study is to evaluate the role of Insulin like growth factor binding protein 4 (IGFBP-4) as a predictor for Placenta accreta spectrum (PAS). This current study included 44 patients indicated for elective caesarean section due to a sonographic diagnosis of placenta previa or placenta previa accreta. Ten ml of maternal blood was collected from all participants before delivery and serum was separated. Slices of placental tissue were taken from all participants and washed of blood. Sera and tissues were immediately stored at -80 ºC. Insulin like growth factor binding protein 4 (IGFBP4) assay was done by enzyme-linked immunosorbent assay for stored sera and homogenates of placental tissue. Placenta or hysterectomy specimens were sent for histopathological evaluation. According to histopathological final diagnosis, patients were classified into 2 groups; placenta accreta spectrum group (study group, n=16) and placenta previa non-accreta group (control group n=28). By ELIZA serum IGFBP4 level was insignificantly higher in placenta accreta group (22.23 ± 6.07 ng/ml) as compared to placenta previa non-accreta group (20.92 ± 3.9 ng/ml) (p=0.942). Placental tissue IGFBP4 level was insignificantly higher in accreta group (24.36 ± 10.9 ng/ml) as compared to non-accreta group (21.8 ± 9.5 ng/ml) (p= 0.472). Placental tissue concentration of IGFBP4 was higher than serum level in both groups. The study suggests a potential association between IGFBP-4 and placenta accreta spectrum. However, larger studies with a greater number of patients are recommended to draw more definitive conclusions about the relationship between IGFBP-4 and PAS.

Keywords: Placenta, Accreta, IGFBP4

Full-length article **Corresponding author's* e-mail: amreahmed@psas.bsu.edu.eg

1. Introduction

Globally, Insulin-like growth factor is composed of two peptides (IGFI and IGF 2), which are important growth factors that play a crucial role in promoting proliferation, survival, and cell differentiation of various body tissues [1,2,3]. Administration of IGFS has shown growth-promoting effects on a variety of tissues, including skeletal growth in experimental animal models and somatic growth in children with growth hormone receptor deficiency [4,5]. IGFS are short-lived unless they are carried by binding proteins that transport them in the circulation and deliver them to specific tissues to affect normal tissue as well as the growth of several cancers. Therefore, the biological effects and functions of IGFS depend not only on IGF concentration but also on the amount of insulin growth factor binding protein (IGFBP) and the specific IGFBP proteases that regulate IGFBP availability [6,7,8].

Among six different IGFBPs, IGFBp-4 is particularly important in locally reducing IGF function [9,10]. Increasing evidence suggests that IGFBP-4 inhibits IGF-induced cell growth in vitro and in vivo [11]. IGFBP-4 has been shown to inhibit cell proliferation primarily through

an IGF-dependent mechanism. The IGF system is complex, and IGFBP-4 is a key component of this system in a variety of body tissues. Furthermore, IGFBP-4 appears to have IGF-independent effects. Although IGF/IGFBP complexes can cross the vascular endothelium, they must also dissociate in the extracellular compartment to release IGH, which interacts with cell surface receptors and triggers biological responses. The IGF/IGFBP complex is disrupted by proteolysis of IGFBP by proteases.

Therefore, proteolysis appears to be an important regulatory mechanism that alters the binding affinity of IGFBP-4 for IGFs. The IGF-dependent IGFBP-4 protease cleaves IGFBP-4 into fragments that bind IGF with little or no affinity and therefore do not inhibit IGF-induced cell proliferation. Proteolysis of IGFBP by proteases in the circulation and extracellular space appears to play a crucial role in increasing the local amount of free IGF required for proliferation and enhanced tissue growth. In this context, the presence of multiple IGFBP proteases in serum has been reported, especially in pregnancy serum [12,13]. Pregnancy-associated plasma protein A (PAPP-A), one of the placental proteins secreted by trophoblast cells during pregnancy, is an IGF-dependent IGFBP-4 protease. Therefore, IGFBP-4 protease activity gradually increases during pregnancy [14]. During pregnancy, foetal growth increases the demand for growth hormones such as IGF. Proteolysis of IGFBP by the specific protease PAPP-A is important for increasing the amount of IGF during pregnancy. Therefore, PAPP-A is able to increase IGF bioactivity and stimulate IGF-mediated cell growth. PAPP-A appears to play an important role in foetal growth [15]. At the same time, normal development of the placenta depends in part on IGF stimulation of trophoblast cell proliferation and trophoblast cell invasion and migration outside the villi. There was a significant correlation between complications such as preeclampsia, and intrauterine growth restriction, as well as IGFBP_4 concentration and pregnancy-related plasma protein A [16]. The placenta accreta spectrum (PAS) includes placenta accreta, placenta accreta, and placenta percreta, which is characterized by excessive extravillous trophoblast invasion [17]. The primary aim of this study was to determine IGFBP-4 levels in the placenta accreta spectrum.

2. Materials and Methods

2.1. Patients

This study was conducted on 44 patients who were delivered by elective Caesarean section (CS) due to placenta accreta or placenta previa as diagnosed by ultrasonography, in Minia University Maternity Hospital, Minia, Egypt, from January 2022 till July 2022. The study was approved by Institutional Review Board of Faculty of Medicine, Minia University (approval No. 213-2022). Written consent was taken from all women before participation in this study. For each patient clinical and demographic data including age, parity, gravidity, gestational age, and number of prior CS were recorded after categorization into either group.

2.2. Blood samples

In the operating room, before elective CS, a 10 ml blood were collected from patients, left for half an hour, and centrifuged at 4000 rpm for 5 minutes. Serum was immediately stored in -80°C freezer.

2.3. Tissue Samples

Tissue slices from freshly removed placenta were taken and washed in normal saline to remove excess blood and were immediately transferred to a -80°C freezer for further biochemical assay.

2.4. Pathological examination

Pathological assessment of placenta was done by examining the delivered placenta or hysterectomy specimens. The specimens were fixed in 10 % neutral buffered formalin and gross examination was performed to detect placental invasion into myometrium. Sections were submitted for histopathological assessment [18]. Diagnosis of placenta accreta was settled when chorionic villi were identified invading into myometrium and was reported with subsequent classification of PAS according to FIGO grading [19].

2.5. ELISA

Serum and tissue homogenate were assayed for IGFBP4 level using human ILGFBP 4 ELIZA kit (Bioassay Technology Laboratory-BT LAB) according to the manufacturer's instructions

2.6. Statistical analysis

Data was collected and arranged for statistical tests. The Mann-Whitney U test was used to measure the relation between variables. Statistical procedures were performed using SPSS® version 25. Statistical significance was determined at a p value of ≤ 0.05 and was 2-sided.

3. Results

3.1. Patients

According to histopathological classification 16 patients were diagnosed as placenta accreta (study group), and 28 patients as non-accreta (control group) (Table 1). Distribution of PAS patients according to FIGO grading was reported (Table 2, Figure 1). The two groups were comparable regarding maternal age, number of previous deliveries, number of abortions and gestational age. The number of previous CS was higher in placenta accreta group 3.37±0.95 (range 2-5) compared to placenta non-accreta group 2.5 ± 1.2 (range 0-5) with a statistically significant difference (p=0.035) (table 3).

3.2. Serum IGFBP4 concentration

Serum IGFBP4 concentration was higher in placenta accreta group 22.23 ± 6.07 ng/ml (range 15.77 – 33.33) compared to placenta previa non-accreta group 20.9 ± 3.9 ng/ml (range 16.54 -33.33), but the difference was statistically insignificant (p= 0.942) (table 4).

3.3. Tissue IGFBP4 concentration

Tissue IGFBP4 concentration was higher in placenta accreta group 24.36 ± 10.9 ng/ml (range 8.08 - 44.48) compared to placenta previa non-accreta group 21.8 ±9.5 ng/ml (range 5.77 - 50.98), but the difference was not statistically significant (p=0.472) (table 5).

Table 1. Distribution of patients according to histopathological classification

Total patients	Accreta group	Non-accreta group
44 patients	16	28
100 %	36.3	63.7

Table 2. Distribution of PAS according to FIGO grading

Grade	*n* (%)
Grade 2	12 (75%)
Grade 3A	3 (18.75%)
Grade 3E	1 (6.25%)

Table 3. Patients characteristics in both study (accreta) group and control (non-accreta) group

	Placenta accreta group (n = 16)	Placenta non-accreta group (n = 28)	P value
	Mean ± SD (Range)	Mean ± SD (Range)	
Age in years	32.75 ± 3.8 (27-38)	31.18 ± 5.56 (21- 39)	0.313
Number of deliveries	3.56 ± 0.89 (2-5)	2.96 ± 1.34 (1-5)	0.123
Number of abortions	1.38 ± 0.95 (0-3)	0.86 ± 1.2 (0-5)	0.06
Gestational age in weeks	36.69 ± 1.7 (32-38)	37 ± 0.54 (36-38)	0.619
Number of previous CS	3.37 ± 0.95 (2-5)	2.57 ± 1.2 (0-5)	0.035*

Analysed by Mann- Whitney U test * Significant difference at P value < 0.05

Table 4. Serum IGFBP4 concentration by ELIZA in placenta accreta group (study group) and placenta previa non-accreta group (control group)

Serum IGFBP4 concentration (ng/ml)	Placenta accreta (n=16)	Placenta praevia non-accreta (n = 28)	p value
	Mean ± SD (Range)	Mean ± SD (Range)	
	22.23 ± 6.07 (15.77 – 33.33)	20.9 ± 3.9 (16.54 -33.33)	0.942

Analysed by Mann-Whitney U test Significant difference at *p* value < 0.05

Table 5. Tissue IGFBP4 concentration by in placenta accreta group (study group) and placenta praevia non-accreta group (control group)

	Placenta accreta (n =16)	Placenta praevia non-accreta (n=28)	p value
Tissue IGFBP4 concentration (ng/ml)	Mean ± SD (Range)	Mean ± SD (Range)	
	24.36 ± 10.9 (8.08 - 44.48)	21.8 ± 9.5 (5.77 - 50.98)	0.472

Analysed by Mann-Whitney U test Significant difference at *p* value < 0.05

Table 6. Correlations between Serum IGFBP4 concentration and tissue IGFBP4 in placenta accreta group (study group), in placenta previa non-accreta group (control group) and in all sample size

Correlations	r value	p value
Serum IGFBP4 concentration and tissue IGFBP4 concentration in placenta accrete	0.323	0.222
Serum IGFBP4 concentration and tissue IGFBP4 concentration in placenta previa non-accreta	- 0.176	0.371
Serum IGFBP4 concentration and tissue IGFBP4concentration in all sample size	0.085	0.583

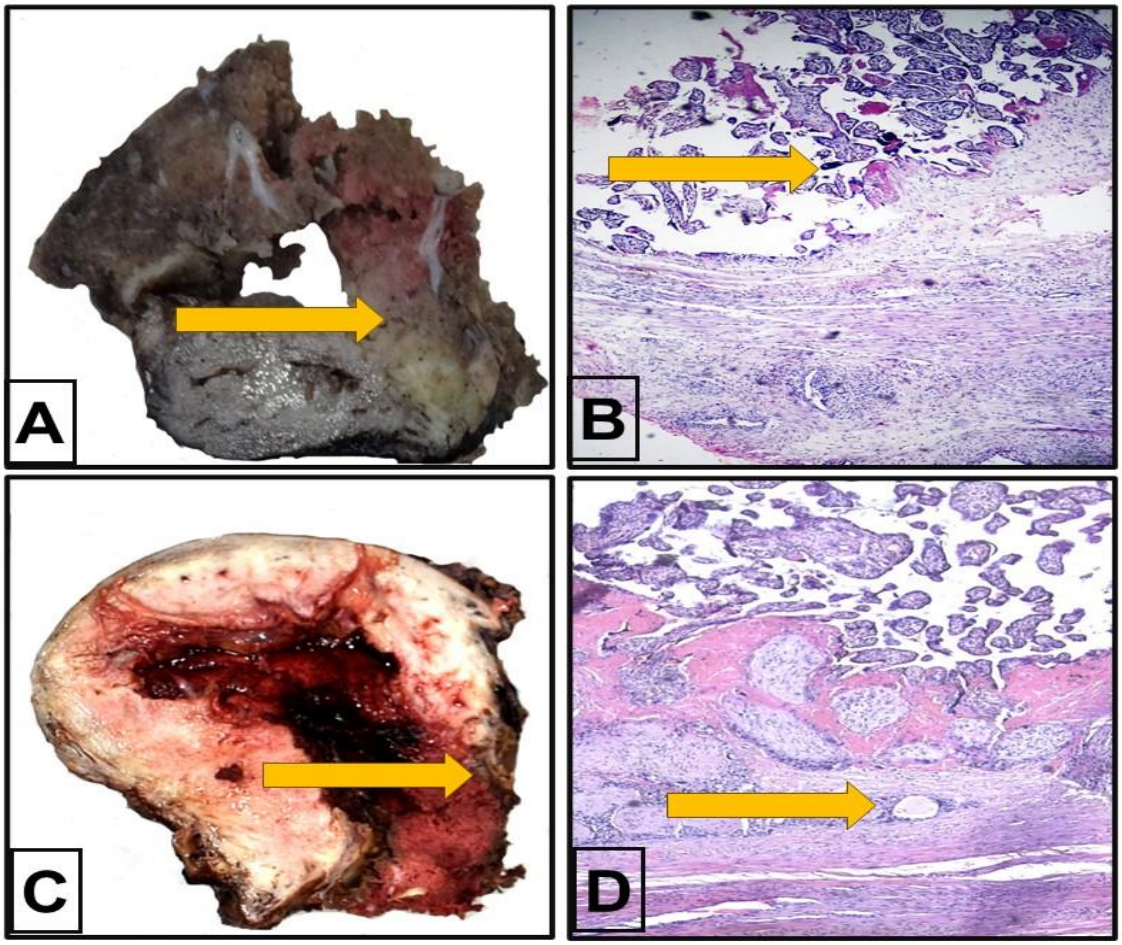

Figure 1. Representative examples of pathology of PAS
(A) A gross image of part of uterine wall with PAS grade 2 showing placental tissue invasion of superficial myometrium (arrow). (B) A haematoxylin and eosin-stained section from a PAS grade 2 showing chorionic villi invade superficial in myometrium (arrow) 100x. (C) A gross image of a bisected uterus showing PAS grade 3 where placental tissue invades deeply into the myometrium (arrow). (D) A haematoxylin and eosin-stained section with showing chorionic villi invade superficial in myometrium in the deep myometrium (arrow) 100x.

3.4. Correlation between IGFBP4 and PAS

There was insignificant positive correlation between serum and tissue IGFBP4 concentration in patients with placenta accreta group (r=0.323, p=0.222) and in all patients included in the study (the sum of both groups) (r=0.085, p=0.583) but there was insignificant negative correlation between serum and tissue IGFBP4 concentration in patients with placenta non-accreta group (r=0.176, p=0.371) (Table 6).

4. Discussion

Insulin-like growth factor binding protein-4 (IGFBP-4) is expressed exclusively by the maternal decidua, is a potent inhibitor of IGF action, and is the second most abundant IGFBP in the placental bed, where the placental trophoblast also expresses IGF-II. The IGF family has important responsibilities for implantation and placental physiology. Giudice et al, findings indicate that multiple elements may be involved in the placental bed during human implantation, including substrates (IGFBP-4), enzymes (PAPP-A), inhibitors (proMBP) and cofactors (IGF-II) (Giudice et al., 2002) [20]. As, PAPP-A has been suggested as a marker for predicting PAS [21] and due to the intimate relation between PAP A and IGFBP4, this prompted us to study maternal serum and placental concentration of IGFBP4 in PAS delivering patients.

The main substrate of PAPP-A is insulin-like growth factor binding protein [22]. PAPP-A increases the local bioavailability of insulin-like growth factor (IGF) by cleavage inhibitors IGFBP-4 and -5 (insulin-like growth factor binding protein-4 and -5; however, its function is unclear) [23]. Low PAPP-A levels are associated with elevated IGFBP protein levels, resulting in low free IGF levels. IGF controls the uptake and transport of glucose and amino acids in trophoblasts and plays a role in the autocrine and paracrine invasion of trophoblasts into the decidua [24]. The role of serum PAPP- A levels in early pregnancy as a PAS biomarker makes it worthwhile to study the role of IGFBP; observations such as these also suggest that further work in the area of PAS cellular energy metabolism may be important.

At the time of submission of this study, we are not aware of any studies that have examined the role of IGFBP4 in predicting PAS. The current study showed a non-significant positive correlation between serum and tissue IGFBP4 elevation in patients with placenta accreta compared with patients without placenta accreta. Due to sample size, this difference may not have yet reached statistical significance. The results of this study showed that IGFBP4 in both serum and placental tissue was higher in the accreta group than in the non-accreta group. However, the difference was not statistically significant. The study found that IGFBP4 levels in placental tissue were higher than those in serum. Future studies with large numbers of patients are needed to draw reasonable conclusions about the association between IGFBP4 and placenta accreta spectrum. Incorporation of the biomarker, IGFBP4, within routine evaluation of high-risk patients for PAS may prove beneficial in predicting these patients.

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