Hyperglycosylated Human Chorionic Gonadotropin (hCG-H) as a Predictor Marker of Placenta Accreta Spectrum

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ABSTRACT

Objectives: The objective of current investigation is to study the relation between hyperglycosylated human chorionic gonadotropin and placenta accreta in the 3rd trimester of pregnancy.

Patients and methods: The 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 taken before delivery and serum was separated. Slices of placental tissue were taken and washed of blood. Sera and tissues were immediately stored in -80°C. Hyperglycosylated human chorionic gonadotrophin (hCG-H) 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.

Results: According to histopathological final diagnosis, patients were classified into 2 groups; placenta accreta spectrum group (study group, n=16) and placenta previa non-accreta spectrum group (control group n=28). Serum hCG-H level was statistically significant higher in placenta accreta group (1.35 ± 0.35 mIU/ml) as compared to placenta previa non accreta group (1.07 ± 0.3 mIU/ml) (p=0.02). Placental tissue hCG-H level was higher in accreta group (1.28±0.24) as compared to non accreta group (1.16±0.33 mIU/gram), but the difference was statistically insignificant (p=0.150).

Conclusion: hCG-H may be a potential biomarker for predicting placenta accreta spectrum. More large-scale studies are needed to define the possibility of the presence of a reliable cut off diagnostic point. Combined with other possible biomarkers they can add to the diagnostic accuracy of already used imaging techniques.

Keywords: Placenta, Accreta, HCG, Hyperglycosylated, Prediction.

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INTRODUCTION

Placenta accreta encompasses various types of abnormal placentation in which chorionic villi attach directly to or invade the myometrium. This leads to failure of separation of the placenta from the uterine wall following delivery of the foetus. It was first described in 1937 by Irving et al and named this condition as morbid placental adherence (1). Various terminologies have been applied to this case; however, recently there is a consensus to be termed as placenta accreta spectrum (PAS) (2). It involves a spectrum from placenta accreta, where placental villi attach to the myometrium, to placenta increta, where the villi invade the myometrium, to placental percreta in which the villi invade at or beyond the serosal surface of the uterus (3).

PAS is a serious complication of pregnancy associated with significant maternal morbidity and mortality, in particular massive obstetric haemorrhage and peripartum hysterectomy (4). Maternal mortality rates up to 7% have been reported to be associated with PAS (5). In the last decade, the incidence of PAS has increased from 0.8 per 1000 deliveries in the 1980s to 3 per 1000 deliveries in the last decade (6). This is primarily due to the increasing percentage of pregnant women undergoing caesarean sections (CS). In the presence of placenta implanted in the lower uterine segment (placenta praevia) and 3 previous CS, a patient would have a 61% risk of PAS (7). Antenatal diagnosis of such cases is the key element to improving maternal and perinatal outcomes by allowing optimal obstetric management. Besides the obstetric history, ultrasound (US) remains the diagnostic standard tool available. Placental lacunae and abnormal colour Doppler imaging are the most helpful

criteria for the diagnosis. Also, there has been interest in magnetic resonance imaging (MRI) for the evaluation of PAS. However, these diagnostic methods are not completely satisfactory due to under-diagnosis or over-diagnosis resulting in poor maternal outcomes (8-9-10).

There is markedly increased risk of morbidity and mortality in PAS cases if the diagnosis is not made antenatally (11). Therefore, there is a need to develop additional methods to improve the antenatal diagnosis of PAS.

A number of diagnostic serum biomarkers have been investigated in PAS. These include human chorionic gonadotrophin (hCG), pregnancy-associated plasma protein-A (PAPP-A) and alpha-foetoprotein (AFP). They have shown variable reliability and variability of measurements (12-13-14). Human chorionic gonadotrophin is not a single biological molecule as it was thought to be (15). The regular form of hCG is produced by syncytiotrophoblast cells covering the chorionic villi. It is a hormone made with primary function of maintaining pregnancy by stimulating progesterone synthesis by the corpus luteum. Its maximum level is reached by 8-10 weeks of gestation and declines as placental steroid synthesis commences (16, 17).

On the other hand, hyperglycosylated hCG (hCG-H) is a glycosylated variant of hCG produced by extravillous trophoblastic cells. It has a critical function in trophoblastic invasion and growth of choriocarcinoma cells and implantation of pregnancy (18, 19). It has been shown that hCG-H works through blockage of apoptosis in cytotrophoblast cells, causing cell growth (20). It has been demonstrated that gestational trophoblastic diseases are regulated by the presence of hCG-H (21), Therefore, the assay of hCG-H is a useful marker in diagnosis and management of gestational trophoblastic diseases and in detection of quiescent gestational trophoblastic disease (15). PAS is characterised by excessive trophoblastic invasion (22). Normal placentae do not proceed beyond the inner third of the myometrium; however, in PAS the placenta proliferates and invades local structures in a similar manner as a malignant tumour. It is reasonably that hCG-H assay may be a useful biochemical marker for diagnosis of PAS. Few studies evaluated second and third-trimester serum levels of hCG-H in pregnant women with PAS (23).

We hypothesised that serum hCG-H would differ in women with PAS than those pregnant women without PAS. Thus, we aim to evaluate the usefulness of serum hCG-H in diagnosis of PAS.

MATERIALS AND METHODS

Patients

This study was conducted on 44 patients who were delivered by elective CS due to placenta accreta or placenta previa as diagnosed by ultrasonography, in Minia University Maternity Hospital, Minia University, 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, in addition to subsequent surgical findings (Table 2).

Blood samples

Prior to elective CS, 10 ml blood were sampled from patients and centrifuged at 4000 rpm for 5 minutes within one hour of sampling. Serum was immediately stored in -80^{O}C freezer.

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^{o}C freezer for further biochemical assay.

Pathological examination

Specimens (delivered placenta or hysterectomy) were fixed in 10% neutral buffered formalin. Detailed gross examination was performed, tissue sections were taken, and processed as usual (24). Histopathological evaluation was performed and the presence of placental invasion into myometrium was reported with subsequent classification of PAS according to FIGO grading (25).

ELISA

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

Statistical analysis

Data was collected and arranged for statistical tests. Mann-Whitney U test was used to measure the relation between variables. Statistical procedures were performed using SPSS® Release 16.0. Statistical significance was determined at *p* value of ≤0.05 and was 2-sided.

RESULTS

Patients

Sixteen patients were diagnosed histopathologically as placenta accreta (the study group table 1) and 28 patients as non-placenta accreta group (figure 1). Both placenta accreta and placenta previa non accreta groups were comparable regarding maternal age, number of previous deliveries, number of abortions and gestational age. However, number of previous CS

was higher in placenta accreta group 3.37± 0.95 (2-5) compared to placenta previa non accreta group 2.57 ± 1.2 (0-5) and the difference was statistically significant (p=0.035) (table2).

Table 1: Distribution of PAS according to FIGO grading

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

Table 2: Demographic data in Placenta accreta group (study group) and Placenta previa non accreta group (control group)

Demographic data	Placenta accreta (n = 16)	Placenta previa non accreta (n = 28)	P value
	Mean ± SD (Range)	Mean ± SD (Range)	
Age (years)	32.75 ± 3.8 (27-38)	31.18 ± 5.56 (21- 39)	0.313
No of deliveries	3.56 ± 0.89 (2-5)	2.96 ± 1.34 (1-5)	0.123
No of abortions	1.38 ± 0.95 (0-3)	0.86 ± 1.2 (0-5)	0.06
Gestational age	36.69 ± 1.7 (32-38)	37 ± 0.54 (36-38)	0.619
No 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

hCG-H measurements

Serum hCG-H level was higher in placenta accreta group 1.35 ± 0.35 (0.75 - 1.82) compared to placenta previa non accreta group 1.1+-0.38, and the difference was statistically significant (p= 0.02) (table 3).

Table 3: Serum hCG-H concentration in placenta accreta group (study group) and placenta previa non accreta group (control group)

Serum hCG-H concentration (mIU/ml)	Placenta accreta (n=16)	Placenta previa non accreta (n = 28)	p value
	Mean ± SD (Range)	Mean ± SD (Range)	
	1.35 ± 0.35 (0.75 - 1.82)	1.07 ± 0.3 (0.47 –1.71)	0.02*

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

Placental tissue hCG-H level was higher in accreta group 1.28 ± 0.24 (0.85-1.68) compared to placenta previa non accreta group 1.16±0.33 (0.44-1.96), but the difference was statistically insignificant (p= 0.150) (table 4).

Table 4: Tissue hCG-H concentration in Placenta accreta group (study group) and Placenta previa non accreta group (control group)

Tissue hCG-H concentration (mIU/ml)	Placenta accreta (n =16)	Placenta previa non accreta (n=28)	p value
	Mean±SD (Range)	Mean±SD (Range)	
	1.28±0.24 (0.85-1.68)	1.16±0.33 (0.44-1.96)	0.150

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

There was no statistically significant difference between serum hCG-H concentration and tissue hCG-H concentration in the case of placenta accreta (p= 0.221) and placenta previa non-accreta (p=0268). While in all cases serum hCG-H concentration was statistically higher than tissue hCG-H concentration (p=0.033) Table 5.

Table 5: Correlations between serum hCG-H concentration and tissue hCG-H 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 hCG-H concentration and tissue hCG-H concentration in placenta accreta	0.324	0.221
Serum hCG-H concentration and tissue hCG-H concentration in placenta	0.167	0.268

previa non-accreta		
Serum hCG-H concentration and tissue hCG-H concentration in all sample size	0.322	0.033*

Table 5: Validity of serum HCG test to detect placenta accreta:

Cut off value	AUC	*p* value	Sensitivity	Specificity	Positive predictive value	Negative Predictive value
1.28	0.712	0.013*	56.25	85.7	69.2	77.4

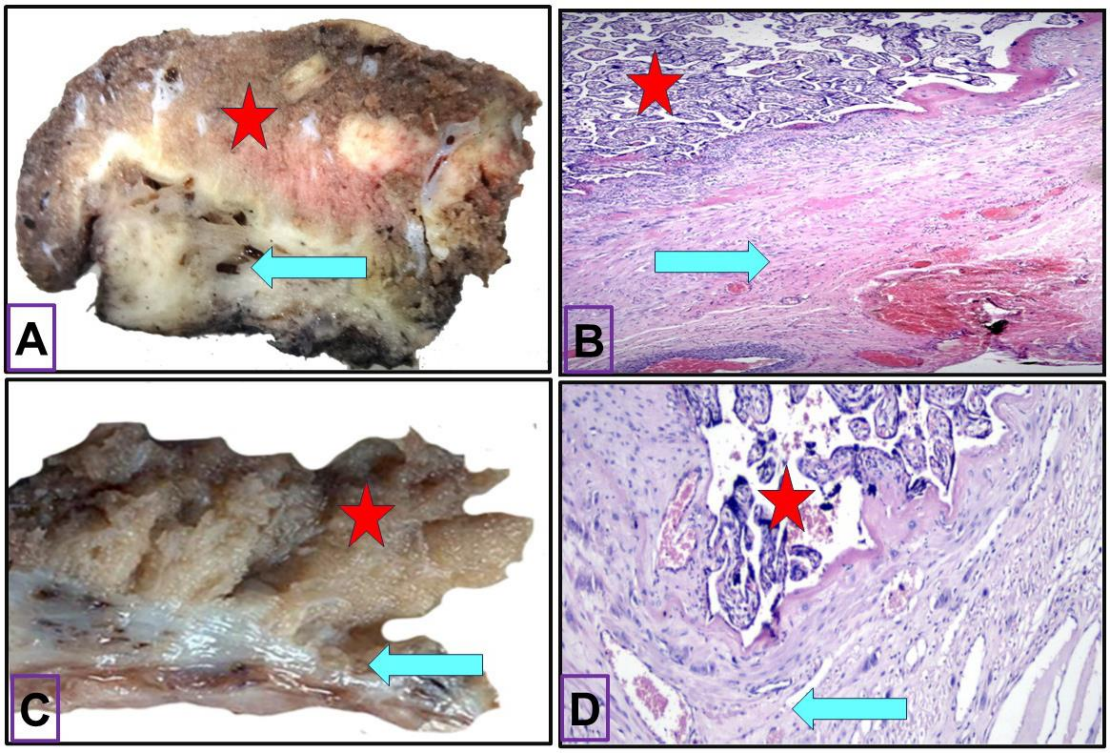

Figure (1): Representative example of PAS

A and B show representative images of PAS grade 2. **A** is a gross image showing placental tissue (asterix) invades superficial in myometrium (arrow). **B** is a hematoxylin and eosin-stained section showing direct extension of chorionic villi (asterix) into superficial myometrial fibers (arrow). **C and D** show representative images of PAS grade 3. C is a gross image showing placental tissue (asterix) invades deep in myometrium (arrow). **D** is a hematoxylin and eosin-stained section showing direct extension of chorionic villi (asterix) into deep myometrial fibers (arrow).

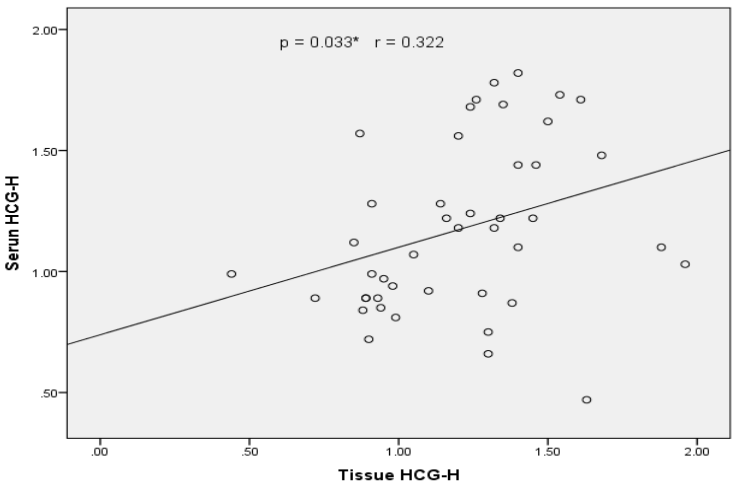

Figure (2): Correlations between serum hCG-H concentration and tissue hCG-H in all sample size

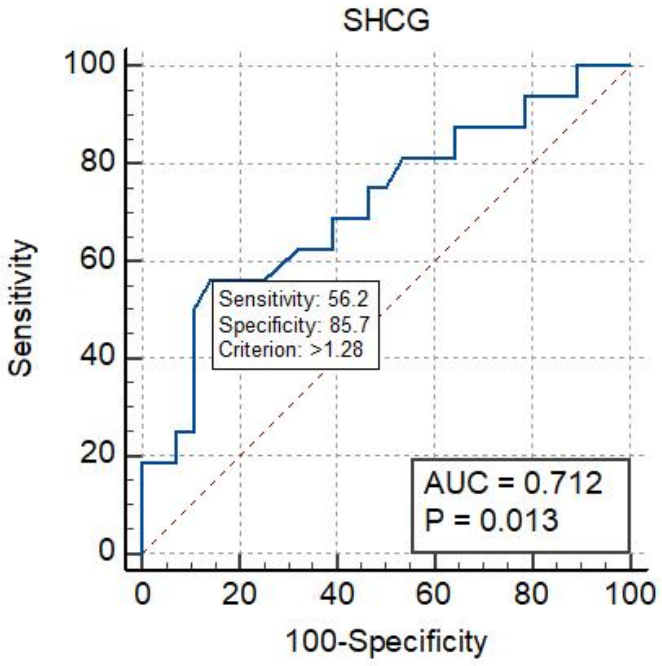

Figure 3: ROC curve for validity of serum hCG-H in predicting PAS.

DISCUSSION

In the last few decades, the incidence of placenta accreta spectrum has been increased due to the increasing rate of CS (26). In this study there is highly significant increase in number of previous CS in the confirmed histopathological accreta group compared to those of non accreta group P=0.007. These results go with the general agreement that the number of previous CS is the most important antenatal risk factor for PAS.

Antenatal diagnosis of PAS is essential for reducing the maternal morbidity and mortality and for improving the maternal and foetal outcome. Although ultrasound and Doppler examinations have improved antenatal diagnosis of PAS, there is still a need to develop another method to achieve better diagnosis.

Several serum biomarkers including hCG, PAP A and AFP have been investigated. Human chorionic gonadotrophin is a pregnancy specific glycoprotein which is secreted by trophoblast at the time of implantation (27). The regular form of hCG is produced by syncytiotrophoblast with primary function of maintaining the pregnancy.

However, the glycosylated form of hCG (hCG-H) is produced by extra villous cytotrophoblast. This form is mainly involved in trophoblastic invasion and implantation of pregnancy.

This study shows that maternal serum hCG-H level in the third trimester pregnancy is significantly higher in accreta group than in non-accreta group P 0.02 (Table3). This result agrees with Shalal et al whom stated that hCG-H showed

higher serum level in pregnant women at 36 weeks gestation with placenta accreta compared to those with placenta praevia and normal pregnancy. They stated that hCG-H with an optimal cut point of (3) IU/L predicted placenta accreta with 90% specificity, 76.7 sensitivity and 81,1% accuracy. So, they concluded that hCG-H could be a good diagnostic tool for PAS (28).

Regarding the predictive value of hCG-H for placenta accreta, the present study showed a specificity of 85.7%, but low sensitivity of 56.2. These data agree to some extent with those of Shalal et al. On other hand, Einerson B.D. et al showed that hCG-H levels in the 2^{nd} and 3^{rd} trimesters of pregnancy were lower in PAS than without PAS (23).

It seems that clinical applications and diagnostic value of hCG-H need further chemical characterisations of its structure and biological functions. There is a consensus that hCG-H has an important role in the process of invasion of trophoblast. It is also reasonable to suggest that hCG-H might represent a good marker for PAS.

It would be very valuable if a multicentric study could be conducted on large number of patients at different time segments of pregnancy with different depth of myometrial invasion to conclude the value of hCG-H measurements as a tool for prediction and diagnosis of PAS.

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