Clinical Implication of Serum and Immunohistochemistry Expression of Human Placental Growth Hormone in Ectopic Pregnancy

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ABSTRACT:

BACKGROUND:

Ectopic pregnancy causes major maternal morbidity and mortality. Human Placental growth hormone shows a non-pulsatile secretion pattern and short serum half-life making it of major advantage compared to the standard marker human chorionic gonadotropin.

OBJECTIVE:

To evaluate the clinical implication of serum human placental growth hormone variant and immunohistochemistry as a possible biomarker for the course of ectopic pregnancy.

PATIENTS AND METHODS:

This is a cross sectional study carried out in the department of Obstetrics and Gynecology of Al- Yarmouk Teaching Hospital for the period from January 2020 to October 2020, It includes 50 pregnant women in first trimester with confirmed ectopic pregnancy, maternal serum β -human chorionic gonadotrophin titer was measured and human placental growth hormone variant level was analyzed for each patient using enzyme-linked immunosorbent assay and tissue specimen were taken from serum human placental growth hormone variant negative and positive cases and examined by immunohistochemistry.

RESULTS:

Serum human placental growth hormone variant was shown to be positive in 28 cases of ectopic pregnancy and negative in 22 cases, β-human chorionic gonadotropin levels were significantly higher in the serum of the patient with human placental growth hormone variant positive group (p-value = 0.001). Serum human placental growth hormone variant was positive in all specimens of cases examined by immunohistochemistry even in serum negative cases.

CONCLUSION:

Human Placental growth hormone was shown to be present both in serum and tissue of ectopic pregnancy patients. Its short half-life makes it of a great clinical advantage as a biomarker for ectopic pregnancy course and monitoring response to treatment.

KEYWORDS: β-human chorionic gonadotropin, Ectopic pregnancy, Human placental growth hormone variant.

INTRODUCTION:

Ectopic pregnancy (EP) occurs when a fertilized oocyte implants outside the main cavity of the uterus. This places the woman at risk of physical harm, psychological morbidity and death [1]. Up to 73.9% of women with an EP may be diagnosed on TVS assessment and 94% are diagnosed before emergency surgical intervention[2]. However, it is difficult to estimate the current incidence of EP from available hospitalization because the number of EP cases requiring hospitalization has decreased [3]. It is one of the leading causes of pregnancy related maternal deaths and accounts for about 10% of maternal mortality during the first trimester [4].

The woman with EP may report abdominal distention with pain and shoulder tip pain and vaginal bleeding [1].

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Up to now β-human chorionic gonadotropin (β-hCG) with its long serum half-life is the only standard marker in monitoring the course of EP [5]. Serial β-hCG level assessment is recommended and checked 48 hours later [2].

A biomarker with a short serum half-life would be of great clinical advantage and could reduce number of patient's visit with great patient's comfort [5]. Early diagnosis of EP can be obtained by TVS, serum β-hCG level measurement. Other serum markers of EP have been evaluated from several studies, focusing on proteins associated with placental, endometrial and/or corpus luteal functions, angiogenesis and inflammation [6]. Several biomarkers have been studied, CA-125 level has been found to be higher in patients with viable intrauterine pregnancies. In addition, women with EP have a wide range of CA-125 concentrations; however, the concentrations are lower compared to those with intrauterine pregnancies. Another marker is the percentage of $CD3^+$ T cells which was significantly higher in patients with EP than in women with normal early pregnancy [7].

Growth hormone (GH), primarily secreted from the anterior pituitary, stimulates growth, cell reproduction, regeneration and is a major regulator of postnatal growth. Two GH genes in human encode two versions of GH proteins: a pituitary (GH-N/*GH1*) and a placental GH-variant (GH-V/*GH2*) [8].

Human placental growth hormone (hGH-V) is produced by the GH variant gene on chromosome 17. HGH-V is expressed in the syncytiotrophoblast and extravillous cytotrophoblast layers of the human placenta [9]. During pregnancy, its concentration increases progressively in maternal blood, whereas pituitary GH level decreases [10]. HGH-V is detectable in the fetal tissues, cord blood and amniotic fluid [11].

AIM OF STUDY:
To evaluate the clinical implication of serum Human placental growth hormone variant and immunohistochemistry expression as a possible biomarker for the course of ectopic pregnancy.

PATIENT AND METHOD:
This is a cross-sectional study carried out in the department of Obstetrics and Gynecology of Al- Yarmouk Teaching Hospital for the period from 1st of January 2020 to 1st of October 2020.
The study protocol was approved by Scientific

Counsel of Obstetrics and Gynecology Specialization / Iraqi Board for Medical Specializations.

Informed consent was obtained from women admitted to the gynecological ward and from women attending consultation clinic upon enrollment into the study.

The study included 50 pregnant women in first trimester with confirmed ectopic pregnancy attending the consultation clinic and inpatient ward in the gynecological department. The diagnosis of ectopic pregnancy was confirmed by clinical evaluation (abdominal pain and /or vaginal bleeding), positive pregnancy test (serum β-hCG titer), positive visualization of adnexal mass on transvaginal ultrasound, and all patients that underwent laparotomy with histopathlogical sample.

Women with heterotopic pregnancy, women who had blood transfusion within 6 months of the study, women using intrauterine contraceptive device at the time of conception and history of previous ectopic pregnancy were excluded.

Detailed history was taken and information about demographic and clinical characteristics using semi-structured questionnaire which includes information about age, parity, last menstrual period, age of marriage, use of contraception, history of previous ectopic pregnancy, previous history suggestive of PID (abdominal pain, vaginal discharge) and abdomino-pelvic surgery. General and systemic examination and measurement of vital signs were done.

The studied groups were investigated by ultrasonography for aiding the diagnosis.

Maternal blood samples were collected at the time of admission and sent to the laboratories for the blood group and cross match, full blood count, β-hCG titer measured by an immune- radiometric assay, the detection limit for this assay was 0.9 mIU/ml and hGH-V assay. Blood samples were taken from surgically treated patient on day 1 for hGH-V assay. In patient treated medically series serum samples were collected at different points at admission, day 1 and day 4. Tissue samples from ten surgically treated patients (5 from cases that tested positive for serum hGH-V and 5 from negative cases) sent for hGH-V analysis using immunohistochemistry (IHC).

Sample collection and preparation: Five ml of venous blood were collected from each patient

placed in sterile tubes which were labeled with the patient's name allow samples to clot for two hours at room temperature and centrifuged for 15 Minutes at 1000 ×g and serum was collected carefully and immediately stored at -20°C or -80°C.

Test principle**:** the assay employs the quantitative sandwich enzyme immunoassay technique Catalog Number: CSB-EL009408HU.

Antibody specific for GH2 has been pre-coated onto a microplate. Samples and standards are pipetted into the wells with a Horseradish Peroxidase conjugated antibody specific for GH2. Followed by removing any unbound reagent by washing, then adding a substrate solution to the wells and color develops in proportion to the amount of GH2 bound in the initial step. The color development is stopped and the intensity of the color is measured. Detection range: 133pg/ml-4000pg/ml.

Immunohistochemistry of hGH-V: A panel of monoclonal antibodies raised against hGH-V was tested on placental and EP tissue to screen for a suitable set-up. The (IHC) was performed on tissue samples from surgically treated patients.

Statistical analysis:
The data were incorporated into Microsoft excel sheet 2016, and loaded into SPSS v23. After checking and cleaning, the studied variables were presented using table (frequency, relative frequency, mean and standard deviation) and graphs accordingly. The statistical significance of associations between related categorical data was tested using Chi square test. Parametric statistical tests like 2 sample t test were used to find out statistical significance of differences between 2 independent numerical variables means. P value less than 0.05 was considered as discrimination point for alpha error (significance) in all used statistical tests.

RESULT:
Serum hGH-V was positive in 28 cases while the remaining 22 cases were negative. Thirty-five cases treated surgically of which 19 cases were positive for serum hGH-V. In medically treated cases which were 15 cases, 9 of them were positive for serum hGH-V. Table1 shows patient's characteristics for the cases included in the study; the result showed that the mean gestational age was 49.16 ±7.9 days, in hGH-V positive group (55±7.9 day) which was significantly higher than that of hGH-V negative group (43.3±4.7 day), p value=0.001. Regarding the parity 50% of hGH-V positive group were nulliparity compared to 45.5% in hGH-V negative group and this was not significant. The mean β-hCG at presentation was 1807±374 and was significantly higher in hGH-V positive group (1979±279mU/ml) than that of hGH-V negative group (1589± 370mU/L), p value=0.001. There was no significant difference in maternal age, BMI and parity between the hGH-V positive and negative cases, (p value>0.05) in all conditions.

Table 1: Patients' characteristics of EP cases.

Variables	Mean ± SD	hGH-V positive	hGH-V negative	p-value
Number of cases	50	28	22	
Maternal age / years	27.7±5.7	28.18±6.09	27.05±5.30	0.493
Gestational age/ days	48.16±7.9	52.6±7.9	43.3±4.7	0.001
BMI (kg/m^2)	29.7±4.1	29.24±4.13	30.17±4.15	0.434
Parity (Nulliparity %)	48%	50%	45.5%	0.749
β-hCG at admission (mU/mL)	1807±374	1979±279	1589±370	0.001

P-value < 0.05 were considered statistically significant

Table 2 shows hGH-V was detected in the patient's blood in 28 out of the 50 cases with concentrations between 143-296.3 pg/ml. For β- hCG concentrations in hGH-V positive cases, the range was 1456-2702 mU/mL while in all cases the range was 684-2702 mU/ml. In the 35 cases that treated surgically, 19 of them were hGH-V positive with mean of 268.57 and mean β- hCG 1927.21. The remaining cases were hGH-V negative with mean β- hCG 1797.18. Regarding cases that treated medically hGH-V was positive in 9 cases with mean of 264.61 and mean β- hCG was 1801.33 and the mean β- hCG was 1467.33 in hGH-V negative cases that treated medically.

Table 2: Serum levels of hGH-V and β-hCG in EP cases and modes of treatment at presentation.

variables	n	β-hCG (mU/ml)			hGH-V (pg/ml)		
		mean	median	range	mean	median	range
EP	50	1807.76	1830	684-2702	150.40	252.85	0-296.3
hGH-V positive	28	1979.2	1953	1456-2702	268.57	274.2	143-296.3
hGH-V negative	22	1589.45	1739.5	684-1958	0	0	0
surgical intervention	35	1867.77	1611.58	684-2702	146.81	264	0-296.3
hGH-V positive	19	1927.21	1715.90	1456-2702	270.45	277.2	143-296.3
hGH-V negative	16	1797.18	1944	684-2250	0	0	0
Medical intervention	15	1667.73	1830	690-1748	158.76	254	0-284.4
hGH-V positive	9	1801.33	1775	1958-1972	264.61	265	251.7-284.4
hGH-V negative	6	1467.33	1672	690-1850	0	0	0

Table 3 shows that in 19 cases that tested positive for serum hGH-V and treated surgically, 7 of them changed to negative hGH-V 24 hours after surgical treatment while in medically treated cases, 9 of them were hGH-V positive and 5 of them changed to negative after 24 hours. There was no significant difference between rate of changes in hGH-V from positive to negative after 24 hours of treatment whether this treatment is medical or surgical (p value=0.350)

Table 3: Association between types of treatment and changes in serum hGH-V level after 24 hours of treatment.

	HGH-V positive	Change to negative		Not changed		
		N.	Row N%	N.	Row N%	p-value
Surgical	19	7	36.8%	12	63.2%	0.350
medical	9	5	55.6%	4	44.4%	

Figure 1 shows that in medically treated EP cases which were 15 cases, β-hCG level decreased by > 15% on day 4 after methotrexate administration and progressively below detection level.

No additional MTX administration was required. hGH-V levels were measured in serum from serial samples of medically treated patients hGH-V level changed to negative at Day 4 after single-dose regimen MTX.
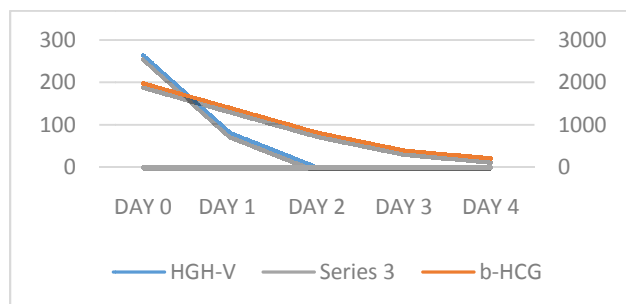

Figure 1 : Serum hGH-V and β-hCG before and after medical treatment measured at day 1 and 4 for ectopic pregnancy treated with methotrexate (MTX); β-hCG and hGH-V (y-axis, right and left)

Figure 2 shows hGH-V expression in tissue obtained from EP, ten histopathological samples of EP were analyzed by IHC (5 from the hGH-V serum positive and 5 from the serum-negative cases, selected by random). hGH-V could be localized to the syncytiotrophoblast in all 10 cases independent of its detection in the serum. The IHC staining showed a linear cytoplasmic staining and sparing of the nuclei.
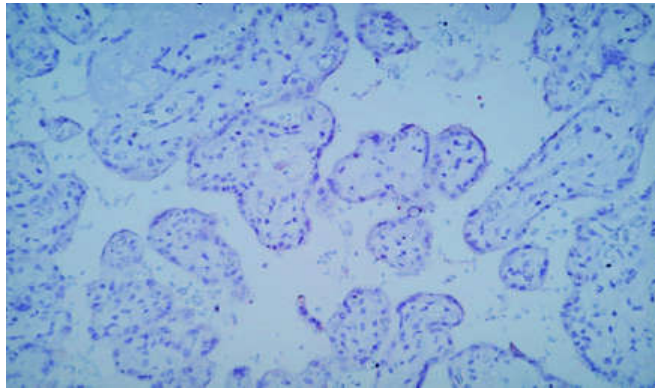
Figure 2: HGH-V expression in ectopic pregnancy (Immunohistochemistry)

DISCUSSION:

In the current study, no significant difference was noticed regarding maternal age, BMI and parity between hGH-V positive and negative ectopic pregnancy cases (p-value 0.493), (p-value 0.434) and (p-value=0.749) respectively as shown in table 1, these observations were in accordance with a case control study done by **kwaku** et al. they showed that majority 66% of maternal ages range of cases were 25-34years and they found no significant difference regarding maternal age (p-value= 0.654) [12]. Another study done by **Audry** et al. found in their prospective cohort study that there was no significant association regarding maternal age and BMI and EP, p-value=0.34 and p-value=0.87 respectively [13].

In a study done by **Arslan** S. et al, no significant association found between cases of EP regarding parity [15] and this agrees with the current study. The same study found no significant association regarding GA between cases of EP [14], this disagrees with the present study in which there was a significant association between GA and hGH-V positive and negative cases of EP. This difference may be related to differences in method of study and difference in sample size. The levels of β-hCG were significantly higher in hGH-V positive cases than negative cases of EP: this agrees with a study done by **Hubner C**. et al which aimed to investigate human placental growth hormone (hGH-V) in ectopic pregnancy [5]. In the present study, the mean serum hGH-V was comparable to a study done by **Hubner C**. et al, with mean hGH-V was 0.07ng/ml and range was 0-0.5ng/ml and mean hCG was 10800 IU/L [5]. No significant difference was found between type of treatment

and change of hGH-V positive cases to negative after 24 hrs as shown in table 3, and this was in agreement with **Hubner** et al in which 59 women with EP were studied and received surgical and medical treatment and hGH-V were below detection level after 24 hrs. [5].

Figure 1 showed that the mean initial β-hCG level decreased by > 15% on day 4 after methotrexate administration and progressively below detection level while hGH-V level changed to negative at Day 4 after medical treatment. This was in accordance with a study done by **Helmy S**. et al a retrospective cohort study. They found that average half-life clearance of β- hCG was 82.5± 50.2 SD in patients with steadily declining serum β-hCG levels [15]. This was also comparable to a retrospective study done by **Dhar H**. et al, they found that average β- hCG level on day 4 was 1350 mU/ml and average time of resolution of EP was 32 days for single dose MTX [16]. Immunohistochemistry (IHC) was performed on ten samples, five from the hGH-V serum-positive and five from the serum-negative cases. HGH-V could be localized to the syncytiotrophoblast in all ten cases regardless of hGH-V detection in the serum. The pattern of distribution was linear with cytoplasmic staining and nuclear sparing. This result is in accordance with the pattern described in a study done by **Hubner** et al [5].

CONCLUSION:

Human Placental growth hormone (hGH-V) was shown to be present both in serum and tissue of EP patients. Its short half-life makes it of a great clinical advantage as a biomarker for ectopic pregnancy course and monitoring response to treatment.

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