



The diagnostic utility of placental growth factor in ectopic pregnancy

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Abstract

Purpose Ectopic pregnancy is a condition of enormous gynaecological priority. It occurs when fertilized ovum implants outside the uterine cavity. Ectopic pregnancy is estimated to be 1–2% of all-natural conception. This study aims to evaluate the role of the placental growth factor in the diagnosis of ectopic pregnancy.

Methods A case–control study was conducted in (Baghdad) teaching hospital in (Baghdad, Iraq) over one year. The study includes 240 women: 120 women with first trimester (5–10) weeks viable intrauterine pregnancy, and 120 women with ectopic pregnancy.

Results There is a significant association observed in the placental growth factor mean (PLGF), which is lower in ectopic pregnancy than in intrauterine pregnancy. The range of PLGF was less than 50 pg/mL in ectopic pregnancy, while it was up to 800 pg/mL in intrauterine pregnancy. In an ectopic pregnancy with a cut-off PLGF level of ≤ 15.5 pg/mL, the PLGF had a sensitivity of (92.5%), a specificity of (82.5%), a positive predictive value of (91.8%), a negative predictive value of (83.5%), and an accuracy of (90%). Different factors can affect PLGF, like the parity and body mass index.

Conclusion The serum level of the placental growth factor seems to be a promising biomarker for diagnosing ectopic pregnancy because a highly significant difference was found between healthy and ectopic pregnancy.

Keywords Placenta growth factor · Pregnancy · ectopic · Body mass index · Parity

Introduction

Ectopic pregnancy (EP) occurs when a fertilized egg attaches outside the uterus. It is not an uncommon condition in developed countries as it counts for 1–2% of live births. This percentage can increase to up to 4% in women using assisted reproductive technology [1]. Additionally, EP was found to be a leading cause of death among pregnant women during the first trimester with a 10% death rate [2].

Different factors have been found to cause EP; however, in more than 50% of cases, no recognized risk factors are identified [3]. Patients with EP can be asymptomatic or can present with different symptoms or signs. However, vaginal bleeding and abdominal pain are the chief complaints.

Nevertheless, only less than half of all cases exhibit both of these symptoms [4]. The combination of aching pelvic pain, mild vaginal spotting in the first trimester, and secondary amenorrhea may point towards a pregnancy outside the uterus. Nevertheless, these symptoms may occur as a consequence of early miscarriage or even in a healthy intrauterine pregnancy [5].

EP continues to be challenging in diagnosis [6]. Most of deaths related to EP occur shortly after admission in a health facility or even in the community; thus, EP is a relevant public health issue [7]. The diagnosis of EP depends principally on history and physical examination. Moreover, pelvic ultrasound (US) facilitates early diagnosis before rupture or other complications. However, the US is operator dependant and not always available. Different tests are used to diagnosis EP like human chorionic gonadotropin (hCG) level and serial hCG values. Nonetheless, these tests should not be used solely. Instead, these tests should be used in combination with the evaluation of symptoms, clinical judgement, and even repeated ultrasound to diagnose EP. Even though most of EP cases have hCG curves within normal limits, it

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is crucial to reassess the patient in case of hCG fluctuation near the extremes before any intervention [6, 8].

Placental growth factor (PLGF) is a pro-angiogenic protein secreted during pregnancy, mainly from placental trophoblast cells. It belongs to the family of vascular endothelial growth factors. PLGF has a vital role in controlling trophoblast growth and differentiation. During pregnancy, PLGF has been found at the implantation site and affects the surrounding cells, specifically endothelial cells. This action is mediated through flt-1 (VEGFR1), and flk-1/KDR (VEGFR2) receptors enhancing local blood supply development. Convenient placental blood vessel development is essential for compatible embryonic development [9]. Normally, as a consequence of implantation, a boost in secretion of PLGF is observed; this increment is reflected systemically by measuring the serum level [10]. Different factors are presumed to influence PLGF like age, parity, and body mass index (BMI). Although the literature mentioned that a lower level of maternal serum PLGF in early pregnancy correlates with a higher risk of having pre-eclampsia in late pregnancy [11], the diagnostic utility of PLGF is still under investigation and its effect on inflammatory reaction is still vague [12].

Thus, this study aims to evaluate the role of placental growth factor in the diagnosis of EP.

Patients and methods

This study is a prospective case–control study conducted in the Emergency department and Obstetrics and Gynaecology department of Baghdad teaching hospital, Baghdad, Iraq. This study was conducted over one year, starting from March 2016 to the end of March 2017.

A sample of 240 pregnant women enrolled in this study, 120 healthy women with intrauterine pregnancy of 5–10 weeks, and 120 women with ectopic tubal pregnancy. The second group was further subdivided into two subgroups: ruptured and un-ruptured tubal ectopic pregnancy at the same gestational age.

This study was approved by the Obstetrics and Gynaecology Committee of the Iraqi Board for Medical Specialization and the Obstetrics and Gynaecology department of Baghdad Teaching Hospital.

All participating women were informed about the nature of the study, its benefits, and the procedures used. Verbal and written consent was obtained from each woman.

Patient collection

Patients presenting to the Emergency department or Obstetrics and Gynaecology department of Baghdad Teaching Hospital with a positive pregnancy test and a complaint

of abdominal pain, vaginal bleeding or both were checked against the inclusion and exclusion criteria. Those who met the inclusion criteria and did not align with the exclusion criteria were enrolled in the study.

Control group

Inclusion criteria

1. Gestational age of (5–10) weeks.
2. Singleton viable pregnancy.

The gestational age was determined depending on the absolute dating of the last menstrual period and confirmed by transvaginal ultrasound. Early gestational age (5–7 weeks) undergoes regular follow-ups later to confirm the viability by repeated transvaginal ultrasound.

Exclusion criteria

1. Gestational age less than 5 weeks and more than 10 weeks.
2. Abortion.
3. Multiple pregnancy.
4. Nonviable pregnancy.
5. Patients who undergo any hormonal therapy.
6. Patients with medical or chronic diseases.

Patient group

The diagnosis of EP was presumed depending on the positive pregnancy test (serum hCG level) and the visualization of an adnexal mass on transvaginal ultrasound; the final diagnosis of EP was made by laparotomy or laparoscopy with confirmed histopathological diagnosis.

Eventually, women in patient and control groups were divided into two groups: 1st group of 120 healthy pregnant women with single intrauterine pregnancy (5–10 weeks).

2nd group of 120 women diagnosed to have EP which subdivided to:

- (a) Ruptured EP of 101 patients.
- (b) Un-ruptured EP of 19 patients.

Sample collection

Once the EP was diagnosed, 5 mL of venous blood was drawn into a plain tube before any operative intervention. It was left then for a few minutes until clotting occurred where the sera of blood separated by centrifuging them at 2163.33 g for 15 min. The serum samples were kept below –20 °C until they were studied.

Regarding the control group, the blood samples were collected from patients with healthy, viable pregnancies with similar gestational age to the EPs.

Statistical analysis

A computer statistical software was used to analyse the patients' data. The data were computerized by Statistical Package for Social Science (SPSS) version 21. Descriptive statistics showed as (mean + standard deviation) and frequency showed as a percentage. The data were normalized and verified by Kolmogorov Smirnov's analysis. Multiple prospect tables were done and proper statistical tests were performed. Fischer exact test was used for categorical variables and *t* test was used to compare two means. ROC curve was used to clarify the validity test. The result of significance was set at <0.05 in all statistical analyses. The results are displayed as tables.

Results

A total of 240 pregnant women were included in the present study, of which 120 were healthy pregnant women with a mean age of 27.5 ± 8.1 years and 120 were women with EP with a mean age of 25.7 ± 6.9 years. No significant difference in age was observed between healthy and EP women ($p=0.6$). The mean gestational age of healthy pregnant women was 6.6 ± 1.3 weeks while the mean gestational age of EP women was 7 ± 1.2 weeks. Similar to the age, no significant difference in mean gestational age was observed

Table 1 Distribution of age and gestational age in the healthy and ectopic pregnant women

Variable	Healthy pregnant control group		Women with ectopic pregnancy		<i>p</i>
	No.	%	No.	%	
Age					0.6
< 20 years	21	17.5	27	22.5	
20–29 years	48	40.0	57	47.5	
30–39 years	45	37.5	33	27.5	
≥ 40 years	6	5.0	3	2.5	
Mean ± SD	27.5 ± 8.1		25.7 ± 6.9		0.2
Gestational age					0.4
5th week	30	25.0	12	10.0	
6th week	24	20.0	27	22.5	
7th week	42	35.0	39	32.5	
8th week	12	10.0	27	22.5	
9th week	9	7.5	12	10.0	
10th week	3	2.5	3	2.5	
Mean ± SD	6.6 ± 1.3		7 ± 1.2		0.1

between healthy and EP women ($p=0.1$). All these findings are shown in Table 1.

Moreover, as displayed in Table 2, there was no significant association between healthy pregnant women and women with EP regarding parity ($p=0.18$) and body mass index (BMI) ($p=0.01$). However, the mean BMI of women with EP (28.1) was higher than the BMI of healthy pregnant women (24.9).

When assessing the hCG and PLGF of the patients, there was a highly significant association between the lower mean of hCG and pregnant women with EP ($p<0.001$). Additionally, a highly significant association was observed between the lower mean of PLGF and women with EP ($p<0.001$) (Table 3).

When we assessed the best cut-off points and the corresponding validity tests values for PLGF level in the prediction of EP women from healthy pregnant women in the first trimester, we found that a cut-off PLGF level of ≤ 15.5 pg/mL had acceptable validity results (92.5% sensitivity, 83.5% specificity, 91.8% positive predictive value (PPV), 83.5% negative predictive value (NPV) and 90% accuracy).

Table 2 Distribution of parity in the healthy and ectopic pregnant women

Variable	Healthy pregnant control group		Women with ectopic pregnancy		<i>p</i>
	No.	%	No.	%	
Parity					0.18
Nulliparous	24	20.0	21	17.5	
1	24	20.0	18	15.0	
2	36	30.0	42	35.0	
3	30	25.0	12	10.0	
4	6	5.0	12	10.0	
5	0	–	9	7.5	
6	0	–	6	5.0	
Mean ± SD	1.7 ± 1.1		2.2 ± 1.6		0.1
BMI					0.01
Normal	27	67.5	15	37.5	
Overweight	10	25.0	14	35.0	
Obese	3	7.5	11	25.0	
Mean ± SD	24.9 ± 1.3		28.1 ± 1.2		<0.001

Table 3 Distribution of HCG and PLGF according to healthy and ectopic pregnant women

Variable	Healthy	Ectopic	<i>t</i> test	<i>p</i>
	Mean ± SD	Mean ± SD		
HCG	$27,616.8 \pm 20,000.8$	2466.2 ± 1001.1	7.9	<0.001
PLGF	21.2 ± 9.9	11.3 ± 5.3	10.5	<0.001

Table 4 Distribution of PLGF mean according to age of ectopic pregnant women

Age groups	PLGF Mean \pm SD
< 20 years	46.9 \pm 25.7
20–29 years	42.3 \pm 21.5
30–39 years	37.3 \pm 21.8
\geq 40 years	82.1 \pm 32.4
ANOVA (<i>p</i> value)	0.2

Table 5 Distribution of PLGF mean according to parity of ectopic pregnant women

Parity groups	PLGF Mean \pm SD
Nulliparous	38.4 \pm 20.9
1	30.3 \pm 23.7
2	43.8 \pm 23
3	41.4 \pm 23.9
4	44.9 \pm 18.3
5	52.8 \pm 10.4
6	58.8 \pm 37.4
ANOVA (<i>p</i> value)	< 0.001

Table 6 Distribution of PLGF mean according to gestational age of ectopic pregnant women

Gestational age groups	PLGF Mean \pm SD
5th week	30.7 \pm 27.1
6th week	33.5 \pm 18.9
7th week	49.8 \pm 23.3
8th week	48.2 \pm 25.1
9th week	50.1 \pm 16.2
10th week	18.4 \pm 9.6
ANOVA (<i>p</i> value)	0.3

When we assessed the best cut-off points and the corresponding validity tests values for hCG level in the prediction of EP women from healthy pregnant women in the first trimester, we found that a cut-off hCG level of \leq 4250 had high validity results (90% sensitivity, 90% specificity, 90% PPV, 90% NPV and 90% accuracy).

While there were no significant differences observed in PLGF mean according to age group of women with EP ($p=0.2$) (Table 4), highly significant differences were observed in PLGF mean according to parity of women with EP ($p<0.001$) (Table 5).

Although there were no significant differences observed in PLGF mean according to the gestational age of women with ectopic pregnancy ($p=0.3$) (Table 6), highly significant differences were observed in PLGF mean according to BMI of women with EP ($p<0.001$) (Table 7).

Table 7 Distribution of PLGF mean according to BMI of ectopic pregnant women

BMI	PLGF Mean \pm SD
Normal	40.8 \pm 22.3
Overweight	33.1 \pm 22.2
Obese	29. \pm 25.1
ANOVA (<i>p</i> value)	< 0.001

Table 8 Distribution of HCG and PLGF according to the rupture of ectopic pregnancy

Variable	Ruptured Mean \pm SD	Un-ruptured Mean \pm SD	<i>t</i> test	<i>p</i>
HCG, mIU/mL	2420.5 \pm 1000.1	2599.5 \pm 998.8	0.09	0.4
PLGF, pg/mL	11.3 \pm 5.3	12.7 \pm 5.1	1.36	0.1

No significant difference was observed in the mean hCG between ruptured and un-ruptured EPs ($p=0.4$). However, the mean hCG of un-ruptured EP was higher than the ruptured EP. No significant difference was observed in mean PLGF between ruptured and unruptured EP. Additionally, the mean PLGF in un-ruptured EP was higher than ruptured (Table 8).

Discussion

Despite the increase in EP incidences, we notice a marked decrease in its mortality rate. This is mainly imputed to earlier diagnosis and the advances of diagnostic methods. Besides, the evolution in the surgical approach to EP allowed earlier intervention, prohibited major complications and life-threatening events, and maintained fertility for possible future pregnancy [13]. Moreover, it is crucial to identify the main factors that may lead to EP, which could be considered the first step for proper management. Indeed, multiple factors are considered as relative risks for the development of EP. Theoretically, all causes that interfere and delay the blastocyst migration to the endometrial cavity can lead to an EP [14].

The main finding in the present study was a highly significant decrease in the level of serum PLGF in the EP patients compared to that in the healthy first-trimester pregnant women, which is in agreement with Daponte et al. [15] study which revealed that the concentration of PLGF was markedly less in women with a missed abortion (16.25 \pm 4.73 pg/mL) and women with EP (14.60 \pm 3.42 pg/mL) when compared to women with viable intrauterine pregnancy (21.64 \pm 5.68 pg/mL; $p=0.001$). In his study, Petrelli et al. [16] mentioned that patients EP had marked lower concentrations of PLGF

compared to threatened abortions and physiological gestation. Additionally, Horne et al. [17] revealed that the concentration of PLGF was imperceptible in patients with ectopic tubal pregnancy. Similarly, it was either imperceptible or lower in patients with miscarriage than in women with viable intrauterine pregnancy.

The current study revealed that the PLGF is a predictor of EP with a higher sensitivity than specificity. Simultaneously, the positive predictive value was better than the negative predictive value with the area under the curve (AUC = 0.90) and the best cut-off value at ≤ 15.5 pg/mL. This high sensitivity can make PLGF a useful screening tool for suspected EP in emergency departments or other healthcare facilities. The elevated PPV reinforces this. Nevertheless, PLGF had a suboptimal capability for ruling out EP due to average specificity.

When we reviewed the published studies in this field, we recognized that Daponte et al. [15] found that PLGF, with a cut-off point of 15.7 pg/mL, had a high accuracy in diagnosing EP (AUC = 0.822). Similarly, Shafik A [18] found that PLGF had a sensitivity of (92.3%) and a specificity of (92.3%) with slightly better NPV than PPV and the best cut-off value at 20 pg/mL.

Regarding the hCG, the current study concluded that a cut-off hCG level of ≤ 4250 had an appropriate predictive value. This can help physicians predict EP in susceptible patients due to its relevant sensitivity, specificity, PPV, and NPV. These values are better than those found by Shafik [18] where the cut-off hCG level of ≤ 3024 IU/ml gave 57.7% sensitivity, 61.5% specificity, 60% PPV, and 59.3% NPV.

Regarding the relation between PLGF mean and parity of EP women, our study found that the mean PLGF increased significantly when the parity increased. Simultaneously, Masoud's study [19] found that when the parity increased, the mean PLGF increased but not at a significant level.

Malak et al. [13] revealed that the mean BMI was 23.8 ± 1.0 (healthy weight) in the EP group, which is not in agreement with the current study's mean BMI of 28.1 ± 1.2 (overweight). Moreover, Moini et al. [14] found that the mean BMI was (25.9 ± 3), which is less than that of the present study. This may be due to differences in sample size collection in addition to the differences in habitual diet.

The current study found that highly significant differences were observed in PLGF mean according to the BMI of women with EP. When the BMI (obesity) increased, the level of the PLGF decreased, which agrees with Zera et al. [20] who found that higher BMI was associated with lower sFlt-1 value and lower PLGF. This indicates that obese women may have an abnormality in angiogenesis by relatively low levels of sFlt-1 and low levels of PLGF.

Limitations of the study

The PLGF test is not available in the hospital where the study was conducted. Thus, we had to prepare this test for the hospital laboratory at our expense. Furthermore, no previous studies on this factor were done in our hospital or our country to compare with. Additionally, limited recourses were available on this topic for more detailed information and comparison.

Conclusion

The serum level of PLGF seems to be a promising biomarker for diagnosing EP as highly significant difference was found between healthy pregnancy and EP. Thus, further research with larger sample sizes to evaluate the effect of other parameters on the PLGF level like parity and BMI are needed.

Author contributions AAZ: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation, Visualization and Project administration. MME: Conceptualization, Software, Validation, Writing—Original Draft, Writing—Review and Editing, Visualization and Supervision.

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Code availability Not Applicable.

Compliance with ethical standards

Conflict of interest We have no conflicts of interest to disclose.

Ethics approval This study was approved by the Iraqi Board's Obstetrics and Gynaecology committee for medical specialization and the Department of Obstetrics and Gynaecology of Baghdad Teaching Hospital. Approval Number: 365 Date: 15/06/2017". The Iraqi Board that functions under the Ministry of Education is considered the higher authority that supervises scientific research and contains an ethics committee to approve studies on humans.

Consent to participate The patients consent to the publication has been taken.

Consent for publication The consent to the publication has been taken.

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