

## Serum Progesterone & CA125 Levels as an Aid in the Prediction of Ectopic & Intrauterine Pregnancy

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### ABSTRACT:

#### BACKGROUND :

Prompt diagnosis of ectopic pregnancy is a difficult dilemma, and reliable predictors for diagnosis and differentiation of ectopic pregnancy from early pregnancy miscarriage are needed.

#### OBJECTIVE:

To evaluate the diagnostic value of serum progesterone and CA-125 levels in ectopic pregnancy and early intra uterine miscarriage.

Study design: A prospective case control study

Setting: The study was carried out in the department of obstetrics and gynecology of AL-Yarmouk teaching hospital (Baghdad – Iraq) from February 2011 to February 2012

#### PATIENTS AND METHODS:

This study was carried out on sixty pregnant women in their first trimester. Twenty women diagnosed as unruptured ectopic pregnancy; treated by laparotomy, twenty women diagnosed as inevitable miscarriage; treated by curettage and twenty women with healthy pregnancy of a comparable gestational age as control group; follow up of this group was done to ascertain uneventful pregnancy with no complications.

We compared the serum levels of progesterone and CA-125 in all groups at the time of presentation and 24h after surgical intervention in first and second groups.

#### RESULTS :

There was a significant decrease in mean post operative progesterone level in women with ectopic pregnancy and miscarriage groups with significant difference ( $P < 0.0001$ ). The study observed that the mean level of progesterone in women with ectopic group ( $6.76 \pm 3.63$  ng/ml) was lower than that in miscarriage group ( $15.17 \pm 6.01$  ng/ml) and control group ( $17.58 \pm 4.57$  ng/ml) with significant difference ( $P < 0.0001$ ). This study also observed that the mean level of CA-125 in women with miscarriage group ( $117.07 \pm 94.30$  U/ml) was higher than that in ectopic group ( $38.11 \pm 28.79$  U/ml) and control group ( $30.51 \pm 16.10$  U/ml) with statistically significant difference ( $P < 0.0001$ ).

There was a significant decrease in mean level of post operative CA-125 in women with ectopic pregnancy and miscarriage groups with significant difference ( $P < 0.0003$ ).

#### CONCLUSION:

Combined measurement of both serum progesterone and CA-125 can be sensitive predictors in diagnosis and differentiation of ectopic pregnancy from inevitable miscarriage and normal pregnancy.

**KEY WORDS:** ectopic pregnancy, miscarriage, progesterone, CA125.

### INTRODUCTION:

Ectopic pregnancy (EP) is one of abnormal gestation that implantation occurred outside endometrial uterine cavity, which tubal pregnancy is the most common site<sup>(1)</sup>. The IVF

and related treatments increase the likelihood of EP, reaching up to 1-3%, which twice the normal rate<sup>(2)</sup>. The etiology of EP may be due to tubal factor, zygote abnormalities ovarian factors, exogenous hormones like Progestin only oral contraceptive<sup>(3)</sup>, ovulation induction: 10-15% of EP occurs after IVF<sup>(4)</sup>. IUCD, Smoking & Increasing age.<sup>(3,5)</sup>

These may be investigated through repeated serum  $\beta$ -hCG measurements are

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taken, *transvaginal sonography (TVS)*, while Laparoscopy remains the gold standard for the diagnosis of EP<sup>(6)</sup>.

Spontaneous Miscarriage is a pregnancy loss spontaneously before the fetus has reached a viable gestational age, at present it is at or before 24 weeks gestation<sup>(2)</sup>.

It can be due to: Embryonic abnormalities<sup>(7)</sup>. Maternal factors (Systemic disease, Maternal infections, Diabetes mellitus, Severe Hypertension, Renal Disease, Thyroid Disease, Abnormalities of the reproductive system, Immunological disorders, Severe malnutrition & Trauma)<sup>(3,7)</sup>.

These may be investigated through: Serum human Chorionic Gonadotrophin as Low non doubling levels of  $\beta$ hCG, or  $\beta$ hCG result that reduce before the expected time of the  $\beta$ hCG peak all are strong indicators of an impending spontaneous miscarriage<sup>(8)</sup>. *Transvaginal sonography (TVS)* allows for precise dating, particularly of very early pregnancy, it's also an efficient and accurate means of verifying expulsion of the gestational sac after medical abortion<sup>(9)</sup>.

Progesterone is secreted during early pregnancy from the ovary by corpus luteum, it is an essential hormone for the establishment and maintenance of pregnancy by inducing secretory changes in the lining of the uterus which is important for implantation of the fertilized ovum<sup>(10)</sup>. The lowest serum Progesterone concentration associated with a viable 1<sup>st</sup> trimester pregnancy is 5.1ng/ml, and a single serum Progesterone measurement of at least 25ng/ml carries 97% likelihood for viable intrauterine pregnancy, being more sensitive than two serial  $\beta$ hCG measurements<sup>(11)</sup>.

CA-125 levels are increased in early pregnancy, levels peak during the 1<sup>st</sup> trimester of pregnancy, between 6-7 weeks and drop to non pregnant values in 2<sup>nd</sup> and 3<sup>rd</sup> trimesters<sup>(12)</sup>. It is also elevated immediately after birth, implicating the disintegration of the maternal decidua (i.e.: blastocyst implantation and placental separation) as a possible cause of the tumor marker elevation<sup>(13)</sup>. The significant increase in serum CA-125 levels was also reported in a group of patients with vaginal bleeding and impending spontaneous miscarriage<sup>(15)</sup>.

Women who have EP (ruptured or unruptured) are more likely to have elevated levels of CA-125 compared with women who have intrauterine pregnancy. An elevation in maternal CA-125 is

dependent on the extent of decidual disruption<sup>(14)</sup>.

Numerous studies have been attempted to demonstrate as a marker for diagnoses ectopic pregnancy with the rationale that ectopic pregnancy had lower CA-125 level than normal pregnancy because placenta cannot grow as well as normal. In addition, the results from these studies were inconclusive in view of diagnostic purpose and predicting value of rupture<sup>(15)</sup>.

### **PATIENTS AND METHODS:**

This prospective control study was carried out in the department of obstetrics and gynecology of Al-Yarmook teaching hospital, Baghdad-Iraq. The study was approved by the Iraqi scientific council of obstetric & gynecologist specialization from February 2011 to February 2012 & Verbal consents were obtained from all patients before enrolling them in the study. It includes 78 pregnant women of reproductive age, in first trimester pregnancy. They were subdivided into three groups as follows:

- Group A: 20 pregnant patients presented with abdominal pain  $\pm$  vaginal bleeding, diagnosed as EP by ultrasonography combined with measurement of serum  $\beta$ hCG. They were treated by laparotomy (salpingectomy) and confirmed by histopathology. Other 18 patients diagnosed with EP were excluded from the study as far as they showed one or more of the exclusion criteria listed below.
- Group B: 20 pregnant patients presented with abdominal pain  $\pm$  vaginal bleeding, diagnosed as inevitable miscarriage by clinical examination, combined with ultrasonography that showed intrauterine gestation. They were treated surgically by curettage and confirmed by histopathology.
- Group C: 20 healthy pregnant women with no risk factors were selected as a control group with a comparable gestational age that was determined by an accurate last menstrual period. A single intrauterine pregnancy was visualized by ultrasonography which also confirms the viability and localization of the pregnancy and excludes other pelvic pathology.

Pregnancy follow up of the pregnant women included in the control group was done in antenatal clinic. The exclusion criteria include women with complete and incomplete miscarriage, Women with ruptured and aborted ectopic pregnancy, Twin pregnancy, Maternal

## PREDICTION OF ECTOPIC & INTRAUTERINE PREGNANCY

disease that elevate serum CA-125 ,Women conceived after ovarian stimulation ,Women with ectopic pregnancy treated by medical or expectant management.

The patients were collected from outpatient clinic and inpatient in the gynecological department of Al-Yarmook hospital. They were chosen by simple collection criteria.

Questionnaire format was done to evaluate many of the dependent variables such as patient age, gestational age, parity, vaginal bleeding, abdominal pain, medical history, drug history and previous obstetric and gynecological history; they were subjected for abdominal and pelvic assessment.

The investigation (laboratory measurements of progesterone, CA-125 and  $\beta$ hCG) we used progesterone enzyme immuno assay kit and CA-125 enzyme immuno assay kit from biocheck, Inc323 Vintage Park drives Foster City, CA94404 , While for  $\beta$ hCG concentration in human serum we use enzyme linked immunosorbent assay (ELISA) test from human Gesellschaft für Biochemica und diagnostica mbH- Germany. All were done in Laboratory department of Al-Yarmook hospital.

### RESULTS:

Table (1) shows the characteristics of the study groups in relation to the means of maternal age , parity ,gestational age & BMI

The mean age in women with EP group was slightly higher than in women with miscarriage group and control group ( $29.30 \pm 5.71$ ,  $27.65 \pm 6.71$  and  $25.90 \pm 5.10$  years) respectively. There was no statistically significant difference in mean maternal age among the three groups ( $P=0.197$ )

When compared to control pregnancy with mean value of  $8.85 \pm 1.35$  week. There was statistically significant difference in the mean gestational age among the three comparable groups ( $P<0.0001$ ).

The mean parity in women with miscarriage group was higher than that in women with EP group and control group ( $2.25 \pm 2$ ,  $1.90 \pm 1.59$  and  $1.10 \pm 0.97$ ) respectively. There was no statistically significant difference between the mean parity among the three comparable groups ( $P=0.069$ ).

The mean BMI nearly equal in the three groups ( $25.8 \pm 2.95$   $\text{kg/m}^2$  in women with EP group,  $25.65 \pm 3.1$   $\text{kg/m}^2$  in women with miscarriage group and  $25.65 \pm 3.84$   $\text{kg/m}^2$  in women with control group). There was no statistically significant difference in mean BMI ( $P=0.98$ ) among the three comparable groups.

**Table 1: characteristics of the study groups.**

	Ectopic pregnancy		Miscarriage		Control		P
	No	%	No	%	No	%	
Maternal age Mean $\pm$ SD	29.30 $\pm$ 5.71	20-43	27.65 $\pm$ 6.71	16-38	25.90 $\pm$ 5.10	19-37	0.197
Gestational age Mean $\pm$ SD	6.95 $\pm$ 1.61	5-10	9.55 $\pm$ 1.00	6-10	8.65 $\pm$ 1.35	6-10	0.0001*
Maternal parity Mean $\pm$ SD	1.90 $\pm$ 1.59	0-5	2.25 $\pm$ 2.00	0-6	1.10 $\pm$ 0.97	0-3	0.069
BMI (Kg/m <sup>2</sup> ) Mean $\pm$ SD	25.80 $\pm$ 2.95	21.0-32.0	25.65 $\pm$ 3.10	21.0-32.0	25.65 $\pm$ 3.84	19.0-32.0	0.986

\* Significant using Pearson Chi-square test for difference between proportions or ANOVA test for difference among more than two independent means at 0.05 level of significance.

Table (2) shows the distributions' of the hCG , progesterone & CA125 in the studied groups

For hCG, there was a statistically significant difference between mean pre-operative and post-operative hCG in women with miscarriage group ( $P<0.001$ ) and in women with EP group ( $P<0.004$ ) .

For progesterone, there was a significant difference between mean pre-operative and post-operative

progesterone in women with EP group and miscarriage group ( $P<0.0001$ ) for both.

For CA125, there was statistically significant difference between mean pre-operative and post-operative CA-125 in women with miscarriage group and EP group ( $P<0.001$ ) for both

**PREDICTION OF ECTOPIC & INTRAUTERINE PREGNANCY**

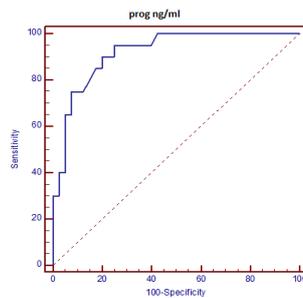
**Table 2: Distributions' of the hCG , progesterone & CA125 in the studied groups.**

	Ectopic pregnancy		Miscarriage		Control		p
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	
hCG (IU/L) Pre-operative	3053.4±3158.1	154.0-9506.0	8376.6±6549.3	197.0-17603.0	35567.2±26269	3266.0-79331.0	0.0001*
Post-operative 24h after	1933.8±2252.3	103.0-6322.0	4890.6±5114.3	123.0-16004.0	-	-	0.023*
P	0.004*		0.001*		-		
Prog (ng/ml) Pre-operative	6.75±3.63	2.2-16.3	15.17±6.01	3.9-23.2	17.58±4.57	8.9-25.3	0.0001*
Post-operative 24h after	5.05±2.66	2.1-12.2	10.68±4.52	2.9-16.9	-	-	0.0001*
P	0.0001*		0.0001*		-		
CA-125 (U/ml) Pre-operative	38.11±28.79	9.1-116.9	117.07±94.30	12.9-310.1	30.51±16.10	5.5-61.3	0.0001*
Post-operative 24h after	28.79±23.55	6.8-94.2	65.83±46.15	6.5-162.1	-	-	0.003*
P	0.001*		0.001*		-		

\* Significant using Pearson Chi-square test for difference between proportions or ANOVA test for difference among more than two independent means at 0.05 level of significance

**Sensitivity and specificity of progesterone in ectopic pregnancy.**  
The ROC curve demonstrated a significant discriminatory ability of low progesterone level

for the diagnosis of ectopic pregnancy. Cut off Value for Progesterone among women with ectopic pregnancy versus controls.



**Figure 1: receiver operator characteristic curve (ROC) of low progesterone for the diagnosis of ectopic pregnancy.**

**Sensitivity and specificity of CA-125 in miscarriage.**

The ROC curve demonstrated a significant discriminatory ability of high CA-125 level for the diagnosis of miscarriage.

Cut off Value for CA 125 among women with miscarriage versus controls .

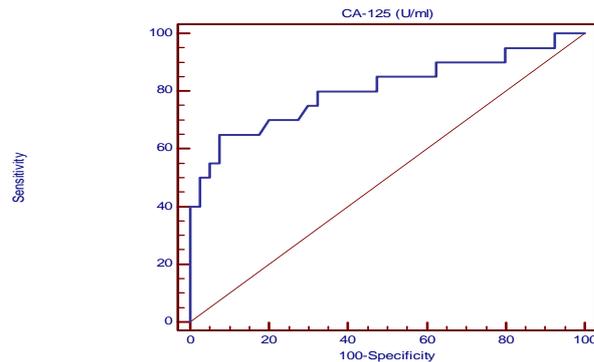


Figure 2: Receiver operator characteristic curve (ROC) of high CA-125 levels as diagnostic test for miscarriage.

When using a combination of both of cut-off (90%), specificity (85%), positive predictive values (progesterone  $\leq 11.7$  ng/ml and CA-125  $\leq 36.7$  U/ml) for the diagnosis of EP, value (85.7%) and negative predictive value (89.4%). significant result was obtained with sensitivity

Table 3: Relation between cut-off value of progesterone and CA-125 levels and type of pregnancy.

	Type of pregnancy		
	EP	Miscarriage	Control
progesterone $\leq 11.7$ ng/ml + CA-125 $\leq 36.7$ U/ml	18	4	2
Total	20	20	20

A combination of both progesterone and CA-125 measurements showed higher differential accuracy than using either analysis alone.

**DISCUSSION:**

The diagnosis of an early ectopic pregnancy remains difficult, and about 40% of cases the initial diagnosis is erroneous and a nonviable intrauterine pregnancy is later found<sup>(16)</sup>. The investigation process may be long and thus involve significant psychological morbidity. Nearly half the patient (47%) required at least three visits in order to diagnose or exclude ectopic pregnancy<sup>(17)</sup>.

Investigation of new serum markers could result in early diagnosis, leading to timely implication of medical treatment and preservation of tubal rupture and its complications and allowing for subsequent preservation of patient fertility<sup>(18)</sup>.

In the present study the mean level of progesterone in women with EP was significantly lower than the mean level of progesterone in women with inevitable miscarriage and normal pregnancy ( $6.76 \pm 3.63$ ,  $15.17 \pm 6.01$  and  $17.58 \pm 4.57$  ng/ml) respectively with ( $P < 0.001$ ).

In the Abdul-Hussein MM et al study, the receiver operating characteristic (ROC) analysis, comparing ectopic pregnancy versus normal

pregnancy group, the ROC curves demonstrated a significant discriminatory ability of decreased CA-125 levels for diagnose ectopic pregnancy and the cut-off point of this study was CA-125 level of equally or less than 30 IU/ml., which had the highest efficiency of test. Similar findings have previously been reported.<sup>(15)</sup>

The present study also in agreement with Daponte et al,<sup>(19)</sup> observed decrease of progesterone in women with EP, but are not discriminate enough to be diagnostic in isolation. In the present study, we found that serum progesterone can be used as a valuable marker to distinguish ectopic pregnancy from intra uterine pregnancy and miscarriage, but to improve specificity; we found that using other marker (CA-125) has the ability to discriminate ectopic pregnancy from miscarriage.

The present study observed that the mean level of CA-125 in women with inevitable miscarriage was significantly higher when compared with the mean levels in women with EP and women with intrauterine pregnancies ( $117.07 \pm 94.30$ ,

38.11±28.79 and 30.51±16.10 Iu/ml respectively, with (P<0.0001).

Schmidt et al study , They concluded that a single serum measurement of CA-125 in symptomatic 1<sup>st</sup> trimester pregnant patients failed to discriminate EP from miscarriage and normal pregnancy which disagree with our study <sup>(20)</sup>.

The present study in agreement with Fiegler et al, they observed that all women with symptoms of imminent miscarriage had elevated level of CA-125

Derwish et al study , They observed that the level of CA-125 in women with miscarriage had no significant difference when compared with women of normal pregnancies and the value of CA-125 in women with history of recurrent miscarriage was still unclear and cannot be recommended as routine bases which disagree with our study <sup>(21)</sup>.

The present study in agreement with Ayaty et al study , they observed that the mean CA-125 level was higher in women with threatened miscarriage that finally aborted (58.17±7.25Iu/ml) than those in women with threatened miscarriage that pregnancy continued and did not abort .

Finally the present study was in consistent with Katsikis et al . They observed that combined measurement of progesterone and CA-125 levels is useful in discriminating EP from inevitable miscarriage and normal gestation <sup>(22)</sup>.

**CONCLUSION:**

Combined measurement of serum progesterone and CA-125 levels have higher differential accuracy of ectopic pregnancy from inevitable miscarriage or normal gestation.

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