

## EVALUATION OF THE IMPORTANCE OF BIOCHEMICAL PARAMETERS IN RELATION TO ULTRASONOGRAPHIC FINDING IN ECTOPIC PREGNANCY DIAGNOSIS

Dragan Lončar

The implantation of the fertilized egg outside the uterine cavity leads to the development of ectopic pregnancy. The incidence of ectopic pregnancy is 1/100 births. The most common place of ectopic implantation of the fertilized ovum is the oviduct (98%) with predilection for the ampullar part of the Fallopian tube. The aim of this study was to determine the predictive importance of beta-hCG and progesterone concentration compared to ultrasonographic finding in the ectopic pregnancy diagnosis.

We examined 24 patients with ectopic pregnancies which we divided according to the days of amenorrhea into two groups: the first group with the total of 28 patients from 16–42 days and another group of 8 patients with amenorrhea longer than 42 days. The control group was comprised of 20 patients with vital intrauterine pregnancy, gestational age of 42–52 days. Blood samples for quantitative determination of hormones were collected on three occasions after 48 hours in the forenoon time in the examined and control group of pregnant women. Ultrasonographic examinations of all pregnant women were carried out immediately after blood sampling, with the transvaginal approach using "make loop" option, and measurements with an accuracy of 0.1 mm.

Mean values for beta-hCG range from 698–1774 mIU/ml in the first group of pregnant women, and in the second group of 1896 mIU/ml to 4410 mIU/ml with a statistically significant difference compared to the values in the control group ( $p < 0.001$ ). The concentration of progesterone in the first group of women ranging from 41–70 nmol/l, and in the second group of 76–94 nmol/l which is also the statistically significant difference compared to the control group ( $p < 0.002$ ). We have shown that ultrasonographic finding with its parameters reliably predicts the values of biochemical parameters both in normal intrauterine pregnancy and in the case of ectopic pregnancy.

Embryo viability and implantation place condition the values of biochemical parameters, which makes establishing the correct diagnose difficult by following only these markers. It is necessary to combine the growth dynamics of these hormones with ultrasonographic finding as the gold standard in diagnosing ectopic pregnancy. *Acta Medica Medianae 2011;50(3):16-21.*

**Key words:** ectopic pregnancy, human chorionic gonadotropin, progesterone, ultrasonography

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### Introduction

Implantation of the fertilized egg outside the uterine cavity leads to the development of ectopic pregnancy. The incidence of ectopic pregnancy is 1/100 births (1). The most common site of ectopic implantation of the fertilized ovum is the oviduct (98%) with predilection for the ampullar part of the Fallopian (Fallopian) tube (2). About 17% of women achieve their first pregnancy as ectopic, and around 40% of these women will not be able to have regular spontaneous pregnancy later (3). After surgeries, 50% of women will no longer become pregnant, and the risk of repeated

ectopic pregnancy is 10–15%. After the micro-surgery with the ectopic pregnancy 1/3 of women will never become pregnant, 1/3 of women will again have an ectopic pregnancy and 1/3 of women will have a regular spontaneous pregnancy (4). Human chorionic gonadotropin (hCG) is a glycoprotein secreted by syncytiotrophoblast cells. In ectopic pregnancy, the concentration of beta-hCG is lower in 85% of the cases than the level of beta-hCG found in normal pregnancy at a similar gestational age (5). In 50% of women with ectopic pregnancy, the level of beta-hCG has a discontinuous growth alternating with phases of decline (6). In the case of ectopic pregnancy, corpus luteum secretes a smaller amount of progesterone than in normal pregnancies of the same gestational age. However, not even the known value of progesterone can differentiate abnormal pregnancy inside the uterus from an ectopic pregnancy (7). Low level of progesterone shows the suspected viability of the pregnancy. An important method as a supplement to the

quantitative determination of beta-HCG and progesterone is a transvaginal ultrasonography, which can identify pregnancy in the uterus already with the concentration of beta-hCG of 1500 mIU/ml in 70% of cases, and always when the level of beta-hCG exceeds 2500mIU/ml ie. about 5 to 6 weeks after the last menstrual period (8).

### Aim

The aim of this study was to determine the predictive significance of biochemical parameters in relation to ultrasonographic finding in the diagnosis of ectopic pregnancy.

### Methods

A prospective, observational study was carried out at the Department of Gynecology and Obstetrics, Clinical Center Kragujevac in the years 2009 and 2010. During the research we used a clinical-experimental study model. The study was approved by the Ethics Committee of Clinical Centre of Kragujevac. In the research period, 24 patients were hospitalized with suspected ectopic pregnancy. The algorithm of establishing the ectopic pregnancy diagnosis in all patients consisted of the following procedures, or criteria: absence of menstruation, absence of gestational sac in the uterine cavity (confirmed by ultrasound examination), the increase in levels of beta-hCG, and/or histological verification of curettage from the uterus. Patients were divided according to the days of amenorrhea into two groups: the first group with the total of 16 patients from 28–42 days and another group of 8 patients with amenorrhea longer than 42 days. The control group was comprised of 20 patients with vital intrauterine pregnancy, gestational age of 42-52 days.

Blood samples were collected on three occasions after 48 hours in the forenoon time in groups of patients. Ultrasonographic examinations of all pregnant women were carried out immediately after blood sampling, with the transvaginal approach using "make loop" option, and measurements with an accuracy of 0.1 mm. Quantitative measurements of beta-hCG level were determined from venous blood of patients using the commercial test of the company DPC-USA. Tests were based on the analytical immunochemiluminescence assay and were realized by using the automated analyzer IMMULITE 2000 Manufacturer of analyzer is also the firm Diagnostics Product Corporation (DPC), Los Angeles, California, USA. The assessment of progesterone concentration was performed in the Laboratory for Nuclear Medicine in the Clinical Center of Kragujevac by applying the radioimmunoassay method (RIA) that uses marked progesterone with a J-125 (reagent set "INEP" - Zemun, Serbia). We performed ultrasonographic examinations of pregnant women by transvaginal probe 6.5 MHz, apparatus GE Volusion 730 3D/4D Ultrasound System, Northern Virginia (Washington, USA).

All the obtained results were deposited into the unique data base with required logistic control. Statistical analysis included calculation of mean values and standard deviations (SD) for each numerical parameter and analysis of the obtained value in relation to the subgroups (t-test, Mann-Whitney-u) using the statistical program SPSS 17.

### Results

Growth dynamics of beta-hCG concentration in the examined groups of pregnant women with ectopic pregnancy is shown in Tables 1 and 2. Statistically significant difference in their distribution is demonstrated in relation to the concentration of this parameter in the control group of pregnant women, Table 3.

Table 1. The display of beta-hCG concentration (mIU/ml) in the examined sample, the first group of pregnant women

First group 28- 42 days of amenorrhea	N	Mean	Standard Deviation	T	Df	P
b-hCG <sub>1</sub>	16	698.18	61.78	-19.53	15	0.000
b-hCG <sub>2</sub>	16	1160.00	147.87	4.32	15	0.001
b-hCG <sub>3</sub>	16	1774.25	334.32	9.26	15	0.000

b-hCG<sub>1,2,3</sub> - levels of b-hCG collected on three occasions after 48 hours

Table 2. The display of beta-hCG concentration (mIU/ml) in the examined sample, the second group of pregnant women

Second group >43 days of amenorrhea	N	Mean	Standard Deviation	T	Df	P
b-hCG <sub>1</sub>	8	1896.25	197.11	-11.53	7	0.000
b-hCG <sub>2</sub>	8	2946.25	269.21	4.68	7	0.002
b-hCG <sub>3</sub>	8	4410.87	798.05	6.77	7	0.000

b-hCG<sub>1,2,3</sub> - levels of b-hCG collected on three occasions after 48 hours

Table 3. The display of beta-hCG concentration (mIU/ml) in the examined sample, the control group of pregnant women

Control group 42-52 days of amenorrhea	N	Mean	Standard Deviation	T	Df	P
b-hCG <sub>control 1</sub>	20	3472.75	599.40	-22.58	19	0.000
b-hCG <sub>control 2</sub>	20	6227.15	527.70	-2.31	19	0.032
b-hCG <sub>control 3</sub>	20	1054.,65	782.98	23.08	19	0.000

b-hCG<sub>control 1,2,3</sub> - levels of b-hCG collected on three occasions after 48 hours

Table 4. The display of progesterone concentration (mIU/ml) in the examined sample, the first group of pregnant women

First group 28-42 days of amenorrhea	N	Mean	Standard Deviation	T	Df	P
Prg <sub>I</sub>	16	41.43	1.75	-31.00	15	0.000
Prg <sub>II</sub>	16	46.31	1.25	-27.80	15	0.000
Prg <sub>III</sub>	16	70.62	1.66	37.46	15	0.000

Prg<sub>I, II, III</sub> - levels of progesterone collected on three occasions after 48 hours

Table 5. Display of the progesterone concentration (nmol/l) in the examined sample, the second group of pregnant women

Second group > 43 days of amenorrhea	N	Mean	Standard Deviation	T	Df	P
Prg <sub>I</sub>	8	76.87	1.64	-13.99	7	0.000
Prg <sub>II</sub>	8	91.25	2.37	7.44	7	0.000
Prg <sub>III</sub>	8	94.50	4.56	5.88	7	0.001

Prg<sub>I, II, III</sub> - levels of progesterone collected on three occasions after 48 hours

Table 6. Display of the progesterone concentration (nmol/l) in the examined sample, the control group of pregnant women

Control group 42-52 days of amenorrhea	N	Mean	Standard Deviation	T	Df	P
Prg <sub>control 1</sub>	20	113.80	2.60	-19.20	19	0.000
Prg <sub>control 2</sub>	20	208.20	285.96	1.30	19	0.209
Prg <sub>control 3</sub>	20	157.05	2.91	49.24	19	0.000

Prg<sub>control 1,2,3</sub> - progesterone levels collected on three occasions after 48 hours

Table 7. The display of ultrasonographic parameters in the examined groups of pregnant women

Ultrasonographic finding					
First group N=16; 28.- 42. days of amenorrhea		Second group N=8; >43. days of amenorrhea		Control group N=20; 42.-52. days of amenorrhea	
GS <sub>1</sub> mm/SD/p	FH i /ili YS <sub>1</sub>	GM <sub>2</sub> mm, SD/p	CRL <sub>2</sub> mm/SD/p	GM <sub>Control</sub> mm/SD/p	CRL <sub>Control</sub> mm/SD/p
3.62±1.02 p=0.000	-/-	24.12±1.12 p=0.000	6.65±1.06 p=0.000	25.22±.2.12 p=0.003	8.09±1.13 p=0.001
11.50±1.15 p=0.209	-/+	30.25±1.38 p=0.000	9.00±1.30 p=0.068	28.10±2.54 p=0.002	10.29±1.63 p=0.000
18.12±1.36 p=0.000	+/+	35.37±1.06 p=0.000	12.62±1.06 p=0.000	30.05±2.90 p=0.000	14.55±2.01 p=0.004

\*GS-(Gestational Sac); CRL (Crown Rump Length); YS (Yolk Sac); FH (Fetal hearth)

Tables 4 and 5 show the distribution of the progesterone level in the examined sample of pregnant women in different periods of amenorrhea during the ectopic pregnancy. Statistically significant difference in secretion of this hormone is demonstrated in relation to their levels at the intrauterine pregnancy, Table 6.

The importance of information about the pregnancy viability and place of implantation of a fertilized egg that we followed by transvaginal ultrasonography are presented in Table 7. The statistically significant difference is shown in morphological parameters of early gestation, which is fully consistent with the levels of examined

biochemical parameters ( $p < 0.001$ ) comparing the ectopic and intrauterine pregnancy.

Ultrasonographic parameters of pregnancy viability and place of implantation of ovulum reliably predict the quantitative values of measured hormones in the examined pregnant women.

## Discussion

The key to the interpretation of quantitative beta-hCG value is not in its number, but in the growth dynamics (2, 9). In a normal pregnancy, beta-hCG increases so that the average value doubles every two days. For this reason, the beta-hCG test is usually repeated two days after the first test to see if the "beta" properly "doubles". As the pregnancy progresses and the value of beta-hCG grows, the "doubling" time also grows (10). Pregnancies that end with miscarriage or ectopic pregnancy show lower values as a rule and a slower time of growth, although some normal pregnancy may also have lower values of hCG (10). Some believe that a shorter, "doubling" time represents multiple fetuses, which is not true according to some researches, although it has been observed that women with multiple pregnancy have generally higher values of beta-hCG than women with singleton pregnancies (11, 12). One should be careful and not too fond of calculations with numbers in interpreting the results of these tests (13).

In our study, we presented mean values with standard deviations of b-hCG and progesterone and the number of pregnant women examined by weeks of pregnancy, specifically from 4th to 9th weeks. We see that the mean values for b-hCG range from 698-1774 mIU/ml in the first group of pregnant women with a statistically significant difference compared to the values in the control group ( $p < 0.001$ ). In the second group of pregnant women we found values in the range of 1896 mIU/ml to 4410 mIU/ml and confirm a significant difference in the level of beta-hCG ( $p < 0.001$ ) compared to the control group with regular intrauterine pregnancy with the gestation of 6-9 weeks. The progesterone concentration in the first group of women ranges from 41-70 nmol/L, and in the second group of 76-94 nmol/L which is also the statistically significant difference compared to the control group ( $p < 0.002$ ) (Tables 1-6). The progesterone concentration increases progressively after ovulation, reaching the plateau in the next seven days (luteal phase of menstrual cycle) and, if fertilization has occurred, the values of the serum progesterone fluctuate within the plateau during the 6-7 weeks and then progressively increase (14, 15). A medium growth can be observed from the 7th week, and from 8th week a significantly increased secretion of progesterone. The absence of difference in concentrations of progesterone in the 4th and 5th week is realistically expected, because at this stage of pregnancy place of the progesterone creation is corpus luteum (corpus luteum). Significant difference between the 5th and 6th weeks that is 6th and 7th we explain by the ongoing placenta-tion, so the concentration of progesterone is of dual origin - from the corpus luteum and placenta. A

significant increase in the 8th and 9th weeks, we interpret by the increase of secretive capacity of the endocrine placenta. Some authors describe a temporary decline in progesterone concentration between the 5th and 9th weeks, others do not record this fall, but they do not also record the significant increase in progesterone concentrations until the 9th week (12, 16). McCord et al. find progressive increase from the 6th to 9th weeks (17). In their statements, Mol et al. found that during the first four weeks of pregnancy the progesterone concentration increases, that over the following weeks it does not significantly decrease referring to the placental "contribution" in the later stages of pregnancy (7). The importance of determining progesterone can be reduced by the statements of some authors about the existence of daily variations of progesterone concentration in the same pregnant women (16). Mol et al. discuss the fluctuations of progesterone during the day without any concrete evidence and conclusions (7).

Our results, presented in this study, show that current fluctuations are not significant. We have shown statistically significant difference in levels of b-hCG and progesterone levels between the examined and control group of pregnant women in the given gestational framework. The growth dynamics of biochemical parameters in our study is consistent with other studies which confirms the value of the applied algorithm in the ectopic pregnancy diagnosis (17, 18). Ultrasound examination after 5-6 weeks gives much better foundations for predicting the outcome of pregnancy, than only monitoring the value of beta-hCG and progesterone (19). We have shown that ultrasonographic finding with its parameters reliably predicts the values of biochemical parameters in all the examined groups of pregnant women. Viability of pregnancy and implantation place condition the values of biochemical parameters, which makes establishing the correct diagnose difficult by following only these markers (19-21). Transvaginal ultrasonography, as a gold standard, represents an essential link in the modern protocol of the diagnostics of ectopic pregnancy (22, 23) (Table 7).

## Conclusion

Proper growth of biochemical markers with high probability shows the normal course of pregnancy. Growth dynamics of beta-hCG and progesterone values is a more significant parameter of prediction than the specific quantitative values. Ectopic pregnancy is a clinical entity that the clinician sets a number of dilemmas and concerns. Quantitative determination of hormone levels is not a reliable diagnostic procedure, especially not in a single measurement. It is necessary to combine growth dynamics of these hormones with clinical examination and ultrasonographic finding, which remains a gold standard in ectopic pregnancy diagnostics. Numerous scientific reports, as well as the results of our research, have shown especially high level correlation between the ultrasonographic findings and quantitative values of beta-hCG and progesterone in monitoring pregnant women with ectopic pregnancy.

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## EVALUACIJA ZNAČAJA BIOHEMIJSKIH PARAMETARA U ODNOSU NA ULTRASONOGRAFSKI NALAZ U DIJAGNOSTICI EKTOPIČNE TRUDNOĆE

Dragan Lončar

Implantacija oplođene jajne ćelije van materične šupljine dovodi do razvoja ektopičnog graviditeta. Incidenca ektopične trudnoće iznosi 1/100 porođaja. Najčešće mesto ektopične implantacije oplođenog ovuma je jajovod (98%) sa predilakcijom za ampularni deo Falopijeve tube.

Cilj studije bio je da utvrdi prediktivni značaj koncentracije beta-hCG i progesterona u odnosu na ultrasonografski nalaz u dijagnostici ektopične trudnoće.

Ispitali smo 24 bolesnice sa ektopičnom trudnoćom koje smo podelili prema danima amenoreje u dve grupe i to: prva grupa od ukupno 16 bolesnica od 28 do 42 dana i druga grupa od 8 bolesnica sa amenorejom dužom od 42 dana. Kontrolnu grupu činilo je 20 trudnica sa vitalnom intrauterusnom trudnoćom gestacijske starosti od 42 do 52 dana. Uzorci krvi za kvantitativno određivanje hormona su uzimani u tri navrata nakon 48 sati u prepodnevnom terminu u ispitivanoj i kontrolnoj grupi trudnica. Ultrasonografski pregledi svih trudnica vršeni su neposredno nakon uzimanja uzorka krvi, transvaginalnim pristupom sa korišćenjem "make loop" opcije i merenja sa preciznošću od 0,1 mm.

Srednje vrednosti za beta-hCG kreću se u opsegu 698- 1774 mIU/ml u prvoj grupi trudnica, a u drugoj grupi od 1896 mIU/ml do 4410 mIU/ml sa statistički značajnom razlikom u odnosu na vrednosti u kontrolnoj grupi ( $p < 0,001$ ). Koncentracija progesterona u prvoj grupi ispitanica kreće se od 41-70 nmol/l, u drugoj grupi od 76-94 nmol/l, što je takođe statistički značajna razlika u odnosu na kontrolnu grupu ( $p < 0,002$ ). Pokazali smo da ultrasonografski nalaz sa svojim parametrima pouzdano predviđa vrednosti biohemijskih parametara kako u normalnoj intrauterusnoj trudnoći tako i u slučaju ektopične trudnoće.

Vijabilnost ploda i mesto implantacije uslovljavaju vrednosti biohemijskih parametara, što veoma otežava postavljanje tačne dijagnoze praćenjem samo ovih markera. Potrebno je kombinovati dinamiku rasta ovih hormona sa ultrasonografskim nalazom kao zlatnim standardom u dijagnostici ektopičnog graviditeta. *Acta Medica Medianae 2011;50(3):16-21.*

**Ključne reči:** ektopična trudnoća, humani horionski gonadotropin, progesteron, ultrasonografija