Hormonal Biochemical Markers in Ectopic Pregnancy Monitoring

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Ectopic pregnancy is a polymorphic disorder, with difficult early diagnosis, and in late stages it determines serious complications, targeting obstetrical and vital prognosis of the patient. A precise and early diagnosis is supported on ultrasound and dosage of the β-hCG dynamics. The presence of β-hCG, the empty uterine cavity on ultrasound tests, the detection of a periuterine tumor mass leads to the diagnosis of ectopic pregnancy. The treatment of ectopic pregnancy is a medical and surgical one. The medical treatment is preferred by most clinicians when it is possible. Our study aims to diagnose and monitor the tubal ectopic pregnancy under methotrexate treatment through biochemical marker hormone β-hCG and progesterone dosing in dynamics. Success or failure rate of the conservatively treatment with methotrexate depends on the initial β-hCG value. The purpose to spare fertility of the young women who want to procreate has led us to avoid surgery and give more weight to curative therapeutic behavior.

Keywords: ectopic pregnancy, hormonal biochemical markers, methotrexate, female fertility

Ectopic pregnancy defines all forms of abnormal implantation of the gestational sac elsewhere than the pre-established genetic cavity (uterine cavity): uterine tube, peritoneum, abdominal cavity, ovary, spleen, liver, endocol.

The presence of β-hCG (beta-human chorionic gonadotropin), the empty uterine cavity at ultrasound investigation, the detection of a periuterine tumor mass, establish the diagnosis of ectopic pregnancy. In over 95% of cases, ectopic pregnancies are located in the uterine tube [1,2].

By beta-hCG dosing it is possible to confirm the presence of a pregnancy 6 to 10 days after implanting the fertilized egg [3]. The biological role of hCG consists in maintaining the luteal body during pregnancy. Serum and urinary hCG concentration increases exponentially during pregnancy, doubling about 48 hours later and reaching a maximum about 10 weeks later, then progressively decreases until the 20th week of pregnancy, then stays flat until term [4].

The hCG is a hormone of glycoprotein nature, produced by the placenta, consisting of two different subunits: α and β. The subunit α, consisting of 92 amino acids, is identical to that of LH, FSH and TSH. The β subunit contains 145 amino acids and it is specific for the gonadotropin chorionic [5].

In ectopic pregnancy, doubling of hCG values is recorded much slower, at over 48 h [5]. During pregnancy there is also a progressive increase in the level of progesterone between weeks 9 to 32, often 100 times the pre-pregnancy level [3]. Also, in choriocarcinoma or other gestational trophoblastic disease the levels of β-subunit is high [6]. The basal level of other biochemical markers does not influence the rate of ectopic pregnancy after different assisted reproductive techniques or surgical technique [7,8].

Progesterone is a steroid hormone produced by the yellow ovarian body cells physiologically outside the pregnancy, and during pregnancy by the placenta.

In United States, the incidence of ectopic pregnancy is 1-2% of all pregnancies [9]. Recently, Hoover and collaborators in 2010 in their research in women of reproductive age between the ages of 15-44 have found a frequency of 6.4 per 1000 pregnancies [10]. This incidence was explained by increased frequency of sexually transmitted diseases and in particular Chlamydia trachomatis [11], improving early diagnostic methods, using intrauterine devices [12] tubal infertility with restoration of tubal permeability [13], increase in procreation age of women, assisted medical reproduction techniques.

The treatment of the ectopic pregnancy is medical and surgical. The medical treatment is preferred by most of the clinicians when possible. There are used methotrexate, prostaglandin, progesterone-mifepristone antagonists, potassium chloride, injected hyperosmolar glucose in the extrauterine mass [14,15]. Sometimes the life quality can be influenced by the treatment with methotrexate [16].

The methotrexate is a folic acid antagonist that inhibits the growth of rapid growing tissues, acting at the level of DNA and RNA in the syncytio-trophoblast; prevents the synthesis of the nitrate purinic and pyrimidinic bases which enter the DNA duplex. By blocking these biochemical compounds the protein synthesis is inhibited from the level of the blastocyte.

Surgery can be classic and laparoscopic, practicing salpingectomy as a radical method or salpingostomy, salpingotomy, *tubal milking* as a conservative method.

Our study, aims to diagnose and monitor the tubal ectopic pregnancy under treatment with methotrexate through dosage of biochemical markers in dynamics, β-

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hCG and progesterone. Keeping the fertility in young women who want to procreate has determined us to avoid surgical intervention and to give more weight to the therapeutical behavior for curative purposes.

Experimental part

Over a 5-year period, January2013 to January 2017, we analyzed 348 extrauterine pregnancies hospitalized, diagnosed and treated in SCJU Craiova, Obstetrics – Gynecology IInd Department. The study analyzed women ranged from 17 to 45 years of age. In all the cases, haematological and urinary tract investigations have been made, abdominal and transvaginal ultrasound tests to determine the diagnosis and in order to differentiate the intrauterine pregnancy from the incomplete abortion. Not all patients with extrauterine pregnancy could benefit from the treatment with methotrexate.

A total of 89 cases, 25.5% of the 348 extrauterine pregnancies, were studied. When applying the treatment, certain criteria were taken into account: stable patients from the hemodynamic point of view, pregnancy 1-1.5 cm diameter, no contraindications to methotrexate (haematological, hepatic, renal deficiency), absence of active tubal or hemoperitoneum bleeding signs, β-hCG below 15,000 IU / L, absence or presence of cardiac fetal activity.

The 89 patients, aged between 17 and 45 years, were divided into 2 lots, as follows: patients aged between 17 and 30, 26 cases (29.2%) and patients aged 31 - 45 years, 63 cases (70.8%) (table 1) and (fig. 1).
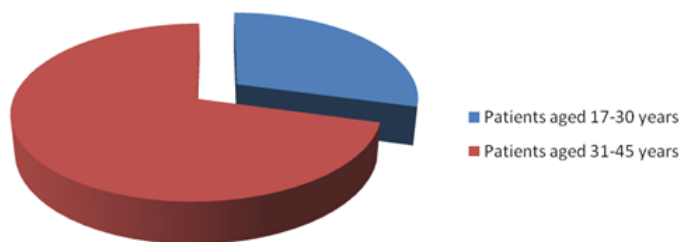

Fig. 1. Case distribution upon patients age

The gestational age of the patients was between 5 and 7 weeks. Age of gestation of 5 weeks was established in 24 cases (27%), of which 20 cases had beta-hCG between 100-1000 IU / L and 4 cases with β-hCG 1000-3000 IU / L. The age of 6 weeks of gestation was established in 33 cases (37%), of which 13 cases had β-hCG between 1000-3000 IU/ L and 20 cases with β-hCG 3000-5000 IU / L. The 7-week gestation age was established in 32 cases (36%),

of which 8 cases had β-hCG ranging from 3000-5000 IU / L and 24 cases with β-hCG 5000-15000 IU / L (table 2).

In the 89 cases, the patients with tubal pregnancy were monitored by dosing in dynamics of the β -hCG and of the plasma progesterone. The dosage of β-hCG has been made in dynamics to track the increase or decrease in values depending on the evolution of the pregnancy. Depending on the initial value of β-hCG, the patients were grouped as follows: 20 cases (22.5%) with β-hCG 100-1000UI / l, 17 cases (19.1%) with β-hCG 1000-3000UI / L, 28 cases (31.4%) with β-hCG 3000-5000UI / L and 24 cases (27%) with β-hCG 5000-15000UI/L (table3), (fig. 2).
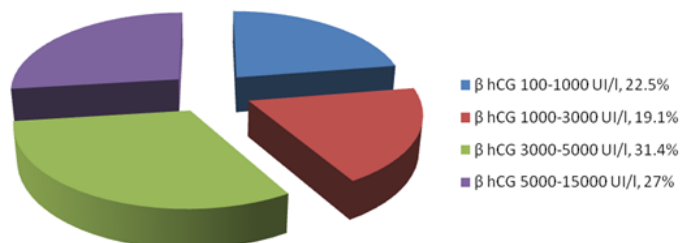

Fig. 2. Cases distribution upon the beta-hCG initial value

The dosage of the progesterone under 5 ng/mL along with ultrasound indicated the ectopic pregnancies in evolution (32 cases, 35.95%). In 57 cases, respectively 64%, the progesteronemy was between 10 and 20 ng/mL (table 4).

The medical treatment applied to these cases was done with intramuscular injection of Methotrexate intra-muscularly in single dose 50mg/m² body surface, repeated as needed. We repeated the dosage of β-hCG on days 4 and 7 after the administration of methotrexate and then weekly until normalization of values.

For the comparison of the statistical samples distribution, we used the χ^2 test, and for the comparison of means, the ANOVA test for multiple samples and the t-Student test for two samples. To test the normality of data distribution we used the Lilliefors test, based on the Kolmogorov-Smirnov test. The nul hypothesis (H0) stated a nonsignificant difference between the means while the alternative hypothesis (H1) stated a significant difference. The statistical verification of the results was performed in Matlab (Mathworks, USA), taking into account a confidence interval of 95%; the value $p < 0.05$ was considered statistically significant. The value $p < 0.05$ of the χ^2 test was considered statistically significant for the evaluation of the distribution β-hCG values, according to the gestational age and values range.

Table 1
CASES DISTRIBUTION UPON PATIENTS AGE

Patients age	Patients aged between 17 – 30 years	Patients aged between 31 – 45 years	Total
Number of cases	26	63	89
Percentage	29.2%	70.8%	100%

Table 2
CASE DISTRIBUTION UPON GESTATIONAL AGE

Gestational age(weeks)	Cases	Percentage (%)	No. of Cases	B-hCG (UI/l)
5	24	27	20	100-1000
			4	1000-3000
6	33	37	13	1000-3000
			20	3000-5000
7	32	36	8	3000-5000
			24	5000-15000
	89	100%		

Table 3
CASE DISTRIBUTION UPON beta-hCG INITIAL VALUE

B hCG (UI/l)	No. cases	%
100-1000	20	22.5
1000-3000	17	19.1
3000-5000	28	31.4
5000-15000	24	27

Table 4
CASES DISTRIBUTION UPON THE PLASMA PROGESTERONE VALUE

Progesterone (ng/ml)	No. cases	%
10 – 20	57	64
Under 5	32	36

Results and discussions

Depending on the age of the patients, we noted the prevalence of ectopic pregnancies between the ages of 31 and 45, 63 cases, respectively 70.8%. The result is over two times higher compared to the age group of 17-30 years, 26 cases, respectively 29.2% (fig. 1). Out of the 89 cases treated with Methotrexate, the positive result of medical therapy was noted in 53 cases (59.5%).

The determination of human chorionic gonadotrophin has established early diagnosis of pregnancy, abortion imminence, gestational age estimation, diagnosis and monitoring of ectopic pregnancies.

In our study, the β-hCG curves returned to normal (below than 10 IU/L) after 6 weeks after the start of the treatment, with serial doses given at 7 days. Returning to normal of the β-hCG curves were based on baseline: in patients with β-hCG 100-1000 IU/L the values decreased progressively reaching normal values after 21 days, in the cases with β-hCG 1000-3000 IU/L they reached normal values after 28 days and in patients with β-hCG 3000-5000 IU/L they reached normal values after 35 days. For the patients with β-hCG above 5000 IU/L, the values decreased progressively reaching normal values after 42 days (fig. 3).

In the 57 cases of evolutionary ectopic pregnancies (64%), the progesteronemy was between 10 and 20ng / mL, and in the 32 cases of out-of-paced pregnancy (36%) it was below 5ng / mL (table 4).

We consider a successful medical therapy the 53 cases (59.5%), which under the treatment with Methotrexate had a decrease in β-hCG values and did not require surgical treatment. In the remaining 36 cases, 40.5%, surgical intervention was involved in tubal abortions with hemoperitoneum (fig. 4). In these cases, it was not necessary to remove the tube but to pour it into the pavilion area, with the postoperative repeat of a dose of Methotrexate intramuscularly 50 mg/m² body surface area.

For the analysis of the mean time recovery of β-hCG to normal values following the treatment with methotrexate, the four samples (fig. 3) were compared both together (ANOVA, $p < 0.001$) and individually (t-Student, $p < 0.05$), resulting significant statistical differences for each of the 4 statistical samples ($p < 0.05$), which confirms the alternative hypothesis H1. The treatment was significantly effective.

The disadvantage of the conservative, classical or laparoscopic surgical procedures is the persistence of the trophoblastic tissue in the trunk or peritoneal cavity. So we resorted to the use of methotrexate as an adjuvant treatment after the conservative surgery, for the destruction of the remaining trophoblastic tissue.

The ectopic pregnancy is a polymorphic disorder, whose diagnosis at early stages is often difficult, and in late stages it causes serious complications, targeting the obstetrical and vital prognosis of the patient. A precocious and early diagnosis is supported on ultrasound and dosing in the β-hCG dynamics.

The serum level of β-hCG is the most accurate biochemical prognostic indicator in the medical treatment with Methotrexate. After Lipscomb et al., the success rate was 92% at an initial serum value of β-hCG below 5000UI/L, while at a concentration above 15000UI/L the rate of success was 68% [17]. According to Menon et al., 2007, a level of β-hCG greater than 5000UI/L is 4 times more likely to be associated with a methotrexate treatment failure than below 5000 UI/L [18].

In our study, the positive result of the medical therapy was 59.5% (fig. 4), over two thirds of the patients, 76 cases (73%), with a β-hCG below 5000UI/L. The success and failure rate of the conservative treatment with methotrexate depends on the β-hCG baseline. The success rate is even higher as the initial value of β-hCG is lower; the failure rate is higher as the initial β-hCG values are higher. The treatment was performed under ultrasound and dynamic monitoring of β-hCG, which returned to normal, below 10 IU/L, after 21 to 42 days after methotrexate administration, based on the initial β-hCG value.

The variation in plasma progesterone concentration between the weeks 5-10 of gestation is minimal. Mol et al. (1998) investigated the serum progesterone levels to differentiate the ectopic pregnancy from intrauterine pregnancy. With levels less than 5ng/mL, the out of paced pregnancy was detected with almost perfect specificity. Values above 20-25 ng/mL were identified in evolution pregnancies [19]. Contraception in patients with previously ectopic pregnancy and gestational diabetes mellitus is mandatory [20].
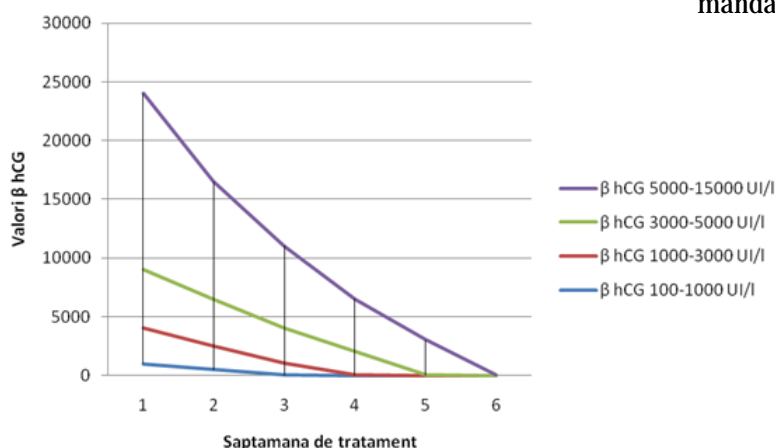

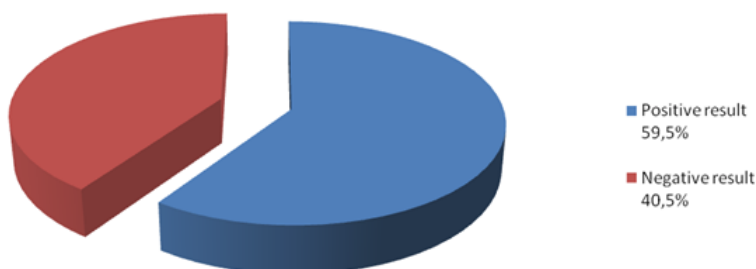Fig. 3. Values of beta-hCG decrease range upon the week of treatment

Fig. 4. Results of the medical treatment with methotrexat

In our study, in 57 cases of ectopic pregnancy (64%), the progesterone ranged between 10 and 20 ng / mL. The progesterone levels cannot differentiate between ectopic pregnancy and intrauterine pregnancy, but can differentiate the pregnancy in evolution from the out of paced pregnancy.

There are authors that carry out studies on new markers for detecting the ectopic pregnancy: the endothelial vascular growth factor (VEGF) [21], the celiac antigenic marker (CA125), the serum creatine kinase, the fetal fibronectin [22]. The serum A inhibitor levels are significantly lower in women with extrauterine pregnancy than in those with normal pregnancies [23]. None of these tests are in the current clinical use being still in the research stage.

The interrelation between β-hCG dosage and plasma progesterone on a quantitative basis differentiated the intrauterine pregnancy in evolution from the out of paced pregnancy; the same aspect being noticed in the tubal ectopic pregnancy, especially after the treatment with methotrexate. These concomitant plasma dosages are especially justified in the case of patients with tubal ectopic pregnancy who wish for maternity rather than surgical removal of the uterine tube.

Conclusions

The study confirms that the most important and affordable biochemical marker for fertile population in our country is the dynamic dosage of β-hCG. The dosage of the plasma progesterone was useful in differentiating the evolutionary pregnancies from those arrest in evolution. The correlations of these dosages with anamnestic data, the clinical examination and the transvaginal ultrasound exploration are the most important diagnostic elements in the tubal extrauterine pregnancy. The β-hCG value is the most important prognostic factor in the disease progression after the administration of methotrexate; the lower the initial value, the better the therapeutics.

The methotrexate treatment was easily accepted by the patients, not being aggressive, it requires minimal hospitalization and it is cheaper compared to surgery. The drug treatment is the only solution for storing the unique tube after a past salpingectomy.

The success rate on the methotrexate treated group of extrauterine pregnancies was over half of the cases (59.5%) and taking into account the maternal desire of women it is preferable to approach the methotrexate therapy and thus to ensure the woman's fertile future.

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