Predictive factors of trophoblastic invasion into the ampullary region of the tubal wall in ectopic pregnancy

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BACKGROUND: Predictive factors of damage to the Fallopian tube may guide the treatment for patients with tubal pregnancy. The aim of this study was to assess the predictive value of the following parameters: gestational age, serum β-hCG concentrations and ultrasound findings. METHODS: A total of 105 patients with ampullary pregnancy undergoing salpingectomy were analysed. Trophoblastic invasion was histologically classified as stage I when limited to the tubal mucosa, stage II when extending to the muscle layer and stage III in the case of complete tubal wall infiltration. We correlated the depth of trophoblastic infiltration into the tubal wall with gestational age, β-hCG concentration on the day of surgery and the type and size of the ectopic mass upon ultrasound. RESULTS: No association was observed between the depth of trophoblastic invasion and gestational age (P = 0.53) or tubal mass diameter (P = 0.43). Trophoblastic invasion was, however, associated with β-hCG concentration (P < 0.001) and with the type of ultrasonographic image (P = 0.001). β-hCG levels of 2400 mIU/ml showed 82.8% sensitivity and 85.5% specificity for stage I, and levels of 5990 mIU/ml showed 82.6% sensitivity and 74.6% specificity for stage III. CONCLUSIONS: Depth of trophoblastic penetration into the tubal wall of the ampullary region of Fallopian tube is correlated with β-hCG concentration and the type of ultrasonographic image; serum β-hCG is the best predictor of the depth of penetration.

Key words: β-hCG/ampullary pregnancy/ectopic pregnancy/first trimester haemorrhage/trophoblastic tissue

Introduction

In view of its high incidence, morbidity and mortality, ectopic pregnancy (EP) is considered to be a true public health problem. EP is the fourth most frequent cause of maternal death in the UK (Lewis and Drife, 2004), and its prevalence reaches 2% among all pregnancies in the USA (Centers for Disease Control and Prevention, 1995).

The increase in the incidence of EP over the last few years has been attributed to the growing number of risk factors such as a higher prevalence of sexually transmitted diseases (pelvic inflammatory disease, chlamydia and gonorrhoea), an increased tubal sterilization practice and subsequent attempted reversal, the more frequent use of assisted reproduction technologies (ARTs) and late primiparity. In addition, there are improved diagnostic methods, especially transvaginal ultrasound and serum measurement of β-hCG (Chow et al., 1987; Coste et al., 1991; Fernandez et al., 1991; Job-Spira et al., 1993; Ankum et al., 1996; Bouyer et al., 2003), which permit the diagnosis of spontaneously regressing EP that would have gone unnoticed in the past.

Fallopian tubes affected by EP frequently show morphologic and functional alterations such as persistent foci of decidual transformation, diverticula or post-inflammatory changes (Green and Kott, 1989). These abnormalities may persist after conservative treatment. In addition, implantation of trophoblastic tissue into the tubal wall may impair oviductal function either by altering the ciliary epithelium or mainly by causing architectural derangement of the wall musculature because of an inflammatory reaction, increasing the risk for a new episode of EP. It is believed that the impairment of tubal function depends on the degree of invasion of the trophoblast into the tubal wall (Green and Kott, 1989).

Some researchers report that deep invasion of trophoblastic tissue into the tubal wall is a common event (Budowick et al., 1980; Dietl et al., 1988), a fact contested by others who believe that trophoblast growth is exclusively intraluminal (Stock, 1985; Pauerstein et al., 1986; Senterman et al., 1988; Stock, 1991). There are still no adequate criteria for the prediction of the depth of invasion of trophoblastic tissue into the tubal wall. The viability of trophoblastic tissue indicates its ability of implantation and infiltration into the tubal wall. The concentration of β-hCG is a precise marker of trophoblast viability and is therefore frequently used for the diagnosis and follow-up of patients with EP (Kiss et al., 1997). Ultrasonography, because
it provides information regarding the location and size of the ectopic mass and also detects free fluid in the abdominal cavity, permits to guide treatment by identifying cases of tubal rupture and by allowing the monitoring of patients managed conservatively (Stovall et al., 1989; Cacciatore, 1990; Menard et al., 1990; Brown et al., 1991; Condous et al., 2005). Some authors have correlated these parameters with the depth of trophoblastic infiltration into the tubal wall (Oktay et al., 1994; Klein et al., 1995; Natale et al., 2003), but the series were small, and we believe that prospective studies involving larger patient populations are needed to confirm their findings.

The main aim of this study was to assess the predictive value of gestational age, serum $\beta$-hCG concentration and the size and type of the ultrasonographic image of EP for the determination of the depth of trophoblastic infiltration into the tubal wall affected by ampullary pregnancy.

Materials and methods

Between 1 January 2000 and 31 March 2004, a prospective study was conducted on patients with a diagnosis of tubal pregnancy in the ampullary region, who were submitted to salpingectomy. The Ethics Committee of the institution approved the research project.

Criteria for inclusion in the study were knowledge of the date of last menstruation, a diagnosis of tubal pregnancy in the ampullary region, radical surgical treatment (salpingectomy), measurement of serum $\beta$-hCG on the day of surgery and description and measurement of the ectopic mass by transvaginal ultrasound. Excluded were cases in which no agreement regarding the location of the tubal pregnancy upon surgical description and histologic analysis could be obtained.

A total of 194 consecutive cases of EP were recorded during the study period. Of these, 82 patients were not included for different reasons: 5 cases were not tubal pregnancies, 10 cases were tubal but not ampullary pregnancies, in 8 cases, no ultrasound image was available, 16 patients did not know the exact date of the last menstrual period, 9 patients were treated by salpingostomy, 8 patients received methotrexate and 26 other patients were managed expectantly; 112 patients fulfilled the inclusion criteria and 8 patients received methotrexate and 26 other patients were managed expectantly; 112 patients fulfilled the inclusion criteria and were selected to participate in the study. Seven cases were excluded because the exact site of implantation of trophoblastic tissue into the Fallopian tube could not be confirmed by anatomicopathologic examination.

Patients with clinical signs suggestive of EP were submitted to the determination of serum $\beta$-hCG concentration and transvaginal ultrasound. Serum $\beta$-hCG was quantified with a two-site immunofluorimetric assay based on the direct sandwich technique (1235 AutoDELFIA Immunoassay System, AutoDELFIA hCG, PerkinElmer, Turku, Finland). The inter- and intra-assay coefficients of variation (CVs) were 5.1 and 3.9, respectively.

The ectopic mass was classified based on the ultrasonographic aspect as follows: (i) tubal ring—para-ovarian formation similar to a gestational sac not containing a viable embryo consisting of an anechoic structure surrounded by a peripheral hypechoic halo. An empty ectopic gestational sac or a sac containing an embryo without cardiac activity or a vitelline vesicle was also classified as a tubal ring. (ii) Solid or complex mass—para-ovarian image suggestive of haematosalpinx or pelvic haematoma intermingled with irregular hypechoic and/or hypechoic areas and (iii) ectopic gestational sac containing an embryo with cardiac activity.

The size of the adnexal mass (in mm) was determined by measuring its longer axis by transvaginal ultrasound (Ecocoe apparatus equipped with a 7.5-MHz transvaginal probe; Toshiba, Tokyo, Japan). A well-trained obstetrician from our department performed all examinations. Gestational age was calculated in weeks from the first day of the last menstruation.

The Fallopian tubes were fixed in 10% formalin and sectioned serially for light microscopic analysis. An average of six sections stained with haematoxylin–eosin was analysed. Histological assessment was performed by a single well-experienced pathologist who was unaware of the clinical and laboratory characteristics of the patients.

Ampullary pregnancies were classified histologically according to the depth of trophoblastic infiltration into the tubal wall (Natale et al., 2003): stage I, trophoblastic infiltration limited to the tubal mucosa (Figure 1A); stage II, trophoblastic infiltration extended to the tubal muscularis (Figure 1B) and stage III, complete tubal wall infiltration with or without rupture of the serosa (Figure 1C).

The three histologic stages were compared with regard to gestational age, serum $\beta$-hCG levels and the type (tubal ring, complex mass and embryo with cardiac activity) and size of the transvaginal ultrasonographic image. The hypothesis of equality between groups was tested by one-way analysis of variance (ANOVA), and when the assumption of normality of the data was rejected, the variable was transformed logarithmically. Multiple comparisons were performed by Bonferroni’s test, and the chi-squared test was used for comparison between proportions. A stepwise logistic regression model was used to select predictors of the degree of trophoblastic invasion. A receiver operating characteristic (ROC) curve was constructed to obtain $\beta$-hCG threshold levels associated with the stages of invasion. The level of significance was set at 5% for all tests.

Results

The age of the patients ranged from 18 to 46 years [mean 29.5 ± (SD) 6.6 years]. A total of 52 (49.5%) patients were white and 53 (50.5%) were non-white. With respect to obstetric history, 33 (31.6%) patients were nulliparous and 7 (6.7%) had a history of EP in the contralateral Fallopian tube. Histologic analysis showed that 29 patients (27.6%) had stage I tubal infiltration, 30 (28.6%) had stage II and 46 (43.8%) had stage III. There was no significant difference in gestational age between the three histologic groups ($P = 0.53$) (Table I).

Serum $\beta$-hCG concentrations on the day of surgery ranged from 325 to 9 050 mIU/ml (11 438 ± 16 651 mIU/ml). One-way ANOVA revealed a significant correlation between the different stages of trophoblastic invasion and $\beta$-hCG levels ($P < 0.001$). Significantly lower values were observed for patients with stage I infiltration compared with stages II and III ($P < 0.05$) and for patients with stage II compared with stage III ($P < 0.05$) (Table II).

There was an association between the stage of trophoblastic infiltration into the tubal wall and the type of ultrasonographic image ($P = 0.001$, chi-squared test). No embryo with cardiac activity was identified in any of the patients with stage I invasion, whereas a complex mass was the most frequent finding in this group (86.2%) (Figure 2). An embryo with cardiac activity was more frequent in patients with stage III invasion compared with the other groups.

The maximum diameter of the ectopic mass upon ultrasonography ranged from 9 to 149 mm. One-way ANOVA
showed no association between the different stages of trophoblastic invasion and the diameter of tubal mass \( (P = 0.43) \) (Table III).

Multivariate logistic regression analysis including the two variables that presented a significant correlation with the stage of trophoblastic invasion in the previous analysis, that is, serum β-hCG concentration and the type of ultrasonographic image, was performed. This analysis showed that β-hCG level was the most important predictor of trophoblastic invasion into the tubal wall.

Using the ROC curve, the threshold (β-hCG concentration) was calculated for stages I and III infiltration (Figure 3A and B). The serum β-hCG level that best predicted stage I trophoblastic invasion was 2400 mIU/ml, with this threshold showing a sensitivity of 82.8%, specificity of 85.5%, positive predictive value of 68.6% and negative predictive value of 92.7% (84.8% accuracy) (Table IV). In addition, cases with serum β-hCG levels <2400 mIU/ml presented a greater chance of being classified as stage I trophoblastic infiltration, with an odds ratio \( (OR) = 28.4 \) [95% confidence interval (CI) = 8.9–90.1]. On the contrary, a serum β-hCG level of 5990 mIU/ml was the best predictor of stage III infiltration with a sensitivity of 82.6%, specificity of 74.6%, positive predictive value of 71.7% and negative predictive value of 84.6% (78.1% accuracy). Cases
Discussion

The early diagnosis of an EP (before rupture) permits conservative, clinical or surgical treatment in order to preserve subsequent patient fertility. However, the conservative approach might be questioned because it is associated with a higher risk of persistence of trophoblastic tissue and a higher recurrence rate when compared with salpingectomy (Yao and Tulandi, 1997). In addition, salpingectomy does not change subsequent patient fertility when the contralateral tube seems to be healthy. Tuomivaara and Kauppila (1988) reported subsequent rates of intrauterine pregnancy and EP of 85 and 9%, respectively, in the presence of a normal contralateral tube irrespective of the type of treatment performed. Fernandez et al. (1998), studying 340 patients submitted to salpingectomy for EP after a mean follow-up period of 73 months, observed a subsequent intrauterine pregnancy rate of 82.1% in patients younger than 30 years with no history of tubal disease, a rate similar to that obtained for another group managed conservatively. Mol et al. (1998) evaluated 135 patients undergoing surgery for EP—56 submitted to conservative treatment and 79 to salpingectomy—and found no significant difference in subsequent intrauterine pregnancy rates between the two groups.

Various factors have been studied in an attempt to predict tubal integrity in patients selected for medical treatment: physical examination, transvaginal ultrasound (presence of a heartbeat, ectopic mass and fluid in the pouch of Douglas), serum \( \beta \)-hCG estimation and haemoglobin assessment. These factors were found to be efficient in predicting the success of treatment and some of them can be used to estimate microscopic lesions in the histologic structure of the Fallopian tubes caused by trophoblast implantation (Oktay et al., 1994; Klein et al., 1995; Natale et al., 2003).

We studied the depth of trophoblast penetration into the tubal wall by comparing it with gestational age, serum \( \beta \)-hCG levels and ultrasonographic features of the ectopic mass. Only ampullary pregnancies were included in this study because they represent the main extraterine site of trophoblast implantation (Stovall and Ling, 1993). Because the different anatomic segments of the Fallopian tube are histologically distinct, we believe that trophoblast penetration might occur in a specific manner in each tubal portion.

In this study, no correlation was observed between gestational age and the stage of trophoblastic invasion into the tubal wall, with gestational age being similar in the three groups with different depths of trophoblastic infiltration as also reported by Natale et al. (2003). The exact gestational age may be difficult to assess, because the date of the last menstrual period is sometimes uncertain. On the contrary, the duration of amenorrhoea at the time of diagnosis does not necessarily indicate the period during which trophoblastic tissue was viable (i.e. with invasion potential).

Cases with serum \( \beta \)-hCG levels <2400 mIU/ml presented a 28.4 times higher probability of superficial tubal invasion compared with the other patients selected. In these cases, tubal damage is probably less intense, and preservation of this tube by conservative treatment might be beneficial if the patient desires subsequent fertility. On the contrary, cases with \( \beta \)-hCG

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**Table IV.** Distribution of the population according to histologic classification and \( \beta \)-hCG levels

<table>
<thead>
<tr>
<th>Stage of trophoblastic infiltration</th>
<th>( \beta )-hCG (mIU/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>&lt;2400</td>
</tr>
<tr>
<td>I</td>
<td>24 (82.8%)</td>
</tr>
<tr>
<td>II</td>
<td>9 (30.0%)</td>
</tr>
<tr>
<td>III</td>
<td>2 (4.3%)</td>
</tr>
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with \( \beta \)-hCG levels >5990 mIU/ml showed a greater probability of being classified as stage III trophoblastic invasion, with an OR = 13.9 (95% CI = 5.3–36.4). Distribution of the population according to histologic classification and \( \beta \)-hCG levels are showed in Table IV.
levels >5990 mIU/ml showed a 13.9 times greater probability of complete tubal infiltration compared with the other cases selected. In these patients, in view of the very satisfactory values of sensitivity (82.6%), specificity (74.6%), positive predictive value (71.7%), negative predictive value (84.6%) and accuracy (78.1%), the high serum β-hCG levels seemed to be associated with the destruction of the tubal wall and the invasion of the muscle layer may impair the complete removal of trophoblastic tissue if a conservative therapeutic approach is used.

These data agree with the findings of Oktay et al. (1994), who studied patients with ampullary ectopic pregnancies and observed higher serum β-hCG concentrations in patients in whom the tubal muscularis was invaded compared with the group presenting no muscular invasion (13 665 ± 2986 mIU/ml and 2169 ± 870 mIU/ml, respectively; P = 0.0001). A serum β-hCG level of 5400 mIU/ml or higher showed an 89% positive predictive value and 94% negative predictive value for the diagnosis of invasion of the tubal muscularis. In the study of Klein et al. (1995), trophoblastic tissue infiltration was restricted to the tubal lumen in 84.6% of patients with serum β-hCG concentrations <2500 IU/l, a significantly higher proportion that observed among patients with higher hormone levels in whom the penetration of the muscle layer was more frequent (P = 0.0045).

Natale et al. (2003) also studied Fallopian tubes affected by EP by anatomopathologic examination. Serum β-hCG concentration was proportional to the degree of tubal invasion, with a significant difference between the groups studied (P = 0.004). Similar to the present study, trophoblastic invasion at least up to the tubal muscularis was observed in all patients with a serum hormone concentration >6000 mIU/ml.

Cacciatore (1990) suggested that the ultrasonographic aspect of the ectopic mass could predict the state of tubal integrity. The author showed that the presence of a tubal ring was associated with tubal integrity, whereas the identification of a heterogeneous and hypechoico adnexal mass was related to rupture of the Fallopian tube in 22% of the cases. Fleischer et al. (1990) studied patients with a diagnosis of tubal pregnancy and associated the identification of a tubal ring by ultrasonography with integrity of the Fallopian tube (68% sensitivity). Mol et al. (1999) evaluated the ability of noninvasive diagnostic tools to predict tubal rupture and active bleeding in patients with tubal pregnancy. Abdominal pain, rebound tenderness on abdominal examination, fluid in the pouch of Douglas detected by transvaginal ultrasound and a low serum haemoglobin level were independent predictors of tubal rupture and/or active bleeding. Detection of an ectopic gestational sac or an ectopic mass by ultrasound reduced the risk of tubal rupture. Abdominal pain was the most sensitive predictor, with a sensitivity of 95%. Natale et al. (2003) related the presence of cardiac activity of the product of conception to the degree of trophoblastic invasion into the tubal wall. Similar to the present study, no case of an embryo with cardiac activity was observed among patients with superficial tubal invasion. The prevalence of this type of ultrasonographic finding increased with increasing depth of invasion. It should be emphasized that the presence of this finding may indicate the possible occurrence of greater tubal damage.

We believe that the definition of factors indicative of the degree of morphologic and functional damage to the Fallopian tube affected by EP may contribute to the choice of treatment for patients who desire subsequent fertility, thus avoiding the removal of less compromised structures and improving the reproductive prognosis. On the contrary, the attempt of preservation of the extensively damaged Fallopian tubes which, in addition to postponing a new pregnancy, markedly increase the morbidity resulting from a new EP episode could be prevented.

Gestational age and the diameter of tubal mass were not efficient in predicting the degree of trophoblastic invasion into the tubal wall or the extent of organ damage. On the contrary, patients with higher serum β-hCG concentrations and the ultrasonographic finding of an embryo with cardiac activity had Fallopian tubes that were more deeply invaded by trophoblastic tissue.

We conclude that β-hCG concentration is the best predictive factor of trophoblastic invasion into the ampullary portion of the tubal wall. High serum β-hCG levels are indicative of higher trophoblast vitality and greater depths of invasion of this tissue. In our opinion, this fact may justify the indication of salpingectomy for cases with serum β-hCG levels >5990 mIU/ml.

References

Trophoblastic invasion in tubal pregnancy


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