Vascular endothelial growth factor and β-human chorionic gonadotropin are associated with trophoblastic invasion into the tubal wall in ectopic pregnancy

Fábio Roberto Cabar, M.D., Ph.D.,[a] *Pedro Paulo Pereira, M.D., Ph.D.,*[a] *Regina Schultz, M.D., Ph.D.,*[b] *Rossana Pulcineli Francisco, M.D., Ph.D.,*[a] *and Marcelo Zugaib, M.D., Ph.D.*[a]

[a] Department of Obstetrics and Gynecology and [b] Department of Pathology, Faculty of Medicine, University of São Paulo, São Paulo, Brazil

Objective: To assess the association between the depth of trophoblastic penetration into the tubal wall with serum concentrations of vascular endothelial growth factor (VEGF) and β-hCG and to assess its predictive value.
Design: Prospective study.
Setting: Tertiary care university hospital.
Patient(s): Thirty patients with ampullary pregnancy undergoing salpingectomy were analyzed.
Intervention(s): 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.
Main Outcome Measure(s): The relation between depth of trophoblastic infiltration into the tubal wall with VEGF and β-hCG serum concentrations on the day of surgery.
Result(s): An association between the depth of trophoblastic invasion and maternal serum concentrations of VEGF and β-hCG was observed. VEGF levels of 297.2 pg/mL showed 100.0% sensitivity and 90.0% specificity for stage I, and levels of 440.1 pg/mL showed 81.8% sensitivity and 88.8% specificity for stage III. Beta-hCG levels of 2590.0 mIU/mL showed 88.9% sensitivity and 80.0% specificity for stage I, and levels of 10,827.0 mUI/mL showed 72.7% sensitivity and 88.9% specificity for stage III.
Conclusion(s): Maternal serum VEGF and β-hCG concentrations are associated with depth of trophoblastic penetration into the tubal wall. (Fertil Steril® 2010;94:1595–600. ©2010 by American Society for Reproductive Medicine.)

Key Words: Ectopic pregnancy, VEGF, β-hCG, tubal pregnancy, first trimester hemorrhage, trophoblastic tissue

Ectopic pregnancy (EP) is considered to be a true public health problem since it is still a major cause of maternal morbidity and mortality, accounting for 9%–13% of all pregnancy-related deaths in the United States (1) and representing the fourth most frequent cause of maternal death in the United Kingdom (2). The increase in the incidence of EP over the last years has been attributed to the growing number of risk factors (3–7) and to the improvement of diagnostic methods (3–5, 8–10).

Vascular endothelial growth factor (VEGF) is a well-known angiogenic factor, which may play a key role in the establishment of a viable pregnancy, participating in the processes of implantation and placentation. VEGF serves as a major modulator of vascular growth, remodeling, and permeability in endometrium, decidua, and trophoblast and of the vascular development of the embryo (11–17). The secretion and expression of VEGF is dependent on local conditions, such as hypoxia (18), and it has been observed that the cellular VEGF production is increased in hypoxic conditions (12, 16, 17, 19). The implantation environment in the oviduct is very different from that of the well-vascularized endometrium, and the production and secretion of VEGF seem to be elevated in EP (20, 21).

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 due to an inflammatory reaction, increasing the risk for a new episode of EP (22, 23). It is believed that impairment of tubal function depends on the degree of invasion of the trophoblast into the tubal wall (24). There are still no adequate criteria for the prediction of the depth of invasion of trophoblastic tissue into the tubal wall. Maternal serum β-hCG concentration seems to be related to the depth of trophoblastic penetration into the tubal wall in ampullary pregnancies (25, 26).

Higher VEGF concentrations may permit deeper invasion of trophoblastic tissue into the tubal wall. The aim of the present study

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Reprint requests: Fábio Roberto Cabar, M.D., Ph.D., Department of Obstetrics and Gynecology, Faculty of Medicine, University of São Paulo, Av. Dr. Enéas de Carvalho Aguiar, 255-10° Andar, São Paulo-SP, 05403-000, Brazil (FAX: 55-11-30698183; E-mail: fabiocabar@uol.com.br).

was to assess the association between the depth of trophoblastic penetration into the tubal wall and serum concentrations of both VEGF and β-hCG in patients affected by ampullary pregnancy.

MATERIALS AND METHODS

Institutional Review Board approval was obtained (Clinical Research Ethics Committee of the University of São Paulo).

Between October 1, 2006, and September 30, 2007, a prospective study was conducted on patients with a diagnosis of tubal pregnancy in the ampullary region who were submitted to salpingectomy at Hospital das Clínicas of the University of São Paulo Medical School. Criteria for inclusion in the study were singleton pregnancies, spontaneous conception, diagnosis of tubal pregnancy in the ampullary region, radical surgical treatment (salpingectomy), and measurement of serum VEGF and serum β-hCG on the day of surgery. Cases in which there was no agreement regarding the location of the tubal pregnancy upon surgical description and histological analysis were excluded. Assessment of gestational age was made based on the last menstrual period. A detailed informed consent was obtained from each patient before the inclusion.

A total of 52 consecutive cases of EP were recorded during the study period. Of these, 22 patients were not included for different reasons: two cases were not tubal pregnancies, five cases were tubal but not ampullary pregnancies, and 15 patients were treated by a conservative approach (clinical or surgical); 30 patients fulfilled the inclusion criteria and were selected to participate in the study. None was excluded.

To confirm the diagnosis, patients were routinely submitted to a serum β-hCG determination and a transvaginal ultrasound was performed. After diagnostic confirmation, if the woman could be enrolled in the protocol, determination of serum VEGF concentration was also performed. Blood samples were collected by peripheral venous puncture in siliconized tubes and were allowed to coagulate at room temperature for 2–6 hours; serum was obtained by centrifugation and stored at −80°C until assays were performed in batches. The serum VEGF was measured in triplicate by commercial ELISA (R&D System, Inc., Minneapolis) specific for the human molecule. Samples were diluted 1:4 with assay diluent and incubated in triplicates in microtiter plates precoated with a monoclonal antibody specific for VEGF at room temperature for 2 hours. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for VEGF was added. After incubation at room temperature for 2 hours and washing, a substrate solution was added. Color development was stopped after 20 minutes at room temperature, and color intensity was read at 450 nm (reference wavelength 540 nm) within 30 minutes. Results were calculated from a standard curve (recombinant human VEGF165; range, 15–1000 pg/mL) generated by a four-parameter logistic curve fit and expressed as picograms per milligram of cytosol protein. The sensitivity of the assay was <5.0 pg/mL, and intra-assay variability was 5.1% at a VEGF concentration of 512 pg/mL.

Serum β-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 were 5.1 and 3.9, respectively.

The fallopian tubes were fixed in 10% formalin and sectioned serially for light-microscopic analysis. An average of 10 sections stained with hematoxylin-eosin was analyzed. To facilitate the identification of tissue invaded by the trophoblast, histological material was also stained with Masson's trichrome to identify muscular fibers. Immunohistochemical staining for human placental lactogen (hPL) was performed to identify intermediate trophoblast and determine the depth of trophoblastic invasion into the tubal wall. 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 (25): stage I, trophoblastic infiltration limited to the tubal mucosa; stage II, trophoblastic infiltration extended to the tubal muscularis; stage III, complete tubal wall infiltration with or without rupture of the serosa (Fig. 1A–1F).

As our data are normally distributed, means ± SD were described. The three histological stages were compared regarding serum VEGF levels and β-hCG concentrations. The hypothesis of equality between groups was tested by one-way analysis of variance (ANOVA). Whenever the ANOVA test showed a significant difference, we used the Bonferroni's post hoc test to determine, specifically, where the significance difference occurred.

The discriminatory power as well as the cutoff values of the serum VEGF and β-hCG to differentiate between stages I and II of trophoblastic infiltration and stages II and III were determined using receiver operating characteristic (ROC) curves. *Z*-statistics were applied to compare the VEGF with the β-hCG ROC curves, and each area under the curve was compared with the value of 0.5, which represented the nullity hypothesis. Positive likelihood ratios, accuracy, sensitivity, specificity, and positive and negative predictive values were calculated based on cutoff points obtained by the ROC curve.

$P<.05$ was considered statistically significant; all tests were two-tailed. Ninety-five percent confidence intervals (CIs) were calculated for the mean differences, for the area under the ROC curves, and for area differences. All statistical analyses were performed on a personal computer with the Statistical Package for the Social Sciences (SPSS, Chicago), version 11.5.1 for Windows, except for the ROC curve analysis, where the software Analyse-it 2.12 was used instead (Analyse-It Software Ltd., Leeds, United Kingdom).

RESULTS

The age of the patients ranged from 17 to 43 years (29.1 ± 5.8 years), and there was no significant difference in mean maternal ages among the three histological groups (stage I, 31.0 ± 7.3; stage II, 27.3 ± 5.3; stage III, 28.7 ± 4.5; $P=.385$). Sixteen patients (53.3%) were Caucasian, and 14 (46.7%) were non-Caucasian. With respect to obstetric history, eight patients (26.7%) were nulliparous and four (13.3%) had a history of EP in the contralateral fallopian tube. Histological analysis showed that 10 patients (33.3%) had stage I tubal infiltration, 9 (30.0%) had stage II, and 11 (36.7%) had stage III. The gestational age ranged from 41 to 56 days (47.9 ± 4.9 days), and there was no significant difference in mean gestational ages among the three histological groups (stage I, 47.9 ± 6.0; stage II, 46.8 ± 4.7; stage III, 48.8 ± 4.3; $P=.669$).

Serum VEGF concentrations on the day of surgery ranged from 167.5 to 783.1 pg/mL (368.8 ± 167.7 pg/mL), and serum β-hCG levels on the day of surgery ranged from 108.0 to 46,165.0 mIU/mL (9345.0 ± 11,028.3 mIU/mL). One-way ANOVA revealed a significant difference in the VEGF concentrations (stage I, 220.7 ± 39.1; stage II, 365.8 ± 81.7; stage III, 505.8 ± 179.6; $P<.001$) and the β-hCG levels (stage I, 1731.2 ± 1085.8; stage II, 9720.8 ± 14,002.8; stage III, 15,961.7 ± 9408.9; $P=.008$) regarding the stages of trophoblastic invasion. According to Bonferroni's test, the mean value of serum VEGF in patients with stage I tubal infiltration was significantly lower than that in stage II ($P=.042$), which was significantly lower than that in stage III ($P<.001$), whereas the mean value of serum β-hCG in patients with stage I tubal infiltration was significantly lower than in that in stage III ($P=.006$).

Using the ROC curve, the cutoff values between stages I and II infiltration and between stages II and III of VEGF and β-hCG concentrations were calculated (Fig. 2A–2D). The comparisons between these curves are depicted in Table 1.

The serum VEGF level that best differentiated stage I from stage II trophoblastic invasion was 297.2 pg/mL; a serum VEGF level of 440.1 pg/mL best differentiated stage II from stage III infiltration.

The maternal serum β-hCG concentration that best discriminated stage I from stage II trophoblastic invasion was 2590.0 mIU/mL, and a serum β-hCG level of 10,827.0 mIU/mL best discriminated stage II from stage III infiltration. The positive likelihood ratios, accuracies, sensitivities, specificities, positive predictive value (PPV), and negative predictive value (NPV) according to the cutoff scores are displayed in Table 2.