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Serum vascular endothelial growth factor as a marker for tubal pregnancy

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate whether a single measurement of vascular endothelial growth factor could distinguish between intrauterine pregnancy and ectopic pregnancy and to correlate the levels of vascular endothelial growth factor with serum levels of progesterone and β-human chorionic gonadotropin in each subgroup.

METHODS: Ninety patients with a positive human chorionic gonadotropin test and either abdominal pain or vaginal bleeding were selected; pregnancies were singletons, spontaneously conceived, 42–56 days of gestational age. All patients had a transvaginal ultrasound examination and were divided into three subgroups: abnormal intrauterine pregnancy, tubal pregnancy, and normal intrauterine pregnancy. Tubal pregnancies were surgically treated and histologically confirmed. Blood samples were collected for the determination of β-human chorionic gonadotropin, progesterone, and vascular endothelial growth factor and their concentrations were compared in each subgroup. Receiver operating characteristic curve was calculated by comparing the subgroup of tubal pregnancy to the other groups. A Fisher discriminant function analysis was performed. The level of significance was 5%.

RESULTS: One-way analysis of variance revealed a significant correlation between the different subgroups and β-human chorionic gonadotropin, progesterone, and vascular endothelial growth factor serum levels ($p<0.001$). Vascular endothelial growth factor concentration was significantly higher for patients with tubal pregnancy than for other subgroups ($p<0.05$). β-Human chorionic gonadotropin and progesterone levels were higher in the subgroup with normal intrauterine pregnancies compared with the subgroups with tubal and abnormal intrauterine pregnancies ($p<0.05$). Serum vascular endothelial growth factor level >188.7 ng/mL predicted tubal pregnancy with 96.7% sensitivity, 95.0% specificity, 90.6% positive predictive value, and 98.3% negative predictive value.

CONCLUSIONS: Serum vascular endothelial growth factor could be a marker in discriminating intrauterine pregnancy from tubal pregnancy; its levels are increased in women with ectopic pregnancy compared with women with normal and abnormal intrauterine pregnancies.

KEYWORDS: VEGF. Pregnancy, ectopic. Vascular endothelial growth factor A. Trophoblasts. Pregnancy, tubal.

INTRODUCTION

Ectopic pregnancy (EP) is considered a true public health problem, as it is still a major cause of maternal morbidity and mortality, accounting for 9–13% of all pregnancy-related deaths[1]. Despite the introduction of highly sensitive assays for the estimation of serum human chorionic gonadotropin (hCG) and an increase in the sensitivity of transvaginal sonography (TVS), it is believed that 40–50% of cases initially are misdiagnosed[2].

Based on the combined use of TVS and serum hCG measurements, a variety of diagnostic algorithms have been proposed in the literature[3,4]; however, the utility of a single hCG measurement to confirm the absence of an EP has been questioned and the measurement of serial hCG values has been proposed[5,6]. Unfortunately, serial hCG values are not practical, especially when the patient presents for an emergency evaluation.

Vascular endothelial growth factor (VEGF) is a well-known angiogenic factor, which might play a key role in the establishment of a viable pregnancy, participating in the processes of implantation and placentation. That substance serves as a major modulator of vascular growth, remodeling,

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and permeability in endometrium, decidua, trophoblast, and also in the vascular development of the embryo[7,8]. The secretion and expression of VEGF is dependent on local conditions, such as hypoxia, and it has been observed that the cellular VEGF production is increased in hypoxic conditions[7-9]. The implantation environment in the oviduct is very different from that of well-vascularized endometrium, and the production and secretion of VEGF may be affected in the EP[10,11].

It would be particularly valuable if there was a reliable serum marker that could differentiate intrauterine pregnancy (IUP) from extrauterine pregnancy in a single measurement. In an emergency setup, it would decrease the time to diagnosis, reduce the possibility of tubal rupture, and diminish the maternal morbidity and mortality. The aim of the present study was (i) to evaluate whether a single measurement of VEGF would allow us to distinguish between IUP (normal and abnormal) and EP and (ii) to correlate the levels of VEGF with serum levels of progesterone and β-hCG in each subgroup.

METHODS

The study was approved by the Clinical Research Ethics Committee of the University of São Paulo.

Ninety patients were selected from a population of women presenting to the Hospital das Clínicas of the University of São Paulo Medical School from October 2006 to September 2007. Women elected had had a positive hCG test and presented with either abdominal pain or vaginal bleeding; all pregnancies were singletons, spontaneously conceived, with accurate assessments of their gestational age (42–56 days from the 1st day of the last menstrual period). A detailed informed consent was obtained from each patient before the inclusion.

All patients had a transvaginal ultrasound examination (Ecocee apparatus equipped with a 7.5 MHz transvaginal probe; Toshiba, Tokyo, Japan) and were divided into three subgroups: (i) abnormal (arrested) IUP (defined as a gestational sac greater than 16 mm of mean diameter without fetal tissue or an embryo greater than 5 mm without embryo cardiac activity); (ii) tubal pregnancy (no evidence of IUP, presence of a adnexal mass, and suboptimal rise of serum hCG levels in 48 h); all tubal pregnancies were surgically treated and were histologically confirmed; they did not receive any treatment with methotrexate before operation; (iii) normal IUP (intrauterine gestational sac, embryo vitality confirmed). Exclusion criterion was non-ampullar tubal pregnancy (surgically confirmed).

Blood samples were collected by peripheral venous puncture before treatment; a total of 15 mL blood was withdrawn (2 mL for β-hCG, 3 mL for progesterone, and 10 mL for VEGF determination). Blood samples for VEGF were collected in siliconized tubes and were allowed to coagulate at room temperature (RT) for 2–6 h; serum was obtained by centrifugation and stored at -80°C until assays were performed in batches. Serum VEGF was measured in triplicate by commercial ELISA (R&D System, Inc., Minneapolis, USA) specific for the human molecule. Samples were diluted in a ratio of 1:4 with assay diluent and incubated in triplicates in microtiter plates pre-coated with a monoclonal antibody specific for VEGF at RT for 2 h. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for VEGF was added. After incubation at RT for 2 h and washing, a substrate solution was added. Color development was stopped after 20 min at RT and color intensity was read at 450 nm (reference wavelength 540 nm) within 30 min. 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 pg/mg cytosol protein. The sensitivity of the assay was <5.0 pg/mL; 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-assay and intra-assay coefficients of variation were 5.1 and 3.9, respectively. Serum progesterone was measured by a solid-phase RIA (1235 AutoDELFIA immunoassay system, AutoDELFIA progesterone; PerkinElmer Life and Analytical Sciences, Finland). The inter-assay and intra-assay coefficients of variation were 1.7 and 2.0, respectively. The sensitivity of the assay was 0.8 nmol/L.

The statistical analysis was performed using SPSS-PC software (version 13.0; SPSS, Chicago, Illinois, USA). Demographic data were compared using one-way analysis of variance (ANOVA) and serum concentrations of VEGF, β-hCG, and progesterone were compared in each subgroup using the Kruskal-Wallis test. Multiple comparisons were performed by nonparametric tests. A stepwise logistic regression model was used to select predictors of the tubal pregnancy subgroup. Receiver operating characteristic (ROC) curve was calculated to discriminate the tubal pregnancy subgroup from other groups. A Fisher discriminant function analysis was performed in order to classify the cases into the different subgroups. The level of significance was set at 5% for all tests.

RESULTS

The age of the patients ranged from 17 to 44 years [mean 29.6 ± (SD) 6.6 years]. A total of 41 (45.6%) patients were white and 49 (54.4%) were non-white. With respect to obstetric history, 18 (20.0%) patients were nulliparous and 8 (8.9%) had a history of EP in the contralateral Fallopian tube. There was no difference in maternal age between the three subgroups (p=0.633), but gestational age was significantly different between the subgroups (p=0.003).

Serum VEGF concentrations ranged from 15.6 to 783.1 ng/mL between all the subgroups. One-way ANOVA revealed a significant correlation between the different subgroups and β-hCG, progesterone, and VEGF serum levels (p<0.001) (Table 1). Serum VEGF concentrations were significantly higher for patients with tubal pregnancy compared with the other subgroups (p<0.05). β-hCG and progesterone levels were higher in the subgroup with normal intrauterine pregnancy compared with the subgroups with tubal and abnormal intrauterine pregnancies (p<0.05) (Table 2).

Multivariate logistic regression analysis was performed, and it showed that serum VEGF level, but neither β-hCG nor progesterone levels, could discriminate tubal pregnancies from intrauterine pregnancies.

Using the ROC curve, the threshold (VEGF concentration) was calculated for discriminating tubal pregnancy. The serum VEGF level that best predicted EP was 188.7 ng/mL, with this threshold value showing a sensitivity of 96.7%, a specificity of 95.0%, a positive predictive value (PPV) of 90.6%, and a negative predictive value of 98.3%. Cases with serum VEGF levels >188.7 ng/mL presented a greater chance of being classified as tubal pregnancy, with an odds ratio (OR)=551.0 [95% confidence interval (CI)=64.7–∞].

A Fisher discriminant function analysis was performed using VEGF, β-hCG, and progesterone levels, and the results are summarized in Table 3. The linear functions could predict the correct subgroup in 82.2% of cases.

DISCUSSION

The increase in the incidence of EP over the past years has been attributed to the growing number of risk factors such as a higher prevalence of sexually transmitted diseases, an increased tubal sterilization practice and subsequent attempted reversal, more frequent use of assisted reproduction technologies, late primiparity, and the use of levonorgestrel as an emergency contraceptive method[12-14].

Table 1. Serum VEGF, β-hCG, and progesterone concentrations.

	VEGF* (median ± SD)	β-hCG* median (range)	Progesterone* (median ± SD)
Tubal pregnancy	368.8 ± 167.7	4641 (108–46165)	6.1 ± 3.9
Evolutive intrauterine	83.6 ± 62.8	45944 (10124–239025)	22.5 ± 6.1
Non evolutive intrauterine	83.4 ± 51.3	6751 (190–76712)	9.7 ± 6.2

Kruskal-Wallis test. *p<0.001.

Table 2. Multiple comparisons between serum concentrations and different subgroups.

	Comparison	Z-value	p
VEGF	Tubal vs. Non-evolutive	6.5	**<0.001**
	Tubal vs. Evolutive	6.6	**<0.001**
	Non-evolutive vs. Evolutive	0.1	0.912
β-hCG	Tubal vs. Non-evolutive	-0.7	0.503
	Tubal vs. Evolutive	-6.0	**<0.001**
	Non-evolutive vs. Evolutive	-5.4	**<0.001**
Progesterone	Tubal vs. Non-evolutive	-1.8	0.070
	Tubal vs. Evolutive	-7.0	**<0.001**
	Non-evolutive vs. Evolutive	-5.2	**<0.001**

Nonparametric tests.
Bold indicates significance is p<0.05.

Table 3. Classification of function coefficients; vascular endothelial growth factor, β-hCG, and progesterone concentrations.

Constant	VEGF	β-hCG	Progesterone
Tubal - 8.1170	0.0335	-1.6×10^{-6}	0.2787
Evolutive - 10.8679	0.0113	-3.1×10^{-5}	0.7414
Non-evolutive - 3.1364	0.0092	-1.9×10^{-6}	0.3402

Fisher discriminant function analysis.

Pregnant patients presenting with vaginal bleeding as an emergency still represent a diagnostic challenge. Transvaginal ultrasound and serum β-hCG and serum progesterone determinations are the most widely used methods for EP diagnosis; nevertheless, ultrasound examination can be helpful just when an intrauterine gestation or an adnexal mass is seen and serial determinations of serum β-hCG can separate normal IUP from an abnormal IUP, but it cannot distinguish an abnormal IUP from an EP[15,16]. Progesterone concentrations are higher in women with normal IUP, but its application to differentiate an EP from an abnormal IUP is not reliable[17].

Vascular endothelial growth factor is indispensable for trophoblast development during vascular development of the[18]. In contrast to hCG and progesterone, which are trophoblast-dependent, this angiogenic factor is produced by both trophoblast and endometrium[9]. This difference is of extreme importance because the main discrimination between abnormal IUP and EP is not the viability of the trophoblast (reflected by low levels of both progesterone and hCG), but fundamentally in the ground of implantation.

Extrauterine implantation environments are very different from those of the endometrium and the hypoxic conditions at the unusual implantation site may cause increased VEGF production[19,20]. Lam P.M. measured the mRNA expression of VEGF and its receptor (KDR and ftl-1) in the implantation and non-implantation sites of the human Fallopian tubes with EP and described that the expression of VEGF was significantly higher in the implantation site of the tube with EP[21]. The authors suggested that VEGF may be the angiogenic factor responsible for the implantation of an EP in the oviduct.

We studied the value of a single measurement of VEGF for differentiating between ectopic and normal/abnormal IUP in a group of pregnant women with vaginal bleeding in the first trimester. As the different anatomic segments of the Fallopian tube are histologically distinct, we believe that different implantation conditions might influence the VEGF production by the trophoblast in each tubal portion; so, in the tubal pregnancy subgroup, only ampullary pregnancies were included, as they represent the main extrauterine site of trophoblast implantation[22,23]. Besides, we decided to delimit the gestational age (42–56 days), as the previous studies included larger periods (5–10 weeks), which might have biased the results. Serum VEGF concentrations ranged from 15.6 to 783.1 ng/mL between all the subgroups. Statistical analysis revealed that the concentration of VEGF was significantly different between the subgroups and that significantly higher values were observed in the patients with EP compared with IUP (normal and abnormal) subgroups ($p<0.001$). We found that serum VEGF levels >188.7 ng/mL could differentiate EP from intrauterine pregnancies with high sensitivity (96.7%), specificity (95.0%), PPV (90.6%), and NPV (98.3%). These data are in accordance with the results already published in the literature.

A prospective study performed in 20 patients with EP found that serum VEGF values were higher in women with EP when compared with those with abnormal IUP[10]. They postulated that serum VEGF levels were more specific and had a higher PPV than serum progesterone levels in differentiating the various types of pregnancies; therefore, this study supported the fact that a serum VEGF level of >200 ng/mL could distinguish intrauterine pregnancies from extrauterine pregnancies with a specificity of 90% and a PPV of 86% and abnormal intrauterine pregnancies from extrauterine pregnancies with a specificity of 80% and a PPV of 86%. These findings were confirmed by other authors; Felemban A. studied 45 pregnant women (EP, normal and abnormal intrauterine pregnancies – 15 cases each group) and stated that the cutoff concentrations of 200 pg/mL for VEGF could distinguish normal intrauterine pregnancies from EP with a sensitivity of 88%, a specificity of 100%, and a PPV of 100%[11]. Between EP and abnormal intrauterine pregnancies, the sensitivity was 87.5%, specificity was 75%, and PPV was 77.8%. A similar study described higher serum VEGF levels in women with EP than in women with intrauterine pregnancies of comparable gestational age[24].

As already shown in other studies, serum progesterone levels could differentiate abnormal (topic or ectopic) from evolutive intrauterine pregnancies. On the one hand, in the subgroup with evolutive intrauterine pregnancies, only 6.7% of cases had serum progesterone levels lower than 15 nmol/L.

On the other hand, progesterone levels were higher than 15.0 nmol/L in 13.3% of cases of the other two subgroups.

This study shows that serum VEGF, but not progesterone, could be a more specific marker in discriminating IUP from EP, as its levels are increased in women with EP compared with women with normal and abnormal intrauterine pregnancies.

This finding can allow earlier and more successful EP diagnosis and treatments in the emergency rooms. Indeed, VEGF antagonists are under investigation for their potential use in disorders characterized by pathological angiogenesis (such as tumor growth) and the inhibition of VEGF action may also be a potential medical treatment for EP[25]. This area deserves further investigation.

AUTHORS' CONTRIBUTIONS

FRC: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – original & draft. **PPP:** Conceptualization, Writing – review & editing, Project administration, Validation, Visualization. **MAO:** Data curation, Writing – original & draft, Investigation. **RPVF:** Supervision, Validation, Visualization, Writing – review & editing.

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