Association of serum levels of vascular endothelial growth factor and early ectopic pregnancy

M.O. Fernandes da Silva, J. Elito Jr., S. Daher, L. Camano, A. Fernandes Moron

Department of Obstetrics of the Universidade Federal de São Paulo, São Paulo (Brazil)

Summary

Background: This study evaluated serum vascular endothelial growth factor (VEGF) concentrations in women with ectopic pregnancy (EP), miscarriage, and normal pregnancy (NP). *Materials and Methods:* This was a case-control study comparing serum VEGF concentrations among 72 women with ectopic pregnancy (n = 35), miscarriage (n = 15), and normal pregnancy (n = 22) matched for gestational age. For the determination of serum VEGF concentration a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) was used. Patients were stratified according to serum VEGF above or below 200 pg/ml. *Results:* The serum level of VEGF was significantly higher in women with EP (median 211.1 pg/ml; range 5-1,017.0 pg/ml) than in women with normal pregnancy (median 5 pg/ml; range 5-310.6 pg/ml) $p < 0.0001$. Serum VEGF concentrations did not show any statistically significant difference between women with miscarriage (median 231.9 pg/ml; range 5-813.7 pg/ml) and EP (median 211.1 pg/ml; range 5-1,017.0 pg/ml). When threshold concentrations of serum VEGF level > 200 pg/ml were used, an EP could be distinguished from a normal pregnancy with a sensitivity of 51.4%, a specificity of 90.9%, and a positive predictive value of 90%. Between EP and miscarriage, the sensitivity was 51.4%, specificity 42.8%, and a positive predictive value of 69.2%. *Conclusions:* Serum VEGF could not distinguish an EP from a miscarriage. However, serum VEGF concentrations could discriminate a normal intrauterine pregnancy (IUP) from an unviable pregnancy (EP or miscarriage).

Key words: Ectopic pregnancy; Miscarriage; Normal pregnancy; VEGF.

Introduction

The incidence of ectopic pregnancy (EP) has dramatically increased over the last two decades and accounts for 1.5% - 2% of all pregnancies [1]. Although the mortality related to EP has decreased significantly, it is the most important cause of maternal death in the first trimester accounting for 9% - 13% of all pregnancy-related deaths [2-4].

Treatment of EP has changed over the years and a conservative approach (medical treatment with methotrexate, expectant management, and salpingostomy by laparoscopy) now predominates [5, 6]. Early diagnosis is important in order to allow conservative treatment options [6-8].

In spite of a high-resolution vaginal ultrasound and highly-sensitive quantitative beta-human chorionic gonadotropin (β-hCG) assays, at first presentation, an EP can be difficult to diagnose at an early stage; 36.4% of all cases do not exhibit adnexal tenderness, and nine percent report no pain [9]. For this reason, a serum biomarker of tubal implantation, which could accurately identify an EP at first presentation, would be a major clinical advance. Several markers have been investigated for early diagnosis of EP [10].

For the establishment of a viable pregnancy, implantation and placentation are the early and crucial processes, both accompanied by angiogenesis, for which vascular endothelial growth factor (VEGF) is mainly accountable and plays a key role [11]. Several authors hypothesized that implantation of the conceptus within the oviduct might increase VEGF production as a form of accommodation to the hypoxic unfavorable environment [4, 8, 12, 13]. Therefore, serum VEGF could distinguish an EP from a miscarriage [8, 12, 13].

The aim of the study was to determine the serum levels of VEGF and compare them in cases of EP, miscarriage, and normal pregnancy (NP).

Materials and Methods

Patients

The study group was comprised of 35 women with EP confirmed by transvaginal ultrasound (TVUS) or at surgery and gestational age under 7.5 weeks. The inclusion criteria were the presence at TVUS of an extra-ovarian adnexal mass in women with a suspected EP (amenorrhea, uterine bleeding, and pain) with positive β-hCG test. The exclusion criteria were non-tubal EP (intrauterine, cervical, cesarean scar, ovarian, interstitial, and abdominal) and the suspect cases of early EP not confirmed by TVUS.

The control group consisted of 15 women with miscarriage and gestational age less than 7.5 weeks. The diagnosis was performed by means of serial β-hCG measurements and by TVUS. The criteria for ultrasound confirmation of a failure pregnancy were the absence of a visible yolk sac with a mean sac diameter of 13 mm, the absence of a visible embryo with a mean sac of 20 mm, the absence of cardiac motion with an embryo measuring five mm or more, or the presence of an empty amnion.

The other control group was composed of 22 women with NP and gestational age less than 7.5 weeks. The TVUS confirmed a viable intrauterine pregnancy.

Revised manuscript accepted for publication November 29, 2012

In all groups, blood samples were collected as soon as anamnesis suggested a possible patient for the study. When there was doubt in diagnosis of any patient, she was followed up with TVUS and serial quantitative β-hCG, until the authors were certain which group could match her.

The three groups: EP (n = 35), miscarriage (n = 15), and NP (n = 22) were matched for gestational age (by date of last menstrual period and ultrasound findings).

This work has been approved by the Ethics Committee of the Universidade Federal de São Paulo. All patients agreed with the study and signed Informed consent.

Serum assay

All blood samples were collected, before treatment, by peripheral venous puncture, and immediately centrifuged at 1,000 rpm for ten minutes, and the supernatants were stored at –80°C until assayed. For the determination of serum VEGF concentration, a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) was used, which involved two kinds of highly specific antibodies (human VEGF) specific for the human molecule.

Statistical analysis

Data are presented as median and range (minimum, maximum). The three groups were compared using the Kruskal-Wallis test and the Mann-Whitney U test with Bonferroni's correction. Results were considered significant when $p < 0.05$. The statistical analysis was performed using SPSS r12.

Results

The mean (± SD) gestational age was similar in the three groups of women*:* 47.6 ± 4.8 days, 48.3 ± 4.9 days, 49.7 ± 4.3 days for the EP, miscarriage and NP groups, respectively.

The serum level of VEGF was significantly higher in women with EP (median 211.1 pg/ml; range 5 – 1,017.0 pg/ml) than in women with NP (median 5 pg/ml; range 5 – 310.6 pg/ml) $p < 0.0001$ (Table 1).

In this study, the median VEGF level among women with EP (median 211.1 pg/ml; range 5 – 1017 pg/ml) and miscarriage (median 231.9 pg/ml; range 5 – 813.7 pg/ml) was not statistically significant (Table 1).

When cut-off concentrations of 200 pg/ml for VEGF were used, EP could be distinguished from NP with a sensitivity of 51.4%, a specificity of 90.9%, and a positive predictive value of 90%. Between EP and miscarriage, the sensitivity was 51.4%, specificity 42.8%, and positive predictive value of 69.2%.

Table 1. — *Serum VEGF concentrations in women with EP, abnormal IUP, and normal IUP. Values are mean ± SD and median values with ranges.*

VEGF (pg/ml)	EP (n = 35)	Abnormal IUP (n = 15)	Normal IUP (n = 22)
Mean	297.5	299.6	39.9
Standard deviation	259.4	278.3	91.4
Median	211.1	231.9	5
Min	5	5	5
Max	1,017	813.7	310.6

$p < 0.0001$ between normal IUP and the other two groups (EP and abnormal IUP).

Discussion

The evidences found in the present study suggest that serum VEGF levels are higher in women with EP than in those with NP of comparable gestational age ($p < 0.0001$). The median of the VEGF serum values in EP was (211.1 pg/ml, n = 35) that is similar to the levels measured by Daniel *et al*. (226.8 pg/ml, n = 20), by Kucera-Sliutz *et al*. (211.2 pg/ml, n = 42), by Mueller *et al*. (203.6 pg/ml, n = 43), by Daponte *et al.* (227.2 pg/ml, n = 27) and differ from the study of Ugurlu *et al*. (55.2 pg/ml, n = 28).

The comparison of serum VEGF concentration between EP and NP demonstrated in several studies that the levels of VEGF are higher in EP [4, 8, 14] similarly to the present results. However, other authors showed no difference between both groups [3].

The current results support, that serum VEGF may distinguish EP from NP. Therefore, early diagnosis of EP could be suspected in a high probability when the serum VEGF concentration is higher.

The crucial point is the discrimination between ectopic and abnormal intrauterine pregnancy. In this work, accordingly to previous studies, serum concentrations of VEGF in women with EP were higher than in those with miscarriage, but these concentrations did not show any statistically significant difference between the two [8, 12-15].

When threshold concentrations of a serum VEGF level > 200 pg/ml were used in previous studies, EP could be distinguished from a NP with a sensitivity of 88%, specificity of 100%, and a positive predictive value of 100% [8], however, in the current study, these corresponding values were 51.4%, 90.9%, and 90%, respectively. For the discrimination between EP and miscarriage, Daniel *et al*. found a sensitivity of 60%, a specificity of 80%, and a positive predictive value of 86%, when a cut-off of 200 pg/ml of serum VEGF concentration was used [12]. Another study found a sensitivity of 87.5%, a specificity of 75%, and a positive predictive value of 77.8% [8]. The corresponding values of another study were 56.1%, 51.2%, and 53.5%, respectively [14]. For discrimination between EP and miscarriage the present authors found a sensitivity of 51.4%, a specificity of 42.8%, and a positive predictive value of 69.2%. On the other hand, serum VEGF levels can distinguish an EP from a NP with a specificity of 90.9% and a positive predictive value of 90%.

Serum VEGF initially seemed to be a very helpful serum marker for EP [8, 12, 13]. Furthermore, other reports showed the limitation of serum VEGF to distinguish an EP from a miscarriage [14, 15].

Recently a study has shown that using a two-step algorithm with four markers (progesterone, VEGF, inhibin A, and activin A), it was possible to achieve 99% accuracy when diagnosing EP [16]. This suggests that even if VEGF is not important alone, it could be helpful in association with other markers.

It is important to point out that TVUS used as a routine diagnostic method for EP demonstrated to have a sensi-

tivity and specificity to detect EP of 90.9% and 99.9%, with positive and negative predictive values of 93.5% and 99.8%, respectively [17].

In the present authors' point of view, serum VEGF measurement could be useful in the diagnosis of EP. In this way a single serum VEGF measure could discriminate a viable from an unviable pregnancy in early stages of gestation. In this phase a single β-hCG measurement could not discriminate an EP from a miscarriage and in this situation repeated β-hCG measurements with intervals of 48 hours are necessary. A single serum progesterone measurement could not discriminate between EP and miscarriage according to meta-analysis [18]. TVUS, sometimes, could not identify the exact site of the implantation in early stages of pregnancy. Despite the fact that serum VEGF concentration is not very specific in the early diagnosis of EP, it could discriminate the viable pregnancy from an unviable one. This aspect is very relevant since it helps the diagnosis of the cases with major risk of complication.

Conclusions

Accordingly to the present results, VEGF levels could not distinguish an EP from a miscarriage. However, serum VEGF concentrations could discriminate a normal from an unviable pregnancy (EP or miscarriage).

Acknowledgments

This work was financially supported by FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo – The Foundation of the Assistance for Research in the State of São Paulo / Brazil).

References

[1] Centers for Disease Control and Prevention. Ectopic pregnancy - United States, 1990-1992. MMWR CDC Surveil summ. 1995, 44, 46.

[2] Nederlof K.P., Lawson H.W., Saftlas A.F., Atrash H.K., Finch E.I.: "Ectopic pregnancy surveillance, United States, 1970-1987". *MMWR CDC Surveil Summ.,* 1990, *39*, 9.

[3] Gerton G.L., Fan X.J., Chittams J., Sammel M., Hummel A., Strauss J.F. *et al*.: "A serum proteomics approach to the diagnosis of ectopic pregnancy". *Ann. N.Y. Acad. Sci.*, 2004, *1022*, 306.

[4] Mueller M.D., Raio L., Spoerri S., Ghezzi F., Dreher E., Bersinger N.A.: "Novel placental and nonplacental serum markers in ectopic versus normal intrauterine pregnancy". *Fertil. Steril.*, 2004, *81,* 1106.

[5] Yao M., Tulandi T.: "Current status of surgical and nonsurgical management of ectopic pregnancy". *Fertil. Steril.,* 1997, *67,* 421.

[6] Tulandi T., Sammour A.: "Evidence-based management of ectopic pregnancy". *Curr. Opin. Obstet. Gynecol*., 2000, *12*, 289.

[7] Elito J. Jr., Reichmann A.P., Uchiyama M.N., Camano L.: "Predictive score for the systemic treatment of unruptured ectopic pregnancy with a single dose of methotrexate". *Int. J. Gynaecol. Obstet.*, 1999, *67,* 75.

[8] Felemban A., Sammour A., Tulandi T.: "Serum vascular endothelial growth factor as a possible marker for early ectopic pregnancy". *Hum. Reprod.,* 2002, *17*, 490.

[9] Tay J.I., Moore J., Walker J.J.: "Ectopic pregnancy". *West J. Med.,* 2000, *173,* 131. Review.

[10] Cartwright J., Duncan W.C., Critchley H.O., Horne A.W.: "Serum biomarkers of tubal ectopic pregnancy: current candidates and future possibilities". *Reprod.,* 2009, *138,* 9. Epub 2009 Mar 25.

[11] Nowacek G.E., Meyer W.R., McMahon M.J., Thorp J.R., Wells S.R.*:* "Diagnostic value of cervical fetal fibronectin in detecting extrauterine pregnancy". *Fertil. Steril.*, 1999, *72*, 302.

[12] Daniel Y., Geva E., Lerner-Geva L., Esched-Englender T., Gamzu R., Lessing J.B. *et al.*: "Levels of vascular endothelial growth factor are elevated in patients with ectopic pregnancy: is this a novel marker?". *Fertil. Steril.,* 1999, *72,* 1013.

[13] Daponte A., Pournaras S., Zintzaras E., Kallitsaris A., Lialios G., Maniatis A.N., Messinis I.E.: "The value of a single combined measurement of VEGF, glycodelin, progesterone, PAPP-A, HLP and LIF for differentiating between ectopic and abnormal intrauterine pregnancy". *Hum. Reprod.,* 2005, *20,* 3163.

[14] Ugurlu E.N., Ozaksit G., Karaer A., Zulfikaroglu E., Atalay A., Ugur M.: "The value of vascular endothelial growth factor, pregnancy-associated plasma protein-A, and progesterone for early differentiation of ectopic pregnancies, normal intrauterine pregnancies, and spontaneous miscarriages". *Fertil. Steril.,* 2009, *91,* 1657.

[15] Kucera-Sliutz E., Schiebel I., Konig F., Leodolter S., Sliutz G., Koelbl H.: "Vascular endothelial growth factor (VEGF) and discrimination between abnormal intrauterine and ectopic pregnancy". *Hum. Reprod.*, 2002, *17*, 3231.

[16] Rausch M.E., Sammel M.D., Takacs P., Chung K., Shaunik A., Barnhart K.T.: "Development of multiple marker test for ectopic pregnancy". *Obstet. Gynecol.*, 2011, *117,* 573.

[17] Condous G., Okaro E., Khalid A., Lu C., Van Huffel S., Timmerman D., Bourne T.: "The accuracy of transvaginal ultrasonography for the diagnosis of ectopic pregnancy prior to surgery". *Hum. Reprod*., 2005, *20*, 1404.

[18] Mol B.W., Lijmer J.G., Ankum W.M., van der Veen F., Bossuyt P.M.: "The accuracy of single serum progesterone measurement in the diagnosis of ectopic pregnancy: a meta-analysis". *Hum. Reprod.*, 1998, *13,* 3220.

Address reprint requests to:
M.O. FERNANDES DA SILVA, M.D.
Department of Obstetrics of the
Universidade Federal de São Paulo
Av. Dr. Paulo de Moraes 1671, ap. 12
Piracicaba SP, CEP 13.400-853 (Brazil)
e-mail: mosf75@uol.com.br