

The importance of some angiogenic markers in spontaneous abortion

**N. Ozturk¹, I. Gozukara², Z. Kamalak², M.A. Gul¹, Z. Bayraktutan³, N.K. Baygutalp¹,
A. Kızıltunc¹, E. Bakan¹, E.U. Karakilic²**

¹ Ataturk University, Faculty of Medicine, Department of Medical Biochemistry, Erzurum

² Nenehatun Obstetrics Hospital, Erzurum; ³ Regional Training and Research Hospital, Department of Biochemistry, Erzurum (Turkey)

Summary

Aim: In this study, the authors aimed to determine the serum levels of vascular endothelial growth factor (VEGF), angiopoietin-1 (ang-1) and angiopoietin-2 (ang-2) factors as indicators of placental angiogenesis and vasculogenesis in abortion cases. **Materials and Methods:** This study was conducted in 40 women who were pregnant for 7-20 weeks and diagnosed with an incipient abortion and 40 pregnant healthy women with similar ages, gestational weeks, and body mass index (BMI) values. Serum VEGF, ang-1, and ang-2 levels were measured with ELISA methods. **Results:** The authors found that the serum VEGF levels were higher and ang-1 levels were significantly lower in pregnant women whose pregnancies failed with abortion, compared to control group. There was no significant difference in terms of ang-2 levels between groups. **Conclusion:** A strong relationship was found between VEGF and ang-1 early pregnancy loss, and significant changes of these factors may also be associated with the physiopathology of abortion incipience. Evaluating these factors may be beneficial for prediction and designing of treatment modalities on spontaneous abortion.

Key words: Abortion; VEGF; Angiopoietin-1 (Ang-1); Angiopoietin-2 (Ang-2).

Introduction

Normal embryonic development and growth is dependent on formation and sustained fetoplacental blood vessels [1]. Angiogenesis and vasculogenesis that are important stages in the development of fetal and placental vascular structures are complex processes that occur as a result of interaction between growth factors and cells. Vasculogenesis is the development of primitive vascular structure from hemangiogenic stem cells. Angiogenesis is the formation of new capillaries from pre-existing vessels. These processes are regulated by many growth factors, so vascular network is controlled on the basis of the interactions of these factors [1-6].

Vascular endothelial growth factor (VEGF) is a multi-functional cytokine. VEGF provides the formation, migration, and proliferation of endothelial cells, and plays a central role in the regulation of placental angiogenesis [2, 3, 7]. Angiopoietins are vascular growth factors which have functions in the regulation of embryonic and postnatal angiogenesis. Angiopoietin-1 (ang-1) and angiopoietin-2 (ang-2), produced by placenta, are important for restructuring of vessels and the endothelial cell survival [1]. Ang-1 mediates stabilization of developing vessels, maintains vessel integrity, and provides angiogenic progression [1, 2, 8]. Despite ang-1 and ang-2 share a similar structure, they have different effects on the same receptors. Ang-2 initi-

ates the vascular regression and cell death by disrupting connections between endothelial and perivascular cells [1, 2, 4, 9]. In the presence of proangiogenic factors such as VEGF, the destabilization caused by the ang-2 results in the formation and progress of new vessels [8].

Approximately 15% of clinically diagnosed pregnancies end in abortion through the first trimester due to different etiologic factors [10]. Recently, researchers have tried to show the relationship between angiogenic factors and recurrent pregnancy loss in a number of studies [1, 11, 12].

In this study, the authors aimed to compare the serum levels of VEGF, ang-1, and ang-2, possible placental angiogenesis and vasculogenesis indicators, between pregnant women diagnosed with incipient abortion and healthy women with similar ages.

Materials and Methods

The local ethics committee approved the study (protocol number: 22.03.2013/8) and participants gave written informed consent. This study included 40 women who were between 7-20 weeks of pregnancy and presented with vaginal bleeding, pain, and cervical dilation complaints and also diagnosed with incipient abortion. Control group consisted of 40 pregnant healthy women with similar ages, gestational weeks and body mass index (BMI) values. Serum samples were stored at -80°C until assay. Serum VEGF levels measured with ELISA kit and an ELISA reader according to the kit insert. Also ang-1 and ang-2 serum lev-

Table 1. — *The clinical characteristic of healthy and incipient abortion pregnant subjects.*

	Control	Incipient abortion	<i>p</i> value
Age (years)	29.76 ± 4.1	29.40 ± 4.3	> 0.05
Gravidity	2.2 ± 0.4	2.1 ± 0.3	> 0.05
Pregnancy weeks (menstruating)	13.4 ± 5.5	12.5 ± 4.4	> 0.05
VEGF (pg/ml)	14.17 ± 4.18	56.66 ± 16.17	< 0.001
Ang-1 (ng/ml)	114.96 ± 15.09	86.91 ± 12.73	< 0.001
Ang-2 (pg/ml)	688.80 ± 131.56	648.76 ± 128.04	> 0.05

VEGF: vascular endothelial growth factor, Ang-1: angiopoietin-1, Ang-2: angiopoietin-2.

els were measured by ELISA kits.

Statistical analyses were performed using the SPSS 20.0 program. Compliance with the normal distribution of parameters was assessed by Kolmogorov-Smirnov test. Independent samples *t*-test was used for evaluation of parameters normally distributed. The correlation analyses between parameters were evaluated by Pearson correlation analysis.

Results

Serum VEGF levels were higher ($p < 0.001$) and ang-1 levels were significantly lower ($p < 0.001$) in pregnant women that had an abortion compared to control group (Table 1). There was no significant difference in terms of ang-2 levels between groups ($p = 0.172$) (Table 1). Again, a significant weak correlation ($p < 0.05$, $r < 0.6$) was found between serum ang-1 and VEGF levels in patient group (Figure 1).

Discussion

Spontaneous abortion (SA), a common finding affecting 20% of pregnancies, usually occurs at an early stage of pregnancy. Until now, various factors have been identified that influence miscarriage such as genetic, endocrine, infectious, anatomical, nutritional and environmental, but exact pathogenesis is not known [13, 14]. However inappropriate angiogenesis and blood flow pattern have been attributed to miscarriage and implantation failure [15, 16]. Inhibition of angiogenesis in pregnant mice results in complete failure of embryonic growth owing to interference with placental and yolk sac formation and embryonic vascular development [17]. Nonetheless some angiogenesis factors like ang-1, ang-2, and VEGF are suggested to have important roles in the abortion cases. In this study, the authors found increased serum VEGF levels and decreased serum ang-1 levels in pregnancies resulting in abortion, compared to the control group. These results suggest that there is a strong relationship between these factors and early pregnancy loss and significant changes of these factors also may be associated with the pathology of abortion incipience.

VEGF is a specific mitogen that has been documented to participate in many phases of the reproductive process such as embryo implantation and placental growth [18, 19]. During early gestation, VEGF is related with oocytes maturation, trophoblastic proliferation, implantation and development of the embryo, angiogenesis of the placenta, and the growth of maternal and fetal blood vessels in the uterus [20, 21]. A crucial role of VEGF in fetal and placental angiogenesis has been also supported from gene

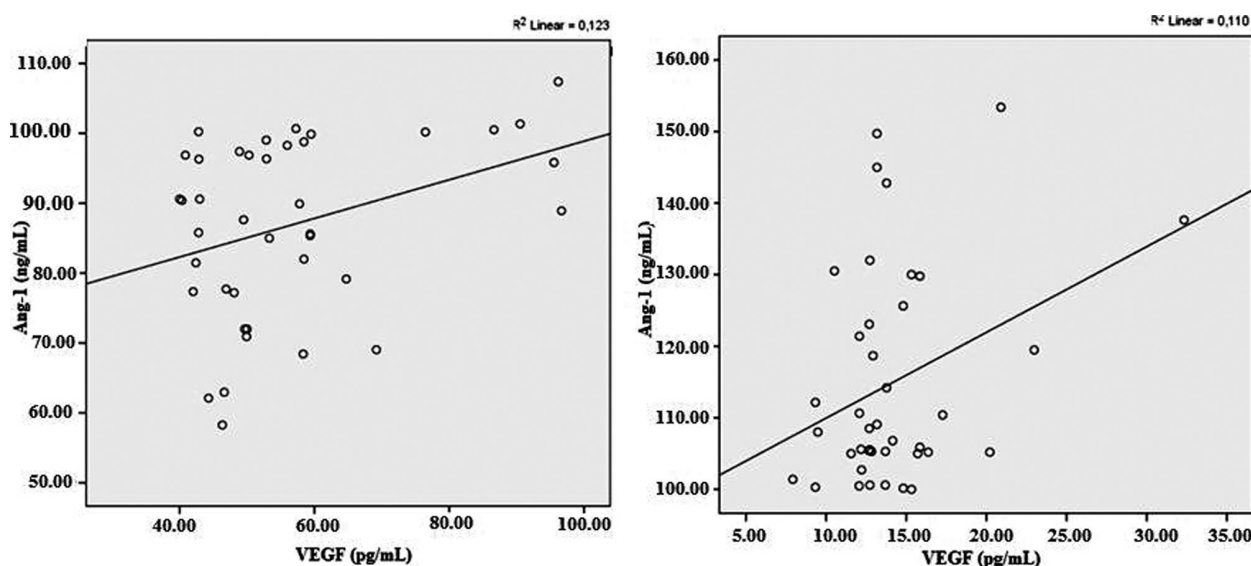


Figure 1. — Scatter plot graph between ang-1 and VEGF levels for the patient group ($p = 0.026$, $r = 0.351$). Scatter plot graph between ang-1 and VEGF levels for the control group ($p = 0.036$, $r = 0.332$).

knockout studies [22, 23]. Pang *et al.* [24] reported that in hypoxia cases, like poor vascularization at the early phases of placental development, maternal serum VEGF levels increase and the pregnancy results in abortion due to endothelial dysfunction. VEGF, that modulates placental vascular development, was also high in patients with abortion in the present series. This finding may be explained as increased blood vessel density in decidua parietals was related with spontaneous human first trimester abortion, similar with previous studies [25]. Increased vascularization is considered a consequence of hypoxia. This often occurs with increased microvascular permeability and increased capillary density under the condition of tissue ischemia and hypoxia [26]. Meanwhile, in vitro experiments have indicated that hypoxia could enhance VEGF secretion [27].

Among other biomolecules, ang-1 maintains vessel integrity and probably plays a role in the later stages of vascular remodeling. Ang-2 is a functional antagonist of ang-1 and leads to vascular dilatation, loosening of cell/cell interactions, and disruption of vessel integrity [28]. From obstetric aspect, circulating levels of ang-1 and ang-2 are associated with poor pregnancy outcomes, and low serum levels of these factors have been reported with predictive for potential consequences in cases of abortion or ectopic pregnancy [8]. Schneuer *et al.* [8] reported that patients with adverse pregnancy outcome have lower levels of ang-2. Similar to these results, in a case-control study by Daponte *et al.* [29] reported that serum ang-1 and ang-2 levels at six to eight weeks of gestation were significantly lower in failed pregnancies compared to normal pregnancies. Researchers concluded that ang-1 and ang-2 levels may be used as biomarkers of ectopic pregnancy and missed abortion by means of a single measurement of their serum levels at six to eight weeks of gestation. In the present study, serum ang-1 concentrations were significantly lower in abortion incipience compared to the control group and it showed positive correlation with serum VEGF levels. Although this correlation was not statistically significant, ang-2 levels were also decreased in abortion incipience group. Similar to the present study, reduction of ang-2 was also observed in women with a history of recurrent miscarriage. The estimated increased VEGF and reduced ang-2 may contribute to the advanced vessel maturation observed in this group of patient [30].

In conclusion, the implantation process of embryo and steadiness of pregnancy seems to depend on proper blood supply to fetal placental tissue that is regulated with angiopoietic factors like VEGF, ang-1, and ang-2. These factors may have a predictive potential for spontaneous abortion and also new treatment modalities, and may have beneficial effect on prevention of SA.

References

- [1] Kappou D., Sifakis S., Konstantinidou A., Papantoniou N., Spandidos D.A.: "Role of the angiopoietin/Tie system in pregnancy (Review)". *Exp. Ther. Med.*, 2015, 9, 1091.
- [2] Zhang E.G., Smith S.K., Baker P.N., Charnock-Jones D.S.: "The regulation and localization of angiopoietin-1, -2, and their receptor Tie2 in normal and pathologic human placentae". *Mol. Med.*, 2001, 7, 624.
- [3] Suri C., Jones P.F., Patan S., Bartunkova S., Maisonpierre P.C., Davis S., *et al.*: "Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis". *Cell*, 1996, 87, 1171.
- [4] Dunk C., Shams M., Nijjar S., Rhaman M., Qiu Y., Bussolati B., Ahmed A.: "Angiopoietin-1 and angiopoietin-2 activate trophoblast Tie-2 to promote growth and migration during placental development". *Am. J. Pathol.*, 2000, 156, 2185.
- [5] Wulff C., Wilson H., Dickson S.E., Wiegand S.J., Fraser H.M.: "Hemochorial placentation in the primate: expression of vascular endothelial growth factor, angiopoietins, and their receptors throughout pregnancy". *Biol. Reprod.*, 2002, 66, 802.
- [6] Demir R., Yaba A., Huppertz B., Hurliman A.K., Speroff L., Stouffer R.L.: "Vasculogenesis and angiogenesis in the endometrium during menstrual cycle and implantation". *Acta Histochem.*, 2010, 112, 203.
- [7] Patton P.E., Lee A., Molskness T.A.: "Changes in circulating levels and ratios of angiopoietins during pregnancy but not during the menstrual cycle and controlled ovarian stimulation". *Fertil. Steril.*, 2010, 93, 1493.
- [8] Schneuer F.J., Roberts C.L., Ashton A.W., Guilbert C., Tasevski V., Morris J.M., Nassar N.: "Angiopoietin 1 and 2 serum concentrations in first trimester of pregnancy as biomarkers of adverse pregnancy outcomes". *Am. J. Obstet. Gynecol.*, 2014, 210, 345.e1.
- [9] Maisonpierre P.C., Suri C., Jones P.F., Bartunkova S., Wiegand S.J., Radziejewski C., *et al.*: "Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis". *Science*, 1997, 277, 55.
- [10] Yakut S., Toru H.S., Çetin Z., Özel D., Şimşek M., Mendilcioğlu İ., Lüleci G.: "Chromosome abnormalities identified in 457 spontaneous abortions and their histopathological findings". *Türk. Patoloji. Derg.*, 2015, 31, 111.
- [11] Banerjee P., Ghosh S., Dutta M., Subramani E., Khalpada J., Roychoudhury S., *et al.*: "Identification of key contributory factors responsible for vascular dysfunction in idiopathic recurrent spontaneous miscarriage". *PLoS One*, 2013, 8, e80940.
- [12] Andraweera P.H., Dekker G.A., Roberts C.T.: "The vascular endothelial growth factor family in adverse pregnancy outcomes". *Hum. Reprod. Update*, 2012, 18, 436.
- [13] Rai R., Regan L.: "Recurrent miscarriage". *Lancet*, 2006, 368, 601.
- [14] Stephenson M.D.: "Frequency of factors associated with habitual abortion in 197 couples". *Fertil. Steril.*, 1996, 66, 24.
- [15] Habara T., Nakatsuka M., Konishi H., Asagiri K., Noguchi S., Kudo T.: "Elevated blood flow resistance in uterine arteries of women with unexplained recurrent pregnancy loss". *Hum. Reprod.*, 2002, 17, 190.
- [16] Quenby S., Nik H., Innes B., Lash G., Turner M., Drury J., Bulmer J.: "Uterine natural killer cells and angiogenesis in recurrent reproductive failure". *Hum. Reprod.*, 2009, 24, 45.
- [17] Klauber N., Rohan R.M., Flynn E., D'Amato R.J.: "Critical components of the female reproductive pathway are suppressed by the angiogenesis inhibitor AGM-1470". *Nat. Med.*, 1997, 3, 443.
- [18] Clark D.E., Smith S.K., He Y., Day K.A., Licence D.R., Corps A.N., *et al.*: "A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation". *Biol. Reprod.*, 1998, 59, 1540.
- [19] Torry D.S., Holt V.J., Keenan J.A., Harris G., Caudle M.R., Torry R.J.: "Vascular endothelial growth factor expression in cycling human endometrium". *Fertil. Steril.*, 1996, 66, 72.
- [20] Jelkmann W.: "Pitfalls in the measurement of circulating vascular endothelial growth factor". *Clin. Chem.*, 2001, 47, 617.
- [21] Su M.T., Lin S.H., Lee I.W., Chen Y.C., Kuo P.L.: "Association of polymorphisms/haplotypes of the genes encoding vascular endothelial growth factor and its KDR receptor with recurrent pregnancy

- loss". *Hum. Reprod.*, 2011, 26, 758.
- [22] Carmeliet P., Ferreira V., Breier G., Pollefeyt S., Kieckens L., Gertsenstein M., *et al.*: "Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele". *Nature*, 1996, 380, 435.
- [23] Ferrara N., Carver-Moore K., Chen H., Dowd M., Lu L., O'Shea K.S., *et al.*: "Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene". *Nature*, 1996, 380, 439.
- [24] Pang L., Wei Z., Li O., Huang R., Qin J., Chen H., *et al.*: "An increase in vascular endothelial growth factor (VEGF) and VEGF soluble receptor-1 (sFlt-1) are associated with early recurrent spontaneous abortion". *PLoS One*, 2013, 8, e75759.
- [25] Vailhe B., Dietl J., Kapp M., Toth B., Arck P.: "Increased blood vessel density in decidua parietalis is associated with spontaneous human first trimester abortion". *Hum. Reprod.*, 1999, 14, 1628.
- [26] Cao Y., Linden P., Shima D., Browne F., Folkman J.: "In vivo angiogenic activity and hypoxia induction of heterodimers of placenta growth factor/vascular endothelial growth factor". *J. Clin. Invest.*, 1996, 98, 2507.
- [27] Hornig C., Barleon B., Ahmad S., Vuorela P., Ahmed A., Weich H.A.: "Release and complex formation of soluble VEGFR-1 from endothelial cells and biological fluids". *Lab. Invest.*, 2000, 80, 443.
- [28] Geva E., Jaffe R.B.: "Role of angiopoietins in reproductive tract angiogenesis". *Obstet. Gynecol. Surv.*, 2000, 55, 511.
- [29] Daponte A., Deligeoroglou E., Pournaras S., Tsezou A., Garas A., Anastasiadou F., *et al.*: "Angiopoietin-1 and angiopoietin-2 as serum biomarkers for ectopic pregnancy and missed abortion: a case-control study". *Clin. Chim. Acta*, 2013, 415, 145.
- [30] Lash G.E., Innes B.A., Drury J.A., Robson S.C., Quenby S., Bulmer J.N.: "Localization of angiogenic growth factors and their receptors in the human endometrium throughout the menstrual cycle and in recurrent miscarriage". *Hum. Reprod.*, 2012, 27, 183.

Corresponding Author:
N. KILIC-BAYGUTALP, Ph.D
Faculty of Medicine, Ataturk University
Department of Medical Biochemistry
25240 Erzurum (Turkey)
e-mail: eczbaygutalp80@gmail.com