

Effect of Fetal Gender on Angiogenesis: Analysis of Angiogenin and Leptin Levels in Second Trimester Amniotic Fluids

Fetal Cinsiyetin Anjiogenez Üzerine Etkisi: İkinci Trimester Amniyon Sıvılarında Anjiogenin ve Leptin Seviyelerinin İncelenmesi

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Geliş Tarihi/Received: 07.04.2013

Kabul Tarihi/Accepted: 10.07.2013

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ABSTRACT Objective: To investigate a possible effect of fetal gender on placental angiogenesis by analysing second trimester amniotic fluid angiogenin and leptin levels in pregnancies with normal karyotype. **Material and Methods:** Angiogenin and leptin have strong angiogenetic properties. Therefore we retrospectively and randomly identified 48 fetuses (24 male and 24 female) with normal karyotype from our perinatology database in order to analyse their amniotic fluid angiogenin and leptin levels. Angiogenin and leptin levels were measured by ELISA. Kruskal-Wallis test was used to compare the clinical characteristics and Mann-Whitney U test was used to compare angiogenin and leptin levels between male and female fetuses. A value of $p < 0.05$ was considered to be significant. **Results:** There were no significant differences in median levels of maternal age, weight, gestational age at amniotic fluid sampling, gravidity, parity and body mass index of the two groups. There were no significant changes in amniotic fluid angiogenin and leptin levels between male and female fetuses. Median angiogenin levels were 15.91 ng/ml (range 9.24- 37.91) for male and 20.91 ng/ml (5.69-45.02) for female fetuses ($p=0.09$); median leptin levels were 15.94 ng/ml (range 2.30-39.36) for male and 13.25 ng/ml (3.28-49.23) for female fetuses ($p=0.3$). **Conclusion:** Fetal gender does not affect the degree of placental angiogenesis.

Key Words: Amniotic fluid; angiogenin; leptin

ÖZET Amaç: Normal karyotipli fetüslerin ikinci trimester amniyon sıvılarında anjiogenin ve leptin seviyelerini inceleyerek fetal cinsiyetin plasental anjiogenez üzerine olası etkisini araştırmak. **Gereç ve Yöntemler:** Anjiogenin ve leptinin güçlü anjiogenetik özellikleri bulunmaktadır. Bu nedenle amniyon sıvılarındaki anjiogenin ve leptin seviyeleri incelenmek üzere perinatoloji veritabanından retrospektif olarak normal karyotipli 48 fetus (24 kız-24 erkek) belirlendi. Anjiogenin ve leptin seviyeleri ELİSA yöntemi kullanılarak çalışıldı. Klinik özellikleri karşılaştırmak için Kruskal-Wallis testi ve dişi ile erkek fetüsler arasında anjiogenin ve leptin seviyelerini karşılaştırmak için Mann-Whitney U testi kullanıldı. $p < 0,05$ anlamlı olarak kabul edildi. **Bulgular:** Gruplar arasında medyan anne yaşı, gravida, parite, vücut kütle indeksi ve amniosentez yapıma haftaları arasında bir fark yoktu. Erkek ve dişi fetüsler arasında amniyon sıvısı anjiogenin ve leptin seviyeleri arasında anlamlı bir fark bulunmadı. Medyan anjiogenin seviyeleri dişi fetüsler için 20,91 ng/ml (5,69-45,02), erkek fetüsler için 15,91 ng/ml (range 9,24-37,91) ($p=0,09$); medyan leptin seviyeleri dişi fetüsler için 13,25 ng/ml (3,28-49,23) erkek fetüsler için 15,94 ng/ml (range 2,30-39,36) ($p=0,3$) olarak bulundu. **Sonuç:** Fetal cinsiyet plasental anjiogenez düzeyini etkilememektedir.

Anahtar Kelimeler: Amniyotik sıvı; anjiyogenin; leptin

Türkiye Klinikleri J Gynecol Obst 2014;24(1):16-9

Angiogenesis means new vessel formation and proliferation from the existing blood vessels. Angiogenesis takes part in physiologic processes like menstruation cycle, wound healing and placental for-

mation in pregnancy. Huge amount of angiogenesis is required for the growth of placenta and development of vascular structures. The major component of the human placenta is the syncytiotrophoblast. It plays a leading role in fetal-maternal exchanges and the secretion of pregnancy specific hormones. Human chorionic gonadotropin (hCG), a glycoprotein secreted by syncytiotrophoblasts, have been shown to be affected by fetal gender. It was demonstrated that maternal serum hCG levels are higher in women carrying a female fetus.¹

Angiogenin is a 14-kDa, non-glycosylated polypeptide that has angiogenetic and ribonucleic activities and plays role in this complex process of angiogenesis.² Angiogenin is one of the most potent inducers of neovascularization.³ The origin of amniotic fluid angiogenin is unknown. Feto-placental unit is thought to be the origin of amniotic fluid angiogenin.⁴

Leptin is secreted from adipocytes and exists in circulation as a 16-kDa monomeric plasma protein. It plays a key role in regulating bodyweight by controlling food consumption, sympathetic nervous system activation and thermogenesis.⁵ Leptin is a pleiotrophic hormone that also plays role in regulation of angiogenesis, inflammation and immunity.⁶ Proliferation of endothelial cells and stimulation of angiogenesis has been shown by leptin administration in vitro and in vivo. Leptin is also synthesized and released by syncytiotrophoblast and fetal fat cells.⁷ Amnion cells also produce and secrete leptin into amniotic fluid.⁸

Considering the possible roles of angiogenin and leptin on angiogenesis of placenta, the aim of the present study is to search whether fetal gender affects the degree of angiogenesis. For this reason we analysed amniotic fluid angiogenin and leptin levels in pregnancies with normal karyotype.

MATERIAL AND METHODS

In the Perinatology Department of Obstetrics and Gynecology Clinic at Dokuz Eylül University, we store approximately 2 ml excess amniotic fluid samples from pregnant women who undergo mid-trimester amniocentesis for various indications after their informed consent are obtained.

Study consisted of non-smoker pregnant Turkish women. Indications for amniocentesis were as follows: increased maternal age, increased risk at first trimester screening test and increased risk at triple test.

Amniocentesis was performed under ultrasound guidance transabdominally in a sterile manner. 2 ml amniotic fluids were centrifuged for 5 minutes at 3000 cycle/min. The supernatants were stored at -80 °C for future investigations.

From the perinatology record database we retrospectively and randomly identified 48 fetuses (24 male and 24 female) with normal karyotype in order to analyse their amniotic fluid angiogenin and leptin levels.

Amniotic fluid angiogenin levels were analysed by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis). The ELISA was validated for amniotic fluid and samples were assayed in duplicate. The ELISA sensitivity for amniotic fluid was 0.026 ng/ml, and the interassay and intraassay coefficients of variation were 4.6% and 2.9%, respectively. The ELISA used is specific for angiogenin and does not cross-react or interact with human interleukin-1c, interleukin-1l3, interleukin-2, interleukin-3, interleukin-4, interleukin-6, interleukin-7, interleukin-8 or tumor necrosis factor α .

Amniotic fluid leptin levels were analysed by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, USA). The ELISA was validated for amniotic fluid and samples were assayed in duplicate. The minimum detectable dose of leptin is typically less than 7.8 pg/mL. The intra- and interassay coefficients of variation were 3.2% and 3.5% respectively. The ELISA used is specific for leptin and does not cross-react or interact with human interleukin-1 α , interleukin-1 β , interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, tumor necrosis factor- α or angiogenin.

The results were analysed by using Statistical Package for the Social Sciences version 22 for Windows (SPSS Inc., Chicago, IL, USA). Kruskal-Wallis

test was used to compare the clinical characteristics and Mann-Whitney U test was used to compare angiogenin and leptin levels between male and female fetuses. A value of $p < 0.05$ was considered to be significant.

RESULTS

Maternal characteristics are shown in Table 1. There were no significant differences in median levels of maternal age, weight, gestational age at amniotic fluid sampling, gravidity, parity and body mass index of the two groups.

There were no significant changes in amniotic fluid angiogenin and leptin levels between male and female fetuses. Median angiogenin levels were 15.91 ng/ml (range 9.24-37.91) for male and 20.91 (5.69-45.02) for female fetuses ($p=0.09$); median leptin levels were 15.94 (range 2.30- 39.36) for male and 13.25 (3.28-49.23) for female fetuses ($p=0.3$) (Figure 1).

DISCUSSION

This study questions whether fetal gender affects the degree of placental angiogenesis. In order to analyse this we measured potent angiogenetic factors, angiogenin and leptin in the second trimester amniotic fluids of fetuses with normal karyotype.

To the best of our knowledge based on PubMed search (angiogenesis, fetal gender, amniotic fluid) we found only one relevant study concerning this subject. In this only study in the literature, Poggi et al. studied angiogenin, IL-6 and IL-10 levels in second trimester amniotic fluids of both term and preterm gestations.⁹ They found angiogenin but not IL -6 and IL-10 levels to be significantly lower in male fetuses both term and preterm cases. Angiogenin levels including all fetuses were 22.2 ng/ml (5.9-66.4) for male and 32.0 ng/ml (11.4-159.2) for female fetuses. They speculated that their findings were somewhat unexpected since male fetuses were more likely to deliver preterm and higher levels of midtrimester amniotic fluid angiogenin have been associated with preterm delivery. So one would expect higher amniotic fluid angiogenin levels in male fetuses. In

	Fetal Gender		*p value
	Male	Female	
Maternal age (year)	34 (29-38)	35 (31-41)	0.73
Maternal weight (kg)	53 (47-68)	59 (49-74)	0.32
Gestational age at amniocentesis (week)	16.5 (15-19)	17 (15-19)	0.20
Gravidity	4 (1-5)	2 (1-4)	0.66
Parity	2 (0-2)	2 (0-2)	0.91
Body Mass Index at amniocentesis (kg/m ²)	24 (17-25)	22 (18-27)	0.37

Data were given as median (range), *Kruskal-Wallis Test.

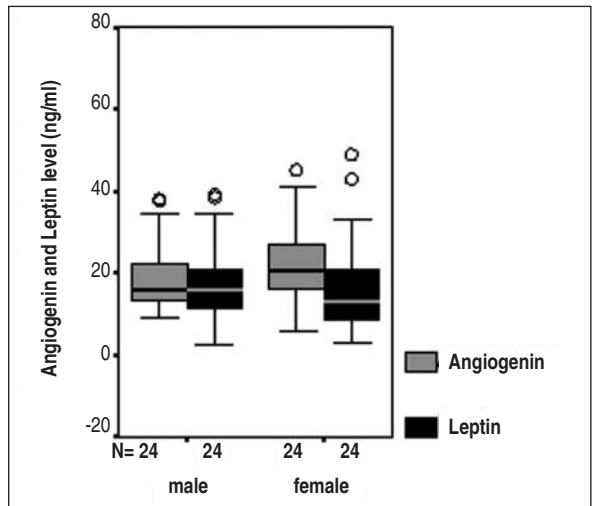


FIGURE 1: Box plot graphs show angiogenin and leptin levels classified by fetal gender.

our study median angiogenin levels were 15.91 ng/ml (range 9.24-37.91) for male and 20.91 (5.69-45.02) for female fetuses ($p=0.09$) regarding normal karyotype. The levels are relatively low in male fetuses but this does not reach statistical significance. The large range of results of angiogenin levels in Poggi et al's study might have caused the statistical significance.

In the literature there are conflicting reports concerning the level of amniotic fluid leptin levels regarding the fetal gender. Cagnacci et al. measured leptin levels at 16 weeks of gestation and found amniotic fluid leptin to be significantly lower in male than female fetuses (7.91 ± 0.36 ng/ml versus 10.45 ± 0.38 ng/ml; $p = 0.0001$).¹⁰ Chan et al. found amniotic fluid leptin levels in the pregnant women carrying a female fetus (5.6 ± 0.3 ng/ml) were sig-

nificantly higher than those carrying a male fetus (4.7+/-0.2 ng/mL) (p=0.004).¹¹ On the other hand, Marek et al. determined amniotic fluid samples of 32 male and 23 female fetuses and demonstrated no gender-dependent differences in leptin and Neuropeptide Y levels.¹² Basbug et al. measured amniotic fluid levels of leptin in normal and fetuses with neural tube defects.¹³ They found the mean leptin levels in female and male fetuses did not significantly differ in amniotic fluids in both groups. Choi et al. measured second trimester amniotic fluid leptin levels in normal and Down syndrome groups.¹⁴ They found amniotic fluid leptin levels to decrease significantly in Down syndrome group. However, there were no significant difference when leptin levels were stratified by gender.

Being male carries an increased risk of spontaneous but not iatrogenic preterm birth. The reasons behind this remain obscure.¹⁵ Male fetal gender is associated with an overall increased risk of pre-eclampsia.¹⁶ If we had managed to find gender dependent difference on angiogenesis we might have speculated that some gestational diseases like preterm delivery, preeclampsia are seen more often in male gender due to a gender related defect on angiogenesis.

As a conclusion, second trimester amniotic fluid angiogenin and leptin levels do not show any significant differences regarding the gender. To elucidate conflicts in literature, studies with larger number of subjects are needed.

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