SECOND TRIMESTER ELEVATION OF MATERNAL SERUM α FETOPROTEIN LEVEL AT TRIPLE SCREEN IS ASSOCIATED WITH POOR PREGNANCY OUTCOME

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ABSTRACT

Objective : Elevation of maternal serum α-fetoprotein in mid trimester is associated with fetal death, preterm delivery, and fetal growth restriction. Placental ischemia may be the common underlying pathogenesis of these outcomes. Thus, we tested angiogenin a potent inducer of neovascularization, in mid trimester amniotic fluid of patients with elevated maternal serum α-fetoprotein values to determine whether α-fetoprotein elevation is due to ischemia with subsequent stimulation of neovascularization.

Study design : We designed a case-control study of singleton gestations undergoing mid trimester aminocentesis for standard genetic indications. Patients with elevated maternal serum α-fetoprotein levels ≥ 2.0 multiple of the median, n = 9 at triple screen were matched with 18 controls. On the basis of maternal age, race and parity, the median elevation of maternal serum α-fetoprotein in the study population was 4.01 multiples of the median (range 2.65 to 7.24) multiples of the median. Inclusion criteria were (1) pregnancy outcome information available, (2) no evidence of fetal structural or chromosomal anomalies and (3) genetic aminocentesis. Amniotic fluid was immunoassayed for angiogenin; sensitivity 0.026 ng/ml. Angiogenin and maternal serum α-fetoprotein values were normalized with use of natural log transformation for statistical analysis.

Results : Angiogenin values were significantly elevated in patients with high maternal serum α-fetoprotein lev-

el [median 31.1 (range 9.2 to 54.6) versus 17.1 (range 9.0 to 29.2) ng/ml, P = 0.02]. Mean gestational age at sampling; maternal age, and year of amniocentesis were not significantly different between the study and control groups (each P > 0.05). There was a significant increase in preterm deliveries and small-for-gestational age neonates in the patients with elevated maternal serum α-fetoprotein levels (each P < 0.01).

Conclusions : Mid trimester amniotic fluid angiogenin levels are significantly elevated in patients with elevated mid trimester maternal serum α-fetoprotein levels. Because angiogenin is a known marker of tissue ischemia, resulting in neovascularization, we hypothesize that elevation of maternal serum α-fetoprotein levels at triple screen is due to placental ischemia.

Key words : Elevated maternal serum α fetoprotein-Angiogenin-Fetoplacental ischemia.

INTRODUCTION

Neovascularization is a common response of tissues to the presence of ischemic damage and chronic inflammation [1]. The polypeptide angiogenin, a known potent inducer of neovascularization [2], plays a role in the vascular development of the fetus and in the neovascularization that accompanies diseases and wound healing [3]. Messenger ribonucleic acid encoding angiogenin is expressed in almost every tissue or cultured cell line examined [2], including normal epithelial cells, fibroblasts, and peripheral blood cells [4,5]. Because patients with elevated maternal serum α-fetoprotein (AFP) in the second trimester have previously been shown to be associated with adverse perinatal outcome, including preterm delivery, intrauterine growth restriction, and fetal death [6,7], we hypothesized that placental ischemia may be the common underlying pathogenesis of elevated maternal serum AFP. Thus, we tested angiogenin in mid trimester amniotic fluid of patients with elevated maternal serum AFP levels to determine whether AFP elevation is due to ischemia with subsequent stimulation of angiogenesis.

MATERIALS AND METHODS

For this case-control study we used a database of women with singleton gestations who underwent mid trimester aminocentesis for standard genetic indications at genetic unit, de-

partments of Obstetrics & Gynecology and Clinical Pathology, Zagazig University, between March 2004 and March 2005. Inclusion Criteria were maternal serum AFP ≥ 2.0 multiples of the median, pregnancy outcome available, gestational age at aminocentesis 15 to 20 weeks and no evidence of fetal structural or chromosomal anomalies. Patients with elevated maternal serum AFP levels (≥ 2.0 multiples of the median) at triple screen were matched with control group patients on the basis of year of aminocentesis, maternal age, and parity. Gestational age was confirmed or established by ultrasonographic fetal biometry at time of aminocentesis (< 20 weeks gestation). SGA was defined as birth weight < 10^{th} percentile for gestational age (8). After aminocentesis the amniotic fluid specimens were centrifuged (100 to 150 g for 9 minutes) to remove cellular components for karyotype determination, and the supernatant samples were frozen at -20°C. Demographic data including gestational age at aminocentesis and delivery, pregnancy complications such as pre labour rupture of membranes, chorioamnionitis, preterm labour, and neonatal birth weight were collected by chart review.

Angiogenin levels were measured in supernatant sample of amniotic fluid by enzyme-linked immunoassay (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 inter-assay and intra-assay 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-1α, interleukin-1β, interleukin-2,3, interleukin-4, interleukin-6, interleukin-7, interleukin-8, tumor necrosis factor- α or tumor necrosis factor-β.

Serum α-feto protein levels were measured by Immulite. Immulite 1000 AFP is a solid phase, two-site sequential chemiluminescent immunometric assay (Diagnostic products corporation "DPC", USA). AFP triple screen which include Estradiole, HCG and AFP for exclusion of mongol or any congenital anomalies in pregnancy to be confirmed by aminocentesis if one of triple screen giving positive results.

Statistical analysis included $\chi 2$ analysis and Fisher's exact test for

categorical variables, and the Mann-Whitney U test for non parametric data, with $P < 0.05$ considered significant. Angiogenin level were normalized with natural log transformation for statistical analysis.

RESULTS

Nine patients with elevated maternal serum AFP values were matched with 18 controls. There were no significant differences in mean maternal age, nulliparity, or female neonatal gender between patients with elevated maternal serum AFP levels and controls (Table I). Mean gestational age at amniotic fluid sampling, year of sampling, and indication for amniocentesis were also not significantly different between the two groups. Gestational age at delivery and neonatal birth weight were significantly lower in patients with elevated maternal serum AFP levels (Table I).

Pregnancy outcome characteristics of the patients with elevated maternal serum AFP levels are shown in Table (II).

Angiogenin was detected in all samples of amniotic fluid. Amniotic fluid angiogenin levels were significantly higher in patients with elevated maternal serum AFP levels compared with controls [median 31.1 ng/ml (range 9.2 to 54.6 ng/ml) versus median 17.1 ng/ml (range 9-29.2 ng/ml)]. $P = 0.02$. Although the average gestational age in the patients with elevated maternal serum AFP levels was preterm, by definition of < 37 weeks, this was due to the patient with a fetal death at 21 weeks.

However, after removal of this patients and her controls, the significant difference remained for both gestational age at delivery and birth weight (37.6 ± 1.2 versus 39.7 ± 1.5 weeks, $P = 0.003$, and 2576 ± 494 gm versus 3650 ± 535 gm, $P < 0.0001$, respectively). In addition, five (55%) of the patients with elevated maternal serum AFP levels were delivered of SGA neonates compared with none of the controls. A significant difference ($P = 0.002$), and a seventh neonate was at the 10th percentile. Also, two patients (22%) with elevated maternal serum AFP levels were delivered preterm compared with none of the controls and ninth neonate attract congenital heart disease compared with none of the controls.

Table (I) : Obstetric characteristics (Values are mean ± SD or number and percent.)

Items	Elevated maternal serum AFP (n = 9)	Control (n = 18)	Significance
Maternal age (yr)	30.2 ± 2.6	30.4 ± 2.1	P = 0.9
Nulliparous	6 (66%)	13 (72%)	P = 0.6
Gestational age at sampling (wk)	18.1 ± 0.9	18.4 ± 1.7	P = 0.6
Gestational age at delivery (wk)	35.8 ± 5.7	39.7 ± 1.4	P = 0.009
Birth weight (gm)	2307 ± 932	3662 ± 505	P < 0.0001
Female neonatal gender	6 (66%)	10 (55%)	P = 0.7

Table (II) : Pregnancy and outcome characteristics of patients with elevated maternal serum AFP levels.

Case	Maternal serum AFP (MoM)	Gestational age at delivery (wk)	Birth weight (gm)	Angiogenin value (ng/ml)	Outcome
1	4.01	21	150	16.9	SGA. FDU. multiple myeloma
2	7.24	34	2012	33.6	SGA. PTL
3	3.86	36	2078	31.1	SGA. PROM. PTL
4	4.44	37	2350	33	SGA
5	2.85	37	2820	37.8	Algohydramnios
6	6.31	38	2230	17.7	SGA
7	2.65	38	2730	16.5	Birth weight at 10^{th} percentile
8	2.78	38	2835	54.6	
9	2.95	40	3510	9.2	Congenital heart disease

MoM. Multiples of median; FDU. Fetal death in utero; PROM. Prelabour rupture of membranes; SGA. Small for gestational age.

DISCUSSION

Patients with elevated maternal serum AFP levels in the early second trimester had significantly levels at the time of genetic amniocentesis, these patients also were significantly more likely to have poor pregnancy outcomes, including stillbirth, fetal growth restriction, and preterm delivery. In addition, because the study patients were delivered earlier. The mean fetal birth weight was significantly lower than that of the control group. Elevated levels of angiogenin have been reported in patients destined to be delivered preterm.

Angiogenesis is mediated by growth factors and precise regulation of the synthesis and degradation of the extracellular matrix. Growth factors that stimulate angiogenesis include angiogenin, tumor necrosis factor-α, and series prostaglandins (11,12).

Macrophages, tumor cells, and platelets produce and release these angiogenic modulators (14,15). Inter-estingly, some of the mediators of angiogenesis are also closely involved in the pathogenesis of preterm delivery tumor necrosis factor-α has been identi-fied as a marker for preterm delivery in mid trimester amniotic fluid (15).

Prostaglandins, which indirectly stimulate angiogenesis are also stimulators of uterine contraction and labour (17).

Our working hypothesis is that an elevation of angiogenin is a marker for placental ischemia. In support of this is the clinical observation that the prevalence of fetal growth restriction is significantly higher among preterm deliveries compared with term deliveries (16,18).

Growth restriction in karyotypically and structurally normal fetuses is considered secondary to placental ischemia in up to two thirds of cases and results from a decreased uteroplacental vascular supply resulting from failed conversion of the spiral arterioles.

It is possible that preterm delivery and growth restriction are two outcomes from a spectrum of potential outcomes after placental ischemia, ranging from fetal death (in cases of severe ischemia or inadequate neovascularization) to term delivery of healthy appropriate-for-gestational-age infants (when neo-

vascularization is successful with minimal damage).

Growth restriction and preterm delivery may have similar etiopathogenic mechanisms, including abnormal placentation, ischemia, and chronic inflammation. These conditions are related and closely linked, as shown by the significant proportion of patients with preterm delivery and SGA neonates (13).

The origin of angiogenin in the amniotic fluid is unknown. The messenger ribonucleic acid for angiogenin is produced by almost all tissues examined, including normal epithelial cells, fibroblasts, and peripheral blood cells (2).

Further studies are needed to identify the source of angiogenin in the amniotic fluid. Further studies include the correlation of mid trimester amniotic fluid angiogenin level with placental lesions to better under stand the pathologic processes, in situ hybridization for cellular localization of angiogenin release, and correlation of mid trimester amniotic fluid angiogenin with maternal serum angiogenin levels. An elevation of mid trimester amniotic fluid angiogenin appear to identify patients at risk for spontaneous preterm delivery.

In conclusion, because angiogenin is a known marker of tissue ischemia resulting in neovascularization, the etiology of elevation of maternal serum AFP levels at triple screen may be due to placental ischemia.

REFERENCES

1- Zetter, B.R. (1988) : Angiogenesis : state of the art. Chest; 93 : 159 – 655.

2- Weremowicz, S.; Fox, E.A.; Morton, C.C. and Vallee, B.L. (1990) : Localization of the human angiogenin gene to chromosome band 14 q 11, proximal to the t cell receptor-α/Y locus. Am. J. Hum. Genet.; 47 : 973 – 81.

3- Furcht, L.T. (1986) : Critical factors controlling angiogenesis : cell products, cell matrix, and growth factors. Lab. Invest.; 55 : 505 – 9.

4- Diaz-Flores, L.; Gutierrez, R. and Varela, H. (1994) : Angiogensis : an update.

Histol. Histopathol.; 9 : 807 – 43.

5- **Rybak, S.M.; Fett, J.W.; Yao, Q.Z. and Vallee, B.L. (1987) :** Angiogenin mRNA in human tumor and normal cells. Biochem. Biophys. Res. Commun.; 146 : 1240 – 8.

6- **Wenstrom, K.D.; Owen, J.; Davis, R.O. and Brumfield, C.G. (1996) :** Prognostic significance of un-explained elevated amniotic fluid alpha-fetoprotein. Obstet. & Gynecol.; 87 : 213 – 6.

7- **Williams, M.A.; Hickok, D.E.; Zingheim, R.W.; Luthy, D.K.; Kimelman, J.; Nyberg, D.A., et al. (1992) :** Elevated maternal serum α-fetoprotein levels and mid trimester placental abnormalities in relation to subsequent adverse pregnancy outcomes. Am. J. Obstet. & Gynecol.; 167 : 1032 – 7.

8- **Williams, R.I.; Creasy, R.K.; Cunningham, G.C.; Hawes, W.E. and Norris, F.D. (1992) :** Fetal growth and perinatal viability in California. Obstet. & Gynecol.; 5 : 624 – 32.

9- **Crandall, B.F. (1981) :** Alpha-fetoprotein: a review. CRC Crit. Rev. Clin. Lab. Sci.; Sept., 127-85.

10- **Brock, D.J.H. (1976) :** Prenatal Diagnosis-chemical Methods. Br. Med. Bull.; 32 : 16.

11- **Cockerill, G.W.; Gamble, J.R.; Vadas, M.A., et al. (1995) :** Angiogenesis : models and modulators. Int. Rev. Cytol.; 159 : 113 – 60.

12- **Ribatti, D.; Vacca, A.; Roncali, L. and Dammaco, F. (1992) :** Angiogenesis under normal and pathological conditions. Haemato-logica; 76 : 311 – 20.

13- **Wennergren, M.; Wennergen, G. and Vilbergsson, G. (1998) :** Obstetric characteristics and neonatal performance in a four-year small-for-gestational age population. Obstet. & Gynecol.; 72 : 615 – 20.

14- **Bauer, J.; Margolis, M.; Schreiner, C.; Edgell, C.J., et al. (1992) :** In vitro model of angiogenesis using a human endothelium-derived permanent cell line. J. Ce.. Physiol.; 153 : 437 – 49.

15- **Ghidini, A.; Eglinton, G.S.; Spong, C.Y.; Jenkins, C.B.; Pezzullo, J.C.; Ossandon, M., et al. (1996) :** Elevated mid trimester amniotic fluid tumor necrosis alpha levels : a predictor of preterm delivery. Am. J. Obstet. & Gynecol.; 174 : 307 – 313.

16- **Hediger, M.L.; Scholl, T.O.; Schall, J.I.; Miller, L.W. and Fischer, R.L. (1995) :** Fetal growth and the etiology of preterm delivery. Obstet. & Gynecol.; 85 : 175 – 81.

17- **Karim, S.M. (1986) :** The prostaglandins. New York : Wiley.

18- **Ott, W.J. (1993) :** Intrauterine growth retardation and preterm delivery. Am. J. Obstet. & Gynecol.; 168 : 1710 – 7.