Original Article

Mid-Trimester Amniotic Fluid High Sensitive C-Reactive Protein Level in the Prediction of Preterm Delivery

Sedigheh Borna[1], Fatemeh Mirzaei[2], Alireza Abdollahi[3] , Akram Sarbiaei[4]

1. Dept. of Obstetrics and Gynecology, Fetal- Maternal Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Dept. of Obstetrics and Gynecology, Kerman University of Medical Sciences, Kerman ,Iran.
3. Dept. of Pathology, Tehran University of Medical Sciences,Tehran, Iran.
4. Dept. of Clinical Laboratory, Imam Hospital laboratory, Tehran, Iran.

ABSTRACT

Background and Objectives: Preterm labor is a serious problem in obstetrics, accounting for 70% of perinatal mortality. High sensitive C - reactive protein (HS-CRP) is a sensitive marker of Inflammation. Our aim in this study was to determine Amniotic fluid hs-CRP concentration and its correlation with pre-Term delivery.

Materials and methods: This prospective study was conducted on 90 pregnant women who underwent genetic amniocentesis between the 15th and 20th weeks of gestation. All patients were followed until delivery. Pateints with abnormal karyotype and iatrogenic preterm delivery for fetal and maternal indications were excluded. The samples were carried immediately to the laboratory of Imam Khomaini Hospital Complex, Tehran, Iran for cytogenetic examination and tested for HS-CRP by turbidimetric method. Non parametric tests and receiver-operating characteristics curve analysis were used for statistical purpose.

Results: The study showed no correlation between amniotic fluid HS-CRP concentrations with preterm delivery. Maternal serum alpha-fetoprotein (AFP) levels were higher in patients delivered preterm compared with term deliveries (*P*=0.036).

Conclusion: Our results implicated that HS-CRP like other acute phase response markers was not as a possible risk marker of preterm delivery.

Key words: Preterm delivery, High sensitive C-Reactive protein, Amniotic fluid, Iran.

Introduction

Preterm labor is a serious problem in obstetrics, accounting for 70% perinatal mortality and almost half of long term neurologic morbidity (1). The physiologic mechanism that initiates preterm labor has not been substantially identified. Placental ischemia and acute inflammation are the most common two

pathologies that have been implicated (2). Compelling clinical and experimental evidence has demonstrated an association among intrauterine inflammation and preterm delivery (3). High sensitive C - reactive protein (HS-CRP) is a sensitive marker of inflammation that remains stable in serum (4). Elevated concentration of HS-CRP in peripheral circulation has been associated with the presence of intrauterine infection (5,6). Recently, investigators have noted elevated fluid HS-CRP concentration among women with intrauterine infections as compared with controls (7, 8).

Our aim in this study was to determine if concentrations of amniotic fluid HS-CRP level obtained during genetic amniocentesis could identify patients at risk for spontaneous preterm delivery.

Materials and Methods

This prospective study was conducted in 2007 during a period of 9 months, involved 110 singleton pregnancies that underwent genetic amniocentesis between the 15th and 20th weeks. Research Ethics approval was obtained before the initiation of the study and written informed consent was obtained from all patients.

Amniocentesis was performed for advanced maternal age, increased risk for aneuploidy (triple test, aneuploid child) to qualify for participation. Subjects were required to 1) have a singleton pregnancy between 15 to 20 weeks of gestation;2) have a known gestational age;3) normal pregnancy course before the procedure;4) absence of congenital malformations or chromosomal abnormality; and 5) be older than 18 years age. Gestational age was based on the last menstrual period (LMP) and confirmed by ultrasound examination, prior to 20 weeks gestation.

If both LMP and ultrasound dating were available, and the two agreed within 14 days, we used the former to assign gestational age. If the two dates differed by more than 14 days, we used the ultrasound date. All the pregnancies were followed until delivery. Women with spontaneous preterm delivery before 37 weeks (preterm delivery group, n=17) and those who delivered at term (term delivery group, n=73) were compared with respect to maternal characteristics, amniotic fluid HS-CRP.

The cases of iatrogenic prematurity, e.g; delivery for abruption, IUGR, maternal medical disease (diabetes, hypertension) were excluded. Amniocentesis was performed with a 21 gauge needle under ultrasonographic guidance with a free- hand technique. The first 0.5 ml of amniotic fluid was collected by using a 5-ml syringe and discharged to avoid maternal contamination, subsequently; 15-20 ml of amniotic fluid was collected by using a 20 –ml syringe and used for the karyotype analysis and hs-CRP concentration measurements.

The amniotic fluid specimens were centrifuged for 10 minutes to obtain the cellular components for the karyotype analysis.

Amniotic fluid supernatant was tested for HS-CRP in a Alcyon 300 (Abbott, USA) automated analyzer by turbidimetric method (high sensitive CRP; Biosystems, Spain kit). The detection limit of kit was 0.06 mg/L and sensitivity was: 60 mA.L/mg at 5 mg/L and measurement interval was 0.06 – 15 mg/L.

Results were reported as mean ± standard deviation (SD) or median with 1st and 3rd quartiles (whenever the data did not appear to have normal distribution) for quantitative variables, and categorized variables were summarized by absolute frequencies and percentages. Continuous variables were compared using the student's t-test or nonparametric Mann-Whitney U test for non-normally distributed variable, and categorical variable were compared using the chi-square or Fisher's exact test, between the two groups. For statistical analysis, the statistical software SPSS version 13.0 windows (SPSS Inc., Chicago, IL) were used. All P values were 2 tailed, with statistical significant by $P \leq 0.05$.

Results

During the study period, 110 patients underwent amniocentesis. A chromosomal abnormality was present in 4 patients. Six of patients were delivered for fetal or maternal indications (preeclampsia, fetal growth restriction and placenta abruption) and 10 patients could not be reached.

Remaining 90 women were included in the study and investigated for the risk of preterm delivery. Indications for amniocentesis included abnormal triple test showing increased risk for Down's syndrome in 71 patients (79%), advanced maternal age in 13 patients (14.4%) and history of chrosomal abnormalies in 6 patients (6.6%).

uE_3: Unconjugated estriol, hCG: Human Chorionic gonodotropin, AFP: Alpha – fetoprotein

MOM:median of multiple.

History of the preterm delivery was higher in the preterm delivery group than term group but was no significant difference between the groups (P Value = 0.656)..Women who deliverd before 37 weeks had a higher median serum AFP concentration than those

who delivered at ≥ 37 weeks median= 1.27 [range, 1.015, 2.505 MOM] vs. median=0.950 [range, 0.680, 1.450 MOM; *P*=0.036].

Receiver-operator characteristic curve analysis was performed to evaluate the screening efficiency of amniotic fluid HS-CRP in predicting preterm delivery.

The sensitivities, specificities, positive predictive value (PPV), negative predictive value (NPV)

and accuracy for amniotic fluid HS-CRP, on the identification of spontaneous preterm delivery at the time of genetic amniocentesis were 30% , 81% , 27%, 83% , 71%, respectively .

There were no significant differences between the groups with respect to maternal age, gestational age at amniocentesis, parity, indication for amniocentesis and location of placenta, maternal hCG, uE3 MOM values. (Table 1).

Table 1: Characteristics of the term and preterm deliveries

Characteristics	Preterm (n=17)	Term (n=73)	*P* value
Maternal age (year)	37(32-39)	36(31-33)	0.897
Parity (n,6SD)	2(1,2.5)	2(1,3)	0.675
History of the preterm birth (n, %)	1 (5.9)	3(4.1)	0.574
Indication of the amniocentesis (n, %)			
Abnormal triple test	12(70.6)	59(80.8)	
Maternal age>35 years	3(17.6)	10(13.7)	0.474
History of aneuploidy	2(11.80)	10(13.7)	
Gestational age at delivery (weeks)	16-20	18-21	0.077
Placental localization			
Anterior (%)	10(58.8)	35(48.6)	0.449
Posterior (%)	4(41.2)	37(51.4)	
Bleeding in the insertion of the needle (%)	1 (5.9%)	3 (4.2%)	0.579
Serum uE$_3$ Level (MOM)	0.81(0.605-1.03)	0.83(0.61-1.11)	0.926
Serum hCG Level (MOM)	1.705(1.243-3.383)	1.57(1.03-2.645)	0.426
Serum AEP Level (MOM)	1.27(1.015-2.505)	0.95(0.680-1.450)	0.036

Discussion

Various biochemical and clinical prognostic markers for use in preterm labor have been advocated.

Amniotic fluid HS-CRP levels in women with preterm PROM are elevated in the presence of intraamniotic infection (9,10). The role of HS-CRP in the setting of an intrauterine inflammatory process is difficult to determine. When an intrauterine inflammation is present very early in gestation; the fetus participated in this process by mounting a subclinical inflammatory response. Several arguments support this theory. First, HS-CRP is a protein with a heavy molecular mass that does not cross the placental barrier. Second, increased concentrations of HS-CRP have been found in the plasma of fetuses in which sepsis was diagnosed at delivery. Last, the fetal liver has been found capable of producing acute- phase proteins in response to an inflammatory insult. (10-13)

In our study we did not find any difference with respect to mean HS-CRP levels between term nd preterm delivery groups, there was no significant difference between the amniotic fluid HS-CRP of

preterm deliveries and patients that term deliveries , but HS- CRP levels higher 1 mg/l had sensitivity 30%, specificity of 81% in the prediction of spontaneous preterm delivery at <37 weeks.

Ghezzi *et al.* found that women in preterm delivery at<37 weeks had a higher median range of amniotic fluid concentration at the time of genetic amniocentesis that those who delivered at the term (11). In this study amniotic fluid HS-CRP concentration of >110 ng/ml had a sensitivity of 80.8% and specificity of 69.5% in prediction of spontaneous preterm delivery (7). While Ozer *et al.* did not find any difference with respect to mean HS-CRP levels between term and preterm deliveries and HS-CRP levels higher than 5 mg/l (75th percentile) had sensitivity of 30%, specificity of 87% in the prediction of spontaneous preterm delivery at <37 weeks (8). In Ozer *et al.* study, there was good correlation with preterm labor and amniotic fluid PAPP-A and interferon gamma T-cell alpha chemoattractant concentrations(8).

In this study we showed that elevated maternal serum AFP in mid-trimester were associated with preterm delivery. Our study confirms previous

findings regarding the relationship between elevated maternal serum AFP and preterm delivery (11).

The etiology of preterm delivery is undoubtedly multifacterial. It is this multifacterial etiology that raised difficulties and differences in determining the effectiveness of various markers in the prediction of preterm delivery (12,14,15).

Conclusion

Our results implicate HS-CRP, like other acute phase response markers including interleulcin-6 and tumor necrosis factor-α was not as a possible risk marker of preterm delivery, particularly delivery before 34 completed weeks gestation. The presence of infection or markers of inflammation alone does not sufficiently explain the occurrence of preterm delivery. Additional work is needed to: 1) identify specific agents causing infections or stimulating a pro-inflammatory response 2) identify factors that mediate the effects of infection and 3) identify factors that modulate maternal and fetal susceptibility to infection and a pro-inflammatory response.

Acknowledgments

References

1. Arias F, Tomich P. Etiology and outcome of low birth weight and preterm infants. Obstet Gynecol 1982; 60(2):277-81.

2. Edwin SS, Romero R, Rathnasabapathy CM, Athaydel N, Armant DR, Subramanian MG. Protein kinase c stimulates release of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase -1 by human decidual cells. J Matern Fetal Neonatal Med 2002; 12(4):231-6.

3. Gomez R, Ghazzi F, Romero R, Munoz H, Tolosa JE, Rojas I. Premature labor and intra-amniotic infection: clinical aspects and role of cytokinase in diagnosis and pathophysiology. Clin perinatol 1995; 22(2):281-342.

4. Kluft C, De Maat MP. Sensitive markers of inflammation make it possible to study the chronic process: the rise of interest in low levels of C-reactive protein. Vascul pharmacol 2002; 39(3): 113-5.

5. Mazor M, Kassis A, Horowitz S. Relationship between C - reactive protein levels and intraamniotic infection in women with preterm labor. J Repord Med 1993; 38(10): 799-803.

6.Potkul PK, Moawad AH, Ponto KL. The association of subclinical infection with preterm labour: the role of C-reactive protein. Am J Obstet Gynecol 1985;153(6): 642-5.

7.Ghezzi F, Franchi M, Raio L, Di Naro E, Bossi G, D·Eril GV, *et al*. Elevated amniotic fluid C-reactive protein at the time of genetic amniocentesis is a marker for preterm delivery. Am J Obstet Gynecol 2002; 186(2): 268-73.

8.Ozer KT, Kovak ZN, Gokaslan H, Elter K, pekin T. Predictive power of maternal serum and amniotic CRP and PAPP-A concentrations at the time of genetic amniocentesis for the preterm delivery. Eur J Obstet Gynecol Repord Biol 2005; 122(2): 187-90.

9.Di Naro E, Ghezzi F, Raio L. C-reactive protein in vaginal fluid of patients with preterm premature rupture of membranes. Acta Obstet Gynecol Scand 2003; 82(12): 1072-90.

10.Thompson PJ, Greenough A, Davies E, Nicolaides KH. Fetal C-reactive protein. Early Hum Dev 1993; 32(2-3): 81-5.

11.Van Rijn M, Van Der Schouw YT, Hagenaars AM, Visser GH, Chiristiaens GC. Adverse obstetric outcome in low-and high –risk pregnancies: predictive value of maternal serum screening. Obstet Gynecol 1999; 94(6): 929-934.

12. Palacio M, Cobo T, Figueras F, Gomez O, Coll O , Cararach V, *et al*. Previable rupture of membranes: Effect of amniotic fluid on pregnancy outcome. European journal of obstetrics and Gynecology and Reproductive Biology. Eu J of Obs & Gyn & Rep Biology. 2008;138(2): 158-163.

13. Shin Park J, Hyun Yoon B, Eun Lee SI, Romero R, Jung H, Park CH. The intensity of the fetal inflammatory response in intraamniotic inflammation with and without microbial invasion of the amniotic cavity. Am J of Obs & Gyn 2007;197(3): 294-e1-297.e6.

14. Cark S, Belfort M, Dildy G, Herbst M, Meyers J, Hankins G. Maternal death in the 21st century: causes, prevention, and relationship to cesarean delivery. AM J of Obs & Gyn 2008; 199(1): 36.e1-36.e5.

15. Lee Si, Romero R, Park CH, Jun J, yoon B. The frequency and significance of intraamniotic inflammation in patients with cervical insufficiency. Am J of Obs & Gyn 2008;198(6): 633.e1-633-e8.