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Original Article

Correlation analysis of AFP and β-hCG on obstetric complications and pregnancy outcomes

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Abstract: Birth defects and obstetric complications are medical problems faced by obstetrics and affect the quality of the population. In order to improve the quality of the population, prenatal screening is an effective means to detect high-risk pregnant women with congenital defects in the fetus or obstetric complications. Serum alpha-fetoprotein (AFP) and free β-human chorionic gonadotropin (β-hCG) are commonly used for screening in children with 21-trisomy syndrome and congenital neural tube defects, but their impacts on obstetric complications and pregnancy outcomes have not yet been fully elucidated. A total of 987 cases of maternal defects were enrolled. Peripheral blood was collected at 15-20 weeks in the second trimester. AFP and β-hCG were detected by time-resolved fluorescence immunoassay. The associations of AFP and β-hCG with maternal gestational age, body mass index (BMI), age, pregnancy outcome abortion, pre-term infants, and low birth weight infants were analyzed. The correlation of AFP and β-hCG with obstetric complications, including pregnancy associated with severe anemia, postpartum hemorrhage, placenta previa, and neonatal asphyxia. AFP was positively correlated with gestational age and BMI ($r = 0.321$, $r = 0.415$, $P < 0.05$). β-hCG was negatively correlated with gestational age and BMI ($r = -0.428$, $r = -0.255$, $P < 0.05$). AFP and β-hCG were associated with miscarriage, premature infants and low birth weight infants, postpartum hemorrhage, placenta previa, and neonatal asphyxia ($P < 0.05$), but not with severe anemia and age ($P > 0.05$). AFP and β-hCG can be affected by gestational age and BMI in pregnant women. Their measured values were related to pregnancy outcomes and obstetric complications, but not to severe anemia.

Keywords: AFP, β-hCG, pregnancy, complications

Introduction

Birth defects and obstetric complications are medical problems faced by obstetrics and affect the quality of the population [1, 2]. With the development and progress of society, the improvement of people's living standards, and the rapid progress in medical diagnosis and treatment technology, people's awareness of prenatal and postnatal care continues to increase. Therefore, the importance of prenatal screening has been increasingly strengthened [3]. Prenatal screening is still difficult in China. The increase in the number of elderly mothers leads to an enhanced risk of neonatal birth defects and obstetric complications [4, 5]. In order to improve the quality of the population, prenatal screening is an effective method to detect pregnant women with congenital defects or possible high-risk of obstetric complications [6]. General prenatal screening is to detect relevant markers from the peripheral blood of women in the first or second trimester. The main tests include 21-triple syndrome [7]. The general serum markers for screening pregnant women include pregnancy-associated protein-A (PAPP-A), AFP, unconjugated estradiol (u-E3), and free human β-hCG, among which the most common markers are AFP and β-hCG [8, 9].

AFP is synthesized by the yolk sac in the early pregnancy, and is mainly synthesized by the fetal liver after 3 months. AFP is the most common fetal serum specific globulin [10]. AFP has the effect of preventing immune rejection [11]. AFP in fetal blood begins to increase at 6 weeks of gestation, reaches a peak at 13 weeks of gestation, and reduces with time. The maternal

blood AFP mainly comes from amniotic fluid and fetal blood and the change trend is similar to that in fetal blood AFP. However, it shows later peak at 28-32 weeks of gestation and declines following time, which is later than in fetal blood [12, 13]. Placental trophoblast placental cells produce human chorionic gonadotropin hCG. Two subunit glycoproteins α and β constitute hCG, which exist in various forms, including intact hCG and β chain hCG [14]. Although different forms of hCG have different activities, β-hCG is a specific type that can be detected [15]. After entering the mother's body, hCG can rapidly increase until the peak at 8 weeks of gestation, then slowly declines to 20 weeks and remains stable. Therefore, the detection rate of β-hCG in the second trimester is high, which has the advantages of high detection rate and good stability [16]. However, the effect of maternal serum markers on obstetric complications and pregnancy outcomes has not been fully elucidated. Therefore, this study was to analyze the relationship between AFP and β-hCG with obstetric complications and pregnancy outcomes.

Materials and methods

General information

A total of 987 cases of maternal from August 2016 to December 2017 in Sichuan Chengdu Jinniu District Maternal and Child Healthcare Hospital were enrolled. All pregnant women were single pregnancy with mean age of 29 ± 5.6 (22-48) years and average gestational weeks of 16 ± 2.3 (15-20) weeks. Inclusion and Exclusion Criteria [4]: the inclusion criteria included monocyesis, Han nationality, met the prenatal serum screening criteria for pregnant women in the second trimester, no history of smoking and drinking, and no fetuses with chromosomal or structural abnormalities. Exclusion criteria included obstetric complications or severe pathological pregnancy that had been identified before screening, *in vitro* fertilization for pregnant women, liver and kidney dysfunction or nephritis, malignant tumors, primary hypertension, primary heart disease, and insulin-dependent diabetes. This study was approved by the Medical Ethics Committee in Sichuan Chengdu Jinniu District Maternal and Child Healthcare Hospital. All selected subjects had signed informed consent.

Main reagents and instruments

AFP/free β-hCG double-labelled detection kit was purchased from PerkinElmer (Finland). Other commonly used reagents were purchased from Sangon (Shanghai, China). The Wallac 1235 fully automated time-resolved fluorescence immunoassay system was purchased from PerkinElmer (Finland).

Methods

Sample collection: All selected subjects were bled 5 ml within 24 hours after admission. The control group collected blood samples at the same time. The sample was centrifuged at 3000 rpm for 15 minutes. The serum was placed in an Eppendorf (EP) tube and stored in a -80°C freezer.

Clinical information: The general clinical data of the selected subjects were collected, including name, date of birth, occupation, contact number, weight, blood pressure, gestational age, and maternal serum prenatal screening records. Body mass index (BMI = weight in kilograms divided by height in meters squared, kg/m^2).

Sample detection: Time-resolved fluorescence immunoassay was used to test AFP and free β-hCG in the study subjects according to the kit instructions. The AFP and free β-hCG in maternal peripheral blood were measured by a fully automatic time-resolved fluorescence immunoassay system.

Statistical analysis

All data was analyzed by SPSS 22.0 software. Measurement data are described as mean ± standard deviation (± SD) and were compared using one-way ANOVA and Pearson correlation analysis. Levels were checked based on $\alpha = 0.05$. $P < 0.05$ was considered as a statistically significant difference.

Results

Analysis of gestational age and AFP in the second trimester

In this study, AFP was tested in pregnancy women at 15-20 weeks. The results confirmed that AFP elevated with the increase of gestational age (**Figure 1**). There was a positive correlation between AFP and gestational age ($r = 0.321$, $P < 0.05$).

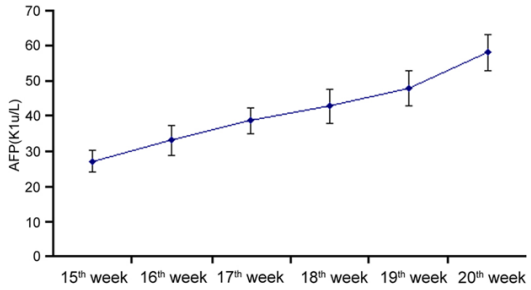

Figure 1. AFP analysis in the second trimester.

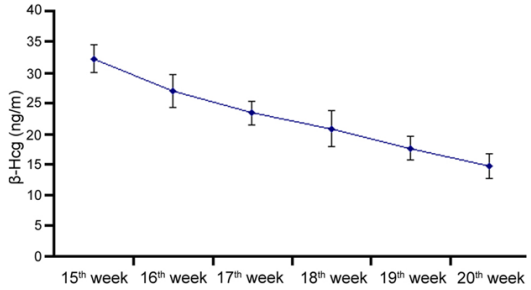

Figure 2. Free β-hCG analysis in the second trimester.

Analysis of gestational age and free β-hCG in the second trimester

In this study, free β-hCG was tested in pregnancy women at 15-20 weeks. The results showed that free β-hCG declined with the increase of gestational age (**Figure 2**). There was a negative correlation between free β-hCG and gestational age ($r = -0.428$, $P < 0.05$).

Relationship analysis between BMI and age with AFP and β-hCG in second-trimester women

The relationship between AFP and β-hCG was further explored with BMI and age in second trimester women. The results demonstrate that there was a positive correlation between AFP and BMI ($r = 0.415$, $P < 0.05$), but there was a negative correlation between β-hCG and BMI ($r = -0.255$, $P < 0.05$). AFP and β-hCG were not related to maternal age ($P > 0.05$) (**Table 1**).

Correlation analysis of AFP with obstetric complications and pregnancy outcomes

The association between AFP with pregnancy outcomes including abortion, pre-term and low birth weight infants, and obstetric complications including severe anemia, postpartum hemorrhage, placenta previa, and neonatal asphyxia were analyzed. The results reveal that AFP is associated with miscarriage, pre-term and low birth weight infants, postpartum hemorrhage, placenta previa, and neonatal asphyxia ($P < 0.05$), but not with severe anemia (**Table 2**).

Table 1. Relationship analysis between BMI and age with AFP and β-hCG in second-trimester women

	AFP	β-hCG
BMI	0.415*	-0.255*
Age	0.123	0.211

*$P < 0.05$.

Correlation analysis of β-hCG with obstetric complications and pregnancy outcomes

The association between β-hCG with pregnancy outcomes including abortion, preterm and low birth weight infants, and obstetric complications including severe anemia, postpartum hemorrhage, placenta previa, and neonatal asphyxia were analyzed. The results revealed that AFP is associated with miscarriage, pre-term and low birth weight infants, postpartum hemorrhage, placenta previa, and neonatal asphyxia ($P < 0.05$), but not with severe anemia (**Table 3**).

Discussion

At present, prenatal screening has attracted wide attention because of its simple, rapid, and non-invasive characteristics. The prenatal screening methods are increasingly diversified, not only serum markers, but also genetic testing [17, 18]. The prenatal screening based on serum markers is more convenient, low-cos, and high accuracy. Therefore, it is the first choice for prenatal screening for pregnant women. It can identify high-risk pregnant women and maximize reduced birth defects [19]. Currently, maternal serum marker AFP and free β-hCG are used to screen congenitally deficient children [20]. However, the correlation between AFP and free β-hCG with general clinical characteristics of pregnant women, including age, gestational age, and BMI, has not been fully understood. This study collected peripheral blood from pregnant women during the second trimester and conducted rigorous screening. In

Table 2. Correlation analysis of AFP with obstetric complications and pregnancy outcomes

Pregnancy outcomes	n (%)	r	Obstetric complications	n (%)	r
Abortion	5 (0.5)	0.452*	Severe anemia	32 (3.2)	0.115
Preterm	58 (5.8)	0.376*	Postpartum hemorrhage	87 (8.8)	0.621*
Low birth weight	62 (6.3)	0.526*	Placenta previa	92 (9.3)	0.386*
			Neonatal asphyxia	78 (7.9)	0.459*

*$P < 0.05$.

Table 3. Correlation analysis of β-hCG with obstetric complications and pregnancy outcomes

Pregnancy outcomes	n (%)	r	Obstetric complications	Obstetric complications	n (%)	r
Abortion	5 (0.5)	0.311*	Severe anemia	Severe anemia	32 (3.2)	0.107
Preterm	58 (5.8)	0.457*	Postpartum hemorrhage	Postpartum hemorrhage	87 (8.8)	0.598*
Low birth weight	62 (6.3)	0.623*	Placenta previa	Placenta previa	92 (9.3)	0.316*
			Neonatal asphyxia	Neonatal asphyxia	78 (7.9)	0.372*

*$P < 0.05$.

order to prevent genetic or environmental factors, the selection range contained Han populations, while it excluded smoking, insulin-dependent diabetes, and *in vitro* fertilization. It has been reported that diabetes may reduce AFP and free β-hCG concentrations, whereas *in vitro* fertilization may affect serum markers due to the necessity of multiple embryo transfer and hormone [21, 22].

This study showed that AFP expression elevated, while free β-hCG expression reduced with the increase of gestational age. It has been reported that as body weight increases, maternal blood volume elevation diluted serum marker concentrations [23]. However, this study revealed that AFP is positively correlated, whereas β-hCG is negatively correlated with BMI in pregnant women. AFP in pregnant women's blood is the lowest in the early pregnancy and reaches peak at 28-32 weeks of pregnancy [12, 13]. Free β-hCG rapidly increased after entering the mother and then slowly declined after 20 weeks until the peak at 8 weeks of gestation [16]. In this study, AFP was positively correlated, and β-hCG was negatively correlated with gestational age. Age is one of the high risk factors for pregnant women. Following the age increase of the mother, the detection rate of children with congenital defects elevated. Moreover, age can lead to false positive or detection rates of the results [24]. This study demonstrated that AFP and β-hCG were not associated with the age of the pregnant woman, suggesting that maternal age is an independent factor affecting children with congenital defects.

This study further analyzed the association of AFP and β-hCG with pregnancy outcomes and postpartum complications. The results found that AFP and β-hCG were associated with miscarriage, premature infants and low birth weight infants, postpartum hemorrhage, placenta previa, and neonatal asphyxia, but not with severe anemia. It showed that abnormal placenta structure results in placental hypoxia, destruction of the placental blood vessels, enhanced trophoblast cells secretion, and synthesis, and sustained utero placental blood vessel hypoxidosis, leading to placental trophoblast cells hypertrophy and excessive secretion of hCG [25]. Enhanced immune response can also lead to increased free hCG synthesis and secretion. Amniotic fluid angiogenin further promotes placental angiogenesis, which can also cause enhanced hCG secretion [26]. Therefore, an increase in β-hCG is a hallmark of obstetric complications or pathological pregnancy, and can eventually lead to adverse pregnancy outcomes [27]. In the case of pregnancy, it can result in an increase in AFP, a miscarriage, preterm infants, or low-quality children. The proportion of postpartum complications, postpartum hemorrhage, placenta previa, and neonatal asphyxia will also increase. Since AFP has many important physiological functions, including transport function, bilateral regulatory function as growth regulators, immune suppression, and apoptosis induced by T lymphocytes. AFP is closely related to the occurrence and development of hepatocellular carcinoma and many kinds of tumors. It presents high concentrations in many kinds of tumors and can

cause misjudgments in the analysis. Therefore, tumor patients are commonly excluded during the selection of research subjects [28, 29]. AFP can enter the maternal blood circulation through the amniotic fluid. In the majority of pregnant women with spina bifida and brainless children, peripheral AFP level is elevated at 16-18 weeks of gestation, thus has higher diagnostic value [30]. This study confirmed the association of AFP with obstetric complications and pregnancy outcomes. This study explored the association of AFP and β-hCG with pregnancy outcomes and obstetric complications, not only provided a theoretical basis for predicting related obstetric diseases, but also extended the clinical application of prenatal screening serum markers. However, the mechanism of AFP and β-hCG on pregnancy outcomes and obstetric complications remains to be further studied.

Conclusion

AFP and β-hCG can be affected by gestational age and BMI in pregnant women. Their measured values are related to pregnancy outcomes and obstetric complications, but not to severe anemia.

Disclosure of conflict of interest

None.

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References

[1] Vogel I and Petersen OB. Prenatal screening for atypical chromosomal abnormalities: past or future? Ultrasound Obstet Gynecol 2018; 51: 434-435.

[2] Tanimura K, Morizane M, Deguchi M, Ebina Y, Tanaka U, Ueno Y, Kitajima K, Maeda T, Sugimura K and Yamada H. A novel scoring system for predicting adherent placenta in women with placenta previa. Placenta 2018; 64: 27-33.

[3] Gruber A, Pacault M, El Khattabi LA, Vaucouleur N, Orhant L, Bienvenu T, Girodon E, Vidaud D, Leturcq F, Costa C, Letourneur F, Anselem O, Tsatsaris V, Goffinet F, Viot G, Vidaud M and Nectoux J. Non-invasive prenatal diagnosis of paternally inherited disorders from maternal plasma: detection of NF1 and CFTR mutations using droplet digital PCR. Clin Chem Lab Med 2018; 56: 728-738.

[4] Wu Y, Ma J, Wang Y, Zhang Y, Hou Y, Zhang C, Sun H, Sun J, Wang Z and Li N. Development of an alpha-fetoprotein and Golgi protein 73 multiplex detection assay using xMAP technology. Clin Chim Acta 2018; 482: 209-214.

[5] Xu Y, Li Y, Song T, Guo F, Zheng J, Xu H, Yan F, Cheng L, Li C, Chen B and Zhang J. A retrospective analysis of 237 Chinese families with Duchenne muscular dystrophy history and strategies of prenatal diagnosis. J Clin Lab Anal 2018; 32: e22445.

[6] Zhao HH, Sun XP, Shi MC, Yi YX, Cheng H, Wang XX, Xu QC, Ma HM, Wu HQ, Jin QW and Niu Q. Molecular analysis-based genetic characterization of a cohort of patients with duchenne and becker muscular dystrophy in Eastern China. Chin Med J (Engl) 2018; 131: 770-775.

[7] Sotiriadis A, Figueras F, Eleftheriades M, Papaioannou GK, Chorozoglou G, Dinas K and Papantoniou N. First-trimester and combined first- and second-trimester prediction of small-for-gestational age and fetuses with late growth restriction. Ultrasound Obstet Gynecol 2019; 53: 55-61.

[8] Inan C, Sayin NC, Dolgun ZN, Gurkan H, Erzincan SG, Uzun I, Sutcu H, Ates S, Atli E and Varol F. Prenatal diagnosis of chromosomal polymorphisms: most commonly observed polymorphism on Chromosome 9 have associations with low PAPP-A values. J Matern Fetal Neonatal Med 2019; 32: 1688-1695.

[9] Kondo Z, Makino T and Iizuka R. Measurement of serum unconjugated estriol and estradiol by high-performance liquid chromatography. J Clin Lab Anal 1990; 4: 410-3.

[10] Aslan D, Karabacak RO and Aslan OD. Maternal serum alpha-fetoprotein levels are normal in Fanconi anemia: can it be a lack of postnatal inhibition of AFP gene resulting in the elevation? Pediatr Blood Cancer 2017; 64.

[11] Birdir C, Fryze J, Frölich S, Schmidt M, Köninger A, Kimmig R, Schmidt B and Gellhaus A. Impact of maternal serum levels of Visfatin, AFP, PAPP-A, sFlt-1 and PlGF at 11-13 weeks gestation on small for gestational age births. J Matern Fetal Neonatal Med 2017; 30: 629-634.

[12] Zhang X, Wang W, He F, Zhong K, Yuan S and Wang Z. Proficiency testing of maternal serum

prenatal screening in second trimester in China, 2015. Biochem Med (Zagreb) 2017; 27: 114-121.
[13] He F, Wang W, Zhong K, Yuan S and Wang Z. The status of quality control investigation and analysis for maternal serum marker of prenatal screening laboratories in China. Clin Lab 2017; 63: 183-188.
[14] Catic A, Gurbeta L, Kurtovic-Kozaric A, Mehmedbasic S and Badnjevic A. Application of neural networks for classification of Patau, Edwards, Down, Turner and Klinefelter syndrome based on first trimester maternal serum screening data, ultrasonographic findings and patient demographics. BMC Med Genomics 2018; 11: 19.
[15] Wang C, Guan D, Chen C, He S, Liu X, Wang C and Wu H. Rapid detection of unconjugated estriol in the serum via superparamagnetic lateral flow immunochromatographic assay. Anal Bioanal Chem 2018; 410: 123-130.
[16] Kor-Anantakul O, Suntharasaj T, Suwanrath C, Hanprasertpong T, Pranpanus S, Pruksanusak N, Janwadee S and Geater A. Normative weight-adjusted models for the median levels of first trimester serum biomarkers for trisomy 21 screening in a specific ethnicity. PLoS One 2017; 12: e0182538.
[17] Xia Y, Yang Y, Huang S, Wu Y, Li P and Zhuang J. Clinical application of chromosomal microarray analysis for the prenatal diagnosis of chromosomal abnormalities and copy number variations in fetuses with congenital heart disease. Prenat Diagn 2018; 38: 406-413.
[18] Zou Z, Huang L, Lin S, He Z and Luo Y. Unusual twinning: additional findings during prenatal diagnosis of twin zygosity by single nucleotide polymorphism (SNP) array. Prenat Diagn 2018; 38: 428-434.
[19] Guo B, Xiao J, Li L, Wang S, Wang L and Liu S. Clinical study of prenatal ultrasonography combined with Tbox transcription factor 1 as a biomarker for the diagnosis of congenital heart disease. Mol Med Rep 2018; 17: 7346-7350.
[20] Pass RF and Arav-Boger R. Maternal and fetal cytomegalovirus infection: diagnosis, management, and prevention. F1000 Res 2018; 7: 255.
[21] Fistouris J, Bergh C and Strandell A. Classification of pregnancies of unknown location according to four different hCG-based protocols. Hum Reprod 2016; 31: 2203-11.
[22] Merz E, Thode C, Eiben B and Wellek S. Prenatal risk calculation (PRC) 3.0: an extended DoE-based first-trimester screening algorithm allowing for early blood sampling. Ultrasound Int Open 2016; 2: E19-26.
[23] Lanes A, Huang T, Sprague AE, Leader A, Potter B and Walker M. Maternal serum screening markers and nuchal translucency measurements in in vitro fertilization pregnancies: a systematic review. Fertil Steril 2016; 106: 1463-1469, e2.
[24] Huai L, Leng J, Ma S, Huang F, Shen J and Ding Y. Replacing alpha-fetoprotein with alpha-fetoprotein-L3 increases the sensitivity of prenatal screening for trisomy 21. Am J Ther 2016; 23: e1754-e1757.
[25] Çınar M, Timur H, Aksoy RT, Güzel Aİ, Tokmak A, Bedir Fındık R and Uygur D. Evaluation of maternal and perinatal outcomes among overweight women who experienced stillbirth. J Matern Fetal Neonatal Med 2017; 30: 38-42.
[26] Wan X, Wen J, Song X, Guo Y, Liu X, Yang B and Lu X. The analysis of second-trimester triple screening for Down syndrome in Chinese normal singleton pregnancies. Scand J Clin Lab Invest 2012; 72: 642-7.
[27] Weintraub AY, Weiss I, Friger M and Hershkovitz R. Pregnancy and delivery outcomes following a pathological second trimester triple test screening result and a normal karyotype. J Matern Fetal Neonatal Med 2012; 25: 2606-9.
[28] Dutton PJ, Warrander LK, Roberts SA, Bernatavicius G, Byrd LM, Gaze D, Kroll J, Jones RL, Sibley CP, Frøen JF and Heazell AE. Predictors of poor perinatal outcome following maternal perception of reduced fetal movements--a prospective cohort study. PLoS One 2012; 7: e39784.
[29] Wang S, Zheng Y, Liu J, Huo F and Zhou J. Analysis of circulating tumor cells in patients with hepatocellular carcinoma recurrence following liver transplantation. J Investig Med 2018; 66: 1-6.
[30] Goto E. Meta-regression analysis to evaluate relationships between maternal blood levels of placentation biomarkers and low delivery weight. Int J Gynaecol Obstet 2018; 142: 148-155.