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Article

Association Analysis between Maternal Serum Biomarkers and Fetal Congenital Heart Disease

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Abstract

Objective: To investigate the association of maternal serum biomarkers alpha fetal protein (AFP), β-human chorionic gonadotropin (HCG) and unconjugated estriol 3 (uE3) in the second trimester with fetal congenital heart disease (CHD) in low risk populations during the screening of Down's syndrome. **Methods**: 109 cases diagnosed with fetal CHD in the second trimester by fetal echocardiography were enrolled as the CHD group. Pregnancy- and gestational-age matched 344 cases without fetal CHD were used as the control group. The values of maternal serum biomarkers HCG, AFP and uE3 were tested and the association with CHD was analyzed. **Results**: The means of HCG multiple of median (MoM) and AFP MoM were higher, while the mean of uE3 MoM was lower in the CHD group than those of the control group ($p < 0.05$). The number of cases with HCG MoM $\geq$85% quantile, AFP MoM $\geq$85% quantile was more, while that with uE3 MoM $\leq$15% quantile was less in the CHD group than that of the control group ($p < 0.05$). The univariate logistic regression analysis showed that fetal CHD was associated with high values of HCG MoM and AFP MoM and low value of uE3 MoM as well as the HCG MoM $\geq$85% quantile, AFP MoM $\geq$85% quantile and the uE3 MoM $\leq$15% quantile. The multivariate logistic regression analysis showed that HCG MoM $\geq$85% quantile and AFP MoM $\geq$85% quantile or the uE3 $\leq$15% quantile were the independent factors of fetal CHD. In addition, the risk of fetal CHD was higher when one situation existed among the HCG MoM $\geq$85% quantile, AFP MoM $\geq$85% quantile and the uE3 MoM $\leq$15% quantile. The risk of fetal CHD was much higher when both the HCG MoM $\geq$85% quantile and AFP MoM $\geq$85% quantile existed or both the HCG MoM $\geq$85% quantile and the uE3 MoM existed $\leq$15% quantile. **Conclusions**: Second trimester maternal serum biomarkers may be useful indicators for fetal evaluation for CHD to screen positive pregnancies without identified chromosomal defects.

Keywords

congenital heart disease; Down's screening; alpha fetal protein; human chorionic gonadotropin; unconjugated estriol 3

Introduction

Fetal congenital heart disease (CHD) is the most common congenital anomaly and also one of the most lethal diseases of congenital anomalies [1]. In recent years, the incidence of CHD in China has increased annually [2]. The prenatal diagnosis of fetal CHD has been an important subject in perinatal medical research. Although the association of fetal CHD with high-risk pregnant women increased significantly, more than 80% of fetal CHD was found in pregnant women without risk factors [3]. Therefore, methods to strengthen the monitoring of low-risk populations and the early detection of fetal CHD has become the focus of research in fetal medicine and ultrasonics. Fetal CHD is closely related to 21-trisomy, 18-trisomy and Down's serology; and triple screening is an important approach to determine the occurrence of chromosomal abnormalities [4]. However, the association between serum biomarkers and fetal CHD in Down's screening requires further investigation. Traditionally, indicators of Down's screening have been associated with serum biomarkers including alpha fetal protein (AFP), β-human chorionic gonadotropin (HCG) and unconjugated estriol 3 (uE3) [5]. These serum biomarkers are synthesized by different organs of the fetus and secreted into the maternal blood via the placenta. AFP is an embryo-specific globulin produced in the embryo by the yolk sac and liver cells. 12 weeks after pregnancy, the liver becomes the main source of AFP. In normal pregnancy, a small amount of AFP enters into the maternal circulation through the placenta [6]. HCG is the gestational hormone produced by placental trophoblast cells, which plays an important role in maintaining pregnancy [5]. uE3, a natural estrogen, is synthesized through the placenta aromatase and secreted into the maternal blood circulation in the free form, which, to a certain extent, can reflect the development of the

fetus [7]. A study has suggested that abnormal serum levels of biomarkers may be associated with fetal CHD during the second trimester of Down's serological screening [7]. The present study was designed to investigate the association of maternal serum biomarkers AFP, HCG and uE3 in the second trimester with fetal CHD in low risk populations during the screening of Down's syndrome and to explore the predictive value of these biomarkers in fetal CHD.

Materials and Methods

Study Subjects and Inclusion and Exclusion Criteria

Selected subjects in this study were pregnant women who received regular prenatal care and final delivery or abortion in Fujian Provincial Maternal and Child Health Hospital of Fujian Medical University and The First Affiliated Hospital of Fujian Medical University from January 2010–January 2020. Approval was granted by the institutional ethics committee of Fujian Medical University ([2015]084-2). This study conforms to the STROBE reporting criteria.

Inclusion criteria in the CHD group were: (1) The pregnant women with a single pregnancy with CHD diagnosed by fetal echocardiography at 18–24 weeks of pregnancy in accordance with the National Ministry of Health diagnostic criteria and classified based on birth defects ICD-10 (China) [8]; (2) The result of Down's screening in the second trimester showed low risk; (3) Results of fluid/umbilical cord blood chromosome examination confirmed no chromosomal abnormalities. Exclusion criteria in the CHD group were: (1) Fetal malformations including fetal chromosomal abnormalities, absence of encephalopathy, encephalocele, open spina bifida, severe thoracic and abdominal wall defects, visceral valgus, and fetal liver tumors. Inclusion criteria in the control group were: (1) Pregnant ages and pregnant weeks were matched with those in the CHD group; (2) The result of Down's screening in the second trimester showed low risk; (3) The results of fetal echocardiography showed no heart malformations and other system abnormalities; (4) Malformations were not found in follow-up to 6 months after birth. Exclusion criteria in both the CHD and control groups were: (1) Pregnant women with fetal CHD risk factors, including those with a gestational age of $\geq$35 years; (2) Conception with assisted reproductive technology; (3) Multiple births or twin pregnancies; (4) Previous history of fetal malformations; (5) Poor control of maternal diabetes mellitus, and phenylketonuria; (6) Having anti-Sjogren's syndrome A or anti-Sjogren's syndrome B antibodies; (7) Medication with teratogen agents such as Salidan, angiotensin-enzyme inhibition (ACEI), retinoic acid and nonsteroidal anti-inflammatory drugs (NSAID); (8) Rubella virus infection in the first trimester; (9) Close relatives having CHD and (10) Liver tumors, liver inflammatory diseases, ovarian tumors.

Methods

Detection of Biomarkers

Serum was separated from blood samples and used for measurement of biomarkers HCG, AFP and uE3 using automatic time-resolved fluoro-immunoassay (PerkinElmer, Waltham, MA, USA). The multiple of median (MoM) values and the corresponding risk values of the serum biomarkers were calculated using the Life-Cycle 3.2 software (PerkinElmer, Waltham, MA, USA). Down's syndrome (DS) risk $>$1:270 was defined for positive, 18-trisomy risk $>$1:350 was defined as positive. The references for HCG MoM, AFP MoM and uE3 MoM were 0.25–2.0, 0.7–2.5 and 0.5–2.0 respectively.

Fetal System Ultrasound and Fetal Echocardiography

In accordance with "Fetal heart ultrasound screening guidelines" published by The International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) (2006) [9] and "Prenatal ultrasound guidelines - Fetal heart echocardiography guidelines" published by Chinese Medical Association Ultrasound Physicians Branch (2012) [10], seven sections of the fetal heart were inspected and superimposed with color Doppler flow imaging (Philips IU22) with combinations of M-mode ultrasound in different sections. The activity curve of the atrioventricular valve and atrioventricular wall were observed in each cardiac cycle.

Chromosomal Examination

Using the ultrasound positioning guided abdominal paracentesis puncture, the amniotic fluid 20 mL, or cord blood 1.5~2.0 mL was collected for G banding staining. The chromosome automatic analyzer (PerkinElmer, Waltham, MA, USA) was used to count 30 karyotypes followed by analysis of five karyotypes. The abnormal karyotype count was doubled. Chromosomal karyotypes were described by the International Nomenclature System for Human Cytogenetics (ISCN).

Statistical Methods

Data were processed using a statistical software package STATA 13.0 (StataCorp, College Station, TX, USA). The qualitative data were expressed as n or % and analyzed by χ^2 test. Median of measurement data was analyzed by Wilcoxon rank sum test, and the statistic value was expressed as z. The *t* test was used to compare the data between the two groups and the statistic value was expressed as F. The odds ratio (OR) and the 95% confidence interval

were calculated by the unconditional single factor binary logistic regression model. The test level was $\alpha = 0.05$.

Results

General Characteristics and Diagnosis of CHD

The classifications of fetal congenital heart disease were as follows: septal class defects, anomalies of large vessels and arterial cones, anomalies of valve system development, anomalies of the venous systems, abnormalities of cardiac position and other forms of heart disease. Of 109 cases with CHD diagnosed by fetal echocardiography, 12 cases were born in full-term delivery whose CHD were confirmed by neonatal echocardiography 3 days after birth; 97 cases chose to terminate their pregnancy, among which 89 cases were confirmed to have CHD by autopsy and 8 cases failed to do so because of the fetal edema induced-heart tissue autolysis. All cases in the CHD group were ruled out for fetal chromosomal abnormalities by checking the amniotic fluid or blood from the umbilical cord. All 334 cases in the control group were confirmed for not having CHD by neonatal physical examination, cardiac auscultation and echocardiography. Follow-up at 6 months after birth confirmed no physical development abnormalities.

The Relationship between Fetal CHD and Serum Biomarkers in Low-Risk Cases with Down's Syndrome

The indices of the two groups were compared, including age, gestational age, body weight and the mean value of MoM of all serum biomarkers in the Down's screening. For the entire population, the 15% and 85% quantiles were 0.6256 and 2.0618 for HCG MoM, 0.5870 and 1.6474 for AFP MoM and 0.6734 and 1.8670 for uE3 MoM respectively. Each serum biomarker was divided into sub-groups of $\leq$15% quantile, 15%–85% quantile and $\geq$85% quantile assigning codes of –1, 0, and +1 respectively. The results showed no significant difference in age, body weight and gestational age between the two groups. The means of HCG MoM and AFP MoM in CHD group were higher than those of the control group, and the mean of uE3 MoM was lower than that of the control group. The number of cases with HCG MoM $\geq$85% quantile, AFP MoM $\geq$85% quantile in CHD group was more than those of the control group. However, the number of cases with uE3 MoM $\leq$15% quantile in CHD group was less than that of the control group. The results suggested that the CHD group was more likely to have higher values of HCG MoM and AFP MoM and lower value of uE3 MoM in the second trimester (Table 1).

The results of the univariate logistic regression analysis showed that fetal CHD was associated with high values of HCG MoM and AFP MoM and low values of uE3 MoM in the second trimester as well as the HCG MoM $\geq$85% quantile, AFP MoM $\geq$85% quantile and the uE3 MoM $\leq$15% quantile. However, no association was found between CHD and the age, gestational age and body weight of pregnant women during screening for Down's syndrome (Table 2).

The results of multivariate logistic regression analysis showed that HCG MoM $\geq$85% quantile (OR: 2.77) and AFP MoM $\geq$85% quantile (OR: 3.63) or the uE3 $\leq$15% quantile (OR: 12.91) were the independent predictors of fetal CHD (Table 3).

The Prognostic Value of HCG, AFP, and uE3 MoM for Fetal CHD in Mid-Pregnancy

The Jorden index was calculated using serum HCG, AFP, and uE3 MoM levels as test variables and the occurrence of fetal CHD as a status variable. The values of HCG, AFP, and uE3 MoM levels that predicted the occurrence of fetal CHD were used as prediction cut-off values. Using the χ^2 test, the sensitivity and specificity of these two criteria were estimated based on the actual incidence of CHD. As demonstrated in Table 4, the specificity of serum HCG, AFP, and uE3 MoM as predictors of fetal CHD was 84.4%, 85.3%, and 95.5%, respectively, and their predictive value and accuracy were high. The receiver operator characteristics (ROC) curve was plotted and the Jorden index was calculated using HCG, AFP, uE3 MoM two-by-two and three combined predictors as the test variables and the presence or absence of fetal CHD as the status variable, with the total resistance score with the highest predictive value serving as the predictive threshold, as shown in Fig. 1. The Jorden index was greatest when the three combined predictors were 0.403, with a value of 1.494, indicating that the greatest predictive value was achieved at this time; consequently, 0.403 was used as the predictive threshold for the combined predictor of HCG, AFP and uE3 MoM, and a χ^2 test was performed with the actual incidence of fetal CHD, which had a specificity of 85.2% and a sensitivity of 68.2%. The combined predictor had the highest predictive value ($p < 0.05$) for the development of fetal CHD, while also having the largest area under the ROC curve (0.797). Therefore, the predictive value of the combination of the three predictors was greater than that of any single indicator or the combination of two indicators.

Discussion

Fetal CHD is closely related with chromosomal abnormalities [2]. Common chromosomal abnormalities such as 21-trisomy, 18-trisomy and trisomy 13 are associated with different degrees of fetal CHD [11]. Down's screening is the simplest, noninvasive method of assessing the chromosomal aberrations described above. MoM values were used to standardize serum markers for clinical eval-

Table 1. Comparisons of general characteristics and values of biomarkers.

		Control (N = 334)	CHD (N = 109)	Statistic value	p value
Age (years)	Mean ± SD	28.23 ± 4.06	27.95 ± 3.27	F = 0.41	0.5246
	M (Q1~Q3)	28.23 (25.38~30.44)	27.71 (26.13~30.05)		
	Min~Max	19.00~46.59	21.75~37.00		
Weight (kg)	Mean ± SD	53.83 ± 7.20	54.11 ± 6.95	F = 0.13	0.7180
	M (Q1~Q3)	53.00 (49.00~57.55)	54.00 (51.20~57.40)		
	Min~Max	36.50~90.00	22.00~77.00		
Gestation (weeks)	Mean ± SD	17.65 ± 0.96	17.56 ± 0.71	F = 0.78	0.3784
	M (Q1~Q3)	17.50 (17.20~18.30)	17.57 (17.14~18.00)		
	Min~Max	15.00~20.50	15.86~19.71		
HCG	Mean ± SD	1.27 ± 0.97	1.95 ± 1.41	z = 4.57	<0.0001
	M (Q1~Q3)	1.12 (0.80~1.46)	1.70 (0.84~2.34)		
	Min~Max	0.25~10.94	0.43~6.62		
HCG	–1	53 (15.87%)	13 (11.93%)	z = 4.84	<0.0001
	0	252 (75.45%)	59 (54.13%)		
	1	29 (8.68%)	37 (33.94%)		
AFP	Mean ± SD	1.01 ± 0.80	1.46 ± 0.61	z = 7.49	<0.0001
	M (Q1~Q3)	0.91 (0.65~1.15)	1.26 (0.96~1.99)		
	Min~Max	0.40~12.70	0.57~2.74		
AFP	–1	62 (18.56%)	2 (1.83%)	z = 8.25	<0.0001
	0	249 (74.55%)	64 (58.72%)		
	1	23 (6.89%)	43 (39.45%)		
uE3	Mean ± SD	1.33 ± 0.50	0.84 ± 0.53	z = 9.00	<0.0001
	M (Q1~Q3)	1.23 (0.96~1.69)	0.68 (0.48~1.02)		
	Min~Max	0.48~2.91	0.23~3.02		
uE3	–1	14 (4.19%)	52 (47.71%)	z = 9.28	<0.0001
	0	261 (78.14%)	51 (46.79%)		
	1	59 (17.66%)	6 (5.50%)		

CHD, Fetal Congenital Heart Disease; HCG, β-human chorionic gonadotropin; AFP, alpha fetal protein; uE3, unconjugated estriol 3.

Table 2. Univariate logistic regression analysis evaluating the association between CHD and biomarkers.

Factors	Coefficient	SEM	Statistic value	p value	OR (95% CI)	Total evaluation	
						Chi square	p value
HCG –1 *vs.* 0	0.0465	0.3416	0.02	0.8916	1.05 (0.54, 2.05)	36.20	<0.0001
HCG 1 *vs.* 0	1.6955	0.2871	34.88	<0.0001	5.45 (3.11, 9.57)		
AFP –1 *vs.* 0	–2.0754	0.7320	8.04	0.0046	0.13 (0.03, 0.53)	57.60	<0.0001
AFP 1 *vs.* 0	1.9843	0.2939	45.58	<0.0001	7.27 (4.09, 12.94)		
uE3 –1 *vs.* 0	2.9449	0.3378	76.01	<0.0001	19.01 (9.80, 36.85)	83.83	<0.0001
uE3 1 *vs.* 0	–0.6531	0.4550	2.06	0.1512	0.52 (0.21, 1.27)		
Age	–0.0185	0.0291	0.41	0.5238	0.98 (0.93, 1.04)		
Weight	0.0056	0.0155	0.13	0.7173	1.01 (0.98, 1.04)		
Gestation	–0.1092	0.1238	0.78	0.3777	0.90 (0.70, 1.14)		

OR, odds ratio; CI, Confidence interval; SEM, Standard Error of Mean.

uation. MoM (Multiples of the median) is a ratio that represents the value of a serum marker in a pregnant woman divided by the median value of a normal pregnant woman in the same gestational week. Traditionally, indicators of Down's screening have been serum biomarkers including HCG, AFP and uE3 [5]. These serum biomarkers are synthesized by different organs of the fetus and secreted into the maternal blood via the placenta. AFP is an embryo-specific globulin produced in the embryo by the yolk sac and liver cells. HCG is the gestational hormone produced by placental trophoblast cells, which plays an important role in maintaining pregnancy. uE3, a natural estrogen, is syn-

Table 3. Multivariate logistic regression analyses evaluating the association between CHD and biomarkers.

Factors	Coefficient	SEM	Statistic value	*p* value	OR (95% CI)	Whole evaluation	
						Chi square	*p* value
HCG –1 *vs.* 0	0.1308	0.3807	0.12	0.7312	1.14 (0.54, 2.40)	8.08	0.0176
HCG 1 *vs.* 0	1.0203	0.3612	7.98	0.0047	2.77 (1.37, 5.63)		
AFP –1 *vs.* 0	–2.0233	0.7918	6.53	0.0106	0.13 (0.03, 0.62)	22.07	<0.0001
AFP 1 *vs.* 0	1.2893	0.3543	13.24	0.0003	3.63 (1.81, 7.27)		
uE3 –1 *vs.* 0	2.5578	0.3695	47.91	<0.0001	12.91 (6.26, 26.63)	49.79	<0.0001
uE3 1 *vs.* 0	–0.0941	0.4952	0.04	0.8493	0.91 (0.34, 2.40)		

Table 4. Stratified analysis of the relationship between fetal CHD and serum biomarkers.

Risk factors	Control %, OR (95% CI)	CHD %, OR (95% CI)
HCG ≥85th	5.70, Reference	26.73, 6.03 (3.12–11.65)
AFP ≥85th	4.14, Reference	29.36, 9.63 (4.73–19.58)
uE3 ≤15th	1.79, Reference	37.74, 32.96 (15.14–78.64)
HCG ≥85th and AFP ≥85th	2.34, Reference	17.19, 8.60 (4.63–17.73)
HCG ≥85th and uE3 ≤15th	0.89, Reference	25.00, 36.33 (17.92–75.49)
APF ≥85th and uE3 ≤15th	0, Reference	26.67, NA

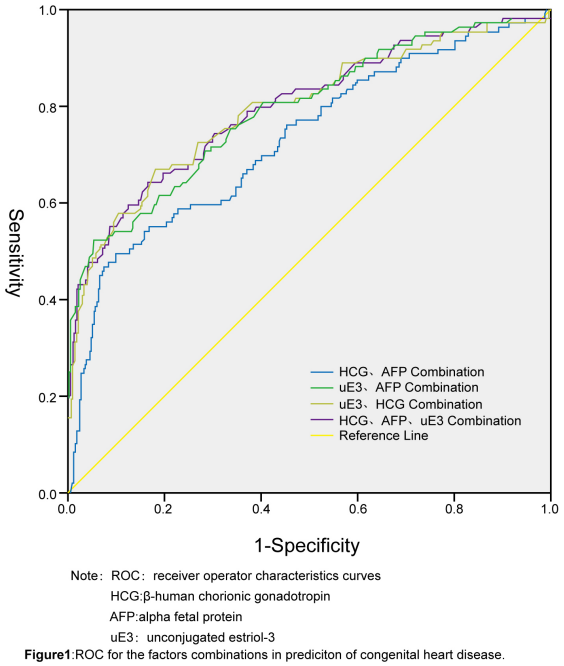

Fig. 1. ROC for the factors combinations in prediction of congenital heart disease. Note: ROC, receiver operator characteristics curves; HCG, β-human chorionic gonadotropin; AFP, alpha fetal protein; uE3, unconjugated estriol-3.

thesized through the placenta aromatase and secreted into the maternal blood circulation in the free form, which, to a certain extent, can reflect the development of the fetus [5]. Thus, the concentration of maternal serum biomarkers, to some extent, reflects the growth of the fetus and placental function. Based on the genetic susceptibility of the embryo, a variety of pathogenic factors can induce embryonic genetic abnormalities, including chromosomal abnormalities or gene mutations, resulting in abnormal organ structure and function. Functional abnormalities are reflected by the abnormal changes of the corresponding serum biomarkers produced by the fetus, which can identify high-risk pregnancies which require further examination. Minor abnormalities of fetal organs cannot be recognized earlier by ultrasound. Thus, abnormal serum biomarkers not only indicate an increased risk of fetal chromosomal abnormalities, but also an increased risk of adverse pregnancy outcomes. In recent years, the non-invasive prenatal test has grown in popularity in some developed regions of China, but due to the price, it cannot be widely adopted (the cost of $191.8 is not covered by the minimum social medical insurance program). In most of China, Down's serology screening is free and can be aggressively promoted. Down's serology screening can be used to predict adverse pregnancy outcomes in China because serum markers used in Down's serology screening not only assist in determining the risk of chromosomal abnormalities in the fetus, but an increasing number of studies indicate that they are predictive of adverse pregnancy outcomes. Therefore, the non-invasive prenatal test will not completely replace Down's serology screening in China for the time being.

Studies have been shown that fetal CHD is related to specific biomarkers such as β-HCG and AFP. DeVore *et al.* [11] and Souter *et al.* [12] found a close association between serum β-HCG in pregnant women having Town's syndrome with fetal CHD. Bravo-valenzuela *et al.* [5] reported that abnormal elevated AFP during pregnancy usually indicates a poor pregnancy outcome, which can be explained by the increased incidence of fetal CHD. In a case-control study, Jelliffe-Pawlowski *et al.* [13] studied the relationship between CHD and maternal serum levels of AFP, β-HCG and uE3 in the second trimester in a low risk fetal population, and found that the CHD group had higher lev-

els of AFP and β-HCG and lower levels of uE3 than those in the normal group. Compared to the normal group, the CHD group tends to have higher levels of AFP MoM and β-HCG MoM and lower levels of uE3 MoM with the OR value of 1.8–2.4 and 95% CI of 1.1–7.8 [7,13,14]. These results suggested that abnormalities of AFP, β-HCG and uE3 MoM in the second trimester may be indicative of the increased risk of fetal CHD. In the present study, the univariate and multivariate logistic regression analyses demonstrated that the existence of one or more than one of the three situations among AFP MoM $\geq$85% quantile, β-HCG MoM $\geq$85% quantile and uE3 MoM $\leq$15% quantiles increased the risk of fetal CHD. These results suggested that HCG MoM $\geq$85% quantile, AFP MoM $\geq$85% quantile and uE3 MoM $\leq$15% quantile are independent risk factors for fetal CHD. This suggests that these maternal mid-pregnancy serum markers are associated with fetal congenital heart disease.

In regard to the pathophysiological processes explaining the observed relationship between CHD and AFP and HCG, evidence suggests that there might be both correlational and causal components. The development of the early embryonic liver is closely related to the development of the diaphragmatic mesenchyme and the primitive heart in both time and space [15,16]. During the third week of the embryo, the cells of the ventral endoderm in the caudal part of the foregut near the yolk sac proliferate and grow rapidly ventrally, extending and aggregating to become liver buds, which are located in the dorsal-caudal side of the primitive heart. At this time, the liver buds are located in the dorsal and caudal side of the primitive heart, and the ventral side of the liver buds and the primitive heart are surrounded by transverse mesenchyme filling. The primitive heart begins to develop as early as day 15 of the embryo in the mesoderm, so cardiac teratogens, while affecting the development of the fetal heart, may also cause fetal liver abnormalities and impaired function, which results in abnormalities of fetal liver synthesis of AFP as well as the conversion process of uE3.

Fetal CHD-related metabolism and viral infections can affect both cardiac and mid-pregnancy serum marker levels. Genetic metabolic studies [17] have found that a certain degree of vitamin B and folate deficiency can affect the folate synthesis pathway and lead to cardiac developmental abnormalities. HCG levels; growth-associated factors such as activin A and activin receptor-like kinase (ALK2) [18] have been associated with cardiac abnormalities, and these factors have also been shown to affect serum AFP and HCG levels [19,20]. Viruses associated with CHD such as cytomegalovirus enhancers can greatly increase the efficiency of the AFP promoter in hepatocytes, leading to a significant increase in the synthesis of AFP by hepatocytes [21]. Therefore, changes in serum markers can be considered as concomitant events in the development of CHD.

Levels of AFP or HCG have been shown to be indicative of an increased risk for intrauterine growth retardation and/or preterm birth [15,16]. These same outcomes are more common in pregnancies with CHD [17]. It is hypothesized that changes in maternal serum markers in mid-pregnancy may reflect fetal cardiac development to some extent. Combined with the results of this study, fetuses with CHD may be due to abnormalities in metabolism, viral infections and other factors that lead to a significant increase in the synthesis of AFP in the fetal liver such as impaired placental barrier leading to an abnormal increase in the concentration of AFP, HCG in the maternal serum, and varying degrees of placental dysfunction leading to abnormalities in the synthesis of uE3 manifested as a decrease in the concentration of uE3 in the maternal body. When one or more of AFP MoM $\geq$1.6474, HCG MoM $\geq$2.0618, uE3 MoM $\leq$0.6734 is present in Down's serologic screening, the chance of fetal congenital heart anomalies is increased, and ultrasonography of the fetal heart should be emphasized in order to prevent omission of the diagnosis of congenital heart disease.

Since fetal CHD is the most likely missed congenital deformity [15], prenatal diagnosis of CHD is essential. Fetal echocardiography is currently recognized to be the main method for the diagnosis of fetal CHD. The principles in "Prenatal ultrasound guidelines-fetal echocardiography guidelines" published by the Chinese Medical Association Ultrasound Physicians Branch in 2012 [10] state that fetal echocardiography is required for the high-risk population with fetal CHD. However, we found in our clinical practice that more than 80% of fetal CHD occurs in low-risk pregnant women. Therefore, methods to increase the monitoring of low-risk populations and the earlier detection of CHD is essential. Down's serological screening provides a way to improve the diagnosis of fetal CHD in low-risk populations. For the high-risk pregnant women identified by Down's screening, chromosomal abnormalities are checked and obstetrics system ultrasound is recommended with a focus on fetal heart development. For the low-risk cases in Down's screening, the MoM values of the serum biomarkers should be studied. If one or more than one of the three situations among AFP MoM $\geq$1.6474, HCG MoM $\geq$2.0618 and uE3 MoM $\leq$0.6734 exists, detailed systematic ultrasound and fetal echocardiography examinations should be performed.

Due to the limitations of our study design, we did not include smoking status during pregnancy, maternal living environment, and lifestyle and dietary habits as exclusion confounders. We excluded groups with high risk factors for fetal congenital heart disease from the selection of our case group, resulting in selection bias. As a result, the findings of this investigation are limited in determining serum indicators in the low-risk category for fetal congenital heart disease.

Conclusions

The combination of maternal serum biomarkers in the second trimester and fetal echocardiography is valuable to improve the detection of the incidence of CHD in low-risk populations.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available due to the privacy of the hospital database but are available from the corresponding author on reasonable request.

Author Contributions

HJ, YL and XH designed the research study. HJ and YL collected data. HJ wrote the manuscript. YL analyzed the data. XH reviewed and revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

The study was consented by the patients and granted by the institutional ethics committee of Fujian Medical University ([2015]084-2).

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Conflict of Interest

The authors declare no conflict of interest.

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