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A risk prediction model for fetal hypospadias by testing maternal serum AFP and free beta-HCG

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ABSTRACT

Objective: We aimed to determine the correlation between serum alpha-fetoprotein (AFP), the free human chorionic gonadotropin beta subunit (free beta-HCG) in pregnant women during the mid-trimester, and fetal hypospadias, and tried to establish a risk prediction model.

Methods: A multi-center case-control study was conducted. We divided the fetuses into two groups (69 with fetal hypospadias and 62 without fetal hypospadias). Time-resolved immunofluorescence was used to determine the serum levels of AFP and free beta-HCG. The best cut -value and area under curve (AUC) were determined based on the Receiver operating characteristics (ROC) curve, and the diagnostic value of AFP and free beta-HCG was evaluated.

Results: The level of AFP in the study group was 1.14 multiple of Median(MOM), which was higher than in the control group (0.96 MOM); the difference was significant ($Z = 2.831$, $P = .005$). Similarly, the free beta-HCG level was 1.30 MOM in the study group, and was also significantly higher than in the control group (0.84 MOM; $Z = 3.131$, $P = .004$). We used the AFP and free beta-HCG levels separately to predict fetuses with hypospadias, and the AUCs were 0.644 (95% Confidence interval (CI): 0.5500.737, $P = .005$) and 0.659 (95% CI: 0.5650.752, $P = .002$), respectively. According to the ROC curve, the best cut-off values for AFP and free beta-HCG were 0.945 MOM and 1.275 MOM, respectively. When we combined the AFP level with the free beta-HCG level, the AUC was 0.700 (95% CI: 0.610–0.789, $P < .001$), with a sensitivity of 0.551 and a specificity of 0.855.

Conclusion: Combined screening for fetal hypospadias with maternal serum AFP and free beta-HCG levels during the early second trimester has high sensitivity and specificity, which can be used as a new marker.

1. Introduction

Fetal hypospadias is a common congenital malformation of the male external genitalia that is characterized by abnormal opening of the urethra to the penis, ventral scrotum, or perineum.

It has been reported that hypospadias is caused by single genes, including *WT1, SF1*, and *BMP4* [1]. Others believe that the *GSTT1, HOXA4, IRX5, IRX6, EYA1* [2], *CYP1A1, GSTM1,* and *GSTT1* genes, combined with maternal genetic polymorphisms [3], are components of risk factors (both genetic and environmental), such as endocrine-disrupting chemicals [4–6], smoking, low birth weight, maternal hypertension, and pre-eclampsia, but most risk factors remain unknown [2]. The existing literature indicates that the incidence of hypospadias is between 1 in 300 and 1 in 125 male births [7]. The incidence of fetal hypospadias in the northeastern Iranian city of Mashhad is 0.4% [8]. The incidence of fetal hypospadias is increasing worldwide. The clinical diagnosis of hypospadias is mainly based on the observation of abnormal urethral openings by the clinician after birth. Prediction of fetal hypospadias by maternal serum alpha-fetoprotein (AFP) and free human chorionic gonadotropin beta subunit (free beta-HCG) levels have rarely been reported. [9] At present, the combination of maternal serum AFP and free beta-HCG levels is widely used in screening for fetal Down's syndrome and open neural tube defects. Our study adopted a case-control retrospective study method. On the basis of double-screening for Down's syndrome, healthy pregnant women and pregnant women diagnosed with hypospadias fetuses were selected to explore the diagnostic value of AFP and free beta-HCG for fetal hypospadias.

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2. Materials and methods

2.1. Materials

A multi-center case-control study was conducted. We retrospectively analyzed 501,628 women with singleton gestations in five prenatal screening centers with gestational ages ranging from 15 to 20^{+6} weeks. The gestational age was calculated from the last menstrual period. In women with irregular menstrual cycles, we determined the gestational age by B-mode ultrasound. Among the women, 141,338 were recruited from the Prenatal Screening Center of Yuhang District Maternal and Child Health Hospital of Hangzhou City (China) between October 2008 and September 2018;a total of 254,475 were recruited from Hangzhou Obstetrics and Gynecology Hospital (Hangzhou Maternal and Child Health Hospital); 28,969 were recruited from the Obstetrics and Gynecology Hospital Affiliated to Zhejiang University (China); 52,026 were recruited from Zhejiang Xiaoshan Hospital; and 24,820 were recruited from Fuyang District Maternal and Child Health Hospital of Hangzhou. The study group consisted of 69 pregnant women diagnosed with hypospadias by unaided inspection at birth (including 55 simple hypospadias cases and 14 cases complicated with other malformations, including syndactyly and polydactyly, which do not affect the AFP and free beta-HCG levels). The control group was formed as follows. First, we screened the samples by matched maternal age, gestational age at the time of testing, fetal gender, and maternal body mass index. We then randomly selected 69 cases (seven were deleted for incomplete materials) from the above samples at a ratio of 1-to-1. The control group included 62 pregnant women with normal infant development during the same period of follow-up. There were no significant differences in maternal age, gestational age at the time of testing, or maternal body weight between the case and control groups ($P > .05$), as shown in Table 1. Thus, we believed the AFP and free beta-HCG levels were comparable between the two groups. The study was approved by the Ethics Committee and the approval number was No. 2018-004-01.

2.2. Diagnostic and exclusive criteria

Hypospadias is a congenital malformation that is characterized by an abnormal opening of the urethra to the penis, ventral scrotum, or perineum. Clinically, according to the position of the urethral opening, fetal hypospadias can be divided into the following types: (1) penile head; (2) penile body; (3) penile scrotum; and (4) perineal. The diagnosis can be made by the unaided eye after birth. The exclusion criteria include the following: urethral fissure; penile curvature; redundant prepuce with normal urethral opening; pseudohermaphroditism; twins and multiple pregnancies; combined with other medical conditions, such as insulin-dependent diabetes mellitus and severe pregnancy complications; smoking; test tube infants; follow-up results of Down's syndrome, trisomy 18, neural tube defects, and other birth defects; and incomplete information.

2.3. Sample collection

We collected 3–5 mL of fasting venous blood from each subject. The serum samples were separated 30 min later. We obtained a 1–2 ml blood sample by a centrifugal tube and placed the sample in a refrigerator at 2–8 °C. Samples were transported at a low temperature and sent for examination within 1 week. Surplus serum samples were stored in a refrigerator at −80 °C after prenatal screening.

2.4. Time-resolved fluorescence immunoassay

We used a 1235 Auto DELFIA automatic time-resolved fluorescence immunoassay analyzer (PerkinElmer, USA) for detection with a double labeling kit (AFP/free β-HCG),. The levels were expressed as a MOM. The detection steps were per the manufacturer's instructions.

2.5. Establishing different models to compare the screening efficiency of hypospadias

The risk calculation model (life cycle-like risk truncation scheme) was constructed based on AFP and free beta-HCG levels and compared with the original single truncation scheme using the AFP MOM or free beta-HCG MOM alone. The MOM values of AFP and free beta-HCG were shown to obey the multiple normal distribution f (AFP MOM and free beta-HCG MOM). According to the risk calculation model [10], the distribution parameters of each index was calculated, and the distribution likelihood was calculated as the hypospadias risk. Similarly, the following three models were constructed: model 1, AFP MOM value only; model 2, free beta-HCG MOM value only; and model 3, AFP and free beta-HCG together.

2.6. Statistics

We used software Excel 2007 to establish the database. IBM SPSS 21.0 (USA) was used for statistical processing. The one-sample Kolmogorov-Smirnov test was used to determine whether or not the data were normally distributed. Age, weight, and gestational age were normally distributed and expressed as $\bar{x} \pm s$. The results of AFP and free beta-HCG had a skewed distribution and were expressed as the median and percentile [M (P2.5, P97.5)]. The basic data between the study and control groups were compared using an independent samples *t*-test. The rank-sum test was used to compare the AFP and free beta-HCG levels. The ROC curve was drawn and the best truncation values, area under the ROC curve (AUC), and Youden index of AFP and free beta-HCG were calculated. We believe that the risk model with the larger AUC and higher sensitivity had better diagnostic value. A *P* value < .05 was statistically significant.

3. Results

3.1. AFP and free beta-HCG levels in the two groups

The AFP level of the study group was 1.14 (0.65–3.40) MOM, which was higher than the control group (0.96 [0.55–1.94] MOM; $Z = 2.831$, $P = .005$). The free beta-HCG level in the study group was 1.30 (0.33–19.41) MOM, which was higher than the control group (0.84 [0.37–3.48] MOM; $Z = 3.131$, $P = .004$, Table 2).

Table 1
Basic data of each group.

Group	n	Age(year)	Gestational age(day)	Body weight(kg)
Control	62	27.40 ± 3.19(26.59–28.21)	118.60 ± 5.14(117.29–119.90)	54.23 ± 7.75(52.26–56.20)
Hypospadias	69	28.04 ± 3.18(27.28–28.81)	118.01 ± 5.32(116.73–119.29)	56.84 ± 8.74(54.73–58.93)
t		2.160	0.639	1.796
P		0.248	0.524	0.075

Table 2
serum AFP and free beta-HCG levels in two groups.

Group	n	AFP(MOM)	Free β-HCG (MOM)
Control	62	0.96(0.55–1.94)	0.84(0.37–3.48)
Hypospadias	69	1.14(0.65–3.40)	1.30(0.33–19.41)
z		2.831	3.131
P		0.005	0.004

3.2. ROC curve analysis of hypospadias predicted by AFP or free beta-HCG alone

As shown in Table 3, the AUC of hypospadias predicted by AFP was 0.644 (95% CI: 0.550–0.737, $P = .005$) and the AUC of hypospadias predicted by the free beta-HCG was 0.659 (95% CI: 0.565–0.752, $P = .002$). According to the ROC curve, the best cut-off values of AFP and free beta-HCG for screening fetuses with hypospadias were 0.945 MOM and 1.275 MOM, respectively. The sensitivity, specificity, and Youden index were 0.739, 0.484, and 0.223 for AFP, and 0.522, 0.790, and 0.312 for beta-HCG, respectively.

3.3. Combination of AFP and free beta-HCG to predict fetal hypospadias

The life cycle-like risk calculation method was used, as follows [10]:
Age equation [11]: $risk_{age} = 0.000627 + exp^{-16.2395 + 0.286*(age - 0.5)}$.
where $risk_{age}$ is the age risk value and age is the age at the expected date of confinement.

The likelihood ratio calculation is as follows: $LR_{multinorm} = \frac{\text{likelihood of hypospadias}}{\text{likelihood of controls}}$

The likelihood formula for a two-dimensional normal distribution was as follows:

$$f(x,y) = \frac{1}{2\pi\sigma_X\sigma_Y\sqrt{1-\rho^2}}\exp\left(-\frac{1}{2(1-\rho^2)}\left[\frac{(x-\mu_x)^2}{\sigma_X^2} + \frac{(y-\mu_Y)^2}{\sigma_Y^2} - \frac{2\rho(x-\mu_X)(y-\mu_Y)}{\sigma_X\sigma_Y}\right]\right)$$

where σis the standard deviation of the corresponding indicators, ρ is the correlation coefficient of the two indicators, and μ is the sample mean. X denotes the logarithm of the AFP MoM value and Y is the logarithm of the free-beta HCG MoM value.

The hypospadias risk value was calculated as follows:

$$risk_{hypospadias} = \frac{1}{LR_{multinorm} \times risk_{age}}$$

The AUC of hypospadias predicted by AFP together with the free beta-HCG was 0.700 (95% CI: 0.610–0.789, $P < .001$), with a sensitivity of 0.551 and a specificity of 0.855 (Table 3 and Fig. 1).

3.4. Comparison of different screening methods

The life cycle-like risk truncation schemes compared with the AFP MoM or free beta-HCG MoM single truncation scheme are shown in Fig. 1. The diagnostic value of the combined prediction of AFP and free beta-HCG for fetal hypospadias was higher than AFP or free beta-HCG alone.

4. Discussion

Hypospadias is a common congenital malformation of the male external genitalia that is characterized by an abnormal urethral opening to the penis, ventral scrotum, or perineum. Hypospadias is one of the common malformations of the human urogenital system. Hypospadias is difficult to diagnose in early and mid-pregnancy, and is mainly diagnosed after birth based on visual inspection. Therefore, there is an urgent need for the early diagnosis of hypospadias. At present, the combined detection of maternal serum AFP and free beta-HCG is widely used in prenatal screening of fetal trisomy 21 and 18 syndromes, open neural tube defects, abdominal fissures, and umbilical cord bulges. There are rare reports about screening for fetal hypospadias based on maternal serum AFP and free beta-HCG levels. [9] Therefore, this study mainly discussed the correlation and diagnostic value of AFP and free beta-HCG levels with fetal hypospadias.

Our results showed that the serum free beta-HCG MOM level in the hypospadias group was higher than the control group ($P < .05$). We thus concluded that the serum free beta-HCG level is related to hypospadias. The AUC of free beta-HCG screening for hypospadias was 0.659 (95% CI: 0.565–0.752, $P = .002$). When the best cut-off value of free beta-HCG was 1.275 MOM, the sensitivity, specificity, and Youden index were 0.522, 0.790, and 0.312, respectively. Therefore, we believe that the serum free beta-HCG is associated with hypospadias; however, Schneuer [12] showed that the serum free beta-HCG level in early pregnancy complicated by hypospadias in New South Wales was 0.88 (0.66–1.40) MOM compared with controls (0.92 [0.65–1.38] MOM, $P = .83$). In Western Australia, the serum free beta-HCG level was 0.84 (0.63–1.28) MOM in pregnant women with fetal hypospadias, while the control group was 0.88 (0.59–1.30) MOM ($P = .76$). There was no significant difference between the study and control groups (all $P > .05$). The results suggest that there is no correlation between maternal serum free beta-HCG levels and fetal hypospadias or cryptorchidism in early pregnancy, which is in contrast to our results. We thought that the gestational age of sampling in the Schneuer study [12] was earlier than the current study and this was a possible reason for the opposite result. We chose a gestational age ranging from 15 to 20^{+6} weeks because urethra formation was completed and the serum free beta-HCG was relatively stable during that time; furthermore, pregnant women undergo other prenatal screening during that period (thus reducing the frequency of blood withdrawals and medical visits).

The level of the AFP MOM in the hypospadias group was higher than in the control group ($P < .05$). The AUC of AFP screening for fetal hypospadias was 0.644 (95% CI: 0.550–0.737, $P = .005$). When the best cut-off value of AFP for screening hypospadias was 0.945 MOM, the sensitivity, specificity, and Youden index were 0.739, 0.484, and 0.223, respectively. Therefore, our study also indicated that AFP screening for fetal hypospadias was clinically meaningful. Previously, Papp [9] reported that only 12.8% (5/39) of fetuses with hypospadias were detected by maternal serum AFP MOM levels when the AFP (> 2.5 MOM) was taken as the cut-off value. In this study, if the AFP cut-off value was > 2.5 MOM, only 4 of 69 cases of hypospadias were diagnosed with a sensitivity of 5.80% (4/69), which was lower than the Papp findings [9]. Hence, localized AFP MOM truncation values are

Table 3
Value of AFP、free β-HCG in diagnosing Hypospadias.

Screening methods	AUC	95%CI	*P*	Critical value	Sensitivity	Specificity	Youden index
AFP	0.644	0.550–0.737	0.005	0.945	0.739	0.484	0.223
free β-HCG	0.659	0.565–0.752	0.002	1.275	0.522	0.790	0.312
AFP + free β-HCG	0.700	0.610–0.789	< 0.001	1/1018	0.551	0.855	0.406

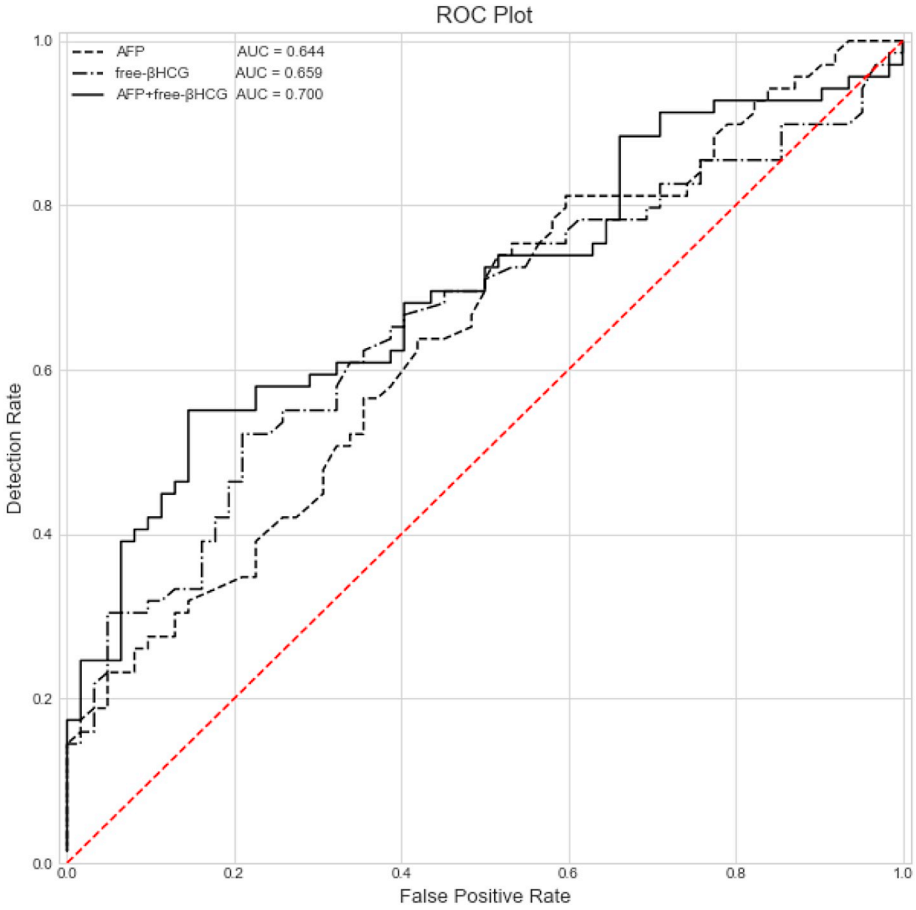

Fig. 1. ROC curve of three methods in diagnosis of hypospadias.

recommended to improve the sensitivity and specificity of AFP in predicting fetal hypospadias. We chose 0.945 MOM as the best cut-off value, and a relatively high sensitivity result was obtained.

Our results demonstrated that the highest AUC (0.700) for predicting fetal hypospadias was obtained when the AFP and free beta-HCG levels were combined, which was higher than the 0.644 and 0.659 for AFP and free beta-HCG alone, respectively. The sensitivity for predicting fetal hypospadias with AFP and free beta-HCG was 0.551, which was lower than 0.739 and higher than 0.522 of free beta-HCG, but the specificity was 0.855, which was higher than 0.484 of AFP and 0.790 of free beta-HCG. In 2009, Carlson [13] reported that advanced maternal age was associated with increased severity of hypospadias. In 2016, Carlos [14] reported that maternal age is a risk factor for hypospadias. Therefore, we believed the maternal age is associated with the risk of fetal hypospadias. In our study, based on maternal age, combining serum AFP and free beta-HCG gave rise to a higher AUC, specificity, and a moderate sensitivity. Thus, we concluded that during gestational age from 15 to 20^{+6} weeks, when the proper cut-off value was used, combining serum AFP with free beta-HCG to predict fetal hypospadias has better diagnostic value in south China.

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Disclosure

No conflicts of interest are declared.

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