Am J Transl Res 2026;18(2):1103-1113
www.ajtr.org /ISSN:1943-8141/AJTR0166608

Original Article

Fetal echocardiography combined with serum biomarkers for early diagnosis of congenital heart disease and birth defect risk prediction

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Received June 5, 2025; Accepted September 26, 2025; Epub February 15, 2026; Published February 28, 2026

Abstract: Objective: To evaluate the significance of fetal echocardiography in the early diagnosis of congenital heart disease (CHD) and its use as an early warning system for birth defects when combined with maternal serum biomarkers. Methods: A retrospective study was conducted at the Maternal & Child Care Center of Qinhuangdao between 2022 and 2025. A total of 260 pregnant women with suspected fetal cardiac abnormalities who underwent prenatal examinations were included. Based on postnatal diagnosis, participants were divided into a CHD group (n=41) and a non-CHD group (n=219). Diagnostic efficacy was compared between the four-chamber view with cranial deviation (FCV-CD) alone and FCV-CD combined with sequential segmental analysis (SSA). Maternal serum β-human chorionic gonadotropin (β-hCG) and N-terminal pro-B-type natriuretic peptide (N-proBNP) levels were analyzed, as well as their correlations with CHD. The predictive performance of single diagnostic strategy and combined strategies (ultrasound + biomarkers) was also evaluated. Results: Combining FCV-CD with SSA substantially improved CHD detection, attaining a significantly higher sensitivity. Compared to the non-CHD group, the CHD group showed notably lower maternal β-hCG levels and higher N-proBNP levels (both $P<0.001$). Logistic regression analysis confirmed significant correlations between β-hCG, N-proBNP, and CHD. FCV-CD combined with SSA demonstrated excellent diagnostic precision, and further integration with biomarkers increased specificity to 92.24%. Conclusions: The integration of FCV-CD and SSA significantly improved the diagnostic efficacy of fetal echocardiography for CHD. Incorporating maternal β-hCG and N-proBNP further improved diagnostic specificity, providing an effective early warning system for congenital heart defects.

Keywords: Echocardiography, sequential segmental analysis, congenital heart disease, four-chamber view with cranial deviation, serum biomarkers

Introduction

Congenital heart disease (CHD) is a prevalent birth defect, accounting for approximately one-third of all congenital malformations and representing a major cause of infant mortality and disability [1]. Despite advancements in perinatal medicine and cardiac surgery, early diagnosis of CHD remains challenging, as certain complex anomalies are only detected postnatally, thereby delaying timely intervention [2-4]. Improving prenatal detection and refining risk assessment for CHD have thus become critical clinical priorities.

Fetal echocardiography (FE) is the primary modality for prenatal CHD screening, given its high diagnostic accuracy [5]. The four-chamber view with cranial deviation (FCV-CD) allows preliminary assessment of cardiac symmetry and hemodynamics, although its standalone application may miss major arterial and conotruncal malformations [6]. In contrast, sequential segmental analysis (SSA) systematically evaluates atrioventricular and ventriculo-arterial connections, thereby improving the detection of complex CHD [7]. However, factors such as small fetal heart size, fetal position variations, and operator experience may still limit the reliability of ultrasound alone [8].

Recently, maternal serum biomarkers, such as β-human chorionic gonadotropin (β-hCG) and N-terminal pro-B-type natriuretic peptide (N-

https://doi.org/10.62347/YFPZ1807

proBNP), have gained increasing attention for their potential value in CHD risk prediction. Altered maternal serum levels in maternal serum of CHD pregnancies may reflect placental dysfunction or compensatory fetal cardiac changes [9, 10]. However, the diagnostic value of combining these biomarkers with ultrasound remains to be further validated. Existing studies, both domestic and international, predominantly focus on ultrasound techniques or serum biomarkers as independent diagnostic approaches [11, 12], with limited evidence supporting optimized multimodal diagnostic strategies. In particular, further clinical validation is needed to improve diagnostic sensitivity and reduce missed diagnoses.

Accordingly, this study retrospectively evaluated the diagnostic performance of FCV-CD combined with SSA in prenatal CHD detection, while also incorporating maternal serum β-hCG and N-proBNP measurements. The aim was to establish a foundation for a multimodal prenatal early-warning system that integrates echocardiographic findings with serologic biomarkers for CHD.

Patients and methods

Study population

A retrospective analysis was conducted on 350 pregnant women with suspected fetal cardiac abnormalities who underwent prenatal examinations at our hospital between January 2022 and January 2025. After applying strict inclusion/exclusion criteria, 260 cases were ultimately enrolled. This study was approved by the Ethics Committee of the Maternal & Child Care Center of Qinhuangdao and complied with the World Medical Association Declaration of Helsinki.

Inclusion and exclusion criteria

Inclusion criteria: individuals who underwent prenatal echocardiographic screening (including FCV-CD and SSA); individuals who met the following diagnostic standards for suspected cardiac abnormalities: Structural anomalies including asymmetric four-chamber view (ventricular diameter ratio >1.5 or <0.6) and great vessel abnormalities; functional indicators including cardiomegaly (cardiac-thoracic ratio >0.33) and valvular regurgitation (jet length >5 mm); rhythm abnormalities including sustained tachycardia (>180 bpm) or bradycardia (<100 bpm) lasting ≥10 minutes; individuals with stable vital signs and the ability to comply with medical procedures; singleton pregnancy; complete follow-up data including both prenatal and postnatal echocardiographic results; availability of maternal serum β-hCG and N-proBNP measurements.

Exclusion criteria: contraindications for echocardiography; pre-existing cardiac diseases, chronic gynecologic conditions, or pregnancy complications; history of substance (alcohol) dependence.

Based on postnatal neonatal echocardiography results (interpreted independently by two blinded pediatric cardiologists), 41 cases with confirmed fetal CHD were assigned to the CHD group, while 219 cases served as controls. The specific screening and grouping process is shown in **Figure 1**.

Methods

Measurement of serum β-hCG and N-proBNP levels: Fasting venous blood samples (5 mL) were collected from pregnant women during prenatal visits at 15-21 weeks of gestation. After centrifugation at 3,000 rpm, serum was separated and stored at -70°C until analysis. Serum β-hCG levels (Wenzhou Kemiao Biological Technology Co., Ltd., Cat. No.: KME-Hu010775) and N-proBNP levels (Shanghai Jihe Biotechnology Co., Ltd. Cat. No.: JH-H10303) were measured using enzyme-linked immunosorbent assay (ELISA), strictly following the manufacturer's protocols.

Rationale for timing of assessments: Serum biomarkers were measured at 15-21 weeks of gestation, corresponding to the peak placental secretion phase of β-hCG and the period of early fetal cardiac adaptation reflected by N-proBNP. Fetal echocardiography was performed at approximately 24 weeks to ensure optimal visualization of cardiac structures. This interval aligns with standard prenatal screening protocols, where early biomarker assessment supports subsequent targeted imaging. Previous studies have demonstrated the predictive stability of these biomarkers during mid-gestation (15-24 weeks) for congenital anomalies [13].

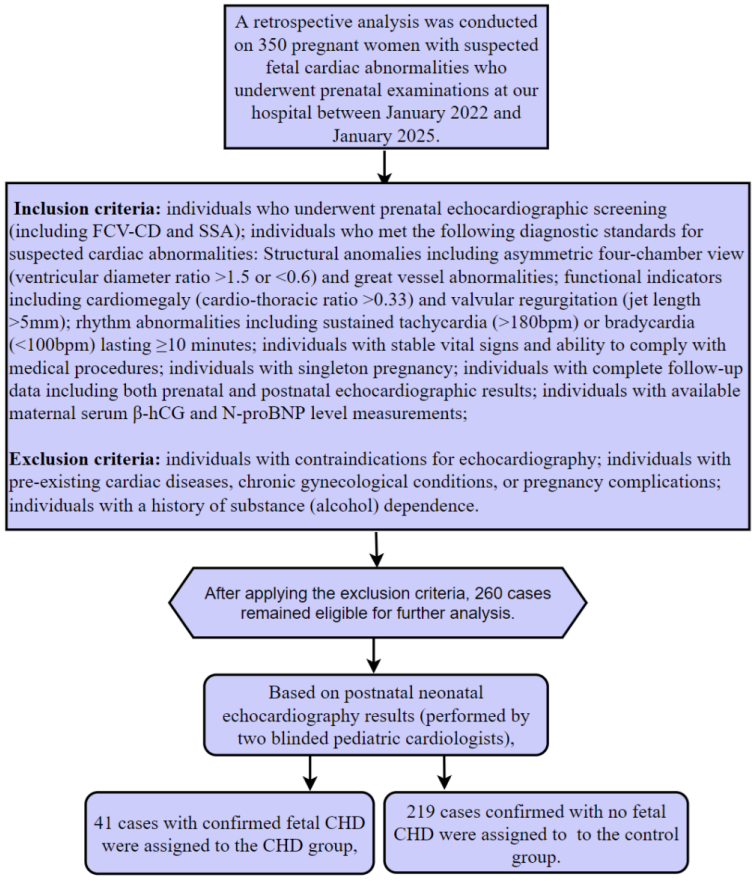

Figure 1. Screening and grouping process. Notes: CHD: congenital heart disease; FCV-CD: four-chamber view with cranial deviation; SSA: Sequential segmental analysis.

Echocardiographic examination [6, 14]: All examinations were performed using a Siemens Antares color Doppler ultrasound system equipped with an M3S cardiac probe (frequency 3-6 MHz; output power <120 mW/cm^2). Prior to examination, the room temperature was maintained at 26°C. Prior to scanning, health education and psychological counselling were provided to ensure maternal cooperation and improve compliance.

FCV-CD: The pregnant woman was positioned in the supine or lateral decubitus position. Following routine examination of the placenta and amniotic fluid, fetal cardiac evaluation began with an upper abdominal transverse section to establish anatomical orientation. The cardiac mode was then selected to determine cardiac position and apex orientation, with appropriate image magnification. The probe was gradually advanced from the fetal cephalic side with slight rotational adjustments to acquire right/left ventricular outflow tract views and three-vessel (including trachea) views. Upon image acquisition, the probe was rotated 90° to sequentially obtain short-axis views of the great arteries, the four-chamber view, and aortic/pulmonary arch views. In cases of suboptimal fetal positioning, the pregnant woman was instructed to ambulate under supervision until proper fetal positioning was achieved, after which ultrasound image acquisition was repeated.

Sequential segmental analysis (SSA): For cases with suspicious findings on initial four-chamber view screening, SSA was additionally performed. Image resolution parameters were optimized, and systematic assessment was conducted from the abdominal level upward to assess atrial morphology, visceroatrial relationships, ventriculoarterial alignments, and ventriculoarterial connections. Particular attention was paid to the development of semilunar valves and to the spatial relationships of the great arteries.

After both echocardiographic techniques were completed, all suspected CHD cases underwent comprehensive follow-up. Postnatal echocardiography was subsequently performed to confirm neonatal cardiac anatomy, allowing comparison of the diagnostic performance of FCV-CD alone versus its combination with SSA.

Data collection

Comprehensive clinical data were collected for all participants, including the maternal age, gestational age at ultrasound examination, body mass index (BMI) at the time of ultrasound examination, parity, comorbidities (e.g., diabetes mellitus and hypertension), place of residence, laboratory test results (β-hCG and

N-proBNP), and postnatal fetal diagnostic outcome.

Outcome measures

Primary outcome measures: (1) Categorical detection: Comparison of the diagnostic performance of FCV-CD alone versus FCV-CD combined with SSA for identifying different categories of fetal cardiac anomalies. (2) Single diagnostic modality performance: Evaluation of the screening efficacy of three independent modalities - maternal serum β-hCG, N-proBNP level, and fetal echocardiographic examination. (3) Multimodal diagnostic value: Investigation of the clinical application potential of a combined diagnostic strategy integrating echocardiography with serum biomarkers (β-hCG + N-proBNP).

Secondary outcome measures: (1) Baseline characteristics: Comparison of maternal characteristics between CHD and non-CHD groups, including age, gestational weeks, BMI, parity, history of diabetes mellitus, history of hypertension, and place of residence. (2) Serum biomarker levels: Comparison of maternal serum β-hCG and N-proBNP levels between CHD and non-CHD groups. (3) Logistic regression analysis: A multivariate logistic regression was adopted to explore the independent correlation between serum β-hCG/N-proBNP levels with fetal CHD risk. (4) Diagnostic efficacy: Using postnatal neonatal echocardiography as the golden standard, the diagnostic accuracy of FCV-CD alone was compared with FCV-CD + SSA for fetal CHD. Additionally, the performance of a multimodal diagnostic strategy integrating FCV-CD + SSA, β-hCG, and N-proBNP was evaluated using a multivariate Logistic regression model. The optimal predictive cut-off was determined by receiver operating characteristic (ROC) curve analysis (maximum Youden index), with cases classified as positive if their predicted probability exceeded the cut-off.

Statistical analyses

Data were analyzed using SPSS 20.0 (IBM Corp, Armonk, NY, USA), and figures were generated with GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA). Categorical variables were described as frequencies and percentages [n (%)], with between-group comparisons conducted using chi-square tests (χ^2). The normality of continuous variables was assessed using the Kolmogorov-Smirnov test, confirming that all continuous variables followed a normal distribution and were presented as mean ± standard deviation (SD); the between-group comparisons were conducted using independent-samples t-tests. Logistic regression analysis was employed to examine the associations between β-hCG/N-proBNP levels and CHD occurrence. The diagnostic value of single and combined diagnostic strategies (ultrasound + serum biomarkers) was evaluated using ROC curve analysis. The optimal cut-off value was determined by the maximum Youden index. A two-sided *P* value <0.05 was considered significant.

Results

Comparison of baseline data between CHD and non-CHD groups

Baseline characteristics of the study population are presented in **Table 1**. No significant differences were observed between the CHD (n=41) and non-CHD (n=219) groups. Specifically, mean maternal age was comparable (32.31±4.22 years vs. 31.64±5.23 years; P=0.892), as were gestational age at examination (24.33±2.98 vs. 24.75±2.22 weeks; P=0.296) and BMI (22.99±3.01 vs. 23.19±3.17 kg/m^2; P=0.704). Distribution of parity, comorbidities, and geographic factors also showed no significant variation between groups (all *P*>0.05).

Comparison of diagnostic performance between FCV-CD and FCV-CD + SSA for fetal CHD

The diagnostic efficacy of the FCV-CD alone versus its combination with SSA was systematically evaluated (**Table 2**). Using postnatal neonatal echocardiography as the reference standard, FCV-CD alone detected 27 of 41 CHD cases (sensitivity 65.9%), with 14 false negative cases. By contrast, the combined approach (FCV-CD + SSA) identified 37 cases (sensitivity 90.2%), demonstrating a 24.3% absolute increase in sensitivity (P=0.003) and a 71.4% reduction in false negative (from 14 to 4). The sensitivity improvement was primarily driven by anatomic complexities: double outlet right ventricle increased from 3 to 7 cases (+133%,

Table 1. Comparison of baseline data between CHD and non-CHD groups

	CHD group (n=41)	Non-CHD (n=219)	t/χ^2	P
Age (years)	32.31±4.22	31.64±5.23	0.137	0.892
Gestational age at ultrasound examination	24.33±2.98	24.75±2.22	1.048	0.296
BMI at ultrasound examination	22.99±3.01	23.19±3.17	0.380	0.704
Multiparas			1.645	0.199
Yes	18	120		
No	23	99		
History of hypertension			1.53	0.216
Yes	8	27		
No	33	192		
History of diabetes mellitus			1.500	0.221
Yes	10	36		
No	31	183		
Place of residence			0.503	0.478
Urban areas	20	120		
Rural areas	21	99		

Notes: CHD: congenital heart disease; BMI: body mass index.

Table 2. Comparison of diagnostic performance between FCV-CD alone and FCV-CD + SSA for fetal CHD

Diagnostic Metric	FCV-CD alone	FCV-CD + SSA	Difference (95% CI)	*P*-value
Sensitivity	65.9% (27/41)	90.2% (37/41)	+24.3% (8.1-40.5%)	0.003
Specificity	88.6% (194/219)	91.8% (201/219)	+3.2% (-1.5-7.9%)	0.18
False negative cases	14	4	-10	-
False positive cases	25	18	-7	-
PPV	64.3% (27/42)	80.4% (37/46)	16.10%	0.098
NPV	89.5% (194/217)	94.7% (201/212)	5.20%	0.042

Notes: FCV-CD: four-chamber view with cranial deviation; SSA: sequential segmental analysis; CHD: congenital heart disease; PPV: Positive Predictive Value; NPV: Negative Predictive Value.

P=0.031), tricuspid regurgitation from 4 to 7 cases (+75%, P=0.025), and hypoplastic left heart syndrome from 5 to 8 cases (+60%). No detection difference was observed for atrioventricular septal defect (5 vs. 5 cases) or pooled rare anomalies (10 vs. 10 cases combining atrial septal aneurysm, aortic valve stenosis, tetralogy of Fallot and endocardial cushion defect). Details can be found in **Table 3**.

Among the 219 non-CHD cases, the combined strategy showed a marginally higher specificity (91.8% vs. 88.6% for FCV-CD alone; P=0.18), though this difference did not reach statistical significance. Notably, the combined approach also reduced misdiagnosed cases by 28% (from 25 to 18) and improved both positive predictive value (PPV: 64.3% to 80.4%) and negative predictive value (NPV: 89.5% to 94.7%).

Comparison of serum β-hCG and N-proBNP levels

The CHD group demonstrated significantly lower maternal serum β-hCG levels (0.64±0.25 mIU/mL vs. 1.04±0.29 mIU/mL, P<0.001) but notably higher N-proBNP levels (107.65±15.79 ng/L vs. 89.50±8.87 ng/L, P<0.001) compared to the non-CHD (**Figure 2**; Supplementary Table 1).

Associations between serum β-hCG/N-proBNP levels and fetal CHD

Logistic regression analysis was conducted with fetal CHD status as the dependent variable (1= present, 0= absent) and β-hCG and N-proBNP levels as independent variables. Both biomarkers were significantly associated

Table 3. Comparison of detected CHD type between FCV-CD alone and FCV-CD + SSA (n=41)

CHD Classification	FCV-CD alone	FCV-CD + SSA	Absolute Increase	Relative Increase	*P*-value
Total Detected Cases	27	37	+10	+37.0%	0.002
Atrioventricular septal defect	5	5	0	0%	1.000
Double outlet right ventricle	3	7	+4	+133%	0.031
Hypoplastic left heart syndrome	5	8	+3	+60%	0.125
Tricuspid regurgitation	4	7	+3	+75%	0.025
Other complex CHD	10	10	0	-	-

Notes: For subgroups with baseline detection ≥3 cases, Fisher's exact test was performed. CHD: congenital heart disease; FCV-CD: four-chamber view with cranial deviation; SSA: Sequential segmental analysis.

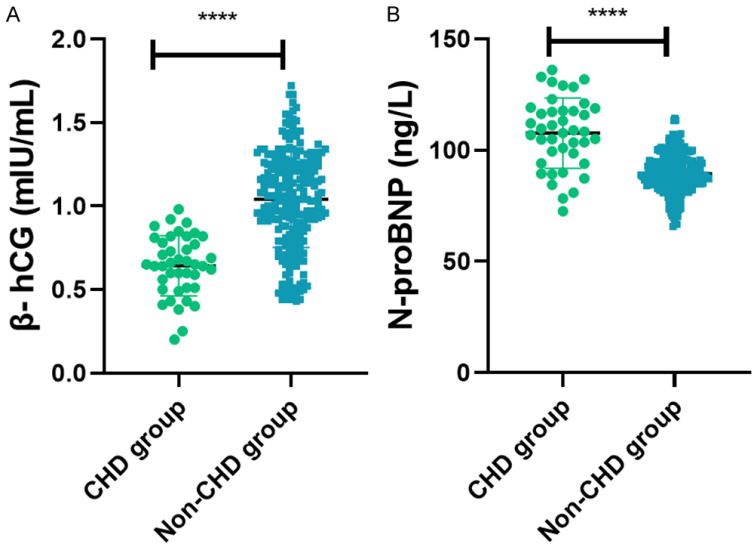

Figure 2. Comparison of serum β-hCG (A) and N-proBNP (B) levels between CHD and non-CHD groups. Notes: ****P<0.0001. CHD: congenital heart disease; β-hCG: beta-human chorionic gonadotropin; N-proBNP: N-terminal pro-B-type natriuretic peptide.

with fetal CHD. Lower β-hCG levels were inversely correlated with CHD risk (OR=0.046; 95% CI: 0.011-0.194; P<0.01), while N-proBNP levels were positively correlated with CHD risk (OR=1.071; 95% CI: 1.039-1.104; P<0.001) with fetal CHD (**Table 4**).

Diagnostic performance of individual and combined approaches using β-hCG, N-proBNP, and echocardiography (FCV-CD) for fetal CHD

The combined diagnostic strategy integrated echocardiographic findings (FCV-CD + SSA), β-hCG, and N-proBNP. The optimal cut-off probability was 0.42 determined by ROC curve analysis using the maximum Youden index (0.898). Cases with predictive probabilities that exceeded this threshold were classified as positive.

For individual serum biomarkers, optimal cutoff values were also determined. β-hCG: 0.82 mIU/mL (sensitivity 85.37%, specificity 65.37%, Youden index 0.507); N-proBNP: 98.5 ng/L (sensitivity 53.66%, specificity 92.21%, Youden index 0.459). These thresholds, summarized in **Table 5** and illustrated in **Figure 3**, provide quantitative references for distinguishing fetal CHD from non-CHD pregnancies.

As shown in **Figure 3** and **Table 5**, echocardiography alone demonstrated strong diagnostic efficacy, with sensitivity of 90.24%, specificity of 92.21%, and an AUC of 0.912 (0.872-0.943). The combined diagnostic strategy achieved the best overall performance, with sensitivity of 97.56%, specificity of 92.24%, accuracy of 93.08%, and an outstanding AUC of 0.971 (0.944-0.988). This combined approach demonstrated significant improvements over all individual methods (vs. β-hCG: ΔAUC=0.195, P<0.001; vs. N-proBNP: ΔAUC=0.239, P< 0.001; vs. echocardiography: ΔAUC=0.0589, P=0.001).

Discussion

Congenital heart disease (CHD) is a leading cause of infant mortality and morbidity [15], and early diagnosis followed by timely intervention can substantially improve clinical outcomes [16]. Despite advances in prenatal imaging technologies, particularly fetal ultra-

Table 4. Associations between serum β-hCG/N-proBNP levels and fetal CHD

	B	S.E.	Wals	df	Sig.	Exp (B)	95% C.I. For EXP (B)	
							Lower limit	Upper limit
β-hCG	-3.080	0.735	17.570	1	<0.001	0.046	0.011	0.194
N-proBNP	0.069	0.016	19.307	1	<0.001	1.071	1.039	1.104

Notes: β-hCG: beta-human chorionic gonadotropin; N-proBNP: N-terminal pro-B-type natriuretic peptide; CHD: congenital heart disease.

Table 5. Diagnostic performances of individual and combined approaches using β-hCG, N-proBNP, and echocardiography

Metric	β-hCG	N-proBNP	Echocardiography	Joint
Cutoff value	0.82 mIU/mL	98.5 ng/L	-	-
Sensitivity	85.37%	53.66%	90.24%	97.56%
Specificity	65.37%	92.21%	91.81%	92.24%
Accuracy	68.38%	85.29%	91.54%	93.08%
AUC (95% CI)	0.776 (0.722-0.824)	0.732 (0.675-0.784)	0.912 (0.872-0.943)	0.971 (0.944-0.988)
ΔAUC (vs. joint)	-0.195	-0.239	-0.0589	-
P-value for AUC comparison (vs. joint)	<0.001	<0.001	0.001	-

Notes: β-hCG: beta-human chorionic gonadotropin; N-proBNP: N-terminal pro-B-type natriuretic peptide; ROC: Receiver operating characteristic curve; AUC: Area under the curve.

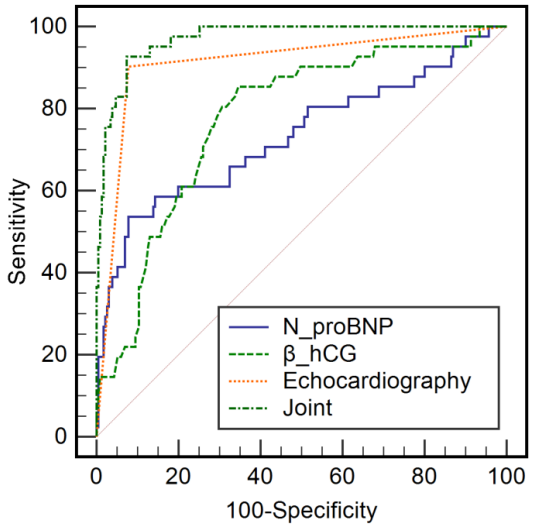

Figure 3. ROC curves for individual (β-hCG, N-proBNP, echocardiography) and combined diagnostic approaches for fetal CHD. Notes: ROC: Receiver operating characteristic; β-hCG: beta-human chorionic gonadotropin; N-proBNP: N-terminal pro-B-type natriuretic peptide; CHD: congenital heart disease.

sound and echocardiography, the sensitivity and specificity of these modalities, when used alone, still have notable limitations [16-19]. This study investigated the diagnostic value of fetal echocardiography for early CHD detection and evaluated the clinical utility of combining echocardiographic findings with serum biomarkers for birth defect early warning.

Our study demonstrated that the integrated echocardiographic approach combining FCV-CD and SSA significantly improved CHD detection sensitivity from 65.9% (standalone FCV-CD) to 90.2%, with a 24.3% absolute increase, while maintaining high specificity (91.8%). This combined strategy reduced missed diagnoses by 71% (from 14 to 4 cases) and improved both PPV (64.3% to 80.4%) and NPV (89.5% to 94.7%; P=0.042). These results are consistent with the findings of Narasimhan *et al.* [20], who demonstrated that advancements in imaging technologies, particularly when combining multiple views, have enhanced diagnostic accuracy. While also providing a robust evidence base for developing personalized treatment strategies and improving clinical outcomes. The results of the current study also align with findings from Sabatino et al. [21], who emphasized the critical role of multi-view integration for accurate assessment of right heart structures. Although our study employed a two-dimensional echocardiographic approach rather than three-dimensional techniques, the synergistic effect of FCV-CD and SSA similarly achieved a significant improvement in diagnostic sensitivity (+24.3%). This finding is consistent with the literature's assertion that "a single imaging plane cannot comprehensively display the tricuspid valve apparatus". The concordance between our observations and these previous findings underscores the universal value of

adopting multiple complementary views in cardiac imaging, whether through advanced 3D modalities or optimized 2D protocol combinations, particularly for complex structural evaluations in CHD.

While advances in imaging technologies have improved structural assessment, complementary serum biomarkers may provide additional functional insight. Notably, the FCV-CD and SSA combination showed superior detection for specific CHD subtypes, with significant improvements for double outlet right ventricle and tricuspid regurgitation, underscoring its value for complex anomalies. This advantage may be attributed to SSA, which systematically evaluates the structural segments of the heart and their connections, thereby compensating for the limitations of the Four-Chamber View alone in diagnosing complex malformations. As pointed out by Mozumdar et al. [22], advancements in ultrasonography have substantially enhanced the comprehensiveness and accuracy of fetal cardiac structural assessments. These results align with the systematic segmental evaluation provided by SSA, which addresses the limitations of using four-chamber views alone [23]. However, four CHD cases remained undetected, which may be attributed to: (1) the late gestational onset of certain cardiac anomalies, which only become hemodynamically significant in later pregnancy stages [24]; and (2) operator-dependent factors, such as suboptimal fetal positioning or limited acoustic windows, which can compromise image quality [25]. These missed cases highlight the biological and technical constraints of current prenatal screening paradigms, reinforcing the need for serial sonographic evaluations in high-risk pregnancies and the potential complementary role of emerging technologies like fetal echocardiography or circulating biomarker panels, to enhance detection sensitivity

In serum biomarker analysis, the CHD group exhibited significantly lower β-hCG and higher N-proBNP levels, a finding consistent with prior research on its role in cardiac stress [26]. Logistic regression confirmed both biomarkers as independent predictors of CHD. The observed β-hCG reduction reflects placental dysfunction in CHD, where abnormal fetal hemodynamics impair syncytiotrophoblast function, evidenced by histologic findings of decreased villous vascular density and trophoblast apoptosis [27]. Conversely, elevated N-proBNP level directly reflect fetal cardiac stress, with structural anomalies like outflow tract obstructions mechanically activating myocardial NPPB gene transcription by cAMP pathways [28], and these levels correlate with CHD severity. This dual biomarker pattern captures both placental malperfusion (β-hCG) and compensatory cardiac adaptation (N-proBNP), thereby offering supplementary pathophysiologic perspectives. The findings align with the large-scale study conducted by Alanen et al. [29], which reported notable decreases in first-trimester PAPP-A and β-hCG levels among various severe CHD subtypes. Both studies support the "placenta-heart axis" hypothesis, connecting abnormal placental secretory proteins (PAPP-A/β-hCG) with CHD. However, in this investigation, biomarkers alone were less effective than echocardiography, thereby reinforcing the notion that biomarkers should be used as supplementary rather than primary diagnostic tools [30].

The combination of echocardiography and biomarkers demonstrated the highest diagnostic effectiveness, characterized by a sensitivity of 97.56%, specificity of 92.24%, and an AUC of 0.971. Unlike simple parallel (any positive) or serial (all positive) testing, this study weighted each diagnostic tool's contribution: ultrasound provided structural evidence, whereas biomarkers introduced biological insights. This approach enhanced sensitivity without excessively compromising specificity, making it clinically valuable by minimizing missed CHD cases without causing excessive false positives (and unnecessary interventions). These findings align with the multicenter study by Wie *et al*. [31], which reported an 85.9% detection rate for major CHD but only 34.5% overall detection rate with mid-trimester ultrasound screening, underscoring the limitations of isolated ultrasonographic screening. A meta-analysis of 859 CHD cases revealed that ultrasound detection alone had a sensitivity of only 76%, with extremely high heterogeneity (I^2=100%), emphasizing the need for combining non-invasive methods [31]. Similarly, our study demonstrated that the combination of β-hCG, N-proBNP, and ultrasound improved the diagnostic AUC to 0.971. Serum biomarkers, with their standardized testing advantages, provide an effective complement to ultrasound screening, particularly for resource-limited regions.

Clinically, these findings hold particular relevance for scenarios where ultrasound results are inconclusive or higher diagnostic specificity is required. Combining biomarker profiling with ultrasound can enhance diagnostic accuracy. Moreover, the reduction in false negatives likely offsets the costs associated with delayed CHD management, while fewer false positives could reduce unnecessary invasive testing.

This study has several limitations that should be considered when interpreting the results. The relatively small sample size and retrospective design may have limited the generalizability of our findings and introduced selection bias, particularly for rare cardiac anomalies. Additionally, the single-timepoint measurement of serum biomarkers precluded assessment of dynamic changes throughout gestation. We also could not systematically analyze all possible confounding factors or perform subgroup analyses by specific CHD types. Most importantly, while the protocol demonstrates strong diagnostic performance in this high-risk cohort with abnormal screening ultrasounds, its applicability to general populations with lower disease prevalence may be limited. These limitations underscore the need for validation in larger prospective studies that incorporate longitudinal biomarker assessment and more comprehensive confounding factor analysis.

Conclusion

An integrated ultrasonographic approach, combining FCV-CD and SSA, represents a reliable diagnostic method for fetal CHD, with serum β-hCG and N-proBNP measurements serving as valuable adjunctive tools. This multimodal screening strategy provides a novel paradigm for early CHD detection, enabling intervention to improve fetal outcomes.

Acknowledgements

This work was supported by Medical Science Research Project in Hebei Province (20251270).

Disclosure of conflict of interest

None.

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Supplementary Table 1. Comparison of serum β-hCG and N-proBNP levels between CHD and non-CHD groups

	CHD group (n=41)	Non-CHD (n=219)	t	P
β-hCG (mIU/mL)	0.64±0.25	1.04±0.29	8.543	<0.001
N-proBNP (ng/L)	107.65±15.79	89.50±8.87	10.410	<0.001

Notes: CHD: congenital heart disease; β-hCG: beta-human chorionic gonadotropin; N-proBNP: N-terminal pro-B-type natriuretic peptide.