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**AN OBSERVATIONAL STUDY ANALYZING THE TREND OF CARDIOLOGIST DRIVEN FETAL ECHOCARDIOGRAPHY IN INDIAN SCENARIO WITH A COMPARISON OF PRENATAL VERSUS POSTNATAL FINDINGS: LESSONS LEARNED DURING A LONGITUDINAL ASSESSMENT OF FETAL CARDIA.****Cardiology**

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ABSTRACT

Background and aims: Fetal echocardiography stands as the diagnostic pivot for serial assessment of cardiovascular anatomy and hemodynamics of fetus from first trimester to postnatal life. It offers high sensitivity in accurate detection of cardiac development and lesions during intrauterine life. The gold standard remains as postnatal or autopsy validation of antenatal findings. Our objective was data analysis of the factors determining referral pattern to pediatric cardiologist, confirmation of the diagnosis and appropriateness of parental counseling and pregnancy management strategies. **Methods and results:** an observational study retrospectively reviewed all pregnant women reporting for fetal echocardiography to tertiary pediatric cardiac center from February 2021 to July 2023. Relevant factors for referral to pediatric cardiology were tabulated. Fetal echocardiography protocol was adapted from American Institute of Ultrasound in Medicine guidelines with Extended Cardiac Echocardiographic Examination protocol. Fetal echocardiographic results were categorized into simple, severe and functional defects. The fetuses were reviewed postnatally by neonatal echocardiography. Total of 330 pregnant ladies reported for fetal echocardiography in our department, 268 (81.2%) of them were referred cases. The most common reasons for referral were intra-cardiac echogenic foci (ICEF) in 87 (32.5%) cases followed by suspected congenital heart disease (CHD) in 80 (29.9%), maternal illness in 39 (14.6%), extra-cardiac anomaly 22 (8.2%), family history of CHD 18 (6.7%), fetal arrhythmia 11 (4.1%), isolated valve regurgitation seven (2.6%), assisted reproduction three (1.1%) and cardiac mass in one (0.3%) case. We detected cardiac abnormality in 113 (34.2%) fetuses. Of those, 55 (48.7%) had severe CHD, 32 (28.3%) had simple defect and 26 (23%) had functional heart defect. 70 (62%) were born with congenital heart disease; of which 15 (21.4%) were operated at the appropriate postnatal age. **Conclusions:** fetal echocardiography by pediatric cardiologist delivers additive diagnostic value and facilitates appropriate parental counseling from mid gestation, helps in proper perinatal triaging and continued cardiac care from fetus- to newborn.

KEYWORDS

Birth Defect, Congenital Heart Disease, Fetal Echocardiography, Prenatal Ultrasound

INTRODUCTION

The domain of pediatric cardiology underwent giant transformations in diagnosis, anatomical and hemodynamic analysis and treatment starting from 20th century. The pioneers including Maude Abott, Einthoven, Robert Gross, Helen Taussig, Richard Van Praagh, Robert Anderson and innumerable clinicians adopted clinical observations, cardiac autopsy, animal studies, cardiac catheterizations and surgical outcomes to understand congenital heart disease (CHD). Concurrently, echocardiography progressed to permit non-invasive study of the heart. Inge Edler (physician) and Hellmuth Hertz (physicist) collaborated to develop the first cardiac ultrasonic in Lund in 1923 [1]. Assessment of the fetal cardia was always the 'holy grail' to decrease the impact of CHD. Obstetrician Ian Donald and engineer Tom Brown developed the first such prototype and introduced ultrasounds in pregnancy in 1956 in Glasgow [2].

Before the epoch of echocardiography, diagnosis of CHD in children depended primarily on tripod of clinical examination, ECG, and chest x-ray. For final validation, cardiac catheterization, surgery and autopsy were the standard available modalities [3]. During the previous four decades, echocardiography emerged as the first line diagnostic modality with high sensitivity and specificity for screening, diagnosing and triaging CHD in both antenatal and postnatal life. [4]. This consequently led to increased survival of CHD and successful transition to adulthood with emergence of a novel cohort of adult congenital heart disease (ACHD) accomplished by simultaneous developments in the fields of cardiothoracic surgery, anesthesia, and super-specializations in pediatric cardiology [3,4]. Prenatal ultrasounds especially TIFFA (Targeted Imaging for Fetal Anomalies) scan between 18 -22 weeks of gestation followed by fetal echocardiography at 22 -24 weeks offered a formidable tool for diagnosis of fetal CHD with reported high sensitivity, specificity and a diagnostic accuracy of 99.82% from Indian studies in low- and middle-income countries (LMIC) setups [5]. Although not foolproof, both false -positives and false -negatives were diagnosed when compared with postnatal echocardiography and certain lesions were missed

altogether [4,5]. Major reasons for such disparities are attributed to lack of appropriate instruments, transducer frequency, expertise of examiner, tertiary versus remote centers, maternal obesity, abdominal scar, gestational age, fetal position and amniotic fluid volume [4]. Thus, fetal echocardiography stands as the pillar supporting fetal cardiac morphological and physiological appraisal in utero to delivery. The physician must be aware of its variable sensitivity in different gestational ages, different risk levels and different echo views [4,6]. Consequently, prenatal counseling, customized care of individual pregnancy, delivery planning, engaging the perinatal team in high-risk cases ensures a smooth transition from in-utero to ex-utero environment improves fetal survival and morbidity. In limited scenarios, the option of termination may be discussed with families in LMIC setups in critical fetal CHDs with poor long -term prognosis and treatment outcomes [7]. Our objective was data analysis of the factors determining referral pattern to pediatric cardiologist for fetal echocardiography, confirmation of the diagnosis, appropriateness of parental counseling and pregnancy management strategies, and impact of counselling on outcome.

METHODS

A retrospective review of electronic medical database of all pregnant women referred for fetal echocardiography and/or self- referrals at a tertiary pediatric cardiac center and its outreach clinic from February 2021 to July 2023 was performed. Fetal echocardiography was done by an experienced pediatric cardiologist using Philips ultrasound machine EPIQ CVx 9.0.1 with Transducer frequency 4-6 MHz following the previously described guidelines and protocols [6].

The indications for fetal echocardiography and the final result after fetal echocardiography were collected. Detailed counseling was done considering the final diagnosis and as per the severity of lesion. Management plan and pregnancy outcomes were explained. With a written therapeutic protocol, they were referred back to treating obstetricians. Serial fetal echocardiography was advised in severe and functional CHD; the remainder were followed up with post natal echocardiography.

The fetal cardiac lesions were categorized into structural and functional defects. Functional defects were defined as cardiac involvement in absence of structural heart diseases (examples valvar regurgitation, ductal restriction, arrhythmias, chamber asymmetry and cardiomegaly, pericardial effusion etc). Structural defects were further divided into simple versus severe defects according to complexity of CHD. Severity was graded as simple when score was ≤ 3 and severe when score ≥ 3 [8].

Statistical analysis

Data were analyzed by using IBM SPSS 25 version. Quantitative data were expressed as mean and standard deviation, whereas qualitative data were expressed as frequency and percentage. Chi-square Pearson's test was used for categorical variables.

RESULTS

Total 330 pregnant women underwent fetal echocardiography during the study period. Maternal age at time of echocardiography was 28.9 ± 4.6 years (mean $\pm$ SD, range 19 - 52 years). The gestational age at time of scan was 25 ± 4.2 weeks (mean $\pm$ SD; range 16 to 38 weeks). 62 (18.8%) women came for routine fetal echocardiography whereas 268 (81.2%) were referred to pediatric cardiologist post abnormal USG/TIFFA scans.

The most common reasons for referral were intracardiac echogenic foci (ICEF) in anomaly scan in 87 (32.5%) followed by suspected CHD in 80 (29.9%) in the cohort. Additional reasons for referral were maternal illness in 39 (14.6%) : including gestational diabetes 15 (5.6%), hypothyroidism 12(4.5%), ANA positivity six (2.2%), bad obstetric history four (1.5%) , maternal COVID one, and fibroid uterine one. extra cardiac abnormalities in 22 (8.2%): including single umbilical artery eight (2.9%), polyhydramnios four (1.5%), pericardial effusion three (1.1%), diaphragmatic hernia two, nasal bone defect two, ascites one, brain ventriculitis one, and oligohydramnios one. Family history of CHD in 18 (6.7%) : all with affected siblings. Fetal arrhythmia in 11 (4.1%), isolated valve regurgitation in seven (2.6%), assisted reproduction (IVF) three (1.1%), and fetal cardiac mass in one case. Table 1.

Table 1: Baseline characteristic of the patients underwent for fetal echocardiography and indications

Variables	Mean $\pm$ SD or N(%)
Maternal age (Y) mean $\pm$ SD	28.96 $\pm$ 4.66
Gestational age (W) mean $\pm$ SD	24.94 $\pm$ 4.21
Routine fetal echo	62 (18.8)
Referral for fetal echo	268 (81.2)
Indication for referral fetal echo	
Echogenic foci	87 (32.5)
Suspected CHD	80 (29.9)
Maternal illness	39 (14.6)
Extra cardiac abnormality	22 (8.2)
Family H/o CHD	18 (6.7)
Fetal Arrhythmia	11 (4.1)
Isolated Valve regurgitations	7 (2.6)
IVF	3 (1.1)
Cardiac mass	1 (0.3)

N (%); number (percentage), Age (Y); years, age (W); weeks, echo; echocardiography, CHD; congenital heart disease, H/o; history of, IVF; in vitro fertilization.

In our study group, 217 (65.6%) fetuses had normal cardiac versus 113 (34.2%) fetuses with cardiac abnormalities. Severe fetal CHD was diagnosed in 55 (16.6%), simple in 32 (9.7%) and functional defects in 26 (7.9%), Table 2.

Tables 2: Number of heart defect recognized by fetal echocardiography and its distribution

Total abnormal heart (n=113)		
Severe defect 55 (48.7)	Simple defect 32 (28.3)	Functional defect 26(23)
AV canal defect 10(8.9)	Isolated VSD 21(18.6)	Isolated TR 12(10.6)
HLHS 8(7.1)	VSD+ TR 6(5.3)	Atrial ectopic rhythm 6(5.3)

DORV 7(6.1)	VSD+MR 1(0.9)	Ductal restriction 5(4.4)
TGA 6(5.3)	ASD secundum 1(0.9)	Pericardial effusion 2(1.8)
Pulmonary atresia 4(3.5)	Mild aortic stenosis 1(0.9)	Dilated all chamber with dysfunction 1(0.9)
Hypoplastic RV+TV 4(3.5)	Aneurysm IAS in LA 1(0.9)	
DILV 2(1.8)	Mesocardia with bicuspid aortic valve 1(0.9)	
TOF 2(1.8)		
Cardiac mass 2(1.8)		
Dysplastic TV 2(1.8)		
TA+VA discordance 2(1.8)		
Coarctation of Ao 1(0.9)		
Aortic stenosis 1(0.9)		
Restricted PFO 1(0.9)		
Single ventricles 1(0.9)		
Idiopathic atrial calcification 1(0.9)		
AV dissociation+3-degree block 1(0.9)		

N(%); number percentage, AV; atrioventricular, HLHS; hyoplastic left heart syndrome, DORV; double outlet right heart, TGA; transposition of great artery, RV; right ventricle, TV; tricuspid valve, DILV; double inlet left heart, TOF; tetralogy of fallot, VA; ventriculoarterial, Ao; aorta, PFO; patent foramen ovale, AV; atrioventricular, VSD; ventricular septal defect, TR; tricuspid regurgitation, MR; mitral regurgitation, ASD; atrial septal defect, IAS; intra-atrial septal, LA; left atrium, LV; left ventricle.

Amongst the ICEF sub-group, 15 (17.2%) had cardiac abnormality; eight simple, two severe and five functional defects. (Figure 1).

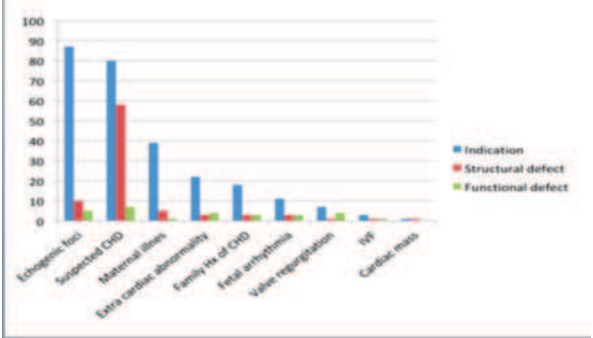


Figure 1. Detection of cardiac abnormality among the referral indication

CHD: congenital heart disease; Hx: history; IVF: in vitro fertilization

In the suspected CHD referrals, 65 (81.2%) were confirmed to cardiac lesions; 14 simple, 44 severe, and seven functional defects.

Within the maternal comorbidity group, six (15.3%) had abnormal fetal echo; four simple defects (all VSD), one severe and one functional defect. Likewise, 22 (8.2%) cases had simultaneous extra-cardiac abnormality; seven (31.8%) of this sub-group had cardiac abnormality; three simple and four functional defect.

18(6.7%) had history of CHD in previous child. With such history recurrence in next pregnancy in our cohort was six (33.3%); two simple defect, one severe and three functional defect.

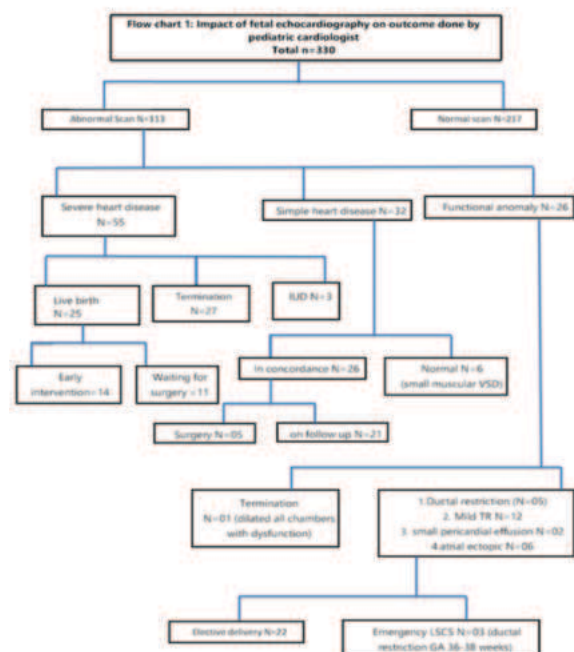
11 (4.1%) had fetal arrhythmia; In this faction, six (54.5%) had fetal heart defects; three severe and three functional defect.

Isolated valve regurgitation was documented in seven (2.6%) in which five (71.4%) has abnormality; one severe and four functional defects. Three (1.1%) IVF pregnancies were referred for fetal echocardiography; in such case one had functional defect. Remaining one cardiac mass had same finding.

The Pearson's chi-square test conducted using variable of maternal age ≥ 35 years and ≥ 35 years to normal versus abnormal, the p value was 0.037 at 95% confidence interval showed significant association between maternal age and fetal heart disease whereas no significant association existed between maternal age and types of defect, $p=0.183$ (P value is considered significant as $P \leq 0.05$).

All abnormal ($n=113$) fetal echocardiography were followed by postnatal echocardiography to validate the diagnosis.

Among the abnormal fetal echocardiography group, 28 (24.8%) pregnancies were terminated (all complex CHDs), five (4.4%) lost to follow-up and three (2.6%) intrauterine fetal death occurred (IUFD) (Idiopathic PA calcification, complete heart blocks and tricuspid dysplastic valve with hydrops fetalis). Figure 2 (flow charts).



N=number; UID: intra uterine death; VSD: ventricular septal defect; TR: tricuspid regurgitation; LSCS: lower segment cesarian section; GA: gestational age

Postnatal, total 70 (62%) of fetuses were born with CHD; of which 15(21.4%) had severe CHD necessitating surgical intervention after birth. six (5.3%) cases were false positive (VSDs spontaneous closure). The accuracy of fetal echocardiography compared with postnatal echocardiography had 100% sensitivity and 97% specificity with negative predictive value 100% and positive predictive value 92% (after excluding the cases lost to follow up, IUFD, termination of pregnancy). Table 3.

Table 3: Accuracy of fetal echocardiography

	POST NATAL		
	CVS DISEASE	NO CVS DISEASE	
CVS DISEASE	70	6	76
NO CVS DISEASE	0	217	217
	70	223	293

CVS: cardiovascular disease.

Sensitivity 100%,

Specificity = 97%,

Positive predictive value = 92 %,

Negative predictive value = 100%,

DISCUSSION

Clinical fetal echocardiography was practiced in India from 1980s and its high specificity and diagnostic accuracy was authenticated by several meta-analyses [4]. Prenatal ultrasound scan remains as the first indicator of suspicion of heart disease in fetus. The standard of care adopted in developed nations is confirmation by a pediatric cardiologist with incorporation of antenatal counseling. Due to heterogeneous socio-economic-legal factors, the majority of such fetuses in India are cared for by radiologists and fetal medicine specialists in antenatal life with obstetricians as primary care -givers [9]. As per literature, 67,385 babies are born in India every day [Key Data]. UNICEF India. Available from: <https://www.unicef.org/india/key-data>. The incidence of CHD is 0.9% live births. Correlating these two statistical data, it is obvious that most CHDs get missed during pregnancy. These children usually may represent major CHD group. The entire benefit of antenatal diagnosis of critical CHD is lost in such a scenario even in this third millennium AD.

In our cohort, where all the final fetal echocardiography was performed by expert pediatric cardiologist, 113 (34.2%) fetuses of total number of 330 pregnancies were confirmed to have fetal cardiac diseases; of which 48.7% were severe, 28.3% simple and 23% functional defects. The European registries showed overall prenatal detection rate of CHD as 25%, with France having the highest rate of 48% [10]. Another multicentric cohort study showed prenatal detection rate of fetal CHD 14.9-25.2% with major CHD detected in 28.8 - 53.6% and minor CHD 46.4 -71% respectively [11]. In few previous studies, up to 78% prenatal detection rate has been mentioned [4,9]. The differences in the detection rates of fetal CHD in other nations have been attributed to the variations secondary to different ethnicities [3]. This is in contrast to the on ground factors in India. The average gestational age at time of detection of prenatal CHD was 25 weeks with mean $\pm$ SD (25 ± 4.2) weeks and range 16 to 38 weeks, consistent with previous reports [4,9-11]. The optimum time for fetal echocardiography should be performed at 18 to 22 weeks of gestation, although certain types of CHD can be detected as early as 12 to 14 weeks of gestation [4,9,11].

The commonest indication for referral in our cohort was ICEF in 87 (32.5%) in anomaly scan, of which 15 (17.2%) fetuses had cardiac abnormality. In literature review, 2647 fetuses with ICEF were evaluated with an aneuploidy seen in three, cardiac anomalies in 3.7%. Interestingly, the prevalence of CHD was significantly higher in fetuses with right sided ICEF [12]. Hence, ICEF may not only be a soft marker for genetic anomaly in fetus but also an underlying cardiac lesion in few.

In this study, 80 (29.9%) women were referred for suspected CHD on prenatal USG which was confirmed in 65 (81.2%). This data correlates with similar researches on suspected CHD in antenatal USG where confirmation rate was 70% -71.7% after fetal echocardiography [11,13,14]. Incorporating all possible echo views and merging the knowledge of postnatal cardiology to fetal echocardiography, we observed that the indications for fetal heart screening were appropriate in cases where prenatal ultrasound accomplished good evaluation of fetal heart. Still it missed some cardiac defects [4,9]. In our study, positive cardiac finding was higher than those without cardiac abnormality found in prenatal anomaly scan.

American Heart Association (AHA) statement recommends referral to pediatric cardiologist if there are maternal morbidities, extra cardiac fetal abnormality and familial history of CHD [15]. In this study, we identified 39 (14.6%) cases were referred due to maternal illness out of which six (15.3%) fetuses had cardiac abnormality the common defect was VSDs in diabetic mothers. Consistent with previous report, in high- risk pregnancies especially maternal diabetes, the most common finding was VSD in 10 fetuses [11]. Whereas other reasons for referral were extra cardiac abnormality in 8.3%, family history of CHD 6.7% (all siblings), and fetal arrhythmia in 4.1% of which seven (31.8%), six (33.3%) and six (54.5%) fetuses were found to have cardiac defects respectively. The indications of referral were consistent with previous studies [7,13,14,15]. Survey from Brazil showed reasons for referral were maternal illness 81 (30%), extracardiac abnormality 39 (17%), history of CHD 26 (10%), and fetal dysrhythmia 10 (10.7%), of which fetal cardiac abnormality were found in 1.2%, 15%, 7.1% and 65.5% respectively [16]. Similarly, scientific statement from AHA also showed diabetes mellitus especially pre-conceptual diabetes was associated with nearly five-fold higher risk of fetal heart abnormality than normal population, history of sibling with CHD had 20-35% risk

of fetal cardiac malformation, Fetal CHD was more common in extra cardiac abnormality with an incidence rate of 20-45% and Bradyarrhythmia in fetus correlated with CHD was present in 55% in literature [15]. These finding were comparable to our study. However, in systematic reviews, superior diagnostic sensitivity for CHD in high risk selected populations was found during second trimester scan [9]. In this study, small number of pregnant women were referred due to isolated valve regurgitation seven (2.6%), IVF three (1.1%) and cardiac mass in one case, pattern consistent with other studies [4,9,10,11].

In this study 113 heart defects were identified, of which severe defects were found in 48.7%. They included complete atrioventricular (AV) canal defect in 8.9%, hypoplastic left heart (HLHS) in 7.1%, double outlet of right ventricle (DORV) in 6.1%, transposition of great artery (TGA) in 5.3%, pulmonary atresia (PA) with VSD in 3.5%, hypoplastic right ventricle in 3.5%, double inlet left ventricle (DILV), tetralogy of Fallot (TOF), cardiac mass and dysplastic tricuspid valve in 1.8% each. Similarly, 28.3% fetuses had simple defect, most common simple defect was VSD (18.6%). Functional defects were diagnosed in 26%. Their distribution was isolated tricuspid regurgitation in 10.6% followed by atrial ectopic in 5.3% and ductal restriction in 4.4% as demonstrated in table 2. These findings were similar to previous reports [13,14,16]. A large cohort, 249070 fetuses were evaluated, overall prenatal detection of CHD was 71.5%; the most commonly identified CHD was VSD in 21.3%, AV canal defect in 9.5%, HLHS in 7.8%, TGA in 7.7%, pulmonary atresia in 2.7%, tricuspid atresia in 1.6% [17]. Another large population based study done by QU et al. showed 70% of major congenital heart defect was detected prenatally, the most frequently diagnosed CHD was DORV in 81%, HLHS in 80%, TGA 58%, AV canal defect in 54%, PA in 56%, TOF in 50% and septal defect in 12% of fetuses CHDs [18]. These finding were higher than current study. The factor that may influence the prenatal detection rate is sample volume included in different studies and registries. Interestingly, prenatal detection rate of CHD varies significantly between countries even after using the same screening policies [10]. Further more, recent literature review meta-analysis showed using different scan protocols of fetal echocardiography provide great diagnostic potential and visibility of lesions [19]. Hence there is a possibility to increase prenatal detection rate.

Furthermore, diagnosis made by prenatal fetal echocardiography had perfect strength of agreement with postnatal CHD, high sensitivity and specificity. The detection rate of complex CHDs like TGA, DORV, HLHS, TOF, AV canal defect, septal defects and other abnormality of heart has highest rate of agreement with 100% negative predictive value and right heart abnormality has 99% negative predictive value that there is 1% chance of false negative result [20]. However, it also states that DORV and septal defects have higher rates of false positivity when compared to rest. We found less than one percent case of coarctation of aorta, mild aortic stenosis, restricted PFO, idiopathic arterial calcification, atrioventricular discordance, aneurysmal interatrial septum, mesocardia, single ventricle, thickened atrio-ventricular valve with ectopic rhythm. These findings were similar to previous study [4,7,9,11,13-15,17-20].

Undoubtedly the challenges in prenatal detection of CHD can be minimized with high sensitivity and specificity of fetal CHD, using totalitarian scan protocol; BCEE, ECEE, 4CV, OTV+3VT has highest diagnostic accuracy and provides more detail anatomical and functional of the fetal heart [4,9,19,20]. Consistent with the current study, the sensitivity and specificity of fetal echocardiography detecting CHD was 100% and 97% respectively with positive and negative predictive value was 92% and 100% respectively. As basic prenatal USG may miss significant fetal cardiac lesions, some researchers have advocated fetal echocardiography in every pregnancy [9,15]. This is nearly impossible in our nation where many pregnant women still lack access to basic medical care.

CONCLUSIONS

Data analysis of fetal cardiology practice in a pediatric cardiac tertiary center in national capital of India offered glimpses into current scenario prevailing in Northern India and ideas for redressal. The current study unequivocally concludes that fetal cardiac evaluation in India is working on a disorganized, chaotic fashion.

Instead by adopting Beckhard model of teamwork incorporating GRPI

(Goals, Roles, Procedures and Interpersonal relationships); a suspicious primary anomaly/TIFFA scan done by non-cardiologists can be evaluated subsequently through fetal echocardiography by trained pediatric cardiologists to offer satisfactory add-on diagnostic value and appropriate parental counseling and perinatal management. However, due to dearth of referrals and referees, we propose few simple strategies to lessen the burden of CHD in India. Firstly, societal awareness about CHD creation through effective use of media. Secondly, an all -inclusive perinatal cardiology team approach may overcome our lack of trained manpower. Suspected CHD in anomaly/TIFFA scan, high risk pregnancies, family history of CHD, advanced maternal age may be used as red flags for fetal referral to pediatric cardiologist. Earlier detection of fetal heart disease allows effective utilization of government welfare schemes and health insurance. Postnatal evaluation and longitudinal follow up in multiple centers across India will add to our scientific knowledge.

Limitations

The study was conducted at a tertiary center. Referral bias to pediatric cardiologist was present. Sample size in this study is low compared to our population size. As every fetus was referred back to it's primary obstetrician, there were variations in standards of care. Being an observational study, short to mid -term follow up data beyond immediate neonatal period is not uniform. Medical termination of pregnancy was decided by the family and obstetric team irrespective of severity scale scoring by pediatric cardiologist. The autopsy findings of terminated pregnancies were not available to the pediatric cardiologist.

We acknowledge that this study was designed to observe the patterns of referral for fetal echocardiography to pediatric cardiologist and whether this improved benefit to CHD in affected fetuses. We still do not have any hard data to compare accuracy between fetal echocardiography done by expert pediatric cardiologist and expert radiologist/fetal medicine specialist. However, a combined approach of imaging followed by post diagnostic counseling and postnatal echo was better when pediatric cardiologist was involved in our limited patient cohort.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethics

approved by medical ethics committee

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