

Study on the polymorphism of methylenetetrahydrofolate reductase C677T gene as the genetic predisposition of congenital heart disease in North Indian population



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ABSTRACT

Background: The etiology of congenital heart disease (CHD) is still not known properly. The variant alleles in the methylenetetrahydrofolate reductase (MTHFR) C677T gene help in elevating the serum homocysteine level which is an independent predisposing factor for generating CHD. **Aims and Objectives:** The aim of the present study was to analyze the role of polymorphism of MTHFR C677T gene polymorphisms in CHD patients of Varanasi, Uttar Pradesh, North India. **Materials and Methods:** The present study included 36 unrelated CHD patients along with their parents. At the same time, 40 healthy control samples were included in the study. MTHFR 677 C>T genotype was identified by polymerase chain reaction followed by restriction digestion restriction fragment length polymorphism mechanism. **Results:** There was a significant difference observed in MTHFR C677T gene polymorphism between the cases and controls ($P < 0.001$). Among the different etiological factors, increased maternal age, family history, and teratogens are the major ones. Maternal MTHFR C677T genotype and periconceptional folate intake have important ascription toward CHD formation. **Conclusion:** The results designate that MTHFR C677T polymorphism has a substantial association with the development and progression of CHDs. Folate supplementation might be the probable management strategy for reducing the risk of CHDs as maternal MTHFR C677T polymorphism has an important contribution.

Key words: Congenital heart disease; Homocysteine; Methylenetetrahydrofolate reductase; Polymorphism

INTRODUCTION

Congenital heart disease (CHD) refers to a group of problems associated with the structure of the heart and function due to abnormal heart development before the birth of a child. It is composed of several malformations in the heart including ventricular septal defect, atrial septal defects, tetralogy of Fallot, patent ductus arteriosus,

and transposition of greater vessels.¹ CHD is the most common birth defect that represents nearly one-third of all the congenital abnormalities and it poses a tremendous financial burden to the affected families.²⁻⁴ According to the American Heart Association, approximately 1% of infants (~40,000) born each year having this genetic anomaly in the United States.⁵ With a believed incidence rate of 8–12/1000 live births, nearly 180,000 children are born with CHD

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every year in India.^{6,7} According to a large population-based study, the incidence of CHD has increased over the past 60 years, from 1% to 9–10% in urban areas whereas in rural populations, the figure reaches around 4–6%.⁸ Out of these patients about one fifth suffers from critical CHD requiring early intervention. Studies revealed that approximately 10% of infant mortality in India is caused due to CHD. Although proper screening, diagnosis and optimum treatment strategies can save the life of 90% neonatal patients born with CHD.

The raised level of homocysteine (Hcy) in maternal blood and amniotic fluid was correlated with CHD in the embryo.^{9–11} A deficiency in folate, Vit B₁₂ or Vit B₆ may enhance the Hcy level which may impair the endothelial vasomotor function and damage coronary arteries. Periconceptional folic acid intake is supposed to reduce the risk of CHD in the newborn. Regardless of recent advancement in CHD diagnosis, experts are yet to fully understand the underlying causes of CHDs. Therefore, growing research interest has been generated to find out the genetic susceptibility factors to CHD. In recent years, considerable numbers of studies have been done on the polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene.^{12–14}

5,10-MTHFR gene (On-line Mendelian Inheritance in Man - OMIM accession number: 607093) is situated on the short arm of chromosome 1 (1p36.22).¹⁵ MTHFR enzyme converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate that acts as the methyl donor for remethylating of Hcy to generate methionine. Therefore, the proper function of MTHFR is essential for DNA synthesis, methylation, and maintenance of Hcy level. One of the polymorphisms in MTHFR is C677T which is a thermolabile variant with reduced activity. MTHFR C677T mutation at exon 4, leads to the alteration of the amino acid alanine to valine at position 226 in the protein.¹⁶ The most common mutated form of MTHFR is C677T, whereas the mutant allele TT can increase the probability of a high Hcy level.^{17–19}

The previous studies have been done for detecting the association of Hcy enzyme gene polymorphisms and CHD with contradictory results.^{20–22} Moreover, scanty reports are available from the Indian scenario as the majority of studies from Asia are confined to China, Japan, and the Sri Lankan population.^{23,24} Limited studies have been done from North, Western, and South India.^{25,26} This initiated us to detect the association of genetic polymorphisms of the Hcy gene.

Aims and objectives

The present study aimed to determine the etiology of the genes responsible for CHDs originating from North India

by investigating MTHFR (C677T) gene polymorphism for the development of CHD through a case–control study.

MATERIALS AND METHODS

Selection of study subjects

A total of 36 clinically diagnosed cases with CHDs were chosen from the outdoor, Department of Pediatrics, Sir Sunderlal Hospital, BHU, Uttar Pradesh, India. All the subjects were visited in the hospital from the surrounding rural areas of Varanasi city. Majority of the patients were of North Indian background. The diagnosis of non-syndromic CHD and classification of different types of cardiac defects had been performed by a pediatrician based on clinical and echocardiographic findings (Supplementary Table 1). A total of 40 age- and sex-matched healthy controls without any heart-related complications were taken for the study. The CHD parents were between the ages of 20 and 36 years. The average age of the CHD cases was 1.34 ± 0.53 years at the time of investigation.

Detailed family history, lifestyle, and socioeconomic conditions were collected from all the subjects. Their mothers were enquired to collect demographic data, such as childbearing age, history of CHD in the family, and drug exposure during pregnancy. The relevant biochemical parameters were noted from their medical reports.

Maintenance of ethical background

Informed written consents were collected from the parents of the affected children with CHD. In the same way, consent forms were also obtained from all the healthy controlled subjects enrolled in this investigation. The study has been conducted according to the Helsinki declaration. The research protocol was approved by the Institutional Ethics Committee ethical and research advisory committee of the institution for the final permission.

Clinical condition of the studied subjects

CHD is a structural defect inside the newly born baby since birth and it broadly divided into two types, acyanotic and cyanotic heart diseases. The clinical presentation mainly depends on type of lesion and age of the child. During this study, all clinically suspected baby of CHD were finally confirmed by 2 D Echocardiography. Presenting features of most of the child was fever, rapid respiratory rate, poor weight gain, inability to feed properly, excessive sweating during breastfeeding, bluish discoloration of lips or whole body, repeated chest infection, and rarely seizures and unconsciousness. Subsequent clinical examination revealed mostly following findings such as tachycardia, tachypnea, low pulse volume, hepatomegaly, signs of congestive heart failure, and

murmur on auscultation. Diagnosis was made clinically and with the help of relevant investigation.

Sample collection

4–5 ml of blood samples were collected in an ethylenediaminetetraacetic acid (EDTA) vacutainer. Sampling was done by an expert phlebotomist from both the CHD patients along with their parents and control subjects. Samples were preserved in -20°C freezer for future use.

Genomic DNA isolation

Genomic DNA was extracted from peripheral blood leukocytes using the modified salting out method. Precisely, 4–5 ml of blood samples were collected in the EDTA vacutainer. Fifteen milliliters of 0.9% NaCl were mixed with the blood sample in a polypropylene tube. After centrifugation at 5000 rpm for 5 min, Sucrose-MgCl₂ solution was added with the supernatant. Then successively, Tris- EDTA (pH 8.0) and NaCl treatment were carried on. Sodium dodecyl-sulfate (20%) was added and mixed properly. Chilled chloroform and sodium perchlorate (5M) were added sequentially. After that, centrifugation was done at 5000 rpm for 5 min. The aqueous layer was separated with a cut tip and transferred to another test tube. Finally, a double the amount of chilled isoamyl alcohol and chilled absolute alcohol were added to the aqueous layer. A viscous layer was generated in between and it was resuspended with cut tips. DNA appeared as thread-like solid substance and resuspended in nuclease-free water. It was stored at -80°C for future use.

Quantitative analysis of DNA by spectrophotometry

Absorbance based DNA quantification of nucleic acid was done with NanoDrop-1000 Spectrophotometer (Thermo Scientific; Thermo Fisher Scientific, Waltham, MA). For each sample, a duplication test was done. The ratio of sample absorbance at 260 nm and 280 nm is used to assess the purity of sample DNA (normal value ~ 1.8). A lower 260:280 ratio may indicate the presence of contaminants such as protein, phenol, and salts. With the reference to

nanodrop quantification results, we took 30 ng of DNA. In certain cases, we eluted the solution by adding distilled water to obtain the final concentration of 30 ng/ μl .

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to detect the polymorphism

To amplify the desired region of MTHFR at 677, PCR was carried out using Thermal Cycler (Veriti™ 96-well Thermal Cycler). Briefly, the PCR master mixture was prepared by adding 12.5 μl Taq Polymerase^{PM} (Qiagen PCR kit; Hilden, Germany), 1.25 μl of each forward and reverse primers (20 pmoles), and 8.0 μl of Dnase-Rnase free water. Two microliters of individual DNA samples were added in each PCR tube.

PCR products were digested at 37°C for 2 h by adding 1.0 μl restriction enzyme (RE) (10 units/ μl), 2.0 μl buffer, 18.0 μl Dnase-Rnase free water, and 10.0 μl of PCR product to make the final reaction volume of 25.0 μl . Each gene polymorphism was carried out using PCR with their corresponding primers, REs, PCR reaction mixtures and reaction conditions (Table 1). All the results were repeated twice in double-blind.

Agarose gel electrophoresis

The products of restriction digestion were observed in 3% agarose gel. The PCR amplified products and restricted fragments were visualized under ultraviolet light after staining with ethidium bromide. The size of the PCR and RE products was determined by comparing it with a 1 kb DNA ladder which was used as a DNA marker.

Statistical analysis

All the data and statistical analysis were done using Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics, v 25.0) software. Allelic frequencies were determined by gene counting methods. Hardy-Weinberg equilibrium was applied to the case group. Alleles and genotypes distribution was verified for deviation from the Hardy-Weinberg equilibrium by Chi-squared test. The

Table 1: The PCR reaction conditions and corresponding RE products for MTHFR C677T polymorphism

Gene polymorphism	Chromosomal position	Primers	PCR reaction conditions	PCR product	RE product
MTHFR C677T	1p36.22	Forward: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' Reverse: 5'-AGGACGGTGCGGTGAGAGTG-3'	Initial denaturation: 94°C , 8 min Denaturation: 94°C , 1 min Annealing: 63°C , 1 min Extension: 72°C , 1 min (40 cycles) Final extension: 72°C , 7 min	198 bp	HinfI WT: 198bp HT: 198, 175, 23bp MT: 175, 23bp

PCR: Polymerase chain reaction, RE: Restriction enzyme, MTHFR: Methylenetetrahydrofolate reductase, WT: Wild-type, HT: Heterozygous type, MT: Mutant type, sec: Seconds, Min: Minutes

odd-ratios (OR) for different association models were tested with 95% confidence interval (CI) and concurrently P-values were calculated. A value of $P < 0.05$ was considered as statistically significant. Pearson Chi-square test was conducted for testing the significance of observed interactions.

RESULTS

Socio-demographic characteristics of the studied subjects

Demographic data revealed that in certain cases, family history and drug exposure during early pregnancy directed to cause CHD among the infants. Most of the patients come from lower to middle- income socio-economic background with medium educational qualification. The details of the socio-demographic characteristics of the studied subjects are described in Table 2. We obtained 16 (44.4%) children in the age group ranging from 0 to 3 months which constitutes the majority of the studied subjects followed by eight (22.2%) children from 6 to 9 months of age. Among the studied CHD children, there were 55.6% male and 44.4% female. Varanasi is a city where most of the population belong to Hindu ethnicity. The studied subjects were mainly the residents of remote areas surrounding Varanasi. We obtained 61.1% of children with Hindu ethnicity.

The literacy rate and awareness about folate intake during pregnancy were not satisfactory among the mothers of CHD children (Table 2). We observed 18 (50%) mothers with primary educational qualifications among which 77.8% mothers were not aware of taking periconceptual folic acid intake. Out of the total 36 mothers of CHD subjects, 69.4% did not take folic acid supplements during their pregnancy (Table 3).

In the present study, 69.4% mothers had an average age >30 years, whereas 30.6% mothers had an average age <30 years. We observed the impact of some unhealthy lifestyle including drinking strong tea or coffee, smoking, tobacco chewing, and alcohol consumption. Nearly 33.3% mothers were reported to take strong tea or coffee during their pregnancy followed by passive smoking (19.4%). Nearly 19.4% cases were investigated to have family history of CHD (Table 4).

Polymorphism study in MTHFR gene

On digestion with Hinf I (New England Biolabs, USA) RE, produced 175 and 23 bp fragments for TT condition (homozygous polymorphic) and 198, 175, and 23 bp fragments for CT condition (heterozygous polymorphic). An undigested RFLP product length of 198 bp was retained by the wild type (Figure 1).

Association between MTHFR C677T polymorphism and CHD

Out of 36 CHD patients, we observed 14 were heterozygous type (CT) and four were homozygous mutant (TT) type. The

Table 2: Socio-demographic characteristics of children with congenital heart disease (n=36)

Age (months)	Total (%)	Male	Female
0–3	16 (44.4)	11	5
3–6	5 (13.9)	2	3
6–9	8 (22.2%)	3	5
9–12	7 (19.4%)	4	3
Total	36	20 (55.6%)	16 (44.4%)
Ethnicity	Frequency	Percentage	
Hindu	22	61.1	
Muslim	8	22.2	
Others	6	16.7	
Socio-economic class			
Upper class	0	–	
Upper middle class	3	8.3	
Middle class	5	13.9	
Lower middle class	12	33.3	
Lower class	16	44.4	
Maternal Education level			
Illiterate	6	16.7	
Primary	18	50	
Secondary	9	25	
Graduate	3	8.3	

Table 3: Association between maternal education and periconceptual folic acid

Maternal education	Periconceptual folic acid was taken	Periconceptual folic acid was not taken	Total (%)
Illiterate	2 (33.3)	4 (66.7)	6 (16.7)
Primary	4 (22.2)	14 (77.8)	18 (50)
Secondary	5 (55.6)	4 (44.4)	9 (25)
Graduate	2 (66.7)	1 (33.3)	3 (8.3)
Total	11 (30.5)	25 (69.44)	36 (100)

Pearson Chi-square=4.2, P=0.24, df=3

Table 4: Maternal risk factors and association with congenital heart disease

Maternal risk factors	Frequency	Percentage
Maternal age (in years)		
<30	11	30.6
>30	25	69.4
Unhealthy lifestyle		
Drinking strong tea or coffee	12	33.3
Passive smoking	7	19.4
Smokeless tobacco	5	13.9
Drinking Alcohol	2	5.6
Adverse condition during previous pregnancy		
Abortion	2	5.6
miscarriage	8	22.2
stillbirth	1	2.8
Family history	7	19.4

frequency of C allele in CHD patients was 0.89 and frequency of T allele was 0.50. In contrast, the frequency of C and T allele was 1.0 and 0.05, respectively, in control samples $P < 0.001$. The value is quite significant in case of association between polymorphism of MTHFR C677T with CHD. The genotype frequencies are enlisted in Tables 5 and 6.

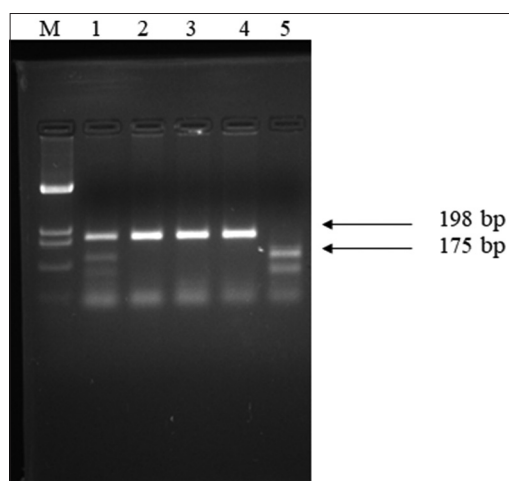


Figure 1: Polymerase chain reaction products for methylenetetrahydrofolate reductase C677T polymorphism and restricted fragments on 3% agarose gel electrophoresis. Lane M represents the DNA ladder, lane 1: heterozygous type (198 bp, 175 bp, 23 bp), lane 2, 3, 4: wild type (198 bp), lane 5: homozygous mutant (175 bp, 23 bp) fragments

Distribution of maternal MTHFR genotype

Out of 36 CHD patients, 12 (33.3%) had MTHFR polymorphism along with their mother ($P=0.20$). In contrast, among the control samples, in 85% cases both mother and baby were wild type ($P=0.15$). We got only six patients where both mother and baby were wild type for the MTHFR genotype (Table 7).

DISCUSSION

The ethnicity of the population is one of the most important characteristics for conducting genetic studies. The North-East Indian population is the admixture of several migrant residents and it has not been well studied for genetic polymorphisms in the etiology of CHD. Hyperhomocysteinemia is assumed as a major causative agent for CHD. The prevalence of hyperhomocysteinemia in Indian newborns is approximately 8.6%.²⁷ Hence, the study on genetic elements attributed to hyperhomocysteinemia is very relevant. Here, we have conducted the pilot study to analyze the influence of polymorphisms of MTHFR gene observed at 677 nucleotide position in the gene sequence among a study population of thirty-six CHD patients along with their parents as well as forty control subjects. We observed the polymorphic variants of the MTHFR gene

Table 5: Distribution of MTHFR genotypes and allele frequencies in the studied group

Subjects	Genotype frequencies			Allele frequencies	
	CC (%)	CT (%)	TT (%)	C (%)	T (%)
Case (n=36)	18 (50.0)	14 (38.9)	4 (11.1)	0.89 (0.025)	0.50 (0.014)
HWE	p (0.694)+q (0.306)=1				
Control (n=40)	38 (95.0)	2 (5.0)	0	1 (2.50)	0.05 (0.125)
HWE	p (0.975)+q (0.025)=1				

HWE: Hardy-Weinberg equilibrium (C vs. T: OR=0.09, 95% CI=0, 1349.61, $P > 0.05$; CC+CT vs. TT: OR=0.00, 95% CI=0, infinity, $P > 0.05$; CC vs. TT: OR=0.00, 95% CI=0, infinity, $P > 0.05$; CT vs. TT OR=0.00, 95% CI=0, infinity, $P > 0.05$; CC vs. CT+TT: OR=0.053, 95% CI=0.11, 0.253, $P < 0.001$), MTHFR: Methylenetetrahydrofolate reductase

Table 6: Distribution of MTHFR genotype in the studied subjects

Genotype	Subjects		Crude OR	P-value
	Case (n=36)	Control (n=40)		
CC	18 (50.0)	38 (95.0)	0.47	0.26
CT	14 (38.9)	2 (5.0)	7.00	0.06
TT	4 (11.1)	0	---	---
C allele frequency (%)	0.89 (0.025)	1 (2.50)	0.89	0.79
T allele frequency (%)	0.50 (0.014)	0.05 (0.125)	10.0	<0.001

MTHFR: Methylenetetrahydrofolate reductase, OR: Odds ratio

Table 7: Distribution of MTHFR polymorphism among the CHD patients and their mothers

MTHFR gene polymorphism	Subjects (% frequency)		P-value
	Case (n=36)	Control (n=40)	
Both mother and baby are wild type	18 (50.0)	34 (85.0)	0.33
Mother is wild and baby is polymorphic	6 (16.67)	2 (5.0)	0.15
Mother and baby both are polymorphic	12 (33.3)	4 (10.0)	0.20

MTHFR: Methylenetetrahydrofolate reductase, CHD: Congenital heart disease

were more predominant in CHD cases in comparison to control samples ($P < 0.001$).

In the present study, the majority of the patients were coming from lower-middle class families and the possible reason might be the easy accessibility of Government Hospital. Another probable explanation could be the early screening of pregnant mothers of higher socio-economic class using echocardiography. Most of the mothers of CHD patients came to know about the diagnosis during the postnatal period. It might be due to low education among the mothers. In the present study nearly 16.7% mothers were illiterate and the majority (50%) had educational qualification up to primary-school level (Table 3).

In the studied subjects, more than half (69.4%) of the mothers were >30 years of age; therefore, advanced maternal age can be a major risk factor. Special care and awareness should be taken when women become pregnant above 30 years of age. There were seven mothers who had family history of CHD whereas eight mothers had previous history of miscarriage. Antenatal screening is suggested for the mothers with previous abnormal history of pregnancy including recurrent miscarriage, abortion, and preterm pregnancy.

Lifestyle of the pregnant mothers also plays a key role in the development of the fetus. Unhealthy lifestyle is a risk factor for forming CHDs. For more than a decade, the preventive role of maternal multivitamin supplements containing folic acid on the occurrence of neural tube defects and congenital heart defects has been studied.^{28,29} In our study, association was observed between no folic acid supplementation in periconceptional period and maternal education. Therefore, at-risk mothers with the lower education should be advised to take periconceptional folic acid supplementation. We have also attempted to establish the influence of maternal MTHFR genotype in the progression of CHD (Table 7).

The role of MTHFR C677T polymorphism in the formation of CHD is still doubtful. Some scientists believe that the C677T mutation in the MTHFR gene is a potential risk factor for the formation of CHD. Earlier, the possible role of the hcy level and MTHFR genetic polymorphism was investigated in the Turkish population.³⁰ Consequently, no significant role of MTHFR polymorphism was found among the Pakistani population for the development of CHD.³¹ Previously, few studies have been reported from the Indian scenario. Matam et al.,³² studied three well known genetic polymorphisms including MTHFR (C677T), PON1 (Q192R), and ACE (I/D) in the South Indian population and shown that there was a strong correlation between T allele of MTHFR and the occurrence of CHD. One study on the North Indian

population suggested that polymorphism in the MTHFR gene may contribute up to 60% reduction in enzymatic activity.³³ Another study done by Dhar et al.,³⁴ described the probable positive correlation between MTHFR gene polymorphism with CHD in the East Indian population. Nair et al.,³⁵ reported the role of MTHFR mutation on the regulation of the level of Hcy, which could be a major risk factor for CHDs in the Indian population. One more study done in the South Indian population depicted the significant contribution of MTHFR gene polymorphism in elevating plasma Hcy level.³⁶ The polymorphism study was also done earlier from the North Indian population where hyperhomocysteinemia was found to be associated with TT homozygous state.³⁷ A significant association was also observed from other parts of India.³⁸ On the other hand, some opposing results are also obtained in the Indian scenario. Kumar et al.,³⁹ reported the role of MTHFR A1298C is more prominent in comparison to MTHFR C677T. Another study done on Indian population revealed that there is no significant difference in frequencies of MTHFR C677T genotypes observed between stroke patients and the control subjects.⁴⁰

In this present study, a few limitations have to be considered. It has provided only the genetic association for MTHFR (C677T) gene polymorphisms among the North-Indian CHD patients as compared to control subjects. Apart from MTHFR gene polymorphisms, there are several other genes that need to be analyzed such as T-Box Transcription Factor 5, NK2 Homeobox 5, and GATA binding protein 4 which have significant roles in ameliorating the clinical severity of CHD.^{41,42} The association of polymorphisms in genes related to different enzyme systems is more strongly correlated with CHD formation in comparison to the isolated polymorphism. Although, due to shortage of time we have considered only MTHFR C677T gene polymorphisms. Apart from that, the present study was hospital-based; thus, large scale population-based data could not be generated. One of the major drawbacks of the present study was the limited sample size which was not even satisfactory to perform the statistical analysis to improve the strength of results observed. A family history of CHD also plays an important role in the manifestation of this disease. Although a detailed pedigree analysis was not possible due to financial constraints. Correlation between clinical data and molecular data was lacking. In addition, we failed to analyze the MTHFR m-RNA levels in both CHD and control subjects. Repetitive studies with a large sample size are also required to better understand the etiological factors.

CONCLUSION

The present study suggests a probable genetic mechanism in the formation of CHD, concerning the maternal

genotype of folate metabolizing enzyme, MTHFR. In the term of application for prenatal diagnosis purpose, mothers with polymorphic variants for MTHFR C677T are more susceptible to have babies with CHD. Periconceptual supplementation of folate for the mothers having polymorphic allele can be a better management strategy to reduce the incidence of CHDs in newborn babies. Mothers with medical complications should be properly counseled before conception to reduce the chance of having children with CHD. On the basis of the study, we can presume that MTHFR gene mutation does have some clinical significance with respect to prognosis. The identification of genetic predispositions may contribute to the development of personalized risk prediction and health-care strategic planning.

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Authors Contribution:

AP- Concept and design of the study, performed the experiments, analyzed the data and wrote the manuscript; **AA-** Contributed immensely during sample collection and provided clinical details; **AAP-** Concept, coordination, statistical analysis, and interpretation of the data and revision of the manuscript. The authors have read and approved the manuscript.

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SUPPLEMENTARY

Supplementary Table 1: Distribution of different types of CHDs in the studied subjects

Types of CHDs	No. of Subjects	Percentage
VSD	11	30.56
TOF	8	22.22
ASD	6	16.67
AVSD	4	11.11
PDA	3	8.30
TGA	2	5.56
PS	1	2.78
BAV	1	2.78

VSD: Ventricular septal defect, ASD: Atrial septal defect, AVSD: Atrioventricular septal defect, PDA: Patent ductus arteriosus, TOF: Tetralogy of fallot, TGA: Transposition of the great arteriosus, PS: Pulmonary stenosis, BAV: Bicuspid aortic valve