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**ORIGINAL RESEARCH ARTICLE**

# STUDY OF PREVALENCE, NON GENETIC RISK FACTORS AND NKX2.5 GENE CORRELATION OF CONGENITAL HEART DISEASES IN KANPUR AND PERIPHERY

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**Abstract:**

**Background:** The estimated birth prevalence of CHD is 8-12/1000 live births with a significant geographical difference**.** In India, over 180,000 children are born with CHD every year with state wise variation and contribute to 10% of the present infant mortality. Nearly one thirds of the CHD are critical requiring intervention in the 1st year of life. Most of the CHD are thought to be multifactorial and result from a combination of genetic and non genetic risk factors.
**Materials and Methods:** A total of 200 CHD cases and 200 as control subjects were studied. Risk factors were examined separately. Nkx2.5 gene correlation was studied by using conventional PCR.
**Main Findings:** The prevalence of CHD is reported in this study is 8.48/1000 live births. Total 200 cases of CHDs, VSD-40%, ASD-31%, TOF-11%, PDA-10%, PS-03%, AS-03%, COA-01% and TA-01% are reported. Total 200 cases of CHD patient's mothers medical history was analyzed and noted history of abortions in 65 cases, pregestational diabetes in 60 cases, obesity in 50 cases, family history with CHD in 40 cases, maternal usage of drugs in 15 cases, advanced maternal age in 15 cases and smoking and drinking alcohol in 8 cases.
**Conclusion:** Both Genetic and Non -genetic risk factors are associated to CHDs. Nkx2.5 gene is the master regulator for process of cardio genesis. Nkx2.5 Gene is mostly associated to Atrial Septal Defects, Tetralogy of Fallot and Ventricular Septal Defects. Building a healthy life habits, maternal counseling for periconceptional control of blood glucose, adequate weight maintenance, and avoidance of stress is needed to prevent CHD.

**Keywords:** Congenital Heart Diseases, Non Genetic Risk Factors, Nkx2.5 Gene

## INTRODUCTION:

Congenital heart disease (CHD) is defined as a gross structural abnormality of the heart and major blood vessels that causes significant functional impairment [1]. The estimated birth prevalence of CHD is 8-12/1000 live births with a significant geographical difference [2]. In India, over 180,000 children are born with CHD every year with state wise

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variation and contribute to 10% of the present infant mortality [3]. Nearly one thirds of the CHD are critical requiring intervention in the 1st year of life. Most of the CHD are thought to be multifactorial and result from a combination of genetic and non genetic risk factors. According to recent update report of the American Heart Association, atrial septal defect (ASD), ventricular septal defect (VSD), tetralogy of fallot (TOF), patent ductus arteriosus (PDA), pulmonary stenosis, aortic stenosis, coarctation of aorta, and atrioventricular septal defect accounts for 85% of all CHDs.

The congenital heart defects are often life threatening during infancy, and infants born with this disorder are at much higher risk (~12) of mortality especially within the 1st year of life [4]. The genetic basis for many of these defects remains elusive, mutations in genes encoding core cardiac transcription factors have emerged as major contributors to many forms of congenital heart disease [5]. Many of the genes associated with CHD, including NKX2-5, GATA4, TBX5, NOTCH1, and TBX20, were identified using early genetic techniques [6].

The non genetic risk factors like rubella during pregnancy, pregestational diabetes, Certain medications taken during pregnancy like( thalidomide, angiotensin-converting enzyme (ACE) inhibitors, statins, the acne medication isotretinoin and lithium),smoking, drinking alcohol during pregnancy, and heredity are commonly associated to CHD. Consequently, we aim to reducing the burden of disease and consolidate our knowledge on multifactorial causes of CHDs and pave a way for further research regarding CHDs.

## MATERIAL AND METHODS:

Total 200 cases of CHD were taken from Rama Hospital, Rama University, and peripheral region of Kanpur, U.P, during the period of 2019 to 2021.The Genetic study was carried out in Central Research Laboratory, Rama Medical College, Rama University, Kanpur, UP.

### Data Collection

The common parameters like age, sex and stage of heart disease were recorded. All cases were thoroughly examined by chest x-ray, electrocardiogram, and 2D echocardiography. Family history of any heart abnormality, history of multiple abortions, nutrition and drug intake, any other patho-physiological conditions, and parity status of mother were recorded for analysis. Age of below 5 years is included in this study. The normal children of equivalent age group were taken as control.

### Inclusion criteria.

1. Below 5 years of age groups are included in this study.

2. Only Kanpur and peripheral region CHD cases are included in this study.

### Exclusion criteria

1. Age more than 5 years is excluded.

2. If there is no data on history of mother was excluded.

Questionnaire form was prepared and noted the medical history of mother. Parents are also investigated. The molecular analysis of Nkx2.5 Gene was done by using conventional PCR. The Institutional Ethical Committee of Rama Medical College and Hospital approved this study.

**RESULTS AND DISCUSSION:** During the study period number of births in Kanpur and peripheral region noted is 23580. Total number of CHDs reported 200. The prevalence of CHD is reported in this study is 8.48/1000 live births. In the worldwide the estimated birth prevalence of CHD is 8/1000 live births is generally accepted with a significant geographical differences. In India various authors reported the prevalence of CHD is given in table 1.

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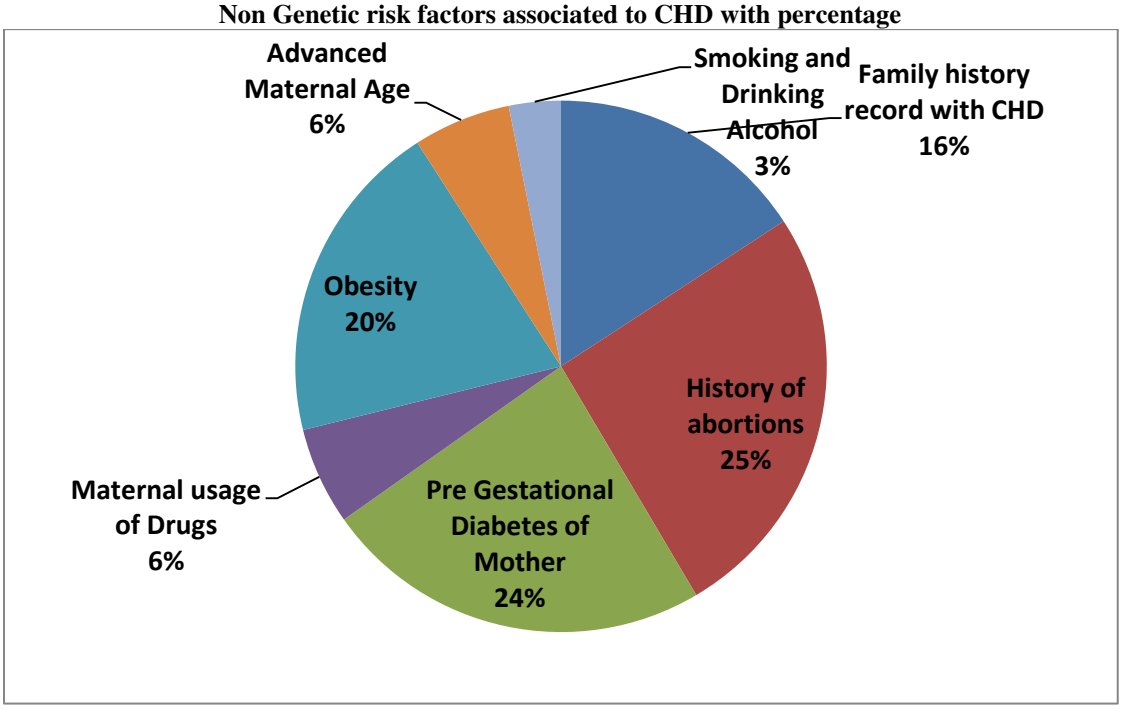
**Table 1-Prevalence of CHD reported by various Authors**

| Authors/Year | State | Prevalence |
|---|---|---|
| Tank et al. 2004 [7] | Bombay | 2.2 to 50.89/1000 live births |
| Smitha et al.2006 [8] | Karnataka | 6.6 to 13.06/1000 live births |
| Kapoor and Gupta 2008 [9] | Kanpur | 26.4/1000 live births |
| Kiran B et al.2016 [10] | Karnataka | 1.9/1000 live births |
| Anitha Saxena et al.2016 [11] | North India | 8.07/1000 live births |
| Rajeev Bhardwaj et al.2016 [12] | Himachal pradesh | 6.37/1000 live births |
| Rajkumar Motiram et al.2018 [13] | Maharashtra | 10.13/1000 live births |
| Suhail Naik et al. 2019 [14] | Kashmir | 5.3/1000 live births |
| In Present Study | Kanpur | 8.48/1000 live births |

The prevalence of CHD in Kanpur U.P is 26.4/1000 live births reported by Kapoor R and Gupta, 2008[9]. But in present study the prevalence of CHD in Kanpur reported is 8.48/1000 live births.

Total 200 cases of CHD patient's mothers medical history was analyzed and noted history of abortions in 65 cases, pregestational diabetes in 60 cases, obesity in 50 cases, family history with CHD in 40 cases, maternal usage of drugs in 15 cases, advanced maternal age in 15 cases and smoking and drinking alcohol in 8 cases.

**Non Genetic risk factors associated to CHD with percentage**




Developments in neonatal and pediatric cardiovascular surgery have improved the prognosis of CHD. Current challenges in the primary prevention of CHD are accurate identification of modifiable maternal risk factors. This

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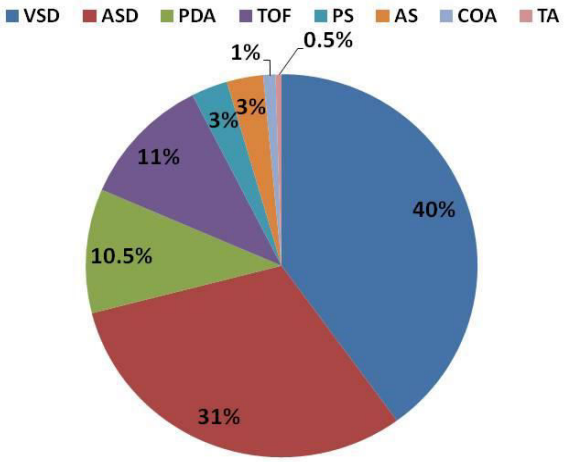
study aimed to identify possible associations between risk factors and CHD in a Kanpur population. Analyzing the risk factors, we found a high association between diabetes and CHD. Vascular disruption and oxidative stress associated with increased blood sugar levels may lead to increased risk of CHD in diabetes mellitus. Next positive association was abortions, obesity, family history with CHD, increased maternal age, maternal usage of drugs and smoking and drinking alcohol. Ramkumar et al. 2018 states that maternal age, prepregnancy BMI, uncontrolled diabetes, caffeine intake, consanguineous marriages, stress and folic acid deficiency are reliable significant risk factors contributing to CHD in south Indian population [15]. Similar studies are done in North Indian population by Saxena A et al. 2016 report an incidence of 0.87% [11].

**The Percentages of Variables in Cases and Controls**

| Risk factors | Cases (n=200) | | Controls (n=200) | |
|---|---|---|---|---|
| | Count | % | Count | % |
| Pregestational Diabetes | 60 | 30.0% | 20 | 10.0% |
| Abortions | 65 | 32.5% | 25 | 12.5% |
| Obesity | 50 | 25.0% | 16 | 8.0% |
| Family History with CHD | 40 | 20.0% | 2 | 1.0% |
| Maternal Usage of Drugs | 15 | 7.5% | 0 | 0.0% |
| Advanced Maternal Age | 15 | 7.5% | 0 | 0.0% |
| Smoking and Drinking Alcohol | 8 | 4.0% | 2 | 1.0% |

Total 200 cases of CHDs male children's are 106 and female children's are 94. The various types of congenital heart defects are identified. The ventricular septal defect is noted in 80 cases, atrial septal defect in 62 cases, tetralogy of fallot in 22 cases, patent ductus arteriosus in 21cases, pulmonary stenosis in 06 cases, aortic stenosis in 06 cases, coarctation of aorta in 02 cases and tricuspid artesia in 01case.

**Figure 1: Types of Congenital Heart Defects with percentage**



ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Kapoor R and Gupta S, 2008 CHD in Kanpur reported VSD was the most common heart defect (21.3%), ASD (18.9%), PDA (14.6%), AVSD (10.3%) and TOF (4.6%) [9]. In present study we reported VSD in 40%, ASD in 31%, TOF in 11%, PDA in 10%, PS in 03%, AS in 03%, COA and TA in 01%.

**Molecular Analysis:**

NKX2.5 Gene Polymorphism

**Isolation of DNA:** The DNA was extracted with the use of Qiagen Kit by following user manual protocol. The obtained DNA was estimated through Agarose gel electrophoresis and by the use of spectrophotometer (nanodrop).Concentration of DNA was estimated with the use of 1% Agarose gel electrophoresis and the quality assurance of DNA was also calculated with the use of standard spectrophotometer (nanodrop) at 260 nm and 280 nm ratio. Molecular characterization of polymorphism Nkx2.5 Gene, the main objective was to estimate molecular analysis of Nkx2.5 gene polymorphism associated to Congenital Heart Diseases at Kanpur and peripheral region.

**Primers for polymorphism:**

Following pairs of oligoes are being used for the amplification of NKX 2.5 gene;

| S.N. | Forward Primer | Reverse Primer | Tm ($^{o}$C) |
|---|---|---|---|
| **1.** | 1F 5′-AATGGGGGGCTACGGTCT-3′ | 1R 5′-CGTTAGGGGTGTGTGAAGC-3′; | **56** |
| **2.** | 2aF 5′-CCAGGGAGAGGAAAGTCTTG-3′ | 2aR 5′-CAGGACGGGCACAGCTACTC-3′; | **54** |
| **3.** | 2bF 5′-AGAACCGACGCTACAAATGC-3′ | 2bR 5′-GAGATCCCTCCGGAAAGAAG-3′ | **51** |

The PCR conditions were 95$^{o}$C for 3min, 35 cycles of 95$^{o}$C for 30s, 52$^{o}$C for 30s, 72$^{o}$C for 1.20 min and final extension at 72$^{o}$C for 5min.

**List of NKX2.5 Gene mutations reported in previous studies**

| Mutation - Nucleotide Change | Mutation - Amino Acid Change | CHD Phenotype | Reference |
|---|---|---|---|
| C554T | Gln149ter | ASD, VSD | [16] Benson D.W.,et al. Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. J. Clin. Invest. 1999; 104:1567–1573. |
| A677G | 167Glu | VSD | [17] Reamon-Buettner S., Borlak J. Somatic NKX2-5 mutations as a novel mechanism of disease in complex congenital heart disease. J. Med. Genet. 2004; 41:684–690. |
| G554T | Trp185Leu | ASD, VSD | [18] Sarkozy A., et al. Spectrum of atrial septal defects associated with mutations of NKX2.5 and GATA4 transcription factors. J. Med. Genet. 2005. |
| C735T | Gln187Ter | VSD | [19] Liu X.Y., et al. Novel NKX2-5 mutations in patients with familial atrial septal defects. Pediatr. Cardiol. 2011; 32:193–201. |
| T607C, A65C | Leu144Pro, Gln22Pro | ASD, VSD and TOF | In Present Study |

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Draus J M 2009 states that Nkx2.5 gene on chromosome 5q34 consists of two exons which encode a 324 amino acid protein [20]. This homeobox transcription factor is expressed during early cardiac morphogenesis and serves as a master regulatory protein. Because of its critical role in cardiogenesis, Nkx2.5 has been a prime candidate in studies to identify the genetic basis of structural Congenital Heart Defects. In present study 200 cases of CHDs Nkx2.5 Gene is mostly associated to Atrial Septal Defects, Tetralogy of Fallot and Ventricular Septal Defects.

**CONCLUSION:**

Both Genetic and Non -genetic risk factors are associated to CHDs. Nkx2.5 gene is the master regulator for process of cardio genesis. Nkx2.5 Gene is mostly associated to Atrial Septal Defects, Tetralogy of Fallot and Ventricular Septal Defects. Building a healthy life habits, maternal counseling for periconceptional control of blood glucose, adequate weight maintenance, and avoidance of stress is needed to prevent CHD.

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**Conflict of Interest:** The authors report no conflicts of interest.
**Ethical approval:** The study was approved by the Institutional Ethics Committee.

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ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

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