Original Article

Epidemiology of Cardiomyopathy - A Clinical and Genetic Study of Dilated Cardiomyopathy: The EPOCH-D study

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Abstract

Background: Dilated Cardiomyopathy (DCM) is a genetic disorder where a heterogeneous group of cardiac-muscles are involved and is characterized by ventricular dilatation, impaired systolic function, reduced myocardial contractility with left ventricular ejection fraction (LVEF) less than 40%. Our study aims to report the Demographic, Clinical and Genetic profile of Indian Dilated Cardiomyopathy patients. **Methodology:** All patients were recruited with prior written informed consent and are of Indian origin. **Results:** In a total of 80 DCM patients, the prevalence was higher among males. In males, mean age of onset was comparatively less than females. In this cohort, 40% had familial inheritance. Sixty two percent of DCM patients belong to NYHA functional class II with ejection fraction (EF) ranging between 21-30% and, around one third of the patients had atrial fibrillation (AF). Genetic screening revealed a novel splice site mutation LMNA (c.639+ G>C) and a rare variant MYH7 (c.2769 C>T) in a patient and insilico analysis of both variants suggested functional changes that were considered pathogenic. We report 3% and 4% occurance of variants, each in LMNA and MYH7, where as reported frequencies of these genes are 6% LMNA and 4% MYH7. **Conclusions:** DCM is often familial and all possible candidate genes should be screened to identify mutations. Such type of exercise may help in the identification of mechanistic pathways. Next generation sequencing platforms may play an important role in this respect in future.

Key words: Dilated cardiomyopathy, epidemiology, India, genetics

Introduction

Dilated cardiomyopathy (DCM) is a disease of the heart muscle, primarily affecting the left ventricle and characterized by ventricular dilatation, impaired systolic function, and reduced myocardial contractility.[1] It has a heterogeneous phenotype, and the causes can be genetic, toxins, infections, metabolic disease, and idiopathic.

Some studies indicate that 30–35% of idiopathic DCM may have a family history[2] of DCM. Diagnosis of any case of DCM should be consistently reviewed for the family history. If the family history reveals possible cardiac death at a comparatively immature age, congestive heart failure, unexplained heart disease, arrhythmias, syncope, or sudden death, even without an obvious cardiac history, hereditary disease must be considered positive. Since echocardiographic screening and electrocardiography (ECG) are more sensitive than family history, detailed screening of first-degree family members is recommended. Most of the familial DCM have an autosomal dominant inheritance, but autosomal recessive, X-linked and mitochondrial inheritance is also present in some cases.[3]

More than 40 genes are known to cause DCM out of which 25% of the familial and 18% of the sporadic cases are due to mutations in the Titin gene.[4] The LMNA gene coding for lamin A/C protein and MYH7 gene encoding myosin heavy chain beta (MHC-β) isoform have been found to share 6% and 4% of the mutations, respectively.[5] The lamin A/C protein is a nuclear protein which provides nuclear stability and is located on chromosome 1q22. The mechanism by which these mutations result to DCM is not known. MHC (MYH7) is a sarcomeric protein, which is located on chromosome 14q12 and comprises 40 exons. MYH7 generates movements by transferring energy from the hydrolysis of ATP into sliding of myofilaments. MYH7 mutations lead to a late age of diagnosis and have incomplete penetrance in adults.[6]

Since very little is known about the epidemiology of cardiomyopathy in India, our study aimed for a series of

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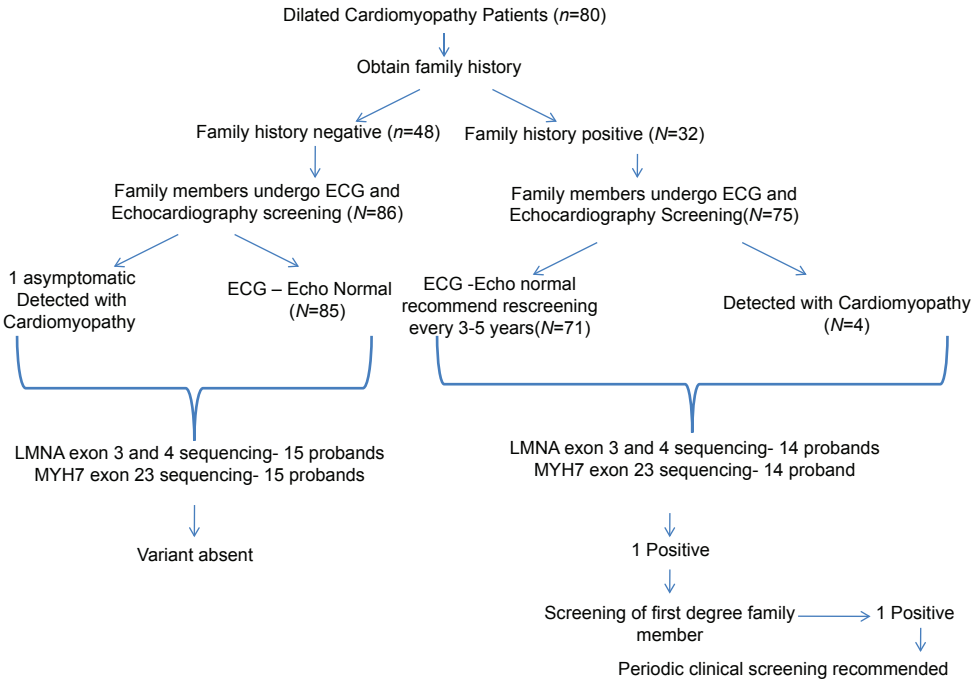

Figure 1: Sample stratification of the patients.

prospective clinical and genetic studies of Cardiomyopathy patients, and here we present the data on DCM.

Methods

We prospectively followed a cohort of cardiomyopathy patients out of which 80 were DCM patients (EPidemiolOgy of CardiomyopatHy-DCM study [EPOCH-D study]), who were followed up for 3 years. The total patient sample stratification is given in Figure 1. The inclusion criteria for DCM patients was Left ventricular ejection fraction (LVEF) <40% and absence of evidence of coronary artery disease by either coronary angiography or stress imaging. Patients with intrinsic valve disease, congenital malformations, severe hypertension, insulin-dependent diabetes, and drug-induced cardiomyopathy, amyloidosis, and myocarditis were also not included in this study. The controls for the study comprise of 83 age, sex, and ethnic group matched individuals.

Written consent was obtained from all the patients and their first-degree family members who took part in the study for clinical and genetic screening. Approval was taken from the ethics committee of both the participating institutes. Clinical screening for DCM includes 12 lead ECG and two-dimensional echocardiography. Pedigrees were constructed to obtain detail family history regarding disease or any other condition. Symptomatic changes or types of symptoms related to disease such as dyspnea, orthopnea, paroxysmal nocturnal dyspnea, appetite, edema, activity level, chest pain, palpitations, and syncope or presyncope were also recorded. Treatment history has recorded. The patients were kept under clinical and telephonic follow-up for 3 years.

Five milliliter intravenous blood sample was taken from patients and their family members who consented to participate in the study. DNA was isolated through phenol-chloroform method for genetic testing.[7] Selected regions of two major genes (MYH7 and LMNA) which are hotspot regions were sequenced for the identification of variants. Sequencing of exon 23 of MYH7 gene and exons 3 and 4 of LMNA gene was done by the Sanger method (ABI 3730 XL) for patient samples and if any variants were detected then sequencing was followed in family members also. Titin was not sequenced since titin mutations as a cause of DCM had not been reported at the time of designing of the study.[8]

Results

A total of 80 patients who met the inclusion criteria for DCM were included in the study. Thirteen patients did not participate in the study out of which six were not interested, and seven were nonrespondents.

The demographic details are described in Table 1. Of 80 patients, 49 (61.3%) were male. The mean age was 41.7 ± 16.5 years. The mean age of onset of symptoms in males was 34.07 ± 12.7 years, and in females it was 39.75 ± 12.8 years. The majority of patients were in New York Heart Association (NYHA) Class II (62.5%). The mean ejection fraction was 26 ± 19.68%. LVEF less ≤ 30% was observed in 56 (70%) patients, between 31% and 40% in 19 (23.75%) patients and ≥40% in 5 (6.25%) patients. Ninety percent of our patients were on ACE inhibitors or angiotensin receptor blockers, and 51% were on β blockers. Twenty-one percent were on digitalis, and all were on diuretics. The survival rate of our patients was 95% over the follow-up period of 3 years, and the majority of them belonged to NYHA Class II. One patient had a heart transplant, and four patients

Table 1: Demographics characteristics of DCM cases

Variables	Cases, *n* (%)	Familial (%)	Sporadic (%)
Numbers of individuals	80 (100)	32 (40)	48 (60)
Gender			
Male	49 (61.3)	21 (65.62)	28 (58.33)
Female	31 (38.8)	10 (31.25)	21 (43.75)
Present age (years), mean	41.7±16.5	44.32±16.50	40.12±16.52
Age of onset (years), mean	36.3±12.8	40.36±12.77	35.66±12.96*
Male	34.07±12.7	35.37±12.77	33.17±12.96
Female	39.75±12.8	53.16±16.61	38.77±12.96
Age wise distribution (years)			
0-20	06 (7.5)	03 (9.37)	03 (6.25)
21-30	14 (17.5)	04 (12.5)	10 (20.83)
31-40	24 (30.0)	11 (34.37)	13 (27.08)
40-50	17 (21.3)	06 (18.75)	11 (22.91)
50 above	19 (23.8)	11 (34.37)	8 (16.66)
NYHA classes			
NYHA Class I	01 (1.25)	01 (3.12)	0
NYHA Class II	50 (62.5)	17 (53.12)	33 (68.75)
NYHA Class III	18 (22.5)	07 (21.8)	11 (22.91)
NYHA Class IV	11 (13.75)	07 (21.8)	04 (8.33)

*P=0.01. NYHA: New York Heart Association, DCM: Dilated cardiomyopathy

died during the period of 3 years. Atrial fibrillation (AF) was present in 23 (29%) patients, Mitral regurgitation was present in 68 (85%) patients, and Tricuspid regurgitation was present in 52 (65%) patients [Table 2].

Echocardiographic screening was done in 161 relatives out of which 87 were males and 74 were females. The mean age of these family members was 30 ± 16.49 years. Familial DCM was present in 40% of the patients (defined as occurrence in a patient plus one more family member). Five family members were asymptomatic prior to echocardiographic screening. There was no consanguineous family in our cohort. All the familial cases in our studied samples show an autosomal dominant inheritance.

There were significant differences between familial and sporadic cases in respect to age of onset (sporadic = 35.66 ± 12.96 vs. familial = 40.36 ± 12.77 years) and left bundle branch block (LBBB) (familial = 53. 12% vs. sporadic = 25%) but there were no differences in AF and EF. There were no differences in NYHA functional class.

LMNA (lamin A/C) exon 3 and 4 were sequenced in 29 patients, and exon 23 of MYH7 (MHC) was also sequenced in 25 patients by Sanger Sequencing. Mutations were found in only one family. In this family, we found a novel splice site mutation LMNA (c. 639 + G > C) and a rare variant MYH7 (c. 2769 C > T) in a male proband aged 45 and on screening of his family members, his son aged 20 was also found to be a carrier of same mutations. The proband parents are dead, the cause of death is not known, but as reported by the proband his mother had a sudden death at the age of 65. The son is currently asymptomatic. Both these variants were found to be pathogenic by insilico analysis.

Discussion

Many patients with DCM are labeled as idiopathic. As more and more genetic studies are being done, familial causes are being considered important. In our study, most of the patients were young, NYHA Class II and has a survival rate of 95%. The male-female ratio was 1.5:1. Forty percent of the patients in our study were found to have familial Cardiomyopathy with an autosomal dominant pattern of inheritance. Mutations were picked up in only one family for LMNA and MYH7 in the patient and his son. Sanger sequencing was used, and next generation sequencing techniques were not used to sequence the entire genome. Titin mutations were also not looked for.

In our study, the number of males is higher compared to females. Similar results have been reported in number of studies.[9,10] Ushasree *et al*. has also reported the male-female ratio as 2:1 and suggested that female hormones could act as cardioprotective agents.[11]

In the studied population, earlier age of onset is observed in sporadic DCM compared to familial cases whereas in most of the reported studies familial DCM has an earlier age of onset.[12,13] Since most of the studies seem to suggest that familial DCM have an earlier disease onset, our study variation can only be explained as a chance occurrence.

Left bundle branch block was present in 46.25% of the cases. The frequency of LBBB was significantly higher in familial DCM as compared to nonfamilial DCM. In a study from Eastern India, LBBB was present in 21% of the patients.[14] Ntusi *et al*. and Kurbanov *et al*. found that LBBB was higher in sporadic cases compared to familial DCM.[13,15] In a study by Arbustini *et al*., both familial and sporadic DCM cases had almost equal frequency of LBBB.[12]

Atrial fibrillation is a potential risk for sudden cardiac death in DCM patients. Twenty-nine percent of our cases had AF. In the Eastern India, study by Paul R *et al*. AF was present in 15.7% of the cases.[16] Mitral regurgitation was present in 85% of our patients out of which 30.8% had AF. Left ventricular end-diastolic volume was more than 110 ml in 85% of the patients, and 92.5% of the cases had left ventricular end-systolic volume more than 50 ml.

In our study, 40% cases were of familial origin and 5 family members who were earlier asymptomatic were diagnosed with DCM after the Echocardiography screening. In 2004, prevalence of familial DCM was considered to be 20–35% which rose up to 48% in 2013.[17] Most of the individuals who are detected with DCM are not considered to have familial DCM until the detailed familial history has been asked. Therefore, importance should be given to detailed family history as the age of onset can vary from infancy to late 70s within the same family.[3] In 90% of the familial cases of

Table 2: Clinical characteristics of DCM cases

ECG abnormalities	Total (%)	Familial (*n*=32) (%)	Sporadic (*n*=48) (%)
LBBB			
Present	37 (46.25)	17 (53.12)	20 (25)*
Atrial fibrillation			
Present	27 (33.75)	11 (34.37)	16 (33.33)
Heart rate			
<60 beats/min	12 (15)	08 (25)	04 (8.3)
60-100 beats/min	47 (58.75)	21 (65.62)	26 (54.16)
>100 beats/min	21 (26.25)	11 (34.37)	10 (20.83)
Echocardiographic measurements			
Ejection fraction	26.43±19.62	26.22±19.59	26.92±19.51
LVes volume (ml)	140.5±57.90	133.5±16.98	144.47±58.53
LVed volume (ml)	200.7±68.22	203.48±68.26	199.1±69.82
Regurgitation			
Mitral			
Trivial	20 (25)	07 (21.87)	13 (27.08)
Mild	23 (28.75)	10 (31.25)	13 (27.08)
Moderate	23 (28.75)	09 (28.12)	14 (29.16)
Severe	02 (2.5)	02 (6.25)	0
Tricuspid			
Trivial	16 (20)	07 (21.87)	09 (18.75)
Mild	17 (21.25)	07 (53.12)	10 (20.83)
Moderate	19 (23.75)	09 (28.12)	10 (20.83)
Severe	0	0	0
Aortic			
Trivial	05 (6.25)	02 (6.25)	03 (6.25)
Mild	02 (2.5)	0	02 (4.16)
Moderate	0	0	0
Severe	0	0	0

*P=0.01. LBBB: Left bundle branch block, DCM: Dilated cardiomyopathy;,ECG: Electrocardiogram

DCM, the inheritance is autosomal dominant in which both male and female have equal chances of inheriting the disease. But there are cases where autosomal recessive (10%) and sex-linked genes (5%) are involved. An autosomal recessive pattern is more common in certain ethnic groups and infantile varieties. Sex-linked types are associated with mutations of the dystrophin gene and linked with muscular weakness and a rise of muscle enzymes. Therefore, first-degree relatives should undergo ECG and echocardiography screening.

A novel splice site mutation LMNA (c. 639 + G > C) and a rare variant MYH7 (c. 2769 C > T) were detected in a proband and his son. The rare variant was earlier reported in an Indian HCM family (www.ensemble.org) and also in a European cohort 0f 1000 genome project. For the novel variant human splice finder predicted the elimination of natural donor splice site leading to the formation of a new cryptic donor site which is located seven nucleotide upstream of native splice site with a consensus value of 80.63 (Soumi *et al.*, 2015, in press).

Titin gene was not sequenced due to the large size and unavailability of information while the study was designed. Twenty-five percent of the mutations leading to familial DCM are present in titin (TTN) gene.[5] Next to titin (TTN), LMNA and MYH7 genes with a mutation frequency of 6% and 4%, respectively,[5] are involved. Some of the common mutations causing familial DCM include lamin A/C, MHC-β, MHC-α, actin, alpha-actinin-2, desmin, sarcoglycan, troponin T, alpha-tropomyosin, titin, and phospholamban. There are also genetic polymorphisms associated with increased risk of developing DCM. The clinical heterogeneity observed is due to causation by multiple genes, with gene-environment interactions altering penetrance and phenotypic expression of disease. We report 3% and 4% occurrence variants each

Table 3: Indian studies on genetics

Study	Number of patients of cardiomyopathy	Genetic details
Rai *et al*., 2008[18]	51 DCM patient	Association of ACE I/D polymorphism demonstrated with the risk of HCM, DCM, and RCM. DCM patients with ID genotype showed a decreased LVEF
Rai *et al*., 2009[19]	61 DCM patients	130 patients diagnosed with HCM or DCM (69 with HCM and 61 with DCM) screened for mutations in the MYH7 gene. A novel mutation p.Gly377Ser was identified in the MYH7 gene associated with DCM
Tanjore *et al*., 2010[20]	97 DCM patients	MYH7 gene in HCM and DCM patients had common genetic variations in exons 7, 12, 19 and 20. The data suggested a dose effect of the mutant protein playing a role, where the heterozygous condition lead to hypertrophy and homozygous condition lead to DCM
Jadhav *et al*., 2012[21]	DCM case report	Case report of an Indian family with an Emery-Dreifuss myopathy with DCM, due to a novel LMNA mutation
Matsa *et al*., 2013[22]	115 DCM patients	Three functional polymorphisms of endothelial NOS3 gene were genotyped to identify their role in DCM. NOS3 may be synergistically functioning in DCM via excessive production of NO in cardiomyocytes resulting in decreased myocardial contractility. This suggests that DCM is an end result of oxidative stress
Matsa *et al*., 2014[23]	115 DCM patients	One of the genes linked to DCM is EDN1. Heterozygote patients with insertion variation (+138A) were found to exhibit four-fold increase risk of DCM
Rani *et al*., 2014[24]	147 DCM patients	A novel private mutation (R144W) in troponin T was associated with familial DCM in one family
Das *et al*., 2015 (current study)	80 DCM patients	40% familial cardiomyopathy. A novel splice site mutation LMNA (c. 639+G > C) and a rare variant MyH7 (c. 2769 C > T) found in one family

DCM: Dilated cardiomyopathy, ACE: Angiotensin converting enzyme, I/D: Insertion/deletion, HCM: Hypertrophic cardiomyopathy, RCM: Restrictive cardiomyopathy, NOS: Nitric oxide synthase, EDN1: Endothelin 1, LVEF: Left ventricular ejection fraction

in LMNA and MYH7; whereas reported frequencies for these genes are 6% LMNA and 4% MYH7. Indian studies are limited, but previous Indian studies have reported an association of lamin A/C, MYH7, and ACE polymorphism with DCM [Table 3]. Data on Titin mutation are not available from India but with the advancement of DNA sequencing technologies, new candidate genes can be identified which may have a major contribution in disease. One of the limitations is the small sample size of our study. Full Sequencing of LMNA and MYH7 gene could not be performed in all the patients which might have provided better insights, but screening these hotspot regions of these two genes strongly suggests the involvement of these genes in the pathogenesis of disease.

Conclusion

In our study, most of the patients were young, and the mean age of onset was 36.3 ± 12.8 years. Forty percent of the patients had familial DCM, and few of the relatives of these patients were asymptomatic prior to echocardiography screening. Therefore, we suggest that the patients should be counseled for family screening even if the family history is negative.

Familial DCM accounted for at least 40% of the patients of idiopathic cardiomyopathy in this study. The inheritance pattern was autosomal dominant and mutations in one family were found in the LMNA and MYH7 gene. Next generation sequencing would probably pick up more mutations. These data suggest that family screening should be done for all cases of idiopathic DCM.

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