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Association of Polymorphic Antioxidant Enzymes with Dilated Cardiomyopathy

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ABSTRACT Despite considerable public awareness and technological advances that foster early diagnosis and aggressive therapeutic interventions, heart failure, which results as a final outcome from an underlying cardiovascular disorder remains a critical and an unsolved problem. Among the various cardiomyopathies, Dilated cardiomyopathy with an obscure etiology is known to be the leading cause of heart failure and sudden cardiac death among young adults and children. Studies related to the molecular basis of the condition have implicated oxidative stress pathways, apart from primary disease causing sarcomeric, cytoskeletal and mitochondrial gene mutations in the disease onset. The present study aims to evaluate the role of oxidative stress markers in 97 DCM patients and 105 control individuals to identify specific electromorphic association of superoxide dismutase, catalase and alpha-1-antitrypsin with the disease. Our study has revealed an association of SODA2, catalase HPII and AAT 'M' and 'Z' alleles with DCM, thereby resulting in inefficient scavenging of the free radicals, which may confer decreased protection against oxidative stress induced tissue injury in the disease pathogenesis. The involvement of SOD and AAT in apoptotic pathway and as immunomodulators is also emphasized.

INTRODUCTION

Chronic heart muscle disorders causing deterioration of the myocardium are defined as cardiomyopathies. As per WHO classification, cardiomyopathies are categorized as hypertrophic, dilated, restrictive, obliterative and arrhythmogenic right ventricular dysplasia/ cardiomyopathy, depending upon the anatomic, haemodynamic and pathophysiological features. Among these, dilated cardiomyopathy (DCM) is the most prevalent (80%) but poorly understood group of disorders characterized by left ventricular dilation, impaired systolic function, reduced myocardial contractility and inefficient pumping of the heart (Seidman 2001).

DCM being a heterogenous disorder, is inherited as an autosomal dominant/ autosomal recessive or X-linked trait with age related penetrance and variable expressivity, suggesting the role of genetic and environmental variables in its etiology. However, heart failure in cardiomyopathies is also accompanied by increased free radical generation and lipid peroxidation and is accompanied by a deficit in antioxidant enzymes. Patients with idiopathic or ischemic dilated cardiomyopathy were known to exhibit abnormalities of a range of markers of increased oxidative stress, which contribute to the contractile dysfunction, increased incidence of fatal arrhythmias, and a risk of sudden death (Dogan Yucel et al. 1998). Therefore, oxidative stress in cardiac and vascular myocytes could possibly evaluate the extent of tissue injury in myocardium.

SOD is an important antioxidant that acts in defense against oxidative stress induced tissue injury and lipid peroxidation (Petkau 1975; Zimmerman 1973). Decreased Cu, Zn-SOD activity in the heart had been correlated with increased accumulation of reactive oxygen species and lipid peroxidation (Chen 1994), which in turn promotes apoptotic cell death. Inflammatory reactions such as myocarditis induce the production of hydrogen peroxide, a potentially harmful oxidizing agent. Catalase, also an

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antioxidant enzyme, promotes the conversion of hydrogen peroxide (H2O2) to water and molecular oxygen. Since, catalase and SOD reactions are synergistic in nature (Guo 1993) , the altered SOD/ CAT ratio may also be consistent with increased lipid damage associated with tissue injury and inflammation.

AAT, an acute phase reactant, antioxidant, immunomodulator protein plays, a pivotal role as a protease inhibitor of matrix metalloproteinases, elastase, collagenase etc. Increased levels of AAT are known to prevent the tissue damage induced by proteases at the site of injury (Moraga 2000). On the other hand, a deficiency in AAT may lead to an un-inhibited tissue injury during inflammation and promote the exacerbation of the disease (DeMeo 2004).

Hence the present study is aimed at evaluating the association of polymorphic antioxidant enzymes like superoxide dismutase (SOD), catalase and AAT with DCM as susceptibilityand modifier markers in the exacerbation of the disease condition.

MATERIALS AND METHODS

Sample Source

Ninety seven dilated cardiomyopathy patients referred to cardiology units of CARE Hospital, Mahavir Hospital and Niloufer Hospital for children, Hyderabad, India, over a period of three years were included in the study. 105 age and sex matched healthy blood donors with no previous history of cardiovascular disorders in them or in their family members were considered as control subjects for comparative analysis. Blood sample was obtained from the patients as well as the control subjects for various biochemical analysis.

Methodology

Phenotyping of SOD, Catalase and AAT was carried out in 8% PAGE, following the methods of Beauchamp and Fridovich (1971), Gregory and Fridovich (1974) and Davies procedure (1964) respectively, wherein, achromatic zones of SOD on a blue background were identified as SODA1-1, SODA2-1 and SODA2-2 . Similarly, catalase phenotyping (HPI, HPI-II and HPII) was based on the development of brown color at the site of enzyme activity in the presence of hydrogen peroxide and O-dianisidine, while, alpha-1-antitrypsin phenotypes were identified on PAGE after immunofixation with the specific AAT antisera (Sigma, U.S.A).

Statistically, test of risk estimates, chi square analysis, Hardy Weinberg allelic expectations and odd's ratio, was carried out to evaluate the possible association of specific phenotypes with the disease condition.

RESULTS

The frequency distribution of SOD, catalase and AAT phenotypes is presented in Table 1, wherein, the frequency of SOD2-2, SOD2-1 and SOD1-1 phenotypes were reported to be 78.09%, 13.3% and 8.57% in controls and 39.17%, 48.4%, 12.37% in DCM group, revealing a preponderance of SOD2-1 and 1-1 phenotypes in the DCM. Similarly, the distribution of catalase HPI, HPI-II and HPII phenotypes were found to be 75.2%, 13.3%, 11.4% in the control group individuals and 54.6%, 17.5% and 27.8% in patients, indicating a predominance of HPII phenotype in the disease group, while the frequency distribution of AAT phenotypes was found to be 32.38% FF, 4.76% MM, 0.95% ZZ, 42.85% FM, 7.61% FS, 11.4% MZ in control group and 10.3%

Table 1: Frequency distribution of superoxide dismutase, catalase and alpha-1-anti trypsin phenotypes in control and dilated cardiomyopathy groups.

	Control		*DCM*	
	n	*%*	*n*	*%*
SOD Phenotypes				
SOD2-2	82	78.09	38	39.17
SOD2-1	14	13.3	47	48.4
SOD1-1	9	8.57	12	12.37
Catalase Phenotypes				
HPI-I	79	75.2	53	54.6
HPI-II	8	13.3	17	17.5
HPII-II	12	11.4	27	27.8
AAT Phenotypes				
FF	34	32.38	10	10.3
MM	5	4.76	7	7.21
ZZ	1	0.95	2	2.06
SS	-	-	-	-
FM	45	42.85	22	22.68
FZ	-	-	-	-
FS	8	7.61	6	6.18
MZ	12	11.4	14	14.4
MS	-	-	-	-
ZS	-	-	1	1.03
Total	105	-	97	-

FF, 7.21% MM, 2.06% ZZ, 22.68% FM, 6.18 FS, 14.4% MZ, 1.03% MS in dilated cardiomyopathy. The above results indicate an increased preponderance of AAT 'MM, MZ, SZ' and ZZ' phenotypes in DCM.

The odds test of risk estimates was also carried for the markers (Table 2) wherein, the relative risk estimates of SOD phenotypes were found to be 0.13 (χ^2 31.64) in 2-2 vs 2-1, 0.34 (χ^2 4.83) in 2-2 vs 1-1, 0.18 (χ^2 30.18) in 2-2 vs 2-1/1-1 and 2.51 (χ^2 2.97) in 2-1 vs 1-1, with a significant association of SOD2 allele in DCM. Similarly, the relative risk of catalase phenotypes in the disease group in comparison to the control was also estimated, wherein, the risk of HPI vs HPI-II was 0.29 (χ^2 9.76), HPI vs HPI-II/ II showed a risk of 0.39 (χ^2 9.51), with a significant association of catalase HPII allele. The relative risk estimates of AAT revealed a risk of 5.13 (χ^2 6.55) with FF vs MM/ZZ/SS, and 0.24(χ^2 4.36), with MM vs FF/ ZZ/SS comparisons. Thus, a significant association of 'Z' allele encoding for deficient protein was observed with DCM.

Table 3 gives the allelic frequencies of SOD, catalase and AAT in the control and disease group. The allelic frequency of SOD1 and 2 alleles was reported to be 0.16 and 0.84 in controls and

Table 2: Relative risk estimates of superoxide dismutase, catalase and alpha-1-anti trypsin phenotypes in dilated cardiomyopathy group in comparison to control group.

	Control	*DCM*		χ^2
	n	*n*	*RR*	
SOD Phenotypes				
2-2	82	38	-	-
2-2 vs 2-1	14	47	0.13	31.64**
2-2 vs 1-1	9	12	0.34	4.83*
2-2 vs 2-1/1-1	23	59	0.18	30.18**
2-1 vs 1-1	9	12	2.51	2.97
Catalase phenotypes				
HpI-I	79	53	-	-
HpI-I vs HPI-II	12	27	0.29	9.76**
HPI-I vs HPII-II	14	17	0.55	2.2
HPI-I vs HPI-II/HPII-II	26	44	0.39	9.51**
HPI-II vs HPII-II	14	17	1.85	1.52
AAT Phenotypes				
FF	34	10	-	-
MM	5	7	-	-
ZZ	1	2	-	-
SS	-	-	-	-
FF vs MM/ZZ/SS	6	9	5.13	6.55*
MM vs FF/ZZ/SS	35	12	0.24	4.36*
ZZ vs MM/FF/SS	39	17	0.21	1.46
SS vs FF/MM/ZZ	40	19	-	-
FF vs FM/FZ/FS	53	28	1.79	1.88
MM vs FM/MZ/MS	57	37	0.46	1.52
ZZ vs FZ/MZ/SZ	12	15	0.62	0.13
SS vs FS/MS/ZS	8	7	-	-

*p<0.05; **p<0.01

Table 3: Allelic frequency estimates of superoxide dismutase, catalase and alpha-1-anti trypsin in control and dilated cardiomyopathy groups.

	Phenotypic frequencies						*Alleles*	*Allelic frequencies*	
	Control			*DCM*				*Control*	*DCM*
	Obs	*Exp*	χ^2	*Obs*	*Exp*	χ^2			
SOD Phenotypes									
2-2	82	75.43	0.57	38	38.99	0.02	2	0.84	0.63
2-1	14	27.03	6.28*	47	43.99	0.07			
1-1	9	2.43	17.7**	12	12.99	3.01	1	0.16	0.37
Total	105	-	24.5**	97	-	3.1		-	-
Catalase Phenotypes									
HPI-I	79	70.4	1.04	53	38.9	5.03*	I	0.82	0.64
HPI-II	14	9.07	2.67	17	44.8	17.3**			
HPII-II	12	3.4	21.7**	27	14.06	11.9**	II	0.18	0.36
Total	105	-	25.4**	97	-	34.2**		-	-
AAT Phenotypes									
FF	34	30.25	0.745	14	10.68	0.16	F	0.576	0.355
MM	5	11.90	0.0008	15	15.18	0.31	M	0.319	0.448
ZZ	1	0.36	0.36	3	1.187	0.02	Z	0.066	0.154
SS	-	0.16	0.16	-	0.205	0.20	S	0.038	0.041
FM	45	37.95	0.645	34	25.47	0.01			
FZ	-	6.6	6.6**	-	7.125	7.12**			
FS	8	4.4	2.94	7	2.964	5.50*			
MZ	12	4.14	14.92**	23	8.493	6.63**			
MS	-	2.76	2.76	-	3.533	3.53			
ZS	-	0.48	0.48	1	0.988	0.0001			
Total	105	-	29.6**	97	-	23.4**			

*p<0.05; **p<0.01

0.37 and 0.63 in DCM group. While, the allelic frequency of catalase HP-I and HP-II was found to be 0.82 and 0.18 in control group and 0.64 and 0.36 in DCM, with a significant deviation from Hardy Weinberg expectation in the DCM group, further strengthening the association of HPII allele with DCM. With regard to AAT phenotypes the allelic frequencies of F, M, Z, and S alleles were found to be 0.57, 0.31, 0.06, 0.038 in control and 0.35, 0.44, 0.15, 0.041 in DCM respectively, further strengthening the association of M and Z alleles with DCM.

DISCUSSION

Myocardial injury and myocardial inflammation (myocarditis) are the two important components in the pathogenesis of DCM. The uninhibited tissue injury, may consequentially lead to myocardial inflammation resulting in reduced cardiac output, thus exacerbating the disease condition to heart failure. The vital enzymes involved in oxidative stress induced tissue injury and inflammatory responses were studied for their role as susceptible markers/ gene modifiers in the etiopathogenesis of DCM and their role in the disease progression to heart failure.

Superoxide dismutase protects oxygen-metabolizing cells against harmful effects of superoxide free radicals. Cu-Zn SOD/ SODA is reported to be a polymorphic enzyme with three identifiable *SODA 1-1, 2-1, and 2-2* phenotypes. SODA2 allele is known to encode for a highly unstable protein product, with reduced enzyme activity. Our study suggests an implication of SODA2 allele coded protein in DCMs. Earlier studies have suggested that SOD, in conjunction with nitric oxide at high concentrations, lead to apoptotic cell death (Brockhaus 1999) and since DCM is associated with apoptosis/ tisuue injury of myocardial cells, such an association can be justifiable.

Catalase, an antioxidant, promotes the conversion of hydrogen peroxide (H2O2), to water and molecular oxygen. Catalases are designated as HP-I and HP-II isozymes in the order of increasing anodic mobility (Claiborne 1979). HP-I is bi- functional and has both catalytic and peroxidative activity whereas HP-II has only catalytic function. The study had shown an association of HPII allele, which encodes for a monofunctional enzyme that lacks peroxidase activity, thus HPII association may be explained on the basis of inefficient scavenging of free radicals during oxidative stress, thus conferring susceptibility and decreased protection against free radical induced tissue injury in the disease condition. Further, the synergistic activity of SOD and catalase can justify such an *association of SOD2 and HPII* alleles.

Alpha-1-antitrypsin (AAT) plays a central role as an acute phase reactant as well as a protease inhibitor of matrix metalloproteinase, collagenase, elastase etc in controlling the extra cellular matrix (ECM) protein degradation and appears to be involved in regulation of the immune system. It is also an important enzyme in maintaining the protease-antiprotease homeostasis and acts in defense against active tissue degradation induced by various proteases at the site of injury and inflammation (Moraga 2000). Normal AAT activity is encoded by the 'M' allele, while other variants reported are known to be less functional. An *association of 'Z'* allele with DCM was observed, which can be explained on the basis of uninhibited tissue breakdown and apoptosis of myocytes, culminating in disease exacerbation and heart failure (DeMeo 2004). Thus the present study highlights a significant association of antioxidant *SOD2* allele, catalase *HPII* and antiprotease *AAT 'Z'* with the disease, indicating the modifier role of these genes in the pathogenesis of DCM.

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REFERENCES

Beauchamp C, Fridovich I 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analyt Biochem,* 44: 276-287.

Brockhaus F, Brune B 1999. Overexpression of CuZn superoxide dismutase protects RAW 264.7 macrophages against nitric oxide cytotoxicity. *Biochem J,* 338: 295-303.

Chen Q, Ames BN 1994. Senescence-like growth arrest induced by hydrogen peroxide in human diploid fibroblast F65 cells. *Proc Natl Acad Sci,* 91: 4130–4134.

Christine E Seidman, Schönberger J 2001. Many Roads Lead to a Broken Heart: The Genetics of Dilated Cardiomyopathy. *Am J Hum Genet,* 69(2): 249–260.

Claiborne A, Fridovich I 1979. Purification of the o-dianisidine peroxidase from Escherichia coli B. Physicochemical characterization and analysis of its dual catalatic and peroxidatic activities. *J Biol Chem,* 254 (10): 4245-4252.

Davies BJ 1964. Disc electrophoresis II. Method and application to human serum proteins. *Ann New York Acad Sci,* 121: 404-427.

DeMeo DL, Silverman EK 2004. Alpha 1-antitrypsin deficiency, 2: genetic aspects of alpha(1)- antitrypsin deficiency: phenotypes and genetic modifiers of emphysema risk. *Thorax,* 59(3): 259-264.

Faber JP, Poller W, Weidinger S, Kirchgesser M, Schwaab R, Bidlingmaier F, Olek K 1994. Identification and DNA sequence analysis of 15 new alpha 1-antitrypsin variants, including two PI*Q0 alleles and one deficient PI*M allele. *Am J Hum Genet,* 55: 1113-1121.

Gregory EM, Fridovich I 1974. Visualization of catalase on acrylamide gels. *Anal Biochem,* 58(1): 57–62.

Guo DM, Panakkezhum D. Thomas GD, Lopaschuk S, Mark JP 1993. Superoxide dismutase (SOD) – catalase conjugates- role of hydrogen peroxide and the Fenton reaction in SOD toxicity. *J Biol Chem,* 268(1): 416-420.

Moraga F, Janciauskiene S 2000. Activation of Primary Human Monocytes by the Oxidized Form of 1-Antitrypsin. *J Biol Chem,* 275(11): 7693-7700.

Petkau A, Chelack W, Pleskach S, Meeker B, Brady C 1975. Radioprotection of Mice by Superoxide Dismutase. *Biochem Biophys Res Commun,* 65: 886.

Zimmerman RL, Flohe U, Weser, Hartmann HJ. 1973. Inhibition of lipid peroxidation in isolated inner membrane of rat liver mitochondria by superoxide dismutase. *FEBS Letters,* 29: 117-120.