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AAT: A Comparative Study in HCM and DCM

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ABSTRACT Cardiomyopathies are the sub-acute, chronic disorders of the myocardium that result in cardiac muscle injury thus disrupting the normal contractile function of the heart. HCM and DCM are inflammatory disorders where the role of AAT as a disease marker and cardiac remodeler has been identified. AAT acts as a major serine protease inhibitor and immunomodulator with high degree of polymorphism. Its main role is inhibition of the matrix metalloproteinases, collagen and the enzyme elastase apart from ECM and microfibrillar components degradation. The present study aims to evaluate the role of AAT, in 83 HCM, 97 DCM patients and 100 Control individuals to identify its association with cardiomyopathy. Our results implicate the role of the Z and S alleles in the etiopathogenesis of HCM and DCM, and also their role in cardiomyocyte remodelling through ECM changes. Thus decreased production of AAT may lead to further damage of the myocardiocytes.

INTRODUCTION

Cardiomyopathies are diseases that affect the heart muscles. The World Health Organization (WHO 1995) defines cardiomyopathies as "diseases of the myocardium associated with cardiac dysfunction." (cited from Gaetano et al. 2004).

Cardiomyopathies are of five main types: hypertrophic, dilated, restrictive, arrythmogenic right ventricular cardiomyopathy and unclassified cardiomyopathies of which HCM and DCM form major part of the cardiomyopathies. This paper deals with a comparative role of alpha – 1 – antitrypsin (AAT) in HCM (Hypertrophic Cardiomyopathy) and DCM (Dilated Cardiomyopathy) cases.

AAT is a secretory glycoprotein that functions as the major serine protease inhibitor in human serum playing a central role as an inhibitor of matrix metalloproteinase, collagenase, elastase, extra cellular matrix (ECM) protein and microfibrillar components degradation by a dominant negative effect. 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, apart from being an acute phase reactant and/or immunomodulator enzyme (DeMeo and Silverman 2004).

AAT is inactivated by cleavage and complex formation with target proteases, or by oxidation of methionine at the reactive site (Moraga et al. 2000). Neutrophil elastase is known to be involved in cartilage destruction in rheumatoid arthritis despite the local presence of alpha-1-protease inhibitor (Christophe and Joseph 1996) by the release of matrix metalloproteinases involved in chondrogenesis.

Alpha-1-antitrypsin (AAT) is known to be highly polymorphic. Over 200 allelic variants of this gene have been identified and 34 of them have been associated with a quantitative or functional deficiency of circulating AAT (Russo et al. 2009). AAT is mapped onto chromosome 14q32.1 (Schroeder et al. 1985; DeMeo and Silverman 2004).

Biosynthesis of AAT is controlled at the Pi locus by a pair of genes, which are co-dominant in their expression. Normal AAT activity is encoded by the 'M' allele. Other variants are less functional and are termed A to L and N to Z, depending on whether they are more proximal or more distal to the M band on electrophoresis. The presence of deviant bands on the electrofocusing can signify the presence of alpha-1-antitrypsin deficiency with Z and S alleles encoding for a deficient protein (DeMeo and Silverman 2004).

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Since HCM and DCM is characterized by inflammatory responses and myocarditis, a study on AAT as an indicator in protease-antiprotease homeostasis to prevent tissue injury hence AAT polymorphism is studied to identify the susceptibility alleles in the disease pathology of cardiomyopathies. Further, as MMP (Matrix Metallo Proteinases) and MIP's (Macrophage Inflammatory Protein) homeostasis, components of ECM, maintain cell to cell communication and integrity of myocytes which form the sarcomere, can be remodelled by AAT, hence as a genetic remodeler, its role in remodelling is also examined in cardiomyopathies. The genetic polymorphisms of this glycoprotein and its association with HCM at the quantitative and qualitative levels are a major area of research interest (Jeppson and Frazen 1982; Johnson et al. 1999), which may help in specific allele association and its role of encoded protein in cardiomyopathies can be delineated.

MATERIALS AND METHODS

The present study includes HCM and DCM patients referred to cardiology units of CARE Hospitals, Mahavir hospitals and Niloufer hospitals, Hyderabad. Blood samples from 97 DCM patients and 83 HCM patients; 100 healthy control individuals matched for age and sex were obtained to carryout various biochemical and molecular analysis.

Phenotyping of AAT was carried out on 8% PAGE gel by following Davies (1964) protocol. Later immunofixation using anti AAT anti sera, obtained commercially from Sigma Aldrich, USA was taken up.

RESULTS

Table 1 gives the frequency distribution of AAT phenotypes in the controls, HCM and DCM patients. The phenotypic distribution in control group was 35.0% of FF, 15.0% of MM, 2.0% of ZZ, 38.0% of FM, 10.0% of MZ whereas 18% and 14.4% of FF, 13.2% and 15.4% of MM, 6% and 3% of ZZ, 47% and 35% of FM, 6% and 7.2% of FS, 4.8% and 23.7% of MZ was observed in HCM and DCM respectively. An increased preponderance of the Z and S alleles was seen in both HCM and DCM cases.

Table 1: The frequency distribution of AAT phenotypes in controls, hypertrophic cardiomyopathy and dilated cardiomyopathy cases.

Phenotypes	*Control*		*HCM*		*DCM*	
	n	*%*	*n*	*%*	*n*	*%*
FF	35	35	15	18.07	14	14.43
MM	15	15	11	13.25	15	15.46
ZZ	2	2	5	6.02	3	3.09
SS	-	-	-	-	-	-
FM	38	38	39	46.98	34	35.05
FZ	-	-	1	1.20	-	-
FS	-	-	5	6.02	7	7.21
MZ	10	10	4	4.81	23	23.71
MS	-	-	-	-	-	-
ZS	-	-	3	3.61	1	1.03

Table 2 gives the test of significance of AAT phenotype in the HCM and DCM patients compared to the control group individuals. As seen from the table, the Z homozygous allele in HCM (ZZ = 20.9**) and the heterozygote Z allele in DCM (FZ = 7.12**, MZ = 6.63**) and S alleles (FS = 5.50*) exhibit a significant association with the disease group.

Table 2: Phenotypic frequencies of Alpha-1-anti trypsin in control hypertrophic cardiomyopathy and dilated cardiomyopathy cases.

Phenotypes	*Control*			*HCM*			*DCM*		
	Obs	*Exp*	χ^2	*Obs*	*Exp*	χ^2	*Obs*	*Exp*	χ^2
FF	35	29.16	1.16	15	16.80	0.19	14	10.68	0.16
MM	15	15.21	0.002	11	12.62	0.20	15	15.18	0.31
ZZ	2	0.49	4.65	5	0.83	20.9**	3	1.187	0.02
SS	-	-	-	-	-	-	-	0.205	0.20
FM	38	42.12	0.40	39	29.13	3.34	34	25.47	0.01
FZ	-	-	-	1	7.47	5.60	-	7.125	7.12**
FS	-	-	-	5	3.58	0.56	7	2.964	5.50*
MZ	10	5.46	3.77	4	5.46	0.94	23	8.493	6.63**
MS	-	-	-	-	-	-	-	3.533	3.53
ZS	-	-	-	3	0.79	6.10*	1	0.988	0.0001
Total	100	-	9.982	83		37.83**	97	-	23.4**

*p<0.05; **p<0.01

Thus, the above results indicate an increased frequency of the Z and S alleles with the disease condition with the association being stronger and enhanced in DCM and HCM. Since the S and Z variants of the alpha 1 antitrypsin allele system code for decreased enzyme activity such an association can be justified, as cardiomyopathies are a result of inflammatory responses and cardiac remodelling seen commonly in cardiomyopathies, can account in specific AAT allele encoded product playing a role in extracellular matrix, since AAT acts as the substrate metalloprotein component of extracellular matrix.

Table 3 represents the test of Odds ratio. However the odds risk estimate for the specific allelic association is nullified in the table reflecting the small sample size and large range of class intervals (CI) and classification of phenotypes based on the high degree of polymorphisms.

Table 3: Odds risk estimate of the phenotype in cardiomyopathies compared to control group.

	Odds Ratio	*Class Interval (CI)*	*p*
Genotype/HCM			
FF vs MM	0.584	0.194 - 1.751	0.413
FF vs ZZ	0.171	0.020 - 1.180	0.083
FF vs FM	0.418	0.183 - 0.943	0.034
MM vs FM	0.715	0.265 - 1.914	0.610
MM vs MZ	1.833	0.377 - 9.351	0.607
ZZ vs MZ	6.250	0.613 - 80.393	0.161
Genotype/DCM			
FF vs MM	0.400	0.139 - 1.141	0.093
FF vs ZZ	0.267	0.027 - 2.287	0.350
FF vs FM	0.447	0.191 - 1.035	0.061
MM vs FM	1.118	0.438 - 2.854	0.970
MM vs MZ	0.435	0.136 - 1.373	0.181
ZZ vs MZ	0.652	0.070 - 6.727	1.000

DISCUSSION

Increased levels of AAT were observed in acute inflammatory conditions and are known to prevent the tissue damage induced by proteases at the site of injury. Local regulation of AAT may be important in maintaining the protease-antiprotease homeostasis in preventing tissue damage induced by proteases in the microenvironment of an injury and/or inflammation (Moraga and Janciauskiene 2000).

Alpha-1-antitrypsin, one of the major serine proteinase inhibitors in human pl**as**ma, inhibits over expressed proteinases during inflammation. Proteinase activity is tightly regulated by these inhibitors under normal physiological conditions, but this equilibrium may be impaired in some pathological conditions, where oxidative inactivation of the inhibitor may occur. Indeed, oxidation of alpha-1-antitrypsin results in loss of its anti-neutrophil elastase activity and uncontrolled degradation of connective tissues. Also, its oxidized form activates primary human monocytes, thus contributing to the inflammatory process. Oxidized alpha-1-antitrypsin dr**as**tically impairs its ability to counteract elastase activity (Banfi et al. 2008) thus leading to the disease phenotype and similar mechanism may operate in cardiomyopathies.

Alpha1 antitrypsin (AAT) inhibits neutrophil elastase, as well as cathepsin G, limiting tissue degradation when these enzymes are released from inflammatory cells. It is also seen that AAT reduces Neutrophil adhesion to matrix protein, inhibits neutrophil chemotaxis and limits tissue degradation when tissue proteinases are released from inflammatory cells, thus playing an important role in inflammatory responses and tissue damage. Apart from losing its inhibitory function, oxidized AAT also influences the recruitment of leukocytes by stimulating human neutrophil migration and degranulation, thus playing a role in tissue damage or membrane damage (Noriko et al 2009). A similar mechanism can also be seen in HCM and DCM as inflammatory responses are commonly observed in cardiomyopathies.

Further the decreased levels of alpha-1-antitrypsin in IPAH (Idiopathic Pulmonary Arterial Hypertension) patients can tip the elastase-antielastase balance unfavourably towards accelerated tissue breakdown and predispose to cardiac vascular remodelling. In addition to elastase inhibition, alpha-1-antitrypsin could also prevent endothelial cell apoptosis by inhibiting caspase-3 activity. The decrease of alpha-1-antitrypsin may induce apoptosis of cardiomyocytes, which could result in arteriolar occlusion. Impairment of vascular and endothelial homeostasis was thought to play a major role in the initiation and development of Pulmonary Arterial Hypertension (PAH) (Yu et al. 2007). A similar kind of remodelling can be seen in the case of cardiomyopathies where a HCM phenotype progressively develops into a DCM phenotype by a phenomenon known as phenotypic plasticity, hence justifying the role of AAT and specific allelic association with cardiomyopathies.

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