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PRINT: ISSN 0972-3757 ONLINE: 2456-6360
Int J Hum Genet, 9(3-4): 263-267 (2009)
DOI: 10.31901/24566330.2009/09.03-4.08

Pro and Anti-Oxidants in Cardiomyopathy

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KEYWORDS Dilated Cardiomyopathy. Hypertrophic Cardiomyopathy. Malondialdehyde. Nitric Oxide, Ceruloplasmin

ABSTRACT Free radicals play an essential role in maintaining the physiological condition of the body and oxidative stress is a result of an imbalance between free radicals and protective endogenous antioxidants, mostly associated with inflammatory disorders. Since cardiomyopathy is also an inflammatory disorder, the role of pro and anti oxidants is executed. Overall 180 cases (83 HCM and 97 DCM cases) and 100 healthy volunteers were included in the study. 5ml of venous blood samples were collected for the analysis of Malondialdehyde, Nitric oxide and Ceruloplasmin levels. MDA was found to be high in HCM whereas NO and Cp levels were high in DCM. Based on the findings it can be concluded that, Hypertrophic cardiomyopathy can results due to an imbalance in pro-oxidants while Dilated cardiomyopathy is an end result of oxidative stress. Hence the pro-oxidants MDA and NO, and antioxidant ceruloplasmin levels, may serve as prognostic indicators in hypertrophic and dilated cardiomyopathy, with failing hearts.

INTRODUCTION

Cardiomyopathy is a heart muscle disorder that affects specifically ventricular systolic and diastolic functions as a result of structural and functional alterations in the ventricles. As per WHO (2003) they are classified as (1) Hypertrophic cardiomyopathy (HCM), (2) Dilated cardiomyopathy (DCM), (3) Restrictive cardiomyopathy (RCM), (4) Obliterative cardiomyopathy (OCM) and (5) Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C).

Hypertrophic cardiomyopathy is a common primary myocardial disorder associated with considerable morbidity and mortality. It is characterized by increased left ventricular mass with myocyte and myofibrillar disarray that occurs in the absence of an apparent hemodynamic burden and with variable clinical and morphologic expression. Dilated cardiomyopathy, a common form of cardiomyopathy, on the other hand, is characterized by a dilated left ventricle (LV) and systolic dysfunction that commonly results in congestive heart failure (CHF) (Richardson et al. 1996; Maron et al. 2006).

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Oxidative stress induced by oxygen derived free radicals, is a disturbance in the pro-oxidant and antioxidant balance, leading to potential tissue damage. Hearts with increased antioxidant capacity have been reported to be more resistant to in vivo and in vitro oxidative stress (Yucel et al. 1998). On the other hand, heart failure in Cardiomyopathies is accompanied by increased free radical generation and lipid peroxidation and a relative deficit in 'antioxidant reserve' that may contribute to the decompensated state (Yucel et al. 1998). Oxygen free radicals are directly involved in oxidative damage of proteins, lipids and nucleic acids in ischemic tissue leading to cell death. The peroxidative damage to cellular constituents such as membrane lipids and proteins is the major threat in conditions with increased oxidative stress (Freeman and Crapo 1982).

Malondialdehyde (MDA), an end-product of lipid peroxidation results in oxidative damage of cells and tissues, and is an indicator for such damage. It is also known to form DNA adducts, with guanine being the preferred target, leading to mutagenic lesions and point mutations (Benamira et al. 1995). Increased Malondialdehyde levels were also observed in hypertrophic cardiomyopathy (Reena et al. 2004), CVD like ischemia (Bir et al. 2006) and in post myocardial infarcted dilated cardiomyopathy patients (Yucel et al. 1998).

Nitric oxide (NO) is an important signaling molecule with multiple functions such as blood

vessel dilatation, neurotransmission, immune defense, and cell death regulation/apoptosis. The role of NO in the cardiovascular system is to maintain a vasodilator tone, inhibit platelet aggregation and adhesion and promotes modulation of smooth muscle cell proliferation. It acts mainly against oxidative stress. On the contrary, excess NO may act as a free radical (RNS) and lead to endothelial dysfunction and vasodilatation as seen in chronic heart failure patients (Goishvili et al. 2005).

Ceruloplasmin (Cp), an antioxidant is a copper carrying plasma protein and functions as a copper transporter. Ceruloplasmin is a protein of the á2-globulin fraction of human blood serum and contains 95% of serum copper. Cp is considered as an extra cellular antioxidant and has an important biological role in scavenging oxygen derived free radical that initiate lipid peroxidation (Mateescu et al. 1995).

Since reactive oxygen species is known to induce tissue damage, an investigation of the role of pro-oxidants like malondialdehyde, nitric oxide and antioxidants like ceruloplasmin in the pathogenesis of hypertrophic and dilated cardiomyopathies was taken up.

MATERIALS AND METHODS

The present study was approved by institutional ethical committee of CARE Hospitals. Standard clinical, electrocardiographic and echocardiographic criteria were used for diagnosis and samples were collected from the cardiology unit of CARE and Niloufer Hospitals, Hyderabad. Overall 180 cases (83 HCM and 97 DCM cases) and 100 healthy volunteers without any history of heart and systemic disorders were included in the study. Venous blood samples were collected with anticoagulant (EDTA) for the analysis of plasma malondialdehyde (MDA), and without EDTA for the analysis of serum nitric oxide (NO) and ceruloplasmin (Cp) levels. MDA was quantified following Dahle et al. (1962) protocol, while NO and Cp levels were assayed based on Green et al. (1961) and Ravin et al. (1982) procedures respectively. Student's 't' test was carried out for inter and intra group comparisons at 5% and 1% level of significance.

RESULTS

The *mean levels* of *MDA* were found to be (X ± S.D:346.7 ± 183.2) in controls and (X ± S.D 1090.5 ± 600.4) in HCM groups respectively, with a significant increase in the patient group (t: 11.6). The *mean levels* of *NO* were found to be (X ± S.D:3.43 ± 1.87) in controls and (X ± S.D:4.31 ± 2.97) in HCM groups respectively. The increase in the mean level of NO in HCM was found be significant (t: 2.43). *Ceruloplasmin*, an antioxidant was found to be elevated in the HCM group (X ± S.D:39.97 ± 37.5) in comparison to control group (X ± S.D:32.44 ± 15.20). However the variation was not found to be significant (t: 1.83). Thus highlighting an imbalance in the pro-oxidant mechanism leading to/consequent to the disease in HCM (Table 1).

Similarly, the *mean levels* of *MDA* in the disease group (X ± S.D: 785.30 ± 520.07) were significantly high (t: 7.9) when compared to the healthy group individuals (X ± S.D: 346.7 ± 183.2) indicating the role of oxidative stress mediated tissue injury and lipid peroxidation in DCMs. The *mean levels* of *NO* were also found to be significantly high (X ± S.D: 6.7 ± 3.78) (t: 7.73) in the disease group when compared to the control group individuals (X ± S.D: 3.43 ± 1.87). The *mean levels* of *Cp* when examined were found to be (X ± S.D: 59.70 ± 27.01) in DCM and (X ± S.D: 32.44 ± 15.20) in control groups, the difference was found to be significantly high (t: 8.76), indicating a tilt in pro and antioxidant homeostasis leading to oxidative stress, thus DCM is an end result of oxidative stress (Table 2).

HCM Vs DCM: The *mean level* of *MDA* was

Table 1: Quantitative level variation of Malonaldehyde, Nitric Oxide and Ceruloplasmin in control and hypetrophic cardiomyopathy groups.

Parameter	*Control*			*HCM*			*t*
	$\bar{X}$	*S.D*	*n*	$\bar{X}$	*S.D*	*n*	
MDA(nM/dl)	346.7	183.2	100	1090.5	600.4	83	11.6**
NO(µM/L)	3.43	1.87	100	4.31	2.97	83	2.43*
Cp(mg/dl)	32.44	15.20	100	39.97	37.5	83	1.83*

*p<0.05; **p<0.01

Table 2: Quantitative level variation of Malonaldehyde, Nitric Oxide and Ceruloplasmin in control and dilated cardiomyopathy groups.

Parameter	*Control*			*HCM*			*t*
	$\bar{X}$	*S.D*	*n*	$\bar{X}$	*S.D*	*n*	
MDA(nM/dl)	346.7	183.2	100	785.301	520.07	97	7.9**
NO(μM/L)	3.43	1.87	100	6.7	3.78	97	7.73**
Cp(mg/dl)	32.44	15.20	100	59.703	27.013	97	8.76**

*p<0.05; **p<0.01

Table 3: Quantitative level variation of Malonaldehyde, Nitric Oxide and Ceruloplasmin in Dilated and Hypetrophic cardiomyopathy groups.

Parameter	*HCM*			*DCM*			*t*
	$\bar{X}$	*S.D*	*n*	$\bar{X}$	*S.D*	*n*	
MDA(nM/dl)	1090.5	600.4	83	785.301	520.07	97	3.63 *
NO(μM/L)	4.31	2.97	83	6.7	3.78	97	4.63 *
Cp(mg/dl)	39.97	37.5	83	59.703	27.013	97	4.06 *

*p<0.05; **p<0.01

found to be (X ± S.D:785.301 ± 520.07) in DCM and (X ± S.D 1090.5 ± 600.4) in HCM groups respectively, with a significant increase in the HCM group (t: 3.63). The *mean level* of *NO* was found to be (X ± S.D:6.70 ± 3.78) and (X ± S.D:4.31 ± 2.97) in both DCM and HCM groups respectively. The increase in the mean level of NO in DCM was found be significant (t: 4.63). Similarly c*eruloplasmin*, an antioxidant was found to be elevated in the DCM group (X ± S.D:59.703 ± 27.013) in comparison to HCM group (X ± S.D:39.97 ± 37.5), where the variation was found to be significant (t: 4.06). Such comparisons clearly pinpoint and strengthen the difference in the etiology of HCM and DCM with varied prognostic indicators of HCM/DCM (Table 3).

DISCUSSION

Oxidative stress leads to production of various oxidants as by products which may damage protein, lipids and DNA (Halliwell and Gutteridge 1999). In the present study, a significant increase in the levels of MDA in both HCM and DCM group as compared to the control group is observed. Increased levels of MDA are an indicator of lipid peroxidative damage, which can be suggested as a sensitive marker and can be correlated to the severity of the condition (Diaz-Velez et al. 1995; Halliwell and Gutteridge 1999). Lipid peroxidation leads to production of mutagenic lipid epoxides, lipid hydroperoxides, lipid alkoxyl and peroxyl radicals and enols (Packer and Glazer 1986). An increased level of MDA was observed in HCM when compared to DCM, reflects the pro-oxidant associated mechanism in HCM. The release of free radicals may cause cellular alteration by inhibiting protein synthesis, inactivating enzymes, cross linking of proteins and DNA, thereby leading to loss of membrane functions (Naito et al. 2002) and myocyte architecture. It is also possible that raised levels of MDA could be resulting in the disease process itself rather than the causative effect.

Nitric oxide levels were significantly raised in both HCM and DCM compared to the controls in our study. Excess of NO may cause cell injury and generate cytotoxic species such as ONOO and H_2O_2, which may play an active role in atherosclerosis in endothelial cells and facilitate platelet aggregation and plaque formation (Warren et al. 1994). Raised NO levels could thus indicate a direct role in the muscle oxidative stress since NO is directly released from the endothelial cells. Raised levels could also indicate a high immunodulator role enhancing the secretion of factors like TNFa, cytokines, cGMP and various other factors modifying the pathogenesis of the condition (Rakhit and Marber 2001). In DCM, the levels of NO were found to be higher, than in HCM in our study. A study by Goineau et al. (2001) revealed an endothelium-dependent and independent vasodilation enhancement very early in DCMs, demonstrating increased sensitivity of vascular smooth muscle to nitric oxide. Therefore, the vascular muscle almost always tends to remain dilated, hence the elevated levels of NO in DCMs is accountable. Since NO can play a critical role in the regulation of integrated cardiac and vascular function and

homeostasis, such an increase can be emphasized in cardiomyopathy on similar lines.

Significant increase in the levels of ceruloplasmin was observed in DCM groups compared to control and HCM group individuals, which suggests a tilt in the pro/antioxidant homeostasis in DCM. The possible role of other antioxidants, besides ceruloplasmin, cannot be ruled out in case of HCM (Reena et al. 2004). In DCM, the high levels of Cp may be in response to increased pro-oxidant levels. Cp controls membrane lipid peroxidation probably by direct oxidation of cation thus preventing their catalysis of lipid peroxidation (Yamashoji and Kajimoto 1983). Elevated levels of Cp have been speculated to be a risk factor for cardiovascular disorders (Reunanen et al. 1992) and atherosclerosis (Fox et al. 1995) based on its pro-oxidant properties. Ceruloplasmin synthesis is known to be upregulated at the transcriptional level as an acute phase reactant in several inflammatory and autoimmune conditions (di Patti et al. 2004) and since DCM is also considered as an inflammatory disease, such an elevation is justifiable.

In the case of HCM, the anti-oxidant (Cp) levels are low, hence may fail to neutralize the pro-oxidants effect. Other antioxidants reserves besides Cp may be playing a role in aetiopathology of the condition. On the other hand, in case of DCM, the antioxidant levels seem to be increased and these levels may be in response to the increased membrane susceptibility to peroxidation, thereby conferring protection to the heart muscle from the further damage. Thus the variation in the levels of the pro/antioxidants may culminate to failing hearts in cardiomyopathy.

CONCLUSION

In conclusion, HCM can be a result of imbalance in pro-oxidant mechanism while DCM as end result of oxidative stress. The pro-oxidant MDA and NO, and antioxidant Cp levels in hypertrophic and dilated cardiomyopathy, may serve as prognostic indicators of heart failure, as oxidative stress can be implicated in the pathogenesis and progression to heart failure in DCM/HCM hearts.

ACKNOWLEDGEMENT

The financial support from the DBT and DST, New Delhi, India is acknowledged.

REFERENCES

Benamira M, Johnson K, Chaudhary A, Bruner K, Tibbetts C et al 1995. Induction of mutations by replication of malondialdehyde-modified M13 DNA in Escherichia coli: determination of the extent of DNA modification, genetic requirements for mutagenesis, and types of mutations induced. *Carcinogenesis (Lon.)*, 16: 93-99.

Bir LS, Suleyman D, Simin R, Mehmet K 2006. Increased Malanoldialdehyde levels in Chronic stage of Ischemic stroke. *Tohoku J Exp Med*, 208(1): 33-39.

Dahle LK, Hill EG, Hollman RT 1962. The thiobarbituric acid reaction and auto oxidation of poly unsaturated fatty acid methyl esters. Arch. Biochem. *Biophys*, 98: 256-261.

Diaz-Velez CR, Garcia-Castineriras S, Mendoza-Ramosa E, Hernandez-Lopez E 1995. Increased malondialdehyde in peripheral blood of patients with congestive heart failure. *Am Heart J*, 131: 146-152.

di Patti MC, Persichini T, Mazzone V, Polticelli F, Colasanti M. et al 2004. Interleukin- 1beta up-regulates iron efflux in rat C6 glioma cells through modulation of ceruloplasmin and ferropotin-1 synthesis. *Neurosci Lett*, 363(2): 182-186.

Fox PL, Mukhopadhyay C, Ehrenwald E 1995. Structure, oxidant activity, and cardiovascular mechanisms of human ceruloplasmin. *Life Sci*, 56: 1749–1758.

Freeman BA, Crapo JD 1982. Biology of disease: free radicals and tissue injury. *Lab Invest*, 47(5): 412-426.

Goishvili N, Kakauridze N, Sanikidze T 2005. The role of oxidative metabolism disturbance in the development of NO-related endothelial dysfunction during chronic hearth failure. *Georgian Med News*, 122: 65-68.

Goineau S, Pape D, Guillo P, Ramee MP, Bellissant E 2001. Increased sensitivity of vascular smooth muscle to nitric oxide in dilated cardiomyopathy of Syrian hamsters (Bio TO-2 strain). *J Cardiovasc Pharmacol*, 37(3): 290-300.

Green LC, Wanger DA, Glogowski J, Skipper PL, Wishnok J et al 1982. Analysis of nitrate, nitrite and ^{15}N nitrate in biological fluids. *Annal Biohem*, 126: 131-138.

Halliwell B, Gutteridge JMC 1999. *Free Radicals in Biology and Medicine.* 3rd Edition. Oxford: Oxford University Press.

Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D et al 2006. American Heart Association Council on Clinical Cardiology, Heart Failure and Transplantation, Council on Clinical Cardiology, Heart Failure and Transplantation Committee, Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups, and Council on Epidemiology and Prevention. Contemporary definitions and classifications of the cardiomyopathies: an American Heart Association scientific statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation*, 113: 1807-1816.

Mateescu MA, Chahine R, Roger S, Atanasiu R, Yamaguchi N et al 1995. Protection of myocardial tissue against deleterious effects of oxygen free radicals by ceruloplasmin. *Arzneimittel-Forsch Drug Res*, 45: 476–480.

Naito Y, Takagi T, Uchiyama K, Handa O, Tomatsuri N et al 2002. Suppression of intestinal ischemia-reperfusion injury by a specific peroxisome proliferator-activated receptor-gamma ligand, pioglitazone, in rats. *Redox Rep*, 7: 294-299.

Packer L, Glazer AN 1986. Oxygen radicals in biological systems. *Meth Enzymol*, 186: 113-128.

Rakhit RD, Marber MS 2001. Nitric oxide : an emerging role in cardioprotection? *Heart*, 86: 368-372.

Ravin N.A 1961. An improved calorimetric enzymatic assay of ceruloplasmin. *J Lab Clin Med*, 58: 161-162.

Reunanen A, Knekt P, Aaran RK 1992. Serum ceruloplasmin level and the risk of myocardial infarction and stroke. *Am J Epidemiol*, 136(9): 1082–1090.

Richardson P, McKenna W, Bristow M, Maisch B, Mautner B et al 1996. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the definition and classification of cardiomyopathies. *Circulation*, 93: 841-842.

Reena TR, Annapurna SD, Ushasree B, Pratibha N, Narasimhan C et al 2004. Pro-Oxidant Malondialdehyde and anti-oxidant ceruloplasmin levels in hypertrophic cardiomyopathy. *Indian Heart J*, 56: 72–73.

Warren JB, Pons F, Brady AJB 1994. Nitric oxide biology: implications for cardiovascular therapeutics. *Cardiovasc Res*, 28: 25-30.

Yamashoji S, Kajimoto G 1983. Antioxidant effect of caeruloplasmin on microsomal lipid peroxidation. *FEBS Lett*, 152(2): 168–170.

Yucel D, Aydogdu S, Cehreli S, Saydam G, Canatan H et al 1998. Increased oxidative stress in dilated cardiomyopathic heart failure. *Clin Chem*, 44: 148-154.