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A Study on Epidemiological Factors And Biochemical Changes in Dilated Cardiomyopathy

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Abstract
Background*: Dilated Cardioyopathy (Dcm) is the commonest form of myocardial disease seen in children. It represents a common expression of myocardial damage that has been produced by a variety of yet un established myocardial insults. It is important to realize that the prognosis of children with untreatable cardiomyopathies is dismal in our country where cardiac transplantation and palliative procedures are not routinely available.*
Materials And Methods*: Blood samples were collected from 13 cases and 16 controls for this study to analyse the biochemical parameters like lactic dehydrogenase (LDH), Ceruloplasmin (Cp) and Malondialdehyde (MDA).*
Results: *Levels of MDH and LDH were found to be elevated in children suffering with dilated cardiomyopathy whereas there was no significant variation in the levels of ceruloplasmin (Cp) activity.*
conclusion: *The study concludes that the estimation of biochemical markers is necessary in Dilated cardiomyopathy cases apart from other non invasive modalities.*
Keywords*: Biochemical changes,Ceruloplasmin (Cp), Dilated cardiomyopathy (DCM), Lactic dehydrogenase (LDH), Malondialdehyde (MDH)*

I. Introduction

Dilated Cardiomyopathy represents a common expression of myocardial damage that has been produced by a variety of yet unestablished myocardial insults[1]. Western studies shows a prevalence of 10/10000 lives births in newborn period, where as for all children the prevalence is 36.5/100000 for dilated cardiomyopathy and 2.5/100000 for hypertrophic cardiomyopathy with the prevalence not estimated in Indian context. Since we differ in geographical variation, living habitat and diet compared to western population the epidemiological aspect of cardiomyopathies is necessary for better understanding of the disease.

What we know about the aetiology, causative factors, risk factors regarding the cardiomyopathy is only a tip of iceberg as there is much lacunae to be filled in. Much research is presently going around the world regarding oxidants/antioxidants in causation of disease. Cardiomyopathy constitutes a group of diseases often of unknown aetiology and is defined as intrinsic disease of myocardium in which there is no structural deformity of the heart.

Dilated Cardioyopathy (Dcm) is the commonest form of myocardial disease seen in children. It represents a common expression of myocardial damage that has been produced by a variety of yet unestablished myocardial insults. It has been speculated that an episode of subclinical viral myocarditis initiates any auto immune reaction that culminates in the development of full blown dilated cardiomyopathy. Abnormalities of both humoral and cellurar immunity have been documented[1].Various studies have shown the association between cardiomyopathy and infection like coxsackie B virus, cytomegalovirus, human immune deficiency virus and mumps virus.

Gross examination shows typically considerable ventricular enlargement of one or both ventricles. Left ventricular mass is often increased but not disproportionately and not sufficiently to return the mass/volume ratio to normal. The greater the dilatation of ventricles the less likely is the walls to be thickened.Mural thrombi are found at post mortem examination clots are most often found in the apex of the left ventricle and in left atrial appendage.Microscopically there is degeneration of sarcolemma or myocytolysis.Fibrosis is more typically seen in the sub endothelium and papillary muscles.

D echo is an important tool for diagnosis of dilated cardiomyopathy. It usually demonstrates a dilated, thin walled, and globally hypokinetic left ventricle. The right ventricle may also be affected and very rarely may be enlarged without enlargement of left ventricles. No echocardiographic features differentiates myocarditis from other dilated cardiomyopathy.

Biochemical Parameters Studied In Dilated Cardiomyopathy

Malondialdehyde: (Mda) Malondialdehyde, a common product of lipid peroxidation is formed in the free form in large amounts during unstimulated peroxidation of microsomes . Aldehydes produced from such processes leads to deleterious effects in cells and cell constituents (Packer and Glazer, 1986)[2].Such damage is observed in diseases like chronic renal failure, myocardial infarction, cirrhosis etc, (Mahlouz, I986)[3]. In the light of above observations MDA levels were estimated in dilated cardiomyopathy to examine the lipid peroxidative damage in cardiomyopathies.

Ceruloplasmin: (Cp) Ceruloplasmin is the major copper binding protein of human plasma. It acts as an anti oxidant by preventing the accumulation of oxygen derived free radicals which initiates lipid peroxidation [Wachowitzetal 1990][4].Hence the study of these proteins may highlight its association as a defensive factor in the etiology of DCM.

Lactic Dehydrogenase: (LDH) Lactic dehydrogenase, a glycolytic enzyme catalyzes the reversible conversion of pyruvate to lactate with the reoxidation of NADH .The function of lactic acid can be preferentially modified by differences in the properties of Lactic dehyrogenase isoenzymes.(Orten and Newhas, 1975)[5]. Market and Muller (1985)[6] demonstrated the occurrence of five electrophoretically different zones of lactic dehydrogenase activity in various tissues referred to as LDH 1 — LDH5.Each Isoenzyme consists of four subunits and are the result of random combination of two kinds of polypeptide chains namely heart(H)and muscle(M) controlled by two loci[6]. Different proportions of H and M subunits impose differences in the net catalytic properties of lactic dehydrogenase from various tissues (Good friend and Kaplan, 1964)[7].Cardiac muscle and erythrocytes are rich in lacticdehydrogenase- I ,which is associated with oxidative metabolism while liver and skeletal muscle are rich in lactic dehydrogenase 5 which is associated with anaerobic glycolysis. (Orten and Newhans, 1975)[5]. Elevated levels of lactic dehydrogenase have been reported in liver diseases ,renal diseases anaemia and muscular disorders (Henry, 1986)[8].There is no information available on the changes in the total activity or of its fraction in DCM, hence the study of this parameter.

II. Objectives

1) To study the epidemiological factors such as sex, blood groups and parental consanguinity in cardiomyopathy.
2) To determine the variation in the quantitative levels of biochemical factors related to metabolism of heart muscle like Lactate dehydrogenase and products of lipid peroxidation like Malondialdehyde which is an oxidant and the levels of ceruloplasmin an antioxidant.

III. Materials And Methods

Study Design: - Place Of Study: Niloufer hospital and Osmania General Hospital, Hyderabad.
Period Of Study: August 2014 toJan 2015.
Sample Size: 13 patients and16 controls matched for age and sex.
Inclusion Criteria: -1) AGE: 1-12 years
2) Both sexes
3) All children presenting with congestive heart failureexcluding other causes of congestive heart failure.

Description Of Manoeuvre:- In this study, 13 patients admitted in Niloufer hospital and Osmania General Hospital ,(both which are teaching institutes under Osmania Medical College), with features of congestive heart failure were evaluated for cardiac dysfunction and proved to have dilated chambers with global hypokinesia. Having selected a detailed history was elicited with particular reference to initial symptoms and age of onset and detailed clinical examination was done as per the proforma. Samples were collected from both 16 normal and 13 patients for laboratory analysis of MDA, LDH and Cp.3 ml of venous blood was collected from each individual for serum analysis of LDH and Cp and 2 ml blood was collected in a vial containing 1 mg/ml of EDTA for the analysis of MDA in plasma. Samples were collected after written consent from the parents or responsible guardians of patients.

Methodology 1: Levels of MDA were estimated in the plasma samples of both healthy and DCM individuals following the protocol of Dahleetal (1962)[9]. The principal involved in this technique is based on the reaction of MDA, the end product of lipid peroxidation with 2 thiobarbituric acid to form a pink coloured complex.

Methodology 2: Serum Cp (mg/1) activity was determined based on Ravin's (1961) protocol[10]. The principal involved is paraphenylene diamine, a polyamine when heated with serum reacts with Cp,an oxidase present in

the serum to form a stabled coloured complex. The intensity of this coloured complex is proportional to the concentration of Cp present in the serum sample.

Methodology 3: The principal involved in the estimation of LDH activity is based on the formation of pyruvate dinitrophenyl hydrazoe,using lactate and reduced forms of NAD as substrate and cofactor. The change in intensity of the colour as pyruvate formed is measured spectrophotometrically at 440 nm.(Varley 1984)[11]. A standard curve was prepared by plotting the Optical Density (OD) against the activity of the enzyme. The difference between test and control was converted into units of enzyme activity of the .sample by inferring from the standard curve. Similarly isoenzymes of LDH were separated electrophoretically using PAGE (Davies ,1964) [12] and by appropriate staining procedure(Capture technique).

IV. Results And Discussion

There were 13 cases in this study. One was right sided dilated cardio myopathy. Two cases had thrombi, all other had global hypokinesia. One died during hospital admission.Among the cases that were followed up over a period of six months one had normal 2D echo findings.Consanguinity (4 out of 13) was observed in dilated cardiomyopathy cases.

Table No 1 - Abo Blood Group Distribution In Disease And Control

		DCM		CONTROL		
BLOOD GROUP	MALE	FEMALE	TOTAL	MALE	FEMALE	TOATAL
O	4	4	8	6		6
A	2		2	1	3	4
B	1		1	2	2	4
AB	2		2		2	2
TOTAL	9	4	13	9	7	16

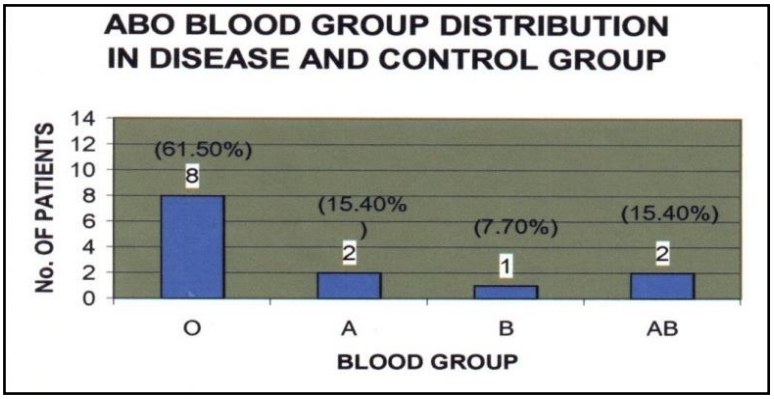


Figure No 1

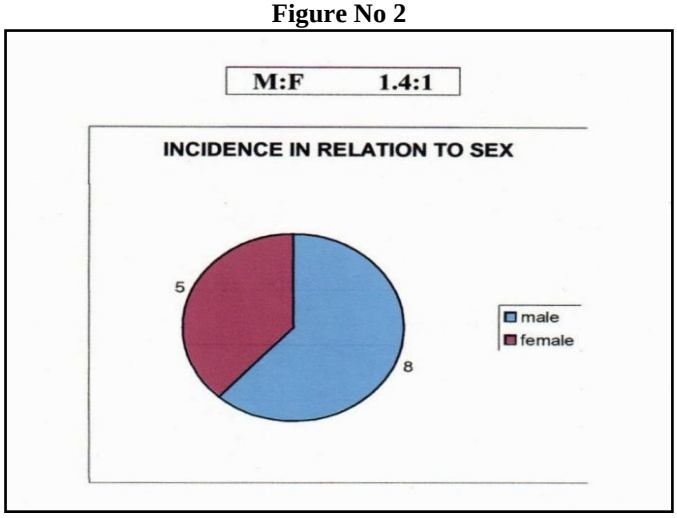
Evidence for the association of genetic factors with DCM comes from the ABO blood group studies wherein 61.5% of patients were of group '0' in comparison to control subjects (37.5%). However the sample size is too small to conclude on the ABO blood group association.

Mean Age Of The Condition: The mean age was found to be 5.69 years in DCM cases as compared to the normal children whose mean age was 10.6 years which was found to be significant ($t=5.99$, $p<0.01$) Indicating that DCM can occur in all age groups and the early onset may be truly genetic in nature as these patients are not exposed to any environmental triggering factors.

Table No 2 - Incidencein Relation To Sex:

CARDIOMYOPATHY				CONTROL			
	x	SD	n	x	SD	n	t value
Total	5.6923	3.4067	13	10.625	2.3345	16	*5.99
Male	6	3.7512	8	10.5556	2.4037	9	2.83
Female	5.2	3.1146	5	10.7143	2.43	7	3.142

Figure No 2



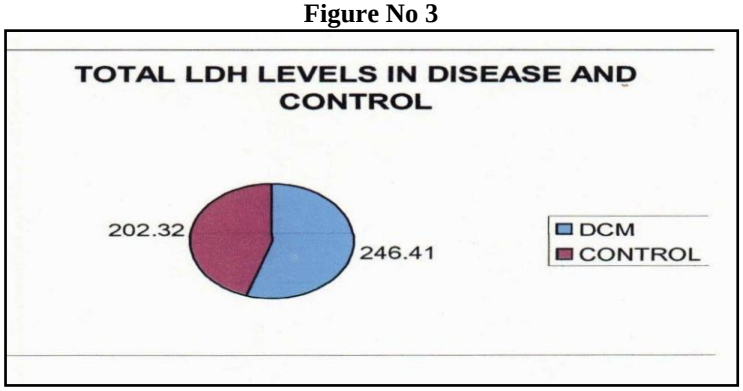
Further DCM was found to occur equally in both sexes with no deviation in sex ratio FIG 2 & TABLE NO 2

Table No 3- Lactate Dehydraogene Activity In Disease And Control Group

DCM				CONTROL			t value
	X	SD	n	x	SD	n	
TOTAL	246.41	13.63	13	202.32	6.54	16	2.98*
FRACTIONS							
LD H1	0.3715	0.021	13	0.3648	0.021	16	1.121
LD H2	0.3921	0.0151	13	0.367	0.018	16	0.231
LD H3	0.091	0.0103	13	0.118	0.018	16	2.11
LD H4	0.0302	0.0044	13	0.05	0.0044	16	0.995
LD H5	0.0241	0.016	13	0.0111	0.0112	16	1.721
SEX VARITATION							
MALE	266.51	16.731	8	181.315	9.881	9	4.78*
FEMALE	224.891	20.85	5	234.761	12.2	7	2.69*

$p<0.01$; $p<0.05$

Figure No 3



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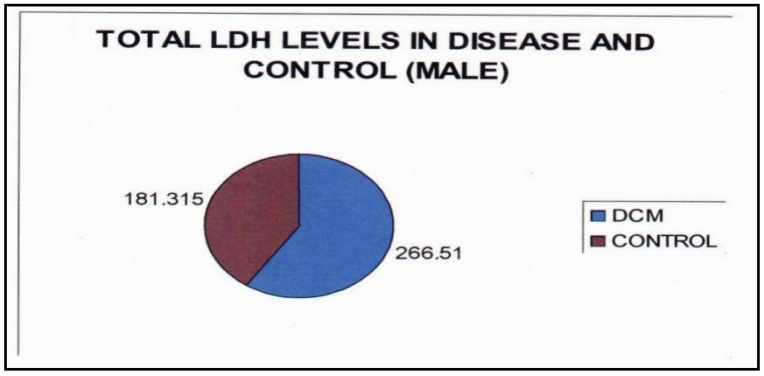
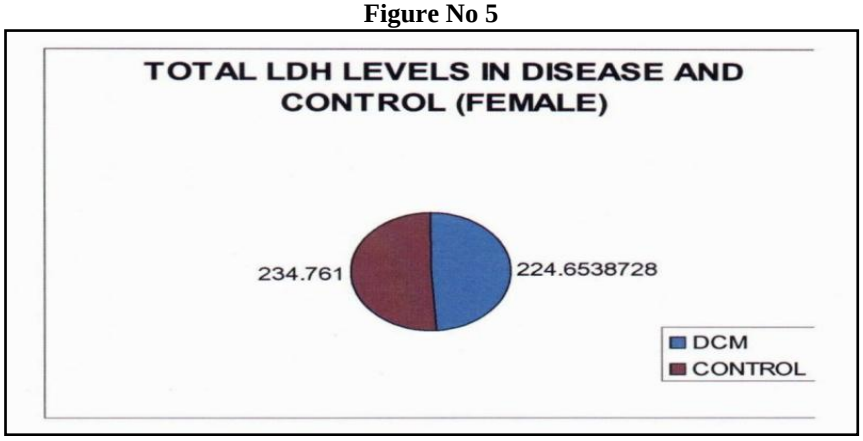


Figure No 5



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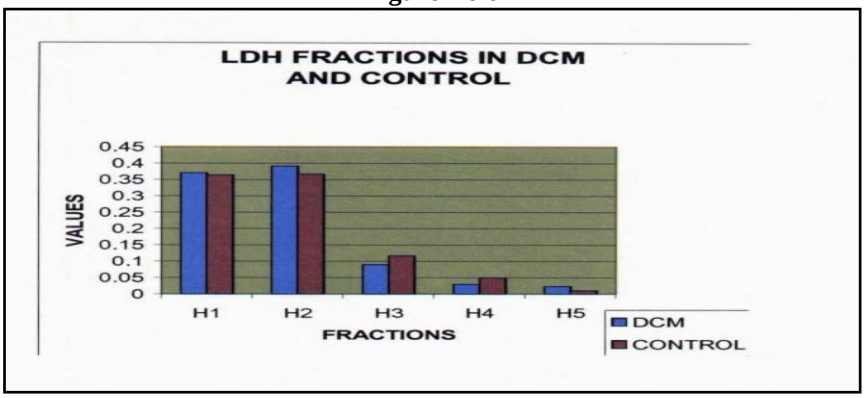
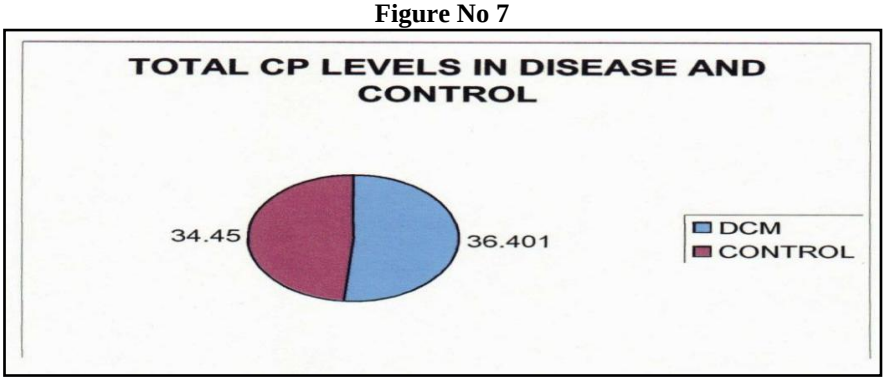


The mean LDH activity was found to be increased in DCM cases compared to the control group with the increase being statistically significant ($t=2.98$, $p<0.05$) . Significantly elevated levels were also observed in male DCM patients compared to male control Subjects ($t= 4.98$, $p<0.01$). This could be explained based on The availability of oxygen which in turn determines the direction of metabolic pathway of pyruvate either towards the formation of lactate or to energy yielding Kreb's cycle. That is changes in LDH activity depends on the degree of changes in the activity of individual LDH fraction which results due to random combination of heart (h) and muscle (m) polypeptide chains which in turn leads to differences in net catalytic properties of LDH activity in various tissues (Good friend and Kaplan 1986)[7] further the increased levels of LDH are in confirmation to earlier studies in myopathies (Henry 1986)[8].

Table No 4- Mean Ceruloplasmin Levels In Disease And Control Group

	DCM			CONTROL			
	X	SD	n	x	SD	n	t value
TOTAL	36.401	24.965	13	34.45	19.48	16	0.228
MALE	31.348	20.2889	8	34.32	18.98	9	0.603
FEMALE	44.4858	32.456	5	35.00	21.41	7	0.685

Figure No 7

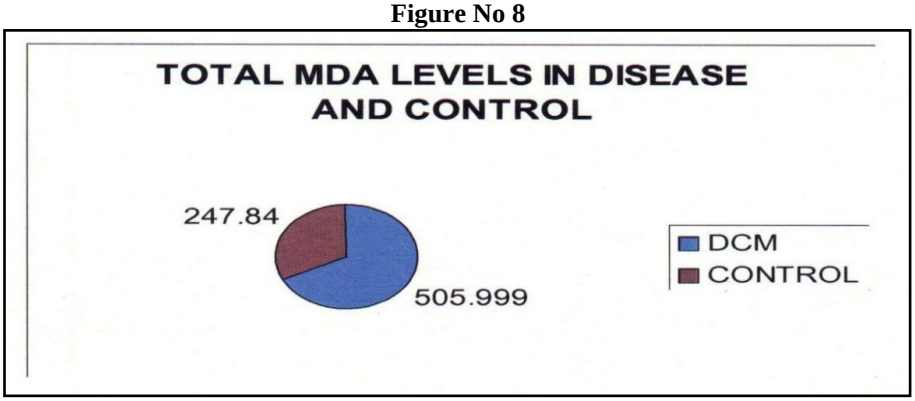


Ceruloplasmin is a copper binding alpha-2 macro globulin which acts as an antioxidant by preventing the accumulation of oxygen derived free radicals which could otherwise initiate lipid peroxidation (Gerlietal 1992)[13]. In the present study the mean activity of cp was found to be slightly increased in the DCM cases compared to the control group. However this difference was found to be statistically insignificant (t=0.228, p<0.05). Similarly cp levels were found to vary with respect to sex where in an increase of cp was found in female DCM patients compared to female normal subjects while reverse trend was observed in male patients. However the variation is statistically insignificant (t= 0.603: 0.0685, p,0.05). The role of cp as an antioxidant in DCM is still obscure and further investigations are required on these lines.

Table No 5- Mean Malondehyde Levels In Disease And Control Group (Lipid Peroxidation)

	DCM			CONTROL			
	X	SD	n	x	SD	n	t value
TOTAL	505.999	361.097	13	247.84	94.04	16	2.651*
MALE	523.006	389.914	8	248.94	90.14	9	1.924
FEMALE	478.783	351.818	5	237.02	107.13	7	1.562

Figure No 8



The mean MDA levels in DCM patients was found to be significantly increased by two folds compared to the control group (t=2.651, p<0.05). Sex variation of MDA levels was also observed but is statistically insignificant. Elevated levels of MDA in DCM cases in comparision to the control subjects indicate the peroxidative damage of membrane lipids. Higher levels of MDA can also cross link with amino thiol group of proteins causing further tissue damage in cardiomyopathies. Sex wise comparison of MDA levels in DCM and control groups revealed elevated levels in male patients compared to females. However no such significant variation of MDA levels was observed with respect to gender.

V. Conclusion

Early onset of condition in children observed with mean age being 5.69 years which is significant when compared to normal (10.6 years) subjects.Dilated Cardiomyopathy occur equally in both sexes but sample size is too small to draw any conclusion.High parental consanguinity (4/13), observed in dilated cardiomyopathy cases indicating the possible involvement of recessive genes. However, the sample size is too small to comment in this aspect.There is a possibility of group O+ve (61.5%) blood group association with Dilated Cardiomyopathy.Elevated levels of Malondialdehyde in Dilated Cardiomyopathy cases confirm the lipid peroxidative mechanisms of tissue damage and generation of free radicals which are known to be deleterious agents in causation of disease.Malondialdehyde could be one of the risk factors associated with Dilated Cardiomyopathy.There is no significant variation in Ceruloplasmin activity indicating that its role as an antioxidantmay be negligible in Dilated Cardiomyopathy. The mean lactic dehydrogenase activity was found to be increased in Dilated Cardiomyopathy cases compared to control group which was statistically significant. Elevated levels of lacticdehydrogenase activity observed in Dilated Cardiomyopathy cases can be explained in the direction of metabolic pathways of pyruvate based on the availability of oxygen which is in confirmation with earlier studies.

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