



HISTOPATHOLOGICAL CHANGES SEEN IN THE HEART SAMPLES OF DCM PATIENTS FROM NORTH INDIA - A CADAVERIC STUDY

Anatomy

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ABSTRACT

Dilated cardiomyopathy is a disease of cardiac muscle, characterized by dilatation and impaired contractility of left or both ventricles, with progressive development of congestive heart failure and occurrence of serious arrhythmic events. The main aim of our study is to find out the histopathological changes seen in heart samples of DCM patients from North-India. For our study, we have collected 60 heart samples (10 were of DCM patients and 50 control samples). Tissues were subjected to Haematoxylin and Eosin and Masson's Trichrome staining to observe any specific changes in the heart samples of DCM patients. The findings range from minimal variation in myocyte size to typical features of myofiber loss, interstitial fibrosis and marked variation in myofiber size, fibrosis and deposition of collagen fibres. Diffuse interstitial reactive fibrosis can be observed in DCM patients. Thus myocardial fibrosis can lead to heart failure and act as substrate for cardiac arrhythmias.

KEYWORDS:

Dilated Cardiomyopathy, Interstitial fibrosis, Myocytes, Collagen fibres, Haematoxylin and Eosin staining and Masson's Trichrome.

INTRODUCTION - Dilated cardiomyopathy (DCM) is a disease of the cardiac muscle, characterized by dilatation and impaired contractility of the left or both ventricles, with progressive development of congestive heart failure and occurrence of serious arrhythmic events. Diagnosis is made by exclusion of underlying cardiac diseases, such as coronary artery disease, valvulopathies or arterial hypertension. A great variety of factors (toxic, infectious, metabolic, immunologic etc.) have been etiologically implicated in DCM. This correlation is of great importance, as myocardial damage may be reversible in some of these cases. However, DCM is often characterized, as "idiopathic", as no etiologic factor is revealed, despite constant diagnostic research. Exact prevalence of DCM in India is not known. The incidence of dilated cardiomyopathy discovered at autopsy is estimated to be 4.5 cases per 100,000 populations per year, whereas the clinical incidence is 2.45 cases per 100,000 populations per year [1]. The disease is reported to be more prevalent and aggressive in Blacks and in females, while a few other reports had shown a preponderance of males. The main objective of our study is to evaluate the histopathological changes in the heart samples in patients with dilated cardiomyopathy in North-Indian population.

MATERIAL AND METHODS - The present study was carried in the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi (U.P), over a period of one and a half year starting from November 2013 to June 2015. 60 human hearts were taken for the study. The age was in the range of 10-70 years. We procured heart samples from Department of Forensic Medicine and Department of Anatomy, Institute of Medical Sciences, BHU, Varanasi (U.P). Out of 60 samples only 10 were of DCM patients. All necessary consents were taken prior to the commencement of our study. Hearts with gross morphological variations were excluded from study. Control and Dilated cardiomyopathy hearts were stained with Haematoxylin and Eosin staining and Masson's Trichrome. A tissue sample of size 3x2 mm was taken from DCM heart samples as well as from normal heart samples. For the light microscopic study, fixation of the tissues was done in 10% formaldehyde and then tissues were subjected to dehydration. The tissues were placed in 70% alcohol for overnight then were transferred into 90% alcohol and kept for 3 hours. Subsequently the tissues were put in Rectified spirit for 1 hour. After that the tissues were placed in Absolute alcohol I and Absolute alcohol II for 45 minutes respectively. Thereafter the tissues were put in Xylene I for 20-25 minutes and then in Xylene II. After the above procedure, tissue samples were transferred into jars containing paraffin wax. We gave three changes in wax for 30 minutes each. Thereafter, with the help of Leukhart's embedding boxes or L pieces, the paraffin blocks were prepared. Tissues were sectioned (3-5 sections/specimen) using a microtome (Rotary Microtome) with 5µm thin sections. For the cardiac

tissue, the specimens were stained with standard staining like Haematoxylin and Eosin (H&E) and Masson's Trichrome (MT) staining to observe the fibrous and elastic fibres of the connective tissue. The histological examination was carried out at a magnification of 10X, 40X and 100X (Nikon, Japan). For Haematoxylin and Eosin staining method, Harris Haematoxylin and Eosin Yellowish dye was used.

Staining procedure (Haematoxylin and Eosin) [2]

The slides were placed (with paraffin sections) in coplin jar and add Xylene. The slides were kept for 30 minutes and paraffin wax was removed. The slides were placed in open for few minutes to make them dry. The slides were placed again in the jar and Absolute alcohol was poured and left it for 2 minutes. The slides were placed successively in solution of alcohol of gradually decreasing strengths (95%, 70%, 50%) for 2 minutes in each. Then washed thoroughly with distilled water. Haematoxylin was added in coplin jar and left for at least 5 minutes. The slides were rinsed in distilled water and examined under the microscope in low magnification to confirm whether sections were overstained or not. The slides were dipped in Acid alcohol and rinsed in distilled water. The slides were then dipped in Ammonia water and rinsed in distilled water. The slides were dipped in eosin for 1 minute and rinsed in distilled water. Thereafter the slides were dipped in alcohol of gradually increasing strength (50%, 70%, 95%). After that slides were taken out and left to dry. Then the slides were transferred in coplin jar containing Xylene. The slides were mounted using Di-n-butyl Phthalate Xylene (DPX).

Staining procedure (Masson's Trichrome) [2]

For Masson's Trichrome staining, the tissues were fixed in Bouin's fluid which was prepared by mixing 1500 ml of saturated picric acid, 500 ml of formaldehyde and 100 ml of glacial acetic acid. Deparaffinization and rehydration was done through 100% alcohol, 95% alcohol 70% alcohol. The slides were washed in distilled water. Rinsed in running tap water for 5-10 minutes to remove the yellow colour. Thereafter staining was done by Weigert's Iron hematoxylin working solution for 10 minutes. Rinsed the same in running tap water for 10 minutes. After that, washed the slides in distilled water. Staining was done in Biebrich Scarlet-Acid Fuchsin solution for 10-15 minutes. The slides were repeatedly washed in distilled water. Differentiated in Phosphomolybdic-phosphotungstic acid solution for 10-15 minutes or until collagen was not red. Then sections were transferred directly (without rinse) to Aniline blue solution and stain for 5-10 minutes. Rinsed briefly in distilled water and differentiated in 1% acetic acid solution for 2-5 minutes. Then slides were washed in distilled water. After that dehydration was done very quickly through 95% ethyl alcohol, absolute ethyl alcohol (these steps will wipe off Biebrich Scarlet-Acid Fuchsin staining). The slides were transferred in coplin jar containing Xylene. Mounting was done by

using Di-n-butyl Phthalate Xylene (DPX).

Staining with Haematoxylin-Eosin and Masson's trichrome, reveals severe hypertrophy of the myocyte fibers as well as fibrosis, no disarray or other signs of HCM, and consistence with DCM.

RESULTS -

Histology of normal cardiac muscle- On histology examination branching cells were seen in cardiac muscle, where in the nucleus was located centrally. Few cardiac muscles were bi-nucleated. Intercalated discs were seen. Individual cardiac muscles were covered with the connective tissue endomysium.

The histologic features of dilated cardiomyopathy - The findings range from minimal variation in myocyte size to typical features of myofiber loss, interstitial fibrosis (Figure 2B), and marked variation in myofiber size. In hearts, the findings were diffuse findings of myocytes, including variation in size, nuclear variation (Figure 1B) and interstitial fibrosis (Figure 1A). Interstitial and replacement fibrosis are also common. Occasionally, there can be fibrofatty change.

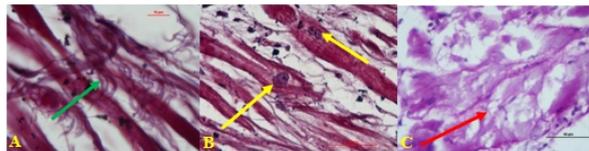


Fig 1 (A). Photomicroscopic heart section from a cardiac explants in a patient with end-stage cardiomyopathy. There is focal interstitial fibrosis (Shown with green arrow). **(B).** Note the variation in nuclear size (Shown with yellow arrow). The change is nonspecific and can be seen in heart failure from any cause. **(C).** Heart section from a cardiac explant in a patient with end-stage cardiomyopathy. Note the intracellular accumulation of amorphous material (basophilic degeneration) (Shown with red arrow). The change is nonspecific and can be seen in heart failure from any cause.

Transmural scars may also occur in dilated cardiomyopathy. Quantitation of collagen has shown up to 4 times the normal collagen concentration, with a decrease in mature cross-linked collagen. Few of the myocytes are hypertrophied, as well as atrophied (Figure 4A). The volume density of myofibrils is reduced, and mitochondrial density is normal, but the mitochondria were more numerous and small.

Histologic examination of myocardial samples has revealed marked disorganization of myocardial fibers (Fig 4B) (involving at least 5% of the tissue surface) in about 95% of patients with DCM (This cellular disarray involves >25% of myocardium in 50% and >50% of the tissue in 25% of the patients and was characterized by oblique and perpendicular arrangement of adjacent muscle fibers. Myofiber disorganization in DCM was not necessarily confined to the most hypertrophied LV segments. Indeed, there was little correlation between wall thickness and extent of cellular disorganization. Thus, cellular disorganization involves an average of 40% of the ventricular septum and 33% of the LV free wall despite differences in the magnitude of hypertrophy in these regions.

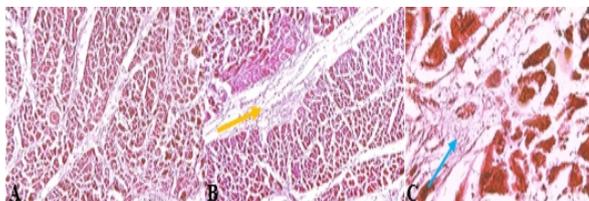


Fig 2. (A). H & E staining 100X of heart tissue (Control) shows normal architecture. **(B).** H & E staining 100X of heart tissue (DCM) shows evidence of fibrosis (orange arrow). **(C).** H & E staining 400X of heart tissue (DCM) shows the deposition of collagen fibres (blue arrow).

Masson's Trichrome staining of heart tissue (DCM) has demonstrated the fibrosis and deposition of collagen fibres which was absent in Controls (Figure 3).

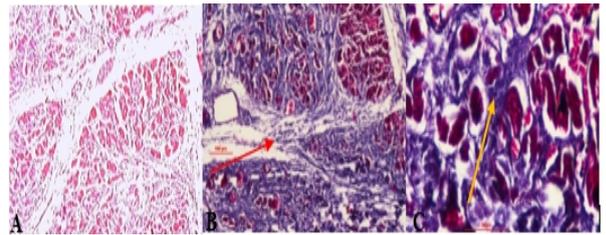


Fig 3. (A). Masson's Trichrome staining 100X of heart tissue (Control) shows normal architecture. **(B).** Masson's Trichrome staining 100X of heart tissue (DCM) shows evidence of fibrosis (Red arrow). **(C).** Masson's Trichrome staining 200X of heart tissue (DCM) shows deposition of collagen fibres (Orange arrow).

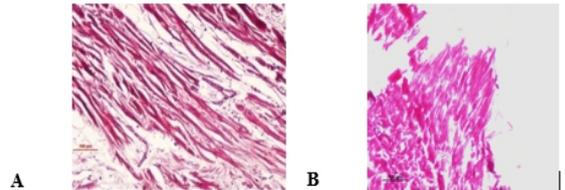


Fig 4 (A). The histology of dilated cardiomyopathy heart shows hypertrophy and degeneration of myocytes (dark red) without disarray. Increases in interstitial fibrosis (pale pink) is evident. **(B).** DCM heart shows marked disorganization of myocardial fibers.

DISCUSSION- DCM is a common cause of congestive cardiac failure (CCF) and is defined by the presence of left ventricular systolic dysfunction with left ventricular dilatation, the absence of coronary artery disease or other causes such as hypertension or valvular pathology [3]. The right ventricle may be involved but is not necessary for the diagnosis. The exact prevalence of DCM in the general population is unknown, but it clearly varies with age and geography and is the most common diagnosis in patients referred for cardiac transplantation [4,5]. Around 30- 50% of cases have a familial component [6,7] and more than 30 genes have been identified, to date, that cause DCM. Most are inherited in an autosomal dominant fashion although some can be autosomal recessive, X-linked or mitochondrial. The actual frequency of familial DCM is probably underestimated.

According to American Heart Association 2006, cardiomyopathies can be classified into- (1) primary cardiomyopathies, which affect the heart alone and (2) secondary cardiomyopathies, which are the result of a systemic illness affecting many other parts of the body. These are then further broken down into subgroups within these two broad categories incorporating new genetic and molecular insights (Table 1)

Primary Cardiomyopathies	Secondary Cardiomyopathies
Genetic (hypertrophic cardiomyopathy; conduction abnormalities: prolonged QT syndrome; Brugada syndrome)	Infiltrative (amyloidosis and Gaucher disease)
Mixed (dilated cardiomyopathy; restrictive cardiomyopathy)	Storage (haemochromatosis and Fabry's disease)
Acquired (inflammatory myocarditis, peripartum, stress cardiomyopathy - "broken heart syndrome" or tako-tsubo)	Toxicity (drugs, alcohol, heavy metals, and chemicals/chemotherapy)

	Inflammatory (sarcoidosis) endocrine (diabetes mellitus; thyroid disorders; hyperparathyroidism), cardiofacial (Noonan syndrome, lentiginosis) neuromuscular/ neurological, nutritional deficiencies, and autoimmune and collagen disorders
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Table 1 : Summary of AHA 2006 classification

The histologic features of dilated cardiomyopathy: the findings range from minimal variation in myocyte size to typical features of myofiber loss, interstitial fibrosis and marked variation in myofiber size. In hearts, the findings were diffuse findings of myocytes, including variation in size, nuclear variation and interstitial fibrosis. Interstitial and replacement fibrosis are also common. Occasionally, there can be fibrofatty change.

CONCLUSION- Fibro-fatty changes take place as a result of myocyte loss due to different causes, such as myocarditis or other noxious stimuli. Fatty infiltration of the myocardium is reported to be a major finding in ethanol-induced cardiomyopathy [8]. A number of antineoplastic agents, such as doxorubicin and cyclophosphamide, may cause extensive myocyte vacuolization [9]. The characteristic histologic findings very likely reflect specific disease processes. As the very structure of the myocytes is distorted in the DCM, the cause may be a defect in the cytoskeletal proteins. It has been suggested that DCM should be termed "cytoskeletalopathy" due to mutations found in dystrophin, actin, and desmin [10]. The contractile force is transmitted to adjacent sarcomeres and myocytes by the cytoskeletal proteins. Normal contractility will be prevented by such defects which ultimately leads to myocyte atrophy. In near future, classification of DCM should be made according to histological findings which is of great help in the detailed and scientific studies of this disease.

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REFERENCES -

1. Rakar S, Di Lenarda A, Poletti A, Bussani R, Silvestri F, Camerini F; Epidemiology of dilated cardiomyopathy: A prospective post-mortem study of 5252 necropsies. *Eur Heart J*, 1997;18(1):117-23.
2. Suvarna SK, Layton C, Bancroft JD; Bancroft's Theory and Practice of Histological Techniques. 7th edition, Elsevier publication, 2012;332-333.
3. Elliott P, Andersson B, Arbustini E, et al.; Classification of the cardiomyopathies: a position statement from the European society of cardiology working group on myocardial and pericardial diseases. *European Heart Journal*, 2008;29(2):270-6.
4. Taylor MRG, Carniel E, and Mestroni L. Cardiomyopathy, familial dilated. *Orphanet Journal of Rare Diseases*. 2006;1(1).
5. Towbin JA, Lowe AM, Colan SD, et al. Incidence, causes, and outcomes of dilated cardiomyopathy in children. *Journal of the American Medical Association*, 2006; 296(15):1867-76.
6. Hershberger RE, Lindenfeld J, Mestroni L, Seidman CE, Taylor MRG, and Towbin JA; Genetic evaluation of cardiomyopathy-a heart failure society of America practice guideline. *Journal of Cardiac Failure*, 2009;15(2):83-97.
7. Towbin JA and Bowles NE; The failing heart. *Nature*, 2002;415(6868):227-33.
8. Davies MJ; The cardiomyopathies: a review of terminology, pathology and pathogenesis. *Histopathology*, 1984;8:363-393.
9. Van Fleet JF, Ferrans VJ; Myocardial diseases of animals. *Am J Pathol*, 1986;12:98-178.
10. Bowles NE, Bowles KR, Towbin JA; The "final common pathway" hypothesis and inherited cardiovascular disease. The role of cytoskeletal proteins in dilated cardiomyopathy. *Herz*, 2000;25:168-175.