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Original article:

A Study on Alteration of Catalase and Protein Carbonyl Levels in Dilated Cardiomyopathy Patients with Heart Failure in a Tertiary Care Medical College and Hospital in Eastern India

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Abstract

Introduction: Various causes may contribute to the pathology of dilated cardiomyopathy (DCM). With this background, the present study was undertaken to investigate any change in oxidant-antioxidant status as indicated by erythrocyte catalase and plasma protein carbonyl levels in DCM.

Objectives: With this background, the present study was undertaken to investigate any change in oxidant-antioxidant status as indicated by erythrocyte catalase and plasma protein carbonyl levels in DCM.

Methods: Erythrocyte catalase and plasma protein carbonyl levels were measured in 59 DCM patients and 51 controls.

Results: There was significant decrease in mean levels of erythrocyte catalase, and increase in mean plasma protein carbonyl levels, in DCM patients compared to controls.

Conclusion: DCM may be associated with decrease in erythrocyte catalase and increase in plasma protein carbonyl levels. Catalase and protein carbonyl might be potential, useful biomarkers of antioxidant and free radical status, respectively, in DCM, for elaboration of treatment strategy and monitoring.

Key words: Catalase, Protein Carbonyl, Dilated Cardiomyopathy

Introduction

Dilated cardiomyopathy (DCM) is a common cardiac diagnosis that may be the consequence of a variety of pathologies [(1)]. Dilated cardiomyopathy is defined by the presence of a dilated and poorly functioning left ventricle in the absence of abnormal loading conditions (hypertension, valve disease) or ischaemic heart disease sufficient to cause global systolic impairment. [(2)]. DCM is an important cause of sudden cardiac death and heart failure and is the leading indication for cardiac transplantation in children and adults worldwide [(3)]. A rigorous work-up can exclude alternative causes of left ventricular dilation and dysfunction, identify etiologies that may respond to specific treatments, and guide family screening. A significant proportion of DCM cases have an underlying genetic or inflammatory basis. Measurement of left ventricular size and ejection fraction remain central to diagnosis, risk stratification, and treatment, but other aspects of cardiac remodeling inform prognosis and carry therapeutic implications [(4)]. Toxic, metabolic, and immunologic causes have each been linked to DCM, as well as hypertension and valvular disease. It is also now increasingly understood that more than one etiology may contribute to DCM

within a given individual [5]. Reactive growth processes in myocytes and architectural rearrangement of the muscle compartment of the myocardium appear to be the major determinants of ventricular remodeling and the occurrence of cardiac failure in DCM [6]. Various parameters have been studied with regard to DCM [7,8,9,10]. With this background, the present study was undertaken to investigate any change in oxidant-antioxidant status as indicated by erythrocyte catalase and plasma protein carbonyl levels in DCM.

Materials and methods

This study was a hospital-based, cross-sectional study conducted in the Department of Biochemistry of a tertiary care medical college and hospital in eastern India. Sample size was considered based on complete enumeration during study period. The study was approved by the local ethics committee and all patients and control subjects gave their informed consent to take part in this investigation.

The duration of the present study was 8 months, and included 59 DCM patients aged 30-40 years attending the outpatient department (group B) and 51 age and sex matched controls (group A). Complete history and physical examination of all subjects were undertaken. Patients with chronic diseases, liver disease, diabetes, renal impairment, acute and chronic inflammatory disease, various malignancies and smokers, alcoholics and those who were on antioxidant drugs were excluded from the study.

After collection of blood samples from each case and control into citrate-containing glass tubes, plasma samples and erythrocyte sediments were obtained by centrifugation at 3000 r.p.m. Erythrocytes were then hemolyzed by diluting with deionized water. Analyses were done in hemolyzed supernatant fractions and plasma. All samples were coded and assayed in a blind fashion by an investigator who was unaware of the subjects' clinical status. Catalase assay method was based on the measurement of the absorbance decrease due to H2O2 consumption at 240 nm [11]. Protein carbonyl (PC) content was spectrophotometrically determined by DNPH assay. Basically, protein carbonyl reacts with 2,4-dinitrophenylhydrazone (DNPH) producing a Schiff base that subsequently produces a corresponding hydrazone, which can be measured spectrophotometrically at 375 nm [12]. Statistical analysis of the data was performed by using Statistical Package for Social Sciences (SPSS) and inferences were drawn. $p< 0.05$ was considered to be significant and $p <0.001$ highly significant.

Results

Blood was drawn from 49 subjects from group A and 54 subjects from group B because 2 subjects from group A and 5 subjects from group B dropped out from the study.

Table 1. Mean erythrocyte catalase and plasma protein carbonyl levels in groups A and B.

Parameters	A	B
Catalase (U/L)	45.1+4.8	41.9+7.3
Protein carbonyl (nmol/mg protein)	0.329+0.031	0.352+0.047

Mean erythrocyte catalase levels (U/L) in groups A and B were 45.1+4.8 and 41.9+7.3 respectively and the difference was found to be significant (P 0.0107) [Table 1].

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Mean plasma protein carbonyl levels (nmol/mg protein) in groups A and B were 0.329+0.031 and 0.352+0.047 respectively and the difference was found to be significant (P 0.0046) [Table 1].

Discussion

Catalases are ubiquitous enzymes that prevent cell oxidative damage by degrading hydrogen peroxide to water and oxygen ($2H_2O_2 \rightarrow 2\ H_2O + O_2$) with high efficiency [13]. Despite our detailed knowledge of its functional mechanisms and its three-dimensional structure, however, several unexpected features of mammalian catalase have been recently discovered [14]. For example, with regard to cardiac tissue, Ivanović-Matić et al, after their study with diabetic mice, hypothesized that an important function of catalase is the suppression of events leading to diabetes-promoted cardiac dysfunction and cardiomyopathy [15]. On the other hand, in line with the present study, Foussal et al found that prevention of mice cardiomyocyte hypertrophy by apelin was associated with increased myocardial catalase activity and decreased plasma lipid hydroperoxide, as an index of oxidative stress [16]. In the present study, mean erythrocyte catalase levels (U/L) in groups A and B were 45.1+4.8 and 41.9+7.3 respectively and the difference was found to be significant (P 0.0107) [Table 1]. Despite an extensive search, no previous literature was found regarding any study regarding serum catalase measurement in cardiomyopathy patients. Thus, decrease of serum catalase in patients compared to controls may indicate lowered antioxidative defense in DCM. This is supported by the conclusion of Kawakami et al, who showed that the antioxidant EUK-8 treatment can prevent and cure murine DCM [17].

The usage of protein carbonyl groups as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated proteins; most of the assays for detection of protein carbonyl groups involve derivatization of the carbonyl group with 2,4-dinitrophenylhydrazine, which leads to formation of a stable dinitrophenyl hydrazone product [18]. Using protein carbonyl as a marker, it could be demonstrated that oxidative damage to proteins correlates well with aging and the severity of some diseases such as diabetes, neurodegenerative diseases, etc [19]. Shirpoor et al demonstrated decline in protein carbonyl and therefore oxidative stress in rats with diabetic cardiomyopathy by the antioxidant vitamin E administration [20]. Banfi et al showed that plasma levels of oxidized proteins are increased in heart failure [21]. In the present study, mean plasma protein carbonyl levels (nmol/mg protein)in groups A and B were 0.329+0.031and 0.352+0.047respectively and the difference was found to be significant (P 0.0046) [Table 1]. Thus, increase of plasma protein carbonyl levels in patients compared to controls may indicate greater oxidative stress in DCM.

Soetikno et al have shown the prevention of DCM by the antioxidant curcumin in a STZ-induced diabetic rat model, and also found that the prevention of DCM by curcumin is associated with the suppression of nitric oxide activation leading to the overgeneration of reactive oxygen species and/or reactive nitrogen species, either through inhibition of protein kinase C or activation of protein kinase B pathways [22].

There are some limitations to these findings. This observational study included patients from a tertiary care medical college and hospital. However, in India, most people come to district, subdivisional, and lower-tier hospitals for treatment. So, results of our study might not reflect the actual scenario of the population as a whole. Probably, a multicentric study would be more beneficial in explaininging the actual statistics. Also, patients were on a number of

medications to control heart failure. However, these treatments are characteristic of patients with DCM and do not affect serum catalase and protein carbonyl levels. Lastly, the total number of patients in the study group was not large enough. Thus, caution should be exercised while extrapolating the present data to other populations.

Despite these limitations, we believe that our study results point toward using catalase and protein carbonyl estimation as an important, potential parameter for DCM. As our findings point to a decrease in the antioxidant enzyme catalase, the problem of oxidative stress in pathogenesis of DCM should be further investigated, and other similar biological parameters to determine oxidative stress should also be assessed. There is a sizeable amount of evidence to suggest that DCM patients are prone to significant alterations at the cellular and molecular level causing structural and functional perturbations in the myocardium, ultimately causing heart failure. Further research is needed so as to ascertain the pathophysiology underlying DCM before suggesting appropriate clinical trials. Advanced understandings into the mechanisms that increase oxidative stress in DCM might lead to newer management strategies. Clinically pertinent studies in this direction might help to further discover the intrinsic mechanisms of DCM.

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