

Hepatic Proteins & Protein Oxidation - Role in Dengue Fever

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Abstract

Protein carbonyl is the biomarker of oxidative stress. An irreversible modification develops, when reactive aldehydes or ketones of ROS reacts with amino acid residues of proteins during oxidation. Dengue virus, the causative agent of dengue fever (DF) and its severe forms can infect different types of cells such as macrophages, endothelial cells and also hepatic cells (site of dengue virus replication) contribute to ROS production. ROS generation has been detected when endothelial cells are stimulated by cytokines, during dengue infection. Thus ultimately leads to loss of membrane function and alterations in the integrity of the membrane carbohydrates, lipids and proteins. It has been suggested that the increased vascular permeability observed in DF is caused by malfunction, a structural destruction of the endothelial & hepatic cells.

Keywords: CRP: C- reactive protein, DF: Dengue fever, PC: Protein carbonyl, ROS: Reactive oxygen species

INTRODUCTION

Globally the incidence of dengue has grown dramatically in the recent years. Every year during the monsoon months and later, many parts of the country witness outbreaks of dengue infection. Classical features of dengue are fever, body ache, rash, thrombocytopenia, hepatic dysfunction and bleeding tendency [1]. In dengue viral infection, there is prolonged activation of T- cells and their apoptosis, has been proposed as a cause of delayed viral clearance [2]. After the virus entry, immune system is activated and the cell produces ROS as a defense mechanism [3]. Oxidants

play a vital role in viral and other diseases, because these are highly unstable and react with lipids, proteins and nucleic acids [4].

Proteins constitute more than 50% of the dry weight of cells, and as such can be considered important targets for the effects of oxidizing species. Proteins are ubiquitous in all cells and tissues and are susceptible to oxidative modification; they can serve as useful markers of oxidative stress [5,6]. ROS leads to oxidation of amino acids by formation of protein – protein linkages resulting in protein fragmentation. Generation of carbonyl derivatives by some of these reactions may serve as markers of oxidative stress in diseased status [7, 8]

Carbonylated proteins can be used as oxidative stress biomarkers according to their special properties including irreversibility, irreparability, stability in physical condition and induction of protein aggregation [9]. The nature of the protein modification can give significant information as to the type of oxidant involved, the present study is aimed to evaluate the hepatic proteins dysfunction and levels of serum protein carbonyl, used as biomarker for the oxidative stress response through dengue virus in dengue fever.

MATERIALS AND METHODS

Thirty dengue diagnosed patients, between the age of 16 and 38 years and with adequate information on clinical and laboratory variables were included retrospectively. The duration of this study was from May-2014 to June-2015, who underwent treatment in Shridevi Institute Of Medical Sciences & Research Hospital, Tumkur. Thirty healthy, age, and sex matched subjects were selected as controls.

History of diabetes mellitus, cardiac disease, patients with any parenteral drug use & past history of fever were excluded in this study. The controls chosen for the study were non dengue healthy individuals of similar age, sex group without liver disease and any other inflammatory disease. Dengue fever was confirmed by immunochromatographic method identifying the antibodies against dengue virus – IgM, IgG and dengue viral antigen – NS1. All the patients were subjected to detailed history, examination, complete blood counts, liver function tests and renal function tests. USG abdomen and chest X- ray done wherever needed.

After taking the serum sample, biochemical analysis were carried out for all parameters, serum proteins using conventional standardized methods on ERBA Chem – 5 semi auto analyzer, diagnostic kits from Erba and protein carbonyl assay by spectrophotometric DNPH method.

Protein carbonyl group assessment

Carbonyl groups in proteins reacts with 2, 4- dinitrophenylhydrazine (DNPH) to form a stable dinitrophenyl (DNP) hydrazones product. This can be assayed spectrophotometrically at an absorbance of 355 nm [10].

Statistical analysis

The statistical analysis was carried out by using the SPSS (Statistical Package for Social Sciences) software. The Student‘t’ test was applied for the statistical analysis

and the results were expressed in mean \pm SD, P values ($p < 0.001$) were considered as highly significant.

RESULTS & DISCUSSION

There were 30 dengue patients included in the study with the serum total protein (8.017 ± 0.778) when compared with controls (6.73 ± 0.415) level were increased. Serum albumin (2.977 ± 0.219 gm/dl) levels were decreased in study group, when compared with controls (3.50 ± 0.305 gm/dl) (Table 1). Serum proteins are affected by capillary permeability, impaired liver functions and inflammation; they are involved in repair and maintenance of immune system along with other body tissues. Liver injury is a common finding in dengue infections and it is mediated by direct infection of hepatocytes and Kupffer cells [11]. Hyper proteinemia is a result of enhanced impairment of the synthetic function of the liver. This may be due to increased need of immune mediators during stress, inflammation & infection by dengue virus and also decreased need for other proteins that are not essential during immune function results in hypo albuminaemia [12, 13].

SGOT levels (108.3 ± 37.8), SGPT levels (114.7 ± 30.09) were increased in study group, when compared with control groups of SGOT levels (23.9 ± 4.18) SGPT levels (24.77 ± 4.739) were statistically significant (Table 1). Increased level of hepatic serum transaminases is possible in dengue fever due to liver parenchymal damage caused by the dengue virus [14]. Estimating the levels of serum transaminases in dengue fever may help in early detection of parenchymal cell damage [15].

Table 1: Mean \pm S.D of plasma hepatic proteins, enzymes and protein carbonyl in healthy individuals (control) & dengue (study) groups

PARAMETERS	Control Group Mean \pm S.D	Study Group Mean \pm S.D
Total Proteins (g/dL)	6.73 ± 0.415	8.017 ± 0.778
Albumin (g/dL)	3.50 ± 0.305	2.963 ± 0.361
SGOT	23.9 ± 4.18	108.3 ± 37.8
SGPT	24.77 ± 4.739	114.7 ± 30.09
CRP (mg/L)	1.3 ± 0.47	4.21 ± 0.588
Protein carbonyl (nmole/ml)	11.69 ± 1.915	21.79 ± 3.31

CRP level significantly elevated in study group (4.21 ± 0.588) when compared with controls (1.3 ± 0.47) (Table 1). CRP is synthesized by the liver and plays an important role in interacting with the complement system in immunologic defense mechanism & develops during inflammation, when acute phase response is triggered by the cytokines, interleukins and tissue necrosis factor released by macrophages and other

cells at the injury site. These factors induce fever and stimulate the liver to produce CRP and other acute phase proteins. CRP levels have been used widely to assess risk factor in the detection and management of chronic infections, inflammatory and other diseases [16, 17].

Serum protein carbonyl levels in the study group (21.79 ± 3.31), when compared with control group (11.69 ± 1.915) were significantly increased (Table 1). Protein carbonyl is produced by oxidative modifications of proteins either by α -amidation pathway, or by oxidation of glutamyl & lysine residues or reactions with aldehydes like malondialdehyde produced during lipid peroxidation and oxidation of glycoproteins [8, 18 & 19]. The oxidative modifications of proteins by ROS are implicated in the etiology or progression of chronic inflammatory diseases and other disorders [20].

CONCLUSION

The diverse studies implicated the liver as a site of dengue virus replication which infects hepatic cells [11, 12 & 21]. This suggests that liver involvement and hepatic protein oxidation may occur during dengue infections, and may outline the possible role played by host immune responses in this process. Elevated levels of protein carbonyl and hepatic protein dysfunctions are evidence of free radical modification of proteins in infection with dengue virus. Thus production of protein carbonyls derivatives is considered an early and stable marker for protein oxidation. Further investigations into the nature of alterations in the oxidative derivatives (protein carbonyl content) may provide a basis for better understanding of pathogenesis and mechanism responsible in the infection of dengue fever.

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