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STUDY OF ASSOCIATION OF OXIDATIVE STRESS, C-REACTIVE PROTEIN AND LIVER ENZYMES IN RELATION TO CHRONIC LIVER DISEASE.

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ABSTRACT **BACKGROUND** : Oxidative stress is a prominent feature in the pathophysiology of both acute and chronic liver disease. Oxidative stress occurs when reactive oxygen species generated from either exogenous sources (e.g. alcohol and acetaminophen) or endogenous sources (e.g. Liver cytochrom P450 enzyme system) and cellular functions (mitochondrial metabolism).Oxidative stress is increasingly recognized as an important factor in the progression of chronic liver diseases of varying etiologies.
AIM: To evaluate oxidative stress status through measurement of Malondialdehyde (MDA) and to analyze association of changes in MDA status with respect to fluctuations in inflammatory marker (CRP) and liver enzymes in CLD.
MATERIALS AND METHODS: The study included 50 Chronic Liver Disease (CLD) cases and 50 age and sex matched controls. Plasma MDA was measured by spectrophotometric method (TBARS) at 531nm, and biochemical parameters like serum ALP, AST, ALT were measured by autoanalyzer(Turbochem-100,model No-4600). Estimation of C-Reactive Protein was done by RHELAX-CRP Slide test on the basis of principle of agglutination.
RESULT- The MDA and CRP levels were increased in CLD cases along with liver enzymes and were statistically significant, There is positive correlation of MDA, CRP with severity of liver disease.
CONCLUSION: It is concluded that MDA, CRP and the transaminases ALT, AST, and ALP levels are higher in the studied liver pathologies as compared to control values

KEYWORDS : MDA, C-reactive protein, nonalcoholic fatty liver, oxidative stress, Aminotransferases ALT & AST), Alkaline phosphatase

INTRODUCTION

The molecular and cellular basis of Chronic Liver Disease (CLD) is not well understood; though, the essential role of the oxidative stress (OS) and the onset of inflammation is being realized. OS is understood to result from a cellular imbalance between levels of oxidants and antioxidants caused by augmented generation of reactive oxygen species (ROS)[1]. Mitochondria and endoplasmic reticulum are the main source of ROS generation; and hepatocytes proteins, lipids and DNA are the principal targets for molecular damage. The popular example is the lipid degradation product Malondialdehyde (MDA) that is generated after ROS mediated lipid peroxidation. It occurs naturally and is considered a marker of OS. ROS degrade polyunsaturated fatty acid and produce MDA. This co mpound is a reactive aldehyde and is one of the many reactive electrophyle species that cause toxicity and stress in cells. OS may result in structural and functional damage or abnormalities in liver. The OS is expected in hepatocytes due to central metabolic function of liver and increased ROS generation during metabolism in hepatocytes.

The physiology of liver involves the filtration and processing of blood as it circulates through the body to oxygenate tissues and collect wastes for metabolism. Liver metabolizes nutrients, detoxifies harmful substances, makes blood clotting proteins, and performs many other vital functions. For being the metabolic centre, liver metabolizes a variety of xenobiotics to their toxic metabolites. The xenobiotic metabolism is carried out by the cytochrome P-450 dependent monoxygenase and in the process ROS are produced. Thus liver is a major organ that is exposed to toxicants and is prone to increased ROS generation accompanied with the obligatory pro-inflammatory changes. Association between ROS generation and pro-inflammatory changes is well known. However, it remains speculative if OS and inflammation at sub-clinical level could form the key basis for liver disorders.

Oxidative stress in mitochondria and endoplasmic reticulum can impact the redox state of cell also and could obligatorily trigger release of pro-inflammatory changes. The redox state of the cell is maintained by cellular antioxidants. To control the level of OS and to maintain a balance between oxidants and antioxidants, the cells are equipped normally with special molecules namely the enzymatic and non-enzymatic antioxidants. The antioxidants are of two types namely preventive or chain breaking. The examples of preventive type are catalase or peroxidase and that of chain breaking type are super oxide dismutase, uric acid, protein bound thiols, vitamin C and E, glutathione, bilirubin.

C-Reactive Protein (CRP) is an annular (ring-shaped) pentamaricprotein found in blood; its levels rise in response to inflammation. CRP is an acute phase protein of hepatic origin that increases following Interleukin-6 secretions by macrophages and adipocytes. Its physiological role is to bind to lysophosphtidyl choline expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the Clq complex [2,3]. CRP is synthesized by liver in response to factors released by macrophages and fat cells (adipocytcs). It is a member of the pentraxin family of proteins that form a group of pattern recognition receptor and are involved in innate immunity [4]. CRP is an acute-phase reactant, synthesized by hepatocytes within 2 hours in response to systemic inflammation, and peaks at 48 hours; and it is helpful for detecting or predicting inflammation. CRP has many pathophysiologic roles in the process of inflammation, including recognizing some foreign pathogens, activating the complement system, initiating the elimination of targeted cells, inducing inflammatory cytokines, and stimulating tissue factor in monocytes. Studies indicate a significant association between higher serum CRP concentrations and higher risk of chronic liver disease mortality.

Liver damage is commonly determined by liver function test (LFT) that evaluates level of enzymes ALT (Alanine transaminase). AST (Aspartate transaminase), Alkaline phosphatase (ALP). gamma glutamyltranspeptidase (GGT), prothrombin time and serum A/G ratio. AST (SGOT) is found in muscles and many other tissues besides the liver, whereas ALT (SGPT) is almost exclusively found in the liver. ALT levels are an easy and inexpensive marker of liver necro inflammation and could be the first-line tool in this process[5]. The elevated amounts of ALT and AST together in the blood signify mostly liver damage. Blood levels of AP and GGT rise when bile flow is slow or blocked. Bile flow problems can be due to a problem in the liver, the gallbladder or the tubes connecting them. Since ALP is also found in bone, it can be elevated in certain medical conditions that affect bone, as well.

In view of above observation in literature, it can be said that OS and inflammation are associated with CLD. However, more studies are needed to validate this association. Exploring the association of OS

with CRP in CLD patients will not only elucidate the importance of OS and inflammation in pathogenesis of disease but will also provide evidence for their utility in improvising relevant clinical management. The OS, CRP together with LFT can offer the potential for large-scale screening of CLD. The study could also provide Opportunity to see sensitivity of CLDs to antioxidant supplemented therapeutic intervention[6]

Aim: To evaluate oxidative stress status through measurement of Malondialdehyde (MDA) and to analyze association of changes in MDA status with respect to fluctuations in inflammatory marker (CRP) and liver enzymes in CLD.

MATERIALS & METHODS

Study Subjects:

Patients attending medicine OPD in Hind Institute of Medical Sciences. Bambanki were enrolled in the study. A total of 50 Chronic Liver Disease (CLD) cases and 50 corresponding (age and sex matched) controls meeting the selection criteria volunteered for study. As per inclusion criteria, CLD cases were the patients of non-alcoholic fatty liver disease, cirrhosis, chronic hepatitis and alcoholic liver disease. Subjects with acute hepatic dysfunction including cholestasis, post-operative conditions, anti-tubercular treatment, and pregnancy and smoking habits were excluded from study as per the exclusion criteria.

METHODS:

Blood samples were collected from the antecubital vein. Rubber tourniquet was applied for less than one minute. The site to be punctured was cleaned with methylated spirit and blood sample (6ml) was taken and allowed to clot, 2 ml serum was separated and stored at -20^0 to estimate the oxidative stress marker (MDA) and inflammatory marker (CRP). Rest of serum are immediately assayed for liver enzyme (ALP,AST & ALT). Plasma MDA was measured by spectrophotometric method (TBARS) at 531nm[7], and biochemical parameters like serum ALP, AST, ALT were measured by autoanalyzer (Turbochem-100, Model No-4600). Estimation of C-Reactive Protein was done by RHELAX-CRP Slide test on the basis of principle of agglutination. The serum is mixed with RHELAX-CRP latex reagent and allow to react. If CRP concentration is greater than 0.6 mg/dl visible agglutination is observed. If CRP concentration is less than 0.6 mg/dl then no agglutination is observed.

RESULT

The study population include 100 subjects. The mean age of cases was 52.16±13.42 years. While the mean age of control was 47.54±9.77 years (Table-I). Among all the cases, 19 (38%) were diagnosed for Liver cirrhosis and remaining 31 (62%) were diagnosed for Non Alcoholic Fatty Liver Disease (NAFLD).

Table I- Distribution of cases & control – Sex and Age wise

Group	Number of Subjects (n)	Male (n)	Female (n)	Age distribution
control	50	29	21	47.54±9.77
cases	50	33	17	52.16±13.42

On comparing the mean ALP values between case & control group it was found that the mean ALP in control group was 72.95 ± 3.76 while in case group it was 81.81±4.03 (Table-II). According to unpaired student's t-test the mean ALP in cases was found to be significantly more than the control group (p<0.001). In this study on comparing the mean AST values between case & control group it was found that the mean AST in control group was 21.46 ±0.57 while in case group it was 31.87±1.12 (Table-II). According to unpaired student's t-test the mean AST in cases was found to be significantly more than the control group (p<0.001). In our study on comparing the mean ALT values between case & control group it was found that the mean ALT in control group was 20.08 ± 0.66 while in case group it was 43.29±1.98 (Table-II). According to unpaired student's t-test the mean ALT in cases was found to be significantly more than the control group (p<0.00l).

Table II- Comparison of ALP, AST, ALT, CRP and MDA between Cases & Control

parameter	Case (mean±SD)	Control (mean±SD)	p-Value
ALP (IU/L)	81.81±4.03	72.95±3.73	<0.001
AST (IU/L)	31.87±1.12	21.46±0.57	<0.001
ALT(IU/L)	43.29±1.98	20.08±0.66	<0.001
CRP(mg/dl)	0.73±0.22	0.30±0.05	<0.001
MDA(nmol/ml)	8.31±0.48	5.95±0.38	<0.001

In our study on comparing the mean CRP values between case & control group it was found that the mean CRP in control group was 0.30 ±0.05 while in case group it was 0.73±0.22 (Table-II). According to unpaired student's t-test the mean CRP in cases was found to be significantly more than the control group (p<0.001).

In our study on comparing the mean MDA values between case & control group it was found that the mean MDA in control group was 5.95 ±0.38 while in case group it was 8.31 ± 0.48.(Table-II) According to unpaired student's t-test the mean MDA in cases was found to be significantly more than the control group (p<0.001).

DISCUSSIONS

The primary laboratory abnormality is the elevated serum AST and ALT levels. However, liver aminotransferase levels are seldom higher than 3 or 4 times the upper limit of normal. The ALT levels are higher than the AST levels in most instances, but the AST level may occasionally be higher than the ALT level, especially in the presence of cirrhosis. A reversal of the ALT/AST ratio to more than 1 had been reported to predict the presence of more advanced fibrosis [8].

Alkaline phosphatases are a group of zinc metalloenzymes that catalyze the hydrolysis of phosphate esters with widespread tissue distribution. As the name implies, this enzyme works best at an alkaline pH, and thus the enzyme is virtually inactive in the blood. Alkaline phosphatases act by splitting off phosphorous, creating an alkaline environment. They are found predominantly in the liver and bone, with intestinal enzymes contributing up to 20% of total activity; other tissue beds include the kidney, placenta, adrenal cortex, lung. Alkaline phosphates are membrane bound glycosylated homodimeric enzymes involved in fundamental biological process. The mean levels of Alkaline phosphates was statistically higher significant. P<0.001 in all liver disease in most of the studies.

Serum amino transferases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) indicate the concentration of hepatic intracellular enzymes that have leaked into the circulation. These are the markers for hepatocellular injury[9].

Higher serum CRP concentrations at baseline were associated with subsequent incidence of liver cancer and death from chronic liver disease. Higher serum CRP is marker for chronic Liver diseases and is an important Prognostic factor[10]

A complex antioxidant system has been developed in mammals to relieve oxidative stress. However, excessive reactive species derived from oxygen and nitrogen may still lead to oxidative damage to tissue and organs. Oxidative stress has been considered as a conjoint pathological mechanism, and it contributes to initiation and progression of liver injury. A lot of risk factors, including alcohol, drugs, environmental pollutants and irradiation, may induce oxidative stress in liver, which in turn results in severe liver diseases, such as alcoholic liver disease and non-alcoholic steatohepatitis[11]

CONCLUSION

It is concluded that MDA, CRP and the transaminases ALT, AST, and ALP levels are higher in the studied liver pathologies as compared to control values. These biochemical changes demonstrate role of oxidative damage in inflammation mediated chronic liver disease. Antioxidants deficiency in liver finds implication in development of the investigated hepatic dysfunction.

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