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Original Research Article

Study of Correlation between Proinflammatory Cytokines and Stress marker enzymes in Alcoholic Liver Disease Patients.

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Abstract:

Background: Alcohol-related liver disease is a major cause of morbidity and mortality worldwide. Chronic alcohol consumption leads to hepatocellular injury, fat accumulation and liver inflammation. Progression of ALD is well characterized and is actually a spectrum of liver diseases, which ranges initially from simple steatosis to inflammation and necrosis (steatohepatitis), to fibrosis and cirrhosis.

Objectives: The study was designed to determine the effect of Pro-inflammatory cytokines & stress marker enzymes in alcoholic liver disease patients with reference to normal healthy individuals.

Methods: 175 alcoholic liver disease patients were enrolled for the study & were compared to 150 normal healthy individuals of the same age from the Outdoor Patient Department of Maharaja Yeshwant Rao Hospital of Indore city. Those fulfilling inclusion & exclusion criteria were enrolled for the study & the blood samples were analysed for TNF-α, IL-6, plasma MDA and SOD.

Results: Significant higher concentrations of TNF-α ($p<0.001$), IL-6 ($p<0.001$), and MDA ($p<0.001$) was demonstrated in patients with alcoholic liver disease when compared with normal healthy individuals. A significantly lower concentration of SOD ($p<0.001$) was demonstrated in patients with alcoholic liver disease when compared with normal healthy controls.

Conclusion: Alcohol consumption causes excessive cytokine production in the liver, leading to inflammatory liver disease. Alcohol-induced liver injury is linked to oxidative stress as observed by decreased levels of SOD and increased levels of MDA.

Keywords: Alcoholic Liver Disease, Pro-inflammatory Cytokines, TNF-α, IL-6 MDA, SOD.

Introduction

Alcohol toxicity is the third most frequent cause of illness and death, contributing to 4.6% of cases of disability-adjusted life years (DALYs) and 3.8% of global fatalities (EASL, 2012) [1]. Chronic alcohol use damages the liver's cells, causes fat accumulation, liver aggravation, and can occasionally result in liver cirrhosis or hepatocellular malignancy. Persistent alcohol consumption results in the pathophysiology of alcoholic liver disease (ALD). The risk of more than 60 major types of diseases is caused by alcoholism, which is one of the most frequently perceived symptoms of alcohol consumption. These and other effects of alcohol consumption have raised alcohol to the third-most important risk factor for illnesses and disabilities worldwide [2]. The liver is the principal organ effected by alcohol damage and the primary site of alcohol metabolism. A spectrum of liver diseases, comprising simple steatosis (fatty liver) to inflammation, necrosis (alcoholic steatohepatitis), fibrosis, and ultimately cirrhosis and hepatocellular carcinoma (HCC), are represented by the progression of alcoholic liver disease (ALD) [3]. (Gao B., Bataller R 2011). Alcohol and alcohol metabolism, dysregulated inflammation, oxidative stress, and altered extracellular matrix (ECM) metabolism all play a role in the development and progression of alcoholic liver disease.

Alcohol-induced liver damage is largely due to inflammation, which is a result of both resident

(such as Kupffer cells) and recruited (such as neutrophils and lymphocytes) inflammatory cells. In contrast to parenchymal cells, which make ROS mostly as a result of electron leakage from metabolic reactions, inflammatory cells are "professional" ROS/RNS producers with the ability to produce significant levels of both species in their milieu. Induction of oxidative stress and inflammation can initiate a vicious cycle that results in progressive tissue damage.

TNF-α belongs to a family of cytokines that stimulates acute inflammation and is a cytokine involved in systemic inflammation. Different cellular types in the body release TNF- α. TNF- α is primarily produced by Kupffer cells in the liver and is a key mediator in a number of physiological processes, including inflammation, cell growth, and apoptosis. Reactive oxygen species (ROS) and inflammatory cytokines secreted by kupffer cells (M. Bilzer et al. 2006) [4] stimulate cells such as hepatocytes, hepatic stellate cells, and endothelial cells (A. M. Diehl, 2005) [5]. Chronic alcohol consumption increases the susceptibility of Kupffer cells to LPS-induced TNF-α production (A. Aldred and L. E. Nagy, 1999) [6]. Patients with ALD had higher serum TNF-α, which is correlated with mortality. TNF-α is associated with the onset of liver damage in ALD. Additionally, ethanol consumption has been shown to cause oxidative stress in the liver and extra hepatic tissues, which is related to an imbalance between the pro-oxidant and antioxidant systems (I.Dupont, et.al.) [7]. The present is carried out to assess Lipid peroxidation, Antioxidant status, TNF-α and IL-6 in the blood of Alcoholic Liver disease patients and Normal healthy subjects.

Material & Methods

The present study was undertaken in the Department of Medical Biochemistry, MGM Medical College Indore (M.P.). The study group comprises of 175 histologically & ultrasound scan proven Alcoholic Liver Disease patients and 150 Healthy Individuals matched for age and dietary habits were treated as controls. The study subjects were randomly selected irrespective of age or occupational status. The age of subjects ranged between 20-70 years and the educational status ranged from illiteracy to post-graduation. Alcoholic liver disease patients selected for the study were, further confirmed by questionnaire, laboratory investigations and clinical findings. The need and probable outcomes of the study were explained, and written informed consent was taken from all the participants.

Inclusion Criteria: Patients with ALD in the age range of 20 to 70 years were diagnosed based on their history of alcohol consumption (amount and duration), clinical examination, abdominal sonography, and, where necessary, liver biopsy.

Exclusion Criteria: Patients suffering from renal disorders, diabetes mellitus, obesity, hypertension, thyroid disease, cardiovascular disease, viral hepatitis, hepatitis A and B, patients with asthma, patients with lung cancer or any other form of cancer, Gout, TB, HIV, malnutrition, malabsorption, and/or patients with any other infectious diseases were excluded from the study.

Blood sampling was, performed in the morning, following a not less than 12 hr. fasting period. 5ml of blood (venous) samples from the ALD patients and healthy normal controls were collected in sterile tubes under aseptic conditions. Biochemical parameters analysed were Plasma MDA, serum SOD, TNF-α and IL-6. The Institutional Ethics Committee granted ethical approval. The statistical packages for social science (SPSS) software, version 20, was used to compute and analyse all of the data. The values are shown as Mean ± SD. $p< 0.05$ is considered as Significant and $p< 0.001$ is considered as highly significant.

Results

Table 1 shows Values of pro-inflammatory cytokines and stress marker enzymes between control and ALD patients. It is evident from the table 1 that the mean values of TNF-α, IL-6, and MDA was found to be higher in alcoholic liver disease patients when compared with the control subjects. The mean value of SOD was found to be significantly lower in alcoholic liver disease patients when compared with the control subjects. The difference in values of TNF-α, IL-6, MDA and SOD in study group and controls was found to be highly significant ($p<0.001$). Table 2 shows Correlation analysis between pro-inflammatory cytokines and stress marker enzymes in ALD patients.

Table 1: Values of Pro-Inflammatory Cytokines and Stress Marker Enzymes Between Control and ALD Patients.

S.No.	VARIABLES	CONTROLS	ALD	p-VALUE
1	TNF-α (pg/ml)	4.17±1.28	15.41±10.74	<0.001
2	IL-6 (pg/ml)	2.30±1.09	17.81±14.69	<0.001
3	P-MDA (nmol/ml)	1.80±0.75	5.71±2.95	<0.001
4	S-SOD (U/g of Hb)	6.83±1.26	4.06±1.38	<0.001

Table 2: Correlation analysis between Pro-inflammatory Cytokines and Stress marker enzymes in ALD patients

Correlation between parameters		Correlation coefficient	
		R	p Value
TNF-α	IL-6	0.73	<0.001
	MDA	0.75	<0.001
	SOD	-0.53	<0.001
IL-6	TNF-α	0.73	<0.001
	MDA	0.77	<0.001
	SOD	-0.66	<0.001
SOD	TNF-α	-0.53	<0.001
	IL-6	-0.66	<0.001
	MDA	-0.65	<0.001
MDA	TNF-α	0.75	<0.001
	IL-6	0.77	<0.001
	HOMA-IR	0.40	<0.001

Discussion

Alcohol either directly or indirectly stimulates Kupffer cells to generate and release TNF-α into the tiny channels (i.e., sinusoids) where the blood passes through the liver. Alcohol also makes hepatocytes more sensitive to TNF-α (Tumor necrosis factor α). The production of TNF-α is one of the first incident of liver damage, which triggers the production of other cytokines that together recruit inflammatory cells, kill hepatocytes and even initiate a healing reaction involving fibrogenesis. TNF-α activates caspase-8, a protease, in the cytoplasm, which cleaves the protein Bid, in response to the release of cytochrome c oxidase from mitochondria. Cytochrome c oxidase activates caspase-3 and causes apoptosis. Similarly, TNF-α induces sphingomyelinase, which increases ceramide, an inhibitor of the activity of the mitochondrial electron transport chain, which leads to an increase in mitochondrial production of ROS, which, in turn, promotes lipid peroxidation and cell necrosis [8]. In the present study the mean values of TNF-α in ALD patients (15.41±10.74) was found to be highly increased when compared with the control subjects (4.17±1.28) (Table-1). The difference in p values ($p<0.001$) of TNF-α in ALD patients and control was found to be highly significant. The results of the present study are in accordance with the work of T. Kitazawa, et al. 2003 [9], McClain CJ et al. (1997) [10]. McClain CJ et al. 1997 [10] reported that ALD patients have high levels of interleukins 6 and TNF-α. And raised levels of TNF-α are correlated with poor prognosis in patients with alcoholic hepatitis.

In the present study the mean values of IL-6 in ALD patients (17.81±14.69) was found to be highly increased when compared with the control subjects (2.30±1.09) (Table-1). The difference in p values ($p<0.001$) of IL-6 in ALD patients and control was found to be highly significant. The results of the present study were consistent with the work of Deviere et al. (1989) [11], Prystupa A et al. (2017) [12]. D. B. Hill, et.al. (1992) [13] also found the elevated serum levels of TNF- α and IL-6 in patients with AH.

Alcoholism is linked to modifications in the oxidant antioxidant system and cell function. In the present study the mean values of MDA in ALD patients (5.71±2.95) was found to be highly increased when compared with the control subjects (1.80±0.75) (Table-1). The difference in p values ($p<0.001$) of MDA in ALD patients and control was found to be highly significant. The results of the present study were in consistent with the work of Nalini G et al., (1999) [14].

The decreased antioxidant enzyme could be due to increase in MDA which can cross link with the amino group of enzyme protein. The elevated free radical and their metabolites decrease the plasma antioxidant status in ALD. Mean values of SOD in ALD patients (4.06±1.38) was found to be decreased significantly when compared with the control subjects (6.83±1.26) (Table-1). The difference in p values ($p<0.001$) of SOD in ALD patients and control was found to be highly significant. Chen YL et al. (2011) [15], Pujar S et al. (2011) [16], Chari S et al. (2003) [17], Janani AV et al., (2010) [18] reported significantly decreased erythrocyte SOD ($p<0.05$) in ALD patients. This result corroborates with previous studies, which shows that as the severity of the alcoholic liver disease increases, serum level of malondialdehyde (MDA) was increased and the serum concentrations of SOD was decreased in ALD patients. Evidence also suggests that the plasma membrane becomes permeable due to severe peroxidative damage, allowing entire cells to leak their cytosolic enzymes [19, 20, 21, 22].

The Pearson correlation was done to observe the significance of relationship in order to measure the strength and direction of relationship of TNF-α, IL-6, P-MDA and SOD in ALD patients.

TNF-α induces mitochondria to increase production of reactive oxygen species. Free radicals initiate lipid peroxidation, which causes inflammation and fibrosis. In this study the strength of correlation of TNF-α with IL-6 & P-MDA was found positive and

statistically highly significant ($p<0.001$). The strength of correlation of TNF-α with SOD was found to be negative (inversely correlated) and statistically highly significant. The present findings were also reported by Craig J. McClain et al. (2004) [23]. TNF- α increases gut permeability & induce oxidative stress. The strength of correlation of IL-6 with TNF-α & P-MDA was found positively correlated and statistically highly significant ($p<0.001$) in this study. The strength of correlation of IL-6 with SOD was found to be negative (inversely correlated) and statistically highly significant ($p<0.001$).

The strength of correlation of P-MDA with TNF-α & IL-6 was found to be positively correlated in ALD patients and statistically highly significant ($p<0.001$) in this study. The strength of correlation of P-MDA with SOD was found to be negative (inversely correlated) and statistically highly significant ($p<0.001$). In this study the strength of correlation of SOD with TNF-α & IL-6 was found negatively correlated (inversely correlated) in ALD patients and statistically highly significant ($p<0.001$).

Conclusion

Numerous alterations in cellular processes and the oxidant-antioxidant system are linked to alcohol intake. Results of present study concluded that the Proinflammatory Cytokines i.e. TNF-α and IL-6 are strongly involved in the pathogenesis of ALD & may help in assessing the severity and prognosis of the disease. In our study the levels of TNF-α and IL-6 was found to be significantly higher in ALD patients when compared with the control group.

Results of current study also shows that patients with alcoholic liver disease have higher plasma levels of MDA and lower levels of SOD activity, this finding may be related to increased exposure to an oxidising environment that can destabilise RBC membrane through lipid peroxidation and result in significant leakage of these intracellular enzymes. Additionally, poor dietary intake of antioxidant minerals caused by increasing alcohol consumption has a negative impact on the activity of these metalloenzymes. All of these factors taken together may serve as a valuable signal for identifying and assessing the severity of alcoholic liver disorders.

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