RESEARCH

COMPARATIVE STUDY OF ANTIOXIDANTS VITAMINS AND ENZYMES IN ALCOHOLIC LIVER DISEASES IN KARNATAKA POPULATION

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Abstract

Background: Consumption of alcohol causes liver damage by several patho-physiological mechanisms which alter the ROS (Rich Oxygen Spices) which has high anti-oxidant vitamins and enzymes. Their values are altered due to excess amount of ethanol. Hence parameters of anti-oxidant indicate the severity of alcohol liver disease. **Materials and Methods:** 35 alcoholic liver disease patients were compared with same number of healthy (controlled) group. Clinical and laboratory investigations were carried out by venous blood plasma vit. E levels by Baker Hatal method, Ascorbic acid by Teitz method and SVD by Beers and seizer method. **Result:** The comparison of non-enzymatic oxidants parameters. Ascorbic acid, vitamin E and comparison of anti-oxidant enzymes SOD, GPX in both groups were statistically highly significant ($p<0.00$). **Conclusion:** Increased oxidant enzymes SOD, GPX except catalase and decreased non-enzymatic oxidants values observed in alcohol liver patients. This pragmatic study will help the physician to treat such alcoholic liver patients treat efficiently to avoid morbidity and mortality because treating alcoholic liver patients is a challenge for clinician globally.

INTRODUCTION

Alcohol is a hepato-toxic that is consumed globally and is associated with a spectrum of liver injury including simple steatosis or fatty liver, alcohol hepatitis fibrosis and cirrhosis. Alcoholic liver disease (ALD) is a general term used to refer to this of alcohol-related liver injuries.[1]

Several studies have demonstrated that consumption of ethanol-containing diets significantly increased hepatic CYP_2E_1 level without significantly affecting plasma alanine transferees(ALT) activity. Hence a potent endogenous anti-oxidant system can prevent potential damage via the excessive expression of CYP_2E_1.[2]

Binge drinking may cause liver injury, as demonstrated by increased blood levels of ALT, aspirate aminotransferees(AST) and / or lactate dehydrogenise (LDH) and lipid accumulation in the alcoholic liver.[3]

It alters metabolic pathways and leads to production of reactive oxygen species (ROS) which has high oxidant properties and also called free radicals.[3] These free radicals cause lipid per-oxidation and it is considered to be the major mechanism of cell membrane destruction and damage of liver.[4] In both physiological and pathological conditions of mammalian tissues free radicals are formed. The un-controlled production of free radicals plays an important role in tissue damage induced by several patho-physiological conditions. Under such conditions there is a variation in oxidant antioxidant profiles because these oxidants and antioxidants try to minimize the damage to liver tissue. Evaluation of these parameter indicates the gravity and degree of liver tissue damage. Hence concentration of anti-oxidant vitamins and antioxidant enzymes were studied in alcoholic liver disease patients and compared with healthy group of people.[5]

MATERIALS AND METHODS

70 (seventy) adult patients regularly visiting Medicine department of faculty medicine science Khaja Banda Nawaz university hospital Kalaburgi-585104, Karnataka were studied.

Inclusive Criteria

Alcoholic liver disease patients aged between 25 years to 50 years.

Exclusion Criteria

Non-alcoholic liver disease patients, malignancy of liver, patients having renal, cardiovascular and other

systemic disease. Immune compromised patients were excluded from the study.

Method

Detailed clinical examination and laboratory investigations were done in both 35 controlled (group-A) and 35 alcoholic liver disease patients (group-B). The venous blood samples were taken from each patient and used for estimation of Ascorbic Acid, SOD (Superoxide Dismutase), GPX (Glutathione peroxide), catalase and MDA (Malondialdehyde) in erythrocytes and vitamin E in plasma. The venous blood samples for the analysis were taken in fasting state and under aseptic conditions. Plasma was separated by centrifugation at 100 rpm 15 minutes. Separated plasma was used for the measurement of the activity of vitamin E. Ascorbic acid levels were estimated in plasma by the method of Teitz (5). Plasma vitamin E levels were estimated by the method of Baker Hetal. SOD (EC1.15.1.1) activity was determined in the hemolysate by the method of Beers and sizer. The activity of Glutathione peroxide GPXEC (1.11.1.9) was measured as described by pagila and valentine in erythrocytes. All reagents used were analytic reagents obtained from Sigma chemicals, St. Louis, Missouri (MO).

The duration of study was June-2021 to May-2021.

Statistical Analysis

Comparison of Non-enzymatic oxidant values and antioxidant values in both groups were compared with z test and significant results were noted. The statistical analysis was carried out in SPSS software. The ratio of the male and females was 2:1.

RESULTS

[Table 1] Comparison of non-enzymatic antioxidants in both controlled and Alcoholic liver disease

- Mean value of Ascorbic Acid (mg/dl) in groups (controlled) was 1.44 (SD± 0.28) and alcoholic liver patients 1.63 (SD± 0.24) in t test 3.04 and p value was highly significant (p<0.03).
- Mean value of vitamin E (mg/dl) 1.40 (SD± 0.39) in controlled group, 1.73 (SD± 0.48) in alcoholic liver disease t test 3.34 and p<0.001 (p value was highly significant).

[Table 2] Comparison of values of antioxidant enzymes in controls and alcoholic liver disease patients.

- Mean value of SOD (U/mg/of protein) 8.04 (SD± 0.55) in controlled, 12.09 (SD± 0.82) in alcoholic liver disease patient and t test was 23.8 and p<0.00 (p value was highly significant).
- Mean value of GPX (U/gm of H) 26.04 (SD± 1.44) in controlled group 43.89 (SD± 1.30) in alcoholic liver disease patients, t test was 44.9 and p<0.00
- Mean value of catalase (nmol H2O2 decomposed / mg protein level) 11.25 (SD± 0.40) in controlled, 10.38 (SD± 0.32) was in alcoholic liver patients, t test was 10.1 and p<0.00(p value was highly significant).

Table 1: Comparison of values of Non-enzymatic parameters in healthy (controls) and alcoholic liver disease patients

Parameters of Non-Enzyme oxidants	Controlled group-A (No. of patients 35) Mean value with SD	Alcoholic liver disease patients group-B (No. of patients 35) Mean value with SD	t test	p value
Ascorbic Acid (mg/dl)	1.44 (± 0.28)	1.63 (± 0.24)	3.04	P<0.03
Vitamin E (mg/dl)	1.40 (± 0.39)	1.75 (± 0.48)	3.34	P<0.001

Both parameters are highly significant (p<0.001)

Table 2: Comparison of antioxidant enzymes in both groups (controlled and alcoholic liver patients)

Parameters	Control group-A No. 35	Alcoholic liver patients No. 35	t test	p value
SOD (U/mg of protein)	8.04 (± 0.58)	12.09 (± 0.82)	23.8	P<0.00
GP (U/gm H)	26.04 (± 1.44)	43.89 (± 1.32)	44.4	P<0.00
Catalase (nmol H2O2 decomposed/mg protein/I	11.26 (± 0.40)	10.38 (± 0.32)	10.1	P<0.00

All three parameters of anti-oxidant enzyme are highly significant (p<0.001)
SOD= Super Oxidedismatase, GPX = Gluta-thioneperoxidase

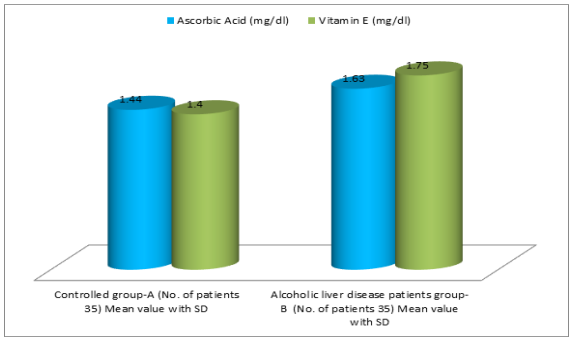

Figure 1: Comparison of values of Non-enzymatic parameters in healthy (controls) and alcoholic liver disease patients

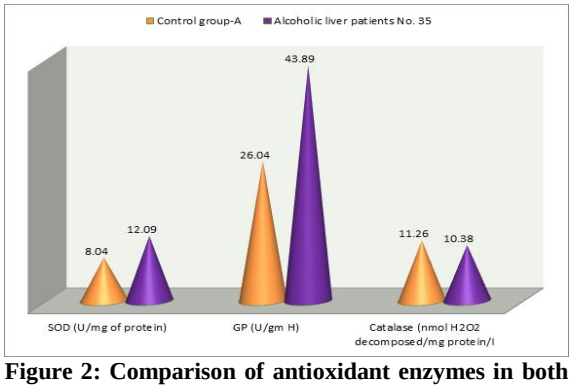

Figure 2: Comparison of antioxidant enzymes in both groups (controlled and alcoholic liver patients)

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DISCUSSION

Present comparative study of anti-oxidant vitamins and enzymes in alcoholic liver diseases in north Karnataka population. The non-enzymatic oxidant values in alcohol disease were compared with healthy (controlled) group. Mean value of Ascorbic acid (mg/dl) 1.44 (± 0.28) in healthy, 1.63 (± 0.24) in alcoholic liver disease patients t test was 3.04 and p<0.003. Mean value of Vitamin E (mg/dl) 1.40 (± 0.39) in controlled, 1.75 (± 0.40) in alcoholic liver disease, t test was 3.34 and p<0.001 [Table 1]. In comparison of anti-oxidant enzymes mean value of SDO (U/mg of protein) 8.04 (± 0.58) in controlled, 12.09 (± 0.80) in alcoholic liver disease, t test was 23.8 and p<0.00. Mean value of GPX (U/gm H) 26.04 (± 1.44) in controlled, 43.89 (± 1.32) in alcoholic liver disease patients, t test was 44.4 and p<0.00. Mean value catalase (nmo1H2O2 decomposed protein /1 min) 11.26 (± 0.40) in healthy (controlled), 10.38 (± 0.32) in alcoholic liver disease, t test 10.1 and p<0.00 [Table 2]. These findings are more or less in agreement with previous studies.[6,7,8,9]

It is widely accepted that oxidation stress plays a central role in alcohol induced pathogenesis. Liver provides the primary site for alcohol metabolism, therefore the effects of alcohol are more pronounced in liver than any other organ. Here detoxification of variety of compounds in our ingested foods or drugs including alcohol by cytochrome P450 molecules uses molecular oxygen and generates ROS. The oxidative damage is further potentiated by alcohol induced decrease in antioxidant enzymes and chemicals particularly glutathione. ROS directly or via its generation via mitochondria are involved in activation of oxidative stress; activation triggers the induction of inflammatory genes and plays a role in initiation and progression of chronic inflammatory diseases. Significant decrease of non-enzymatic oxidant parameters i.e. ascorbic acid, Vitamin E and increase in the antioxidant enzymes, SOD GPX clearly suggests an increase defence against oxidant and non-oxidant damage of liver tissue.[10,11] It is reported that level of erythrocyte MDA (malandialdehyde) was significantly higher in patients with alcohol liver disease due to damage of liver through excess dosage of Ethanol, and ethanol toxicity reduces the catalase levels by generation of excess ROS leading to the production of oxidative stress.[12,13] On the other hand acetaldehyde the metabolic and product of ethanol oxidation by alcohol dehydrogenase or by cytochromes causes the consumption of antioxidants and in activation of antioxidants and responsible for increased generation of free radicals.[14,15]

Chronic alcohol feeding increase AP1 (Activator protein -1) expression in liver Activation of API by chronic alcohol is likely to be important in mediating the inflammatory phase of alcohol induced liver injury as API regulates transcription of genes involved in the inflammatory response.[16,17]

The decreased concentration of measured anti-oxidant enzymes in alcoholic hepatitis could probably be associated with oxidative stress and / or decreased anti-oxidant defence mechanism.[18] GPX (Glutathione peroxidise) activity found to be decreased in alcoholic patients in comparison to healthy subjects. It clearly indicates an imbalance between oxidant and anti-oxidant defensive systems in the body under such pathological scenario.[19]

Vitamin E a potent anti-oxidant and its role as inhibitor (chain breaker) of lipid per oxidation is well established. Alcohol appears to interfere with the body's normal vitamin E content hence patients with alcoholic liver exhibit reduced vitamin E.[20] Hence disease (ALD) can ultimately define the diagnosis according to the typical presence and distribution of hepatic steatosis, inflammation and Mallory-Denk bodies, because of the potential reversible nature of ALD with sobriety regular screening of the alcoholics and early diagnosis are essential.

CONCLUSION

Present comparative study of levels of antioxidants vitamins and enzymes in alcoholic liver disease patients and controlled group. There was significant increased values of antioxidants, vitamins and catalyse activities in alcoholic liver disease patients as compared to normal group because there is increased oxidative stress in alcoholic liver disease patients and to regulate the increased oxidative stress, these vitamins, anti-oxidants and enzymes act as compensatory roles to normalise the liver functions. Hence there is significant increase in the levels of vitamins, anti-oxidant and enzymes. This study demands further patho physiological studies in large number of patients of both sexes at different age groups to confirm these positive findings with latest bio technological methods because exact aetio-pathogenesis of alcoholic liver diseases is still un-clear.

Limitation of study

Owing to the lack of latest technology, less number of patient's, remote location of our institution we have limited findings.

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