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COMPARATIVE STUDY OF OXIDATIVE STRESS AND ANTIOXIDANT VITAMINS STATUS IN ALCOHOLIC LIVER DISEASE - A CASE-CONTROL STUDY

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Abstract

Background

Oxidative stress in the case of ALD can be treated with antioxidants which improve the treatment outcome of liver cirrhosis. It is necessary to determine the levels of vitamins which act as antioxidants.

Objectives

This study aimed to compare the levels of antioxidant vitamins in patients with ALD and the control group.

Materials and Methods

There were 60 controls and 60 ALD patients reporting at the gastroenterology department who were categorized as mild, moderate, and severe ALD. The bilirubin and MDA for oxidative stress were determined which indicated severity. The vitamin C & E levels were determined and compared between the controls and the study groups.

Results

The study included 120 participants. ALD participants had significantly higher weight and BMI compared to controls ($p < 0.001$), and most were male. Alcohol consumption increased with ALD severity, from 976.45±57.45 ml/week in the mild group to 1876.55±45.1 ml/week in the severe group, versus 355.12±50.1 ml/week in controls ($p = 0.002$). Age differences were not significant. Bilirubin in the severe ALD group was 6.4±0.34 mg/dl and in the control group was 0.92±0.2 mg/dl. MDA in the severe ALD group was 14.13±0.56 mg/dl and in the control group was 6.8±0.34 mg/dl. Vitamin C in the severe ALD group was 0.19±0.31 mg/dl and in the control group was 0.71±0.23 mg/dl. Vitamin E in the severe ALD group was 0.32±0.37 mg/dl and in the control group was 0.85±0.43 mg/dl. The difference in each case was statistically significant.

Conclusion

The severity of alcoholic liver disease increases with oxidative stress and with an increase in the bilirubin level. Also, in the case of severe liver disease, there is a significant decrease in vitamin C & E levels.

Recommendation

Vitamin C & E antioxidant therapy should be included in treating ALD.

Keywords: *alcoholic liver disease, MDA, Vitamin C, Vitamin E*
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Introduction

Alcohol abuse is a common problem detected in India, especially in rural areas. Nevertheless, the urban parts of India have also reported liver cirrhosis due to alcoholic abuse. Overall, the liver disorder associated with alcohol is prevalent in India. Alcohol itself and its metabolites are toxic to various organ systems. When this is associated with the deficiencies in the antioxidants it can cause severe hepatic cirrhosis. Study shows that alcohol is hepatotoxic because it initiates lipid peroxidation [1]. The lipid peroxidation causes an increase in the free radicals which leads to cirrhosis of the liver. The liver is infiltrated with leucocytes and free radicals in alcoholic fatty liver disease. Apart from lipid peroxidation, the metabolism of alcohol undergoes two more processes for the generation of oxygen free radicals. The nicotinamide adenine dinucleotide hydrogen and decrease in the beta-oxidation in mitochondria. Both processes cause a decrease in the

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endogenous antioxidants that are glutathione and S-adenosyl-L-methionine [2].

Alcohol-associated liver disease is reversible if there is a cessation in the consumption of alcohol, but the deficiencies of antioxidants cause this process to be irreversible and lead to deleterious complexities. The free radicals are generated when there is lipid breakdown and there is no hydrogen donor (antioxidant) available to stop the chain reaction. Over a long period, this condition worsens as the chain reaction increases the number of free radicals. The free radicals disrupt the lipid cell wall of the hepatocytes. They also interfere with the process of DNA replication and the normal functioning of the hepatocytes. Studies have also shown that the metabolism of ethanol through enzymatic pathways results in an overproduction of NADH and reactive oxygen species, contributing to oxidative stress and liver damage [3-5].

A treatment strategy that completely reverses the inflammation has not been reported. People with alcoholic liver disease are recommended to make lifestyle modifications such as limiting the consumption of alcohol, regular exercise to improve inflammation, and other dietary restrictions to improve the functioning of the liver. The alcohol-related liver diseases are mostly associated with a deficiency of antioxidants [3]. Water-soluble vitamins such as vitamin C and vitamin E are antioxidants that reduce oxidative stress in the body.

Studies are reporting the hepatoprotective role of vitamin E in alcoholic liver diseases [6,7]. People who are deficient in vitamin E tend to develop severe oxidative stress. Although it is known that vitamin E can improve liver cirrhosis with its antioxidant properties, its monotherapy has been studied and the results are contradictory. This makes it necessary to understand the level of deficiencies to determine the exact dosage of these antioxidants. There are various studies regarding the treatment of alcoholic liver disease with antioxidants but there is limited literature regarding the levels of antioxidants in patients with alcoholic liver disease [2,3,8]. This study is conducted to compare the levels of antioxidant vitamins in patients suffering from alcoholic liver disease.

Method

Study design

This case-controlled study.

Study setting

The study was conducted prospectively at the gastroenterology department of Shimoga Institute of Medical Sciences, Shivamogga, Karnataka.

Study Duration

18 months from Dec 2013 to May 2015

Participants

The patients who reported at the gastroenterology department with alcoholic fatty liver disease were included in the study. Since this was a case-control study, the controls were recruited from other departments such as dermatology, ophthalmology, and physiotherapy department. The controls were recruited if they did not have any liver disease in the past including jaundice. The controls were confirmed as not having any gastric surgery in the past. The alcoholic liver disease was confirmed in the participants of the study based on ultrasonography reports. The reports showed an enlarged hepatic vein, portal vein, splenomegaly, echogenicity, and echotexture of the liver along with infiltration of leucocytes and lesions on the liver tissue. The biochemical test included testing of the serum bilirubin level and prothrombin time. The participants with other liver diseases (e.g., hepatitis C, non-alcoholic fatty liver disease), gallstones, liver infections, a history of gastric surgery, or severe comorbidities. Controls with any history of liver disease, including jaundice, were also excluded.

Bias

The study minimized potential bias by using well-matched controls without liver disease and by ensuring comparable demographic characteristics such as age, gender, and BMI across the study groups. Additionally, objective biochemical tests, like serum bilirubin and oxidative stress markers, were used to classify disease severity, reducing subjective bias.

Data collection and Procedure

The blood was withdrawn from the vein and then it was allowed to coagulate, the prothrombin time was calculated. After centrifugation, the serum was separated from which bilirubin was determined. According to the results, the participants were divided into three groups. The participants who had a bilirubin level of less than 5mg/dl and prothrombin time less than 20 seconds were categorized as mild ALD. The patients with bilirubin levels of more than 5mg/dl and prothrombin time less than 20 seconds were categorized as moderate ALD. The patients with bilirubin levels of more than 5 mg/dl and prothrombin time of more than 20 seconds were categorized as severe ALD.

The malondialdehyde was used as a biomarker to measure the oxidative stress associated with alcoholic liver disease. The vitamin C and vitamin E were assayed from the blood.

Statistical analysis

In an Excel sheet data was tabulated. The categorical data such as age, BMI, level of vitamin C & E, serum bilirubin level, and MDA were calculated as average and standard deviation. The data was then compared among the control

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and each category of ALD patients. The p-value was determined. If it was less than 0.05, then it was considered to be significant.

Ethical consideration

The Ethics committee of the institute approved this study and consent was obtained from the participants and the patients of each category.

Results

There were 120 participants in the study. 60 of them were controls recruited from other departments. The other 60 were diagnosed with alcoholic liver disease in the gastroenterology department. 60 of the study participants were categorized according to the severity of alcoholic liver disease. 20 of them were in the mild category, 20 of them in the moderate category, and the remaining 20 were in the severe category. The averages of the control and the study groups were comparable. The weight and BMI of the study group were significantly higher than that of the control group. Overall, the study had a greater number of males suffering from alcoholic liver disease. Table no. 1 shows the comparison between the demographical characteristics of the participants in the study.

Table no.1: Demographical data of the participants

Parameters	Control	Mild group	Moderate group	Severe group	p-value
Age	33.34±8.8	35.35±7.8	32.45±6.7	31.89±3.3	0.2
Weight	72.44±3.4	85.55±7.8	86.77±3.4	89.44±5.3	0.001
BMI	22.1±1.5	29.15±2.3	30.4±4.5	31.6±2.3	0.001
Gender (no. of males)	48/60	15/20	16/20	18/20	0.15
Average consumption of alcohol	355.12±50.1/ week	976.45±57.45/ week	1200.32±65.23/ week	1876.55±45.1/ week	0.002

The bilirubin levels were determined, and it was observed that the study group had a higher level of bilirubin. The severe ALD group had 6.4±0.34 mg/dl and the control group had 0.92±0.2 mg/dl. The difference was found to be statistically significant. Table no. 2 gives the comparison of the level of bilirubin among the control and study groups.

Table no.2: Comparison of bilirubin levels

Sr no.	Category	Average bilirubin level (mg/dl)	p-value
1	Control	0.92±0.2	0.0001
2	Mild group	4.4±0.2	
3	Moderate group	5.3±0.5	
4	Severe group	6.4±0.34	

The malondialdehyde levels were determined to estimate the oxidative stress, it was observed that the study group had higher levels of malondialdehyde. The severe ALD group had 14.13±0.56 mg/dl and the control group had 6.8±0.34 mg/dl. The difference was statistically notable. Table no. 3 gives the comparison of the level of MDA among the control and study groups.

Table no.3: Comparison of MDA level

Sr no.	Category	Average MDA level (nmol/dl)	p-value
1	Control	6.8±0.34	0.0001
2	Mild group	9.35±0.63	
3	Moderate group	10.56±0.65	
4	Severe group	14.13±0.56	

The vitamin C levels were determined, and it was observed that the study group had significantly lower levels of vitamin C. The severe ALD group had 0.19±0.31 mg/dl and the control group had 0.71±0.23 mg/dl. The difference was statistically notable. Table no. 4 gives the comparison of the level of vitamin C among the control and study groups.

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Table no.4: Comparison of vitamin C level

Sr no.	Category	Average vitamin C level (mg/dl)	p-value
1	Control	0.71±0.23	0.001
2	Mild group	0.37±0.21	
3	Moderate group	0.25±0.41	
4	Severe group	0.19±0.31	

The vitamin E levels were determined, and it was observed that the study group had significantly lower levels of vitamin E. The severe ALD group had 0.32±0.37 mg/dl and the control group had 0.85±0.43 mg/dl. The difference was statistically notable. Table no. 5 gives the comparison of the level of vitamin E among the control and study groups.

Table no. 5: Comparison of vitamin E level

Sr no.	Category	Average vitamin E level (mg/dl)	p-value
1	Control	0.85±0.43	0.0001
2	Mild group	0.76±0.34	
3	Moderate group	0.43±0.25	
4	Severe group	0.32±0.37	

Discussion

The study compared oxidative stress markers and antioxidant vitamin levels between 60 patients with alcoholic liver disease (ALD) and 60 controls. The ALD patients were further categorized into mild, moderate, and severe groups based on their bilirubin levels and prothrombin time. A significant difference was observed between the study and control groups in terms of weight, BMI, and alcohol consumption. The ALD group had notably higher BMI ($p < 0.001$), with increased alcohol consumption correlated to disease severity. The findings suggest that heavier drinking and higher BMI may exacerbate the progression of ALD.

In terms of biochemical markers, patients in the severe ALD group had significantly elevated bilirubin (6.4±0.34 mg/dl) and malondialdehyde (MDA) levels (14.13±0.56 mg/dl) compared to the control group (bilirubin: 0.92±0.2 mg/dl, MDA: 6.8±0.34 mg/dl), highlighting increased oxidative stress in more severe cases of ALD. Elevated bilirubin levels indicate impaired liver function, and increased MDA levels reflect enhanced lipid peroxidation, both of which are associated with the progression of liver damage.

Additionally, the levels of antioxidant vitamins (vitamins C and E) were significantly lower in ALD patients. The severe ALD group had the lowest vitamin C (0.19±0.31 mg/dl) and vitamin E levels (0.32±0.37 mg/dl) compared to controls (vitamin C: 0.71±0.23 mg/dl, vitamin E: 0.85±0.43 mg/dl). This reduction in antioxidant vitamins indicates a diminished ability to counter oxidative stress, which further contributes to liver damage and disease progression. These results suggest that antioxidant deficiencies play a critical role in worsening the severity of ALD.

Overall, the study found that oxidative stress increases with the severity of ALD, as indicated by elevated bilirubin and MDA levels, while vitamin C and E levels significantly decrease, highlighting the potential benefit of antioxidant supplementation in managing ALD.

Alcoholic liver disease is commonly found in the middle-class income group or lower-middle-class income groups. In such an individual the habit of heavy drinking leads to ALD. ALD is a chronic disease, the lesions in the liver tissue start appearing after it gets severe. In this study, it is found that the severity of the disease increases in those individuals who consume higher amounts of alcohol. This finding is consistent with the studies that report the hepatotoxicity of alcohol, particularly in those who engage in heavy drinking [9-12].

Alcohol metabolizes into acetaldehyde and it leads to lipid peroxidation, which causes an increase in the free radicals. The free radicals cause inflammation of the hepatic tissue and it also decreases the endogenously available antioxidants. Although there are dietary antioxidants such as vitamin C and vitamin E, in the case of heavy drinkers, the deficiency of these vitamins is widely noticed. A study reported that deficiency increases the severity of alcoholic liver disease [13]. The severity of the disease increases the level of bilirubin, and it also increases the oxidative stress of the body.

In this study, it was found that the level of bilirubin was significantly high in the patients with alcoholic liver disease compared to the controls. This was by the other studies done earlier [14,15]. The research has also found increased oxidative stress in the groups with alcoholic liver disease compared to the control patients. This finding was similar to the findings of the other studies conducted with a similar objective [16,17]. The levels of vitamin C & E were significantly lower in the patients with alcoholic liver disease although the age, gender, and BMI of the controls were comparable to the study group. The dietary antioxidants and cessation of alcohol can completely

reverse alcoholic liver disease [18]. Dietary supplements in patients with alcoholic liver disease are necessary to improve the prognosis of the disease.

Generalizability

The generalizability of the study findings may be limited due to several factors. First, the study was conducted at a single medical center in India, which may not fully represent the broader population in terms of ethnicity, socioeconomic status, or healthcare access. Additionally, the relatively small sample size of 120 participants may restrict the ability to apply these results to a larger population. However, the study's clear findings on the role of oxidative stress and antioxidant deficiencies in alcoholic liver disease (ALD) progression may still be relevant across different populations with similar health profiles, particularly where heavy alcohol consumption is common. Larger, multi-center studies would be needed to confirm the generalizability of these results.

Conclusion

From the study, it can be concluded that the severity of alcoholic liver disease increases with oxidative stress and with an increase in the bilirubin level. Also, in the case of severe liver disease, there is a significant decrease in vitamin C and vitamin E levels.

Limitation

Small cohort was the limitation of the study and to confirm the findings of the study more such studies on large cohorts are required. Also, the beneficial effect of supplementation vitamins is not studied in this research.

Recommendation

Vitamin C & E antioxidant therapy should be included in the treatment of alcoholic liver disease.

Acknowledgment

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List of abbreviation

MDA - Malondialdehyde
ALD – Alcoholic liver disease
BMI – Body Mass Index

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No source of funding received.

Conflict of interest

The authors declare no conflict of interest.

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