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A Study of Copper, Ceruloplasmin, Total Antioxidant Capacity, Iron & Total Iron Binding Capacity in Alcoholic Liver Disease in a Tertiary Care Hospital

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Abstract | **Original Research Article**

Alcohol is the main cause of liver injury. Increase in alcohol consumption in society has resulted in an increase in the number of cases of alcoholic liver disease and liver cirrhosis. Methods: This study was performed in the Department of Biochemistry, SVS Institute of Medical Sciences, Mehboobnagar. Based on the inclusion criteria n=20 patients were selected for the study during the study period. Another group of n=20 patients age and sex-matched were selected randomly to act controls. About 5ml of venous blood will be collected under aseptic precautions in a vacutainer and following assays will be done in serum. The serum copper was estimated by the colorimetric method. Serum ceruloplasmin was estimated by the Copper Oxidase Activity Method. Serum tocopherol can measure by their reduction of ferric to ferrous ions which then form a red color complex with α, α-dipyridyl Tocopherols and carotenes are first extracted into xylene and the absorbance is read at 460nm to measure the carotenes. A correction for the carotenes is made after ferric chloride and reading at 520nm. The serum Iron and total iron binding capacity [TIBC] was estimated by the Ferrozine Method. Results: The means and S.D of serum copper levels in controls is 87 ± 20.9 as compared to alcoholic liver disease 51.4±8.1. The means and S.D of serum ceruloplasmin level in control is 45.5 ± 18.5 as compared to alcoholic liver disease 7.8 ± 3.5. The means and S.D of Vit E in controls is 1.1± 0.4 as compared to alcoholic liver disease 0.8 ± 0.2. The means and S.D of serum Iron in controls is 111.2 ± 22.4 as compared to alcoholic liver disease 38.5± 15.3. The means and S.D of serum TIBC levels in control is 304.4 ± 63.1 as compared to alcoholic liver disease is 186.8± 63.3. Conclusion: Therefore it can be concluded from the above study that estimation of copper, ceruloplasmin, vitamin E, Iron, TIBC may receive as a predictive guide and understanding the pathogenesis, the intervention may be initiated at an early date to avoid the complications.

Keywords: Copper, Ceruloplasmin, Total Antioxidant Capacity, Iron, Total Iron Binding Capacity, Alcoholic Liver Disease.

INTRODUCTION

Alcoholic liver disease (ALD) has been observed to be one of the major medical complications of alcohol abuse. More importantly, alcoholic cirrhosis is now increasingly seen in countries such as Japan and India which traditionally had a low prevalence of the disease. Approximately 80% of heavy drinkers develop steatosis, 10 – 35% develops alcoholic hepatitis, and at least 10% will develop cirrhosis [1]. The role of trace elements in alleviating oxidative stress is well known [2]. Alcohol influences trace elements status and hence produces oxidative stress-related changes in these patients. The etiopathogenesis of alcoholic liver disease is based on the oxidation of ethanol to form acetaldehyde in the liver, which binds to proteins forming antigens leading to tissue injury via antigen-antibody reactions. The reaction also releases NADH and NAD which alter the redox state of the hepatocyte. Alcohol has also been shown to decrease the levels of antioxidant enzymes; all these combine to cause oxidative liver damage[4]. Most trace elements are stored in the liver and hence changes in the metabolism of these elements occur in alcoholic liver disease [2]. Copper is an important component of the number of metalloenzymes which act as oxidases to achieve reduction of molecular oxygen. Its biochemical role is primarily catalytic. More than 90% of copper is transported by ceruloplasmin, which is a positive acute phase reactant. An increase in ceruloplasmin is thus accompanied by an increase in serum copper concentration [2]. Studies have shown that serum copper concentration is increased in liver cirrhosis while it is found to be decreased in acute hepatitis [3]. Since copper acts as a cofactor against hepatic fibrosis, such abnormalities reflect underlying pathology like fibrosis, dysfunction, regeneration or cholestasis [4].

Iron functions as a component of a number of proteins, including enzymes and hemoglobin, whose function is most important for the transport of oxygen to tissues throughout the body for metabolism. Total iron binding capacity (TIBC) is a measurement of the maximum concentration of iron that transferrin can bind. It has been shown to decrease in hepatic iron overload [2]. A number of studies have demonstrated that alcoholic liver disease is often associated with increased serum iron as well as hepatic iron overload by various mechanisms including up-regulation of transferrin receptor expression in the hepatocytes of alcoholic liver disease patients [6]. One such recent study was able to show that alcohol decreases hepcidin transcription causing an increase in absorption of copper [3]. Serum levels of these elements have also been studied in alcoholic liver disease patients. One such study done in Japan found that, as the disease progressed from chronic hepatitis to liver cirrhosis, serum calcium, magnesium, phosphorus and zinc concentrations decrease, while the copper concentration increases [5]. Sufficient studies of this nature have not been carried out in the Indian population. Also, there are some unanswered questions on the link between these trace elements and alcoholic liver disease. A study will, therefore, be conducted on alcoholic liver disease patients of Mahabubnagar City to compare changes in the serum levels of copper, ceruloplasmin, total antioxidant capacity, iron, and total iron binding capacity.

MATERIAL AND METHODS

This study was performed in the Department of Biochemistry, SVS Institute of Medical Sciences, Mahabubnagar. Institutional Ethical committee permission was obtained for the study. Written consent was obtained from all the participants of the study. The inclusion criteria were confirmed cases of Alcoholic liver disease aged >30 years without any significant complications. Excluded criteria were patients with < 30 years of age and older than 75 years of age, patients with concomitant renal disease, smokers, tobacco chewers, malignancies, respiratory diseases, and cardiovascular disease were excluded from the study. Based on the inclusion criteria n=20 patients were selected for the study during the study period. Another group of n=20 patients age and sex-matched were selected randomly to act controls. About 5ml of venous blood will be collected under aseptic precautions in a vacutainer and following assays will be done in serum. The serum copper was estimated by the colorimetric method. Serum ceruloplasmin was estimated by the Copper Oxidase Activity Method. Serum tocopherol can measure by their reduction of ferric to ferrous ions which then form a red color complex with α, α-dipyridyl. Tocopherols and carotenes are first extracted into xylene and the absorbance is read at 460nm to measure the carotenes. A correction for the carotenes is made after ferric chloride and reading at 520nm. The serum Iron and total iron binding capacity [TIBC] was estimated by the Ferrozine Method. Data were analyzed by Microsoft Excel and statistical software. For continues, normal data was summarized by mean ± SD with range. For continues, non-normal data were summarized by median ± IQR (Inter-Quartile Range). The comparison between cases and controls for normal data was done by unpaired t-test. The comparison between cases and controls for non-normal data was done by the Wilson Rank Sum test. All P-values less than 0.05 were statistically significant.

RESULTS

The values of the copper in µg/dl were recorded in patients with alcoholic liver disease and controls. The minimum value for controls is 60 and for cases 40. The maximum value for control is 130 and for cases 64. The means and S.D of serum copper levels in controls is 87±20.9 as compared to alcoholic liver disease 51.4±8.1 and P value <0.0001 which is highly significant in all the above parameters.

Table-1: Shows the comparative data of serum copper levels in control and cases

Test parameter	Copper (µg/dl)				P values
	Minimum	Maximum	Mean	SD	
Cases	130	40	51.4	8.1	
controls	64	60	87	20.9	<0.0001*

The values of serum ceruloplasmin were recorded in mg/dl in patients with alcoholic liver disease. The minimum value for controls is 21 and for cases 1.75. The maximum value for control is 87 and for cases 14.1. The means and S.D of serum ceruloplasmin level in control is 45.5± 18.5 as compared to alcoholic liver disease 7.8± 3.5 and P value is < 0.0001 which is highly significant in all the above parameters.

Table-2: Shows the comparative data of serum ceruloplasmin level in control and cases

Test parameter	Serum ceruloplasmin (mg/dl)				P values
	Minimum	Maximum	Mean	SD	
Cases	1.75	14.1	7.8	3.5	<0.0001*
controls	21	87	45.5	18.5	

The values of vitamin E were recorded in µg/ml in patients with alcoholic liver disease. The minimum value for controls is 0.6 and for cases 0.5. The maximum value for control is 2.1 and for cases 1.2. The means and S.D of Vit E in controls is 1.1± 0.4as compared to alcoholic liver disease 0.8±0.2 and P value is significant in all the above parameters.

Table-3: Shows the comparative data of antioxidant status (Vit E) in control and cases

Test parameter	Vitamin E (µg/ml)				P values
	Minimum	Maximum	Mean	SD	
Cases	0.5	2.1	0.8	0.2	<0.05*
controls	0.6	1.2	1.1	0.4	

The values of serum Iron were recorded in µg/dl in patients with alcoholic liver disease. The minimum value for control is 75 and for cases is 5. The maximum value for control is 151 and for cases 75. The means and S.D of serum Iron in controls is 111.2± 22.4 as compared to alcoholic liver disease 38.5± 15.3 and P value is < 0.0001 in all the above parameters.

Table-4: Shows the comparative data of serum Iron in controls and cases

Test parameter	Iron (µg/dl)				P values
	Minimum	Maximum	Mean	SD	
Cases	5	75	38.5	15.3	<0.0001*
controls	75	151	111.2	22.4	

The TIBC level in controls and cases with alcoholic liver disease were recorded the minimum value for control is 195 and for cases are 77. The maximum value for control is 436 and for cases are 280. The means and S.D of serum TIBC levels in control is 304.4± 63.1 as compared to alcoholic liver disease is 186.8± 63.3 and P value is < 0.01 which is highly significant all the above parameters.

Table-5: shows the comparative study of serum TIBC level in controls and cases

Test parameter	TIBC (mcg/dL)				P values
	Minimum	Maximum	Mean	SD	
Cases	77	280	186.8	63.3	<0.01*
controls	195	436	304.4	63.1	

DISCUSSION

Alcohol is the most frequently used and socially acceptable hepatotoxin Worldwide [7]. Geographic patterns of alcohol intake and the prevalence of alcoholic liver disease are changing constantly and recent reports question the stabilization of its use in western European countries, Canada, and Australia [8, 9]. Approximately two-thirds of adult Americans drink some alcohol [10]. The majority of people drinks light or moderate amounts and do so without problems [11-14] however, a subgroup of drinkers become dependent on alcohol and have the disease of alcoholism or alcohol use disorders [15-16]. Another group of drinkers is alcohol abusers (and problem drinkers) who experience negative consequences of drinking (eg., unemployment, loss of family, or accidental injury or death). These patients are not considered to be alcohol dependent [14, 18]. Failure to recognize alcoholism remains a significant problem and impairs both the prevention and management of alcoholic liver disease (ALD) [18, 19]. The clinical features of tolerance, physical dependence, impaired control, and craving which define alcoholism, as well as their acronym Typical, are suggested as aids to the clinician for making the diagnosis. The alcoholic liver disease presents as a spectrum of clinical signs and pathological changes with a history of acute or chronic alcohol consumption. Alcohol dependence may or may not be seen in all the stages [20]. Alcohol is metabolized in the liver to acetaldehyde and increased alcoholic levels cause increased destruction of liver hepatocytes. The revised threshold of alcohol toxicity on the liver is estimated to be 40g of ethanol daily in men and 20-30 g in women. Although in some liver disease does not develop in most people who drink more than 50g of alcohol daily, a certain amount of daily alcohol is needed to cause alcoholic liver disease [20]. Screening and diagnosis of alcoholic liver disease are more sensitive and specific when done with a CAGE (C-cut down, A-annoyed, G-guilty, E-eye opener) or an (Alcohol Use Disorders Identification Test) AUDIT questionnaire as compared to using clinical and laboratory methods. However many clinicians fail to use these. In the present study, we found significant levels of decrease in serum copper levels, serum Iron, TIBC and antioxidant status [Vit E] in alcoholic liver disease as compared to the normal controls. A study by S Khare et al; measuring the serum iron and TIBC in CLD found the serum iron levels are significantly higher in alcoholic liver disease patients against the results found in this study [21]. The reason could be as the severity of CLD increases the TIBC and serum iron decrease. Fox PL *et al*. studying

ceruloplasmin in cardiovascular disease found levels of cp are inversely related to the development of cardiovascular diseases [22]. Cp acts as a potent catalyst of LDL oxidation and the pro-oxidant activity of cp requires intact structure and a single copper atom at the surface of the protein. Abstinence remains the cornerstone of therapy for ALD. There is also a consensus for the use of corticosteroids and pentoxifylline in severe alcoholic hepatitis, for maintaining good nutritional status, for treating co-morbidities in all forms of ALD, and for liver transplantation in carefully selected patients with end-stage ALD.

CONCLUSION

Within the limitations of the present study, we found that there was a significant decrease in copper, ceruloplasmin, Iron, and TIBC in alcoholic liver disease as compared with controls. There was also a decrease in vitamin E levels in ALD. Therefore it can be concluded from the above study that estimation of copper, ceruloplasmin, vitamin E, Iron, TIBC may receive as a predictive guide and understanding the pathogenesis, the intervention may be initiated at an early date to avoid the complications.

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