Original Article

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The Association of Alcoholic Liver Disease with Vitamin D, Ferritin, and Gamma-Glutamyl Transferase for Improving Diagnosis and Treatment Modalities

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ABSTRACT

Introduction: Progressive fibrosis and cirrhosis, clinically presenting as end-stage liver disease are common outcomes in alcoholic liver disease (ALD) patients. Alcoholic liver disease may take the forms of acute involvement (alcoholic hepatitis) or chronic liver disease (steatosis, steatohepatitis, fibrosis, and cirrhosis). The severity and prognosis of ALD depends on the amount, pattern, and duration of alcohol consumption, as well as on the presence of liver inflammation, diet, nutritional status, and genetic predisposition of an individual. While steatosis is a complete benign disease, liver cirrhosis is associated with marked morbidity, mortality, and life expectancy shortening. The aim of this study was to identify potential biomarkers for progression of alcoholic liver disease. The biomarkers evaluated in this study included serum gamma-glutamyl transferase (GGT), vitamin D, and ferritin.

Methodology: This observational descriptive crosssectional study was conducted in the Department of Biochemistry, in a government medical college of Rajasthan among 100 subjects. Out of these, 50 subjects were patients of alcoholic liver disease and another 50 were normal, age and sex matched healthy volunteers as control group. The circulating level of serum gamma-glutamyl transferase (GGT), vitamin D, and ferritin were analyzed by electrochemiluminescence method.

Results: The circulating levels of serum gamma-glutamyl transferase and ferritin were significantly high in alcoholic liver disease patients compared to controls, ($p< 0.0001$). However, serum level of vitamin D was significantly low in alcoholic liver disease patients compared to controls. A negative correlation between vitamin D v/s GGT (r value= -0.155) and vitamin D v/s ferritin (r value= -0.014) was observed in the study. The p-value for correlation of vitamin D v/s GGT was $p< 0.001$ and vitamin D v/s ferritin was $p< 0.01$.

Conclusion: The study results suggest that elevated and altered biomarkers are associated with pathogenesis and progression of alcoholic liver disease. Increased serum ferritin and GGT levels showed a closer association with severity of ALD compared with level of serum vitamin D, suggesting that serum ferritin level may be a better marker than vitamin D level for predicting the severity of ALD in a clinical setting.

INTRODUCTION

Alcoholic liver disease (ALD) is a leading cause of cirrhosis, liver cancer, and acute and chronic liver failure and as such causes significant morbidity and mortality. Alcohol consumption accounts for approximately 3.8% of all global deaths and 4.6% of global disability-adjusted life-years.[1] Alcoholism is a disease characterized by the habitual intake of alcohol. The definition of alcoholism is chronic alcohol use to the degree that it interferes with physical or mental health, or with normal social or work behaviour and that produces both physical and psychological addiction.

Alcohol is a central nervous system depressant that reduces anxiety, inhibition, and feelings of guilt.[2] Alcoholism can manifest into liver damage from fibrosis to end stage of cirrhosis and may eventually lead to carcinoma of liver. The liver is particularly vulnerable to disease related to heavy drinking, most commonly termed as alcoholic hepatitis or cirrhosis. Chronic consumption of alcoholic beverages is a primary cause of liver injury. Chronic and excessive consumption of alcoholic beverages provokes membrane lipid-peroxidation due to triglyceride accumulation in hepatocytes.[3] Other factors, such as coexistent liver diseases, obesity, metabolic syndrome, and cigarette smoking also contribute to the overall risk of developing ALD.[4]

Alcohol remains most common cause of liver disease in India. Alcoholic liver disease encompasses a clinical histological spectrum, including fatty liver, alcoholic hepatitis and alcoholic cirrhosis. Fatty liver is a benign condition but progression to alcoholic hepatitis and cirrhosis is life threatening. Alcoholic hepatitis is diagnosed predominantly on clinical history, physical examination, and laboratory findings. In our present study, we have focused on alcoholic liver disease and its various complications using biochemical investigations. The study underway can serve as potential diagnostic tools for more specific biomarkers of ethanol-induced diseases. Hence, the study was aimed to evaluate the effect of chronic alcohol consumption on blood, renal, and hepatic biomarkers against worsening Child Pugh criteria.

METHODS

The present study was an observational, descriptive, cross sectional study. The study was conducted in the Department of Biochemistry of a government medical college of Rajasthan, after obtaining ethical clearance from the institutional board and ethical committee. The duration of the study was three years. Patients who agreed to participate in the study were selected. The sample size (n = 100) was derived using single proportion formula taking maximum 50% as the disease prevalence, keeping 5% confidence limit, for level of significance =0.05.

Patients with a history of significant chronic alcohol intake with physical signs of liver disease (jaundice, portal hypertension, complications of portal hypertension) and positive laboratory and radiological finding and age between 30 to 60 years were included in study.

Patients with viral hepatitis, hepatitis B, hepatitis C, post-necrotic cirrhosis, documented seropositivity for HIV, any other form of chronic liver disease, Wilson's disease, hemochromatosis, history of underlying systemic problems, age less than 30 years and more than 60 years were excluded. All subjects were clinically, physically diagnosed by clinician and other biochemical routine investigations that were already done were recorded at hospital. All cases had clinical, biochemical, ultra-sonographic as well as biopsy report. Those receiving calcium and Vitamin D supplementations, taking drugs which affect serum level of ferritin, GGT, and Vitamin D such as Carbonyl iron, Gluconate, Sulphate, Folic acid, Vitamin D_3, Chordiazepoxide, anti-tuberculosis drugs, anti-thyroid etc and patients on steroid therapy and under antibiotic/non-steroidal anti-inflammatory drugs treatment for past six months etc, and patients with other co-morbid illness such as cardiac, respiratory and renal illness were excluded.

A case of alcoholic liver disease was diagnosed in patients with history of significant alcohol intake, physical signs of liver disease and supporting laboratory investigations.[5] Alcoholic cirrhosis was diagnosed by first taking a medical history and discussing a person's history of drinking. Cirrhosis was diagnosed by at least one clinical sign of hepatocellular failure and one of the sign of portal hypertension alongwith at least three ultrasound finding of cirrhosis of liver.[6]

Detailed history was taken and physical examinations were done. Patient was examined for signs of portal hypertension (ascites, splenomegaly, abdominal wall collaterals, and a venous hum), hepatic injury (cutaneous telangiectasia, palmer erythema, finger clubbing, Dupuytren's contracture, and peripheral neuropathy) and feminization (gynaecomastia and hypogonadism). All laboratory investigations including a liver chemistry profile (serum albumin, bilirubin and AST/ALT), complete blood count and prothrombin time were done as routine investigations. Ultrasonogram, barium swallow, and upper gastrointestinal endoscopy were done. Specific analysis of serum Ferritin and Vitamin D were estimated using kit method by electro chemiluminescence (ECL) machine and GGT was estimated by fully autoanalyser by using anamol reagent kits.

Estimation of serum Vitamin D: Vitamin D was estimated by using the Roche diagnostics total assay kit. Vitamin D total assay is a competitive electrochem-iluminescence protein binding assay intended for the

quantitative determination of total 25-OH vitamin D in human serum and plasma. The assay employs a vitamin D binding protein (VDBP) as capture protein, which binds to both 25-OH D3 and 25-OH D2 (Roche diagnostics, Mannheim, Germany).[7] The assay utilizes a 3-step incubation process, which has a duration of 27 minutes. In step 1, the sample is incubated with pre-treatment reagent, which releases bound 25-OH vitamin D from the VDBP. In step 2, the pre-treated sample is incubated with ruthenium labelled VDBP creating a complex between the 25-OH vitamin D and the ruthenylated VDBP. The third incubation step sees the addition of streptavidin-coated micro particles and 25-OH vitamin D labelled with biotin. The free sites of the ruthenium labelled VDBP become occupied, forming a complex consisting of the ruthenium labelled vitamin D binding protein and the biotinylated 25-OH vitamin D. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.[7]

Estimation of Serum Ferritin: Ferritin is the iron storage protein. The ferritin detectable in human serum is in equilibrium with the body's depot iron and hence acts as an indicator for the level of iron stores. Determination of ferritin can be used as an aid in iron metabolism diagnosis, monitoring iron therapy, ascertaining the iron reserves in groups at risk and in the differential diagnosis of anaemia.[8]

Sandwich principle: 1st incubation (9 minutes):10 µL of sample, a biotinylated monoclonal Ferritin-specific antibody, and a monoclonal ferritin- specific antibody labeled with a ruthenium complex form a sandwich complex. 2nd incubation (9 minutes): After addition of streptavidin coated micro-particles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.[8]

Measurement method for serum ferritin and vitamin D: The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by 2 point calibration and a master curve provided via the reagent barcode.

Determination of Gamma Glutamyl transferase: GGT catalyzes the transfer of amino group between L-γ-glutamyl-3 carboxy- 4 nitroanilide and glycylglycine to form L-γ-glutamyl glycyl glycine and 5-amino-2-nitrobenzoate. The rate of formation of 5-amino-2-nitrobenzoate is measured as an increase in absorbance which is proportional to the GGT activity in the sample.[9]

Data obtained through clinical recording forms (CRF) was compiled onto MS Office excel sheet (v.2010, Microsoft Inc, USA). Statistical analysis of data was performed using statistical package for social sciences (SPSS, v.22.0, IBM). Descriptive data like percentage and frequencies of males and females participating in the present study, mean, and standard deviation of numerical data was expressed. Comparison of means of variables like CAL, PI, and DMFT among males and females was done using independent t test.

RESULTS

The present study was conducted on 100 subjects aged between 30 to 60 years, comprising of 50 healthy controls and 50 patients diagnosed of alcoholic liver disease acted as study group. The blood samples of controls as well as study groups were withdrawn and analyzed for serum ferritin, vitamin D, and gamma-glutamyl transferase (GGT).

Table 1 shows that the mean ferritin level was 155.50 ± 23.87 ng/ml in control subjects and it was 341.48 ± 21.43 ng/ml in the subjects of alcoholic liver disease (Figure1). The increase in ferritin level was statistically ($p<0.0001$), depicted in table 1 and figure 1.

Table 1: Comparison of biochemical parameters of the alcoholic liver disease subjects with healthy subjects

Parameters	Healthy Subjects Mean ± SD (N= 50)	Alcoholic liver disease Mean ± SD (N=50)	p value
Ferritin (ng/ml)	155.50± 23.87	341.48± 21.43	<0.0001
Vitamin D (ng/ml)	38.48± 4.29	22.48 ± 4.15	<0.0001
GGT (IU/L)	20.78± 5.29	223.80 ± 12.73	<0.0001

The mean serum vitamin D level was found to be 38.48 ± 4.29 ng/ml in normal healthy subjects (Table 1 and Figure 1). The mean serum vitamin D level was decreased to 22.48 ± 4.15 ng/ml in alcoholic liver disease subjects (study group) as shown in table 1 and figure 1. The highly significant decrease (p<0.0001) in vitamin D level was observed in the study group when compared to healthy controls (Table 1 and Figure1).

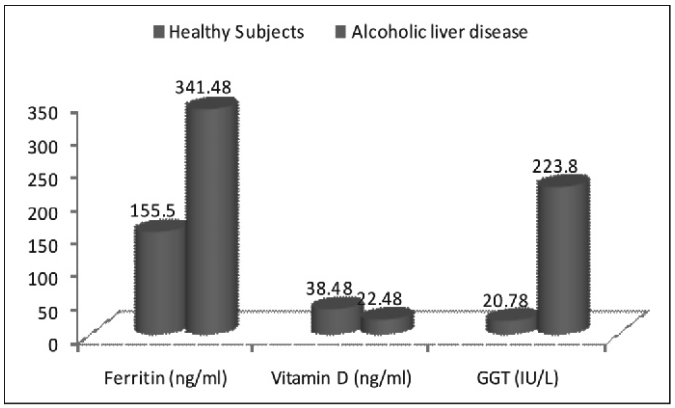

Figure 1: Comparison of mean of biochemical parameters of the alcoholic liver disease subjects with healthy subjects.

The mean ± SD value of serum GGT level in healthy control was 20.78 ± 5.29 IU/L, and in alcoholic liver disease it was found to be 223.80 ± 12.73 IU/L. Serum GGT level showed highly significant increase (p<0.0001) in the study group when compared to healthy control subjects (Table 1 and Figure 1).

DISCUSSION

The effect of serum ferritin level on alcoholic liver disease

Serum ferritin levels were higher in the study group than control groups, these results agree with previous study, which reported increased plasma ferritin level as measured by electrochemical luminescence method in alcoholic liver disease patients.[10] Ferritin is a good indicator of cases of anemia of chronic disease where ferritin is elevated in its capacity as an inflammatory acute phase protein and not as a marker for iron overload. In addition, serum ferritin levels, is generated in excess amounts in alcoholic liver disease or other alcohol related liver disease/damage.[11, 12]

Serum ferritin was increased above 200 micrograms L-1in 64 of 111 alcoholics (58%) (p< 0.01). Twelve of 111 alcoholics (11%) had serum ferritin above 1000 micrograms L-1 (p< 0.01) compared with one of 137 (0.7%) with chronic non-alcoholic liver diseases. The transferrin saturation was increased in 16 of 105 alcoholics (15.2%) in alcoholic liver disease (p< 0.01).[10] It was also noticed that serum ferritin levels were elevated as compared to controls which are quite similar to earlier reports of Jacobsson et al.[12] The values of serum ferritin are associated with GGT and vitamin D as observed by us and may be used as markers in combination for diagnosis for ALD. It has been reported that liver disease has been associated with liver cirrhosis.[12] The finding is well supported by Kristenson et al[11] who suggested that serum ferritin is more frequently elevated in patients with alcoholic liver disease than in patients with other chronic liver diseases such as autoimmune liver diseases and hepatitis C. Because serum ferritin decreases rapidly during abstinence, the measurement of ferritin for the detection of haemochromatosis in patients abusing alcohol should be postponed until the patients are abstaining. Most of the patients with increased serum ferritin have normal transferrin saturation values which can be used to separate them from haemochromatosis.[10, 11, 12]

The effect of serum vitamin D level on alcoholic liver disease

When considering the potential mechanisms that underlie the association between vitamin D, ferritin, and alcoholic liver disease, we theorized that processes related to insulin resistance (IR) and inflammation may be involved. Vitamin D has been reported to play a protective role in IR, which is a feature of metabolic syndrome (MetS), and alcoholic liver disease is characterized as the hepatic component of MetS. Trepo E et al[13] showed that low 25(OH) vitamin D were associated with increased liver damage and mortality, suggesting that vitamin D might be both a biomarker of severity and a potential therapeutic target in ALD (alcoholic liver disease).

The effect of serum GGT level in alcoholic liver disease

Our study also supports the study of Das SK et al[14], Conigrave KM et al[15], and Rogers JT et al[16], who found the same results of ferritin, vitamin D, and GGT in alcoholic liver diseases subjects as compared to healthy control individuals.[17,18] These results were in close agree-ment with the findings of Rogers JT et al[16], who observed an inverse correlation between Vitamin D levels, ferritin, and GGT.[13] Our findings are also in confirmation with the study of Malham et al,[19] who found a similar correlation.

The variation in liver functions with elevated ferritin

levels and corresponding reduction in RBCs and haemoglobin values also revealed risk to liver injury and renal functions related to excessive alcohol intake when compared with control group. Vitamin D is becoming increasingly accepted as an important physiological regulator outside of its classical role in skeletal homeostasis. A growing body of evidence connects vitamin D with hepatic disease.

While pre-clinical experimental data is promising, clinical trials around liver diseases have mostly been under-powered, and further studies will be required to clarify whether vitamin D or vitamin D analogues have beneficial effects on liver disease.

CONCLUSION

Rising levels of gamma glutamyl transaminases and variation in other biomarkers of injury reflect iron as central cofactor in producing injury. Alcohol consumption and iron levels play a key role in the progression of liver disease and pathogenecity. Regular monitoring of these markers and iron overload indicators in alcoholic patients is necessary for better patient management and to mini-mize the morbidity and mortality related to liver injury.

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