ORIGINAL RESEARCH ARTICLE

Association of Severity Serum Iron Indices and High-sensitivity C-reactive Protein with Disease Severity in Men with Alcoholic Liver Disease

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Abstract

Aim and objective: Earlier studies have reported an association between chronic alcohol consumption, iron overload and liver disease. However, there are only limited data from India on serum iron indices and inflammatory markers in alcoholic liver disease. The objective of the study is to estimate serum iron indices and C-reactive protein levels in alcoholic liver disease and their association with disease severity.

Materials and methods: We enrolled 46 alcoholic liver disease cases and 40 controls. Serum iron, ferritin and high-sensitivity C-reactive protein levels were estimated in both groups.

Results: Serum iron, ferritin, transferrin saturation and C-reactive protein were significantly increased in alcoholic liver disease patients compared to controls. Both iron ($r = 0.372$, $p = 0.011$) and ferritin ($r = 0.352$, $p = 0.016$) were positively correlated with model for end stage liver disease score an indicator of severity in alcoholic liver disease. C-reactive protein had a significant correlation with iron ($r = 0.294$, $p = 0.048$), ferritin ($r = 0.483$, $p = 0.001$) and model for end stage liver disease score ($r = 0.344$, $p = 0.019$) in alcoholic liver disease cases.

Conclusion: We conclude that serum iron indices and C-reactive protein are elevated in alcohol liver disease and associated with severity of liver disease.

Clinical significance: Serum iron indices and C-reactive protein can be used as biomarkers for predicting disease severity in alcoholic liver disease.

Keywords: Alcohol liver disease, C-reactive protein, Ferritin, Iron.

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Introduction

The prevalence of alcohol liver disease (ALD) is increasing worldwide including India. In a recent study, it was found that alcohol was the major cause for liver fibrosis and cirrhosis.[1] Previous studies have suggested that asymptomatic alcoholic fatty liver may progress to fibrosis and cirrhosis in 5–15% of the patients despite abstinence and indicated that inflammation and oxidative stress might play a role in progression to cirrhosis.[2,3]

Iron is considered as a risk factor in the progression of many liver diseases.[4,5] Iron and ferritin are known to play a central role in the pathogenesis of ALD.[5] Alcoholic liver disease was found to be associated with elevated serum iron indices and hepatic iron overload.[6] Previous reports have demonstrated that iron overload can predict mortality in patients with alcoholic cirrhosis.[7] The role of ferritin in fibrogenesis of liver parenchyma in patients with ALD has been investigated in earlier studies. Ferritin was shown to be an indirect marker of iron deposition in liver parenchyma in patients with ALD.[5]

C-reactive protein (CRP), a marker of inflammation is found be increased in various disorders including alcoholic liver disease. Previous studies have shown that measurement of serum C-reactive protein was useful in assessing the risk of non alcoholic steatohepatitis.[8] Also studies have demonstrated that CRP can be useful in predicting the prognosis of alcoholic hepatitis and alcoholic cirrhosis.[9]

Even though there are a few reports related to iron overload in alcohol liver disease, there are no reports about serum iron indices and their relation to C-reactive protein and disease severity in Indian population. The present study was designed to study serum iron, ferritin and CRP levels and their association with disease severity in patients with alcoholic liver disease.

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Materials and Methods

The present study was a prospective case control study done at JIPMER hospital, Puducherry for one year from April 2012 to March 2013. The study was approved by the institutional ethics committees for human studies, JIPMER (No SEC/2011/4/9, dated 2/2/2012). Written informed consent was obtained from all the subjects. Forty-six consecutive alcohol liver disease patients who were admitted in medicine ward were recruited in the study after meeting inclusion and exclusion criteria. Controls ($n = 40$) comprised of male healthy

volunteers aged between 18–65 years. They were mostly recruited from the relatives of the patients, residents and staff working in the hospital. Masking was not done for the subjects as it didn't involve any intervention.

Inclusion Criteria

Males aged 18–65 years who were diagnosed as alcoholic liver disease ($n = 46$) based on clinical and ultrasound findings were included in the study. As most of the patients presented with the signs and symptoms of hepatitis and cirrhosis, we included only these two groups in the study.

Exclusion Criteria

Patients with history of diabetes mellitus, pre-existing renal failure, ischemic heart disease, GI bleeding within the past 3 months, co-existent chronic viral hepatitis, those who are on supplements with iron and active infection at any site such as peritonitis, urinary tract infections or pneumonia within the past 2 weeks were excluded. The other causes of liver disease due to viral infection, drugs, malignancies and metabolic disorders like Wilson disease were also excluded from the study.

Sample Size Calculation

The sample size was estimated as '*N*' participants in either group to detect a mean difference of 15 µg/dL in serum iron[10] between the groups at 5% level of significance and 90% power. Sample size was calculated using Open Epi sample size calculator (Emory university, Atlanta, Georgia). The sample size obtained was 46 in each group. Since this work was short term student project, we got only 40 healthy controls during that period.

Blood Collection

5 mL of venous blood was collected from the subjects. 3 mL of sample was collected in a plain tube. Remaining 2 mL of sample was collected in tubes with sodium citrate and the plasma was used for the estimation of prothrombin time. Serum was separated and liver function test parameters were estimated immediately. The remaining sample was stored at −80°C and used for further analysis of the test parameters.

Analysis of Biochemical Parameters

Serum iron and TIBC were estimated by ferrozine method in clinical chemistry analyzer (Olympus AU 400, USA) using reagent kits from Coral diagnostics, India. Ferritin levels were estimated by reagent kits from Siemens using Chemiluminiscence method (ADVIA Centaur CP, Germany). Serum hs-CRP levels were measured using a commercially available quantitative ELISA kit (DBC, Germany). Transferrin saturation was calculated as ratio of serum iron and total iron binding capacity (TS (%) = Serum iron × 100/TIBC). The disease severity was assessed using Model for End stage Liver Disease (MELD) scores.[11] Serum iron, ferritin and liver function test parameters were routine parameters analyzed for patient care services. Two level internal quality control (low and high) was done for all the parameters before the analysis. The samples were analyzed only after the internal quality control was below 1 SD (standard deviation). hs-CRP was analyzed using ELISA where quality control was done with regents provided with the kits.

Statistical Analysis

The Data analysis was done using SPSS software version 16.0 (IBM SPSS Statistics, Armonk, New York, USA). The results were expressed as mean ± S.D and median (range). The normality of the data was tested by Kolmogorov–Smirnov test. The data between cases and controls were compared using independent '*t*' test and Mann–Whitney *U* test. The association between various parameters was assessed by Pearson's Correlation analysis. A p value < 0.05 was considered as significant.

Results

Forty six patients with ALD and 40 controls were included in the study. As compared to controls, serum iron, transferrin saturation, ferritin, high-sensitivity C-reactive protein, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase and prothrombin time were significantly increased and albumin levels were significantly reduced in patients with ALD (Table 1).

Table 1: General characteristics, liver function test parameters, iron indices and hs C-reactive protein levels in controls and alcohol liver disease

Parameters	*Controls (n = 40)*	*Alcoholic liver disease (n = 46)*	*p value*
Age (years)	39.9 ± 6.0	42.4 ± 7.1	0.08
Body mass index (kg/m^2)	23.9 ± 2.2	22.7 ± 2.0	<0.01
Blood glucose (mg/dL)	79 ± 9	82 ± 15	0.13
Blood urea (mg/dL)	21 ± 4	41 ± 22	<0.01
Serum creatinine (mg/dL)	0.8 ± 0.2	1.6 ± 1.1	<0.01
Total bilirubin (mg/dL)	0.8 ± 0.2	7.4 ± 6.3	<0.01
Direct bilirubin (mg/dL)	0.29 ± 0.13	3.9 ± 3.5	<0.01
Aspartate aminotransferase (IU/L)	26 ± 4	127 ± 76	<0.01
Alanine aminotransferase (IU/L)	28 ± 8	64 ± 33	<0.01
Alkaline phosphatase (IU/L)	70 ± 15	146 ± 85	<0.01
Gamma glutamyl transferase (IU/L)	25 ± 10	110 ± 104	<0.01
Total Protein (g/dL)	7.4 ± 0.4	6.1 ± 1.1	<0.01
Albumin (g/dL)	4.4 ± 0.3	2.7 ± 0.4	<0.01
Prothrombin time (seconds)	14.3 ± 1.4	25.1 ± 6.6	<0.01
INR	1.15 ± 0.13	2.12 ± 0.64	<0.01
Iron (µg/dL)	90 (6–184)	160 (13–1139)	<0.01
Total iron binding capacity (µg/dL)	380 (35–646)	367 (20–1621)	0.71
Transferrin saturation (%)	23 (1.57–210.34)	49.8 (2.6–3308.1)	0.01
Ferritin (ng/mL)	61 (6–308)	603 (21–1650)	<0.01
C-reactive protein (ng/mL)	868 (131–8936)	8728 (3867–10714)	<0.01

Table 2: Correlation of iron and ferritin with hs C-reactive protein, MELD score and liver function test parameters in alcoholic liver disease cases ($n = 46$)

	Iron		*Ferritin*	
Parameters	*r*	*p*	*r*	*p*
hs C-reactive protein	0.294	0.048	0.483	0.001
MELD score	0.372	0.011	0.352	0.016
Total bilirubin	0.152	0.312	0.088	0.562
Aspartate aminotransferase	0.07	0.520	0.130	0.390
Alanine aminotransferase	0.421	0.004	0.041	0.786
Alkaline phosphatase	0.080	0.599	0.105	0.489
Gamma glutamyl transferase	0.394	0.007	−0.061	0.687
Albumin	0.001	0.993	−0.125	0.409

Table 2 shows the correlation of iron and ferritin with hs C-reactive protein, MELD score and liver function test parameters. Both iron ($r = 0.372$, $p = 0.011$) and ferritin ($r = 0.352$, $p = 0.016$) were positively correlated with model for end stage liver disease score an indicator of severity in alcoholic liver disease. High-sensitivity C-reactive protein had a significant correlation with iron ($r = 0.294$, $p = 0.048$), ferritin ($r = 0.483$, $p = 0.001$) and model for end stage liver disease score ($r = 0.344$, $p = 0.019$) in alcoholic liver disease cases. Also iron was positively correlated with alanine transaminase ($r = 0.421$, $p = 0.004$) and gamma glutamyl transferase ($r = 0.394$, $p = 0.007$) in subjects with alcoholic liver disease.

Discussion

In the present study we tested the hypothesis whether iron overload and inflammation are associated with the severity of the alcohol liver disease. The key findings in our study are elevated serum iron, ferritin, transferring saturation and C-reactive protein in alcoholic liver disease compared to controls. Both iron and ferritin are significantly associated with C-reactive protein and MELD score in patients with alcoholic liver disease.

Alcohol consumption, liver disease and iron overload are reported to be associated with each other. Chronic alcohol consumption and alcoholic liver disease has been shown to increase the prevalence of iron overload.[6] Several studies have reported contradictory findings related to iron indices in alcoholic liver disease. Husić-Selimović et al. have demonstrated increased serum iron, transferrin saturation and total iron binding capacity in alcoholic liver disease patients compared to controls, where as there was no significant difference in ferritin levels between two groups.[5] Previous investigators have reported significantly reduced iron and higher ferritin levels in patients with alcoholic liver disease patients when compared to controls.[12] In the present study serum iron, transferrin saturation and ferritin were significantly increased in patients with ALD. Previous studies have shown that amino transferases and gamma glutamyl transferase can act as non invasive markers for liver fibrosis.[13] In the current study iron was significantly correlated with alanine transaminase and gamma glutamyl transferase in ALD patients. These findings suggest that alcoholic liver disease is associated with iron overload and were in accordance with earlier reports which demonstrated increased serum iron indices in ALD subjects.[6] In contrast to these studies we didn't find significant difference in total iron binding capacity levels in both the groups. Several investigators have reported that alcohol may enhance iron absorption and storage through its effect on hepcidin.[14] Even though the mechanism of iron overload in alcoholic liver disease was not established from the findings of our study, experimental studies have demonstrated that alcohol down regulates hepcidin expression in liver which in turn leads to elevated expression of iron transport proteins in the intestine there by increasing iron absorption and storage.[15,16]

C-reactive protein (CRP), an acute phase protein is widely studied as a marker of low grade inflammation.[17] Apart from cardiovascular diseases elevated hs CRP levels have been documented in patients with liver disease. Previous studies have indicated that CRP can be used as a noninvasive marker of alcoholic hepatitis in heavy drinkers.[9] Ciećko-Michalska et al. have reported high hs CRP levels in alcoholic liver disease patients and demonstrated that hs CRP is associated with poor prognosis in these patients.[18] In the current study hs CRP levels were significantly elevated in alcoholic liver disease patients compared to non alcoholic subjects without any liver disease. These findings were supported by earlier studies which hypothesized that hs CRP can be used as a predictor of short term mortality in alcoholic liver disease patients.[19]

Alcohol induced iron overload has been linked to liver disease through a common mechanism involving oxidative stress and inflammation. Iron is a pro oxidant and it has been demonstrated that iron overload can generate toxic free radicals via the Fenton reaction that directly damage cellular proteins, lipids and nucleic acids. Free radicals are reported to play a role in the activation and maturation pro inflammatory cytokines resulting in inflammation[20,21] In the present study both iron and ferritin were positively correlated with C-reactive protein and MELD score, an indicator of disease severity in alcoholic liver disease patients. Also hs C-reactive protein was significantly associated with MELD score in these patients. Even though the presence of inflammation is well established in alcoholic liver disease, the results from our study indicate that iron overload associated with inflammation may cause liver injury which in turn increases the severity of the disease which may be associated with the complications related alcoholic liver disease.

The present study is the first study from Indian population to explore the association of iron overload and inflammation with the severity of the alcohol liver disease. The strength of our study was we included fresh cases of alcohol liver disease before any intervention and smokers were excluded as smoking is known to affect some of the test parameters. The main limitation of the study is the small sample size and non inclusion of another control group of non alcoholic liver disease which enables to attribute the differences in parameters to alcoholic liver disease. However, due to logistic constraints we could not include such a group. Liver biopsy was not done in these cases to identify the coexistent hepatitis and cirrhosis as it involves 1% mortality risk. Hepcidin levels were not estimated in these subjects. By measuring hepcidin levels, we could have established the association between alcohol intake and iron overload. Hence non measurement of hepcidin can be considered as one of the limitations of the study.

Conclusion

The present study found increased levels of serum iron, ferritin and CRP levels in patients with alcoholic liver disease, indicating the iron overload and increased inflammation. Significant association between iron, ferritin and CRP levels with MELD score in these patients suggests that iron overload and inflammation might play a role in severity of ALD. Further studies are needed to study the expression of hepcidin in liver specimens in alcohol liver diseases patients to understand the mechanism of iron overload in these subjects. Future clinical trials are needed to investigate whether lowering iron and inflammation will reduce the severity of the alcohol liver disease.

Clinical Significance

At present liver biopsy is considered as gold standard for predicting the disease severity of alcoholic liver disease. This study shows that serum iron indices and C-reactive protein can be used as biomarkers for predicting disease severity in alcoholic liver disease.

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