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SHORT COMMUNICATION

Carbohydrate Deficient Transferrin and Interleukin-6 as Predictors of Fibrosis in Alcohol Cirrhosis

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Abstract The severity of alcoholic cirrhosis depends on the presence of liver inflammation and fibrogenesis. Previous studies have hypothesized that carbohydrate deficient transferrin can be used as marker of liver impairment in alcoholic liver disease patients. The present study was designed to assess whether carbohydrate deficient transferrin is associated with procollagen III peptide and predict fibrosis in alcohol cirrhosis patients. We enrolled 48 patients with alcoholic cirrhosis and 38 healthy controls. Serum carbohydrate deficient transferrin, procollagen III peptide and interleukin-6 levels were estimated in both groups. Serum carbohydrate deficient transferrin, procollagen III peptide and interleukin-6 were significantly increased in alcoholic cirrhosis patients compared to controls. Stepwise regression analysis showed that carbohydrate deficient transferrin (adjusted $R^2 = 0.313$, $\beta = 0.362$, $p = 0.003$) and interleukin-6 (adjusted $R^2 = 0.194$, $\beta = 0.459$, $p = 0.001$) were positively associated with procollagen III peptide when age, duration and amount of alcohol consumption were considered as covariates. We conclude that elevated carbohydrate deficient transferrin and interleukin-6 act as predictors of fibrosis in alcoholic cirrhosis.

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Keywords Carbohydrate deficient transferrin · Procollagen III peptide · Interleukin-6 · Inflammation · Fibrogenesis

Abbreviations

CDT	Carbohydrate deficient transferrin
MELD	Model for end stage liver disease
IL-6	Interleukin-6
INR	International normalized ratio
PCIIIP	Procollagen III peptide

Introduction

Alcohol cirrhosis is associated with considerable morbidity and mortality and its prevalence is increasing worldwide including India in recent years [1]. Apart from the amount and duration of alcohol consumption, the severity of alcohol cirrhosis depends on the presence of liver inflammation and subsequent fibrogenesis [2].

Carbohydrate deficient transferrin (CDT) is a well established marker of alcohol abuse. Previous studies have reported elevated CDT level in alcoholic and non alcoholic liver disease and hypothesized that CDT can be used a marker of liver impairment in these subjects [3, 4]. Also it has been suggested that CDT may not accurately represent alcohol consumption in advanced liver disease [4].

Fibrosis which occurs as a consequence chronic liver disease results in liver insufficiency and cirrhosis [5]. Procollagen III peptide (PCP), a precursor of collagen is considered as a non invasive marker of fibrosis [6]. Earlier studies have demonstrated in increased serum levels of

PCP in alcoholic hepatitis and cirrhosis and concluded that PCP can predict fibrosis in patients with liver disease [7].

Even though liver biopsy is the gold standard to assess the fibrosis and severity of alcoholic cirrhosis, it carries a considerable risk of major complications and procedure-related mortality. Hence non-invasive biochemical markers are needed to assess the severity of cirrhosis. Since there are paucity of studies about predictors of fibrosis in alcohol cirrhosis, the present study was designed to estimate carbohydrate deficient transferrin, interleukin-6 and procollagen III peptide and their association in these patients.

Materials and Methods

The present study was conducted at JIPMER hospital, Puducherry, India. The study protocol was approved by the institute research committee and ethics committee for human studies, JIPMER. Informed written consent was obtained from all the participants.

Males aged 18–65 years who were diagnosed as alcoholic cirrhosis (n = 48) based on clinical and sonographic findings were included in the study. Age matched non-alcoholic men were included as controls (n = 38). The sample size was estimated to detect a difference of 7 U/l in carbohydrate deficient transferrin levels between the groups at 5 % levels of significance and 90 % power using the statistical formula for testing the difference in mean [4].

Patients with history of diabetes mellitus, pre-existing renal failure, ischemic heart disease, co-existent chronic viral hepatitis and active infection at any site such as peritonitis, urinary tract infection or pneumonia within the past 2 weeks were excluded. The clinical disease severity was assessed using Child Pugh and Model for End stage Liver Disease (MELD) scores [8]) using parameters such as total bilirubin, albumin, international normalized ratio creatinine, ascitis and hepatic encephalopathy.

Blood Collection

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

Analysis of Biochemical Parameters

Carbohydrate-deficient transferrin and Procollagen III peptide were estimated using commercially available quantitative ELISA kist (Cusabio Biotech, China). Interleukin-6 levels were measured using a commercially available quantitative ELISA kit (Orgenium, Finland).

Statistical Analysis

The results were expressed as mean ± SD or median (range). The normality of the data was tested by Kolmogorov–Smirnov test. The data was compared between cases and controls using Mann–Whitney U test. Stepwise regression analysis was done to assess the predictors of Procollagen III peptide levels in patients with alcoholic cirrhosis. The data was analyzed using the software SPSS 16. A p value <0.05 was considered statistically significant.

Results

Forty eight men with alcoholic cirrhosis and 38 controls were included in the study. As compared to controls, serum carbohydrate deficient transferrin, procollagen III peptide, interleukin-6, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase and prothrombin time, were significantly increased and total protein albumin levels were significantly reduced in patients with alcoholic cirrhosis (Table 1).

Stepwise regression analysis was used to assess the effect of independent variables on procollagen III peptide levels (PCP) (Table 2). In the first model interleukin-6 was introduced which was significantly associated with PCP. In the second models carbohydrate deficient transferrin was introduced along with interleukin, still both the parameters were significantly associated with PCP. In the subsequent models age, duration of alcohol consumption and amount of alcohol consumption were introduced, still there was significant association between interleukin-6, carbohydrate deficient transferrin and PCP. These results indicate that interleukin-6 and carbohydrate deficient transferrin were predictors of fibrosis in alcoholic cirrhosis. Also carbohydrate deficient transferrin had a significant positive correlation with Child-Pugh score (r = 0.366, p = 0.011) and MELD score (r = 0.299, p = 0.039) in alcoholic cirrhosis cases.

Discussion

The role of carbohydrate deficient transferrin (CDT) in alcoholic cirrhosis is unclear. Some studies have reported that it indicates alcohol abuse where as other studies have demonstrated that it reflects liver damage in cirrhosis patients [4, 9]. In the present study CDT was significantly increased in alcoholic cirrhosis group compared to

Table 1 General characteristics, liver function test parameters, carbohydrate deficient transferrin, procollagen III peptide and interleukin-6 levels in controls and alcoholic cirrhosis

Parameters	Controls (n = 38)	Alcoholic cirrhosis (n = 48)	*p* value
Age (years)	41.3 ± 5.2	42.8 ± 7.3	0.137
Total leukocyte count (cu mm^3)	7567 ± 2048	16,674 ± 9898	<0.001
Blood glucose (mmol/L)	4.32 ± 0.48	4.59 ± 0.79	0.112
Blood urea (mmol/L)	7.26 ± 1.34	14.19 ± 8.13	<0.001
Serum creatinine (μmol/L)	77.47 ± 10.36	138.68 ± 102.75	0.009
Total bilirubin (μmol/L)	13.68 ± 3.51	132.52 ± 108.88	<0.001
Direct bilirubin (μmol/L)	5.22 ± 2.35	54.96 ± 48.32	<0.001
Aspartate aminotransferase (IU/L)	26.13 ± 4.08	126.46 ± 73.91	<0.001
Alanine aminotransferase (IU/L)	27.4 ± 8.01	65.3 ± 32.33	<0.001
Alkaline phosphatase (IU/L)	69.03 ± 15.19	153.33 ± 102.02	<0.001
Gamma glutamyl transferase (IU/L)	25.37 ± 10.13	112.67 ± 104.2	<0.001
Total protein (g/L)	73.86 ± 3.61	61.89 ± 11.02	<0.001
Albumin (g/L)	44.86 ± 2.69	27.54 ± 4.14	<0.001
Prothrombin time (s)	14.3 ± 1.4	25.1 ± 6.4	<0.001
International normalized ratio	1.15 ± 0.13	2.12 ± 0.62	<0.001
Carbohydrate deficient transferrin (ng/ml)	492.25 ± 367.41	1292.29 ± 514.82	<0.001
Procollagen III propeptide (ng/ml)	10.15 ± 5.34	15.36 ± 10.26	0.033
Interleukin-6 (pg/ml)	11.19 (1.65–672.75)	246.42 (18.72–2928.41)	<0.001

Table 2 Stepwise regression analysis to identify the predictors of procollagen III peptide with interleukin-6, carbohydrate deficient transferrin, age, alcohol duration and alcohol amount as covariates in alcohol cirrhosis

Model	Parameters	Adjusted R^2	β	*p* value
1	Interleukin-6	0.194	0.459	0.001
2	Interleukin-6	0.313	0.462	0.001
	Carbohydrate Deficient Transferrin		0.362	0.003
3	Interleukin-6	0.337	0.423	0.001
	Carbohydrate Deficient Transferrin		0.369	0.003
	Duration of alcohol consumption		−0.198	0.110
4	Interleukin-6	0.342	0.426	0.001
	Carbohydrate Deficient Transferrin		0.355	0.005
	Duration of alcohol consumption		−0.219	0.080
	Amount of alcohol taken		0.137	0.264
5	Interleukin-6	0.329	0.433	0.001
	Carbohydrate Deficient Transferrin		0.356	0.005
	Duration of alcohol consumption		−0.254	0.090
	Amount of alcohol taken		0.132	0.286
	Age		0.065	0.659

controls. These findings were supported by previous reports which demonstrated high CDT levels in cirrhosis patients [4]. Earlier studies have related increased CDT levels to metabolic abnormalities of glycoproteins caused by alcohol induced liver damage [10]. Alcohol is known to reduce the activity of liver glycosyl transferase and increase the activity of desialidase which may contribute to the increase in CDT levels [11].

The development of cirrhosis has been attributed to the increased synthesis and deposition of hepatic extracellular matrix components, especially collagen. The amino peptide of type III Procollagen (PCP) is widely used as an index of hepatic necrosis and active fibrogenesis [12]. Previous investigators have demonstrated elevated levels of PCP in alcoholic cirrhosis patients [7]. In the present study PCP levels were significantly elevated in alcoholic cirrhosis cases when compared with controls. Increased PCP in these subjects has been attributed to metabolites of ethanol and inflammation which stimulate collagen synthesis [13].

Inflammation plays a crucial role in the development of alcoholic liver disease and elevation of inflammatory cytokines in alcoholic cirrhosis has been reported by several investigators [14]. Interleukin-6 is known to induce acute phase response and tumorigenesis in liver. There are

contradictory reports regarding the role of IL-6 in alcoholic liver disease. Experimental studies using animal models have revealed the protective role of IL-6 against ethanol induced liver injury [15, 16] where as Stodulska et al. has reported association of elevated IL-6 with disease severity and high mortality in patients with alcoholic liver disease [17]. In the present study IL-6 was significantly elevated in alcoholic cirrhosis cases on comparison with controls. Also we found significant correlation between IL-6 and PCP in cirrhosis cases indicating IL-6 may induce fibrosis in these patients.

To assess the predictors of PCP in alcohol cirrhosis, we did stepwise regression analysis of independent variables like CDT, IL-6, age, duration and amount of alcohol consumption. Both CDT and IL-6 were significantly associated with PCP indicating CDT and IL-6 predicts fibrosis in patients with alcohol cirrhosis.

There are several non-invasive surrogates available for assessing the severity of liver fibrosis which includes FibroTest, Hepascore, FibrometerA, and procollagen III peptide. We used the latter of these as the marker of fibrosis severity in the present study, since the logistics for FibroTest, Hepascore, and FibrometerA were not available. Nonetheless, procollagen III peptide is a valid marker of fibrosis severity and has been validated specifically in patients with alcoholic cirrhosis [18, 19].

The data from the present study concludes that CDT and IL-6 levels are elevated and associated with fibrosis in alcoholic cirrhosis. Further studies are required to assess whether these parameters could be used as prognostic markers in patients with alcoholic cirrhosis and to investigate whether therapeutic interventions targeting these parameters would reduce fibrosis and the disease severity in alcoholic cirrhosis.

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Compliance with Ethical Standards

Conflict of interest None.

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