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PENTRAXIN-3 AS AN EMERGING RELIABLE NON-INVASIVE SERUM MARKER IN THE DETECTION OF ALCOHOLIC FIBROSIS

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Abstract

Alcoholic cirrhosis is the end spectrum of alcoholic liver disease, which includes fatty liver, alcoholic hepatitis, fibrosis, cirrhosis and superimposed hepatocellular carcinoma. Fibrosis is central to the pathology of all types of chronic liver disease. Pentraxin-3 is involved in numerous roles like regulation of inflammation and innate resistance to pathogens. It is strongly expressed on collagen bundles and fibrotic areas. After ethical clearance, demographic and laboratory details were collected from medical records. Leftover samples were used for serum pentraxin-3 estimation using an ELISA kit. The study included a total of 40 participants who were males 20 known cases of alcoholic liver disease and the control group included 20 healthy controls. The average value of serum pentraxin-3 in alcoholic liver disease cases was significantly ($p=0.006$) elevated as compared with healthy volunteers (0.51 ± 0.13ng/ml vs 0.27 ± 0.1ng/ml).This study concludes that serum pentraxin-3 could emerge as one of the novel non-invasive biomarkers in predicting the progression of fibrosis in alcoholic liver disease patients.

INTRODUCTION

Alcoholic liver disease (ALD) is a major cause of alcohol-related morbidity and mortality. Its presentation ranges from fatty liver to hepatitis, cirrhosis, and hepatocellular carcinoma. In South India, the prevalence of alcohol use varies between 33% - 50%, with an increased prevalence among the illiterate and the poor [1]. Alcoholic cirrhosis is the end spectrum of alcoholic liver disease, which includes steatosis to steatohepatitis, alcoholic hepatitis, fibrosis, cirrhosis and superimposed hepatocellular carcinoma. Fibrosis that occurs in alcoholic cirrhosis is a wound-healing response that occurs in all types of chronic liver disease. It is characterized by excessive accumulation of collagen and other extracellular matrix proteins [2]. Albeit, steatosis is visualized in all heavy drinkers, however only 10-20% of individuals might develop cirrhosis. Meanwhile, in compensated patients, advanced fibrosis or cirrhosis is the main predictor for long-term survival and clinically it is highly important to diagnose the advanced fibrosis before decompensation to enhance abstinence and survival.

Pentraxin-3is classified under the pentraxin family and is involved in a mélange of roles like regulation of inflammation and innate resistance to pathogens. It is strongly expressed on collagen bundles and fibrotic areas. It is produced by macrophages, endothelial cells, epithelial cells and fibroblasts. It is strongly associated with human and murine fibroblast differentiation [3]. Previous studies show that plasma pentraxin-3 levels are significantly increased in patients diagnosed with acute myocardial infarction, non-alcoholic steatohepatitis and sepsis [4,5]. Pentraxin-3 is highly populated in fibrotic areas and associated with collagen deposition and further aids the differentiation of monocytes to fibroblasts [6]. Narcisso-Schavion et al. showed that the median pentraxin-3 level is significantly higher in cirrhotic patients than control subjects [7]. However, ALD is a common pathological condition encountered among the Indian population. The majority of

the individual affected with ALD are present with the reversible cirrhosis stage. Non-invasive markers to identify the degree of fibrosis in these patients may help to foresee the occurrence of cirrhosis.

Conventional scoring system includes Child-Pugh score which grades ascites and hepatic encephalopathy which vary according to the physician's judgement. Prothrombin time-INR values in both systems does not sufficiently reflect coagulopathy and hence liver function [8]. Since fibrosis is central to the pathogenesis of alcoholic cirrhosis and pentraxin-3 acts as a valuable marker for fibrosis the present study aims to estimate the levels of pentraxin-3 in ALD and compare them with healthy controls. Further, pentraxin-3 level is also correlated with disease severity scores such as Child-Pugh, Model for End-stage Liver disease (MELD) score and with liver function tests.

MATERIALS AND METHODS

The study was a retrospective analytical study conducted at a tertiary care centre in South India. Ethical clearance was obtained from the Institutional Human Ethics Committee with reference number 18/142. Since only left-over samples were used waiver of consent was obtained from the committee. Anonymity of data was maintained throughout the study.

The study population included two groups – cases which included patients with a diagnosis of alcoholic liver disease and controls which included individuals attending the master health checkup with no clinical or biochemical evidence of liver disease. The participants with cardiovascular diseases, autoimmune diseases and sepsis were excluded from the study.

The study included patients admitted to the Gastroenterology department with a diagnosis of alcoholic liver disease. The total sample size was 40 with 20 ALD cases and 20 healthy controls. The medical records of the patients were accessed to collect demographic details such as age, gender and biochemical tests done which include Total bilirubin-conjugated/direct & unconjugated/indirect, Aspartate transaminase (AST), Alanine transaminase (ALT), Gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP), Total proteins, Albumin, Globulins, Child-Pugh score (based on ascites, hepatic encephalopathy, prothrombin time, total bilirubin and albumin), MELD score (based on total bilirubin, INR and creatinine).Pentraxin-3 levels was measured using a commercially available quantitative enzyme-linked immunosorbent assay(ELISA) kit (Genxbio Health Sciences Pvt Ltd, India).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version - 24 was used for statistical analysis. The data were presented as mean ± SD. The frequencies were calculated for each group, and comparisons were made for categorical variables using Chi-square test. Pearson's correlation test was used to evaluate possible correlations between quantitative variables. *p* value of <0.05 was considered statistically significant.

RESULTS

The study included a total of 40 participants who were males. 20 known cases of alcoholic liver disease and 20 healthy control subjects with negative medical history, normal physical examination, and normal laboratory and radiological examination were recruited for the study.

In this study, the ALD cases displayed significant elevation of total bilirubin, direct bilirubin, AST, GGT and total proteins as compared to the healthy controls. The demographic and clinical characteristics of cases and control groups were shown in Table 1.

Table 1: Demographics and clinical characteristics of the study participants

Parameter	Cases (n=20)	Controls (n=20)	P value
Age (years)	49.6 ± 9.3	42.7 ± 10.4	0.511
Total bilirubin (mmol/l)	191.5 ± 18.8	20.5 ± 1.7	0.001*
Direct bilirubin (µmol/l)	104.3 ± 17.1	3.4 ± 1.7	0.001*
AST (U/L)	70 ± 10	25 ± 5	0.001*
ALT(U/L)	33 ± 8	31 ± 10	0.086
GGT(U/L)	85 ± 12	62 ± 10	0.02*
ALP (U/L)	131 ± 20	74 ± 14	0.249
Total proteins (g/L)	64 ± 8	73 ± 3	0.002*
Albumin (g/L)	26 ± 4	15 ± 3	0.05

*p value < 0.05 considered statistically significant.

The results were expressed as mean ± SD. The comparison was made between cases and control. p< 0.05 was considered as statistically significant.

In this study, among the 20 cases with ALD 14 (70%) subjects displayed Child Pugh score C and 6 (30%) subjects displayed score B. Meanwhile, out of 20 cases of ALD, 7 (35%) subjects elicited MELD score in the range between 30-39, 6 (30%) subjects displayed between the range 20-29 and 7 (35%) subjects elicited in the range between 10-19.

In the present study mean value of serum pentraxin-3 was significantly higher in ALD cases as compared to the healthy controls (0.51 ± 0.13 vs 0.27 ± 0.1ng/mL; p= 0.006). The data were shown in Fig 1.

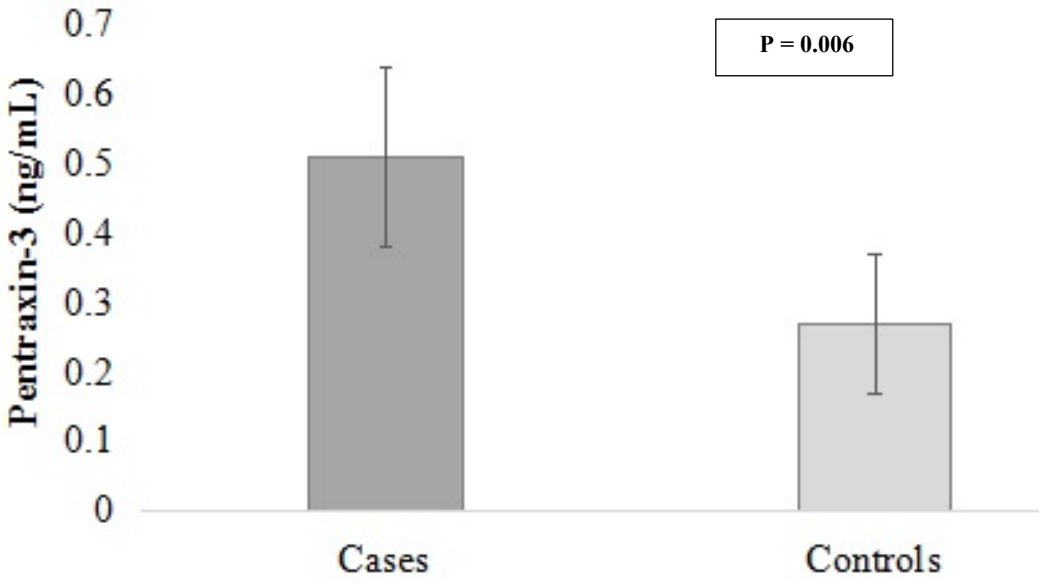

Fig 1: Serum pentraxin-3 levels among the cases and control. The values are represented as mean ± SD. The comparison was between cases and controls.

The association between pentraxin-3 and other biochemical markers was shown in Table 2. The elevated pentraxin-3 levels displayed significant positive correlation with Total bilirubin (r=0.33; p=0.04) and ALT (r=0.4; p=0.04). Further pentraxin-3 displayed a marked negative correlation with total protein and ALP.

Meanwhile, in this study, there was no significant difference in the serum pentraxin level among the ALD cases with Child Pugh score C and B (0.52 ± 0.1 ng/mL vs 0.49 ± 0.1 ng/mL; p =0.275).

Table 2: Correlation of pentraxin-3 with liver function test markers in ALD cases (n=20)

Parameters	Correlation coefficient	P- value
Total bilirubin	0.33	0.04*
Direct	0.2	0.32
Protein	-0.5	0.02*
Albumin	0.2	0.07
ALT	0.4	0.04*
AST	0.2	0.48
GGT	0.01	0.96
ALP	-0.5	0.06

*p value < 0.05 considered statistically significant

DISCUSSION

Alcoholic liver disease (ALD) occupies a major position in the development of chronic liver disease. The quantity and the duration of alcohol ingestion is the cardinal determinant of liver injury as compared to the type of alcoholic beverage. Globally, the mortality is around 5 million as a result of alcohol-related problems [9]. Previous literature shows that ALD progress to fibrosis or cirrhosis in 15% of alcoholics [10]. Further, around 35% of the alcoholics are affected with alcoholic hepatitis and steatohepatitis [11]. Liver fibrosis of various known cause is characterized by high deposition of collagens and extracellular matrix (ECM) proteins in the parenchyma of hepatic cells [12]. Nowadays, fibrosis is most widely used as an endpoint in clinical trials related to liver diseases as per the suggestion by the international fibrosis group [13]. Thus, early detection of liver damage in alcoholics is highly essential for the positive therapeutic outcome. The hallmark "gold standard" method for liver fibrosis staging is a liver biopsy. It is an invasive procedure that leads to marked side effects such as bleeding, pain, kidney puncture and death. In addition, sampling errors are usually common due to the difficulty in obtaining the liver specimen from the complete liver [14,15].

Serum markers of liver fibrosis displays a suitable alternative to liver biopsy, since it is a non-invasive method and offers the dynamic measurement of fibrosis and also cost-effective. Pentraxin-3 is an acute-phase serum protein that is upregulated in inflamed tissues. The C-reactive protein (CRP), which is mainly produced in hepatocytes, by pentraxin-3 level is elevated in liver stellate cells, monocytes and neutrophils [16]. Thus pentraxin 3 is a reliable marker to assess the activation of immune cells and it is highly superior to CRP [17]. Pentraxin-3 has a vital function in tissue healing and repair. Further, its deficiency is associated with higher clotting and fibrin deposition [18]. Hepatic stellate cells (HSC)orchestrate a predominant role in the liver tissue repair. Experimental evidences shows that pentraxin-3 expression is increased in HSC which in turn enhances collagen production and thus reduces the inflammation in the liver [19]. A previous study shows the significant association between pentraxin-3 levels and liver fibrosis stages in patients with non-alcoholic fatty liver disease and alcoholic liver disease [20,21].

In the present study, pentraxin-3 levels are significantly increased in ALD patients as compared to the controls. Our study is in corroboration with the study done by Nandeesha et al. where the pentraxin-3 levels are significantly elevated in alcoholic cirrhosis patients as that of the healthy controls [22]. Apart from ALD, the pentraxin-3 studies are elevated in various disease conditions such as acute myocardial infarction [4], chronic kidney complications [23], acute respiratory failure [24] and in ICU patients affected with infectious agents ARDS and severe infectious diseases affecting patients in intensive care [25].

In the present study, pentraxin 3 levels are increased in ALD subjects with Child Pugh score C and B. Pereira et al. also showed a positive correlation between serum pentraxin-3 levels and the scores associated with severity of liver cirrhosis such as Child-Pugh, MELD and CLIF-SOFA [26]. Further, Nandeesha et al.also showed a positive association between pentraxin-3 level and Child-Pugh score [22]. Our study also revealed a significant positive correlation between total bilirubin and ALT. There is a significant negative correlation between total protein and ALP. However, in the study done Nandeesha et al. there is a positive correlation between pentraxin-3 level and total bilirubin, ALT but it is not significant (Total bilirubin- 0.267, p=0.069; ALT- 0.120, p= 0.422) [22].

Thus early detection of the severity of fibrosis is highly vital in these patients. During the advanced stage of fibrosis, there is a significant loss of complement activity and there is a decrease in bacterial opsonization and enhances its translocation. probably related to deficiencies in the complement system, which impair the elimination of opsonized bacteria and increased bacterial translocation [27,28]. This leads to increased infection in alcoholic cirrhosis patients and also it takes 24-48 hours to know about the nature of the microorganism and thus delays the diagnosis. So it is essential to identify the serum markers for early detection of severity and also for effective treatment.

The major limitation of the study is the small sample size and only male patients are included in the study.

CONCLUSION

The present study data concludes that pentraxin-3 levels are increased in ALD patients and it has a significant association with disease severity. Thus, pentraxin-3 can be used as a reliable diagnostic marker to assess the severity of alcoholic liver fibrosis where invasive techniques such as liver biopsy is not possible.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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