Pentraxin-3 and nitric oxide as indicators of disease severity in alcoholic cirrhosis

H. NANDEESHA*, M. RAJAPPA*, J. MANJUSHA*, P. H. ANANTHANARAYANAN*, T. KADHIRAVAN† and K. T. HARICHANDRAKUMAR‡

*Departments of *Biochemistry, †Medicine and ‡Medical Informatics and Biometrics, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India.*

Accepted: 1 September 2015

ABSTRACT

Recent studies have indicated that pentraxin-3 can be used as a marker to assess the severity of hepatic fibrosis in non-alcoholic steatohepatitis. The present study was designed to assess pentraxin-3, nitric oxide and tumour necrosis factor-α (TNFα) in alcoholic cirrhosis and their association with disease severity. We enrolled 47 alcoholic cirrhosis cases and 32 controls. Serum pentraxin-3, nitric oxide (NO) and TNFα levels were estimated in both groups. Serum pentraxin-3, NO and TNFα were significantly increased in alcoholic cirrhosis patients compared to controls. Pentraxin-3 had a significant positive correlation with TNFα (r=0.303, P=0.039), Child-Pugh score (r=0.394, P=0.006) and MELD score (r=0.291, P=0.047) in alcoholic cirrhosis cases. Also we found positive association between NO with Child-Pugh score (r=0.391, P=0.007) and MELD score (r=0.311, P=0.033) in these cases. Linear regression analysis shows significant association of pentraxin-3 and NO (β=0.375, r^2=0.141, P=0.009). We conclude that elevated pentraxin-3 and NO levels are associated with severity of alcoholic cirrhosis.

KEY WORDS: Inflammation.
Liver diseases, alcoholic.
Nitric oxide.
Tumor necrosis factor-alpha.
PTX3 protein.

Introduction

Alcohol is one of the leading causes of end-stage liver disease worldwide and the prevalence of alcohol-related cirrhosis is increasing in India in recent years.[1] Even though the pathogenesis of alcoholic cirrhosis is unclear, recent studies have implicated the metabolism of alcohol to toxic products, oxidative stress, endotoxins, cytokines, transforming growth factor-β-SMAD, osteopontin and cell signalling pathways like hedgehog signalling in hepatic fibrogenesis and the complications associated with cirrhosis.[2]

Pentraxin-3, an acute-phase protein, has been suggested to play important roles in the innate resistance against pathogens, regulation of inflammatory reactions, and clearance of apoptotic cells.[3] Pentraxin-3 is produced by macrophages, smooth muscle cells and fibroblasts in response to inflammatory mediators such as interleukin–1β (IL-1β) and tumour necrosis factor-α (TNFα).[4] Plasma pentraxin-3 levels have recently been found to be elevated in patients with acute myocardial infarction, non-alcoholic steatohepatitis and sepsis.[5–7] To date there are no reports about pentraxin-3 levels in alcoholic liver disease.

Nitric oxide (NO) is a potent oxidant which has been implicated in the pathogenesis of alcoholic liver disease. Previous studies have demonstrated increased NO levels in patients with decompensated alcoholic cirrhosis,[8] and it has been hypothesised that NO could be used as a non-invasive predictor of liver damage.[9]

As the severity of alcohol-mediated liver disease depends on the continuous presence of liver inflammation and oxidative stress, apart from duration of alcohol intake, the present study was designed to assess the levels of pentraxin-3, nitric oxide, TNFα and IL-1β levels and their association with disease severity in patients with alcoholic cirrhosis.

Materials and methods

The study was carried out at JIPMER hospital, Puducherry, after obtaining approval from the Institute Ethics Committee (Human Studies). After obtaining written informed consent, males aged 18–65 years who were diagnosed with alcoholic cirrhosis (n=47) based on clinical and sonographic findings were included in the study. Age matched non-alcoholic men were included as controls (n=32).

Patients with a history of diabetes mellitus, pre-existing renal failure, ischaemic heart disease, co-existent chronic viral hepatitis and active infection at any site such as peritonitis, urinary tract infection or pneumonia within the previous two weeks were excluded. The disease severity was assessed using Child-Pugh and Model for End Stage Liver Disease (MELD) scores.[10] Child-Pugh score was calculated using biochemical parameters such as total bilirubin, albumin and international normalised ratio (INR), and presence or absence of ascitis and hepatic encephalopathy. The MELD score was calculated using serum bilirubin, creatinine and INR.

Blood collection

Venous blood (5 mL) was collected from the subjects. 3 mL of the sample was collected in a plain tube. Serum was separated and liver function test parameters were estimated

Corresponding author: Dr. H. Nandeesha
Department of Biochemistry, JIPMER, Puducherry, India
Email: nandijipmer@gmail.com

immediately. The remaining 2 mL of the 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

Serum nitric oxide (NO) was estimated using an NO colorimetric assay kit (Oxford Biomedical Research, USA). Pentraxin-3 levels were measured using a commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kit (R& D Systems, USA). Tumour necrosis factor-α and IL-1β levels were measured using a commercially available quantitative ELISA kit (Orgenium, Finland).

Statistical analysis

Results were expressed as mean±SD or median (range). The normality of the data was tested by the Kolmogorov-Smirnov test. Nitric oxide was found to be normally distributed and compared between cases and controls using independent Student's *t*-test. The remaining data, which were not normally distributed, were compared using the Mann Whitney U test. The association between various parameters was assessed by Spearman's correlation analysis. Linear regression analysis was performed to assess the relation between pentraxin-3 and NO levels in patients with alcoholic cirrhosis.

Results

Forty-seven patients with alcoholic cirrhosis and 32 controls were included in the study. As compared to controls, serum NO, pentraxin-3, TNFα, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase and prothrombin time, total leucocyte count, glucose, urea and creatinine were significantly increased and total protein and albumin levels were significantly reduced in patients with alcoholic cirrhosis (Table 1). There was no significant difference in IL-1β levels between the two groups

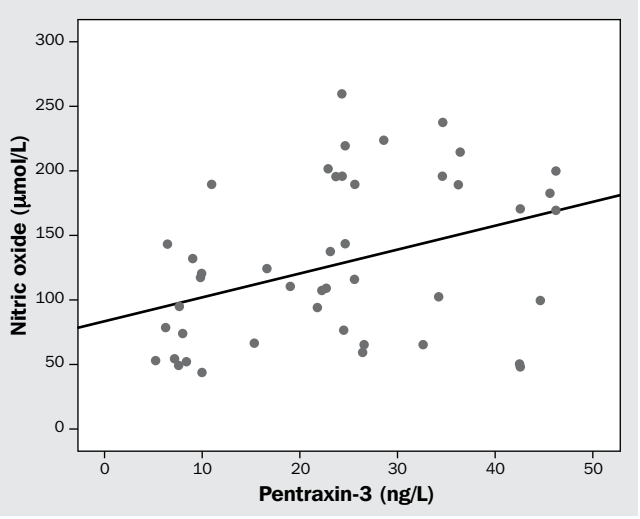

Fig. 1. Linear regression analysis of pentraxin-3 and nitric oxide in alcoholic cirrhosis (β=0.375, r^2=0.141, *P*=0.009).

The association of pentraxin-3 with other biochemical parameters, Child-Pugh score and MELD score is shown in Table 2. Pentraxin-3 had a significant positive correlation with TNFα (*r*=0.303, *P*=0.039), INR (*r*=0.390, *P*=0.007), Child-Pugh score (*r*=0.394, *P*=0.006) and MELD score (*r*=0.291, *P*=0.047) in alcoholic cirrhosis cases. Also we found positive association between NO with Child-Pugh

Table 1. General characteristics, liver function test parameters, pentraxin-3, nitric oxide, tumour necrosis factor-α and interleukin-1β levels in controls and alcoholic cirrhosis.

Parameters	Controls (*n*=32)	Alcoholic cirrhosis (*n*=47)	*P* value
Age (years)	41±6	43±8	0.151
Total leucocyte count (mm^3)	7432±2089	15574±8577	<0.001
Blood glucose (mmol/L)	4.25±0.41	4.58±0.79	0.035
Blood urea (mmol/L)	7.17±1.38	14.23±8.02	<0.001
Serum creatinine (µmol/L)	77.62±9.18	138.05±102.80	0.009
Total bilirubin (µmol/L)	13.25±3.25	135.30±109.76	<0.001
Direct bilirubin (µmol/L)	5.23±2.33	56.10±48.69	<0.001
Aspartate aminotransferase (IU/L)	26.2±4.2	125±75	<0.001
Alanine aminotransferase (IU/L)	27.2±8.5	62±29	<0.001
Alkaline phosphatase (IU/L)	67.7±16	154.7±102.3	<0.001
Gamma glutamyl transferase (IU/L)	26±10	114±104	<0.001
Total protein (g/L)	74.40±3.38	62.08±10.82	<0.001
Albumin (g/L)	44.84±2.56	27.42±3.96	<0.001
Prothrombin time (sec)	14.26±1.51	25.05±6.6	<0.001
INR	1.17±0.13	2.11±0.63	<0.001
Pentraxin-3 (ng/mL)	5.64±4.10	23.46±12.65	<0.001
Nitric oxide (µmol/L)	101.76±29.64	126.36±62.56	0.042
Tumour necrosis factor-α (pg/mL)	8.45 (0.20–55.50)	34.61 (0.39–1000)	0.005
Interleukin-1β (pg/mL)	21.81 (12.57–83.70)	20.40 (14.52–767.49)	0.415

score (r=0.391, P=0.007) and MELD score (r=0.311, P=0.033) in these cases.

Figure 1 shows linear regression analysis of pentraxin-3 and NO in alcoholic cirrhosis. Pentraxin-3 was significantly associated with NO in alcoholic cirrhosis patients (β=0.375, r^2=0.141, P=0.009).

Table 2. Correlation of pentraxin-3 with nitric oxide, TNFα, interleukin-1β and liver function test parameters in alcoholic cirrhosis (n=47).

Parameters	*r*	*P*
TNFα	0.324	0.026
Interleukin-1β	–0.091	0.541
Child-Pugh score	0.438	0.002
MELD score	0.335	0.021
Total bilirubin	0.267	0.069
Aspartate aminotransferase	0.516	0.001
Alanine aminotransferase	0.120	0.422
Gamma glutamyl transferase	0.080	0.594
Prothrombin time	0.395	0.006
INR	0.414	0.004
Total protein	–0.334	0.022
Albumin	–0.019	0.901
Duration of alcohol consumption	0.023	0.912

Discussion

To the best of our knowledge, this is the first study to investigate pentraxin-3 levels in alcoholic cirrhosis. In the present study, pentraxin-3 levels were significantly increased in alcoholic cirrhosis patients when compared with controls. Pentraxin-3 is a member of the long pentraxin family and studies have established that it reflects tissue inflammatory response.[11] Previous studies have hypothesised that inflammatory cytokines, TNFα and IL-1β, play a role in the synthesis of pentraxin-3.[4,12] Pentraxin-3, unlike the classical pentraxins C-reactive protein (CRP) and serum amyloid P component (SAP), is expressed and released by cells of the monocyte-macrophage lineage exposed to inflammatory signals.[4,12] These findings were supported by the data from our study which demonstrated that TNFα was significantly elevated and positively associated with pentraxin-3 in alcoholic cirrhosis patients. In contrast to other investigators, there was no significant elevation of IL-1β in cirrhosis patients or any association with pentraxin-3 in our study.

Earlier studies have confirmed the presence of enhanced oxidative stress in patients with alcoholic liver disease as evidenced by increased lipid peroxidation and reduced antioxidant enzymes.[13,14] Among various markers of oxidative stress, recent studies have focused on the role of NO in the pathogenesis of alcoholic cirrhosis. Several clinical reports have evaluated serum NO levels in cirrhosis and found it to be elevated in these patients.[15] In the current study, NO was significantly elevated in patients with alcoholic cirrhosis when compared with non-alcoholic subjects without cirrhosis. This is consistent with previous observations which reported over-production of NO in cirrhosis patients.[16]

Evidence suggests that interaction between inflammation and oxidative stress is associated with disease severity in alcoholic cirrhosis.[17] In the present study, significant positive association was found between pentraxin-3 and NO level in the cirrhosis group, suggesting oxidative stress enhances tissue inflammation in these patients. Nitric oxide reacts with reactive oxygen species, forming peroxy nitrate radicals which in turn alters cellular functions, enhances inflammatory reactions and fibrinogenesis, thereby increasing disease severity in cirrhosis patients.[18,19] This was supported by the observations in our study as both pentraxin-3 and NO positively correlated with Child-Pugh score and MELD score, indicators of disease severity in cirrhosis. Also, pentraxin-3 was significantly associated with prothrombin time, total protein and aspartate transaminase levels, suggesting elevated pentraxin-3 levels are related to severe tissue damage in patients with alcoholic cirrhosis.

The main limitation of the present study was that liver biopsy could not be performed, unless there was an indication in patients with cirrhosis for assessment of the histological severity of cirrhosis and to rule out coexistent hepatitis. This is an invasive procedure with potentially serious complications and is therefore not acceptable without clinical indication. Finally, our study has a cross-sectional design and prospective interventions with antioxidants are needed to demonstrate the causal role of oxidative stress in alcoholic cirrhosis.

The data from the present study conclude that NO and pentraxin-3 are elevated in alcoholic cirrhosis and their association enhances disease severity in these patients. In the present study, we propose that pentraxin-3 can be used as a marker of severity of cirrhosis, in cases where liver biopsy is not possible. Further prospective longitudinal studies are needed to investigate whether or not pentraxin-3 and NO can be used as prognostic markers in cirrhosis patients. □

This work was supported by a grant from JIPMER intramural fund sanctioned to the corresponding author. We thank Miss T Durgadevi, laboratory technician, for her technical support during this study.

References

1 Das SK, Balakrishnan V, Vasudevan DM. Alcohol: its health and social impact in India. *Natl Med J India* 2006; **19** (2): 94–9.
2 Seth D, Haber PS, Syn WK, Diehl AM, Day CP. Pathogenesis of alcohol-induced liver disease: classical concepts and recent advances. *J Gastroenterol Hepatol* 2011; **26** (7): 1089–105.
3 Okutani D. The role of long pentraxin 3, a new inflammatory mediator in inflammatory responses (in Japanese). *Nihon Rinsho Meneki Gakkai Kaishi* 2006; **29** (3): 107–13.
4 Alles VV, Bottazzi B, Peri G, Golay J, Introna M, Mantovani A. Inducible expression of PTX3, a new member of the pentraxin family, in human mononuclear phagocytes. *Blood* 1994; **84** (10): 3483–93.
5 Latini R, Maggioni AP, Peri G *et al*. Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction. *Circulation* 2004; **110** (16): 2349–54.

6 Yoneda M, Uchiyama T, Kato S *et al*. Plasma pentraxin 3 is a novel marker for nonalcoholic steatohepatitis (NASH). *BMC Gastroenterol* 2008; **8**: 53.
7 Muller B, Peri G, Doni A *et al*. Circulating levels of the long pentraxin PTX3 correlate with severity of infection in critically ill patients. *Crit Care Med* 2001; **29** (7): 1404–7.
8 Siqueira C, Carneiro M, Pedro AJ, Rocha P. Elevated nitric oxide and 3′,5′ cyclic guanosine monophosphate levels in patients with alcoholic cirrhosis. *World J Gastroenterol* 2008; **14** (2): 236–42.
9 Husi -Selimovi A, Huski J, Vukobrat-Bijedi Z, Mesihovi R, Gribajcevi M. The role of nitric oxide and ferritin in the pathogenesis of alcoholic liver disease: a controlled clinical study. *Bosn J Basic Med Sci* 2009; **9** (3): 204–9.
10 Kamath PS, Kim WR; Advanced Liver Disease Study Group. The model for end-stage liver disease (MELD). *Hepatology* 2007; **45** (3): 797–805.
11 Mantovani A, Garlanda C, Bottazzi B. Pentraxin 3, a non-redundant soluble pattern recognition receptor involved in innate immunity. *Vaccine* 2003; **21** (Suppl 2): S43–7.
12 Lee GW, Lee TH, Vilcek J. TSG-14, a tumor necrosis factor- and IL-1-inducible protein, is a novel member of the pentaxin family of acute phase proteins. *J Immunol* 1993; **150** (5): 1804–12.
13 Loguercio C, Federico A. Oxidative stress in viral and alcoholic hepatitis. *Free Radic Biol Med* 2003; **34** (1): 1–10.
14 Paradis V, Kollinger M, Fabre M, Holstege A, Poynard T, Bedossa P. *In situ* detection of lipid peroxidation by-products in chronic liver diseases. *Hepatology* 1997; **26** (1): 135–42.
15 Such J, Frances R, Perez-Mateo M. Nitric oxide in patients with cirrhosis and bacterial infections. *Metab Brain Dis* 2002; **17** (4): 303–9.
16 Lluch P, Torondel B, Medina P *et al*. Plasma concentrations of nitric oxide and asymmetric dimethylarginine in human alcoholic cirrhosis. *J Hepatol* 2004; **41** (1): 55–9.
17 Siegmund SV, Brenner DA. Molecular pathogenesis of alcohol-induced hepatic fibrosis. *Alcohol Clin Exp Res* 2005; **29** (11 Suppl): 102S–109S.
18 Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 2000; **275** (4): 2247–50.
19 Wiest R, Groszmann RJ. The paradox of nitric oxide in cirrhosis and portal hypertension: too much, not enough. *Hepatology* 2002; **35** (2): 478–91.