BRIEF COMMUNICATION

A preliminary study of inflammatory markers in non-alcoholic steatohepatitis patients

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It was reported that C-reactive protein (CRP) levels increase in parallel with the progression of chronic liver diseases, such as chronic hepatitis and liver cirrhosis. Inflammatory markers, such as high sensitive C-reactive protein (hsCRP), ferritin, transferrin, albumin, alpha-1 acid glycoprotein (AAG), alpha-2 macroglobulin (AMG), alpha-1 anti-trypsin (AAT) and lipoprotein a [Lp(a)] were measured in coronary artery disease patients (CAD) and CAD patients with non-alcoholic steatohepatitis (NASH). In the present preliminary study an attempt was made to study whether there is an increase in the levels of CRP in CAD patients associated with NASH. CAD patients showed an increase in CRP and serum ferritin levels. In CAD patients with NASH along with an increase in the levels of serum ferittin ($p < 0.001$), the levels of serum AMG and ceruloplasmin (CP) were also increased ($p < 0.01$). The CAD patients with NASH had a higher proportion of diabetes, hypertension and dyslipidaemia compared to CAD patients. But how this difference contributes to the elevation in acute inflammatory markers particularly AMG and CP levels in CAD patients with NASH cannot be explained. This study shows that a substantial number of CAD patients may be associated with NASH. Non-invasive simple parameters that reflect the degree of inflammation and fibrosis of the liver in patients with NASH would facilitate improved understanding and treatment of the disease. Further studies may be necessary to evaluate the percentage of NASH patients progressing to CAD.

Keywords: *C-reactive protein*; *alpha-1 acid glycoprotein*; *alpha 2 macroglobulin*; *alpha 1 anti trypsin*; *lipoprotein a*; *coronary artery disease*; *non-alcoholic steatohepatitis*

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The C-reactive protein (CRP) is an important test for diagnosis and monitoring patients with inflammatory conditions, such as inflammatory bowel disease, acute pancreatitis and hepatocellular carcinoma (1–3). Since non-alcoholic steatohepatitis (NASH) is an inflammatory condition of the liver, an increased CRP level may be expected. Gupta et al. (2) reported that the expression of CRP is upregulated in alcohol-induced acute liver injury; and serial measurements of serum CRP levels are useful in assessing the clinical activity of alcoholic hepatitis. Recently, it was reported that the CRP levels increase in parallel with the progression of chronic liver diseases (CLDs), such as chronic hepatitis and liver cirrhosis (4, 5). We investigated whether there is an added increase in the levels of CRP in coronary artery disease (CAD) patients associated with NASH.

Methods

Patients were recruited from the cardiac centre of High Tech Hospital, which is affiliated to Vinayaka Deemed Medical University, Salem, Tamil Nadu, India. The study was approved by the Ethics Review Board of the Institution.

In this study, 68 consecutive non-cirrhotic patients with clinical and biochemical diagnosis of CAD were taken. Of these, 36 CAD patients diagnosed with CAD had a mean age of 42.8 ± 4.6 years. These patients had no history of alcohol intake or other liver diseases. Thirty-two patients with a mean age of 55.9 ± 5.6 years were diagnosed to have CAD with non-alcoholic fatty liver disease (NAFLD). The diagnosis was based on ultrasonographic diagnosis along with the estimations of aspartate amino transferase (AST) and alanine amino transferase (ALT) (AST/ALT ratio < 1). Out of these 32

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patients, 18 patients with NASH were confirmed by liver biopsy. Twenty-eight age-matched controls were included in the study.

Fasting blood glucose and two-hour post-prandial glucose levels were measured to diagnose diabetes mellitus (ADA, American Diabetes Association criteria) at baseline and at every three-month follow-up during the study period. In these patients, liver disease was excluded by performing liver function tests and imaging studies. In all patients, the following parameters were evaluated: age, sex, family history (DM or CLD), presence of vascular disease (hypertension, ischaemic heart disease, cerebrovascular disease and peripheral vascular disease), presence of obesity, presence of central obesity (waist circumference, waist:hip ratio), dyslipidaemia (total cholesterol [ULN – upper limit of normal – 200 mg/dl], HDL cholesterol [ULN 60 mg/dl], LDL cholesterol [ULN 100 mg/dl], triglyceride [ULN 150 mg/dl], Apo-A1 lipoprotein [normal range: 120–176 mg/dl], Apo-B lipoprotein [normal range: 63–114 mg/dl], lipoprotein-a [normal range: up to 30 mg/dl]), hyperuricemia (uric acid level [ULN 7.2 mg/dl]).

Diabetes was diagnosed on the basis of the use of insulin and/or oral hypoglycemic drugs, fasting plasma glucose level >126 mg/dl and/or two-hour plasma glucose level >200 mg/dl. Hyperlipidaemia was diagnosed when fasting lipid values were above the 95th percentile of the normal range on at least two occasions. Hypolipoproteinemia was diagnosed when lipoprotein Apo-A1 and Apo-B levels were below 95th percentile of the normal range. Hypertension was defined as systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg. Obesity was defined as body mass index (BMI) >25 kg/m^2. Central obesity was defined in males as waist circumference >90 cm or waist-to-hip ratio >0.9 and in females as waist circumference >80 cm or waist-to-hip ratio >0.85.

Acute inflammatory markers: high sensitive C-reactive protein (hsCRP), ferritin, transferrin, albumin, alpha-1 acid glycoprotein (AAG), alpha2 macroglobulin (AMG), alpha 1 anti trypsin (AAT) and Lipoprotein(a) (Lp(a) were measured using standard methods.

Statistical analysis was performed using ANOVA. *p*-Value was considered statistically significant if it was <0.05.

Table 1. Clinical and laboratory characteristics of patients with CAD, CAD patients with NASH and controls

Parameters	CAD (*n* =36)	CAD+ NASH (*n* =18)	Control (*n* =28)
Gender (M/F)	24/12	10/8	18/10
BMI (weight in kg/ height^2 in m)	28.6+3.3	29.5+3.5	27.2+4.1
Obesity[a]	8	12	6
Smoking[a]	14	9	21
Hypertension[a]	18	19	
Diabetes mellitus[a]	4	8	5
Hyperlipidaemia[a]	15	15	10
Hypercholesterolaemia[a]	5	15	
Hypertriglyceridaemia[a]	9	12	
AST(U/L)	25±3	35±2	20±1
ALT(U/L)	24±4	68±8	21±1
Bilirubin(mg/dl)	0.92±0.25	1.05±0.30	0.95±0.2
Prothrombin time (PT) (seconds)	12.35±0.95	12.80±0.85	12.80±0.75
Albumin (g/dl)	4.85±0.14	4.65±0.15	4.20±0.15

[a]Refers to number of patients.

Note: AST, aspartate amino transferase; ALT, alanine amino transferase; values are means±standard deviation.

Results

Table 1 describes the clinical and laboratory characteristics of patients with CAD, CAD patients with NASH and controls. Levels of hs CRP were increased significantly in patients with CAD associated with NASH (p <0.0001) compared to control subjects (Table 3). CAD patients showed an increase in CRP and serum ferritin levels (Table 2). The levels of serum ferritin were also elevated (p <0.001), but along with an increase in

Table 2. Serum acute phase protein levels in patients with coronary artery disease and controls

APP no. (*N*)	CAD (*n* =36)	Controls (*n* =28)	*p*-Value
CRP (mg/dl)	4.75±0.18	1.68±0.28	<0.0001
Ferritin (ng/ml)	93.12±65.52	81.25±55.20	<0.001
Transferrin (mg/dl)	299.80±44.55	275.45±44.4	NS
Albumin (g/dl)	4.40±0.28	4.72±0.29	NS
AAG (mg/dl)	92.5+20.65	93.20+22.05	NS
AMG (mg/dl)	170.04±40.92	168.0±41.76	NS
Ceruloplasmin (mg/dl)	32.5±6.72	31.5±.65	NS
Haptoglobin (Hp) (mg/dl)	104.4±56.78	104.06±56.6	NS
AAT (mg/dl)	169.64±45.80	161.06±46.48	NS
Lp(a) (mg/dl)	13.06±9.61	12.01±.50	NS

Note: CRP, C-reactive protein; AAG-alpha-1, acid glycoprotein; AMG, alpha 2 macroglobulin; AAT, alpha-1 anti-trypsin; Lp(a), lipoprotein a; CAD, coronary artery disease; NASH, non-alcoholic steatohepatitis; NS, not significant; values are means±standard deviation.

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Table 3. Serum acute phase protein levels in coronary artery disease patients with NASH and controls

APP No. (*N*)	CAD + NASH (18)	Controls (28)	*p*-Value
CRP (mg/dl)	5.26 ± 0.16	1.68 ± 0.28	<0.0001
Ferritin (ng/ml)	168.33 ± 58.20	81.25 ± 55.20	<0.001
Transferrin (mg/dl)	302.0 ± 46.5	275.45 ± 44.4	NS
Albumin (g/dl)	4.12 ± 0.22	4.72 ± 0.29	NS
AAG (mg/dl)	89.05 ± 21.5	93.20 ± 22.05	NS
AMG (mg/dl)	220.04 ± 41.92	168.0 ± 41.76	<0.05
Ceruloplasmin (mg/dl)	48.5 ± 6.78	31.5 ± 5.65	<0.01
Haptoglobin (Hp) (mg/dl)	111.2 ± 58.6	104.06 ± 56.6	NS
AAT (mg/dl)	165.05 ± 46.38	161.06 ± 46.48	NS
Lp(a) (mg/dl)	13.56 ± 7.66	12.01 ± 7.50	NS

Note: CRP, C-reactive protein; AAG-alpha-1, acid glycoprotein; AMG, alpha-2 macroglobulin; AAT, alpha-1 anti trypsin; Lp(a), lipoprotein a; CAD, coronary artery disease; NASH, non-alcoholic steatohepatitis; NS, not significant; values are means ± standard deviation standard error.

AMG and CP in CAD patients with NASH ($p < 0.01$) (Table 3).

Discussion

Levels of CRP are elevated in inflammatory conditions, including CAD. We found that the serum concentrations of CRP in CAD patients with NASH were significantly increased compared to control subjects. The level of this acute inflammatory marker did not show any significant difference between CAD patients and CAD patients with NASH. Isolated and non-specific increases in serum ferritin levels are frequently found in the absence of iron overload and are associated with inflammation, liver necrosis and alcohol abuse (6). Bacon et al. (6) reported abnormal results of serum transferrin and ferritin levels in patients with NASH, but none of their patients had histologic evidence of hemochromatosis.

Subsequent studies of patients with NASH have shown increased serum ferritin levels in 53–62% of patients and elevated transferrin saturation in 11–22% (7, 8). A high serum ferritin level associated with increased liver iron concentration but normal transferrin saturation is typical of a polymetabolic syndrome. A French group has suggested it as a new iron overload entity, possibly related to the insulin resistance syndrome (9, 10). Fargion et al. (10) documented that hyperferritinaemia with normal transferrin saturation is a hallmark of glucose or lipid metabolism disorder and that patients with increased serum ferritin, normal transferrin saturation, a mild iron overload and multiple coexisting metabolic alterations are at high risk of developing NASH. In addition, they observed no correlation between liver iron concentration and histological grade, and stage within a group of patients with NASH. The findings in our study of high serum ferritin and normal transferrin saturation in patients with NASH are in agreement with the results of previous studies. The increase in ferritin level may be due to a synergistic induction of synthesis due to increased iron stores and steatohepatitis. It is also possible that an increase in ferritin occurs as a result of acute phase response.

As a new finding, we observed increased serum ceruloplasmin (CP) and AMG levels in patients with NASH. The importance of this as a part of acute phase reaction is unclear. CP exhibits oxidase activity. Also, oxidation of Fe^{2+} to Fe^{3+}, catalysed by CP, may be important for the binding of iron to transferrin. Increased serum CP concentration in CAD patients with NAFLD may be due to high serum ferritin levels with normal transferrin saturation (11).

There have been several reports that the serum concentrations of acute phase proteins (APP) with high sialic content (Lp(a), AAG, Haptoglobin (Hp) and AAT) show variations in post menopausal women on hormone replacement therapy (12). Min et al. (13) suggested that although there was no difference between the serum concentration of both Lp(a) and APR in patients with hypoalbuminaemia and those with normoalbuminaemia, there were significant differences in the serum AAT and Hp concentrations.

Normal serum concentrations of Lp(a), AAG, Hpt and AAT in our patients could be explained by different kinetics of acute phase reactants or a different time of increase or decrease. Also, these substances may not have a role in the pathogenesis of NASH.

In conclusion, increased serum CRP, ferritin, CP and AMG concentrations were observed in patients with CAD associated with NASH. The CAD patients with NASH had a higher proportion of diabetes, hypertension and dyslipidaemia than CAD patients without NASH. But how this difference contributes to the elevation of acute inflammatory markers, particularly AMG and CP, in CAD patients with NASH cannot be explained. We show that a substantial number of CAD patients may be associated with NAFLD and NASH. Non-invasive simple parameters that reflect the degree of inflammation and fibrosis of the liver in patients with NASH would facilitate improved understanding and treatment of the disease. Further studies may be necessary to evaluate the percentage of NASH patients progressing to CAD.

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The authors have not received any funding or benefits from industry to conduct this study.

References

1. Neuschwander-Tetri BA. Evolving pathophysiologic concepts in non-alcoholic steatohepatitis. Curr Gastroenterol Rep. 2002; 4: 31–6.
2. Gupta S, Slaughter S, Akriviadis EA, Valenzuela R, Deodhar SD. Serial measurement of serum C-reactive protein facilitates evaluation in alcoholic hepatitis. Hepatogastroenterology 1995; 42: 516–21.
3. Shiota G, Umeki K, Okano J, Kawasaki H. Hepatocyte growth factor and acute phase proteins in patients with chronic liver diseases. J Med. 1995; 26: 295–308.
4. Shima M, Nakao K, Kato Y, Nakata K, Ishii N, Nagataki S. Comparative study of C-reactive protein in chronic hepatitis B and chronic hepatitis C. Tohoku J Exp Med. 1996; 178: 287–97.
5. Bell H, Skinningsrud A, Raknerud N, Try K. Serum ferritin and transferrin saturation in patients with chronic alcoholic and non-alcoholic liver disease. J Intern Med. 1994; 236: 315–22.
6. Bacon B, Farahvash M, Janney C, Neuschwander-Tetri B. Non-alcoholic steatohepatitis: an expanded clinical entity. Gastro-enterology 1994; 107: 1103–9.
7. George DK, Goldwurm S, McDonald GA, Cowley LL, Walker NI, Ward PJ, et al. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. Gastroenterology 1998; 114: 311–8.
8. Moirand R, Mortaji AM, Loreal O, Paillard F, Brissot P, Deugnier Y. A new syndrome of liver iron overload with normal transferrin saturation. Lancet 1997; 349: 95–7.
9. Mendler MH, Turlin B, Moirand R, Jouanolle A-M, Sapey T, Guyader D, et al. Insulin resistance-associated hepatic iron overload. Gastroenterology 1999; 117: 1155–63.
10. Fargion S, Mattioli M, Fracanzani AL, Sampietro M, Tavazzi D, Fociani P, et al. Hyperferritinemia, iron overload, and multiple metabolic alterations identify patients at risk for non-alcoholic steatohepatitis. Am J Gastroenterol. 2001; 96: 2448–54.
11. Gerok W, Gross V. Protein secretion, degradation and function. In: Bircher J, Benhamou JP, Mclntyre N, Rizetto M, Rodes J, editors. Oxford textbook of clinical hepatology. 2nd ed., vol. 1. New York: Oxford University Press; 1999, p. 346–67.
12. Tuck CH, Holleran S, Berglund L. Hormonal regulation of lipoprotein(a) levels: effects of estrogen replacement therapy on lipoprotein (a) and acute phase reactants in postmenopausal women. Arterioscler Thromb Biol. 1997; 17: 1822–9.
13. Min WK, Chun S, Hwang SH, Park H. No relationship between serum lipoprotein(a) and albumin concentrations in patients with acute phase response. Ann Clin Biochem. 1999; 36: 617–21.

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