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ORIGINAL ARTICLE

ADAMTS13 deficiency, despite well-compensated liver functions in patients with noncirrhotic portal hypertension

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Abstract
Background We have reported A disintegrin and metalloprotease with thrombospondin type 1 motif, member 13 (ADAMTS13) deficiency in noncirrhotic intrahepatic portal hypertension (NCIPH) patients of European origin with preserved liver function. We aimed to study ADAMTS13–von Willebrand factor (vWF) imbalance in Indian patients with NCIPH.
Methods Twenty-nine cases with NCIPH [22 males; 29 years (13–58); Child's A, 23; B, 6], 22 disease controls with cryptogenic chronic liver disease [15 males; 46 years (18–74); Child's A, 9; B, 9; C, 4] and 17 healthy controls [14 males; 32 years (27–45)] were enrolled in the study. We measured ADAMTS13 antigen and activity (by collagen binding assay (CBA) and by fluorescence resonance energy transfer [FRET] assay), and vWF antigen levels in plasma of study patients.
Results ADAMTS13 activity by CBA in NCIPH patients (32 %, 5 % to 100 %; median, range; p-value <0.001) and disease controls (36 %, 5 % to 144 %; p=0.001) was significantly lower than in healthy controls (87 %; 60 % to 148 %). ADAMTS13 antigen and activity by FRET assay were also lower in cases and disease controls. ADAMTS13 activity (by CBA) to antigen ratio was lower in NCIPH and disease controls than in healthy controls. Of 29 NCIPH patients, 3 (all in Child's A) had severe ADAMTS13 deficiency (<10 % ADAMTS13 activity), and 8 (Child's A, 7; B, 1) had moderate ADAMTS13 deficiency (10 % to 25 % activity). Conversely, vWF antigen and vWF:ADAMTS13 ratio were higher in patients with NCIPH and in disease controls than in healthy controls.
Conclusions This study validates the finding of ADAMTS13 deficiency in NCIPH despite preserved liver functions in an Indian population suggesting its involvement in pathogenesis of NCIPH.

Keywords Endothelial activation · Liver disease · von Willebrand factor

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Introduction

Thrombosis of the intrahepatic microcirculation, a common feature of cirrhosis, is linked to progression of cirrhosis. In a study of 61 explant livers, intimal fibrosis (highly suggestive of healed thrombosis) was detected in hepatic vein branches in 70 % of livers and in portal vein branches in 36 % of livers. While hepatic vein lesions were associated with focal parenchymal extinction, portal vein lesions were associated with

variation in size of cirrhotic nodules and with history of variceal bleeding [1].

In cirrhosis, the disease process initially affects the hepatocyte and followed by thrombosis of the hepatic microcirculation. In contrast, in idiopathic noncirrhotic intrahepatic portal hypertension (NCIPH), an isolated microangiopathy, the initial and main pathology is occlusion of intrahepatic portal vein radicles [2, 3]. Previously recognized as common in India [4], NCIPH remains an important cause of portal hypertension at our centre [5–7].

There is a 'dynamic' component of portal hypertension [8], and endothelial/platelet derived factors, important in primary hemostasis, may play a role in this. A disintegrin and metalloprotease with thrombospondin type 1 motif, member 13 (ADAMTS13) is a von Willebrand factor (vWF) cleaving protease, deficiency of which causes formation of platelet microthrombi in thrombotic thrombocytopenic purpura (a thrombotic microangiopathy) [9]. Reduced ADAMTS13 levels as well as elevated vWF levels are reported in portal hypertension [10–12]. We recently reported ADAMTS13 deficiency in NCIPH in predominantly Caucasian patients [13]. While ADAMTS13 levels decline in advanced cirrhosis [12], we reported ADAMTS13 deficiency in NCIPH patients who have preserved liver function, suggesting a role for ADAMTS13 deficiency in pathogenesis of NCIPH [13].

The aim of our study was to analyze ADAMTS13/vWF balance in portal hypertensive patients with NCIPH (cases), cryptogenic chronic liver disease (disease controls) and healthy controls in India.

Methods

This case–control study was done to compare plasma levels of ADAMTS13 and vWF in NCIPH patients (cases), cryptogenic chronic liver disease patients (disease controls) and healthy volunteers (healthy controls). All study cases and disease controls had portal hypertension (diagnosed by presence of gastrooesophageal varices on endoscopy). Informed consent was obtained from patients prior to recruitment into the study.

Case selection, demography and evaluation of NCIPH patients (cases) and cryptogenic chronic liver disease patients (disease controls)

We recruited 29 NCIPH patients (22 males; age 29 years (13–58) median (range) and 22 cryptogenic chronic liver disease patients (15 males; age 46 years (18–74)) under treatment at our unit into the study. Seventeen healthy volunteers (14 males; age 32 years [27–45]) were also enrolled for the study. Forty-eight percent of cases, 41 % of disease controls and 65 % of healthy controls were from southern India; 41 % of cases, 50 % of disease controls and 6 % of healthy controls were from eastern India. Rest of the study patients belonged to northern India.

NCIPH case definition was as per five defined criteria [14]: (1) presence of portal hypertension, (2) patent portal and hepatic veins at time of diagnosis of NCIPH, (3) absence of cirrhosis or severe fibrosis on liver biopsy, (4) exclusion of conditions causing cirrhosis such as alcoholic liver disease and viral hepatitis and (5) exclusion of conditions that may cause portal venous lesions mimicking NCIPH on histology such as sarcoidosis or congenital hepatic fibrosis. All NCIPH cases included in this study met these five criteria. We excluded patients who were post-liver transplant or had hepatic malignancy. In addition, we also excluded patients who had predominant histological features of another disease process (for example, histological features of autoimmune hepatitis or nonalcoholic fatty liver disease) in addition to histological features suggestive of portal venous insufficiency.

Diagnosis of study patients

All patients with NCIPH underwent liver biopsy (transjugular 17; percutaneous 6 and perioperative 6). The decision to perform transjugular or percutaneous liver biopsy was made on a case-to-case basis, depending on the bleeding parameters. The perioperative liver biopsies were obtained during splenorenal shunt surgery (five patients) or during splenectomy done to treat hypersplenism (one patient). In patients who underwent transjugular liver biopsy, 3 (2–5) median (range) of biopsy cores, 13 mm (5–16) in length and containing 10 (4–15) portal tracts, were obtained. Similarly, in patients who underwent percutaneous liver biopsy, 2 (1–3) cores, 12 mm (10–20) in length and containing 7 (6–15) portal tracts, were obtained. Perioperatively, wedge as well as Tru-Cut liver biopsies were obtained. Liver histology showed portal fibrosis (mild, 20 patients; moderate, 4 patients), portal vein ectasia (16 patients), perisinusoidal/perivenular fibrosis (11), thickened portal vein (4), hypoplastic portal tract (2) and atretic portal vein (1). Of the 15 NCIPH patients who underwent hepatic venous pressure measurements, 5 (30 %) had hepatic venous pressure gradient (HVPG) ≤5 mmHg.

Cryptogenic chronic liver disease was defined as intrahepatic portal hypertension with no identifiable cause of portal hypertension prior to liver biopsy and negative work up for etiology of portal hypertension (e.g. history of ethanol intake, serology tests for hepatitis B and C, tests for autoimmune liver disease, tests for copper and iron overload, and other investigations performed on a case-to-case basis). Liver biopsy done in 9 of the 22 patients with cryptogenic chronic liver disease (transjugular biopsy, 7; percutaneous, 2) showed cirrhosis (5 patients) and bridging fibrosis (3 patients). In 1 patient, the biopsy was inadequate for analysis.

Initial presentation of patients with NCIPH was variceal bleed (13 patients), hypersplenism (7), incidental (4), anemia

(2) and ascites, pedal edema and splenomegaly (1 each). Initial presentation of patients with cryptogenic chronic liver disease was variceal bleed (8 patients), ascites (5), hypersplenism (3), incidental (2), splenomegaly (2) and dyspnea and fatigue (2).

Of the 29 patients with NCIPH, two patients had coexistent adult celiac disease (in both patients, celiac disease was diagnosed after the diagnosis of NCIPH)—one patient (male; 35 years; from Tamil Nadu) had iron and vitamin B_{12} deficient anemia and the other patient (male; 32 years; from Bihar) had iron deficiency. One patient (male; 31 years) had bilateral avascular necrosis of the hip (diagnosed 2 years after the diagnosis of NCIPH). He had no history of steroid intake and did not have any other medical conditions predisposing to avascular necrosis of the hip. One patient (male; 45 years) from West Bengal (i.e. the Gangetic Plain) had skin changes consistent with arsenicosis (keratosis and melanosis of palms and soles) and increased arsenic level in nail of 0.8 mg/kg (normal 0.02–0.5 mg/kg) [15].

Baseline demographics and laboratory parameters of patients with NCIPH and cryptogenic chronic liver disease are shown in Table 1.

Besides routine evaluation for liver disease etiology and severity ascertainment in cases and disease controls, we assayed plasma ADAMTS13 and vWF levels in the study subjects.

ADAMTS13 and vWF assays

For ADAMTS13 and vWF assays, platelet poor plasma derived from citrated blood was divided in aliquots and stored at −80 °C till processing. ADAMTS13 antigen was measured using Imubind® ADAMTS13 ELISA kits (American Diagnostica Inc., Stamford, USA); to facilitate comparison of activity and antigen values, the results were converted to a percentage using the mean normal value. Due to a lack of standardization, ADAMTS13 activity was assayed by two previously described in-house methods [16], firstly by estimating residual collagen binding activity of purified vWF (collagen-binding assay (CBA)) and secondly by fluorescence resonance transfer (FRET) assay with vWF73 substrate. The normal values were as follows: ADAMTS13 activity by CBA (55 % to 160 %), ADAMTS13 activity by FRET assay (60 % to 123 %) and ADAMTS13 antigen (64 % to 136 %) [16]. vWF antigen was measured in plasma by an automated coagulation analyser using an immunoturbidimetric method, and the normal values were 50–150 IU/dL. vWF:ADAMTS13 ratio was calculated as vWF (IU/dL) divided by ADAMTS13 antigen.

Categorising ADAMTS13 and vWF levels as per severity of liver disease

ADAMTS13, vWF levels and vWF:ADAMTS13 ratio in the study cases and disease controls were correlated with severity of liver disease (assessed by model for end-stage liver disease (MELD) score and by Child's class), hepatic venous pressure gradient (HVPG) and platelet counts.

Statistical methods

Discrete variables were expressed as numbers and percentages, and continuous variables were expressed as median and range. Non-parametric tests were used to compare between two groups (Fisher's exact, Mann–Whitney *U*/Kruskal–Wallis tests for unrelated samples, Wilcoxon signed ranks test for related samples and Spearman's correlation coefficient (ρ) for two continuous variables).

To facilitate analysis, patients with ADAMTS13 activity of <5 % were taken as having an activity of 5 %. Patients were grouped based on ADAMTS13 activity by CBA to have severe (<10 % activity), moderate (10 % to 25 %) and mild (25 % to 55 %) deficiency. The magnitude of difference in ADAMTS13 and vWF levels in cases and disease controls as compared to healthy controls was expressed as ratio of means, and the confidence interval of this ratio was calculated by bootstrapping (percentile method) with 1,000 re-samples using R software. SPSS version 15 was used for statistical analysis. A p-value of <0.05 was considered as statistically significant. The study was approved by institutional research and ethics committee.

Table 1 Baseline characteristics of patients with idiopathic noncirrhotic intrahepatic portal hypertension and cryptogenic chronic liver disease

Patient characteristics[a]	NCIPH (n=29)	Cryptogenic CLD (n=22)	p-value
MELD score	10 (6–13)	11.5 (6–23)	0.02
Child's score	5 (5–8)	7 (5–13)	0.001
Child's class (A/B/C)	23/6/0	9/9/4	0.007
Platelet count (×10^5/cmm)	0.53 (0.09–3.44)	0.33 (0.15–3.16)	0.038
Hepatic venous pressure gradient (mmHg)	7 (1–12), n=15	13.5 (7–20), n=6	0.003

NCIPH noncirrhotic intrahepatic portal hypertension, *CLD* chronic liver disease, *MELD* model for end-stage liver disease

[a] Values are either numbers (discrete) or median and range (continuous)

Results

ADAMTS13 activity and antigen levels and activity to antigen ratio

ADAMTS13 activity measured by both CBA (32 %, 5 % to 100 % vs. 87 %, 60 % to 148 %; *p*-value <0.001) and FRET (50 %, 14 % to 114 % vs. 98 %, 71 % to 123 %; *p*-value <0.001) assays and ADAMTS13 antigen (63 %, 30 % to 111 % vs. 102 %, 73 % to 143 %; *p*-value <0.001) were significantly lower in patients with NCIPH when compared to healthy controls (Fig. 1). Reduced ADAMTS13 activity by CBA (36 %, 5 % to 144 %; *p*-value=0.001) and FRET assays (49 %, 5 % to 125 %; *p*-value <0.001) and ADAMTS13 antigen (66.5 %, 3 % to 228 %; *p*-value=0.003) were also seen in patients with cryptogenic chronic liver disease.

ADAMTS13 activity was significantly lower with CBA assay as compared to FRET assay in patients with NCIPH (*p*-value=0.001) and cryptogenic chronic liver disease (*p*-value=0.005) but not in healthy controls (*p*-value=0.14).

Low ADAMTS13 activity by CBA was seen in 19 (66 %) NCIPH patients compared to none in healthy controls (*p*-value <0.001). Of the 29 NCIPH patients studied, based on ADAMTS13 activity by CBA, mild, moderate and severe ADAMTS13 deficiencies were seen in 8 (28 %), 8 (28 %) and 3 (10 %) patients, respectively. Of the 22 patients with cryptogenic chronic liver disease, 15 patients had low ADAMTS13 activity on CBA [mild ADAMTS13 deficiency 7 (32 %), moderate 2 (9 %) and severe 6 (27 %)]. None of the study patients had obvious evidence of hemolysis, e.g. schistocytes.

ADAMTS13 specific activity (activity to antigen ratio for CBA (0.5, 0.07–1.89 vs. 0.81, 044–1.45; *p*-value=0.01)) was lower in NCIPH patients than in healthy controls, which was not the case with FRET assay (0.83, 0.3–1.66 vs. 0.91, 0.61–1.31; *p*-value=0.2). ADAMTS13 activity to antigen ratio for CBA (0.57, 0.09–2; *p*-value=0.8) and FRET assays (0.78, 0.31–2; *p*-value=0.48) in patients with cryptogenic chronic liver disease was similar to NCIPH patients.

vWF antigen levels

vWF antigen levels in NCIPH patients (*n*=23, 172.3, 59.4–289.4 IU/dL) were significantly higher as compared to healthy controls (*n*=17, 73.1, 38.2–135.9 IU/dL; *p*-value <0.001). Similarly, vWF levels were also high in patients with cryptogenic chronic liver disease (*n*=17, 215.8, 18.9–313.8 IU/dL; *p*-value <0.001; Fig. 2). Fifteen of the 17 cryptogenic chronic liver disease and 16 of the 23 NCIPH patients had elevated vWF levels (>150 IU/dL), as compared to none of the healthy control.

Analysis of ADAMTS13–vWF imbalance in patients with portal hypertension (i.e. in cases and disease controls)

vWF:ADAMTS13 ratio was higher in NCIPH (3.1, 1.3–7.8; *p*-value <0.001) and cryptogenic chronic liver disease (2.7, 0.29–97; *p*-value <0.001) as compared to healthy controls (0.7, 0.2–1.7; Fig. 3).

Mean ADAMTS13 activity by CBA in patients with NCIPH and cryptogenic chronic liver disease was 45 % (95 % CI 34 % to 58 %) and 50 % (95 % CI 34 % to 70 %), respectively, of the healthy controls, (Fig. 4). In contrast, mean vWF antigen levels in patients with NCIPH and cryptogenic chronic liver disease, when compared to healthy controls, were 237 % (95 % CI 190 % to 297 %) and 273 % (95 % CI 21 % to 350 %), respectively. Similar results were noted in Child's A

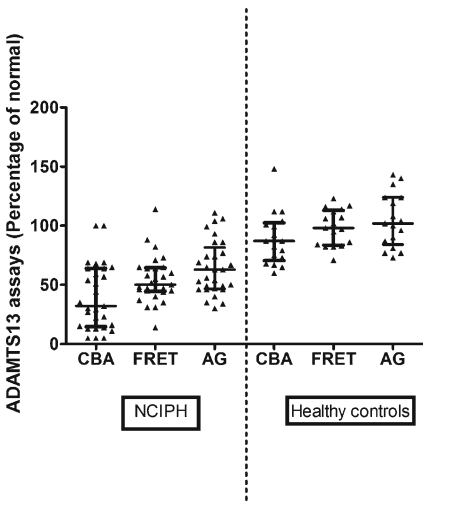

Fig. 1 Results of various assays for ADAMTS13 antigen and activity (expressed as percentage of normal) in 29 patients with NCIPH (idiopathic noncirrhotic intrahepatic portal hypertension; *left panel*) and 17 healthy controls (*right panel*). *NCIPH* idiopathic noncirrhotic intrahepatic portal hypertension; *ADAMTS13* A disintegrin and metalloprotease with thrombospondin type 1 motif, member 13; *CBA* collagen-binding assay; *FRET* fluorescence resonance transfer assay; *AG* antigen assay

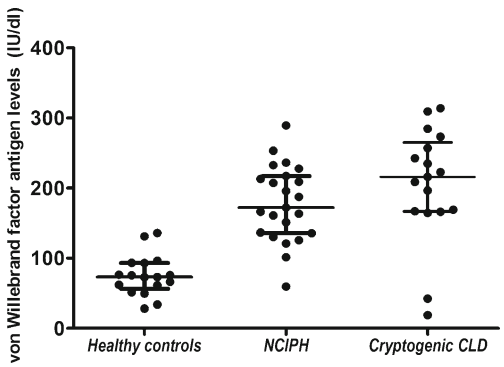

Fig. 2 von Willebrand factor antigen levels in healthy controls (*n*=17) and patients with NCIPH (*n*=23) and cryptogenic CLD (*n*=17). *NCIPH* idiopathic noncirrhotic intrahepatic portal hypertension, *CLD* chronic liver disease

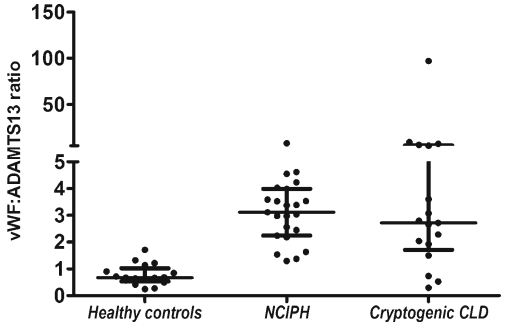

Fig. 3 vWF (IU/dL):ADAMTS13 antigen ratio in healthy controls (n=17), and patients with NCIPH (n=23) and cryptogenic chronic liver disease (n=17). *vWF* von Willebrand factor; *ADAMTS13* A disintegrin and metalloprotease with thrombospondin type 1 motif, member 13; *NCIPH* idiopathic noncirrhotic intrahepatic portal hypertension; *CLD* chronic liver disease

NCIPH patients [ADAMTS13 activity 44 % (95 % CI 32 % to 57 %) and vWF antigen level 240 % (95 % CI 200 % to 300 %)].

ADAMTS13 activity by CBA showed a significant and moderate negative correlation with vWF levels in patients with cryptogenic chronic liver disease (ρ=0.5; p-value=0.05), which was not seen in NCIPH patients (ρ=0.27; p-value=0.2). Of the 51 portal hypertensive patients studied (29 NCIPH and 22 cryptogenic chronic liver disease), only 4 patients (3 NCIPH patients) had normal ADAMTS13 activity and normal vWF levels.

Correlating ADAMTS13 levels to severity of liver disease

Of the nine patients with severe ADAMTS13 deficiency, all three NCIPH patients were in Child's class A (Child score 5 in 2 and 6 in 1; MELD score of 8, 11 and 12); in contrast, only one of the six cryptogenic chronic liver disease patients was in Child's class A (p-value=0.05). Seven (88 %) NCIPH patients with moderate ADAMTS13 deficiency and six (75 %) NCIPH patients with mild ADAMTS13 deficiency were in Child's class A.

Table 2 depicts ADAMTS13 activity in portal hypertensive patients classified according to Child's class. In NCIPH patients, ADAMTS13 activity by CBA was similar in patients with Child's class A and Child's class B (p-value=0.4). In contrast, cryptogenic chronic liver disease patients showed a decreasing trend in ADAMTS13 activity by CBA with worsening liver functions (p-value=0.07).

ADAMTS13 activity by CBA did not correlate with MELD scores in either NCIPH patients (ρ=−0.084; p-value=0.7) or patients with cryptogenic chronic liver disease (ρ=−0.3; p-value=0.16). There was no statistical difference in MELD score in NCIPH patients with severe ADAMTS13 deficiency (10.3; 8–12), moderate deficiency (10, 8–13), mild deficiency (7, 6–12) and patients with normal ADAMTS13 activity (10.5, 6–13; p-value=0.12).

Correlating vWF antigen levels to severity of liver disease

Table 2 depicts vWF antigen levels in portal hypertensive patients. Both in NCIPH patients (p-value=0.26) and in patients with cryptogenic chronic liver disease (p-value=0.7), vWF antigen levels were similar despite advancing Child's class. There was no correlation between vWF antigen levels and MELD score in NCIPH patients (ρ=0.35; p-value=0.13) and in patients with cryptogenic chronic liver disease (ρ=0.1; p-value=0.8).

Correlating vWF:ADAMTS13 ratio to severity of liver disease

In NCIPH patients, vWF:ADAMTS13 ratio did not correlate with MELD score (ρ=0.01, p-value=1) or Child's score (ρ=

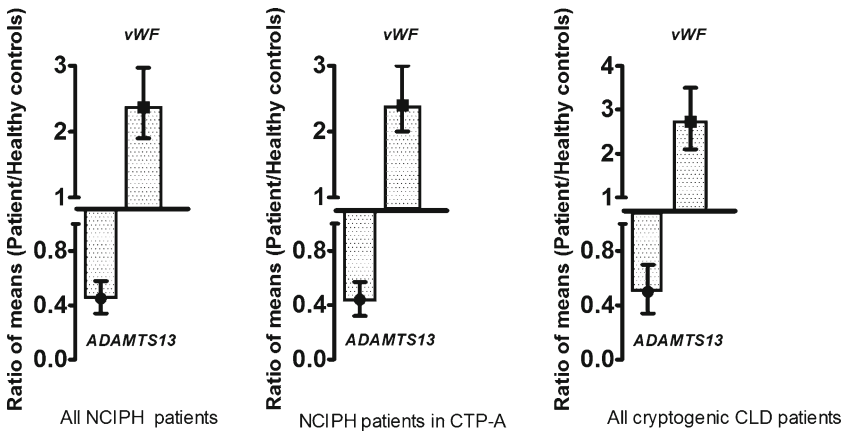

Fig. 4 The graph shows decreased ADAMTS13 and increased vWF levels in NCIPH patients (*left panel*), cryptogenic chronic liver disease (CLD) patients (*right panel*) and NCIPH patients in Child's class A (*middle panel*; n=23) as compared to healthy controls. The *centre* represents the ratio of means, and the *horizontal bars* represent 95 % confidence interval. *ADAMTS13* A disintegrin and metalloprotease with thrombospondin type 1 motif, member 13; *vWF* von Willebrand factor; *NCIPH* idiopathic noncirrhotic intrahepatic portal hypertension; *CLD* chronic liver disease; *CTP-A* Child's class A

0.15; p-value=0.5). The vWF:ADAMTS13 ratio was similar in patients with Child's A (p-value=0.4; Table 2).

In cryptogenic chronic liver disease patients, vWF:ADAMTS13 ratio correlated with MELD score (ρ=0.5; p-value=0.04) and Child's score (ρ=0.56; p-value=0.02). The ratio increased with increasing Child's class in patients with cryptogenic chronic liver disease (p-value=0.03; Table 2).

Correlating ADAMTS13, vWF antigen and vWF:ADAMTS13 ratio to HVPG and platelet count

In 15 NCIPH patients, who had HVPG measured, ADAMTS13 activity by CBA (ρ=0.24; p-value=0.43), vWF antigen level (ρ=0.28; p-value=0.32) and vWF:ADAMTS13 ratio (ρ=0.12; p-value=0.7) did not correlate with HVPG. In the 4 (all Child's class A) NCIPH patients with moderate (3) and severe (1) ADAMTS13 deficiency, HVPG was 4, 4, 6 and 12 mmHg. In the 6 cryptogenic chronic liver disease patients with HVPG measured, there was no correlation noted between HVPG and ADAMTS13 activity by CBA (ρ=0.35; p-value=0.5).

There was no correlation between ADAMTS13 activity by CBA (ρ=0.33; p-value=0.08), vWF antigen (ρ=0.093; p-value=0.7) and vWF:ADAMTS13 ratio (ρ=0.36; p-value=0.09) to the platelet count in NCIPH patients. In patients with cryptogenic chronic liver disease, as well, there was no significant correlation between vWF (ρ=0.16; p-value=0.5) or ADAMTS13 activity (ρ=0.32; p-value=0.14) to platelet count.

Discussion

In our study, patients with portal hypertension had reduced ADAMTS13 activity and elevated vWF levels. We also noted a significant association of low ADAMTS13 activity and NCIPH. The single most important observation in the current study is that ADAMTS13 deficiency occurs in NCIPH patients, despite well-preserved liver functions. This validates a similar finding which we reported in cohort of NCIPH patients from the UK [13].

Of 29 NCIPH patients studied in the current report, all 3 patients with severe ADAMTS13 deficiency (i.e. <10 % activity by CBA) were in Child's A and had MELD scores of ≤12. In our previous report, we described 5 NCIPH patients (of 18 NCIPH patients studied) with severe ADAMTS13 deficiency (by CBA) having MELD scores of 12, 12, 14, 16 and 21 [13]. In contrast, in a study reported by Uemura et al. [12], of 109 patients with cirrhosis (96 of whom had hepatitis C), severe ADAMTS13 deficiency (i.e. ≤3 % of activity by vWF multimer assay) was reported in 5 patients, all of whom were in Child's class C (Child's score 13) [10–14].

In the study by Uemura et al. [12], severe ADAMTS13 deficiency was only seen in 5 of 41 patients with Child's C, while no patient with Child's A (35 patients) or Child's B (33 patients) cirrhosis had severe ADAMTS13 deficiency. This suggests that in patients with cirrhosis, the advancing liver disease leads to reduced ADAMTS13 secretion [12] (the main source of ADAMTS13 being hepatic stellate cells [17, 18]) or increased consumption.

The finding of severe ADAMTS13 deficiency in 8 NCIPH patients in our two reports (current report and Mackie et al. [13]), despite having well-preserved liver functions (MELD score ≤14 in 6), suggests that ADAMTS13 deficiency may be a primary event which predisposes to NCIPH.

In our previous study [13], we performed a detailed investigation of this phenomenon in NCIPH patients. We found no correlation between vWF and ADAMTS13; ADAMTS13 inhibitors and antibodies to ADAMTS13 were not significantly increased in NCIPH patients compared to controls [13]. Sustained ADAMTS13 deficiency was noted in four NCIPH patients in our previous report. In 1 NCIPH patient with severe ADAMTS13 deficiency, half-life studies using ADAMTS13 replacement by fresh frozen plasma infusions showed that

Table 2 ADAMTS13 activity and vWF levels in patients with idiopathic noncirrhotic intrahepatic portal hypertension and cryptogenic chronic liver disease

	Child's class	Number of patients	ADAMTS13 activity by CBA (%)[a]	vWF antigen[a,b]	vWF:ADAMTS13 ratio[a,b]
NCIPH	A	23	30 (5 to 100)	192 (59–253)	3.3 (1.3–7.8)
	B	6	46 (16 to 69)	136 (101–289)	3 (1.4–4)
Cryptogenic chronic liver disease	A	9	100 (5 to 114)	196 (42–309)	2 (0.3–5)
	B	9	35 (5 to 70)	209 (18–314)	2.7 (0.5–7.1)
	C	4	5 (5 to 62)	229 (197–285)	7.5 (3–97)

NCIPH noncirrhotic intrahepatic portal hypertension; *ADAMTS13* A disintegrin and metalloprotease with thrombospondin type 1 motif, member 13; *CBA* collagen binding assay; *vWF* von Willebrand factor; *vWF:ADAMTS13 ratio* ratio of vWF antigen to ADAMTS13 antigen

[a] All values are depicted in median and range

[b] vWF antigen levels were done in 23 NCIPH patients (Child's A, 18; Child's B, 5) and in 17 cryptogenic chronic liver disease patients (Child's A, 8; Child's B, 5; Child's C, 4)

plasma levels of ADAMTS13 activity and antigen were increased by 50 % for 72 h [19]. Deficient ADAMTS13 levels in plasma normalized after liver transplant in another NCIPH patient and remained normal 15 months later. These findings suggest that ADAMTS13 deficiency in NCIPH is not due to accelerated consumption of ADAMTS13. We proposed that primary ADAMTS13 deficiency, either congenital or acquired, predisposes an individual to development of NCIPH [13].

Elevated vWF levels in peripheral blood correlate with development of portal hypertension, hepatic decompensation and mortality in patients with cirrhosis [10, 20]. In the current study, elevated plasma vWF antigen levels in portal hypertensive patients did not correlate with liver disease severity. A possible explanation is lack of patients with advanced liver disease (only four patients with Child's C) included for the current study.

It has been proposed that reduced ADAMTS13 activity accompanied by an increase in its substrate (ultra-large multimers of vWF) in cirrhosis predisposes to formation of platelet microthrombi [12]. In our previous report on NCIPH patients, we noted ultra-large vWF multimers in 4/11 (36 %) patients tested, compared to none of the controls [13]. We proposed that imbalance of (decreased) ADAMTS13 and (increased) vWF may drive the portal venular obliteration in NCIPH, and this mechanism may also be important in determining disease progression in cirrhosis [21]. We proposed a role for ADAMTS13/vWF imbalance in causing portopulmonary hypertension in a patient with NCIPH [19]. The majority (43 of 47 (92 %)) of patients with portal hypertension in the current study had an imbalance of ADAMTS13–vWF levels.

Sequestration of platelets onto splenic endothelium in patients with portal hypertension is observed in hypersplenism [22]. In NCIPH, sequestration of platelets, presumably aggregating with vWF, within the intrahepatic small branches of portal vein, may lead onto the formation of fibrin-poor platelet-rich thrombi, which occlude these portal vein branches. We speculate that such a process may occur in portal hypertension due to any cause.

Hepatic stellate cells, the main source of ADAMTS13 synthesis [17, 18], are located at a hepatic sinusoidal level (these cells reside in the subendothelial space between hepatocytes and sinusoidal endothelial cells) [23]. Hence, it is likely that the lowest levels of ADAMTS13 in the circulation will be in the presinusoidal portal vein branches in the liver. Selective occlusion of portal vein radicles in NCIPH further suggests that pathogenic factors operant in the gut–liver axis are important. We found over-representation of intestinal disease (adult-onset celiac disease and a case of ulcerative colitis) in a small cohort of NCIPH patients, supporting the above hypothesis [24]. Levels of inflammatory cytokines which can stimulate ultra-large vWF multimer release and inhibit ADAMTS13 synthesis [25, 26] are elevated in the sera of celiac disease patients [27]. In the current study, two NCIPH patients had adult-onset celiac disease, while one had arsenicosis (arsenic contamination of ground water is reported in Gangetic Plain in India) [28]—both of these factors have the potential to increase vWF levels [25, 26, 29], especially in the portal circulation. Various pathogenic mechanisms have been explored in NCIPH [30], and we propose that low ADAMTS13 contributes significantly in a subset of these patients.

We chose portal hypertensive patients with cryptogenic chronic liver disease as disease controls in our study. These are patients who did not have any cause for portal hypertension, prior to liver biopsy. A limitation in our study is that only 9 of the 22 patients with cryptogenic chronic liver disease underwent liver biopsy. Another limitation of our study is that the NCIPH cases and disease controls were not well matched in terms of liver disease severity (as assessed by Child's score). While cryptogenic chronic liver disease is a common mimic of NCIPH at our centre [6, 7], any etiology of cirrhosis can account for cryptogenic cirrhosis. In the current study, of the 6 patients with cryptogenic chronic liver disease who had severe ADAMTS13 deficiency, 1 was in Child's A, 2 in Child's B and 3 in Child's C.

Diagnosis of NCIPH on needle liver biopsies is challenging. The key points in making this diagnosis are the lack of significant liver fibrosis in a patient with unexplained portal hypertension and evidence of portal microangiopathy, which may be present (for example, portal vein ectasia was seen in liver biopsy in 16 of the 29 NCIPH cases [55 %] in the present study).

At present, ADAMTS13 assays lack standardization with no internationally recognized reference material [16, 31]; hence, we measured ADAMTS13 activity by two different methods (CBA and FRET assays). In the current study, ADAMTS13 activity levels were more significantly decreased in both NCIPH patients and patients with cryptogenic chronic liver disease (CLD) when assayed by CBA compared to FRET assay. However, this difference in ADAMTS13 activity levels between assays was not noted in healthy controls. We had made a similar observation in our previous report on ADAMTS13 levels in NCIPH patients in UK. The discrepancy could be explained by a number of factors and has been observed in some patients with congenital thrombotic thrombocytopenic purpura and other thrombotic microangiopathies [16]. The CBA utilises full-length vWF as a substrate, whereas the FRET assay uses a 73-amino acid substrate based on the ADAMTS13 cleavage site in the A2 domain of vWF. Since there appear to be multiple interactions between the various structural domains of ADAMTS13 and vWF, it is possible that structural changes such as truncation of ADAMTS13, change in glycosylation, or binding of substances that obscure attachment sites or alter the protein

conformation could lead to decreased cleavage of the natural vWF substrate but not detectable with the FRET substrate. The similarity of findings in the cryptogenic chronic liver disease patients further raises the intriguing possibility that there is an overlap in their pathogenesis with that of NCIPH in India.

ADAMTS13-deficient individuals may exist within the population who are at increased risk of developing NCIPH. In such individuals, increased expression of vWF in the portal circulation in response to stimuli such as celiac disease may predispose to formation of platelet microthrombi in portal venules and cause occlusion of these venules—manifesting as NCIPH. The perturbed vWF:ADAMTS13 ratio may lead to platelet thrombi to form at the confluence of portal vein branches with hepatic arterial inflow. Thus, in NCIPH, the disease remains limited to smaller branches of the portal venular bed, and this also explains the prolonged nature of the disease in contrast to sudden illness of thrombotic thrombocytopenic purpura. There was no evidence of hemolysis in any of the study patients.

We postulate that the same mechanistic pathway of ADAMTS13–vWF imbalance may also be important in cirrhosis of varying etiology; however, the sequence of events is different. While raised vWF levels in patients with cirrhosis are noted in Child's A, B and C (i.e. early as well as advanced disease), ADAMTS13 deficiency occurs only very late in the disease (Child's C) [12]—probably a consequence of poor hepatic synthetic function, with advancing cirrhosis.

Further studies of ADAMTS13–vWF imbalance are needed in NCIPH, cirrhosis and other causes of portal hypertension, in particular, to analyze whether this imbalance affects the natural history of these disorders. Studies of ADAMTS13–vWF levels in the general population, as well as in other vascular liver disorders like extrahepatic portal vein obstruction and Budd–Chiari syndrome are also needed. Interventions to normalize the reduced ADAMTS13 levels and elevated vWF levels in portal hypertensive patients have the potential to retard ongoing microcirculatory occlusion within the liver, and thus favorably influence the natural history of these patients.

Conflict of interest AG, PLA, SCN, IM, BR, JM, SNK, CEE, and EE all declare that they have no conflicts of interest.

Ethics statement The study was performed in a manner to conform with the Helsinki Declaration of 1975, as revised in 2000 and 2008 concerning Human and Animal Rights, and the authors followed the policy concerning informed consent as shown on Springer.com.

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