Variations in Hepatic Vein Pressure Gradient and Liver Stiffness in the Histological Categories of Non-cirrhotic Portal Hypertension

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Research Article

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Abstract

Introduction: Non-cirrhotic portal hypertension (NCPH) usually has lower liver stiffness (LS) and hepatic pressure gradient (HPVG), than cirrhosis. At times, it can have elevated LS and HVPG and be confused with liver cirrhosis. In this study we aimed to categorize those entities of NCPH with higher values of LS and HVPG for the prognostic relevance.

Patients & Methods: This study included liver biopsy cases diagnosed as NCPH (2015-2021). Age and gender-matched controls of cryptogenic cirrhosis (n=127) were included. Parameters were obtained from the electronic records. The histological categories were: Obliterative portal venopathy (OPV), OPV with bridges (OPV-F), incomplete septal cirrhosis (ISC), nodular regenerative hyperplasia (NRH), mega sinusoids with fibrosis (MSF) and unclassified.

Results: Mean age of study participants (n=173) was 39 (27.5-50) years. LS and HVPG were significantly lower than the cirrhosis {8 (6-12) vs 16 (14-17) Kpa; and 8 (6.9-11.7) vs. 27 (23-36), p<0.001. NCPH categories were: OPV {45, (26%)}, OPV- {37, (21.4%)}, ISC {20, (11.6%)}, NRH {19, (11%)}, MSF {19, (11%)}, and unclassified (33, (19%)). Elevated HVPG noted in OPV-F {15(12-16)} and ISC {12 (9-14)} mmHg and correlated with fibrosis quantity. A HVPG >10 mmHg was: OPV (0/45, 0%), OPV-F (29/37, 78.3%), ISC (13/20, 65%), NRH (8/19, 42%), MSF (1/19, 5.2%), and unclassified (1/33, 3%) with p<0.001. Higher values of TE recorded in MSF and NRH, as compared to other categories.

Conclusions: OPV-F and ISC have more fibrosis contents and higher HVPG values than the other categories of NCPH. Whereas, MSF and NRH have spuriously high liver stiffness.

Introduction:

The development of portal hypertension is one of the most severe outcomes of liver diseases. Cirrhosis is by far the most frequent cause of portal hypertension [1]. Portal hypertension in the absence of cirrhosis but with mild or moderate alterations of liver histology is being increasingly recognized. Owing to the heterogeneity of causes and histological findings, a substantial number of terms have been used, and non-cirrhotic portal hypertension is the commonest and most widely used term [1, 2]. Non-cirrhotic portal hypertension (NCPH) is defined as the presence of portal hypertension in the absence of cirrhosis or significantly advanced fibrosis [2].

Liver stiffness measurement using transient elastography (TE-LSM; FibroScan) is routinely done in patients with chronic liver disease. A TE of < 10 kPa with portal hypertension (PH) can distinguish cirrhosis PH from non-cirrhosis PH [3].

Hemodynamic studies can also differentiate between NCPH and cirrhosis. Studies revealed that an average (5 mmHg) or near-normal (10 mmHg) hepatic venous pressure gradient (HVPG) is firmly against the diagnosis of cirrhosis in such cases [4].
However, since the NCPH is a broad term with many histological features and subcategories. [5–8] At times, it can have elevated LS and HVPG and be confused with liver cirrhosis. [8, 9] In this study, we aimed to categorise those entities of NCPH that may show variations in LS and HVPG and have higher LS and HVPG values for prognostic relevance.

**Patients And Methods:**

1. **Patients with NCPH:** This study was a prospective assessment of the retrospective cohort. It included patients with non-cirrhotic portal hypertension diagnosed based on liver histology (June 2010 and June 2020) and with available information on TE and HVPG. Institutional ethics committee Institute of Liver and Biliary Sciences, New Delhi) provided waiver of informed consent and approved the study-IEC/2020/82/MA11. This observational study was designed, conducted, and written in accordance with the STROBE guidelines.

The clinical, laboratory, and radiological data were obtained from electronic records. A diagnosis of NCPH was made based on the VALDIG criteria [2]. Liver biopsies were reviewed by three experienced pathologists (SD, CB, and AR). A biopsy of ≥ 2 cm with ≥ 10 portal tracts with available information on TE and HVPG was considered adequate. Patients with outflow vascular liver disorders, cases of cirrhosis, infiltrative or space-occupying lesions, and inadequate material in tissue blocks were excluded.

The categories of NCPH based on histological characteristics [5–8] that were used for the categorization into appropriate entities are tabulated in Table 1. Cases were classified a priori into six categories: obliterative portal venopathy (OPV), obliterative portal venopathy with fibrosis (OPV-F), incomplete septal cirrhosis (ISC), nodular regenerative hyperplasia (NRH), megasinusoids with fibrosis (MSF), [Fig. 1] and unclassified [Fig. 2].

2. **Patients with Cirrhosis:** Age- and gender-matched cryptogenic cirrhosis patients with available records of TE and HVPG were considered the control group. All patients were of Child-Turcotte-Pugh Class A and had at least one feature of portal hypertension.

3. **Fibrosis Quantification:** Image analysis of the liver biopsies was performed, as described. [10] Briefly, images of a Masson's trichrome-stained section were taken at 2 × Objective (DP71 microscope, Olympus Corporation, Shinjuku-ku, Tokyo, Japan), using a colour camera (U-CMAD3). Using NIS-Elements AR 4.10 with 64-bit Windows 95-compatible software. The total biopsy area and the fibrosis area of the optimised images were analysed using annotations and the measurement tool. Large portal tracts and subcapsular areas were excluded by manual selection. The quantified data were saved as Microsoft Excel spreadsheets. The following formula was used to quantify the level of fibrosis: fibrosis percentage = (the sum of the pixels in Masson's Trichrome stained areas/total area pixels) × 100.

4. **Enzyme Linked Immunosorbent assay:** PIIINP levels in serum were measured using an indirect immunofluorescence technique as a surrogate marker of fibrosis (Human PIIINP ELISA Kit, Lot: B8RLF132K3). Stored serum samples were taken from the national liver disease biobank in Delhi, India.
5. **Statistical Analysis**: The categorical variables were presented in the form of a number and a percentage, while the continuous variables were presented as median values. The quantitative variables' associations were analysed using the independent t-test (for two groups) and ANOVA test (for more than two groups). The association of the qualitative variables in nature was analysed using the chi-square test or Fisher's exact test. The data entry was done in the Microsoft Excel spreadsheet, and the final analysis was done using Statistical Package for Social Sciences (SPSS) software version 21.0. For statistical significance, a p-value of less than 0.05 was considered significant.

**Results:**

1. **Basic characteristics**

   A total of 173 patients had the diagnosis of non-cirrhotic portal hypertension during the study period, fulfilling the inclusion and exclusion criteria. Age and gender matched compensated cirrhosis of cryptogenic aetiology (n = 127) was considered for the comparison. The hepatic venous pressure gradient (mmHg) of the study subjects was 8 (6–12), whereas in cirrhosis cases, it was 16 (14–17) with p < 0.001. Liver stiffness in the NCPH cases was 8 (6.9–11.7) kPa, significantly less than compensated cirrhosis cases {27 (23–36) kPa; p value < 0.001}. Basic parameters in both groups are summarized in Table 2.

2. **Histological categories of NCPH**

   In the majority (73.03%) of cases, acinar architecture was found to be maintained. NCPH were classified as follows by three pathologists: OPV 45 (26%), OPV-F 37 (21.4%), ISC 20 (11.6%), NRH 19 (11%), MSF 19 (11%), and unclassified 33 (19%). In 30 patients, other histopathological features were also noted: 22 had steatosis, 4 had extramedullary hematopoiesis; 2 had mild siderosis, 4 had chronic hepatitis with minimal activity, 1 had cholestasis, and 1 had Mallory-Denk bodies.

3. **Radiological Features in various Categories**

   Radiologically, collaterals were significantly higher in OPV-F (87.7%) and OPV (81%), as compared to ISC (42.86%), NRH (55%), sinusoidal dilatation (47.06%), and MSF (40%) with P < 0.001. Liver-irregular outlines, coarsened echotexture, and cirrhotic appearance were significantly higher in ISC (85.7%) as compared to OPV-F (62.07%), OPV (52.63%), NRH (65%), sinusoidal dilation (35.29%), and NCPF (33.33%) (P value = 0.024). Table-3. Distribution of splenomegaly, ascites, the prominent splenoportal axis, peripheral pruning of the portal vein, umbilical/periumbilical, vein recanalization, the nodular surface of the liver, caudate lobe hypertrophy, and portal vein thrombosis were comparable in various categories.

4. **HVPG and TE in NCPH**

   As expected, the HVPG and TE were significantly lower in NCPH cases than cirrhosis, as shown in Table 1. Among the NCPH cases, those patients with OPV-F and ISC had a higher HVPG than the rest of the categories like OPV, NRH, MSF, and unclassified [Fig. 3A]. Further, we noted significant liver pressure (HVPG > 10 mmHg) in various categories: OPV (0/45, 0%), OPV-F (29/37, 78.3%), ISC (13/20, 65%), NRH
(8/19, 42%), MSF (1/19, 5.2%), and unclassified (1/33, 3%) with p < 0.001. Contrarily, TE was higher in MSF and NRH as compared to other categories [Fig. 3B]. Significantly, high TE (> 10 kPa) among the NCPH categories was: OPV (3/45, 6.6%), OPV-F (29/37, 40.5%), ISC (4/20, 20%), NRH (15/19, 78.9%), MSF (14/19, 73.6%), and unclassified (2/33, 6%) with p = 0.001.

5. HVPG correlates with fibrosis quantity in NCPH

Cirrhosis patients had more fibrosis than NCPH: 12% (IQR: 9-16.5) vs 1.9% (IQR: 1-3.7%), respectively, with p < 0.001. The quantity of fibrosis was higher in the OPV-F and ISC categories of NCPH, as shown in [Fig. 4A]. Further, we used the non-invasive marker PIIINP in serum samples from cirrhosis and NCPH cases. A total of 98 samples of cirrhosis and 86 of NCPH (OPV: 22, OPV-F: 20, ISC: 10, NRH: 9, MSF: 12, and unclassified: 13) were available in the biobank. Cirrhosis cases had higher PIIINP 541 (347–771) ng/dl as compared to NCPH 122 (85–170) ng/dl, p < 0.001. PIIINP was also prominently higher in OPV-F and ISC than the other categories of NCPH [Fig. 4B]. In the NCPH cases, HVPG correlated with quantitative fibrosis with r = 0.751, p < 0.001 (Fig. 3C).

TE, on the other hand, was not linked to actual fibrosis in liver tissue (r = 0.15, p = 0.06; Fig. 3D). It suggests a spuriously elevated TE in MSF and NRH.

Discussion:

In this study, we found that the subset of cases under NCPH can have elevated HVPG and TE. Upon careful examination of the histology of NCPH cases, we found that those cases of OPV with bridging and incomplete septal cirrhosis can have high HVPG. This is linked to a higher fibrosis content in these groups. While patients with MSF and NRH may have higher TE values, the fibrosis content was not found to be higher in these patients. It suggests that TE in these categories is spuriously elevated.

Hemodynamic studies revealed that in NCPH cases, HVPG remains normal or near normal, and TE is usually less than in cirrhotic cases [3, 4]. However, there are a few cases where HVPG and TE levels in the NCPH can be elevated [9]. But these studies are based on small sample sizes. This is by far the largest sample size study in which we have classified NCPH and demonstrated that OPV-F, ISC, and NRH can have high HVPG and NRH and MSF can have falsely high TE. These cases must be classified histologically because their prognosis is better than that of cirrhosis.

Previously, a study of NCPH from our group showed the median hepatic venous pressure gradient (HVPG) and liver stiffness (LS, Fibroscan) values were 8 mm of Hg (range: 5–20 mm of Hg) and 9.2 kPa (range: 4.4–26.3 kPa), respectively [7]. This study indirectly indicated that a subset of patients with NCPH can have high HVPG and TE. Another study by Singh L et al found that TE ranged from 4.7 to 43.5 kilopascals with a mean value of 12.5 kilopascals, suggesting some fibrosis within hepatic lobules in most of the cases [11]. They did not find cirrhosis in any of the cases. But none of these studies indicated the possible association of any particular histomorphology in NCPH cases with elevated HVPG and TE, and sample sizes in both studies were relatively smaller than this study.
HVPG was found to be higher in OPV-F and ISC. Both conditions are characterised by the presence of incomplete fibrous septae, isolated collagen bundles in the hepatic parenchyma, abnormal spacing between portal tracts and veins, and crowding of reticulin fibres between adjacent zones of hyperplastic parenchyma, along with other histological features mentioned in Table 1 [9, 12]. Crowding of reticulin fibres, scattered collagen bundles, and hyperplasia of hepatocytes with the higher fibrosis contents that we noted in this study can cause intrahepatic flow resistance and elevation of HVPG in these conditions.

NRH and MSF were the two conditions where the TE was found to be falsely higher than expected. NRH represents an adaptive hyperplastic reaction of hepatocytes to the mechanical or functional abnormalities of the hepatic microvasculature due to imbalances between arterial and portal venous blood flow and is characterised by diffuse transformation of the hepatic parenchyma into small regenerative nodules in the absence of significant fibrosis [13]. Laharie et al., in a study on 30 patients with a histologically reconfirmed diagnosis of NRH, found that the fibroscan was not helpful in patients with NRH. Because the results did not correlate with portal or perisinusoidal fibrosis on biopsy, and nearly half of the NRH patients without significant portal fibrosis have elevated Fibroscan values, falsely suggesting a liver fibrosis [14]. The increased fibroscan value could be due to an architectural remodel in NRH. Sinusoidal dilatation is seen in the liver parenchyma of NCPH cases and is defined as a sinusoidal lumen that is wider than one liver cell plate [15]. This alteration generally affects multiple lobules within liver biopsy specimens and is associated with a thin rim of perisinusoidal fibrosis. Sinusoidal dilatation is interpreted as a non-specific feature of impaired portal vein flow in the setting of [16]. Wide spaces with plasma content in the lumen and a thin rim of fibrosis along the sinusoids can altogether cause a falsely raised TE.

The study has some limitations. The mechanistic aspect is lacking in this study, as to why only certain conditions of NCPH have elevated HVPG. Though we quantified the fibrosis and assessed it with non-invasive tests. We are planning to explore the molecular pathology of different entities in future studies. Nonetheless, this study provides an important piece of information, regarding the high liver pressure in certain entities of NCPH. The pathologist can carefully examine and categorise these entities to advise clinicians on disease prognosis and avoid mislabeling NCPH as cirrhosis.

Declarations:

Contributions: CB and SKS made the study concept and design; CB, SD, AR acquired the data; CB, SD and MKS did the analysis and interpretation of data; CB and SD drafted of the manuscript; MKS, SKS did the revision of the manuscript for important intellectual content; SKS provided administrative, technical, or material support and study supervision.

Ethics declarations

Conflict of interest: All authors declared no conflict of interest.
Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (and/or in case humans were involved).

References:

3. Liver Stiffness by Transient Elastography to Detect Porto-Sinusoidal Vascular Liver Disease With Portal Hypertension.


Tables:

Table-1: Histological Categories and Characteristics

<table>
<thead>
<tr>
<th>Categories</th>
<th>Histological Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obliterative portal-venopathy (OPV)</td>
<td>Thickening of the vessel wall, occlusion of portal vein lumen, and vanishing of portal veins Characterized by segmental, conspicuous subendothelial thickening of large and medium-sized portal vein branches Phlebosclerosis- defined as a partial or complete obliteration of the portal vein lumen in the setting of a fibrous portal tract</td>
</tr>
<tr>
<td>Obliterative portal-venopathy with bridging fibrosis (OPV-F)</td>
<td>Above features With fibrous bridging</td>
</tr>
<tr>
<td>Incomplete Septal Cirrhosis (ISC)</td>
<td>Incomplete, thin, perforated, or blind-ended septa, intermittently delimit rudimentary nodules, although complete cirrhotic-type regenerative nodules are not seen</td>
</tr>
<tr>
<td>Nodular Regenerative Hyperplasia (NRH)</td>
<td>Distortion of the typical lobular architecture by nodules of hyperplastic hepatocytes surrounded at the periphery by compressed atrophic cell plates &amp; condensed reticulin network but without significant fibrosis Occasionally centered around portal tract and curvilinear sinusoidal dilatation in areas of atrophy seen</td>
</tr>
<tr>
<td>Mega Sinusoids and Fibrosis (MSF)</td>
<td>Nonzonal sinusoidal dilatation (mega sinusoids) and perisinusoidal fibrosis as highlighted by a trichrome stain.</td>
</tr>
<tr>
<td>Unclassified</td>
<td>Portal tract fibrosis, portal tract remnants Dilated/herniated portal veins Approximation of vascular structures</td>
</tr>
</tbody>
</table>

Table-2 Basic parameters in both groups.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-Cirrhotic Portal Hypertension (n=173)</th>
<th>Cirrhosis (n=127)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39 (27.5-50)</td>
<td>41 (34-48)</td>
<td>0.061</td>
</tr>
<tr>
<td>Male: Female, M/F</td>
<td>114:59</td>
<td>82:45</td>
<td>0.433</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.1 (9.5-12.7)</td>
<td>7.8 (6.9-9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total leucocyte count (mm³) x10^3</td>
<td>4 (2.7-5.9)</td>
<td>4 (2.2-7.5)</td>
<td>0.903</td>
</tr>
<tr>
<td>Platelet (mm³)x10^3</td>
<td>70 (49-96)</td>
<td>53 (32-91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>1.3 (1-2.5)</td>
<td>3.2 (1.7-6.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.6 (2.8-4)</td>
<td>3.1 (2.5-3.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.7 (0.5-0.9)</td>
<td>0.8 (0.6-1.2)</td>
<td>0.074</td>
</tr>
<tr>
<td>International normalized ratio</td>
<td>1.2 (1.1-1.4)</td>
<td>1.5 (1.3-1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hepatic venous pressure gradient, mmHg</td>
<td>8 (6-12)</td>
<td>16 (14-17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Liver Stiffness, kPa,</td>
<td>8 (6.9-11.7)</td>
<td>27 (23-36)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table-3: Association of Radiology with Various Categories of NCPH
<table>
<thead>
<tr>
<th>Radiology</th>
<th>OPV (n=45)</th>
<th>OPV-F (n=37)</th>
<th>ISC (n=20)</th>
<th>NRH (n=19)</th>
<th>MSF (n=19)</th>
<th>unclassified (n=33)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenomegaly, (%)</td>
<td>35 (79)</td>
<td>34 (93)</td>
<td>20 (100)</td>
<td>13 (68)</td>
<td>12 (63)</td>
<td>23 (70)</td>
<td>0.089</td>
</tr>
<tr>
<td>Varices/collaterals, (%)</td>
<td>38 (84.4)</td>
<td>32 (87.7)</td>
<td>15 (74)</td>
<td>10 (55)</td>
<td>9 (47)</td>
<td>13 (40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ascites, (%)</td>
<td>13 (28)</td>
<td>10 (27)</td>
<td>2 (10)</td>
<td>7 (37)</td>
<td>8 (42)</td>
<td>12 (36)</td>
<td>0.282</td>
</tr>
<tr>
<td>Splenoportal axis, (%)</td>
<td>29 (65)</td>
<td>26 (70)</td>
<td>15 (75)</td>
<td>10 (53)</td>
<td>7 (35)</td>
<td>16 (50)</td>
<td>0.171</td>
</tr>
<tr>
<td>Peripheral pruning PV, (%)</td>
<td>4 (8)</td>
<td>6 (17)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (10)</td>
<td>0 (0)</td>
<td>0.224</td>
</tr>
<tr>
<td>Liver-irregular outlines/coarsened echotexture, (%)</td>
<td>18 (40)</td>
<td>18 (50)</td>
<td>17 (85.7)</td>
<td>12 (65)</td>
<td>3 (16)</td>
<td>9 (27)</td>
<td>0.025</td>
</tr>
<tr>
<td>Caudate lobe hypertrophy, (%)</td>
<td>10 (22)</td>
<td>9 (23)</td>
<td>0 (0)</td>
<td>4 (20)</td>
<td>2 (11)</td>
<td>3 (16)</td>
<td>0.118</td>
</tr>
<tr>
<td>Portal vein thrombosis, (%)</td>
<td>4 (10)</td>
<td>6 (16)</td>
<td>2 (10)</td>
<td>0 (0)</td>
<td>2 (11)</td>
<td>0 (0)</td>
<td>0.116</td>
</tr>
</tbody>
</table>

**Figures**
Figure 1

Histomorphology of NCPH categories: Obliterative portal venopathy: A. A densely fibrotic portal tract with absent portal vein profiles. The portal tracts have rounded borders (200X, HE). B. Densely fibrotic portal tract with sclerotic type of fibrosis in the portal tract (200X, MT). Obliterative portal venopathy-Fibrosis: C and D. Fibrotic portal tract with absence of portal vein (arrow) and increased periportal fibrosis (double arrow) (100X, MT). Incomplete septal cirrhosis: D. Incomplete septal cirrhosis showing a band of perforated
fibrous tissue that terminates blindly (arrows, 100X, HE). E. Reticulin stain showing thin perforated septa (arrows, 100X, Reticulin stain). **Nodular Regenerative Hyperplasia** F and G. nodules showing hyperplastic hepatocytes at the centre surrounded by atrophic hepatocytes at the periphery (100X, Reticulin stain). **Megasinusoids with fibrosis:** G. Sinusoidal dilatation within the lobule showing markedly dilated sinusoids known as megasinusoids (200X, HE). H. Perisinusoidal fibrosis (200X, HE).

![Image of histomorphological features](image)

**Figure 2**

Histomorphological features of unclassified with variable combination of: A. Approximation of vascular structures. B. Dilated and herniated portal vein seen in portal tract. C. Another portal tract showing herniated portal vein D. Portal tract remnant comprising of only a bile duct.
Figure 3

A. HVPG and B. TE in NCPH categories
Figure 4

A. Fibrosis and B. PIIINP in various categories of NCPH C. Positive Correlation between HVPG and Fibrosis quantity D. No correlation between TE and Fibrosis quantity among the NCPH categories