Gastroenterology and Hepatology From Bed to Bench

ORIGINAL ARTICLE

The association between clinical symptoms, laboratory findings and serum endothelin 1 concentrations, in cirrhotic patients with and without hepatopulmonary syndrome

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ABSTRACT

Aim: This study evaluated the association between serum endothelin- 1 level and symptoms, clinical examination, laboratory and cardio-respiratory parameters, in patients with cirrhosis compared to controls.

Background: Cirrhosis is associated with significant portal, pulmonary and systemic vascular abnormities. Recent studies have suggested that endothelin -1 may have a significant role in the regulation of vascular tone.

Patients and methods: In this case – control study, subjects that had been evaluated and diagnosed with biopsy-proven cirrhosis and age-matched controls with no evidence of cardio-vascular or liver disease were recruited. Review of medical records, routine laboratory investigations and cardio-respiratory investigations including echocardiography to look for evidence of hepato-pulmonary syndrome were performed.

Results: 50 patients were subjects were recruited. The most common aetiology of the cirrhosis was chronic hepatitis B viral infection. 7/50 cases had evidence of the hepatopulmonary syndrome. Among the patients with evidence of the hepatopulmonary syndrome, dyspnoea (100%) and cyanosis (90%) were the most common of the symptoms and signs recorded. Pao_2 and arterial – alveolar oxygen gradients were the most sensitive tests in the diagnosis of hepatopulmonary syndrome. Orthodoxy specificity was 100%. The median concentration of serum endothelin-1 in cases with hepatopulmonary syndrome was 1.06+/- 0.015 pg/ml (range 0.92 – 1.21), in cases of sub-clinical hepatopulmonary syndrome, 2.49+/- 0.08 (4.05- 0.93) in patients with cirrhosis but no evidence of hepatopulmonary syndrome criteria 0.85+/-0.74(1.06-0.64) in controls.

Conclusion: There was a significant difference in serum endothelin- 1 levels between patients with cirrhosis and controls, but not between patients with cirrhosis complicated by hepatopulmonary syndrome and controls.

Keywords: Endothelin -1, Gastrointestinal symptom, Hepatopulmonary syndrome.

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Introduction

The liver is an essential organ of the human body and its diseases have many effects on human health. Endothelin 1 is a potent vasoconstrictor peptide. It is produced by the cholangiocytes and endothelium. It mediates its effects through membrane bound receptors, present in the luminary endothelium, causing vasoconstriction and activating pro- inflammatory and pro-fibrotic

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pathways. Increased endothelin (ET)-1 activity may contribute to the complications of cirrhosis and portal hypertension (1). Portal hypertension is a common clinical syndrome characterized by a increased portal blood flow to the systemic circulation, bypassing the liver. Recent studies have reported that humoral factors play an important role in the pathogenesis of portal hypertension, possibly by increasing vascular resistance at both the intra-hepatic and porto-collateral sites or affecting splanchnic vasodilation with a concomitant increase in porto-collateral blood flow (1,14).Portal hypertension (PHT) is responsible for severe complications of cirrhosis such as the development of oesophageal varices, ascites, renal dysfunction and hepatic encephalopathy. The endothelium is actively involved in the pathophysiology of portal hypertension and the hyperdynamic circulation of cirrhosis (3).

The respiratory system can be compromised by the presence of liver disease. Liver disease can lead to the development of the hepatopulmonary syndrome (HPS). This syndrome is defined by the presence of hepatic cirrhosis, arterial blood deoxygenation (PaO_2< 80 mmHg) and intrapulmonary arterial dilation. Sub-clinical HPS is defined as intra pulmonary arterial dilation without other criteria in the presence of cirrhosis without arterial blood de-oxygenation (Table 1). Symptoms can include dyspnoea and signs can include cyanosis. Serum ET -1 level are increased in patients with liver cirrhosis.

This study assessed patients with cirrhosis for the presence of HPS. Patients with cirrhosis were reviewed and evaluated for the presence of HPS with three parameters; double contrast echocardiography, pulmonary function tests including arterial blood gases and plain chest radiography. In addition we measured serum concentrations of ET-1 in controls and patients with cirrhosis and compared concentrations with clinical symptoms, laboratory findings and respiratory and non-respiratory features of cirrhosis to assess if there is a positive association between ET-1 and HPS.

Patients and Methods

In this case–control study, patients with cirrhosis confirmed by biopsy were recruited from the Gastroenterology Department of Taleghani Hospital in 2004. Patients with known cardiovascular and respiratory diseases were excluded from study. A physical examination was performed on all patients and the presence of clubbing, central and peripheral cyanosis, spider angioma, telangiectasia, jaundice, collateral veins in the abdomen, clouding of consciousness, splenomegaly, dyspnea, peripheral oedema, palmer erythema, oliguria, anuria and pleural effusions was noted. Clinical notes were reviewed including the results of routine blood tests and viral, biliary, autoimmune, metabolic studies establishing the aetiology of cirrhosis.

Table 1. Upright and supine arterial oxygenation status in patients with cirrhosis

	Mean	Maximum	Minimum
Po2 Supine	80	163	44
Po2 up Right	81.35	159	43
O2 Saturation Supine	92	99	66
O2 Saturation up Right	91.5	99	65
PCo2 Supine	29.7	46	20
PCo2 up Right	30.59	43	20
Hco3 Supine	18.4	28	9.8
Hco3 up Right	18.9	26	9

Echocardiography, pulmonary function tests and plain chest x-ray were performed on patients. Contrast enhanced echocardiography was performed by two cardiologists from the Cardiology department of Taleghani Hospital (agitated saline was injected

into the anterior cubital vein of right hand and after 5 beats, all sides of heart were evaluated. Intrapulmonary shunting was defined as the presence of opacity on the left side of the heart after 5 beats. Intra-cardiac shunt was defined as the presence of opacity immediately after injection.) Arterial blood gases were performed in all patients in the supine position and then, after one hour, oxygen saturation and gradient, arterial blood saturation, and orthodoxy were evaluated in the vertical position. Other tests performed included a full blood count, liver function tests, serum creatinine, prothrombin time, partial thromboplastin time, albumin and other routine tests measured in all patients. Cirrhosis was classified according to the Childs-Pugh classification. Ascites fluid was tested for protein, albumin and white blood cells.

Patients fulfilling all three diagnostic criteria of HPS, including hepatic cirrhosis, arterial blood deoxygenation (Po2< 80 mmHg) and intrapulmonary arterial dilation were classed as clinical HPS cases. Those who had intra-pulmonary arterial dilation without other criteria were defined as subclinical hepatopulmonary cases.

Serum ET – 1 level were evaluated using the endothelin-I assay kit (L)-IBL code number 27165. Descriptive data and possible associations between clinical symptoms, laboratory finding and hepatopulmonary syndrome with serum endothelin-1 levels were analyzed using Chi-squared test (c^2), analysis of variance (ANOVA) and Pearson's coefficient of correlation. A probability of less than 0.05 was considered significant. The control group was chosen among physician, Nurses and other personnel's of Taleghani hospital who were negative for any specific disease and matched by sex and age of patients.

Results

100 subjects were recruited (50 subjects with cirrhosis and 50 controls. In the cirrhosis group, 17 patients were female and the median age was 48 years (range 22 to 76). In the control group 24 patients were female and the median age was 47.8 (range 22 to 76). On review of medical records the aetiology of the cirrhosis was found to be viral in 25 patients (18 had hepatitis B viral infection (HBV) and 7 had hepatitis C infection (HCV) and 5 had autoimmune cirrhosis, 1 was Secondary biliary cirrhosis , 1 was hemocromatosis , and 16 were considered idiopathic after other causes had been excluded. Four patients had Child Pugh class A, 28 Child-Pugh class B and 18 Child-Pugh class C cirrhosis.

Symptoms reported included dyspnoea (17/50, 34%) and oliguria (8 cases, 16 %). On clinical examination 41 (82%) had ascites, 39 (78%) had jaundice, 17(34%) had cyanosis, 12 (24%) had clubbing, splenomegaly was found in 11(22%) patients, palmer erythema in 20(40%), collateral veins 13(26%), spiderangioma in 21(42%). Of the 41 patients with ascites, 5 cases were grade 1, 14 were grade 2, 16 were grade 3 and 6 were grade 4. Table 2, shows the frequency of different symptoms in cirrhotic patients. Among the 50 patients with cirrhosis, 10(18.5%) met clinical HPS criteria and 7(13%) met sub-clinical HPS criteria. No significant association between was found between splenomegaly, ascites, oedema, jaundice, oliguria, collateral veins and the presence of HPS. HPS was more common in patients with Child-Pugh class C cirrhosis (6/18 (33.4%)), than among patients with Child-Pugh Class B (1/28 (3.6%)) and Child-Pugh Class A (0/4) and. Pao_2 and arterial – alveolar oxygen gradients were found to be the most sensitive tests in the diagnosis of HPS.

Orthodoxy specificity was 100%. Laboratory findings were shown in table 3.The median level of ascites albumin was 3.6 units, WBC 5.3 units and total protein 30.9 units. Of the 29 cirrhotic patients with Po_2< 80 mmHg, 4 cases had Po_2 < 60 mmHg.

Table 2. Laboratory findings in case and control group

Laboratory test	Case	Control	Normal Rang
White Blood Cell/mm^3	5126	5800	4400 – 11000/
Hemoglobin(g/dl)	10.1	13.8	F:12.3-15.3 M:14-17.5
Total protein(g/dl)	5.94	6.7	6.0 -8.5
Platelet/mm^3	79968	135000	130000-400000
Na(mEq/L)	137.22	...	136-142
K(mEq/L)	4.1	...	3.8-5
Ca(mEq/dl)	10.84	...	8.5-10.3
P(mEq/dl)	5.31	...	2.3-4.7
PT(second)	23	13	10-13
PTT(second)	37.98	24	21-33
INR	2.2	1.1	1.1- 1.3
BUN(mg/dl)	24	21	8 – 23
Cr(mg/dl)	1	0.9	0.6 – 1.2
Endothelin-1(pg/ml)	2.29	0.85	-
ALT(U/L)	75.5	47	0 - 48
AST(U/L)	78.8	31.5	0 - 42
ALK(U/L)	357	195	20 - 130
Albumin(g/dl)	2.29	4.64	3.2-4.5
Total Bilirubin(mg/dl)	2.36	0.692	0.5-1.2

The median serum concentration of ET-1 for all subjects was 1.16 pg/ml (range 0.73 to 26.6). The median concentration among patients with cirrhosis was 26.6 pg/ml. lower concentrations of serum ET-1 were present in normal controls, with a median of 0.01pg/ml. Among patients with cirrhosis and HPS the median concentration was 1.07 pg/ml. The mean concentration of ET-1 level in cirrhotic cases without HPS (2.29 pg/ml) was significantly higher than control group (0.85 pg/ml). There was no significant difference between serum ET-1 level in the HPS (P=0.466 add name of test), and sub-clinical HPS patient groups (P=0.503), compared to controls and patients with cirrhosis but no evidence of HPS. Interestingly, serum ET-1 concentrations were higher in cirrhotic patients that do not have HPS syndrome compared to control subjects. Therefore, no statistical significant correlation was found between the levels of ET-1 and clinical and sub clinical HPS.

Table 3. Demographic and clinical characteristics of patients assessed for the presence or absence of HPS

	HPS	NO HPS	P -value
Mean age	51.29	48.26	0.9
Sex			0.594
male	4	29	
Female	3	14	
Origin of cirrhosis			0.927
HBV	4	14	
HCV	1	6	
Autoimmune hepatitis	0	5	
Secondary Billiary	0	1	
Metabolic	0	1	
Idiopathic	2	14	
PBC	0	1	
Alcoholic	0	1	
Child classification			0.012
A	0	4	
B	1	27	
C	6	12	
Ascites			0.349
Grade I	0	5	
Grade II	2	12	
Grade III	3	13	
Grade IV	2	4	
negative	0	9	
Clubbing			0.002
Positive	5	7	
Negative	2	36	
Vascular			0.017
(spiderangioma)	6	15	
Positive	1	28	
negative			
Dyspnea			0.002
positive	7	15	
negative	0	28	

Discussion

HPS is characterized by a clinical trial that includes liver disease and/or portal hypertension, abnormal arterial oxygenation, and presence of intrapulmonary vascular dilatation (IPVDs). In this study we established the prevalence of HPS in a population of cirrhotic patients. The most common cause for cirrhosis was chronic HBV infection. Patients were assessed clinically and investigated with plain chest radiography, arterial blood gas

analysis and contrast echocardiography. 14 % of all cirrhotic patients had evidence of HPS. Symptoms of dyspnoea and clinical signs (clubbing, spiderangimota and collateral veins) were associated with the presence of HPS. Dyspnoea was more common in patients with HPS (57%of cases) compared with "subclinical HPS" (8%) and patients without HPS (6%).In our series, double contrast in patients with IPVDS was 13(26%) and without IPVDS was 37 (74%). In 13 cases (cirrhosis patients) ECO cardiography double contrast were positive and in 37 cases were negative.

HPS was more common among patients with advanced, Child-Pugh Class C, liver cirrhosis. The best parameter for the assessment of HPS was found to be altered oxygenation, i.e. a PaO_2 of less than 70 mmHg .In our study, 7 cases with HPS had Pao_2 of less than 70 mmHg.

Our results are comparable to other published studies. In a comparable North American study, forty consecutive outpatients with biopsy-proven cirrhosis had contrast echocardiography, a lung perfusion scan, and arterial blood gases analyzed. The causes of cirrhosis in the this study were: hepatitis C (26%), alcoholic liver disease (21%), hepatitis C plus alcoholic liver disease (15%), cryptogenic causes (18%), hepatitis B, which may be coincident with hepatitis D (15%) and miscellaneous (5%) (37). Fifteen of 40 cirrhotic cases (38%) had echocardiogram studies in keeping with HPS. Seven patients with positive echocardiogram results had gas exchange abnormalities and could be considered to have HPS (7 of 40) (35). The Child-Pugh score correlated significantly with the severity of HPS (36, 41). Cyanosis was the only clinical indicator associated with the presence of HPS, and surprisingly the proportion of patients with HPS did not correlate with the severity of cirrhosis (52, 53).

The serum ET-1 concentration results suggest that cirrhosis is associated with higher concentrations than normal controls. This is in keeping with previous studies. In our study the concentration of ET-1 was neither predictive of the severity of cirrhosis nor the presence of HPS. This may reflect sample size and a wide range of serum ET-1 concentrations. Alternatively this suggests cirrhosis may be associated with elevated serum ET-1 concentrations, but that other factor, such as expression endothelin receptors influence the development of HPS. Indeed, cirrhosis may be associated with elevated serum concentrations of ET-1 compared to controls, but the development of HPS may be dependent upon endothelin receptor expression rather than absolute serum ET-1 concentrations.

In conclusion this study suggests that serum ET-1 concentrations are elevated in patients with cirrhosis compared to controls. In addition HPS is not associated with elevate serum concentrations ET-1 compared to cirrhosis with HPS.

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