PULMONARY ARTERIAL PRESSURE IN LIVER TRANSPLANT CANDIDATES. A CENTRE EXPERIENCE AND REVIEW OF LITERATURE

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PULMONARY ARTERIAL PRESSURE IN LIVER TRANSPLANT CANDIDATES. A CENTRE EXPERIENCE AND REVIEW OF LITERATURE (Abstract): Porto-pulmonary hypertension (POPH), characterized by portal hypertension associated with high pulmonary vascular resistance (PVR), represents an important complication in liver cirrhotic (LC) patients. Our study aimed to determine the association between biological profile and pulmonary hypertension in a group of patients on the liver transplant awaiting list. **Material and methods**: The current 3-year prospective study analyzed 60 LC patients admitted for liver transplant evaluation and regular follow-up. All patients underwent biochemical evaluation and LC severity was evaluated by using MELD score and Child-Pugh class. Pulmonary hypertension was determined by standardized echocardiographic evaluation, most important markers being mean and systolic pulmonary artery pressure (mPAP, sPAP). **Results**: Mean age was 50.55 ± 8.3 years, 83.3% males. sPAP was significantly higher in women (24.08±7.76 *vs*. 27.33±13.7 mmHg, p=0.040). The presence of ascites was directly correlated with the severity of pulmonary hypertension (p=0.033) while mPAP was higher in patients with autoimmune LC. mPAP correlated with MELD score (r=0.832, p=0.030), fibrinogen level (r=0.887, p=0.021) and bilirubin level (r=0.758, p=0.045). During the follow-up period, mPAP decreased significantly in patients that received liver transplant. However, mPAP was not a mortality risk factor for the awaiting liver transplant patients. **Conclusions**: Our study revealed that fibrinogen and total bilirubin levels, as parameters of cirrhosis severity, are in direct correlation with mPAP. Furthermore, refractory ascites in end-stage cirrhosis patients could be associated with a higher risk of pulmonary hypertension. Further larger studies on POPH incidence and follow-up in liver cirrhosis are warranted. **Keywords**: PULMONARY HYPERTENSION, LIVER TRANSPLANT, LIVER CIRRHOSIS, ASCITES.

Pulmonary hypertension, characterized as mean pulmonary arterial pressure (mPAP) more than 25 mmHg at rest or more than 30 mmHg during exercise, is often characterized by a progressive and sustained increase in pulmonary vascular resistance that eventually may lead to right ventricular failure (1).

Liver cirrhosis (LC) is one of the numerous conditions that can cause pulmonary hypertension. The association between liver cirrhosis and pulmonary disease consists of a series of vascular changes in the pulmonary vessels due to alterations in distal pulmonary arteries with intimal proliferation, medial hypertrophy, fibrotic changes, plexiform lesions and in situ thrombosis (2). The pathogenesis is still unclear although some of the mechanisms were identified as increasing nitric oxide (NO) levels which determine excessive pulmonary vascular dilatation with secondary ventilation perfusion mismatch (V/Q ratio), intrapulmonary arterio-venous shunt or limited oxygen diffusion (3). There are no medical effective therapies for porto-pulmonary hypertension (POPH), the only reliable treatment being the liver transplant. In the absence of liver transplant the median 24-month survival rate is 23% (4).

POPH represents a form of pulmonary hypertension that is associated with portal hypertension characterized by high pulmonary vascular resistance (PVR) as a consequence of pulmonary vasoconstriction and remodeling. The POPH prevalence in cirrhotic patients is 2-14% (5, 6). The diagnosis is made by right cardiac catheterization by determining the mPAP, the left-sided filling pressures and elevated PVR (>250 dyne/s/cm-5) in patients with cirrhosis and portal hypertension (7, 8). The severity of POPH can be stratified in different risk classes, depending on the mPAP: mild form 25 - 35 mmHg; moderate form 35 - 45 mmHg; severe form >45 mmHg (9). mPAP between 35 and 50 mmHg is associated with an increase in mortality of 50% after liver transplant10. Other studies suggest that in patients with severe form of POPH the mortality is estimated to almost 100% (11). Considering this, liver transplant is indicated only to those who respond to vasodilatory medical treatment with the aim of lowering mPAP below 35 mmHg and a PVR below 400 dyne/s/cm-5 (9). After liver transplant, the pulmonary hypertension is resolved within 6 months at which time medication may be interrupted. In the severe form, mortality after liver transplant is estimated at 42% at 9 months and 71% at 36 months (12, 14). Pulmonary hypertension and cirrhosis are more common among women and patients who also suffer from autoimmune hepatitis and about 5% of the patients with end-stage liver disease will develop pulmonary hypertension (12, 15, 16).

There are no clear clinical factors that determine the risk of POPH in patients with advanced liver disease. Also, the predictors or biological mechanisms responsible for the development of POPH in these patients remain unknown (9, 16, 17). Therefore, the aim of the study was to determine the frequency of POPH in patients with LC on the liver transplant awaiting list and to analyses the patients' biochemical profile in order to identify the relations between biological factors and the severity of the pulmonary hypertension.

MATERIAL AND METHODS

In the current study we have analyzed 60 cirrhotic patients admitted in our institution for liver transplant evaluation and regular follow-up. Patients with other conditions that could lead to portal or pulmonary hypertension or with any pre-existing cardiovascular abnormalities were excluded from the study. The study was conducted as a cross-sectional analysis for a period of three years. The local ethical committee approved the study, and a written informed

consent was obtained from all the patients. The past medical history, clinical examination and laboratory details were obtained from the medical records.

Diagnosis of POPH and patient evaluation

PVR, measured traditionally by right heart catheterization, is an essential parameter of the pulmonary hypertension. However, Doppler echocardiography is an accepted method to evaluate the pulmonary pressure with comparable results. In cirrhotic patients in evaluation for liver transplant this echocardiographic method decreases the requirement of extensive invasive measurements. Our protocol study involved the measurement of the pulmonary pressure by the peak systolic velocity of the tricuspid regurgitation flow and the acceleration time of the pulmonary systolic flow. The current guidelines were used to evaluate all cavities and cardiac volumes. All echocardiographic measurements were repeated three times by the same cardiologist expert. The patients were considered to have pulmonary hypertension by sPAP > 40 mmHg. mPAP was calculated from sPAP (mPAP = 0.61 sPAP + 2mmHg) (18).

All patients were evaluated by abdominal ultrasound and upper digestive endoscopy.

Liver cirrhosis was diagnosed based on clinical signs, biological, ultrasound and endoscopical changes suggestive for portal hypertension and advanced liver fibrosis. MELD score and Child-Pugh score were used to evaluate LC severity.

Biochemical investigations included complete blood count, serum bilirubin, albumin, total protein, prothrombin time/international normalized ratio, liver enzymes (alkaline phosphatase, aspartate transaminase, alanine transaminase, gamma glutamyl transpeptidase), lipid panel, renal function, inflammatory marks such as C reactive protein and fibrinogen.

Statistical analysis

Statistical analysis was performed by using the *SPSS version 21.0* software (SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as mean ± SD and categorical variables as percentages. Biochemical parameters in cirrhotic patients with and without POPH were compared. Quantitative variables with normal distribution were compared using the *Student's t* test. For non-parametric variables, we have used *Mann-Whitney* test. The *Kolmogorov-Smirnov* test was used to check the normality of the data distributions. Chi square test (*Fisher* exact test for small samples) for categorical data was exploited. *Spearman's* correlation coefficient was used to investigate possible correlations between the POPH and different clinical and biological parameters. Statistical significance was considered as p-value ≤ 0.05.

RESULTS

Mean age was 50.55 ± 8.3 years (range 26-63 years), most of them males 83.3%. The first three etiologies of LC were viral B infection (45%), viral C infection (26.7%) and chronic alcohol consumption (16.7%). All patients diagnosed with alcoholic LC had more than 6 months of alcohol abstinence. The majority of patients were classified as Child-Pugh class B (43.3%) with a mean MELD score of 16.9 ± 5.5.

Out of all patients, 5 (8.3%) of them were diagnosed with POPH. In the POPH group 3 were males (60%), viral B infection and autoimmune hepatitis being the most frequent etiologies while the severity

of liver disease was classified in Child-Pugh class B and with mean MELD score 19.9 ± 1.8. The baseline characteristics of the patients included in the study are presented in first table. Alcoholic cirrhosis patients did not present POPH. Among the POPH patients, sPAP values varied from 42 to 55 mmHg.

TABLE I
Baseline characteristics

Parameter	All patients (n = 60)	POPH group (n = 5)	Control group (n = 55)	p-value
Gender, male, n (%)	50 (83.3)	3 (60)	47 (85.4)	0.144
Age, years, mean ± SD	50.55±8.35	54.4±6.22	50.20±8.47	0.641
Etiology of cirrhosis, n (%) HBV HCV Alcohol Autoimmune Other	 27 (45) 16 (26.7) 10 (16.7) 4 (6.7) 3 (5)	 2 (40) 0 (0) 1 (20) 2 (40) 0 (0)	 25 (45.4) 16 (29.1) 9 (16.3) 2 (3.6) 3 (5.6)	 0.246
Child-Pugh class A/B/C, n	12/26/22	0/3/2	12/23/20	0.483
Child-Pugh score, mean ± SD	8.68±2.11	9.40±0.54	8.62±2.19	**0.015**
MELD score, mean ± SD	16.96±5.5	19.0±1.82	16.8±0.78	**0.044**
Creatinine (mg/dl), mean ± SD	0.97±0.54	0.71±0.08	0.99±0.56	0.202
Albumin (g/l), mean ± SD	3.24±0.74	3.33±0.61	3.23±0.75	0.457
Total Bilirubin (mg/dl), mean ± SD	3.37±3.29	3.66±1.47	3.35±3.43	0.365
Fibrinogen (mg/dl), mean ± SD	271.8±92.1	341.2±93.2	264.7±89.9	0.077
ALT (UI/L), mean ± SD	59.6±51.3	34.0±13.6	62.2±53.1	0.090
AST (UI/L), mean ± SD	81.9±55.1	74.6±44.9	82.7±56.3	0.418
GGT (UI/L), mean ± SD	96.9±84.4	81.2±71.9	98.6±86.1	0.518
Ascites, n (%)	39 (65)	5 (100)	34 (61.8)	**0.033**
HRS, n (%)	7 (11.7)	1 (20)	6 (10.9)	0.396
Encephalopathy, n (%)	15 (25)	2 (40)	13 (23.6)	0.418
Hepatocellular carcinoma, n(%)	15 (25)	0 (0)	15 (27.3)	0.173

There was a statistically significant difference between sPAP in women compared with males (24.08±7.76 *vs*. 27.33±13.7, p=0.040) although this difference has not reach the statistical significance for calculated mPAP (16.91±5.29 *vs.* 19.42±8.1, p=0.140).

Regarding ascites, there has been found a direct correlation between the presence of ascites and the severity of pulmonary hypertension (p=0.033) (fig. 1). There was no difference between the LC etiology and the risk of POPH, although mPAP was higher in patients with autoimmune LC.

Regarding the severity of hepatic cirrhosis assessed by Child-Pugh functional classification, 19.7 % of the patients were in class A, in class B 39.3 % and in class C 36.1 %. A correlation between the severity of pulmonary hypertension (reflected by

the value of sPAP) and Child-Pugh class was investigated. The results revealed that sPAP values were not significantly correlated with Child-Pugh class (p = 0.840).

Regarding all biochemical parameters, there was significant direct correlation between mPAP and MELD score (r=0.832, p=0.030), fibrinogen level (r=0.887, p=0.021) and bilirubin level (r=0.758, p=0.045).

During the follow-up period, 20 patients (33.3%) died, and 10 patients (16.7%) received a liver transplant. Increased mPAP was not a risk factor for mortality on the awaiting liver transplant list.

DISCUSSION

POPH is a recognized subtype of pulmonary arterial hypertension and a complication of LC. However, the majority of the cirrhotic patients did not associate this complication. In fact, even though this complication is in direct relation with portal hypertension, not all the cirrhotic develop POPH (5, 19). In our study, 8.3% of the patients with portal hypertension developed POPH, data that is within the prevalence range reported in the literature (9). Although in our study there were more males with cirrhosis that developed POPH, there was no significant difference observed across gender in the incidence of POPH (p=0.144). However, a study performed in the United States reported a greater incidence of pulmonary hypertension in cirrhotic women11. In our study we found no relation between gender, age, LC etiology and the diagnosis of POPH, results that are rather similar with other reported data (8, 9). However, our result is different with results from other studies that suggested that females are at higher risk of developing POPH (16).

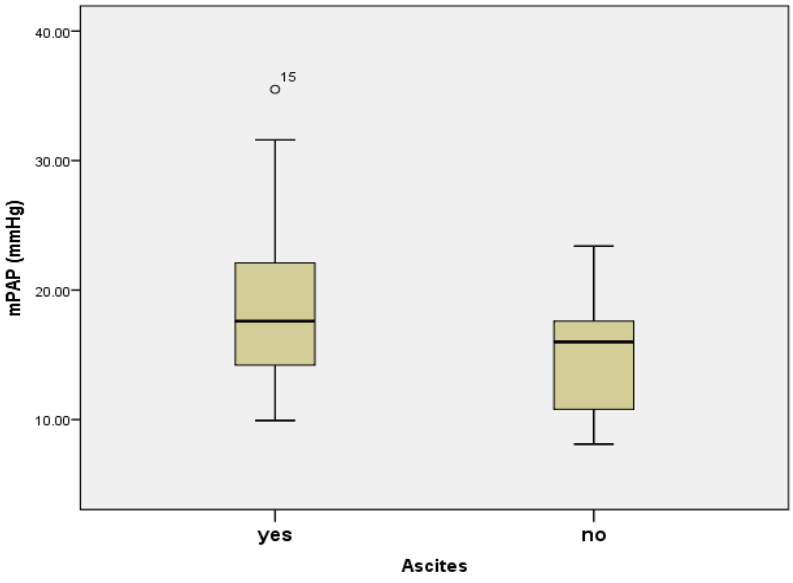

Fig. 1. The relationship between ascites and mPAP

Our study results demonstrated that the severity of LC is related with the development of POPH. We showed that the patients with POPH had a higher Child-Pugh class

and we reported a direct positive correlation between mPAP and the MELD score.

Ascites represents a marker of severity of portal hypertension. In our study, ascites was present in 65% of the patients and had a direct relationship with the severity of the pulmonary hypertension. Other studies showed no correlation but suggested that POPH was more common in patients with refractory ascites and LC possibly due to endothelin-1 excess in the pulmonary circulation (20).

Regarding female patients with POPH and autoimmune cirrhosis, the pulmonary pressure normalized 1 week after the liver transplant and remained normal at 1 month suggesting a good outcome and prognosis. The mechanism of these findings is not fully understood. In many studies, a strong association has been found between portal hypertension and pulmonary hypertension, whether or not liver disease was present. This led to the idea that normalizing the portal pressure could help lowering the pulmonary pressure. Findings in other studies are in concordance with this hypothesis showing improvements and even normalization of the pulmonary and portal pressure after liver transplant. The anatomopathological abnormalities in POPH are rather similar with those described in idiopathic PAH. The most important histological abnormality includes proliferative arteriopathy and necrotizing arteritis, occlusion of the vascular lumen by the smooth-muscle cells or the endothelial cells, and in-situ thrombi formation secondary to a systemic inflammation. The porto-systemic shunts could determine the shunting of the vasoactive substances (endothelin-1, serotonin, interleukin 1, glucagon and secretin) from the splanchnic to the pulmonary circulation. These vasoactive mediators are bypassing the liver metabolism and negatively influence the pulmonary vasculature (21, 22). We demonstrated a direct strong correlation between fibrinogen level as a marker of systemic inflammation and the mPAP. These mechanisms suggest that reversing the portal hypertension could lead to a decrease in pulmonary pressure.

Among the examined laboratory parameters, we found a direct correlation between mPAP and the severity of liver disease assessed by MELD score, fibrinogen level and total bilirubin level. Other studies suggest a correlation between hemoglobin and POPH with a lower value of hemoglobin in the POPH group compared to the non-POPH group (20). This study suggests that hemoglobin level is an independent risk factor for the development of POPH. A decreased hemoglobin leads to a significant cardiac output increase that exacerbates the hyperdynamic splanchnic circulation which is known to be a major contributor to portal hypertension (10). However, in our study no correlation was found between the hemoglobin value and POPH.

Our study has some strengths and also several limitations. Thus, it is one of the few studies examining the incidence of POPH in liver transplant patients. However, the relatively small sample size and lack of long-term evaluation represent limitations to our data.

CONCLUSIONS

In our study, we found no association between gender, age, etiology of the hepatic disease and the development of pulmonary hypertension. The severity of the hepatic disease, fibrinogen level and total bilirubin level are in direct correlation with mPAP in cirrhotic patients. Refractory ascites in end-stage cirrhosis patients could be associated with a higher risk of pulmonary hypertension. Further larger studies on

POPH incidence and follow-up in liver cirrhosis are warranted.

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NEWS

RECENT PROGRESS AND CHALLENGES IN *SCREENING* AND CHARACTERIZATION OF UGT1A1 INHIBITORS

Uridine-diphosphate glucuronosyltransferase 1A1 (UGT1A1) is an important conjugative enzyme in mammals that is responsible for the conjugation and detoxification of both endogenous and xenobiotic compounds. Strong inhibition of UGT1A1 may trigger adverse drug/herb-drug interactions, or result in metabolic disorders of endobiotic metabolism. Therefore, both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have recommended assaying the inhibitory potential of drugs under development on the human UGT1A1 prior to approval. Among the human UGTs, UGT1A1 is of particular clinical significance due to its unique activity in the conjugative detoxification of bilirubin, the endogenous by-product of heme metabolism. Also, UGT1A1 plays a pivotal role in endobiotic homeostasis, in addition to its contribution to xenobiotic disposition and detoxification. Small-molecule inhibitors of UGT1A1 may profoundly influence the catalytic activity of UGT1A1, thereby triggering undesirable effects, like drug/herb-drug interactions (D/HDI) or drug/herb endobiotic interactions: protease inhibitors- indinavir, nilotinib, sorafenib, atazanavir, saquinavir, lopinavir, ritonavir, nelfinavir, tyrosine kinase inhibitors nilotinib, regorafenib, sorafenib, pazopanib, lapatinib, erlotinib, gefitinib and icotinib have been implicated in the development of hyperbilirubinemia, for which UGT1A1 inhibition is the most likely cause. Other drugs levothyroxine, ketoconazole, vitamin A, diclofenac, canagliflozin, diflunisal, gossypol, P-Cresol, herbal extract milk thistle, saw palmetto, echinacea, green tea, epigallocatechin gallate, garlic, ginseng, black cohosh, and valerian, fatty acids, flavonoids, quinine or lignans. Unfortunately, currently commercially available substrates for UGT1A1 (such as bilirubin, estradiol, and etoposide) have different limitations, such as poor selectivity, poor chemical stability, or their use for high-throughput screening (HTS) is unfeasible. Notably, the design principles are already available and experience in the design and development of specific fluorescent substrates for UGT1A1 will surely assist us and other researchers in developing new specific fluorescent substrates for other UGTs. For instance, 1-naphthol was found to be a good fluorescent substrate for UGT1A6, which can be used for HTS of UGT1A6 inhibitors using recombinant enzyme as an enzyme source. However, 1-naphthol can be conjugated by other human UGTs as well, limiting its applications to the recombinant enzyme, rather than use in more complex system such as HLM. Very recently, a set of fluorescent 7-hydroxycoumarine derivatives have been developed as specific substrates for UGT1A10 (an extrahepatic UGT) and at least two of them, appear to work well in tissue preparations. All these findings are very helpful for the design and development of fluorescent probes for different human UGTs, and we hope that more practical fluorescent probes for human UGT enzymes will be successfully developed and used in the near future (Xia LV, Yangliu XIA, Moshe Finel, Jingiing Wu, Guangbo GE, Ling Yang. *Acta Pharmaceutica Sinica B* 2019; 9(2): 258-278).