UNIVERISTY OF MEDICINE AND PHARMACY CRAIOVA
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CARDIOVASCULAR CHANGES IN HEPATIC CIRRHOSIS

PhD THESIS

- ABSTRACT -

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CONTENT

Key words*: liver cirrhosis, cardiomyopathy, cardiovascular changes, echocardiography, ventricular dysfunction*

INTRODUCTION

Hepatic cirrhosis is a chronic, degenerative, irreversible, diffuse liver disease characterized by gradual deterioration of the liver function, including blood circulation, which leads in time to fibrosis and implicitly to alteration in liver architecture, representing a major health problem, both by weight and by complications.

The consequences of hepatic dysfunction occurring during the progression of the disease are reflected on other organs or apparatus, most importantly related to the cardiovascular apparatus. The mechanisms for producing cardiovascular changes in cirrhosis are insufficiently clarified to date, which is the source of many discussions.

Of the whole range of changes in liver cirrhosis, in this PhD thesis I have approached a complex study of cardiovascular disorders occurring during disease progression, a field with many aspects, not yet studied.

The originality of this thesis is to highlight from the subclinical stage the cardiovascular changes in cirrhosis, the new methods of echocardiography (speckle-tracking, tissue doppler), their correlation with the clinical context, the ethology, the degree of severity of the cirrhotic patients, the determination of how these changes can contribute to favorable prognosis of patients by making appropriate, adequate therapeutic decisions, but also by formulating public health decisions.

PART I. THE STAGE OF KNOWLEDGE

CHAPTER 1. HEPATIC CIRRHOSIS – theoretical notions

The "Stage of knowledge" part represents a synthesis of the recent data from the scientific literature with reference to the many aspects of this paper: etiological and epidemiological data of cirrhosis, factors involved in its pathophysiology, as well as diagnostic methods: ultrasound, computed tomography , nuclear magnetic resonance, superior digestive endoscopy, biopsy and hepatic scintigraphy. Also in this chapter I have highlighted the most frequent complications occurred during the disease namely: ascites and spontaneous bacterial peritonitis, hepatic encephalopathy, esophageal varices bleeding.

CHAPTER 2. CARDIOVASCULAR CHANGES IN HEPATIC CIRRHOSIS

Symptoms of liver cirrhosis and cardiovascular changes specific to cirrhosis occurring in advanced stages are presented in this chapter. Hepatic cirrhosis induces cardiovascular changes, including hyperdynamic circulatory syndrome and cirrhotic cardiomyopathy.

Cirrhotic cardiomyopathy (CCM) is the term used to describe structural and functional cardiac changes that occur in patients with cirrhosis. It is characterized by electrophysiological abnormalities, systolic and/or diastolic dysfunction as well as chronotropic failure (IC), all of which in the absence of known cardiac disease.

There are two stages in the evolution of CCM: a subclinical one, which can be underestimated for a long time, being accidentally discovered by the doctor during a routine exam, and another clinically manifest, the cardiac affection dominating the clinical picture, raising the problem for both the diagnosis as well as treatment.

PART II. PERSONAL CONTRIBUTIONS

THE PURPOSE OF THIS STUDY. ESTABLISHED OBJECTIVES.

This study aimed at identifying cardiovascular changes in cirrhotic patients with various stages of disease progression and it highlighed the correlations between the severity of cardiac changes and the degree of hepatic impairment.

OBJECTIVES: complex assessment of patients with liver cirrhosis by laboratory analysis, echographic and electrocardiographic parameters; establishing correlations between electrocardiographic changes (the duration of QTc interval, AF), echocardiographic (the degree of systolic and diastolic dysfunction of the LV), laboratory (NT pro BNP, TNI), and the degree of liver disease assessed by the Child-Pugh score and MELD score.

THE STUDIED LOT AND THE WORKING METHOD

The present study was a prospective, run over a 3-year period, consisting of a representative batch of cases, 102 patients already diagnosed with liver cirrhosis of various etiologies, found in different disease states. Of these, a subset of 40 patients were the control group, consisting of people of the same age without known hepatic impairment.

During the course of the study some patients died due to the advanced stage of the disease involving a number of major complications (spontaneous bacterial ascites and spontaneous bacterial peritonitis, esophageal varicose veins, portal encephalopathy, CMC, CMD) or their low compliance. Samples were taken for histological and immunohistochemical analysis from the deceased patients (18). Patient necropsy was performed in the Prosecutor's Department of the Pathology Anatomy Laboratory of the same hospital. For the control group, normal fragments were collected from 10 deceased patients without associated cardiac pathology. Harvesting of the tissue fragments was followed by the usual histopathological processing consisting of the inclusion of Hematoxylin-Eosin in paraffin and staining.

INCLUDING CRITERIA: Diagnosis of liver cirrhosis confirmed by complete analysis of clinical and paraclinical data, regardless of the stage of the disease or its complications; over 18 years of age

EXCLUSION CRITERIA: hemodynamic instability; minor or major collagen diseases; age under 18; shock states; severe diseases of the endocrine system; medicines that could disrupt cardiac or hepatic function; related hepatocellular diseases: hepatocellular carcinoma, TIPS; severe pulmonary disease that may lead to secondary pulmonary hypertension (bronchial asthma, silicosis, chronic pulmonary cord) over time.

The inclusion visit to the study required full examination of the patients. Thus patients were investigated both clinically and paraclinically (cardiologically and gastroenterologically).

RESULTS AND DISCUSSIONS

The results consist of highlighting a whole series of cardiovascular changes , throughout the evolution of liver cirrhosis: both biochemical, electrocardiographic and ultrasound, most of which correlate with the severity of cirrhosis, estimated by the Child and Meld score.

ILLUSTRATION OF BIOCHEMICAL CHANGES IN PATIENTS OF THE STUDIED LOT

According to the literature, serum levels of TnI are increased in some patients with alcoholic cirrhosis. In the control group we obtained an average serum TnI of 0.0133 compared to 0.0011, that of the hepatic cirrhosis lot, the standard deviation between the two being 0.0337 versus 0.0010. Using the Student t test, we demonstrated that there was a significant difference between the TnI values measured in subjects in the study group and those in the control group, those in the study group having a mean greater than the others ($p = 0.024 < 0.05$).

Over the years, two types of molecular biomarkers have been studied in literature in cirrhotic patients as indicators of LV dysfunction: ANP and BNP, their plasma concentrations being significantly increased in patients with cirrhosis complicated with ascites, and very little in the preascites phase. Clinical trials in patients with liver cirrhosis have demonstrated elevated serum levels of BNP and NT proBNP, these being correlated with the severity of cirrhosis, abnormal cardiac structure and function. However, the highest correlations of BNP levels are with the final diastolic pressure, demonstrating that diastolic relaxation is one of the major determinants of BNP secretion. Analyzing the two batches, we obtained an increased mean value of NTproBNP in the test group 425.54 pg / ml compared to the control group where the value of 46.83 pg / ml was recorded. Using the Student t test, we demonstrated that there was a significant difference between the NT proBNP values measured in subjects in the study group and in the control group, those in the study group having the mean greater than the others ($p = 0.023 < 0.05$).

Among patients in the different Child-Pugh classes, there are significant differences in the mean value of NT proBNP, the ANOVA test result being <0.001. Patients in Child C class

have the highest values, those in the Child B class have intermediate values, and those in Child A have the lowest values.

ILLUSTRATION OF ELECTROCARDIOGRAPHIC CHANGES IN PATIENTS OF THE STUDIED LOT

The heart rhythm disorders have been **the first aspect** of electrocardiographic changes that I followed in patients with liver cirrhosis. I found that most of the patients had no rhythm disturbances during the follow-up, almost 10% had ventricular extrasistoles, and 12.20% had atrial fibrillation, with no significant gender differences. (p Chi square = 0.276> 0.05).

From the point of view of the severity class, although there are important percentage differences between the three Child-Pugh classes in terms of rhythm disorders, they do not exceed the statistical significance threshold, the result of the square Chi test being p = 0.254> 0.05.

The second view of electrocardiographic changes that I have followed in patients with liver cirrhosis is **cardiac frequency**. According to literature data, it is increased in patients with liver cirrhosis, irrespective of their etiology. Using the Student t test, I demonstrated that there was a significant difference between the heart rates of subjects in the study group and those in the control group (p = 0.000203 <0.001), those in the study group having a higher average than the others (81.05 vs. 68.60 bpm). This had an increased prevalence among elderly (> 55years) patients with a higher severity class (Child C). There were no significant gender differences.

The third aspect of the electrocardiographic changes I observed in the cirrhotic patients included in the study is **the duration of the QT interval**. In the literature, elongation (> 440 s) occurs in noncirrhotic patients with portal hypertension and in 30-60% of patients with cirrhosis depending on the severity of liver dysfunction. With the Student t test, we demonstrated that there was a significant difference between the QT interval values of the subjects in the study group and those in the control group (p = 0.000082 <0.001), those in the study group having a mean higher than the others (446.68 vs. 417.20 s). In men, QT prolongation is more common (50 vs. 32), but the QT interval is higher in women (456.81 vs. 440.20), but there are no significant differences (t Student being p = 0.099 <0.05). No significant difference was identified in age or gender concerning the mean QT interval measured in cirrhotic patients.

ILLUSTRATION OF ECOCARDIOGRAPHIC CHANGES IN PATIENTS OF THE STUDIED LOT

THE SYSTOLIC FUNCTION

The systolic function of the LV is evaluated by several types of global and regional function parameters. Numerous studies have shown strong correlations between the systolic function expressed by LVEF, cardiac volumes and the degree of liver cirrhosis. When liver cirrhosis is at a compensated stage, LVEF is normal and remains relatively preserved until late in the progression of the disease.

Following the statistical analysis of the two lots we obtained a mean value of LVEF (Simpson method) of 50.41% for the test lot compared to 55% for the control lot, the standard deviation being 10.40 for the respective test 5.05 for the control. Through the Student t test, we demonstrated that **there is a significant difference** between LVEF of subjects in the study group and those in the control group, those in the study group having a lower mean LVEF than the others ($p = 0.009 < 0.05$).

At present there is a whole range of new echocardiography techniques that can highlight some LVEF changes even at rest. These techniques include 2D-STE that allows the assessment of both global and segmental contractility. We have demonstrated with the Student t test that **there is a significant difference** between LVEF, measured in the apical section of 4 chambers (2D-STE method) of the subjects from the study group and those in the control group, those in the study group having mean LVEF lower than the others ($p = 0.000000014 < 0.001$).

Depending on the longitudinal global contraction (GLS), by performing the Student t test, we demonstrated that there is a significant difference between the GLS values of subjects in the study group and those in the control group (-19.93 vs. -24.10) , those in the study group having a lower average GLS value than the others ($p = 0.000017 < 0.001$).

THE DIASTOLIC FUNCTION

Numerous studies have shown that diastolic dysfunction is the first marker of myocardial function impairment in cirrhotic patients, diastolic parameters being altered as the disease progresses. Diastolic dysfunction leads in time to increased blood volume in LA, which in turn leads to an increase in the transmitral pressure gradient. DDVS can be determined by both

invasively and non-invasively methods (echocardiography). The Doppler examination of the mitral flow was most used in the evaluation of LV diastolic function in hepatic cirrhosis. Using this method, we found that 38 patients had diastolic dysfunction, the remaining 34 having normal LV diastolic function.

However, conventional Doppler indices have clear limits (age and loading conditions), and rarely allow accurate differentiation between normal and pseudonormal diastolic type. At present, the most sensitive and reproducible echocardiographic technique for assessing the dynamics of LV filling is TDI. TDI is a method that uses ultrasound, which records the motion of the myocardium by the Doppler technique, thus evaluating the function segmentation. Recently, the ratio of E / E 'was considered to be the most important parameter of the LV diastolic function. Evaluating the patients in the group studied by this method, LV diastolic dysfunction was observed in 44 cases, the vast majority of them (34) having a slight grade I, 8 grade II and 2 grade III. There was an increased incidence of diastolic dysfunction among female patients (68.11%), rural (68.18%), and over 55 years (54.55%). Depending on the severity class, most patients with diastolic dysfunction were classified as Child C (63.64%), the rest in Class B (22.73%) and A (13.64%).

IMMUNOEXPRESSION OF COLAGEN 1 AND 3, MMP-1 AND TIMP-1 IN DILATATIVE CARDIOMIOPATHY

The histopathological study of 18 cases from deceased patients diagnosed with DCM revealed variations in cellular and nuclear dimensions for myocardocytes, the presence of cytoplasmic spaces filled with basophilic amorphous material. At the MEC level variable collagen fibrosis was present, with distribution both around the muscles and individual myocardial cells.

The collagen 1 immunoexpression was identified in all the analyzed cases of DCM, the markings being observed cytoplasmally, predominantly in the perimisium and cardiac endomysium. At this level, signals were observed in the collagen fibers, vascular walls and cellular elements, represented by fibroblasts, the intensity of the reactions being moderate / increased. In the case of vessels, the markings were uniform and continuous, regardless of the size of the structures. Also, markers were identified in the basal membranes of myocardial sarcophagi, the reactions being continuous or discontinuous depending on the direction of

sectioning of the tissue. In these cases we found at the sarcolem level a moderate /weak intensity of reactions in the case of hypertrophic myocarditis and an increased reaction in atrophic myocarditis. At the same time, we found the presence of signals of collagen 1 in the cytoplasm of the myocardocytes, especially near intracytoplasmic vacuoles or attached to the sarcolem.

The collagen 3 immunoexpression had a similar distribution to collagen 1, the markings being observed in all cases. However, markings at the perimisium, endomisium and sarcolem levels had an increased intensity in all cases. Also, the markings were more intense and more numerous in the cytoplasm of myocardocytes, irrespective of their hypertrophic or atrophic status.

In the case of the control groups, for both types of collagen analyzed, the intensity of the signals was similar to that of the dilated cardiomyopathy at the perimisium, endomysium and sarcolemes of the myocardocytes. However, the number of positive MEC and intracytoplasmatic levels was inferior. The appearance was statistically significant for both collagen 1 ($p < 0.0001$, t-Student test) and collagen 3 ($p < 0.001$, t-Student test).

Immunoreactions of MMP-1 and TIMP-1 were observed in all cases investigated (DCM and control) in the cytoplasm of stromal elements, represented by endothelial cells, fibroblasts and rare lymphocytes. The intensity of the reactions was uniform, moderate / intense. In the case of MMP-1, some discontinuous variable intensity markers were observed in sarcolem or cytoplasm of myocardocytes in the control group. In DCM, MMP-1 and TIMP-1 expression in myocardial cells was absent. The statistical analysis showed significantly lower IOD mean values of MMP-1 in DCM compared to control group ($p < 0.01$, t-Student test). In the case of TIMP-1, the values were superior in DCM compared to the control group, but the appearance was insignificant statistically ($p > 0.05$, t-Student test).

The statistical analysis of mean IOD distribution for the markers analyzed indicated a positive linear correlation between collagen 1 and TIMP-1 ($p < 0.01$, Pearson's test). At the same time, negative linear relationships of collagen 1 / TIMP-1 with collagen 3 and MMP-1 were observed, but the aspects were statistically non-quantifiable ($p > 0.05$, Pearson's test).

CONCLUSIONS

1. The highest incidence of hepatic cirrhosis in the studied group was represented by the decade 50-59 years, over half of the patients being part of the young population. Male sex had a larger share in the studied population, predominantly from rural environment, this being a peculiarity of the studied group. The results obtained in the present study indicate that liver cirrhosis, irrespective of its etiology, affects all the social layers, without being influenced by the patients study level or their environment of origin. The etiology of hepatic cirrhosis in the present study was predominantly ethanolic (80.49%), particularly in the male sex, followed by viral etiology, which predominated in female gender. Assessing the severity of liver cirrhosis by using the Child and MELD scores indicated that most hospitalized cirrhotic patients were in the advanced stages of the disease.
2. The present study showed that patients with cirrhosis had echocardiography (higher atrial volume) and biochemical (higher levels of NT proBNP), changes in cardiac dysfunction expression, leading as spectators or actors to liver decompensation and ascites development. It is clinically relevant that plasma proBNP levels are increased proportionally to the severity of cirrhosis. In fact, advanced cirrhosis and high levels of NT proBNP are significantly associated with increased LA volume and signs of cardiac diastolic dysfunction, which characterize morpho-functional changes typically found in the end stage of hepatic disease.
3. Patients with liver cirrhosis develop a whole series of electrocardiographic changes during the course of their disease. Regarding the prevalence of rhythm disorders (especially AF) it is small. Further studies might be beneficial to check for possible mechanisms of protection against AF development in patients with cirrhosis.
4. The results of our study, particularly on other electrocardiographic changes in cirrhotic patients, demonstrated the presence of QT prolongation, the mean being much higher compared to the control group.
5. The systolic function of LV evaluated by LVEF was normal in patients with cirrhosis in this study. Although the LVEF evaluated at rest in the patients studied was normal or even increased, there is still a subclinical myocardial dysfunction in the incipient stages. 2DSTE is a useful and non-invasive method for the detection of cardiac dysfunction in patients with cirrhosis where cardiac insufficiency is not yet clinically evident.

6. The data from this study demonstrated that patients with liver cirrhosis exhibit diastolic dysfunction. In the absence of other risk factors for cardiac disease, this dysfunction could only be attributed to cirrhotic cardiomyopathy. It has also shown that, although diastolic dysfunction is a common event in cirrhosis, it is usually a mild (low grade) and most often correlates with the severity of liver dysfunction.
7. The markers analyzed in this study can be used to quantify the degree of collagen sclerosis at the MEC level. Further expanded studies are needed to analyze the nature of vacuoles at the level of myocardial cytoplasm. MMP1 and TIMP1 immunoexpression supports these proteins as potential therapeutic targets in DCM.

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