UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
THE FACULTY OF MEDICINE

PhD THESIS
-ABSTRACT-

The importance of non-invasive diagnostic methods in the assessment of patients with non-alcoholic fatty liver

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CONTENTS

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Non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), hepatic fibrosis, liver biopsy, non-invasive diagnostic, non-invasive scoring systems, FibroScan, single nucleotide polymorphisms (SNP), HLA antigens

INTRODUCTION

Non-alcoholic fatty liver disease is considered nowadays a major public health problem worldwide. Obesity, type II diabetes and dyslipidemia, in the absence of alcohol consumption, are the most important risk factors for non-alcoholic fatty liver disease, which is revealed by a number of epidemiological studies that have shown independently a strong link between each and every factor mentioned and the disease.

Non-alcoholic fatty liver disease includes several clinical entities, from the hepatic steatosis to the steatohepatitis, fibrosis and even hepatic cirrhosis, issues requiring histological confirmation.

Currently it is accorded great importance to the study of non-invasive diagnostic methods in order to identify imaging techniques and serological markers that correlate with the lesions of steatohepatitis and hepatic fibrosis and to establish clear indications of liver biopsy in patients with non-alcoholic fatty liver.

Serological markers predictive for the hepatic fibrosis and the non-invasive tests proposed as an alternative to liver biopsy and histological examination were not widely accepted and very few are currently validated.

The arguments presented are supporting the scientific research on non-invasive exploration of non-alcoholic fatty liver to identify the patients with a high risk of developing a more aggressive form of the disease and to develop an algorithm for assessing the grading and stageing of the disease.

GENERAL CONTENT

The first chapter of the general content entitled *DEFINITION OF NON-ALCOOLIC FATTY LIVER DISEASE* reviews the terminology used for liver lesions that characterize the non-alcoholic fatty liver disease, steatosis, steatohepatitis and hepatic fibrosis, as well as the last diagnostic criteria for the metabolic syndrome.

In chapter II, *EPIDEMIOLOGY AND ETIOPATHOGENESIS,* there are presented current data on the incidence and the prevalence of non-alcoholic fatty liver disease, data related to the risk factors and major clinical conditions associated with the non-alcoholic fatty liver disease. The actual incidence and prevalence of non-alcoholic fatty liver disease are not fully known, but the available data up to date indicate a rapid increase in disease prevalence alongside with the dramatic increase of the population with obesity and diabetes. The main risk factors associated with the non-alcoholic fatty liver disease are represented by obesity, diabetes, metabolic syndrome, with an insulin resistance as a primary pathogenic mechanism. In this chapter also we review the main pathogenic mechanisms leading to the emergence and evolution of lesions of hepatic steatosis to fibrosis stage. The chapter concludes with data on the involvement of genetic factors in the development of the non-alcoholic fatty liver.

In chapters III and IV, *CLINICAL DIAGNOSIS* and *PARACLINICAL DIAGNOSIS* there are presented the main clinical manifestations of the patients with non-alcoholic fatty liver as well as the main biological and imaging changes supporting the diagnosis of non-alcoholic steatohepatitis.

Chapter V, *HISTOLOGICAL AND IMMUNOHISTOCHEMICAL DIAGNOSIS,* includes data on the characteristics of anatomo-pathological lesions of steatosis, steatohepatitis and fibrosis, as well as the current systems of classification and of evaluation of the disease stage and grade in patients with non-alcoholic fatty liver.

The last chapter of the general content, *NON-INVASIVE DIAGNOSIS* follows the main non-invasive methods (scoring systems and imaging techniques) currently used for the diagnosis of the non-alcoholic fatty liver disease, insisting on their predictive value in the detection of changes in steatosis, steatohepatitis and hepatic fibrosis compared with the histological changes of each type of lesion.

PERSONAL CONTRIBUTIONS

The study conducted by the Centre for Research in Gastroenterology and Hepatology Craiova, between November 2009 - June 2012 included several study groups, with a significant number of patients, on whom we performed a comparative analysis of conventional invasive diagnostic methods, non-invasive testing diagnosis and of exploration of new generation (liver elasticity measurement by transient elastography FibroScan and genetic tests to determine the PNPLA3, MTTP and MnSOD genes polymorphisms and the

identification of HLA antigens that confer susceptibility to the disease emergence).

- ***The role of non-invasive tests in the diagnosis of patients with non-alcoholic fatty liver***

The liver biopsy in some patients allowed establishing more accurately the role of non-invasive tests in the diagnosis of lesions of steatosis, steatohepatitis and liver fibrosis.

The non-invasive tests analyzed on the first study group showed a statistically significant correlation with the lesions of liver steatosis, steatohepatitis and fibrosis revealed on the liver biopsy. Of the scores used to establish or refute the diagnosis of hepatic steatosis, we investigated the utility of *Fatty liver index* (FLI) and the *NAFLD - liver fat score* (NAFLD-LFS). For the *Fatty liver index*, we achieved an accuracy of 96% for excluding the steatosis by FLI <30 (Se 95.83%, Sp 100%, PPV 100%, VPN 50%) and an accuracy of 96% for all hepatic steatosis confirmation FLI ≥ 60 (Se 87.5%, Sp 100%, PPV 100%, VPN 25%). For the *NAFLD - liver fat score,* for the threshold of -0.640, we achieved a sensitivity of 95.83% and a specificity of 100% when the results were reported to the histological lesions of steatosis. For the steatohepatitis scores we obtained a lower accuracy in diagnosing NASH, 60%, with sensitivity, specificity, PPV and VPN of 80%, 40%, 60% and 57%. We have obtained promising results for the *NAFLD fibrosis score.* A *NAFLD fibrosis score* value below -1.455 excluded significant fibrosis with a sensitivity and VPN 100%, and a value above 0.676 confirmed advanced fibrosis with high specificity and PPV of 100% too.

- ***The role of transient elastometry (FibroScan) in the assessment of patients with non-alcoholic fatty liver disease***

The FibroScan liver elasticity measurement has been proposed as an alternative to liver biopsy for determining the fibrosis degree. The present research is consistent with the literature date, with an area under the ROC curve of 0.823 (95% CI, 0.252 to 0.394) ($p < 0.0001$) for excluding significant fibrosis measured value under 6.9 kPa and an area located under the ROC curve of 0.95 (95% CI, 0.426 to 0.475) ($p < 0.0001$) in liver elasticity measured value above 9.4 kPa. By dividing the group of patients with valid results from FibroScan values depending on the elasticity values, we found that most patients (74.48%) had FibroScan results below 7.9 kPa and the age of these patients was significantly lower than of the patients of the other two subgroups ($p = 0.021$). The difference between the presence and the absence of metabolic syndrome and of diabetes was also statistically significant between the patients in the study groups above. Patients with hepatic elasticity below 7.9 kPa had an average BMI 31.87 ± 7.19 kg/m2 lower than the other two groups of patients, statistically significant difference ($p = 0.003$). Similar results with statistically significant differences between the three subgroups, we have obtained by comparing also the waist circumference, the insulin resistance index, the serum triglycerides, the transaminases and the GGT. Instead, we have not obtained statistically significant differences for the serum cholesterol ($p = 0.137$), HDL cholesterol ($p = 0.627$) or for the report GOT / GPT ($p = 0.171$).

- ***The determination of PNPLA3, MTTP and MnSOD genes polymorphisms and their significance in patients with non-alcoholic fatty liver***

For the PNPLA3 rs738409 polymorphism is associated with an increased risk of hepatic steatosis, hepatic steatosis risk allele is the minor allele [G]. In the study group, the [CG] genotype carriers has a 1.7 times higher risk of developing hepatic steatosis, compared with the [CC] genotype carriers OR1, 768 (95% CI, 1.006 to 3.110) (p = 0.046).

The [G] and [T] allele frequency for MTTP rs1800591 polymorphism, as the [T] and [C] allele frequency for MnSOD polymorphism 1183 T > C did not present differences between the study group and the control subjects, indicating that for these polymorphisms there were not found significant associations with the hepatic steatosis. The PNPLA3 polymorphism is associated with an increased risk of hepatic steatosis in patients with BMI <30 kg/m2, compared with the control population, when the risk allele [G] carriers were compared with the [C] allele carriers (p = 0.038). By comparing the subgroup with steatosis without obesity with the subgroup with steatosis and BMI ≥ 30 kg/m2, we have noticed that the [G] allele carriers compared to the [CC] homozigotes in the dominant model, have a 2.5 times higher risk of developing liver steatosis (p = 0.025). For the PNPLA3 polymorphism, G risk allele is significantly associated with the risk of severe fibrosis (p = 0.038), especially in patients with BMI below 35 kg/m2. Regarding the MTTP 493G/T rs1800591 polymorphism, none of the patients with results exceeding 8.7 kPa at FibroScan was not

carrying the TT genotype. However, the GT genotype carriers have a 2 times increased risk of progression to advanced fibrosis stages compared with the control population.

- ***The role of HLA antigens in determining the disease susceptibility in patients with non-alcoholic steatohepatitis***

Regarding the HLA profile of patients with NAFLD, we have found two combinations of genes that seem to influence the disease emergence:

-HLA A24, HLA B15, HLA DR15, HLA DR 16, HLA DQ3, HLA DQ 5 influence the development of non-alcoholic hepatic steatosis in patients without other risk factors

-HLA-A2, HLA-32, HLA B18, HLA B49 and HLA B53 in patients with obesity, metabolic syndrome and insulin resistance

Upon the completion of the research and after the interpretation of results we have reached 17 conclusions clearly presented in the last chapter of the thesis.

The thesis bibliography is very extensive consisting of more than 200 titles that make an overview of the main titles published recently in this pathology.

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