UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

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PhD THESIS

-ABSTRACT-

Prognostic factors and therapeutic interventions in non-alcoholic fatty liver disease

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CONTENTS

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Non-alcoholic fatty liver disease (NAFLD), steatohepatitis (NASH), hepatic biopsy, atorvastatin, losartan, pentoxifylline, ursodeoxycholic acid

INTRODUCTION

Non-Alcoholic Fatty Liver Disease (NAFLD) is defined in Anglo-Saxon literature as referring to the entire spectrum of hepatic pathologies which emerge in the absence of both alcohol consumption in any quantities that might induce hepatic injury and other evident causes for hepatic disease. This spectrum is made up of: degrees of simple steatosis, non-alcoholic cirrhosis, non-alcoholic steatohepatitis and non-alcoholic fatty liver – the main cause of the so called „cryptogenic cirrhosis".

The impact of the non-alcoholic fatty liver from a biological, epidemiological, pathogenic and socio-economical point of view is tightly linked to dyslipidemia, obesity and type 2 diabetes. The real prevalence and incidence of the non-alcoholic fatty liver are not fully known – however the data we have available until now have indicated a fast increase of the pathology's prevalence in parallel with the dramatic increase of cases of diabetes type 2 and obesity.

The diagnosis of non-alcoholic fatty liver is generally made by accident, either when conducting routine biological explorations, when high values of transaminases are observed, or when undergoing transabdominal ultrasonography.

Multiple pharmaceutical agents were used for the management of the non-alcoholic fatty liver. However, most of the trials were undergone for too short a time period to be able to determine the impact on important clinical results (for instance decompensated cirrhosis) and reported rather partial results, such as the levels of serum transaminases or histological observations, often with contradictory results.

The ideal aim of the treatment is the histological resolution of lesions demonstrated by a HBP with the implicit decrease of the risk of evolution towards hepatic cirrhosis. Under usual conditions it is considered as an acceptable treatment results the decrease of hepatic fatty infiltration demonstrated through non-invasive exploration and the normalization of transaminases.

Other accepted aims of the treatment are normalization of the CMI, of total cholesterol levels and of triglycerides levels in serum. The quality of life and cost-effectiveness are also elements that must be considered when treating NAFLD/NASH.

GENERAL INFORMATION SECTION

Chapter I of the general information section, called THE DEFINITION OF THE NON-ALCOHOLIC FATTY LIVER, reviews the terminology used for describing hepatic lesions which characterize the non-alcoholic fatty liver, steatosis, steatohepatitis and hepatic fibrosis, as well as the latest diagnostic criteria for the metabolic syndrome.

Chapter II, EPIDEMIOLOGY AND ETIPATHOGENESIS, presents the actual data regarding the prevalence and the incidence of the non-alcoholic fatty liver, data which is linked to the risk factors and the main clinical conditions associated with the emergence of non-alcoholic fatty liver. The true prevalence and incidence of non-alcoholic fatty liver are not fully known, but the data we have today indicates a rapid increase of the prevalence of this pathology in parallel with the dramatic increase of the number of cases of obesity and diabetes type 2. In spite of the various pathological conditions which might determine NAFLD – such as certain drugs (estrogens, glucocorticoids, methotrexate, tamoxifen, diltiazem, amiodarone), parenteral nutrition, gastro-intestinal surgical interventions, inflammatory intestinal disease, the term of NAFLD is currently reserved for its use within metabolic pathologies with undefined histological modifications.

The main risk factors associated with non-alcoholic fatty liver are represented by the presence of diabetes mellitus, obesity and the metabolic syndrome, all sharing resistance to insulin as their main pathogenic mechanism. This chapter also reviews the main pathogenic mechanisms which lead to the occurrence and evolution of hepatic steatosis lesions up to fibrosis. The chapter finalizes with data regarding the implication of genetic factors in the occurrence of non-alcoholic fatty liver.

Chapters III and IV, CLINICAL DIAGNOSIS and PARACLINICAL DIAGNOSIS, present the main clinical manifestations of patients suffering from non-alcoholic fatty liver, as well as the main biological and imagistic modifications which sustain the diagnosis of non-alcoholic hepatic steatosis.

Chapter V, HISTOLOGICAL DIAGNOSIS, contains data regarding the anatomopathological characteristics of steatosis, steatohepatitis and fibrosis lesions, as well as the present day systems of classification, stage evolution and degree of disease for patients suffering from non-alcoholic fatty liver.

The final chapter of the general information section, THE MANAGEMENT OF THE NON-ALCOHOLIC FATTY LIVER, tracks the main therapeutic methods presently used to treat non-alcoholic fatty liver, stressing the importance of pharmacological therapy.

PERSONAL CONTRIBUTION

Material and methods

Prospective study conducted within the Clinic of Internal Medicine of the Clinical Municipal Hospital Filantropia of Craiova. It was conducted between October 2010 and December 2012 and it included 4 groups, of a significant number of patients (n=202), for which non-alcoholic hepatic steatosis was confirmed by histopathological exam. These groups underwent therapy with various therapeutic agents – ursodeoxycholic acid (a compound with cytoprotective, immunomodulatory and antiapoptotic properties), losartan (an agent that blocks the renin – angiotensin system), pentoxifylline (an inhibitor of TNFα), atorvastatin (an inhibitor of HMGCoa reductase). Our study made a comparatively analysis of their influence on the evolution of the non-alcoholic fatty liver. The average duration of therapy was of approximately 30 weeks. Subjects included in the study were monitored during their treatment at 10 and 20 weeks respectively. Criteria of inclusion and exclusion were made for the distribution of subjects within their study group.

The histological study consisted of the processing of tissue samples collected by punch biopsy with the aim of computing the disease activity score (NAS) which is defined as the sum between the degree of steatosis (0-3), inflammation (0-3) and ballooning (0-2).

The clinical study aimed at establishing a series of demographic, anthropometric, heredocollateral precedents, personal, physiologic and pathologic data on the subjects included in the study.

The biochemical study consisted in determining sanguinary parameters which would allow the evaluation of the glucidic and lipidic metabolisms, the hepatic function and the oxidative stress.

The statistical analysis of the obtained results

The data was processed using the MedCalc v.11.01 software (MedCalc Software bvba, Ostend, Belgium) and the Microsoft Excel software (Microsoft Corp., Redmond, WA, USA), together with the XLSTAT suite for MS Excel (Addinsoft SARL, Paris, France). The obtained information was stocked as Microsoft Excel files, and statistically processed with the aim of analyzing the relations between the clinical and the paraclinical data of patients.

Results and Discussions

During the first stage of the research, 202 subjects with histological confirmed hepatic steatosis lesions were recruited. From the 202 subjects, 120 were male (59.41%) and 82 female (40.59%). The values of the corporal mass index for the subjects included in the study were situated between 18.10 and 52.80, the average value being 28.26.

Most patients included in this study presented one or several risk factors for non-alcoholic fatty liver. Thus, 26.73% fulfilled the diagnostic criteria for the metabolic syndrome and 39.10% suffered from diabetes mellitus type 2.

A comparative evaluation of the patients within the 4 groups was made and the differences which are noticeable under the administered treatment were identified. The

results of the clinical and biological control of the subjects are presented at the T0 moment, corresponding to an evaluation made 24 hours before beginning the treatment and at T1, T2 and T3 moments, corresponding to the intervals of 10, 20 and 30 weeks respectively since the beginning of the treatment.

Following the clinical and the paraclinical explorations on the 202 patients included in our study, their values of the systolic and diastolic tension respectively were found to be far higher than those of other groups (Ts=169.66 ± 12.10, Td = 91.19 ± 8.11, at the beginning of treatment), the values of diastolic tension for the group of dyslipidemic subjects being similar to those of the non-HTA group, while in the case of the dyslipidemic subjects group we can even note a tendency of growth.

From a paraclinical point of view, there were no significant differences between the recorded haemoglobin values for the 4 groups of patients at the beginning and end of the treatment, its average value remaining within normal limits for the entire duration of the treatment.

Changes of the hepatic function and the glucidic and lipidic profile were observed which were compared to the literature data. Our results were found to be in concordance with an entire series of trials.

Shortly, all the 4 drugs examined in the present study led to significant decreases of ALT and GGT values, of which atorvastatin also led to significant decreases of the levels of alkaline fosfatatis, total cholesterol and triglycerides. As it was previously stressed, the contribution to the reduction of CMI to biochemical improvements was less important, at least not in the specific case of this study, but certainly several investigations are needed to firmly establish this as a fact.

On the other hand, the histological evaluation showed significant improvements of 2 of the 3 or 4 components of the NAS score only for patients treated with atorvastatin, losartan and pentoxifylline, while the patients who were administered UDCA showed no statistically significant improvements. From a histological point of view, the studied cases showed average values of the disease activity score between 4.13 and 4.26 for hypertensive

patients and dyslipidemia patients respectively, and an average value lower than 4 for the other 2 groups.

As for the dyslipidemia patients, a value of p of 0.00007 < 0.001 was found and thus a highly significant difference between the initial and the final NAS scores. The patients from the dyslipidemia group, who received treatment with pentoxifylline, presented values that were slightly lower, with a p value of 0.04073 < 0.05, thus a significant difference. This was different from the group of hypertensive patients, which presented a p value of 0.00004 < 0.001, indicating a statistically highly significant difference.

Administering pentoxifylline resulted in a fast and high decrease of ALT levels (82.60 ± 12.82 to 76.90 ± 6.83, p=0,001) and GGT levels (55.27 ± 17.55 to 49.95 ± 8.95, p=0.004) after 10 weeks of treatment – and this was an effect remained stable for the entire period of the study.

By improving biochemical and histological scores on hypertensive patients with NAFLD, losartan proved to be a valuable agent for this group. Regarding the changes of the lipidic metabolism losartan significantly improved the values of cholesterol (274.75 ± 27.76 to 262.32 ± 30.88, p= 0.0036), HDL cholesterol (161.41 ± 24.83 to 155.92 ± 19.21, p=0.0031) and triglycerides (241.71 ± 65.39 to 231.17 ± 45.96, p=0.0021). The evaluation of the hepatic function on patients treated with losartan highlighted the significant decrease of AST values (79.63 ± 17.09 to 75.96 ± 9.85, p=0.01), a highly significant decrease for ALT values (77.93 ± 19.58 to 72.00 ± 11.50, p=0.007), and a statistically not relevant decrease of GGT values.

Ursodeoxycholic acid was tested on NASH patients due to its antiapoptotic and immunomodulatory effects [173, 175] which are not absolutely linked to the decrease of the level of hydrophobic endogenic acids within the bile.

The present study shows that, in comparison with the base levels, after 30 weeks of treatment UDCA leads to significant improvements of the AST levels (88.79 ± 14.60 to 84.44 ± 7.17, p=0.006), ALT levels (81.78 ± 15.12 la 75.07 ± 6.52, p=0.003) and GGT

levels (57.48 ± 18.77 to 57.48 ± 18.77, p=0.001), as well as the improvement of some histological characteristics.

Conclusions

Non-alcoholic fatty liver represents a major cause of morbidity and the risk of this pathology increases proportionally with the increase of the corporal mass index. The incidence of the non-alcoholic fatty liver is continuously rising due to the increase of prevalence of obesity in the modern society.

The present study, undertaken within the Internal Medicine Clinic of the Municipal Clinical Hospital Filantropia of Craiova, from October 2010 to December 2012, included 4 groups, with a high total number of patients, and studied the influence of some therapeutic agents on the evolution of the pathology of the non-alcoholic fatty liver.

Direct conclusions of our research can be summed as following:

1. *The incidence peak for non-alcoholic hepatic steatosis was situated in the 5th age decade, while the sex distribution of patients of our 4 study groups proved a higher prevalence of the pathology of men compared to women (59.41%)*

2. *Most patients included in this study showed the presence of one or several risk factors for the occurrence of non-alcoholic fatty liver. 26.73% of the patients fulfilled the criteria required for the diagnosis of the metabolic syndrome and 39.10% of the patients suffered from diabetes mellitus type 2.*

3. *In this study, the average value of the corporal mass index for patients suffering from hepatic steatosis was of 28.26, with higher values for patients suffering from dyslipidemia and hypertension.*

4. *From a histological point of view, at the beginning of the therapy, the studied cases presented average values of the pathology activity score between 4.13 and 4.26 for hypertensive patients and dyslipidemia patients and an average value under 4 for the other two groups.*

5. *At the end of the study the average values of the pathology activity score were between 2.87 and 2.93 for hypertensive patients and dyslipidemia patients, while for the other two groups the NAS score had an average value under 3.*

6. *All the four administered drugs led to significant decrease of the values of ALT and GGT.*

7. *Atorvastatin produced highly significant decreases of the levels of alkaline phosphatase ($p < 0.0001$), total cholesterol ($p < 0.0001$) and triglycerides ($p < 0.0001$). Histological evaluation showed significant improvements for 2 of the 3 or 4 components of NAS only for patients undergoing atorvastatin, losartan and pentoxifylline treatment, while patients who received UDCA treatment showed no statistically significant improvements.*

8. *The degree of fibrosis was unchanged in all the 4 groups, and only steatosis seemed to benefit from the administration of atorvastatin, losartan and pentoxifylline.*

9. *The use of pentoxifylline is well tolerated, it improves the values of transaminases and the histological scores for NAFLD patients. The use of pentoxifylline caused a high and fast decrease of ALT levels ($p=0.001$) and GGT levels ($p=0.004$) after 10 weeks of treatment, an effect that remained stable for the entire period of the study.*

10. *By improving biochemical and histological scores on hypertensive patients suffering from NAFLD, losartan proved a valuable agent for its group. As for the modifications of the lipidic metabolism losartan significantly improved the values of cholesterol ($p=0.0036$), HDL cholesterol ($p=0.0031$) and triglycerides ($p=0.0021$). The evaluation of the hepatic function on patients treated with losartan highlighted the significant decrease of AST values ($p=0.01$), a highly significant decrease for ALT values ($p=0.007$) and a statistically non-relevant decrease of GGT values.*

11. *Administering ursodeoxycholic acid on patients with NAFLD led to significant improvements of AST levels ($p=0.006$), ALT levels ($p=0.003$) and GGT levels ($p=0.001$) as well as some histological characteristics. Though some benefits were observed by reducing the levels of transaminases and even if the hepatoprotective properties justify its use in numerous chronic hepatic pathologies, the routine use of UDCA when treating NAFLD remains under research due to the absence of consistent effects on hepatic histology.*

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