UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

PhD THESIS

SUMMARY

THE ASSESSMENT OF GLUCIDIC METABOLISM IN PATIENTS WITH KNOWN LIVER DISEASE WITH PROGRESSION TO HEPATOCELLULAR CARCINOMA.

Scientific advisor:

Prof. Univ. Dr. Rogoveanu Ion

PhD student:

Dr. Braia Natalia

CRAIOVA

2014

The General Part
(State of Knowledge)

1.   **Introduction.** Hepatocellular carcinoma (HCC) represents the fifth cause of cancer at a global level with over 800.000 new cases discovered annually and the second cause of death by cancer. The development of HCC is a sequential process that follows the dysplasia-carcinoma evolution, more often than not the base being preexistent chronic hepatic disease, with the persistence of the inflammatory process, fibrosis or cirrhosis.

Different studies regarding the etiology of HCC have identified alcohol consumption, hepatic viruses and alpha toxins as being the greatest risks of causing the progression towards hepatic cancer. In different areas of the world, HCC caused by these factors, either solitary or together presents an important diversity when it comes to the phenotype and genotype, a fact that hinders the determination of the carcinogenic process. All things considered, a characteristic trait is represented by the alteration of carbohydrate metabolism, which can represent a special bond between different genotypes and phenotypes of HCC and can offer important direction for treatment.

2.   **Etiology.** Hepatic carcinogenesis is a multifactorial complex process. Unlike other tumor related pathologies, HCC offers the possibility of identifying the etiological factor in most cases that had a major contribution to the initiation and promotion of tumor genesis. Despite this, there is no singular cause that determines the appearance of HCC, at the base being the cumulative effects determined by the presence of many risk factors in the same individual. The contribution of each of these factors is dependent on the characteristics of studied populations, being largely influenced by genetic susceptibility, geographical area and life style.

3.   **Pathogenesis.** Hepatocellular carcinoma pathogenesis has not been completely elucidated to the present day. Even so, the plurifactorial nature of the hepatic carcinogenesis process is clear. In most cases, the appearance of HCC is mediated by hepatic injury which develops inflammation and fibrosis, both determining the alteration of the liver architecture with the characteristic aspects of liver cirrhosis. The progression of hepatic cirrhosis towards HCC is a complex process, found in tightly related to the base liver ailment. Multiple studies have

investigated possible mechanisms implicated in the development of HCC in patients with cirrhosis. Although, initiation and progression of carcinogenesis can take place even in the absence of hepatic cirrhosis, as in the case of carcinoma associated with the infection with hepatic virus B (HVB). The data obtained up to this day describe hepatic carcinogenesis as a malign, complex and heterogeneous process that has at its core a large range of genetic and epigenetic modifications, and also specific alterations of certain cell signaling pathways.

4. **Diagnosis.** Most cases of hepatocellular carcinoma are asymptomatic, with an insidious character. For this reason it is difficult to diagnose before reaching an advanced stage. Patients developing HCC do not usually present symptoms or signs with the exception of those correlated with chronic hepatic disease that they are suffering from. It can be identified with the appearance of some complications of hepatic cirrhosis such as ascites, encephalopathy, superior digestive hemorrhage, jaundice. These complications are often associated with the tumor extension in hepatic veins and portal vein or tumor induced arteriovenous shunting.

Biological investigations occupy and important role although these test can be much more altered when HCC is accompanied by hepatic cirrhosis or other pathologies. The exceptions being gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) whose high levels suggest the "space occupying" effect of cancer. Alpha fetoprotein (AFP) is a low cost marker and represents an attractive option for screening, being used for early diagnosis, monitoring and recurrence of HCC. Sadly it has a sensitivity of only 40-64% because most tumors don't produce AFP at all or only in advanced stages. Therefore AFP levels can be the subject of erroneous interpretation. When its value is high, AFP has a specificity of 75-91%, and values over 400 ng/mL are considered a good indicator of HCC diagnosis in the appropriate clinical context, especially if it is coupled with correct radiological results. Normal values of AFP do not exclude HCC.

Histologic diagnosis of HCC is necessary pretty rarely nowadays because of the existence of numerous non-invasive imagistic alternatives. Computed tomography (CT), Nuclear magnetic resonance imaging (MRI) and Contrast ultrasonography (CEUS) are widely available globally and have mostly replaced conventional biopsy and angiography in diagnosing HCC.

5. **The Metabolic Syndrome.** In recent years it has become evident that obesity can be complicated by the appearance of HCC. During this time the growing incidence of obesity, especially in developed countries, represented a big part of the reason why the incidence of HCC grew. A meta-analysis containing 11 studies done in the USA, Europe and Asia pointed out an

increase in the risk of developing HCC of 1.89 (95% trust margins of 1.51 to 2.36), and the fact that the tumor, in concordance with the rapid spread of obesity, has become the cause of death by cancer. Additionally, a prospective study done in Europe has proven the tight link between obesity and HCC: relative risk 3.51, 95% trust interval 2.09 to 5.87 ($p < 0.0001$). In another European analysis a relative risk of 1.9 has been reported in obese patients. Moreover, in the USA the incidence of HCC has tripled (from 1.6/100 000 to 4.9/100 000 people) in parallel with the rise in the incidence of obesity. Adding to all this the fact that the high body mass index (BMI) in children raises the risk of developing HCC during adult life, and can even determine de appearance of the disease during childhood.

6. **Obesity-induced HCC. Pathogenic mechanisms.** Multiple unclarities exist regarding the mechanism through which obesity and the metabolic syndrome can determine malignant transformation, although certain factors are taken into account that can play a key role in this transformation: insulin resistance, hyperinsulinemia, growth of cellular signaling dependent of tumor necrosis factor (TNF), alteration of cellular lipids, presence of NAFLD or NASH, and intestinal microbiom.

Insulin resistance is the main factor that binds all the components of the metabolic syndrome together. It also contributes to the accumulation of lipids in hepatocytes, even in the case of subject with no derailments of the glycemic control, and plays at the same time an important role in the development of NAFLD. Hepatic steatosis leads to the development of hepatic steatohepatitis, which will progress towards malignant transformation.

Personal Contribution

The studies performed had the purpose of identifying and assess the risk factors for HCC development, independent factors of alcohol consumption, chronic viral infections or liver toxicity, thus building a profile of a risk population of developing hepatic cancer. With both a prospective and retrospective character, the study includes patients known of suffering from liver disease, the diagnosis of HCC following epidemiological, clinical, biological and imagistic aspects which were correlated with the prognosis of the disease.

- Patient evaluation was followed through the certitude diagnosis of HCC, imposed either by imaging criteria or liver biopsy when necessary;
- Identifying type II diabetes, as a risk factor for HCC in patients with chronic liver disease. The association between diabetes and HCC was determined with the purpose of underlining the necessity of careful monitoring and thus objectifying the increased potential that these patients have in primitive hepatic cancer;
- Identifying and diagnosing HCC in patients without clinically or imagistically evident chronic liver disease;
- Objectifying Non-alcoholic Fatty Liver Disease (NAFLD) and obesity as risk factors in developing HCC, therefore offering a new perspective regarding the patient that might develop hepatic cancer;

1. Materials and Methods

The study was a prospective one and it included patients consecutively committed in the Gastroenterology Clinics and Internal Medicine Clinics of the Emergency County Hospital of Craiova in the period between 1st of January 2008 and 30th of June 2014, with the diagnosis of hepatic disease. All patients provided signed consent, following the Ethics' Committee of UMF Craiova approved protocol. All study procedures were performed according to The Helsinki Declaration; patients were not submitted to any maneuvers beside the normal diagnostic protocols.

Relevant data was extracted from the anamnesis regarding risk factors, place of origin, significant family history and personal history of disease, but also signs and symptoms specific to hepatic disease.

The diagnosis of hepatic cancer (HC) was established according to the EASL guides, requiring at least two simultaneous criteria:

1. Endoscopic presence of esophageal varices;
2. The existence of regeneration nodules determined ultrasonographically;
3. Imagistic proof of splenomegaly (US or CT).

Diabetes was diagnosed on the base of hyperglycemia (>8mmol/L) during at least two determinations, or active insulin treatment, hypoglycemic oral agents, or both. Small alterations in the glucidic metabolism were not taken into account, such as altered tolerance to glucose base on an oral glucose tolerance test.

A HCC diagnosis was established following a combination of high-contrast transactional imaging methods (CT and MRI), with liver biopsy in the case of uncertainty and a control lasting at least 6 months. AFP over 400 ng/mL was considered relevant, without excluding HCC in patients with AFP in normal limits, but with suggestive imaging results.

A number of patients were selected, suffering from obesity and NAFLD who were subjected to liver biopsy. Initially the grade of liver fibrosis was quantified using Fibroscan (Echosense, France). 8 kpa was the considered cut-off value in determining fibrosis. Using imaging methods the size of the tumor was determined, its characteristics, and metastasis. Patients were classified by the "Barcelona Clinic Liver Cancer" staging, for a more precise evaluation and choosing of ideal treatment.

2. Results and discussions

A total of 2718 patients were diagnosed with HC, 71 refused to offer informed consent and 91 did not present themselves for periodical evaluation. In total, 164 cases of HCC were confirmed during the study period. At the moment of diagnosing HCC, diabetes was observed in 54 patients. Diabetes was found in 317 patients without HCC.

The statistic study done showed a significant correlation between diabetes and cirrhosis (p = 0,040 <0,05). Also, the Odds Ratio value OR=2.053 was statistically significant (95% CI = 1.026-4.109, does not contain value 1), underlining additionally that there exists a correlation between diabetes and cirrhosis, which contributes to the appearance of HCC.

Analyzing all the patients with hepatitis, a strong correlation was found between HCC and diabetes – the Square Chi Test was p<0,001, which underlined the fact that and extremely strong correlation exists between the two factors, for patients with hepatitis. Since the Square Chi Test was significant statistically, the Relative Risk (RR) was calculated for the development of

HCC if diabetes is associated with hepatitis, which was 2.894 times larger than when diabetes is not present (95% CI= 2.063 - 4.060).

Of the total of patients diagnosed 23 were selected to undergo liver biopsy – 14 men and 9 women, of ages between 42 and 69 (with a mean of 56 years). All patients were known to be suffering from hepatic steatosis, without clinical signs of cirrhosis and suffering from obesity with a BMI between 25 and 35 kg/m2 (mean BMI - 32 kg/m2) and an abdominal circumference between 95 cm şi 129 cm (mean - 114 cm). In the selected lot, both alcohol consumption and hepatic viral infection were exclusion factors. Three patients presented smoking history. The hepatic function was altered in all patients, as specified by laboratory tests, with high values of bilirubin and transaminases.

Imaging investigations (abdominal US, CT and MRI) permitted the visualization and localization of hepatic formations, characterizing them offered an indicative diagnosis. For the confirmation of the malignancy hepatic biopsy was imposed. At the same time, the history of hepatic steatosis was confirmed after imagistic investigations.

In 11 cases, at the moment of the diagnosis, HCC had metastasized, especially in the lungs and bones. The diameter of the tumors after US examinations was 3.4 cm, 0.3 cm larger than the average in the case of the other cases in study.

According to the "Barcelona Clinic Liver Cancer", almost half of the patients were diagnosed with advanced stages of cancer C and D, the least number of cases being diagnosed in stage A.

Out of the 23 patients evaluated with impulse elastography a predominance of stage F4 fibrosis was noticed in 13 patients, followed by 6 patients in stage F3. When it comes to the kpa value of fibrosis a mean of 15,425 ± 6.46 kpa was noticed.

After microscopically examining of the sections colored in HE, alterations at the level of the cell architecture were discovered, with a few string of hepatocytes left and tumor cells similar to hepatocytes, without maintaining the many of the characteristics of the original structures. Only a few Kupffer cells were present, and the reticulin network was completely absent in the entire study material. Small, deformed cells with an altered nucleus/cytoplasm ratio were often present and the residual trabeculae with pseudoglandular spaces were visualized. Six cases presented the pseudoglandular variant of HCC, with structures similar to glands padded with tumor cells similar to hepatocytes, derived from the dilatation of residual biliary ducts. In these structures, dense

eosinophilic infiltrates and inflammatory detritus was noticed, with few remaining biliary fragments. Nine cases presented trabecular fractures; in these cases much more differentiated trabeculae were observed, with many cellular layers and endothelium. Two out of these cases were described as macrotrabecular, due to them containing more than 8 cellular layers. The four remaining cases were suffering from advanced HCC, with added present necrosis, abundent inflammatory infiltrate and cellular detritus. The cell architecture was severely altered, without trabecular structures remaining or organized epithelium, glands or biliary ducts.

3. Conclusions

- HCC is a major health issue, being the fifth cause of malignant pathology at a global scale, while being one of the few cancers with an etiology at least partially deciphered;

- There is no individual cause determining de development of HCC, at its core being the cumulative effects determined by the presence of multiple risk factors in the same person, which underlines the plurifactorial nature of the carcinogenesis process;

- Numerous cellular signaling pathways have been highlighted which present implications in the initiation and progression of HCC, as well as a high number of genetic and epigenetic modifications associated with HCC. But still, regardless of the complexity of these modifications and their association with different genetic factors, it becomes obvious that there is a common pathway of HCC pathogenesis, in which the repeatedly affected hepatocytes determine a vicious circle of death and regeneration of the cells with a final stage which leads to genomic instability and the initiation of the hepatic carcinogenesis process;

- The risk factor with the major contribution to the development of HCC is represented by hepatic cirrhosis, but the initiation and progression of carcinogenesis can take place in its absence as well;

- Type II diabetes, obesity, smoking, alcohol consumption, alphatoxins are a few of the factors that favor the forming of HCC and the deficit of alpha-1-antitripsine, hemochromatosis, Wilson's disease are rare causes of HCC;

- Most cases of HCC are asymptomatic, with an insidious character, for this reason it is difficult to diagnose before reaching an advanced state;

- Patients who develop HCC don't usually present signs or symptoms with the exception of those correlated with the chronic hepatic disease they are suffering from;

- AFP is a low cost marker and represents an attractive option for screening being utilized for early diagnosis, monitoring and recurrence of HCC;

- The histological diagnosis of HCC is necessary pretty rarely nowadays because of the numerous non-invasive imaging alternatives;

- There still exist multiple unclarities regarding the mechanisms through which obesity and the metabolic syndrome can determine malignant transformation, although certain factors are taken into consideration which might play a key role in this transformation: insulin resistance, hyperinsulinemia, growth of cellular signaling dependent of tumor necrosis factor (TNF), alteration of cellular lipids, presence of NAFLD or NASH, and intestinal microbiom.

- Defining the etiology offers an important benefit in the prevention of the disease, but also in treating HCC;

- Biopsy of the suspect hepatic tumors is done only after the failure of some imaging methods based on contrast agents, mainly because the inconvenience of the technique and the high risk of complications and dissemination of tumor cells, without the exclusion of possibly false negative results;

- Metabolic deregulations are in a continuous growth, with considerable incidence especially when it comes to diabetes, NAFLD and morbid obesity;

- Dieting practices, combined with the provenance of food and drinks and a chance of lifestyle contributes to the spreading of the metabolic syndrome, defined by insulin resistance, diabetes, obesity and eventually acute renal injury due to NASH;

- From the performed study much information was obtained regarding co-factors, including cirrhosis, its complications, history of hepatitis, obesity, diabetes, NAFLD which could be correlated with the HCC diagnosis;

- The proven connections between HCC and diabetes contribute in a significant manner to the understanding of the initiations and progression mechanisms of the tumor processes in general, but especially of those involved in hepatocarcinogenesis;

- The risk of HCC is significant after a few years since the diagnosis of diabetes, this observation bringing arguments against the hypothesis that early manifestations of cirrhosis and the process of carcinogenesis are responsible for the clinical development of diabetes. There is a positive association between the history of diabetes and the appearance of HCC, and this study also indicates that this relation is independent of the contributions of other major risk factors involved in the etiology of HCC;

- This study results are able to offer quantitative support of the hypothesis that subjects with diabetes have a larger prevalence of developing primitive hepatic cancer;

- A link between lifestyle and the development of HCC has been noticed, especially in sedentary lifestyles and daily nutrition which are the main risk factors for obesity and diabetes;

- HCC can develop on a non-cirrhotic liver and in a patient who denies alcohol consumption and does not present chronic viral infections, the study confirming obesity and NAFLD as risk factors in patients with HCC;

- None of the patients developed colangiocarcinoma;

- Regarding the moment of presentation, most patients were already in an advanced stage, this could be attributed to a poor supervision of the population at risk. The monitoring and supervision of patients with obesity and NAFLD should be taken into consideration for a better optimization of the rate of survival in the future;

- In the cases put forward in this study the diagnosis in already advanced stages shows the necessity of implementing programs for screening to identify and monitor risk factors;

- Although the study did not take into consideration ulterior treatment received by the patients diagnosed with HCC, considering that almost half were diagnosed in inoperable stages, the prognosis and life expectancy were low;

- The necessity of epidemiology studies to identify the groups at high risk for NAFLD-HCC can lead to the supervision and improvement of treatment strategies. Adjacent investigation of the mechanisms and the means of HCC development on non-cirrhotic liver with NAFLD might offer important data for the creation of new diagnosis guides, treatment and supervision;

- The next challenge for doctors engaged in the treatment of HCC, will have disease prevention at its core, with the identification of risk factors so they can be controlled accordingly. Volumes of data from many centers at a global level is necessary for a centralization and an efficient correlation of risk factors regarding HCC developed on non-cirrhotic liver.