Hepatocellular carcinoma and metabolic diseases – histological perspectives from a series of 14 cases

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Abstract
Hepatocellular carcinoma (HCC) represents a major health burden, as curative methods only apply to a select small portion of the affected population. Screening programs are ineffective in the absence of established underlying conditions such as viral hepatitis or alcohol abuse resulting in liver cirrhosis. Thus, overweight or obese, diabetic patients as well as non-alcoholic fatty liver disease (NAFLD) cases are often overlooked as potential candidates for HCC development. Current diagnostic methods for HCC are restricted to non-invasive imaging tests; however, the need for accurate predictive or therapeutic markers make histological studies a necessity; the latest guidelines and recommendations demand an increased effort in obtaining pertinent data from immunohistochemical investigations. Our aim was to retrospectively evaluate a series of patients with common symptoms and manifestations of metabolic syndrome who underwent liver biopsy after imaging revealed suspicious liver masses. We describe the major findings of both common histological evaluation and microvessel density evaluated by positive CD34 immunostaining.

Keywords: hepatocellular carcinoma, metabolic syndrome, diabetes, non-alcoholic liver disease, liver biopsy.

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Introduction
Hepatocellular carcinoma (HCC) currently ranks high amongst primary malignancies affecting both genders. Males are more predisposed to developing HCC, with an estimated rate of approximately 2:1 cases [1–3]. With heterogeneous distribution rates in different regions and diverse risk factors, one principal predisposing condition remains universal – underlying chronic hepatic inflammation and subsequent fibrosis. Metabolic disorders are considered today a major health issue at a global scale, with alarmingly increasing incidences of diabetes mellitus (DM), non-alcoholic fatty liver disease (NAFLD) and morbid obesity. It is estimated that diabetic patients have a 2.5-fold increased risk to develop HCC, independent of either alcohol consumption or underlying viral liver disease exists [4]; other more recent data suggest slightly lower relative risk values (2.01, CI: 1.61–2.51) [5]. When combined with existing hepatitis, DM greatly increases the risk of developing HCC. However, NAFLD acts as an independent risk factor for HCC development, without the need for viral or ethanol injury of the liver parenchyma; mild or absent fibrosis can be encountered in some HCC cases [6]. Currently, a connection between metabolic conditions, liver diseases and HCC has been established through populational and genetic studies [7]. Therefore, an entirely new category of potential HCC patients emerges, whom needs to be studied and regularly screened for malignancy.

Aim
We present here a series of 14 cases of HCC patients proven by liver biopsies between 2000 and 2003, who also presented symptoms of metabolic disorders.

Patients and Methods
Patients’ selection
We retrospectively selected all cases with metabolic conditions, who also underwent ultrasound-guided liver biopsy for the diagnosis of liver tumors within the Department of Gastroenterology, University Hospital of Craiova, Romania, between 2000 and 2003, according to guidelines of that period. We have excluded patients with either chronic viral liver hepatitis, alcohol consumption or primary cholangitis. All patients provided signed agreement before the procedure and a standard consent form was provided, according to ethical procedures of that period. No further ethical clearance was required as the retrospective study did not include any personal information.

Patients were screened by ultrasound (US) and were known with liver deficiencies prior to inclusion; local diagnostic protocols required at that time histological
confirmation of malignancy, and therefore they underwent liver biopsy upon detection of a suspicious liver tumor. Patient charts contained demographic data and laboratory values, as well as extensive descriptions of the procedures and in-hospital follow-up.

Liver biopsy and subsequent pathology

Liver biopsy was performed following the standard US-guided technique. ‘Tru-Cut’ needle driven by a spring-loaded biopsy gun was used in all cases, under constant US imaging by placing the transducer adjacent to the insertion region. Adequate biopsy fragments between 2 and 4 mm diameters were obtained and macroscopic evaluation sustained their viability. Tissue was prepared and stored following usual protocols [8]. Both Hematoxylin–Eosin (HE) standard evaluation as well as CD34 immunostaining for vascular patterns was performed in all patients. We had access to resulting slides from all patients, thus allowing for pathology interpretation.

Results

We have included 14 patients in our study – eight men and six women, between 45 and 66 years of age (median age 59 years). All patients were obese, with a body mass index (BMI) between 30 and 35 kg/m² (median BMI 32 kg/m²) and a waist circumference between 98 and 131 cm (median 108.5 cm). Both alcohol consumption and viral liver disease were exclusion criteria for our study. Only one patient had a history of smoking. Clinical jaundice was present in three cases. Liver function was altered in all patients, according to laboratory tests. Demographic data of patients is presented in Table 1.

Ultrasound-guided liver biopsy was performed in all patients, following suspicious imaging on abdominal ultrasound (seven cases, five males), computed tomography (four cases, two males) and magnetic resonance (three cases, one male). No major complications were encountered, with reported headaches in six patients and localized pain in 10 cases. Tumor tissue was successfully harvested in all 14 cases.

Upon microscopic examination of HE-stained cross-sections, we found an altered cellular architecture, with few remaining chords of hepatocytes and tumor cells that resembled hepatocytes, without preserving many of the original features (Figures 1 and 2). Few Kupffer cells were present and the reticulin network was completely absent from all studied material. Small, deformed cells with an altered nucleus to cytoplasm ratio were often present and we found some residual trabeculae with pseudoglandular spaces (Figure 3). Four cases presented the pseudoglandular variant of HCC, with structures similar to acini lined by tumor cells resembling hepatocytes, derived from the dilatation of residual bile ducts (Figure 4). Within these structures, we could observe dense eosinophilic infiltrates and inflammatory debris, with few remaining bile fragments (Figures 5 and 6). Seven cases presented trabecular features; in these, we observed more differentiated trabeculae with several layers of cells and endothelium. Two of these cases were described as macrotrabecular, since we found more than eight layers of cells. The remaining four cases were advanced HCCs, with more necrosis present, abundant inflammatory infiltrate and cellular debris. Cytoarchitectonics was severely altered, with no remaining trabecular structures or organized epithelium, acini or bile ducts.

<table>
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<tr>
<th>Table 1 – Demographic data of the patient group</th>
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<tbody>
<tr>
<td>Parameter</td>
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<tr>
<td>Age (mean ± SD)</td>
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<td>Gender (n, M/F)</td>
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<tr>
<td>BMI (kg/m², mean ± SD)</td>
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<td>Waist circumference (cm, mean ± SD)</td>
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<td>Viral infection</td>
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<td>Alcohol abuse (n)</td>
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<td>Smoking (n)</td>
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<tr>
<td>Jaundice (n)</td>
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<td>GLI (mg/dL, mean ± SD)</td>
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<td>TG (mg/dL, mean ± SD)</td>
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<td>AST (UI/L, mean ± SD)</td>
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<td>ALT (UI/L, mean ± SD)</td>
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<td>GGT (UI/L, mean ± SD)</td>
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<td>ALK (UI/L, mean ± SD)</td>
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<td>BIL (mg/dL, mean ± SD)</td>
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SD: Standard deviation; n: No. of cases; M: Male; F: Female; BMI: Body mass index; IU/L: International Units per liter; GLI: Glycemia; TG: Triglycerides; AST: Aspartate transaminase; ALT: Alanine transaminase; GGT: Gamma-glutamyl transpeptidase; ALK: Alkaline phosphatase; BIL: Bilirubin.
Cellular features were compromised, with some similarities to hepatocytes still present. Vesicular nuclei were predominantly round; however, we found both pleomorphism and hyperchromatism in various degrees. Giant, pleomorphic cells were found in several cases. We could also observe multiple nuclei and altered shapes, sometimes agglutinating into compact masses. This aspect was present in all advanced HCC cases. We identified clear cytoplasm in cells from four of the pseudoglandular HCC and three from the trabecular type. This was probably due to abundant glycogen and fat deposits, also frequent in obese and NAFLD patients.

Immunohistochemical evaluation of microvessel density by CD34 marking showed extensive vascular proliferation (Figure 7). Neoangiogenesis was a common hallmark for all 14 cases we have evaluated. Small, contorted vascular structures of different calibers and shapes were present, with increased density in highly irregular cyt架构onics (Figures 8–10).
Discussion

We have retrospectively evaluated histological aspects of HCC cases with a history of NAFLD. Chronic viral or other causes of hepatitis, as well as those with alcohol consumption, were excluded from the study. All 14 patients evaluated underwent US-guided and liver biopsy was performed with 100% success rate. We have not encountered any serious adverse effects during or after the procedures.

The lack of adequate screening programs for populations at risk or tumor detection in advanced stages of the disease, when therapeutic resources are limited, places HCC in third place worldwide as mortality [9]. Some population groups are at higher risk to develop HCC, as chronic viral hepatitis and subsequent cirrhosis play an important role as etiological agents. Alcohol consumption was also associated with an increased cirrhosis and HCC risk, its synergistic effect with viral infection further strengthening the relationship [10–12].

However, another emerging pathology in the modern age is quickly becoming a concerning risk factor for HCC. Dietary habits, combined with the provenience of food and beverages and a change in lifestyle all contribute towards the rise of metabolic syndrome, defined by insulin resistance, diabetes mellitus, obesity and ultimately liver injury subsequent to non-alcoholic steatohepatitis (NASH)/NAFLD [13]. The mechanisms responsible for the association between metabolic disorders and HCC remain unclear; it has been stated that insulin-like growth factor-1 (IGF-1) overproduction, following hyperinsulinemia, may stimulate cellular proliferation while inhibiting apoptosis within liver parenchyma [14]. Both in vitro and in vivo (animal and epidemiological) studies support this hypothesis [15, 16].

Alpha-fetoprotein (AFP) is the main serological marker used in the screening and diagnosis process of HCC, the only accepted for use in clinical practice. At a threshold value of 20 ng/mL, the sensitivity is only 60%, thus allowing the unnoticed passage of 40% of existing HCCs. For a threshold of 200 ng/mL, sensitivity decreased to 22%, while the sharp decrease of the threshold value leads to a consecutive growth rate of false positives [17]. The inefficiency of AFP as a screening marker has been demonstrated in multiple studies to date. Even when used as a diagnostic marker, the value of the sensitivity is only 66%, with a specificity of 82% [18].

Currently, biopsy of suspicious liver tumors is used only after the unsuccessful use of methods based on imaging contrast agents, mainly due to the inconvenience of the technique and the high risk of complications or dissemination of tumor cells, not excluding the possibility of false negative results (approximately 20% according to some studies) [19–23]. This results in the technique being used in only selected centers and to a small number of potential candidates. However, the latest guidelines recommend the use of liver biopsy in clinical studies designed for identifying novel markers, which would allow for the discovery of novel chemotherapeutic agents [2, 3, 7]. As only one such drug is currently used for systemic or targeted chemotherapy, in advanced selected cases, the need for additional potential targets for therapy is becoming imperative.

Selecting at risk population for additional screening may yield interesting results in future randomized cohort studies. In our study, we have selected a series of cases linked by the existence of a common pathology – diabetes and/or NAFLD – in the absence of common risk factors for HCC, such as viral infection or alcohol consumption. Moreover, cirrhosis-complicating NAFLD is not necessary for HCC to appear; thus, a novel at-risk population emerges from this association – obese, often-diabetic patients with no history of viral hepatitis or regular alcohol ingestion [6]. Finding novel markers for an accurate early detection and description of HCC in such patients is imperative and may be a requisite in future studies. A recent meta-analysis showed a drastic increase for HCC risk of up to 189% for obese and 117% in overweight subjects [24]. Treating insulin resistance and the metabolic pathways associated with these alterations seem a promising path for increased survival of HCC patients; one study using Metformin combined with radiofrequency ablation revealed lower mortality in diabetic patients [25]. It should be however noted that all patients had early HCC, as local ablative techniques are reserved for these categories.
Conclusions

Obesity, diabetes and NAFLD are emerging as important risk factors for HCC; neglected so far as both viral hepatitis and chronic alcohol ingestion were considered prime factors for cirrhosis and subsequent HCC, shifting dietary habits drastically increased their importance in recent years. Selecting this new at-risk population is crucial for an efficient anti-cancer worldwide policy. Novel markers may arise from future histological studies of HCC, with importance both in diagnosis and especially in more targeted and effective chemotherapeutic agents.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

All the authors contributed equally to preparing this paper and share first authorship.

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