Original Paper

Hepatocellular Carcinoma in Diabetic Patients - a Single Center Experience

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ABSTRACT: Introduction: Hepatocellular carcinoma (HCC) represents a major health concern worldwide; identifying risk factors and associated conditions that may lead to its development are therefore of utmost importance to the medical community. An association between diabetes mellitus (DM) and development of HCC on underlying liver conditions has been previously suggested. The aim of our study is to reveal possible significant statistical correlations between the two entities, which might warrant further study of the pathophysiological mechanisms behind this association. Patients and methods: We have prospectively included consecutive cirrhotic patients admitted to our medical clinic over a period of four years, between 2010 and 2014. Diagnostic was established using the EASL criteria. We have documented history of hyperglycemia and any changes of serum values in these patients, evaluating DM patients within the LC lot. From these patients we have selected all patients with imaging suggestive for HCC and established positive diagnosis on the criteria established in the latest EASL guidelines. We have used statistical tests to identify possible correlations between these pathologies. Results: We have identified 2718 consecutive patients with LC and successfully included 2556. Of these, 164 also had HCC. A total of 371 patients had DM – 54 also had HCC while 317 remained cancer-free through our study period. We found positive correlations between the presence of DM and HCC. Subgroup analysis of the HCC cohort revealed a positive association between DM and liver cirrhosis and chronic hepatitis. We did not find positive relationships between DM and overall liver conditions, splenomegaly, hepatomegaly, other significant symptoms, substance abuse and main serum values. Conclusions: We have found several significant correlations between DM and underlying liver conditions in a HCC cohort. Our study, however, did not reveal other significant associations regarding these diseases. Further studies are required to determine the precise role this disease plays in the development and severity of liver diseases.

KEYWORDS: hepatocellular carcinoma, risk factors, diabetes, cirrhosis, chronic viral hepatitis

Introduction

Hepatocellular carcinoma (HCC) remains a major health problem, representing the 5th leading cause of malignancy worldwide [1–3], even though it is one of the few cancers whose etiology has been at least partially deciphered [4]. HCC predominantly appears in patients with cirrhosis of the liver, chronic viral infection or alcoholic liver disease [1–3]. Type II diabetes, obesity, smoking, alcohol ingestion, intake of aflatoxin are some of the recognized risk factors in the development of HCC. Antitrpsin α1 deficiency disease and Wilsson's hemochromatosis are rare causes of HCC development.

However, there is a large percentage of patients with HCC in whom no risk factors are identified [1,2]. Knowing the etiology is easier to prevent and treat HCC with a poor prognosis. In several cohort studies in the literature showed that diabetes increases the risk of development of 2 to 4 times the HCC [5–8]. The liver is an important step in glucose metabolism, so more chronic disease can cause changes such as increasing insulin resistance and reduced insulin secretion [7]. DM has a synergistic action with other risk factors [7,8].

This study wants to show that diabetes is an important risk factor in the development of HCC in patients with cirrhosis.

Patients and methods

In this study, we have prospectively included consecutive patients admitted to the Department of Gastroenterology and Internal Medicine of the University Emergency Hospital of Craiova between January 1st 2010 and June 30th 2014 with a suspected diagnosis of liver cirrhosis (LC). All patients provided informed consent in written form, following the protocol approved by the local Ethics Committee. All study procedures were conducted in accordance with the Declaration of Helsinki; patients were not subjected to any maneuver outside normal diagnostic protocols.

The diagnosis of LC was reached in accordance to the EASL guidelines, which require at least two criteria to be fulfilled simultaneously: 1. the presence endoscopic gastroesophageal varices, 2. the appearance of regeneration nodules on ultrasonography (US) (irregular surface of the liver) or 3. Imaging evidence of splenomegaly (on US or computer tomography).

Diabetes mellitus (DM) was diagnosed based on the presence of hyperglycemia (>8 mmol/L) in at least 2 samples, or active treatment with insulin, oral hypoglycemic agents, or both. No consideration was given to minor alterations in glucose metabolism, such as impaired glucose tolerance based on an oral glucose tolerance test.

A diagnosis of HCC was reached through a combination of cross-sectional contrast-enhanced imaging methods (computed tomography and magnetic resonance), with liver biopsy in uncertain cases and a follow-up of at least six months. Alpha-fetoprotein (AFP) values above 400 ng / mL were also considered as relevant, without excluding HCC in patients with normal AFP and suggestive imaging findings.

Statistical analysis was performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA), together with the XLSTAT add-on for MS Excel (Addinsoft SARL, Paris, France) and IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY, USA) for processing the data.

To show the connections (associations or influences) between two categorical factors we used Chi square test ($\chi2$) for independence, as well as Odds Ratio (OR) and Relative Risk (RR), with 95% CI. Also, we used Student's t test to compare the mean values (± SD) of numerical parameters.

Results

A total of 2718 patients were diagnosed with LC; 71 refused to provide informed consent and 91 were lost during follow-up. In total, 164 cases of HCC were confirmed during the study period. At the time of diagnosis of HCC, DM was observed in 54 patients. We found DM in 317 patients without HCC.

Table 1. Distribution of the study lot.

DM+HCC	HCC+	HCC-	Total
DM+	54	317	371
DM-	110	2237	2347
Total	164	2554	2718
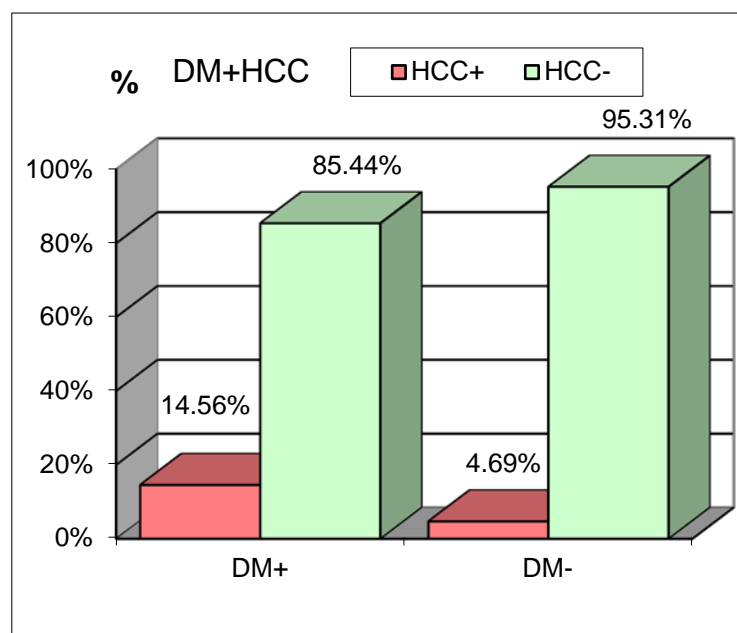
Fig.1. Distribution of DM within the cirrhosis study lot, as per the concomitant presence of HCC. We can observe in the table the significant odds ratio and relative risk values for this association.

Analyzing all the patients with cirrhosis, we found a strong correlation between the occurrences of HCC and DM – Chi square's p-value was <0.001, meaning there is a highly significant relationship. Furthermore, we computed the relative risk (RR) for developing HCC if DM is associated with cirrhosis, which is 3.106 times higher if DM is present (95% CI= 2.197-4.391) (Fig.1 and Table 1).

In the next statistical analysis, we used only patients with HCC, to quantify the relationship between HCC, DM and different other liver pathologies.

Analyzing, for patients with HCC, the relationship between DM and all occurrences of liver disease, we found no statistical proof for such an assumption – Chi square p was 0.219>0.05, while the odds ratio (OR) and relative risk (RR) were close to 1, both with 95% confidence intervals containing the value 1, which shows there is no correlation (Fig.2 and Table 2).

Table 2. Distribution of DM within the HCC study lot, according to the existence of an underlying liver condition.

Liver disease	Yes	No	Total
DM+	44	10	54
DM-	80	30	110
Total	124	40	164
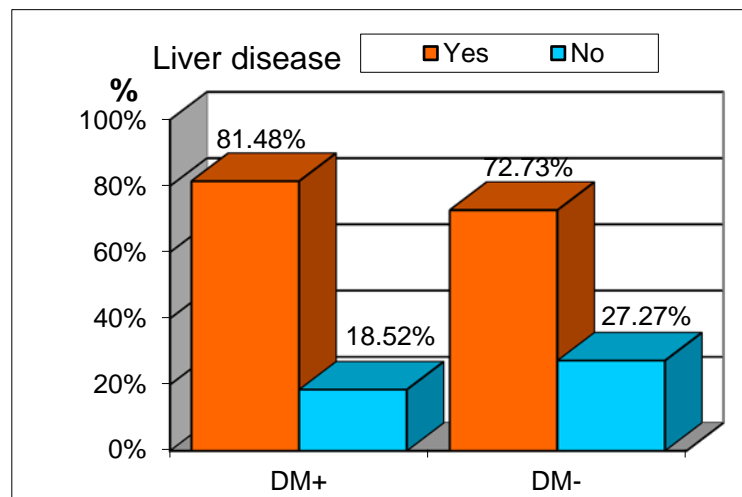
Fig.2. Comparative view of underlying liver disease distribution within the subgroup of patients also having HCC, with and without DM; we could not establish a correlation between DM and liver conditions, when HCC was diagnosed.
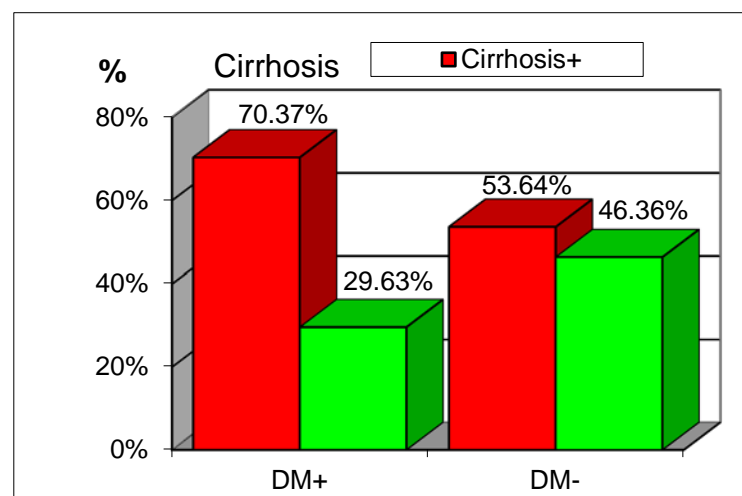
Fig.3. Comparative view of underlying liver cirrhosis distribution within the subgroup of HCC patients, with and without DM; we could observe significant OR but not significant RR, thus establishing a significant correlation between DM and LC.

Table 3. Distribution of DM within the HCC study lot, according to pre-existing liver cirrhosis.

| Cirrhosis	Cirrhosis+	Cirrhosis-	Total
DM+	38	16	54
DM-	59	51	110
Total	97	67	164

There is a statistically significant correlation between DM and cirrhosis (Fig.3 and Table 3), as proved by the value we found using Chi square test, p=0.040 < 0.05. Also, the value for odds ratio, 2.053 was statistically significant

(95% CI=1.026 - 4.109, it doesn't contain the value 1), furthermore stressing there is an association between DM and cirrhosis that contributes to developing HCC.

Analyzing, for patients with HCC, the relationship between DM and medical history of hepatitis without cirrhosis, we found no statistical proof for such an assumption – Chi square p was 0.397>0.05, while the odds ratio (OR) and relative risk (RR) were close to 1, both with 95% confidence intervals containing the value 1, which shows there is no correlation (Fig.4 and Table 4).
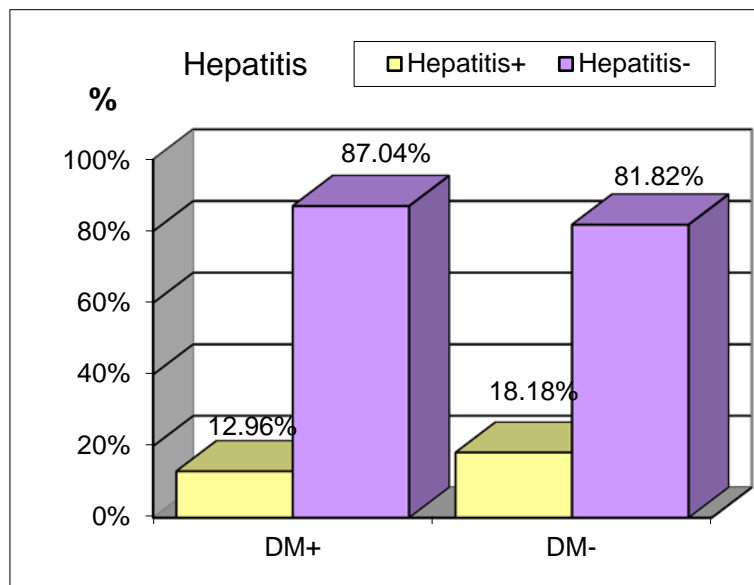
Fig.4. Comparative view of underlying chronic hepatitis within the subgroup of HCC patients, with and without DM; we could not identify any possible relationship between DM and chronic liver inflammation.
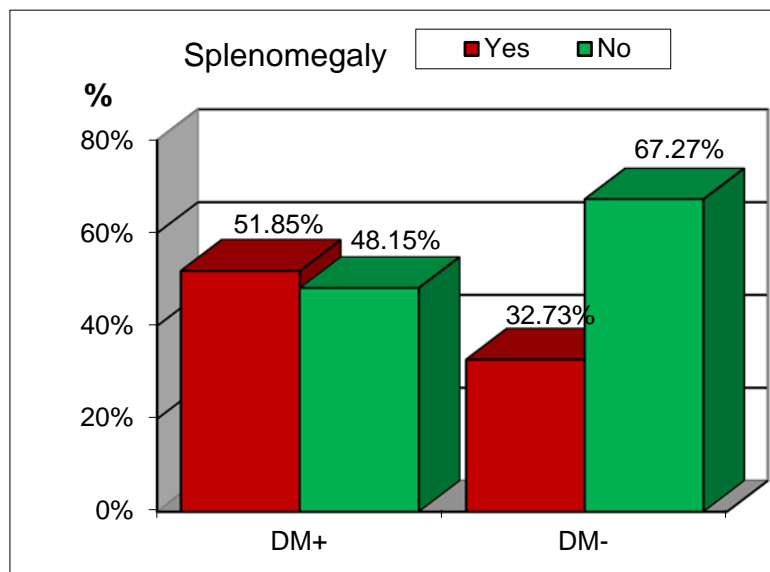
Fig.5. Comparative view of underlying chronic hepatitis within the subgroup of HCC patients, with and without DM; we could not identify any possible relationship between DM and chronic liver inflammation.

Table 4. Distribution of DM within the HCC study lot, according to the existence of hepatitis.

Hepatitis	Hepatitis+	Hepatitis-	Total
DM+	7	47	54
DM-	20	90	110
Total	27	137	164

Table 5. Splenomegaly and DM within the HCC group.

Splenomegaly	Yes	No	Total
DM+	28	26	54
DM-	36	74	110
Total	64	100	164

Patients with HCC and DM had splenomegaly in a significantly higher degree than patients with HCC without DM – Chi square p=0.018<0.05, OR=2.214 (95% CI=1.137-4.309). Results are presented in Fig. 6 and Table 6.

Table 6. Hepatomegaly within the HCC subgroup, divided by the presence or absence of DM.

Hepatomegaly	Yes	No	Total
DM+	39	15	54
DM-	70	40	110
Total	109	55	164
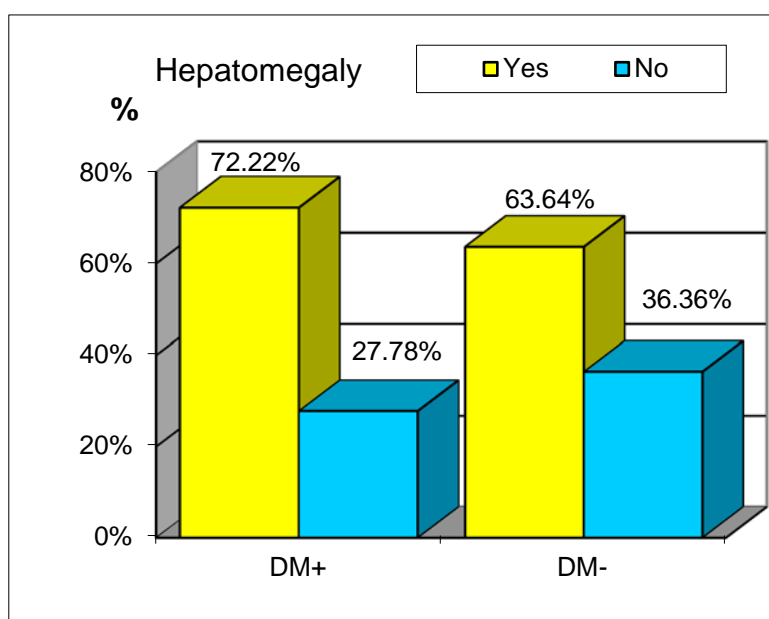
Fig.6. Comparative view of DM and hepatomegaly within the HCC subgroup. No significant relationship between DM and increased hepatic volume was found.
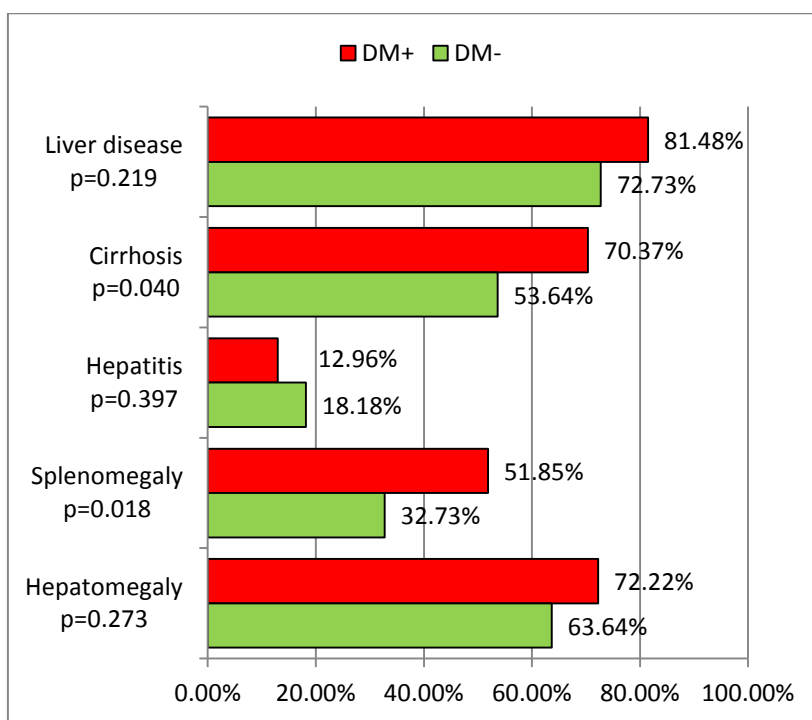
There was no significant difference between patients with HCC and DM and patients with HCC without DM in regards to hepatomegaly (Fig.6 and Table 6); Chi square p=0.273>0.05, OR=1.486 (95% CI=0.730 - 3.025).

An overview of the above results can be found in Table 7 and Fig. 7.

Table 7. Comparative view of Chi square, OR and RR of the association of DM and various other co-existing liver conditions, within the HCC lot.

HCC	Liver disease	Cirrhosis	Hepatitis	Splenomegaly	Hepatomegaly
DM+	44 (81.48%)	38 (70.37%)	7 (12.96%)	28 (51.85%)	39 (72.22%)
DM-	80 (72.73%)	59 (53.64%)	20 (18.18%)	36 (32.73%)	70 (63.64%)
Chi square	p=0.219	**p=0.040**	p=0.397	**p=0.018**	p=0.273
OR (95% CI)	1.650 (0.738 - 3.689)	**2.053 (1.026 - 4.109)**	0.670 (0.264 - 1.699)	**2.214 (1.137 - 4.309)**	1.486 (0.730 - 3.025)
RR (95% CI)	1.120 (0.501 - 2.505)	1.312 (0.655- 2.626)	0.713 (0.281 - 1.807)	1.584 (0.814 - 3.084)	1.135 (0.557 - 2.311)

Fig.7. Summarization of the presence of DM and various other co-existing liver conditions within the HCC lot.

We did not identify any statistically significant differences between patients with and without Diabetes Mellitus in respect to substances abuse (Table 8).

Table 8. Summarization of alcohol and smoking as risk factors for HCC, in accordance with DM. We did not find an increased risk for patients having DM.

Alcohol	No	Occasionally	Yes
DM- (110 cases)	42.73%	22.73%	34.55%
DM+ (54 cases)	42.59%	35.19%	22.22%
p Chi square	0.142	>0.05	NS
Smoking	Non-smoker	Smoker	Ex-smoker
DM- (110 cases)	70.91%	7.27%	21.82%
DM+ (54 cases)	64.81%	12.96%	22.22%
p Chi square	0.477	>0.05	NS

Regarding risk factors such as obesity, dyslipidemia and hypertension, the two groups of patients, with and without DM, are similar. Performing the Chi square test we obtained only p-values higher than 0.05, above the accepted maximum for statistically significant differences (Table 9).

Table 9. Obesity, dyslipidemia and hypertension correlated with presence of DM within the HCC subgroup.

Parameter	DM+ (54 cases)	DM- (110 cases)	p Chi square	Significance
Obesity	22.22%	17.27%	0.122	>0.05 - NS
Dyslipidemia	40.74%	34.55%	0.190	>0.05 - NS
Hypertension	14.81%	8.18%	0.439	>0.05 - NS

Comparing the two studied groups, by mean of the Chi square test, we showed there are no differences regarding subjective symptoms and clinical signs between them, all the results being higher than 0.05 (Table 10).

Table 10. The presence of the most important symptoms within our HCC cohort did not reveal significant correlations with the existence of DM.

Parameter	DM+ (54 cases)	DM- (110 cases)	p Chi square	Significance
Abdominal pain	44.44%	45.45%	0.903	>0.05 - NS
Jaundice	12.96%	11.82%	0.833	>0.05 - NS
Asthenia	72.22%	69.09%	0.681	>0.05 - NS
Loss of appetite	18.52%	27.27%	0.220	>0.05 - NS
Weight loss	20.37%	21.82%	0.832	>0.05 - NS
Ascites	29.63%	22.73%	0.337	>0.05 - NS
Encephalopathy	3.70%	4.55%	0.802	>0.05 - NS
UGI hemorrhage	5.56%	7.27%	0.680	>0.05 - NS

Comparing the numerical parameters investigated for the two groups, with and without DM, we found no statistically significant differences – all the p-values computed through Student's t-test were higher than 0.05 (Table 11).

Table 11. Comparative view of serum values within the HCC cohort, divided by the presence of DM. We could observe no positive relationship. ESR 1h/2h – erythrocyte sedimentation rate; AST – Aspartate transaminase; ALT – Alanine transaminase; TB – total bilirubin; ALK – alkaline phosphatase

Parameter	DM-	DM+	p value – Student's test
Hemoglobin	11.51 ± 2.24	11.35 ± 2.37	0.688 >0.05 - NS
Leukocyte count	7014.10 ± 3436.65	6208.66 ± 2888.59	0.136 >0.05 - NS S
Platelet count	93396.79 ± 93933.04	88146.73 ± 103229.12	0.763 >0.05 - NS
ESR 1h	49.36 ± 30.11	48.53 ± 32.48	0.876 >0.05 - NS
ESR 2h	71.60 ± 31.89	67.65 ± 36.74	0.516 >0.05 - NS
AST	103.90 ± 73.54	95.60 ± 83.43	0.574 >0.05 - NS
ALT	69.13 ± 60.63	79.89 ± 75.52	0.403 >0.05 - NS
TB	2.87 ± 4.56	2.71 ± 3.46	0.816 >0.05 - NS
ALK	7.15 ± 0.75	6.80 ± 0.68	0.158 >0.05 - NS
Urea	51.54 ± 36.85	55.45 ± 40.86	0.577 >0.05 - NS
Creatinine	0.98 ± 0.47	0.97 ± 0.55	0.868 >0.05 - NS

Discussion

Case-control studies up to date confirms that patients with diabetes have an increased risk of developing hepatocellular carcinoma. The association between diabetes and HCC was similar in men and women and in different age groups. The association was also present with early onset in patients with insulin-dependent diabetes, observation that can do to understand basic biological mechanism of developing neoplasia [7-10].

From our study we obtained several information to the co-factors including obesity, previous history of hepatitis and cirrhosis [11,12]. Although patients with cirrhosis develop glucose intolerance, insulin resistance and in an amount diabetes [12-14], these variables were unable to explain the risk of liver cancer in patients with diabetes mellitus. Moreover, none of the factors did not show significant effects and the association with diabetes was also observed in subjects without a history of cirrhosis or hepatitis. The combination of the two observed condition favors information on the occurrence of liver cancer is more likely in patients with a history of diabetes. However, a major long-term condition such as diabetes is unlikely to cause HCC in patients without a history of cirrhosis or hepatitis. With reference to possible biological mechanisms, patients with diabetes, non-insulin-dependent diabetes have insulin resistance and compensatory hyperinsulinemia, high levels of

insulin-like growth factor I (IGF-I) [9,10], that can stimulate liver cell proliferation. However, plasma glucose levels did not influence mortality from liver cancer [14], and the observation that individuals who are likely to have insulin-necessitating diabetes, increased risk of liver cancer which suggests that other mechanisms would may be relevant.

Changes occurred in the liver as fatty degeneration and cirrhosis in subjects with diabetes, which stimulates cell proliferation, may encourage the development of liver carcinogenesis [9]. Another suggested link between hepatitis C and diabetes is recognized as a risk factor for liver cancer [9-12]. The risk of liver cancer was considered after a few years from the diagnosis of diabetes, this observation being against the hypothesis that early manifestations of cirrhosis and carcinogenesis process are responsible for the clinical development of diabetes [11]. There is a positive association between history of diabetes and HCC occurrence and our study also indicates that this relationship is not confused with any of the major risk factors involved in the etiology of HCC [7,10]. A number of possible mechanisms could explain this association. Since a large number of patients are diabetic non-insulin-requiring, this being characterized by hyperinsulinemia, insulin or its precursors may interact with liver cells to stimulate mitogenesis and carcinogenesis [14]. Pathophysiological process may increase insulin resistance in susceptible persons, such as patients with liver disease with an increased prevalence of glucose intolerance [7,8]. It is possible that the mutations occurred in the hepatocytes in the presence of risk factors may lead to diabetes and impaired pancreatic beta-cell function. This hypothesis is supported by our observation that liver damage is higher among HCC in subjects with diabetes than among those without a history of this disease [8,9]. In conclusion, our findings in this study may provide quantitative support to the hypothesis that subjects with diabetes may have an increased prevalence of developing primary liver cancer. This association is also well established regarding epidemiology [11-14].

Conclusions

Because, except the statistically significant relationships between DM and cirrhosis in patients with HCC, we did not find any other differences in patients with and without DM for parameters assessing liver function, liver-related symptoms and clinical signs, or risk factors, we can conclude that only the metabolic changes given by the aforementioned association are responsible for a higher rate of HCC in patients with both cirrhosis and DM.

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