World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v3.i1.24

World J Hepatol 2011 January 27; 3(1): 24-30
ISSN 1948-5182 (online)

BRIEF ARTICLE

First multicenter study for risk factors for hepatocellular carcinoma development in North Africa

Olfa Bahri, Sayeh Ezzikouri, Nissaf Ben Alaya-Bouafif, Fella Iguer, Abdallah Essaid El Feydi, Hafedh Mestiri, Moustapha Benazzouz, Tahar Khalfallah, Rajaa Afifi, Latifa Elkihal, Salah Berkane, Agnes Marchio, Nabil Debzi, Anne Dejean, Pascal Pineau, Hinda Triki, Soumaya Benjelloun

Olfa Bahri, Hinda Triki, Laboratory of Clinical Virology, Institut Pasteur of Tunis, Tunis, BP 1002, Tunisia
Sayeh Ezzikouri, Soumaya Benjelloun, Laboratory of Viral Hepatitis, Institut Pasteur of Casablanca, Casablanca 20100, Morocco
Nissaf Ben Alaya-Bouafif, Laboratory of Epidemiology, Institut Pasteur of Tunis, BP 1002, Tunis, Tunisia
Fella Iguer, Laboratory of Viral Hepatitis, Institut Pasteur of Algiers, El Hamma, Algiers 16108, Algeria
Abdallah Essaid El Feydi, Moustapha Benazzouz, Rajaa Afifi, Latifa Elkihal, Department of Medicine C, Ibn-Sina University Hospital, Rabat 10000, Morocco
Hafedh Mestiri, Tahar Khalfallah, Department of General Surgery, Mongi Slim Hospital, La Marsa 2070, Tunisia
Salah Berkane, Department of Gastroenterology Bologhine University Hospital, Bologhine 16090, Algiers, Algeria
Nabil Debzi, Department of Gastroenterology, Mustapha University Hospital, Algiers 16000, Algeria
Agnes Marchio, Anne Dejean, Pascal Pineau, Nuclear Organization and Oncogenesis Unit, INSERM U993, Institut Pasteur of Paris, Paris 75015, France
Author contributions: Bahri O and Benjelloun S contributed equally to this work; they designed the study and provided financial support; with Pineau P they performed the majority of experiments with Ezzikouri S; Alaya-Bouafif NB performed statistical analysis; Iguer F, El Feydi AE, Mestiri H, Benazzouz M, Khalfallah T, Afifi R, Elkihal L, Berkane S and Debzi N provided the collection of all the human material; Bahri O wrote the manuscript; and Benjelloun S, Pineau P, Marchio A, Dejean A and Triki H were involved in editing the manuscript.
Supported by the "Direction des Programmes Transversaux de Recherches of the Institut Pasteur of Paris (PTR 130)" and the "Tunisian Secretariat for Scientific Research and Technology SERST for Tunisian part of the study (LR: Hépatites et maladies virales épidémiques)"
Correspondence to: Olfa Bahri, MD, Associate Professor, Laboratory of Clinical Virology, Institut Pasteur of Tunis, 13, Place Pasteur, Le Belvédère, BP 74, Tunis 1002, Tunisia. olfa.bahri@pasteur.rns.tn
Telephone: +216-71-843755 Fax: +216-71-791833
Received: October 11, 2010 Revised: December 8, 2010
Accepted: December 15, 2010
Published online: January 27, 2011

Abstract

AIM: To assess the role of the major risk factors for hepatocellular carcinoma (HCC) development in the western part of North Africa.

METHODS: A multicenter case control study was conducted in Tunisia, Morocco and Algeria in collaboration with Pasteur Institutes in these countries. A total of 164 patients with HCC and 250 control subjects without hepatic diseases were included. Prevalences of HBsAg, anti-hepatitis C virus (HCV) and diabetes were assessed. HCV and HBV genotyping were performed for anti-HCV and HBsAg positive patients.

RESULTS: The mean age of patients was 62 ± 10 years old for a 1.5 M:F sex ratio. Sixty percent of HCC patients were positive for anti-HCV and 17.9% for HBsAg. Diabetes was detected in 18% of cases. Odd ratio (OR) and 95% confidence intervals (CI) were 32.0 (15.8 - 65.0), 7.2 (3.2 - 16.1) and 8.0 (3.1 - 20.0) for anti-HCV, HBsAg and diabetes respectively. Multivariate analysis indicated that the three studied factors were independent. 1b HCV genotype and D HBV genotype were predominant in HCC patients. HCV was the only risk factor significantly associated with an excess of cirrhosis (90% *vs* 68% for all other risk factors collectively, $P = 0.00168$). Excessive alcohol consumption was reliably established for 19 (17.6%) cases among the 108 HCC patients for whom data is available.

CONCLUSION: HCV and HBV infections and diabetes are the main determinants of HCC development in North Africa. An active surveillance and secondary prevention programs for patients with chronic hepatitis and nutrition-associated metabolic liver diseases are the most important steps to reduce the risk of HCC in the region.

Key words: Hepatocellular carcinoma; Hepatitis B virus; Hepatitis C virus; Non-insulin-dependent diabetes mellitus; North Africa

Peer reviewers: Byoung-Joon Song, PhD, Senior Investigator, Laboratory of Membrane Biochemistry and Biophysics, National Institute on Alcohol Abuse and Alcoholism, NIH, 5625 Fishers Lane, Room 2S-30, Bethesda, MD 20892, United States; Volker Fendrich, MD, Department of Surgery, Philipps-University, Marburg 35039, Germany; Chin-Hsiao Tseng, MD, PhD, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, China

Bahri O, Ezzikouri S, Alaya-Bouafif NB, Iguer F, El Feydi AE, Mestiri H, Benazzouz M, Khalfallah T, Afifi R, Elkihal L, Berkane S, Marchio A, Debzi N, Dejean A, Pineau P, Triki H, Benjelloun S. First multicenter study for risk factors for hepatocellular carcinoma development in North Africa. *World J Hepatol* 2011; 3(1): 24-30 Available from: URL: http://www.wjgnet.com/1948-5182/full/v3/i1/24.htm DOI: http://dx.doi.org/10.4254/wjh.v3.i1.24

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the 130 major causes of morbidity and mortality in the world[1]. It represents the third leading cause of cancer death in males and the fourth in females with more than 600 000 deaths per year[2]. Geographical distribution of HCC varies throughout the world with an incidence ranging from 2.1 in Central America to 35.5 in Eastern Asia[3]. Globally, three epidemiological zones have been defined according to the age-adjusted HCC incidence per 100 000 habitants per year: low (with less than 5), intermediate (between 5 and 15) and high (higher than 15)[4]. There is a geographic correlation between the incidence of HCC and the prevalence of chronic hepatitis B and C, suggesting that these two viral infections are the most important risk factors of HCC worldwide[5]. In countries where hepatitis C virus (HCV) infection is endemic such as Japan and Egypt, high prevalence of HCV infection is reported among people with HCC. On the other hand, hepatitis B virus (HBV) infection is the major risk of HCC in regions with large populations like China and southern Asia because of the high endemicity of this virus there[1].

In addition to the viral infections largely implicated in HCC development, other factors associated with HCC are well documented. They include toxins and drugs (e.g. alcohol consumption, aflatoxins and anabolic steroid use), cigarette smoking, metabolic liver diseases (e.g. hereditary haemochromatosis, alpha1-antitrypsin deficiency) and steatosis[6,7]. Some of these factors have a direct carcinogenic role while others interact by promoting fibrosis and cirrhosis[8]. The significant association found recently between non-insulin-dependent diabetes (NIDD or type Ⅱ diabetes) and HCC suggests that diabetes is a potential risk factor for HCC development[6,9].

In the western part of North Africa (also called Maghreb), HCC incidence is lower than in sub-Saharan Africa and southern Europe with approximately 1 to 2 cases per 100 000 habitants per year (Globocan 2002, http://www-dep.iarc.fr/). HCC represents 5.9% of the total tumor burden in Morocco and is responsible for 1.1% of cancer deaths in Tunisia[10]. This region belongs, culturally, to the broader Arabo-Muslim world which is different from Europe, Sub-Saharan Africa or Eastern Asia for the characteristics of HCC risk factors. It is characterized by a low consumption of alcoholic drinks, an intermediate endemicity for chronic hepatitis B, low rate of HCV carriage (with Egypt as a major exception) and a recent rise of obesity and NIDD incidences due to lifespan expansion and nutritional transition in populations[11-17]. To our knowledge, the only recent study on HCC in the Maghreb region is a genetic one which demonstrated a lower rate of p53 mutation in comparison with Egypt and Sub-Saharan Africa. No report has yet detailed the respective implications of viral infections or other risk factors such us NIDD in HCC genesis in countries from North Africa. Differently from other parts of the Arabo-Muslim world such as Iran, Egypt and Saudi Arabia, HCC in the Maghreb region has not been described since the 1980s[10-18]. We therefore conducted a multicenter case control study on risk factors for HCC in Tunisia, Morocco and Algeria with the collaboration of Pasteur Institutes in these countries. The most important factors examined were diabetes and HBV or HCV infection.

MATERIALS AND METHODS

Study design and sample size justification

We conducted a case-control study matched by age (± 5 years) and gender in Tunisia, Morocco and Algeria from January 2002 to January 2005. The same protocol was used for recruitment of patients in the three countries. Investigations were approved by the Ethics Committee of the Faculty of Medicine of Casablanca as well as the Algerian and Tunisian Ministries of Health. Informed consent was obtained from each patient. During the study period, 164 cases were recruited from the three countries and we attempted to recruit two matched controls. With this sample size, we determined we would have 90% power, assuming a 7.0% of prevalence of HBV in the general population[11] and an OR of 3 with a 5% significance level. Assuming that the matching was effective, we expected the power to be higher. This level of power would allow for some failure in the recruitment of the targeted number of controls. Power calculations were performed with EPI INFO (version 6.04).

Diagnostic criteria

Diagnosis of HCC was based on imaging showing the characteristic features of HCC and/or, when possible, histological assessment of tissues samples and serum alpha-fetoprotein levels. The presence or not of cirrhosis in the non-tumor liver was considered. A diagnosis of cirrhosis was based on morphological and clinical criteria, ultrasound or computed tomography. For patients with diabetes mellitus, data on onset of the disease, the evolution of

Table 1 Distribution of hepatocellular carcinoma cases and control according to studied risk factors

Risk factor	HCC cases (n = 164)	Controls (n = 250)	Odds ratio
NIDD	18.00% (n = 139)	2.70% (n = 225)	8.0 (3.1 - 20.0)
HBV status			
HBsAg +/anti-HBc +	17.90%	4.00%	7.2 (3.2 - 16.1)
Anti-HBc + alone	15.40%	11.50%	2.1 (1.1 - 4.0)
Anti-HBc +/anti-HBs +	23.50%	14.60%	2.5 (1.5 - 4.4)
Anti-HCV			
Anti-HCV +	60.00%	4.40%	32.0 (15.8 - 65.0)

HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HBV: hepatitis B virus; NIDD: non-insulin-dependent diabetes.

glycemia rates and the type of treatments received were recorded. History of dysmetabolic syndrome or excessive alcohol consumption was also collected from the medical records of each patient. Control subjects were patients admitted during the same period with no hepatic diseases.

Serology and molecular tests

The presence of HBsAg and other serological markers (antibodies to HBs and HBc) was assessed in all patients and controls using commercial kits for enzyme-linked immunosorbent a (ELISA). Antibodies to HCV were assessed using a 4[th] generation ELISA test. Subjects positive for anti-HCV were tested for the presence of HCV RNA by PCR-hybridization (HCV 2.0 Roche); HCV RNA was then genotyped by a commercial kit (Inno-Lipa, Innogenetics) or by partial sequencing in the 5' non-codant region. For HBV infected patients, virus genotyping was performed by PCR - RFLP in the pre-S region as previously described (Bahri *et al* 2006, Ezzikouri *et al* 2008). Ongoing HBV infection was defined on the basis of the presence of both HBsAg and anti-HBc in serum. HCV infection was retained for all patients positive for anti-HCV.

Statistical analysis

Data entry was checked for consistency and accuracy using Epi-data, version 3.0 and analysis was conducted with the Statistical Package for the Social Science (SPSS, version 13.0). The prevalence of HBsAg, anti-HCV and diabetes mellitus was estimated with corresponding 95% confidence intervals (95% CIs). Matched odds ratios (OR) and their 95% were calculated by univariate logistic regression analysis. For each risk factor, those with $P < 0.2$ were considered as statistically significant for further evaluation in the multivariate analysis. The model also included age and gender; participants were matched by these factors. To identify variables independently associated with HCC development, conditional logistic regression analysis (backward logistic regression method) was conducted. The adjusted ORs (AORs) and their 95% CIs based on the final model were used to interpret the results. The statistical significance of the associations was based on a P value of $\leqslant 0.05$.

RESULTS

Demographic characteristics

A total of 164 HCC patients and 250 controls were included in the study; 252 were men (100 HCC patients and 152 controls) and 162 women (64 HCC patients and 98 controls). The sex ratio M/F was 1.53 for HCC cases. The mean age was 62 ± 10.43 years among patients and 58 ± 10.60 among controls. However, it should be noted that the four year difference between mean age of patients and controls may marginally affect the extent of calculated odds ratio.

Prevalence of NIDD and viral markers in studied population

The prevalence of NIDD, HBV markers and anti-HCV in the population studied is shown in Table 1. History of diabetes could be assessed in 139 HCC and 225 control cases. This particular risk factor was present in 27 patients (18%), a rate significantly higher than in controls (2.7%) [odds ratio of 8.0, 95% CI (3.1-20.0)]. Of the 164 HCC patients, 127 were positive for HBsAg and/or anti-HCV (77.7%). Twenty-nine (17.9%) were HBsAg-positive *vs* 9 (4%) of controls ($P < 0.001$). Anti-HBc positivity was observed more frequently among HCC patients than among controls with respectively 56.9% and 30.1% of prevalence ($P < 0.001$). Resolutive HBV infection as defined by the presence of both antibodies to HBs and HBc was found in 32.5% of HCC patients and 14.6% of controls. Ninety-eight cases (60%) and 10 controls (4.4%) were positive ($P < 0.001$) for anti-HCV. Nine patients were positive for the two markers anti-HCV and HBsAg whereas none of the controls had co-infection. Thirty-six HCC patients (22%) had no history of diabetes and had negative serological markers for HBV and HCV. Excessive alcohol consumption was reliably established for 19 (17.6%) cases among the 108 HCC patients for whom data is available. In all cases, no metabolic diseases (haemochromatosis, alpha1-antitrypsin deficiency) or primary biliary cirrhosis were observed. Of the 98 anti-HCV positive patients, HCV RNA was detected in 91 (93%) cases and in the remaining 7 patients was undetectable. Genotypes 1a, 1b and 2 were determined in 17.3%, 63.7% and 14.5% of patients positive for HCV RNA respectively. For the 29 HBsAg positive patients, HBV DNA was detected in 11 (38%) cases; almost all patients were infected by genotype D; genotype A was detected in only one patient.

Multivariate analysis

Table 2 shows the risk factors considered in the multivariate model based on univariate results. The OR for developing HCC in patients with dual infection was estimated to 84.7 [95% IC (4.3-366.9)]. After adjustment for age and gender, there was an association between development of HCC and NIDD, HBV and HCV infection and dual co-infection. No significant interaction between these three risk factors was observed.

Table 2 Risk of hepatocellular carcinoma related to hepatitis B virus and/or hepatitis C virus infections and non-insulin-dependent diabetes

Risk Factor	Adjusted odds ratio	95% IC
NIDD	5.9	1.7 - 19.7
HBsAg +/anti-HBc +	10.6	3.9 - 28.8
Anti-HCV +	33.3	14.1 - 78.8
B/C co-infection	84.7	4.3 - 366.9

HCV: hepatitis C virus; NIDD: non-insulin-dependent diabetes.

Association with cirrhosis

Conclusive evidence on the presence or absence of cirrhosis was reached in 133 HCC patients. Cirrhosis was present in 108 patients (81%). Table 3 shows the prevalence of viral markers and diabetes in HCC patients according to the presence of cirrhosis. Of the 108 HCC patients with cirrhosis, 74 (68.5%) were positive for anti-HCV and 21 (19.5%) were positive for HBsAg; 7 of them had both. NIDD was found in 20 (18.5%) patients: 18 patients were also positive for HBsAg and/or anti-HCV; two patients were negative for both. Nineteen (17.5%) HCC cases with cirrhosis were negative for all factors analysed. HCV was the only risk factor significantly associated with cirrhosis (90% *vs* 68% for all other risk factors collectively, $P = 0.00168$).

Table 3 Prevalence of viral markers and non-insulin-dependent diabetes in hepatocellular carcinoma patients with cirrhosis

	Presence of cirrhosis (n = 108)	Absence of cirrhosis (n = 25)	P
Anti-HCV positive	51	5	0.01
HbsAg Positive	12	2	0.48
HBV and HCV	6	1	0.60
Presence of NIDD			
NIDD alone	2	0	0.81
HCV and NIDD	16	1	0.12
HBV and NIDD	2	1	0.46
HBV, HCV and NIDD	1	1	0.34
Negative for HBV, HCV and NIDD	19	14	$< 10^{-3}$

HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HBV: hepatitis B virus; NIDD: non-insulin-dependent diabetes.

DISCUSSION

WHO estimated that approximately three-quarters (78%) of HCC worldwide are attributable to HBV or HCV[1,19,20]. HBV infection remains the main cause of HCC development, especially in regions with high endemicity of the virus such as China or sub-Saharan Africa where almost 75% of HCC patients are positive for HBsAg[21]. HCC associated with chronic HCV infection is observed more frequently in some countries of Asia and the Near East[22]. However, to date, their association with HCC has not been documented in the developing region of North Africa.

This is the first study on the association between various etiological factors and the risk of development of HCC in North African countries. We detected viral markers for HBsAg and anti-HCV in more than 70% of HCC patients.

Anti-HCV were found in 60% of HCC cases; similar levels were found in North America, Europe (44% to 66% in Italy, 27% to 58% in France, 60% to 75% in Spain) and Asia (over 70% in Japan)[19]. Similar rates were also reported in Egypt despite the marked difference in HCC incidence and viral genotypes[20,21].

In contrast, we observed HBsAg in only 17.9% of our HCC cases whereas the reported rates were higher in other parts of the Near- or Middle-East, like Saudi Arabia, Lebanon, Iran and Turkey where HBV infection remains the most important risk factor of HCC[22-25]. This finding was also markedly different from that reported by the only study conducted in the North African region - a small Tunisian HCC group in the early 1990s with 29 cases - that estimated HBsAg prevalence at 60%[26]. This discrepancy may be explained by the probable decline of HBV infection in recent years due to improvements in hygiene and health standards and the widespread introduction of vaccination against HBV in the region. In addition, we used a fourth generation enzyme assay to detect anti-HCV, a test far more sensitive than first generation tests previously used.

We observed 33-fold and 10-fold increase in HCV and HBV infections respectively compared to the control group. This risk increased 84-fold when patients were co-infected by the two viruses, suggesting a synergy between the two infections. Such positive interactions between HBV and HCV infections have been previously reported[4] but are not constantly present in the Middle-East[22]. The causes of such heterogeneity, presumably linked to the timing of viral infections in a patient's lifespan, are yet to be defined. In a case-control study conducted in Italy, concomitant infection with HBV and HCV was associated with an OR of 165 (95% CI, 81 to 374), while ORs of 17 and 23 were observed with HCV and HBV single positivity[19].

The mechanisms of carcinogenesis in HBV and HCV infection differ. HBV is known to play a direct role in liver cell transformation through direct interactions between viral and cellular proteins or by integration of HBV genome into the host genome[27-31]. We found no significant association between cirrhosis and HBsAg (+) in HCC patients confirming prior research. HCV, in contrast, is thought to promote a fibrotic process progressing to cirrhosis and ultimately to HCC[18,31]. Moreover, it has been recently suggested that several aspects of the HCV life cycle are important in the mechanism of carcinogenesis.

The impact of active replication of the virus and the presence of HCV genotypes 1 and 2 was associated to the development of tumor[32]. In the present work, more than 90% of anti-HCV positive patients were positive for viral RNA and were infected with Subtype 1b (63.7%) or Genotype 2 (14.5%). Both genotypes are prevalent in the region: In Tunisia, Subtype 1b is largely predominant

(79%); Subtype 1a and Genotype 2 are less frequent (5% and 10% respectively)[33]. In Morocco, Subtype 1b and Genotype 2 have been found with a prevalence of 68.4% and 15.8% respectively[34]. For HBV genotypes, our data indicate that genotype D is dominant in HCC patients from the western part of north-Africa, similarly to what was previously reported in HCC patients from other countries of Near- and Middle-East[35,36]. Genotype D was also detected in 80% and 86% of HBV-infected patients from Tunisia and Morocco respectively[37,38].

In addition to HCV and HBV infections, NIDD appears to be associated with HCC in the countries where our study was carried out. After adjusting for age, the association of each risk factor with HCC development remains significant. These findings which confirm previous studies suggest that HCV, HBV and NIDD play an independent role in liver tumorigenesis[39,40]. One may question if diabetes is a risk factor for HCC development or was caused by HCC. However, almost all our patients had onset of diabetes several years before HCC development, suggesting that NIDD would act more as risk factor than a consequence of this tumoral disease. NIDD was frequently associated with cirrhosis in our HCC series and likely plays an indirect role in HCC pathogenesis through predisposing the liver parenchyma to Non-Alcoholic Fatty Liver Disease (NAFLD). This chronic liver disease, frequently diagnosed in western countries[42], is characterized as a chronic necroinflammatory condition that can lead to liver fibrosis, cirrhosis and subsequently to HCC[41]. Recent studies performed in the Middle-East or North-Africa indicate that NIDD, early obesity, metabolic syndrome and NAFLD represent serious public health problems[42-46]. However, the roles of NIDD or NAFLD are still not clear in the development of HCC. Some authors reported NIDD as a risk factor of HCC in some cases from Saudi Arabia and Egypt; this association is not confirmed by studies conducted in Lebanon[47,48]. NIDD has been frequently associated with other risk factors for HCC by promoting cirrhosis[49]. Corroborating these previous results, our study detected anti-HCV among 17 out of the 20 patients, thereby associating NIDD and cirrhosis.

Alcohol consumption was found in only 17.9% of our HCC patients. Similar proportions were reported in other Muslims countries such as Turkey where alcohol abuse was found in only 10% to 16% of HCC patients[20,23]. In countries like Lebanon where an important proportion of the population is not Muslim, excessive alcohol consumption is observed in more than 20% of HCC[23]. However, this risk factor is certainly underestimated because it is not easily revealed by Muslim patients. It is actually admitted that in the Maghreb region alcohol intake plays an important role in different pathologies like psychiatric disorders[50].

In conclusion, our results suggest that HCV and HBV infections and NIDD are the main determinants of HCC development in individuals at risk in North Africa. Preventing the transmission of hepatitis viruses is the most important step to reduce the risk of HCC. However, approximately one fourth of the HCC patients described in our current study were negative for these three risk factors. Other studies should be conducted in the region to estimate the impact of factors such as occult hepatitis B in HCC which are frequently associated with HCC development throughout the world.

COMMENTS

Background

The western part of North Africa (also called Maghreb) is known for a low incidence of hepatocellular carcinoma (HCC), a low consumption of alcoholic drinks, an intermediate endemicity for chronic hepatitis B, a low rate of hepatitis C virus (HCV) carriage and a recent rise of obesity and non-insulin-dependent diabetes (NIDD) incidences. HCC in the Maghreb region has not been described since the 1980s; no report has yet detailed the respective implications of viral infections or other risk factors such us NIDD in HCC genesis in these countries.

Research frontiers

The current study was performed to evaluate the role of hepatitis C, hepatitis B and Diabetes Mellitus in HCC development in the Maghreb region.

Innovations and breakthroughs

This is the first study on the association between various etiological factors and the risk of development of HCC in North African countries.

Applications

This report shows the high implication of HCV and HBV infections and diabetes in HCC development in North Africa. Obtained results incite active surveillance and prevention programs for patients with chronic hepatitis and nutrition-associated metabolic liver diseases, the most important steps to reduce the risk of HCC in the region.

Peer reviews

It is a valuable study that assessed the major risk factors for HCC development in the western part of North Africa. It has an important impact on epidemiology of HCC in the world, giving information about the implications of chronic hepatitis and diabetes for HCC genesis in countries where, so far, no data are available. The article is well written and easy to read. The authors used a matched case-control study with good epidemiological tools to perform their analysis. This study should be completed by others in the region to evaluate other risk factors and to study molecular aspects of HCC.

REFERENCES

1 **Perz JF**, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; **45**: 529-538

2 **Mori M**, Hara M, Wada I, Hara T, Yamamoto K, Honda M, Naramoto J. Prospective study of hepatitis B and C viral infections, cigarette smoking, alcohol consumption, and other factors associated with hepatocellular carcinoma risk in Japan. *Am J Epidemiol* 2000; **151**: 131-139

3 **Oyunsuren T**, Kurbanov F, Tanaka Y, Elkady A, Sanduijav R, Khajidsuren O, Dagvadorj B, Mizokami M. High frequency of hepatocellular carcinoma in Mongolia; association with mono-, or co-infection with hepatitis C, B, and delta viruses. *J Med Virol* 2006; **78**: 1688-1695

4 **Kumar M**, Kumar R, Hissar SS, Saraswat MK, Sharma BC, Sakhuja P, Sarin SK. Risk factors analysis for hepatocellular carcinoma in patients with and without cirrhosis: a case-control study of 213 hepatocellular carcinoma patients from India. *J Gastroenterol Hepatol* 2007; **22**: 1104-1111

5 **Levrero M**. Viral hepatitis and liver cancer: the case of hepatitis C. *Oncogene* 2006; **25**: 3834-3847

6 **El-Serag HB**, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460-468

7 **Chen CH**, Huang GT, Yang PM, Chen PJ, Lai MY, Chen DS,

Wang JD, Sheu JC. Hepatitis B- and C-related hepatocellular carcinomas yield different clinical features and prognosis. *Eur J Cancer* 2006; **42**: 2524-2529
8 **Merle P**. [Epidemiology, natural history and pathogenesis of hepatocellular carcinoma]. *Cancer Radiother* 2005; **9**: 452-457
9 **Amarapurkar DN**, Patel ND, Kamani PM. Impact of diabetes mellitus on outcome of HCC. *Ann Hepatol* 2008; **7**: 148-151
10 **Sebti MF**. [Acute viral hepatitis and hepatitis B virus markers in chronic liver disease and primary cancer of the liver]. *IARC Sci Publ* 1984; 227-236
11 **Triki H**, Said N, Ben Salah A, Arrouji A, Ben Ahmed F, Bouguerra A, Hmida S, Dhahri R, Dellagi K. Seroepidemiology of hepatitis B, C and delta viruses in Tunisia. *Trans R Soc Trop Med Hyg* 1997; **91**: 11-1416
12 **Benjelloun S**, Bahbouhi B, Sekkat S, Bennani A, Hda N, Benslimane A. Anti-HCV seroprevalence and risk factors of hepatitis C virus infection in Moroccan population groups. *Res Virol* 1996; **147**: 247-255
13 **Khalfa S**, Ardjoun H. [Epidemiology of viral hepatitis in Algeria]. *Med Trop (Mars)* 1984; **44**: 247-252
14 **Ezzikouri S**, Chemin I, Chafik A, Wakrim L, Nourlil J, Malki AE, Marchio A, Dejean A, Hassar M, Trepo C, Pineau P, Benjelloun S. Genotype determination in Moroccan hepatitis B chronic carriers. *Infect Genet Evol* 2008; **8**: 306-312
15 **Bouguerra R**, Alberti H, Salem LB, Rayana CB, Atti JE, Gaigi S, Slama CB, Zouari B, Alberti K. The global diabetes pandemic: the Tunisian experience. *Eur J Clin Nutr* 2007; **61**: 160-165
16 **Tazi MA**, Abir-Khalil S, Chaouki N, Cherqaoui S, Lahmouz F, Sraïri JE, Mahjour J. Prevalence of the main cardiovascular risk factors in Morocco: results of a National Survey, 2000. *J Hypertens* 2003; **21**: 897-903
17 **Malek R**, Belateche F, Laouamri S, Hamdi-Cherif M, Touabti A, Bendib W, Nechadi A, Mekideche FZ, Hanat S. [Prevalence of type 2 diabetes mellitus and glucose intolerance in the Setif area (Algeria)]. *Diabetes Metab* 2001; **27**: 164-171
18 **Pineau P**, Ezzikouri S, Marchio A, Benazzouz M, Cordina E, Afifi R, Elkihal L, Khalfallah MT, Mestiri H, Tebbal S, Berkane S, Debzi N, Triki H, Dejean A, Iguer F, Bahri O, Essaid El Feydi AE, Benjelloun S. Genomic stability prevails in North-African hepatocellular carcinomas. *Dig Liver Dis* 2007; **39**: 671-677
19 **El-Serag HB**. Hepatocellular carcinoma and hepatitis C in the United States. *Hepatology* 2002; **36**: S74-S83
21 **Hassan MM**, Frome A, Patt YZ, El-Serag HB. Rising prevalence of hepatitis C virus infection among patients recently diagnosed with hepatocellular carcinoma in the United States. *J Clin Gastroenterol* 2002; **35**: 266-269
21 **el-Zayadi AR**, Badran HM, Barakat EM, Attia Mel-D, Shawky S, Mohamed MK, Selim O, Saeid A. Hepatocellular carcinoma in Egypt: a single center study over a decade. *World J Gastroenterol* 2005; **11**: 5193-5198
22 **Uzunalimoğlu O**, Yurdaydin C, Cetinkaya H, Bozkaya H, Sahin T, Colakoğlu S, Tankurt E, Sarioğlu M, Ozenirler S, Akkiz H, Tözün N, Değertekin H, Okten A. Risk factors for hepatocellular carcinoma in Turkey. *Dig Dis Sci* 2001; **46**: 1022-1028
23 **Ayoola EA**, Gadour MO. Hepatocellular carcinoma in Saudi Arabia: role of hepatitis B and C infection. *J Gastroenterol Hepatol* 2004; **19**: 665-669
24 **Hajiani E**, Masjedizadeh R, Hashemi J, Azmi M, Rajabi T. Risk factors for hepatocellular carcinoma in Southern Iran. *Saudi Med J* 2005; **26**: 974-977
25 **Yaghi C**, Sharara AI, Rassam P, Moucari R, Honein K, Bou-Jaoude J, Slim R, Noun R, Abdul-Baki H, Khalifeh M, Ramia S, Sayegh R. Hepatocellular carcinoma in Lebanon: Etiology and prognostic factors associated with short-term survival. *World J Gastroenterol* 2006; **12**: 3575-3580
26 **Alacacioglu A**, Somali I, Simsek I, Astarcioglu I, Ozkan M, Camci C, Alkis N, Karaoglu A, Tarhan O, Unek T, Yilmaz U. Epidemiology and survival of hepatocellular carcinoma in Turkey: outcome of multicenter study. *Jpn J Clin Oncol* 2008; **38**: 683-688
27 **Triki H**. [Epidemiology of hepatitis B virus, hepatitis C virus and Delta virus in the general population and in liver cirrhosis in Tunisia]. *Arch Inst Pasteur Tunis* 1994; **71**: 403-406
28 **Raza SA**, Clifford GM, Franceschi S. Worldwide variation in the relative importance of hepatitis B and hepatitis C viruses in hepatocellular carcinoma: a systematic review. *Br J Cancer* 2007; **96**: 1127-1134
29 **Gurtsevitch VE**. Human oncogenic viruses: hepatitis B and hepatitis C viruses and their role in hepatocarcinogenesis. *Biochemistry (Mosc)* 2008; **73**: 504-513
30 **Lotz G**, Kiss A, Novák PK, Sobel G, Schaff Z. Hepatitis viruses and hepatocarcinogenesis. *J Physiol Paris* 2001; **95**: 417-422
31 **Hayashi J**, Aoki H, Arakawa Y, Hino O. Hepatitis C virus and hepatocarcinogenesis. *Intervirology* 1999; **42**: 205-210
32 **Abe K**, Edamoto Y, Park YN, Nomura AM, Taltavull TC, Tani M, Thung SN. In situ detection of hepatitis B, C, and G virus nucleic acids in human hepatocellular carcinoma tissues from different geographic regions. *Hepatology* 1998; **28**: 568-572
33 **Djebbi A**, Triki H, Bahri O, Cheikh I, Sadraoui A, Ben Ammar A, Dellagi K. Genotypes of hepatitis C virus circulating in Tunisia. *Epidemiol Infect* 2003; **130**: 501-505
34 **Benani A**, El-Turk J, Benjelloun S, Sekkat S, Nadifi S, Hda N, Benslimane A. HCV genotypes in Morocco. *J Med Virol* 1997; **52**: 396-398
35 **Abdo AA**, Al-Jarallah BM, Sanai FM, Hersi AS, Al-Swat K, Azzam NA, Al-Dukhayil M, Al-Maarik A, Al-Faleh FZ. Hepatitis B genotypes: relation to clinical outcome in patients with chronic hepatitis B in Saudi Arabia. *World J Gastroenterol* 2006; **12**: 7019-7024
36 **Mojiri A**, Behzad-Behbahani A, Saberifirozi M, Ardabili M, Beheshti M, Rahsaz M, Banihashemi M, Azarpira N, Geramizadeh B, Khadang B, Moaddeb A, Ghaedi M, Heidari T, Torab A, Salah A, Amirzadeh S, Jowkar Z, Mehrabani D, Amini-Bavil-Olyaee S, Dehyadegari MA. Hepatitis B virus genotypes in southwest Iran: molecular, serological and clinical outcomes. *World J Gastroenterol* 2008; **14**: 1510-1513
37 **Bahri O**, Cheikh I, Hajji N, Djebbi A, Maamouri N, Sadraoui A, Mami NB, Triki H. Hepatitis B genotypes, precore and core promoter mutants circulating in Tunisia. *J Med Virol* 2006; **78**: 353-357
38 **Ezzikouri S**, Chemin I, Chafik A, Wakrim L, Nourlil J, Malki AE, Marchio A, Dejean A, Hassar M, Trepo C, Pineau P, Benjelloun S. Genotype determination in Moroccan hepatitis B chronic carriers. *Infect Genet Evol* 2008; **8**: 306-312
39 **Hassan MM**, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, Beasley P, Patt YZ. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology* 2002; **36**: 1206-1213
40 **Mori M**, Hara M, Wada I, Hara T, Yamamoto K, Honda M, Naramoto J. Prospective study of hepatitis B and C viral infections, cigarette smoking, alcohol consumption, and other factors associated with hepatocellular carcinoma risk in Japan. *Am J Epidemiol* 2000; **151**: 131-139
41 **Veldt BJ**, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, de Knegt RJ, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *Hepatology* 2008; **47**: 1856-1862
42 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219
43 **Bahrami H**, Daryani NE, Mirmomen S, Kamangar F, Haghpanah B, Djalili M. Clinical and histological features of nonalcoholic steatohepatitis in Iranian patients. *BMC Gastroenterol* 2003; **3**: 27
44 **Adibi A**, Janghorbani M, Shayganfar S, Amini M. First-

degree relatives of patients with type 2 diabetes mellitus and risk of non-alcoholic Fatty liver disease. *Rev Diabet Stud* 2007; **4**: 236-241

45 **Jamali R**, Khonsari M, Merat S, Khoshnia M, Jafari E, Bahram Kalhori A, Abolghasemi H, Amini S, Maghsoudlu M, Deyhim MR, Rezvan H, Pourshams A. Persistent alanine aminotransferase elevation among the general Iranian population: prevalence and causes. *World J Gastroenterol* 2008; **14**: 2867-2871

46 **Judi L**, Toukan A, Khader Y, Ajlouni K, Khatib MA. Prevalence of elevated hepatic transaminases among Jordanian patients with type 2 diabetes mellitus. *Ann Saudi Med* 2010; **30**: 25-32

47 **Abdel WM**, Mostafa M, Salah T, Fouud A, Kandeel T, Elshobary M, Abd Allah OF, Elghawalby N, Sultan A, Ezzat F. Epidemiology of hilar cholangiocarcinoma in Egypt: single center study. *Hepatogastroenterology* 2007; **54**: 1626-1631

48 **Singal AK**, Ayoola AE. Prevalence and factors affecting occurrence of type 2 diabetes mellitus in Saudi patients with chronic liver disease. *Saudi J Gastroenterol* 2008; **14**: 118-121

49 **Davila JA**, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. *Gastroenterology* 2004; **127**: 1372-1380

50 **Kadri N**, Agoub M, Assouab F, Tazi MA, Didouh A, Stewart R, Paes M, Toufiq J, Moussaoui D. Moroccan national study on prevalence of mental disorders: a community-based epidemiological study. *Acta Psychiatr Scand* 2010; **121**: 71-74

S- Editor Zhang HN **L- Editor** Roemmele A **E- Editor** Liu N