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Characterization of hepatocellular carcinoma in Mansoura university Hospitals: A case-control study of risk factors.

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Abstract

Background and aims.

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and is one of the leading causes of cancer related deaths. The aim of this study is to investigate characters and risk factors in the development of hepatocellular carcinoma (HCC) in Mansoura University Hospitals.

Methods.

The study included 220 patients with HCC plus 246 control patients from general population of rural areas of Dakahlia. All the study population was randomly selected. Diagnosis of HCC was based on ultrasonography and confirmed by triphasic computerized tomography and /or triphasic MRI.

Results.

Compared to patients without HCC, patients with HCC were significantly male (76.8% vs. 62.0%), smoker (32.7% vs. 14.2%), diabetes mellitus (24.1%vs. 8.9%), and had a positive hepatitis C virus (HCV) infection (55.9% vs. 13.0%), hepatitis B virus (HBV) infection (5.5% vs.0.4%) and schistosoma infestation (67.7% vs. 44.3%). HCC cases were characterized by, cirrhotic (81.8 %), child B score (47.7), found in right lobe (47.3%), had multifocal lesions (49.1%) and presented with portal vein thrombosis (26.4%). The OR and 95% Confidence interval (CI) of HCC were (OR 36.9, 95% CI 18-75.8) for anti- HCV positive patients and (OR 84.807, 95% CI 8.6- 835.59) for HBsAg positive patients on multivariate analysis. The OR for diabetes (OR 0.31, 95% CI 0.18-0.53), smoking (OR 0.343, 95% CI0.217-0.54) and schistosomiasis (OR 0.376, 95% CI 0.257-0.559) in Univariate analysis.

Conclusions.

Infection with HBV and HCV and schistosomiasis infestation are the major risk factors for the development of HCC in Egyptian patients.

Keywords: Hepatocellularcarcinoma, hepatitis C virus, hepatitis B virus, Schistosomiasis, diabetes mellitus.
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Introduction

Hepatocellular carcinoma (HCC) is one of the few cancers with established major risk factors mainly on top of liver cirrhosis [1-3].

Hepatitis B virus (HBV) is the most common risk factor for HCC worldwide and is believed to account for nearly half of all diagnosed HCCs [4]. exclusion of intra-abdominal surgically-treatable source [2].

The mechanism of HBV related hepatocarcinogenesis is thought to include several factors [5-7]. HBV DNA sequences integrate into the host genome, which may downregulate tumor suppressor genes. Studies have shown that integrated HBV is more frequent in HCCs tissues than in adjacent non-malignant liver tissue, with host integration sites located at cancer-related genes, including the telomerase reverse transcriptase gene, and viral breakpoints detected near the HBxgene [8]. Also, the viral protein HBx may modulate the activity of several cellular factors that regulating cell proliferation, apoptosis, and DNA damage. In addition, cirrhosis, resulting from chronic HBV infection trigger a complex cascade of oxidative stress, hypoxia, necrosis, regeneration, and angiogenesis, which may alter host gene expression [9].

Several studies have confirmed that, sustained viral replication and liver injury are a risk factors for HBV-related HCC, with serum HBV DNA levels directly correlating with the future risk of HCC[10, 11]. Specific variations in the HBV DNA sequence, such as HBV genotype C and basal core promoter mutations have also been associated with a higher HCC risk [12-14].

Hepatitis C virus (HCV) is a principal cause of HCC as a result from chronic inflammation causing injury to the liver parenchyma and subsequent fibrosis [15]. Chronic inflammation leads to increased levels of reactive oxygen species, which damage hepatocytes both at metabolic and genetic levels, finally causing cell death. Compensative liver regeneration, which occurs in a HCV altered environment favors chromosomal instability and irreversible genetic/epigenetic changes, which promote neoplastic transformation of hepatocytes and the progression of malignant clones [16].

It is interesting that, patients with S. mansoni infection tend to retain HBV and HCV for longer periods than those not infected with S. mansoni. In those with hepatosplenomegaly, the cell-mediated immune response

was shown to be markedly depressed causing prolongation of the carrier state of the virus [17].

The association between heavy alcohol abuse and HCC risk has been consistently demonstrated in several studies. Tobacco smoking has consistently been associated with an increased risk of HCC and has been labeled as a risk factor for liver cancer by the World Health Organization International Agency for Research on Cancer [18, 19].

Egypt has a significant prevalence of HBV, HCV, bilharzial infection and smoking [20]. We conducted this study to assess the risk factors of HCC in Egyptian population.

Materials and methods

This is a case control study conducted at Specialized Medical Hospital, Oncology Center and Gastroenterology surgery Center, Mansoura University Hospitals after approval of the institutional review board.

The study included two groups.

1. Group (1) included 220 patients with cirrhosis and HCC.
2. Group (2) included 246 controls.

The sample size was calculated according to the power of study 85% and 95% confidence interval using Epi info. The control group was randomly selected from general population of rural areas of Dakahlia governorate.

The diagnosis of cirrhosis was based on clinical, laboratory and imaging (ultrasonography and confirmed by triphasic CT and /or triphasic MRI) in addition to serum alpha-fetoprotein.

Clinical examination.

Thorough clinical examination was done including, history of contact with canal water, history of drug treatment of schistosomiasis in addition to imaging pattern in form of periportal fibrosis. Written informed consent was obtained from all individuals participated in the study according to the Declaration of Helsinki.

Laboratory investigations.

included; complete blood count, total bilirubin, serum albumin, coagulation profile, serum creatinine, random blood sugar, viral markers (HBsAg, anti-HCV and anti-HIV) were assessed using enzyme linked immunosorbent essay (ELISA), alpha-fetoprotein. Diagnosis of schistosomiasis was made based on indirect haemagglutinition test (IHA) (BilharzioseFumouze Diagnostics /SERFIB, France), Diabetes status was categorized as normoglycemia (fasting glucose <100 mg/dL), impaired fasting glucose (100- 125 mg/dL), and diabetes (≥126 mg/dL or prevalent diabetes).

Evaluation of the severity of liver cirrhosis was assessed in each patient with the Modified CTP score [21]. Staging of HCC according to Barcelona Clinic Liver Cancer (BCLC) classification [22].

Statistical analysis

Comparisons between proportions were performed using chi square test. Comparisons between continuous variables were performed using Mann Whitney non-parametric test. For case control studies, OR examines the increased odds of getting a disease with exposure to certain risk factors vs. non-exposure to that factor using SPSS version 20. P value is considered significant if < 0.05.

Results

General characteristics of the whole study population.

The baseline demographic and social characteristics of the whole study population are shown in **(Table 1)**.

Table 2 shows, the baseline clinical characteristics of the whole study population**.** Diabetes mellitus incidence was present in 53 patients (24.1%) among HCC cases vs. 22 patients (8.9%) in the controls. Anti-HCV were positive in 123 patients (55.9%) and 32 patients (13.0%) for the cases and controls respectively. HBsAg was positive in 12 patients (5.5%) for HCC cases vs. 1 patients (0.4%) for the controls.

Schistosoma antibodies were found in 149 patients (67.7%) of all 220 HCC patients compared to 109 patients (44 %) of sera of all 246 controls (p value <0.001).

As regarding clinical presentation of HCC cases, the majority of patients presented by abdominal pain in 96 patients (43.7%), with patent portal vein in 162 patients (73.6%), mostly in right lobe in 104 patients (47.3%), had multiple lesions in 108 patients (49.1%), on top of cirrhosis in 180 patients (81.8%), had BCLC stage D in104 patients (47.3%) and were child B in105 patients (47.7%).

Table 3 shows the laboratory parameters of the whole study population. Comparison between base line laboratory parameters of HCC cases and controls showed statistically significant decrease in hemoglobin level, platelets count, WBCs, albumin level, whereas a significant increase was found as regarding, serum bilirubin, INR and alpha fetoprotien in HCC cases versus control group (p = 0.001)**.**

Risk Factors for HCC

The risk for HCC development for HBsAg positive patients was12.2 and 84.8 folds higher than non-infected population (OR 12.26, 95% CI: 1.574-95.582) (OR84.8, 95% CI 8.60-835.59) on univariate and multivariate analysis respectively.

The risk for HCC development for anti-HCV positive patients was 8.4 and 36.9 folds higher than non-infected population (OR 8.4, 95% CI: 5.34- 13.3) (OR36.99, 95% CI 18.03-75.88) on univariate and multivariate analysis respectively. on Univariate and multivariate analysis respectively.

The OR for diabetes (OR= 0.31, 95% CI 0.18-0.53), smoking (OR 0.343, 95% CI 0.217-0.54) and schistosomiasis (OR 0.376, 95% CI 0.257-0.559) in Univariate analysis as shown in **(Table 4 and 5).**

Table 1. Baseline social and demographic criteria of both cases and controls.

	Cases N=220	Controls N=246	Total N=466	P-value
Age, years median (IQR)	57.0 (50.0-63.0)	58.0 (50.0-67.0)	58.0 (50.0-65.0)	0.025
Sex				
- **Males, n (%)**	169 (76.8%)	152 (62.0%)	321(69.0%)	0.001
- **Females, n (%)**	51 (23.2%)	93 (38.0%)	144(31.0%)	
Residence				
- **Urban**	94 (42.7%)	18 (7.3%)	112(24.0%)	<0.001
- **Rural**	126 (57.3%)	228 (92.7%)	354(76.0%)	
Diabetes mellitus				
- **Positive**	53 (24.1%)	22 (8.9%)	75 (16.1%)	<0.001
- **Negative**	167 (75.9%)	224 (91.1%)	224 (91.1%)	
HCV				
- **Positive**	123 (55.9%)	32 (13.0%)	155(33.3%)	<0.001
- **Negative**	97 (44.1%)	214 (87.0%)	311(66.7%)	
HBV				
- **Positive**	12 (5.5%)	1 (0.4%)	13 (2.8%)	0.001
- **Negative**	208 (94.5%)	245 (99.6%)	453 (97.2%)	
Schistosomiasis				
- **Positive**	149 (67.7%)	109(44.3%)	258(55.4%)	<0.001
- **Negative**	71 (32.3%)	137(55.7%)	208(44.6%)	

Table 2. Clinical presentation of HCC Cases.

Symptoms at presentation	**No**	**%**
- **Accidental**	76	34.5
- **Abdominal enlargement**	38	17.3
- **Abdominal pain**	96	43.7
- **Others[@]**	10	4.5
Portal vein		
- **Patent**	162	73.6
- **Thrombosed**	58	26.4
Lesion site		
- **Right**	104	47.3
- **Left**	58	26.4
- **Both lobes**	58	26.4
Lesions number		
- **Single**	98	44.5
- **Two**	14	6.4
- **Multiple**	108	49.1
Ascites		
- **Yes**	77	35
- **No**	143	65
Cirrhotic liver		
- **Yes**	180	81.8
- **No**	40	18.2

- BCLC classification for HCC cases		
Stage 0	11	5
Stage A	33	15
Stage B	7	3.2
Stage C	65	29.5
Stage D	104	47.3
CTP Stage		
- A	100	45.5
- B	105	47.7
- C	15	6.8

Table 3. Baseline laboratory characteristics of both cases and controls.

	Cases N=220	**Controls N=246**	**P-value**
Hgb, median (IQR)	11.80 (10.05-13.65)	14.00 (13.10-15.00)	<0.001
Platelets, median (IQR)	120.0 (79.0-171.5)	202.0 (149.0-247.0)	<0.001
WBCs, median (IQR)	5.80 (3.70-7.95)	6.83 (5.42-8.00)	<0.001
Albumin, median (IQR)	3.30 (2.80-3.80)	4.27 (4.02-4.50)	<0.001
Total bilirubin, median (IQR)	1.30 (0.90-2.00)	0.50 (0.40-0.70)	<0.001
INR, median (IQR)	1.24 (1.10-1.40)	1.01 (1.01-1.02)	<0.001
AFP, median (IQR)	46.20 (7.91-975.50)	3.80 (2.40-6.36)	<0.001

Table 4. Univariate analysis of HCC risk factors.

	Univariate		
	OR	**95% C.I. OR**	**P-value**
Smoking	0.343	0.217-0.540	<0.001
DM	0.311	0.182-0.531	<0.001
HCV	8.440	5.344-13.331	<0.001
HBV	12.266	1.574-95.582	0.017
Schistosomiasis	0.376	0.257-0.559	<0.001

Table 5. Multivariate analysis of HCC risk factors.

	Multivariate (Stepwise binary logistic regression)		
	OR	**95% C.I. OR**	**P- value**
HCV	36.997	18.038-75.882	<0.001
HBV	84.807	8.607- 835.593	<0.001
Schistosomiasis	0.128	0.037- 0.246	<0.001

Discussion

Hepatocellular carcinoma frequently occurs among individuals with known risk factors including chronic viral hepatitis, cigarette smoking, alcohol consumption and diabetes [23]. This prospective case control study was performed to assess the association of various etiological factors and the risk of development of HCC. The median age of HCC cases and controls was 57 and 58 years respectively. Most were men 169 (76.8%) for patients and 152 (62.0%) for controls. Analysis of the sex distribution showed that HCC was more common in males than females (76.8% vs 23.2 %). This is in agreement with the study conducted by El-Zayadi et al. where the calculated risk of development of HCC was nearly three times higher in male than in female patients [24]. Predominance of HCC in males may be explained by differences in exposure to risk factors, sex hormones and other X-linked genetic factors may also be important [25]. A wide research was carried out to discover the independent and combined effects of HBV and HCV as a risk factor of HCC [26].

Chronic HBV infection is a key risk factor of HCC and the prevalence of HBV infection mostly mirrors the HCC occurrence. HBV infection accounts for 60% of liver cancer cases in less developed regions but, only 23% in more developed countries [27]. Hepatitis C virus is the major cause of progressive liver diseases and a public health problem worldwide [28]. In the present study, twelve HCC cases (5.5%) and one control (0.4%) were HBsAg +ve. The risk for HCC development for HBsAg +ve patients was 12.2 and 84.8 folds higher than non-infected population (OR 12.2, 95% CI 1.57-95.58) in univariate analysis and (OR 84.8, 95% CI 8.6-835.5) on multivariate analysis. This finding is in harmony with that of Niu et al. who studied the epidemiological risk factors for HCC in southeast china and reported that, population with HBsAg has a 15.39-fold HCC risk in comparison to HBsAg negative cases [27].

Anti-HCV was positive in about 13% of the controls and in 55.9% in cases. The prevalence of HCV among controls in the current study is corresponding to the estimates from studies of HCV in healthy populations; and this is also likely to be due to the older population in our study. Numerous studies have reported increasing HCV prevalence in older age groups in Egypt with rates up to 60% [29-31]. Expected association between HCC and HCV infection was observed in the present study. We reported that, cases with anti HCV has 8.4 risk of developing HCC higher than non-infected population (OR=8.4, 95%CI5.3-13.3) in univariate analysis and (OR 36.9, 95% CI=18 - 75.882) in multivariate analysis.

Our results coincide with Zhang et al. who reported that the prevalence of hepatitis HBsAg and anti-HCV were much higher in HCC patients (63.2% and 11.2% respectively) than in the control patients (5.2%, 3.5%), ORs of HBsAg and HBV infection were 28.82 (95% CI: 11.18-78.78) and 31.22 (95% CI: 13.86-72.15) respectively. Moreover, the risk of HCC significantly increased and showed an additive effect when both HBV and HCV infection were considered (OR = 42.85) [32]. Kumar et al studied the risk factor analysis for HCC in 213 Indian patients and reported that the ORs and 95% CI of HCC were 38.98 (19.55-77.71) for HBsAg positivity, 5.45 (2.02-14.71) for anti-HCV positive and HCV RNA positive [33].

Simonetti et al showed a study in which 151 patients (71%) with HCC were anti-HCV +ve compared with 11 controls (5%) with chronic non-hepatic diseases (OR= 42; 95% CI: 22 to 95). Multivariate analysis showed that anti-HCV was an independent risk factor for HCC (OR= 69; 95% CI: 15 to 308). The analysis also showed that, HBsAg (OR= 8.7; 95% CI: 1.5 to 50) and anti-HBc (OR= 4.2; 95% CI, 1.7 to 11) were risk factors for HCC. [34]. Schiefelbein et al studied HCC risk factors and reported HCV as the most important HCC risk factor [OR 9.7 (95% CI: 3.3–28.0, P <0.01)], but reported that HBV infection showed marginal tendency of increased risk (OR 5.4, 95% CI: 0.9–31.8, P <0.06) [35].

In Egypt, *S. mansoni* was the major cause of liver disease [36]. Resolution of HCV infections is linked to a strong CD4+ and CD8+ Th-1 immune response. Schistosomal infection encourages immune suppression by up-regulation of type 2 responses (Th-2) while down-regulating Th-1 responses, leading to persistence of concomitant viral infections. Co-infection with schistosomiasis and HCV was found to have more severe effects on liver pathology and progression into worsening complications than HCV infection alone [37-39].

In this study, schistosoma antibodies were found in 67.7% of all 220 HCC patients compared to 44 % of sera of all 246 controls. Out of total 123 anti-HCV positive HCC cases, 61 patients (49.6%) were co-infected with schistosomiasis. Our observations coincide with Angelico et al. who concluded that *S. mansoni* and HCV co-infections are highly prevalent among Egyptian patients with chronic liver disease and this association may have mutual interaction, increasing the severity of liver pathology and the risk of HCC [40]. Strickland had shown that 70–90 % of patients with chronic hepatitis, cirrhosis or HCC in Egypt have co-infection of schistosomiasis and HCV [39].

Out of total 12 HBsAg +ve HCC cases, 8 patients (66.7%) were co-infected with schistosomiasis indicating a strong association between HBVand schistosomal co-infection. A systematic review of schistosomiasis and HBV co-infection reported a high prevalence of HBV and S. mansoni co-infection in Egypt ranging from 19.6 to 33.0% [41]. We are strongly in agreement with studies

indicating a positive association between HBV and schistosomiasis and not coinciding with studies rejecting any relationship between schistosomiasis and HBV [42]. The proportion of HCC patients had concomitant HCV and schistosomiasis was 27.7% compared to HCC patients with HCV alone 28.2%. The proportion of HCC patients had concomitant HBV and schistosomiasis were 3.6% compared to HCC patients with HBV alone 1.8%.

Thus, in our study we could prove that there is a strong association between HCV, HBV and bilharzial co-infection. This could be explained by the frequent need of blood transfusion in patients with schistosomiasis and on considering the poor infection control measures in countries where this disease is endemic [43]. However, we could not suggest that the co-infection had increased the incidence of HCC among those patients. OR between all HCC cases and controls (OR 0.37, 95% CI:0.25-0.55).

Chronic alcohol abuse is a well-established risk factor for the development of HCC [44]. But, the association of cigarette smoking and HCC risk is not well established. In the present study we could not report smoking as a risk factor for HCC. Our findings coincide with Mori et al. who found no significant relation between smoking and HCC risk [45]. A meta-analysis of 16 publications to evaluate the epidemiological interactions between HBV and HCV infection, smoking cigarette, and the risk of HCC reported a synergistic effect between smoking and HBV or HCV infection on the risk of HCC [46].

We prospectively examined the association between T2D and HCC risk. We found that 24.1%of HCC patients have T2D compared to 8.9% of non HCC controls (OR= 0.311, 95% CI 0.182-0.531). In the present study, we could not report T2D as risk factors for HCC. In contrast to our results, A meta-analysis published in 2006 reported that, of 13 case-control studies, diabetes was significantly associated with HCC in 9 studies (a pooled odds ratio of 2.5), and of 13 cohort studies, diabetes was significantly associated with HCC in 7 studies (a pooled risk ratio of 2.5). The significant association between HCC and diabetes was independent of viral hepatitis or alcohol use in the 10 studies that examined these factors [47].

The current study has a large sample size, and one of the powerful points is that given the close correlation between smoking and alcohol intake in most epidemiologic studies, it is difficult to exclude the residual confounding effect of alcohol in the association between smoking and HCC. However, in the present study, none of the cases or controls was alcohol consumer and thus the effect of smoking on HCC development could be evaluated without the confounding effect of alcohol intake. The current study has several limitations. One of the limitations of this study is that controls are not matched for age and sex with patients, smoking index was not calculated. We also did not study the effect of T2D duration or additional metabolic factors like obesity, hypertension and dyslipidemia as risk factors for HCC.

Conclusion

Infection with HBV and HCV and schistosomiasis infestation are the major risk factors for the development of HCC in Egyptian patients. Most patients with HCC in our locality were male gender, smoker and diabetic.

References

1. Massarweh, N. N., and El-Serag, H. B. (2017). Epidemiology of hepatocellular carcinoma and intrahepatic cholangiocarcinoma. Cancer Control, 24(3), 1073274817729245.
2. Shiha, G., Mousa, N., Soliman, R., et al. (2020). Incidence of HCC in chronic hepatitis C patients with advanced hepatic fibrosis who achieved SVR following DAAs: A prospective study. Journal of viral hepatitis, 27(7), 671-679.
3. Elbaz, S., Mousa, N., Besheer, T., et al. (2020). Malondialdehyde and C-reactive protein as prognostic markers of hepatocellular carcinoma. British journal of biomedical science, 77(2), 94-96.
4. Kispert, S., and McHowat, J. (2017). Recent insights into cigarette smoking as a lifestyle risk factor for breast cancer. Breast Cancer: Targets and Therapy, 9, 127.
5. But, D. Y. K., Lai, C. L., and Yuen, M. F. (2008). Natural history of hepatitis-related hepatocellular carcinoma. World journal of gastroenterology: WJG, 14(11), 1652.
6. Arzumanyan, A., Reis, H. M., and Feitelson, M. A. (2013). Pathogenic mechanisms in HBV-and HCV-associated hepatocellular carcinoma. Nature Reviews Cancer, 13(2), 123-135.
7. Kew, M. C. (2011). Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. Journal of gastroenterology and hepatology, 26, 144-152.
8. Sung, W. K., Zheng, H., Li, S., Chen, R., et al. (2012). Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nature genetics, 44(7), 765-769.
9. Toh, S. T., Jin, Y., Liu, L., et al. (2013). Deep sequencing of the hepatitis B virus in hepatocellular carcinoma patients reveals enriched integration events, structural alterations and sequence variations. Carcinogenesis, 34(4), 787-798.
10. Liu, J., Yang, H. I., Lee, M. H., et al. (2014). Spontaneous seroclearance of hepatitis B seromarkers and subsequent risk of hepatocellular carcinoma. Gut, 63(10), 1648-1657.
11. Chen, G., Lin, W., Shen, F., et al. (2006). Past HBV viral load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study. Official journal of the American College of Gastroenterology| ACG, 101(8), 1797-1803.

12. Liu, S., Zhang, H., Gu, C., et al. (2009). Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. Journal of the National Cancer Institute, 101(15), 1066-1082.

13. Chan, H. L., Hui, A. Y., Wong, M. L., Tse, A. M., Hung, L. C., Wong, V. W., & Sung, J. J. (2004). Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. Gut, 53(10), 1494-1498.

14. Wong, G. H., Chan, H. Y., Yiu, K. L., et al. S. (2013). Meta-analysis: the association of hepatitis B virus genotypes and hepatocellular carcinoma. Alimentary pharmacology & therapeutics, 37(5), 517-526.

15. Lemon, S. M., and McGivern, D. R. (2012). Is hepatitis C virus carcinogenic? Gastroenterology, 142(6), 1274-1278.

16. Hassan, M. M., Zaghloul, A. S., El-Serag, H. B., et al. (2001). The role of hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients. Journal of clinical gastroenterology, 33(2), 123-126.

17. Toda, K. S., Kikuchi, L., Chagas, A. L., et al. (2015). Hepatocellular carcinoma related to Schistosoma mansoni infection: case series and literature review. Journal of clinical and translational hepatology, 3(4), 260.

18. Grant, B. F., Stinson, F. S., Dawson, D. A., et al. (2004). Prevalence and co-occurrence of substance use disorders and independent mood and anxiety disorders: Results from the national epidemiologic survey on alcohol and related conditions. Archives of general psychiatry, 61(8), 807-816.

19. Lee, Y. C. A., Cohet, C., Yang, Y. C., Stayner, L., et al. (2009). Meta-analysis of epidemiologic studies on cigarette smoking and liver cancer. International journal of epidemiology, 38(6), 1497-1511.

20. Abdel-Wahab, M., El-Ghawalby, N., Mostafa, M., Sultan, A., et al. (2007). Epidemiology of hepatocellular carcinoma in Lower Egypt, Mansoura Gastroenterology Center. Hepato-gastroenterology, 54(73), 157-162.

21. Garcia-Tsao, G. (2016). The Child–Turcotte Classification: From Gestalt to Sophisticated Statistics and Back.

22. Pons, F., Varela, M., & Llovet, J. M. (2005). Staging systems in hepatocellular carcinoma. Hpb, 7(1), 35-41.

23. Boktour, M., Hanna, H., Ansari, S., et al. (2006). Central venous catheter and Stenotrophomonas maltophilia bacteremia in cancer patients. Cancer: Interdisciplinary International Journal of the American Cancer Society, 106(9), 1967-1973.

24. El-Zayadi, A. R., Badran, H. M., Barakat, E. M., et al. (2005). Hepatocellular carcinoma in Egypt: a single center study over a decade. World journal of gastroenterology: WJG, 11(33), 5193.

25. Yu, M. C., Henderson, B. E., Tong, M. J., et al. (1991). Nonviral risk factors for hepatocellular carcinoma in a low-risk population, the non-Asians of Los Angeles County, California.

26. Anwar, W. A., Khaled, H. M., Amra, H. A., et al. (2008). Changing pattern of hepatocellular carcinoma (HCC) and its risk factors in Egypt: possibilities for prevention. Mutation research/reviews in mutation research, 659(1-2), 176-184.

27. Niu, J., Lin, Y., Guo, Z., et al. (2016). The Epidemiological investigation on the risk factors of hepatocellular carcinoma: a case–control study in Southeast China. Medicine, 95(6).

28. Nasser, M., Mahmoud, A. A., Raghda, F., et al. (2018). Recent trends in chronic hepatitis C virus treatment. Medical Journal of Viral Hepatitis, 2(2), 1-6.

29. El-Sadawy, M., Ragab, H., El-Toukhy, H., et al. (2004). Hepatitis C virus infection at Sharkia Governorate, Egypt: seroprevalence and associated risk factors. Journal of the Egyptian Society of Parasitology, 34(1 Suppl), 367-384.

30. Darwish, M. A., Faris, R., Darwish, N., et al. (2001). Hepatitis c and cirrhotic liver disease in the Nile delta of Egypt: a community-based study. The American journal of tropical medicine and hygiene, 64(3), 147-153.

31. Abdel-Aziz, F., Habib, M., Mohamed, M. K., et al. (2000). Hepatitis C virus (HCV) infection in a community in the Nile Delta: population description and HCV prevalence. Hepatology, 32(1), 111-115.

32. Zhang JY1, Dai M, Wang X, et al. (1998). A case-control study of hepatitis B and C virus infection as risk factors for hepatocellular carcinoma in Henan, China. Int J Epidemiol, 27(4):574-8.

33. Kumar, R., Saraswat, M. K., Sharma, B. C., et al. (2008). Characteristics of hepatocellular carcinoma in India: a retrospective analysis of 191 cases. QJM: An International Journal of Medicine, 101(6), 479-485.

34. Simonetti, R. G., Cammà, C., Fiorello, F., et al. (1992). Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis: a case-control study. Annals of internal medicine, 116(2), 97-102.

35. Schiefelbein, E., Zekri, A. R., Newton, D. W., et al. (2012). Hepatitis C virus and other risk factors in hepatocellular carcinoma. Acta virologica, 56(3), 235.

36. Strickland, G. T. (2006). Liver disease in Egypt: hepatitis C superseded schistosomiasis as a result of iatrogenic and biological factors. Hepatology, 43(5), 915-922.

37. Lechner, F., Wong, D. K., Dunbar, P. R., et al. (2000). Analysis of successful immune responses in persons infected with hepatitis C virus. The Journal of experimental medicine, 191(9), 1499-1512.

38. Nasser, M., Ahmed, A. R., Ilham, T. Q., et al. (2019). The role of interleukin-2 and interleukin-4 in disease progression in patients with schistosomiasis combined with chronic viral hepatitis C and chronic viral hepatitis B. Medical Journal of Viral Hepatitis, 3(2), 57-64.

39. Strickland, G. T. (2006). Liver disease in Egypt: hepatitis C superseded schistosomiasis as a result of

iatrogenic and biological factors. Hepatology, 43(5), 915-922.

40. Angelico, M., Renganathan, E., Gandin, C., et al. (1997). Chronic liver disease in the Alexandria governorate, Egypt: contribution of schistosomiasis and hepatitis virus infections. Journal of hepatology, 26(2), 236-243.

41. Hammad, H. A., Fattah, M. M. Z. E., Moris, M., et al. (1990). Study on some hepatic functions and prevalence of hepatitis B surface antigenaemia in Egyptian children with schistosomal hepatic fibrosis. Journal of tropical pediatrics, 36(3), 126-127.

42. Al-Freihi, H. M. (1993). Prevalence of hepatitis B surface antigenemia among patients with Schistosoma mansoni. Annals of Saudi medicine, 13(2), 121-125.

43. Gasim, G. I., Bella, A., & Adam, I. (2015). Schistosomiasis, hepatitis B and hepatitis C co-infection. Virology journal, 12(1), 1-6.

44. Corrao, G., Bernard, V., Zambon, A., et al. (2004). A meta-analysis of alcohol consumption and the risk of 15 diseases. Preventive medicine, 38(5), 613-619.

45. Mori, M., Hara, M., Wada, I., et al. (2000). Prospective study of hepatitis B and C viral infections, cigarette smoking, alcohol consumption, and other factors associated with hepatocellular carcinoma risk in Japan. American journal of epidemiology, 151(2), 131-139.

46. Chuang, S. C., Lee, Y. C. A., Hashibe, M., et al. (2010). Interaction between cigarette smoking and hepatitis B and C virus infection on the risk of liver cancer: a meta-analysis. Cancer Epidemiology and Prevention Biomarkers, 19(5), 1261-1268.

47. El–Serag, H. B., Hampel, H., and Javadi, F. (2006). The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clinical Gastroenterology and Hepatology, 4(3), 369-380.