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Hepatocellular carcinoma and possible related risk factors

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ABSTRACT

Aim: To identify the prevalence, related viral and non viral risk factors for hepatocellular carcinoma (HCC) in Egypt over last 15 years. **Methods:** All HCC patients attending Agouza Liver Center between January 1996 and December 2010 were enrolled in the study. Trend, demographic features of patients (age, gender, and residence), risk factors (HBsAg, HCV-Ab, schistosomiasis and others). **Results:** Over 15 years, 1 759 HCC patients out of 29 000 chronic liver disease patients were diagnosed with an overall proportion of 6.1%. The annual proportion of HCC showed a variation ranged from 5.3% to 6.5%. It was evident that the male proportion was significantly increased 84 % (P-value=0.0001). M/F was about 5:1 with high significant increase among age group of 45-60 years (P-value=0.0001). The distribution of viral hepatitis among HCC patients was: Hepatitis C virus (HCV) infection proportion was 89.8%, hepatitis B virus infection proportion was 1.8%, co infection of HCV and HBV proportion was 1.3%, none B none C proportion was 7.1%. As regards distribution of non viral causes among HCC patients: 8 (0.5%) patients suffered from autoimmune hepatitis, 94 (5.3%) cases were due to non alcoholic steatohepatitis (NASH), and the remaining cases were due to idiopathic causes. **Conclusion:** HCC is an increasing problem. The most related risk factors are viral hepatitis infections; older ages and male gender. NASH is a health problem that must be not ignored as a risk factor for developing HCC.

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KEYWORDS

Poisonous plant;
Toxicological;
Jambudia.

INTRODUCTION

Liver cancer rapidly reduces quality of life and typically causes death within 6 months-1 year from diagnosis.^[1] Globally, it is the fifth leading cause of cancer and the third leading cause of cancer death. This Cancer varies widely in incidence

throughout the world; HCC has increased sharply in the last 5–10 years^[2-4], with an especially high incidence in Egypt^[5]. HCC is the second most frequent cause of cancer incidence and mortality among men and the seventh in women during 2000–2002 in Egypt^[6]. The primary risk factors for hepatocellular carcinoma (HCC) are hepatitis

B virus (HBV), hepatitis C virus (HCV), dietary aflatoxin exposure, and chronic alcohol consumption^[1].

HCV mostly plays an indirect role in tumor development and appears to increase the risk of HCC by promoting fibrosis and cirrhosis^[6].

Although HBV is considered worldwide as a major risk factor for liver cirrhosis and HCC, the prevalence of HBV infection in Egypt has been declining over the last two decades. Elizabeth and Mark concluded that the prevalence of HBsAg among healthy population based samples was 6.7% ($\pm 1.4\%$), with no significant variation over time ($p = 0.59$). When these studies were separated according to age, the pattern was similar; however, prevalence was significantly higher among adults than children ($p < 0.0001$), likely a function of the introduction of the HBV vaccine.^[7] Integration of the viral DNA into host genome was suggested to be the initiating event for HBV-induced carcinogenesis or the HBx protein may inactivate p53 (tumor suppressor gene) leading to development of HCC^[2].

Significant variations occur in the risk for HCC and in the pathological and natural history of the disease. The pathways by which HCC develop are influenced by a variety of environmental and host factors such as the age or gender of the infected person and the genetic characteristics of the virus in cases of HBV or HCV infections. The role of other carcinogens, such as aflatoxin exposure, as additional risk factors for the development of HCC remain to be fully explored in Egypt despite evidence elsewhere that such exposures may damage^[8] the DNA in liver cells and lead to mutations in the p53 tumor suppressor gene.^[10] Critical evaluation of molecular markers of viral (HBV, HCV), environmental (aflatoxin) and genetic factors in well characterized HCC cases and appropriate control groups may provide an improved mechanistic understanding of hepatocarcinogenesis^[11].

Obesity is now widely recognized as a significant risk for the development of many types of cancers. A meta analysis^[10] found that the relative risks (RR) for liver cancer were 1.17 (95% CI = 1.02–1.34) for those who were overweight (BMI = 25–30) and 1.89 (95% CI = 1.51–2.36) for those who were obese (BMI=30).

Contrary to the number of reports on patients with chronic viral hepatitis, only very few cases

of patients with HCC developing due to autoimmune hepatitis (AIH) have been reported^[12].

PATIENTS AND METHODS

Study design

We conducted a retrospective cohort study of chronic liver disease patients by managing their data to examine the utilization and determinants of HCC surveillance.

Collection and management of data

The study was conducted at Agouza Liver Center (ALC) a private institute established to manage patients with liver disease. The center receives patients from almost all regions of Egypt. A specially designed database application was employed for collecting and managing data of the Egyptian patients attending the center during the period from January 1996 and December 2010. A clinical questionnaire was answered by every patient with stress on: age, gender, occupation, residence, history of exposure to known risk factors of viral hepatitis - blood transfusion and parenteral anti-schistosomal therapy, and laboratory findings. Chronic HCV was diagnosed based on elevated serum transaminase levels for at least six months and positive HCV antibody by the second-generation enzyme-linked immunosorbent assay and confirmed by detection of circulating HCV RNA using polymerase chain reaction (PCR) HBsAg was detected by ELISA test (ELISA-Abbot Laboratories), NASH was diagnosed using the following criteria^[13]. (1) histological features of steatohepatitis; (2) intake of less than 20 g ethanol per day; (3) absence of other liver diseases such as autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, and metabolic liver disease such as Wilson's disease and hemochromatosis; and (4) negative for hepatitis B surface antigen and antibody to hepatitis C virus (HCV) and/or negative for HCV RNA on polymerase chain reaction analysis.

The diagnosis of autoimmune hepatitis has been codified by an international panel, and these criteria must now be applied to all patients suspected to have the disease^[14] The *definite* diagnosis requires the exclusion of other similar diseases; laboratory findings that indicate substantial

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immune reactivity; and histologic findings of interface hepatitis. Data of HCC imaging were recorded from ultrasonographic and abdominal spiral computed tomographic (CT) reports.

Diagnosis of HCC was based on histopathological examination and/or detection of hepatic focal lesions by two imaging techniques (ultrasonography and dynamic CT) plus α -fetoprotein level above 200 ng/ml^[15].

Statistical analysis

Data are presented as mean and standard deviation (SD) and percentage. Statistical Package for social science (SPSS) version 13 was used to complete two-sided analyses of descriptive statistics. In the univariate analysis Chi-square test was used to evaluate the crude association between HCC and exposures: demographic and medical history. Odds ratio (OR) and 95% confidence intervals (CI) were calculated to determine the risk and incidence of occurrence of HCC. $P < 0.05$ was considered significant.

RESULTS

29000 patients were enrolled in the current study, 21515 males and 7485 females, their ages range from 30 to 75 years (mean \pm SD = 52.3 \pm 10.67). All suffered from chronic liver disease. They attended ALC during the period from January 1996 and December 2010. 26032 patients suffered from chronic HCV infection, 511 patients suffered from chronic HBV infection, 396

patients suffered from chronic co-infection of HCV and HBV while the remaining 2061 patients suffered from non-viral causes.

The frequency of HCC among the all studied patients was 6.06%. It was noticed that prevalence of HCC ranged from 5.3% to 6.4% along the last 15 years without any significant differences as shown in TABLE 1. It was found that frequency of HCC was significantly increased among patients with ages ranged from 45 to 59 years when compared to other age groups ($p=0.0001$) as shown in TABLE 2 and Figure 1.

TABLE 1: Distribution of HCC cases through 15 years.

| Year | Total Registered cases | HCC cases No (%) |
|-------|------------------------|------------------|
| 1996 | 1922 | 102 (5.31) |
| 1997 | 1961 | 106 (5.41) |
| 1998 | 1934 | 106 (5.48) |
| 1999 | 1957 | 110 (5.62) |
| 2000 | 1965 | 119 (6.06) |
| 2001 | 1888 | 117 (6.20) |
| 2002 | 1846 | 115 (6.23) |
| 2003 | 1846 | 118 (6.39) |
| 2004 | 1877 | 118 (6.29) |
| 2005 | 1966 | 125 (6.36) |
| 2006 | 1890 | 122 (6.46) |
| 2007 | 1943 | 122 (6.28) |
| 2008 | 1967 | 126 (6.41) |
| 2009 | 1979 | 124 (6.27) |
| 2010 | 2059 | 129 (6.27) |
| Total | 29000 | 1759 (6.06) |

TABLE 2 : Comparison between patients with and without HCC in relation to age, sex, etiology of CLD.

| | HCC cases N =1759 | Non HCC cases N =27241 | Odds ratio OR (confidence interval) | X ² value | P value |
|------------------------|----------------------|---------------------------|----------------------------------------|----------------------|---------|
| Age groups : | | | | | |
| 30-44 years no (%) | 349 (19.8) | 14250 (52.3) | Reference | | |
| 45-59 years no (%) | 1018 (57.9) | 10321 (37.9) | 1.8(1.7-1.8) | 758.8 | 0.0001* |
| 60-75 years no (%) | 392 (22.3) | 2670 (9.8) | 3.4(3.1-3.6) | | |
| Sex: | | | | | |
| Males no (%) | 1478 (84.1) | 20342 (74.7) | 1.8(1.5-2.05) | 80.8 | 0.0001* |
| Females no (%) | 281 (15.9) | 6899 (25.3) | Reference | | |
| Causes | | | | | |
| HCV no (%) | 1579 (89.8) | 24453 (89.8) | 1.0(0.9-1.0) | .000 | NS |
| HBV no (%) | 31 (1.8) | 480 (1.8) | 1.0(0.7-1.3) | | |
| HCV+HBV no (%) | 24 (1.4) | 372 (1.4) | 1.0(0.6-1.4) | | |
| Non B Non C no (%) | 125 (7.1) | 1936 (7.1) | 1.0(0.9-1.0) | | |
| Schistosomiasis Cases: | | | | | |
| +ve no (%) | 1116 (63.4) | 22433 (82.4) | 2.07(1.9-2.2) | 386.9 | 0.0001* |
| -ve no (%) | 643 (36.6) | 4808 (17.6) | Reference | | |

Males carry a significant risk to develop HCC when compared to females; (OR: 1.8, CI: 1.5-2.05) as shown in TABLE 2 and Figure 2 (p=0.0001).

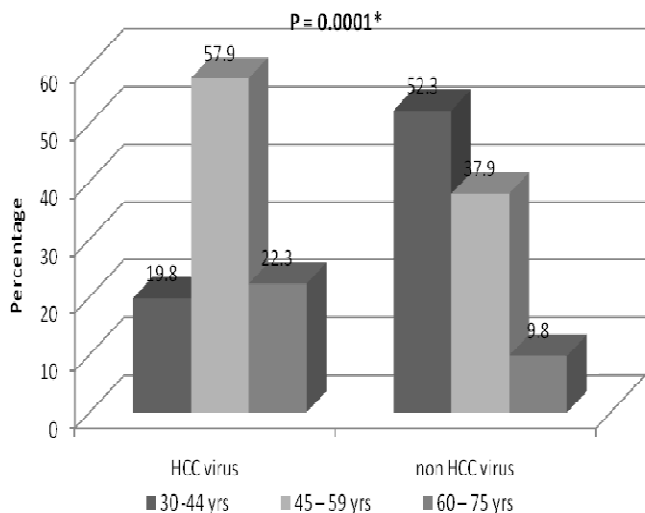


Figure 1 : Comparison between patients with and without HCC in relation to age.

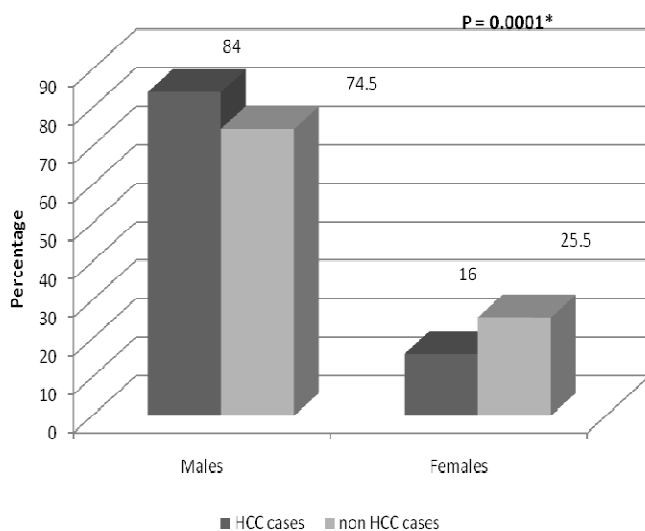


Figure 2 : Comparison between patients with and without HCC in relation to sex.

As shown in TABLE 2, the distribution of HCV; HBV; HCV and HBV and non viral causes was the same among patients with and without HCC. Although HCV represents the highest percentages among patients with HCC, we found that frequencies of HCC among patients with HCV; HBV; co infection of HCV and HBV were the same (6.10%), while the frequency of HCC among patients with non viral causes was the lowest (0.70%) as shown in Figure 4.

As regards distribution of non viral causes among HCC patients: 8 (0.5%) patients suffered

from autoimmune hepatitis, 94 (5.3%) cases were due to non alcoholic steatohepatitis (NASH), and the remaining cases were due to idiopathic causes. Schistosomiasis was significantly higher among patients without HCC as shown in TABLE 2 and Figure 3(p=0.0001).

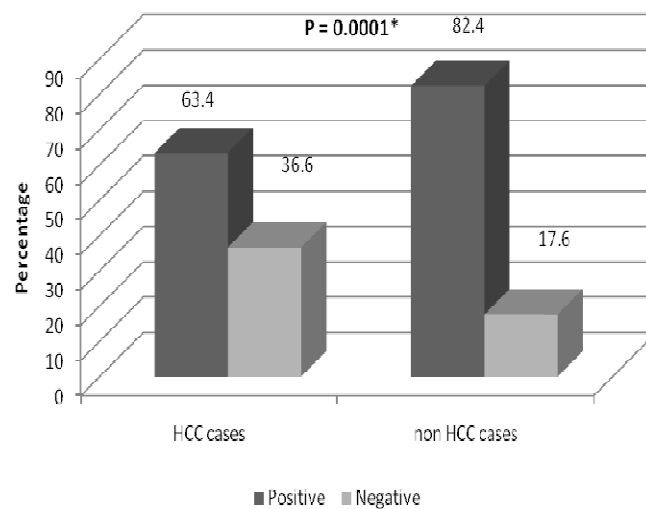


Figure 3 : Comparison between patients with HCC and non HCC in relation to schistosomiasis.

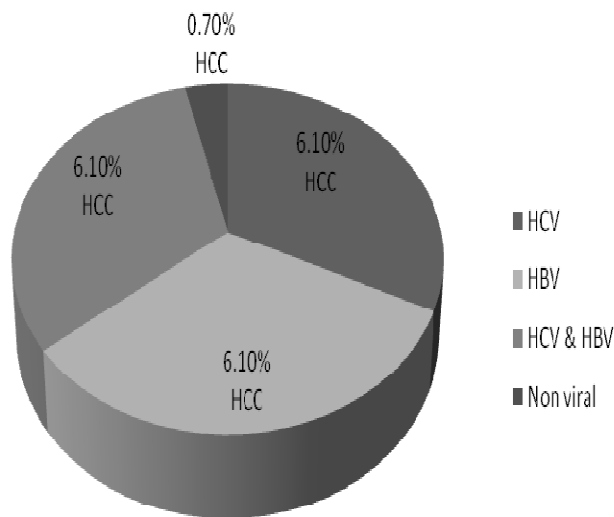


Figure 4 : Frequency of HCC among registered patients with chronic liver diseases.

Single focal lesions were detected among 1028 (58.4%) HCC cases, 790(44.9%) of single focal lesions were detected in right lobe of the liver while 238 (13.5%) lesions were detected in left lobe of the liver. Multiple focal lesions were detected in 731(41.6%). Frequency of multiple focal lesions in right lobe was 23.6 % (415 cases), while it was 1.9% (34 cases) in left lobe of the liver. Multiple focal lesions were detected in 282 cases (16.0%).

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DISCUSSION

In Egypt, it was noticed that HCC is an increasing health problem which threatens millions of Egyptian population due to the high prevalence of HCV infection. So that, we aim to update our Figures which are related to HCC as prevalence and the associated risk factors.

This rising incidence of HCC in Egypt may be explained by the increasing prevalence of risk factors such as the prevalence of hepatitis C virus (HCV)^[9], the contribution of HBV infection, and improvements in screening programs and diagnostic tools^[16], as well as the increased survival rate among patients with cirrhosis allowing time for some of them to develop HCC.

Analysis of age distribution among HCC patients revealed that the most predominant age group (45-59 years). Velazquez et al.^[4], found that cirrhotic patients older than 54 years are at four times greater risk to develop HCC. Asahina and his colleagues concluded that Aging is becoming the most critical risk factor for the development of HCC. Although liver fibrosis was also an important risk factor, they clearly demonstrated that the risk for hepatocarcinogenesis after interferon treatment was significantly higher in older patients at each stage of liver fibrosis except for cirrhosis. Hence, physicians should be aware that older patients can develop HCC regardless of the stage of fibrosis^[17].

In the current study, we found that the ratio between men and women for development of HCC was about 5:1 times which is confirmed by findings of previous study as they concluded that the risk of HCC is 2-7 times higher in men than in women, although this ratio varies across the world.^[18] The explanation for this sex difference might be threefold: firstly, men could have higher rates of environmental exposure to liver carcinogens (such as smoking or alcohol) and hepatitis virus infections; secondly, estrogen effects might suppress interleukin (IL)-6-mediated inflammation in women, reducing both liver injury and compensatory proliferation; thirdly, testosterone effects could increase androgen receptor signaling in men, promoting liver cell proliferation^[19].

Unsurprisingly, we found that the most prevalent cause for HCC development was HCV. This is referred to Egypt's high levels of HCV with different geographic distribution; individuals liv-

ing in rural areas had significantly more anti-HCV seropositivity than those in urban areas (36.1% and 24.7%, respectively). Also individuals living in Cairo and seashore governorates had lower HCV seropositivity (14.7% and 12.7%, respectively) than those living in governorates of Upper Egypt and Lower Egypt (29.3% and 36.3%, respectively)^[9].

We are in need to develop a program for prevention of HCV infection based on learning the modes of HCV transmission in Egypt. The major identified risk factors for acquisition of HCV in

Egypt include intravenous injection, body piercing and invasive health care practice (surgeries catheterization dental practice, etc.) and occupational exposure^[16]. For many individual cases, however, there is no identifiable risk factor, highlighting the critical need for further research on this issue. Due to the large reservoir in this population, HCV is likely to remain prevalent in Egypt for several decades^[20].

Although our patients were above age of 30 years which means that they were not vaccinated against hepatitis B virus, we found that prevalence rates of hepatitis B virus infection alone or accompanied with HCV infection were low. Previous study, published in 2010, showed that prevalence rates have decreased in Egypt < 2%.^[21] A study of village populations in Egypt, published in 1985, revealed an overall HBV prevalence rate of 11.7% and a higher prevalence rate of 20.8% in young adults between the ages of 14-18 years. In these populations, antibodies to hepatitis B core antigen (anti-HBc) were found in nearly 90% of both men and women. Epidemiologic studies have demonstrated a strong association between HBV and HCC. Thus, the incidence of HCC increases parallel the prevalence of HBsAg in all geographic areas.^[22] Generally, in patients who are infected in the first year or two year of life, the interval between initiation of chronic infection and the peak incidence of HCC is 30-50 years^[23,24].

It was noticed that prevalence of HCC was equal among patients with HCV, HBV and co infection HCV and HBV. This is an adding evidence for the great link between viral hepatitis and development of HCC.

A great finding in the current study which must be focused on is the prevalence rate of non viral causes among the studied population. We must do our best to discover these causes in a trial

for HCC prevention program. In this study, viral hepatitis markers were negative in 7.1% of the patients with and without HCC suggesting other strong etiologic factors. El-Serag (2002) found 14.5% of his patients remain without specific risk factors. This could be explained in part by the development of mutant or occult viral infection, or exposure to other risk factors such as aflatoxins and alcohol abuse.^[31] Mohamed et al.^[25] detected a significant higher percent of aflatoxins in the serum of Egyptian patients with HCC compared to their controls; with a twofold increased risk. Aflatoxins may cause mutations in the tumor suppressor gene p53 that act as initiating agents leading to liver cell hyperplasia and HCC. In support of this hypothesis, Kafrawy et al.^[26] documented the presence of p53 codon 249 mutations associated with aflatoxin exposure in a sample of HCC tumor tissues analyzed by gene chip analysis in Egypt.

Another important finding in the current study was that the etiology of hepatocarcinogenesis in 5.3% of HCC patients was NASH. Since cirrhosis is the main risk factor for hepatocellular carcinoma, liver cancer could be simply a complication of end-stage NASH, similar to the situation encountered in other chronic fibrosing liver diseases. However, accumulating evidence suggests that hepatocarcinogenesis may also be related to earlier stages of NAFLD. Case series of patients with HCC and NAFLD as the only identified risk factor strongly suggest that hepatocarcinogenesis is part of the natural history of NAFLD. Based on the known association of NAFLD with insulin resistance and metabolic syndrome. Considering the rapidly increasing prevalence of both conditions in affluent societies, and their significance in the pathophysiology of NASH, a rising incidence of NASH and its complications including HCC can be expected in the mid-term future. Therefore, it is particularly worrying that the most persuasive evidence for an association between NASH and HCC derives from studies on the risk of HCC in patients with metabolic syndrome^[27].

Surprisingly, we found that the frequency of schistosomiasis was significantly increased among patients without HCC. Several studies have postulated that the HCV epidemic in Egypt has disproportionately affected rural populations, which should be reflected in the distribution of HCC cases.^[28] The HCV epidemics in Egypt is

unique. Egypt developed the world's highest rates of HCV infection over a short period of time, largely due to a massive public health campaign. The vast majority of infections among individuals aged 30 years and older can be explained by parenteral anti-Schistosomiasis therapy (PAT) and other iatrogenic exposures.^[29] The anti-Schistosomiasis campaign extended from the 1950's to the 1980's, with peak worked its way through. This suggests that the true burden of liver cancer in Egypt has yet to be realized. While the role of hepatic schistosomiasis has long been controversial, the prevailing view today is that it has limited influence in the etiology of HCC in Egypt. Epidemiological studies of HCC clearly identified HBV, HCV, or HBV/HCV co infection as important, but schistosomiasis could not be identified as a statistically significant independent risk factor^[15].

It was found that few cases of HCC were due to autoimmune liver disease, this is consistent with previous studies.^[12] Several mechanisms explaining the development of HCC from autoimmune liver diseases have been proposed: enhanced progression to cirrhosis through progressive autoimmune hepatitis, decreased antitumor immune responses caused by long-term administration of steroids and immunosuppressants^[30].

Although it is difficult to accurately predict future changes in disease epidemiology, some experts have suggested that the overall global incidence of HCC will continue to rise in the next few years until a plateau is reached in 2015–2020^[31] Subsequent decreases in the rates of

HCC have been predicted, resulting, at least in part, from expected improvements in the control of HBV and HCV infection.^[32] However, as the contributions of HBV and HCV diminish, risk factors such as diabetes and obesity may become increasingly important drivers of future HCC incidence trends.

CONCLUSION

HCC is an increasing^[27] problem. The most related risk factors are viral hepatitis infections; older ages and male gender. NASH is a health problem that must be not ignored as a risk factor for developing HCC. Schistosomiasis has no any significant role in developing HCC. Further studies are required to detect other risk factors like aflatoxins, exposure to environmental toxins, occult or

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mutant viral infections.

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