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ORIGINAL ARTICLE

Virological Profiles of HBV and HCV in Hepatocellular Carcinoma in Egypt and Yemen

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ABSTRACT

Background: *Hepatocellular carcinoma (HCC) is one of the very frequent malignancies that has poor prognosis. Chronic hepatitis B is the most important risk factor in the world particularly in developping countries. In developed world, HCC occurs in the background of chronic infection with HCV or alcohol abuse. Several factors affects the development of HCC in chronic hepatitis patients including viral genotype.* ***Objectives:*** *The aim of this study was to determine HCV and HBV viral profile in Egyptian and Yemeni HCC patients. Additionally, HCV and HBV were genotyped to investigate any possible correlation with HCC development.* ***Methodology:*** *Thirty HCC Egyptian patients admitted to the Hepatology Unit, Medical Research Institute, Alexandria University and thirty HCC Yemeni patients attending the oncology center in Al-Gomhory hospital in Sanaa have been enrolled in this study. Hepatitis B surface Ag (HBs-Ag) was not detected in Egyptian HCC patients but in 36.7% of Yemeni HCC patients. On the other hand, anti-HCV antibodies were detected in 93.3% of the Egyptian HCC patients and in 16.7% only in Yemeni patients. HBV genotyping by direct sequencing of pol gene showed 11 cases of genotype D and 1 case of genotype E among HCC Yemeni patients. HCV genotyping by sequencing of NS5b and 5'UTR showed 21 cases of genotype 4 and 2 cases of genotype 1 and genotype 2 among Egyptian HCC patients.* ***Conclusion:*** *From the current study HCC appears to be significantly associated with HBV and HCV chronic infections in Yemen and Egypt respectively. In Egypt, occult HBV co-infection might escalate the danger of HCC development among HCV patients.*

INTRODUCTION

Hepatocellular carcinoma (HCC) is considered one of the most common primary malignant tumors of the liver in the world [1]. It is rated as the fifth and seventh most common cancer among men and women respectively [2]. It has poor prognosis and ranked as the third leading cause of cancer mortality in the world [1].

Several risk factors have been established for HCC, the pattern of major risk factors varies from one country to another [3]. Chronic hepatitis B infections (CHB) with or without exposure to aflatoxin are accused for more than 70% of HCC cases in hepatitis B virus (HBV) endemic areas [3]. On the other hand, liver cirrhosis developed from chronic hepatitis C infections (CHC) and alcohol abuse is the main background for HCC in developed countries [4].

HCC accounts for about 4.7% of chronic liver diseases in Egypt [5]. It is the second most prevalent cancer among Egyptian men. Hospital based studies in Egypt have showed a rise in the relative frequency of all liver-related cancers from 4% in 1993 to 7.3% in 2003 [6]. This increase was attributed to the emergence of additional risk factors over the same period of time such as HCV and the contribution of HBV infection in addition to the improvement in the diagnostic tools and screening programs of HCC [5].

Before the implementation of HBV vaccine, CHB was widespread and considered the dominant etiologic factor in HCC development. Childhood HBV immunization in Egypt is estimated at 95-100%, therefore HBV-induced HCC is expected to drop regularly over the coming decades [7].

To the best of our knowledge, no published data are available concerning the epidemiology of HCC in Yemen, however a single retrospective study in a medical center reported the admission of 251 confirmed HCC cases over an 8-year period [8].

HBV infection causes injury of liver cells and necroinflammation, followed by hepatocyte proliferation, fibrosis, and cirrhosis. The continuous regeneration during cirrhosis results in increased hepatocyte turnover and accumulation of mutations in its genome that may lead to genetic alterations, chromosomal rearrangements, activation of oncogenes activation, and tumor suppressor genes inactivation [9]. Nevertheless, HBV can induce HCC without liver cirrhosis. HBV can integrate its DNA into host genome acting as a mutagenic agent, causing secondary chromosomal rearrangement and increasing genomic instability [10]. Additionally, HBx is considered to transactivate genes participating in the control of cell proliferation, leading to stimulation of the protein kinase C and nuclear factor kappa B ($NF_{\kappa B}$) pathways, and deregulation of cell cycle control and interference with cellular DNA repair and apoptosis [11].

An important risk factor for HBV-mediated HCC is the presence of HBe-Ag and the amount of HBV in serum (viral load) [12]. Previous reports have proposed that the HBV genome characteristics, including genotypes and certain genetic mutations, are associated with the development of HCC [13, 14]

Being an RNA- virus, HCV is unable to integrate into host genome. Thus HCV is thought to develop HCC via indirect pathways only. Chronic HCV infection can result in cell death, proliferation, and cirrhosis[9]. Additionally, viral protein-host cell interactions may have a role in the development of HCC independent from cirrhosis [15]. Although these interactions are accused in the course of chronic infection and therefore the development of fibrosis and cirrhosis, it was hypothesized that such interactions can play a role in HCC pathogenesis by inducing instability of cell cycle, upregulating oncogenes and losing tumor-suppressor gene functions [15].

HCV genome shows clear heterogeneity due to accumulation of mutations during replication. Six main HCV genotypes and many subtypes were identified by phylogenetic analysis of NS5B and E1 viral regions [16]. In Egypt, HCV-4 has the highest prevalence[16,17]. Although HCV genotypes have been associated with different severity and outcome of the liver disease, the association between different genotypes and subtypes and the development of HCC is not yet confirmed [17].

The aim of this study was to determine HCV and HBV viral profile in Egyptian and Yemeni HCC patients. Additionally, HCV and HBV were genotyped to investigate any possible correlation with HCC development.

METHODOLOGY

Patients:

Thirty HCC Egyptian patients admitted to the Hepatology Unit, Medical Research Institute, Alexandria University and thirty HCC Yemeni patients attending the oncology center in Al-Gomhory hospital in Sanaa have been enrolled in this study. Informed consent was signed by all patients and the study protocol was approved by the institute ethics committee.

HCC was diagnosed by Triphasic CT and Alfa-feto protein (AFP) level. Relevant information were collected from each patient including personal data (Age, gender, smoking, Khat chewing, alcohol consumption) as well as health data (history of blood transfusion, history of parenteral anti-schistosomal therapy, previous surgical interference and dentistry).

Blood samples were collected from all patients and separated sera were stored as aliquots at -20°C and -80°C for further investigations.

Detection of HCV and HBV viral Markers

Commercially available ELISA kits were used according to the manufacturer's instructions to detect antibodies against HCV (Abbott, Murex), HBs-Ag (Abbott, Murex), HBe-Ag (Diasorin), Anti-HBe-antibodies (Diasorin) and Anti-HBc-antibodies (CTK Biotech, Inc).

Nucleic acid extraction

QIAamp® Viral RNA Mini and QIAamp® DNA Mini kits (Qiagen) were used to extract HCV-RNA and HBV-DNA respectively using the spin protocol according to the manufacturer's recommendations.

Nucleic acid amplification and detection

Unless otherwise specified, all polymerase chain reaction (PCR) reagents were obtained from Promega (Madison, WI, USA). All primers used during this study are listed in. (Table 1)

Table 1: Sequence of primer pairs used for PCR

Gene	Primer	Sequence 5' to 3'	Position	T_H °C	Ref
HCV-5' UTR	NF5 (outer sense)	GTG AGG AAC TAC TGT CTT CAC GCA G	47-71	50	[18]
	NR5 (outer antisense)	TGC TCA TGG TGC ACG GTC TAC GAG	324-348		
	KF2 (inner sense)	TTC ACG CAG AAA GCG TCT AG	63-82	50	
	NR4 (inner antisense)	CTA TCA GGC AGT ACC ACA AGG	279-299		
HCV-NS5B	JA 230 (outer sense)	CTA CCA TCA TGG CTA ARA AYG AGG T	8008-8032	50	[17]
	JA 233 (outer antisense)	ATG ATG TTA TGA GCT CCA RGT CRT A	8663-8687		
	JA 231 (inner sense)	TAT GAY ACC CGC TGY TTT GAC	8256-8276	50	
	JA 232 (inner antisense)	CCT GGT CAT AGC CTC CGT GAA	8616-8636		
HBV-*s*	S-sense	AGA ACA TCG CAT CAG GAC TC	159-178	55	[19]
	S- antisense	CAT AGG TAT CTT GCG AAA GC	623-642		
HBV-*c*	C-sense	CTG GGA GGA GTT GGG GGA	1730-1747	55	[19]
	C- antisense	GTA GAA GAA TAA AGC CC	2487-2503		
HBV-*x*	X-sense	CTA GCC GCT TGT TTT GCT CG	1282-1301	55	[19]
	X- antisense	TTA TGC CTA CAG CCT CCT AG	1647-1666		
HBV-*pol*	HBPr 134 (outer sense)	GCT GCT ATG CCT CAT CTT C	414-433	45	[20]
	HBPr 135 (outer antisense)	CAR AGA CAA AAG AAA ATT GG	803-822		
	HBPr 75 (inner sense)	CAA GGT ATG TTG CCC GTT TGT CC	455-477	45	
	HBPr 94 (inner antisense)	GGY AWA AAG GGA CTC AMG ATG	775-795		

T_H = hybridization temperature

Extracted RNA was first reversed transcribed followed by nested PCR to detect HCV-RNA in patients' sera. The primers were derived from the conserved 5' untranslated region (UTR). The nested primers amplify a 237 base pair (bp) amplicon which was detected by ethidium bromide-stained gel electrophoresis.

For genotyping purpose, NS5B gene in HCV-RNA positive patients was amplified using nested PCR degenerate primers.

For the detection of HBV-DNA in patients' sera, Taqman Probe® real-time PCR (Artus® HBV TM RG RCR assay, Qiagen, Hamburg, Germany) was used according to the manufacturer instructions.

For genotyping purpose, *pol* gene in HBV-DNA positive patients was amplified using nested PCR primers.

To detect the presence of HBV-DNA, SYBR green real time PCR (Applied Biosystems) was used to amplify HBV-DNA using primers-pairs specific for *s, c* and *x* genes of HBV.

DNA sequencing and sequence analysis

The amplified HCV-NS5B and HBV-*pol* gene regions were sequenced using ABI PRISM® 310 genetic analyzer and its sequencing software. For HCV-RNA positive patients, where NS5B gene was not amplified, the amplified 5' UTR was sequenced for genotyping purpose.

Statistical Analysis

Statistical analysis was carried out using SPSS statistics software version 23. Qualitative data was expressed by numbers and percent. Pearson Chi-square was used to test the association between the categorical variables. Odds ratio and 95% CI were estimated to determine the factor contributing most to HCC infection coupled with z test for pairwise comparison. In all other applied statistical tests of significance, P value (< 0.05) was considered significant.

RESULTS

The present study group included 30 Egyptian and 30 Yemeni HCC patients. Among the 30 Egyptian HCC patients 76.6% were males and 23.3% were females with a male to female ratio of 3.3:1. In Yemeni patients, 63.3% were males whereas females represented only 36.7% with a lower male to female ratio of 1.7:1 (Figure 1).

Only 10% of both Egyptian and Yemeni patients were aged over 70. Yemeni patients showed a significant higher prevalence at younger ages, where 20% were younger than 40 in comparison to 6.7% of the Egyptian patients (table 2 & figure 1)

Table 2: Distribution of Egyptian and Yemeni HCC patients according to age and sex

	Egyptian HCC				Total (n1=30)		Yemeni HCC				Total (n2=30)		X^2	*P*
	male ($n1_a$= 23)		Female ($n1_b$= 7)				Male ($n2_a$= 19)		Female ($n2_b$= 11)					
	No	%	No	%	No	%	No	%	No	%	No	%		
Less than 40	0	0	0	0	0	0	2	10.5	4	36.3	6	20	4.63	0.032*
40-49	2	8.7	0	0	2	6.7	5	26.3	1	9.1	6	20	1.298	0.255
50-59	10	43.5	4	57.1	14	46.7	4	21.1	5	45.5	9	30	1.128	0.288
60-69	9	39.1	2	28.6	11	36.6	6	31.6	0	0	6	20	1.313	0.252
more than 70	2	8.7	1	14.3	3	10	2	10.5	1	9.1	3	10	0.185	0.667

**p value was considered significant < 0.05 using Pearson chi square*

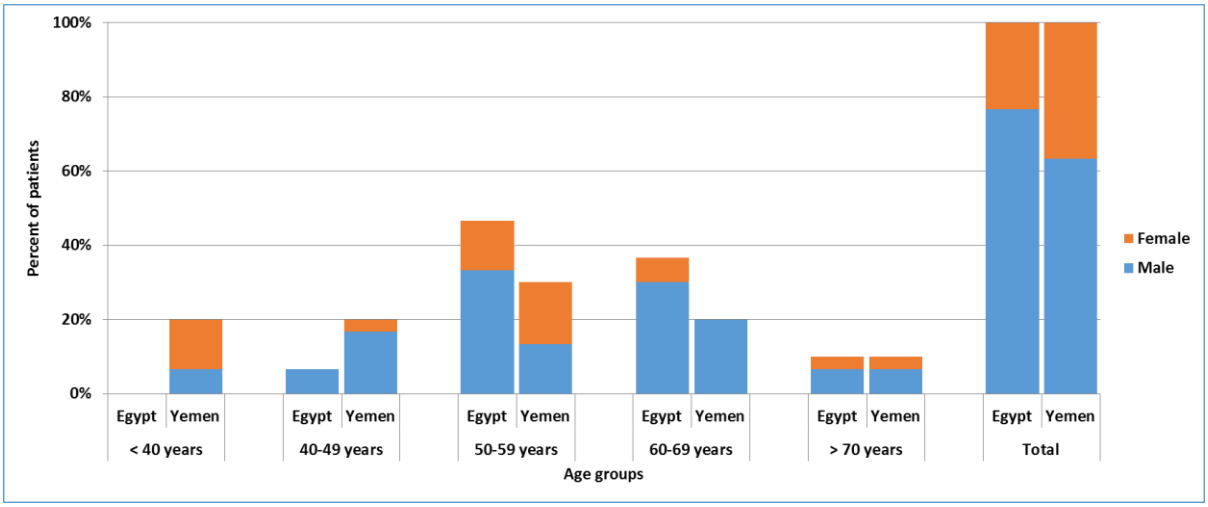

Fig. 1: Distribution of Egyptian and Yemeni HCC patients according to age and sex

Among all non-viral risk factors, only history of parenteral anti-schistosomal therapy (PAT) and khat chewing were statistically significant among Egyptian and Yemeni patients respectively (

Table 3).

Table 3: Exposure to risk factors among Egyptian and Yemeni HCC patients

	HCC Egyptian Patients n1=30		HCC Yemeni Patients n2=30		Z	Odds Ratio & 95% CI	P
	No	%	No	%			
PAT	15	50	5	16.6	2.634	5.00 (1.51 -16.56)	0.008**
Khat Chewing	0	0	23	76.7	3.535	0.01 (0.00 -0.096)	0.004
Smoking	15	50	15	50	0.00	1.00 (0.36 - 2.75)	1.00
Surgery	7	23.3	9	30	0.583	0.70 (0.20 -2.24)	0.56
Dental Intervention	5	16.6	9	30	1.207	0.46 (0.13 -1.62)	0.227
Blood transfusion	5	16.6	3	10	0.752	1.80 (0.39 -8.32)	0.452

**p value was considered significant < 0.05 using Pearson chi square*

**PAT is the most contributing risk factor for HCC infection (odds ratio=5.00, 95% CI [1.51-16.56]) using z test.

Testing HCV as well as HBV viral markers showed that, 93.3% of Egyptian HCC patients were anti-HCV positive and HCV-RNA was positively detected in 86.7% of cases using Taqman probe real-time PCR. On the other hand, anti-HBc were detected in only 60% of patients, of them none showed HBs-Ag seropositivity. In the Yemeni group, anti-HBc and HBs-Ag were detected in 70% and 36.7% of patients respectively. Additionally, HBe-Ag was detected in 13.3% of patients. Anti-HCV was detected in only 16.7% of Yemeni patients (figure 2).

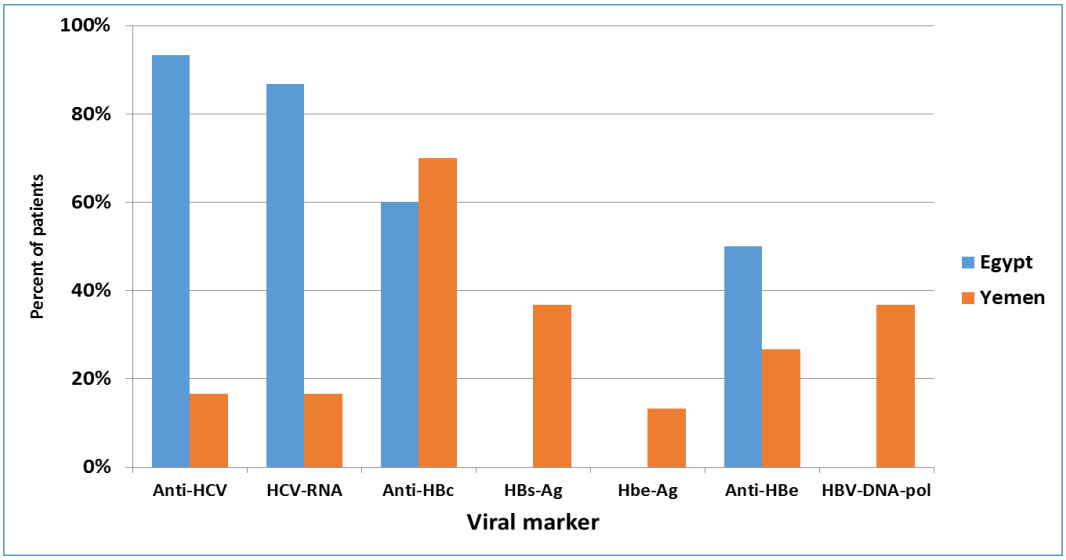

Fig. 2: HBV/HCV viral profile among Egyptian and Yemeni HCC patients

The presence of Anti-HCV antibodies and HCV-RNA was found to be significantly correlated with HCC among Egyptian patients. On the other hand, a significant correlation was detected among Yemini HCC patients with active HBV infection represented as HBs-Ag seropositivity and presence of HBV-DNA (

Table 4).

Table 4: HBV/HCV viral profile among Egyptian and Yemeni HCC patients

	HCC Egyptian Patients n1=30		HCC Yemeni Patients n2=30		X^2	*p*
	No	%	No	%		
HCV Ab	28	93.3	5	16.7	32.59	0.0001*
HCV RNA	27	90	5	16.7	29.53	0.0001*
HBs Ag	0	0	11	36.7	11.13	0.0008*
HBV DNA	0	0	11	36.7	11.132	0.0008*

p value was considered significant < 0.05 using Pearson chi square

For genotyping purpose, HCV-NS5b gene was amplified using nested PCR (Figure 3) followed by DNA sequencing. In cases where HCV-NS5b was not successfully amplified, 5' UTR was sequenced instead (figure 4). Twenty one cases out of 26 HCV-positive Egyptian patients showed infection with HCV-4

genotype; most of them were subgenotyped as HCV-4a (figure 5).

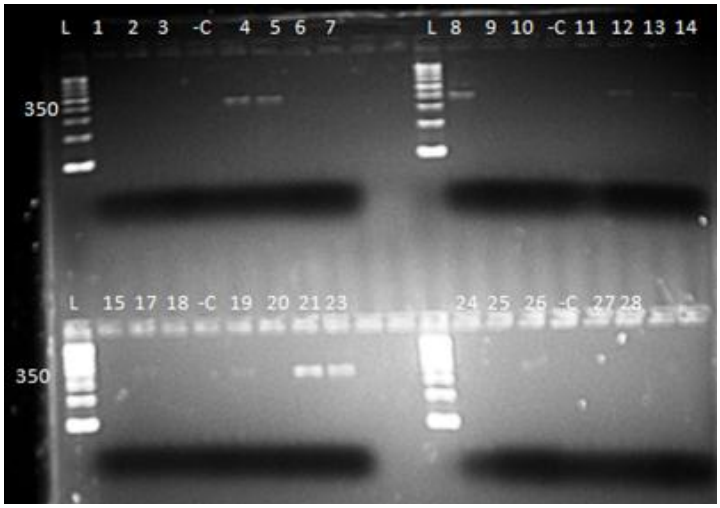

Fig. 3: Ethidium bromide stained agarose gel showing PCR amplification of HCV-NS5B gene. (Amplicon has size of 350bp, -c negative control)

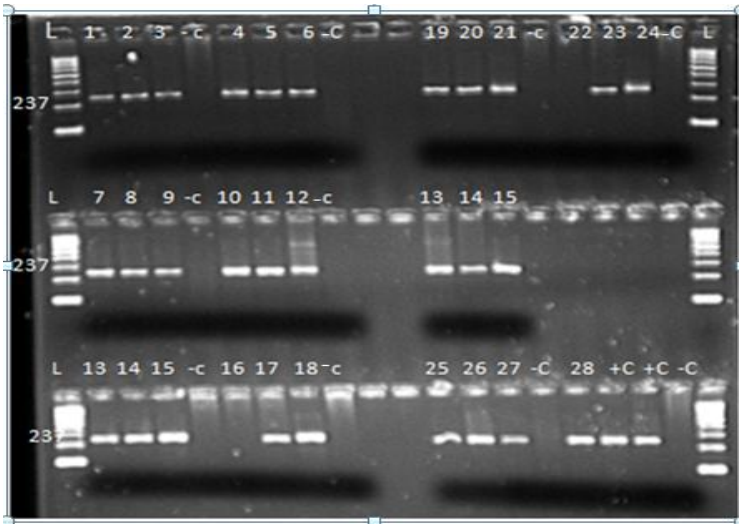

Fig. 4: Ethidium bromide stained agarose gel showing PCR amplification of HCV-5'UTR. The size of the amplicon is 237bp. (+c positive control, -c negative control)

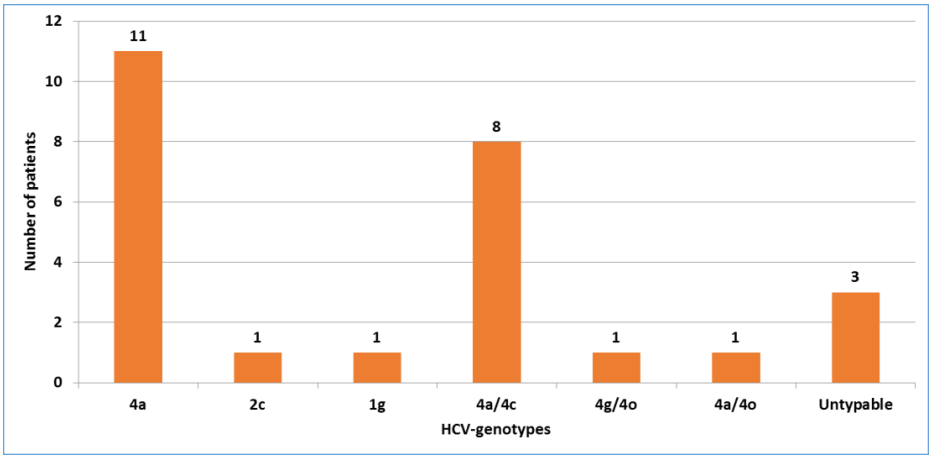

Fig. 5: HCV genotyping and sub-genotyping of 26 Egyptian HCC patients by sequencing of NS5a and 5' UTR regions

Amplified *pol* gene (figure 6) was used for genotyping of HBV infections in Yemeni patients, where 10 out of 11 HBV-*pol* positive patients were HBV-D genotype and only one patient showed HBV-E genotype (figure 7).

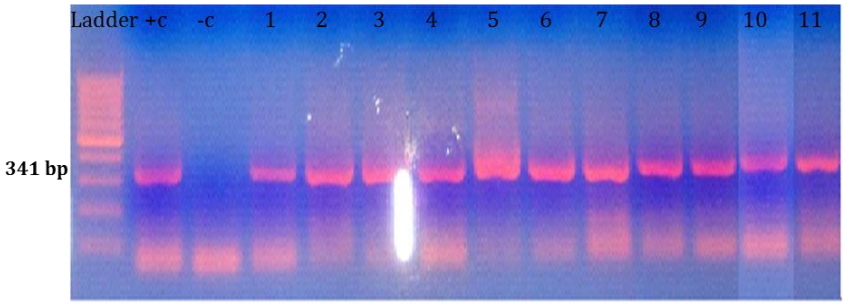

Fig. 6: Ethidium bromide stained agarose gel showing amplification of HBV-*pol* gene by PCR. (Amplicon size 341 bp, +c positive control, -c negative control)

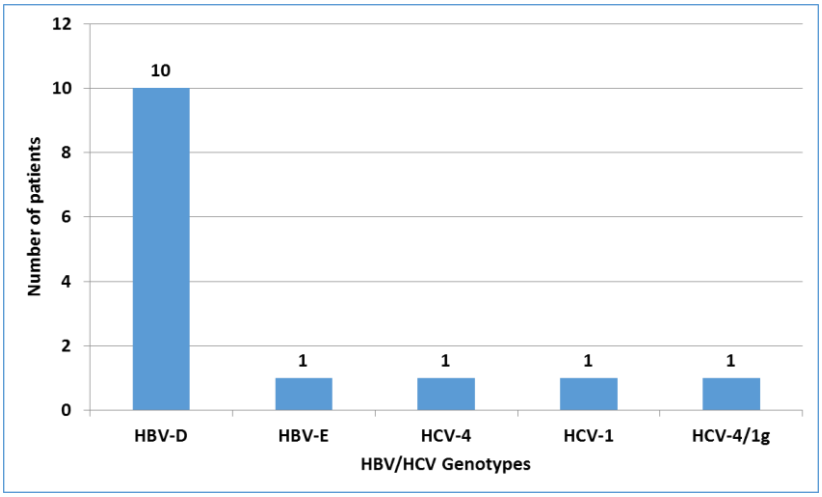

Fig. 7: HBV and HCV genotyping in HCC Yemeni patients by direct sequencing HBV-*pol* and HCV-5' UTR regions

Detection of HBV *s*, *c* and *x* genes by SYBR green real time PCR were positive in 77.7%, 55.5% and 83.3% of Egyptian patients and 36.7%, 36.7% and 30% of Yemeni patients respectively (Figure 8).

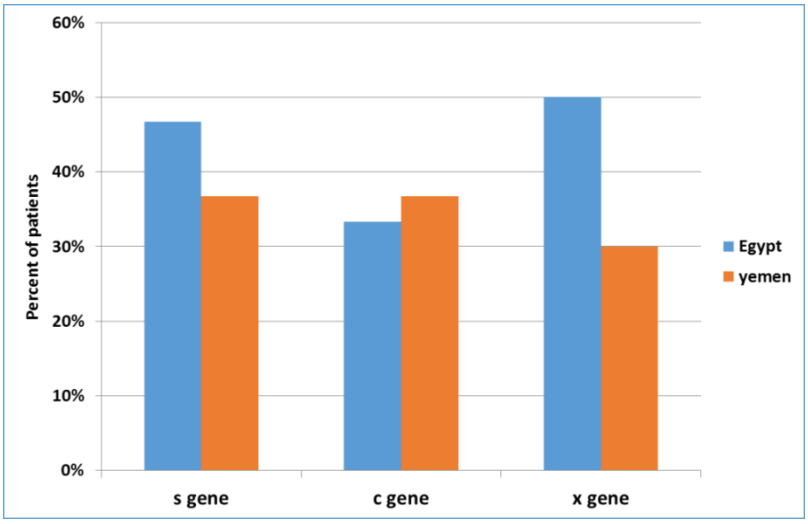

Fig. 8: Distribution of *s*, *c*, *x* genes in Egyptian and Yemeni HCC patients

DISCUSSION

In Egypt liver cancer constitutes 13% of all cancers and is considered the second most common malignancy after bladder cancer in males and breast cancer in females[21]. On the other hand, HCC is the sixth commonest cancer in Yemen according to Aden cancer registry report [22]. In 2008, GLOBOCAN ranked HCC as the most common cancer in Yemen among newly diagnosed men with an incidence and mortality of 11.8% and 13.9% respectively [8].

Generally, incidence of liver cancer is higher two to four times in males than in females. This may be a result of sex-specific differences in exposure to risk factors. Males are more likely infected with viral hepatitis, consume alcohol, smoke cigarettes and have increased liver iron stores. Nevertheless, the large difference between male and female incidence rate no longer occurs among high-risk HCC populations like China, Japan and Korea [23].

Previous studies showed high male to female incidence ratio of 7:1 and 4:1 in Egypt and Yemen respectively [5,8]. Among the 30 Egyptian HCC patients included in this study, 23 patient (76.6%) were males with male to female ratio of 3.3:1, while in the 30 Yemeni patients, 19 (63.3%) out of 30 were males with a still lower ratio of 1.7:1.

Generally, the peak age of female incidence rates is 5 years more than that of male rates In low-risk population, the highest age-specific rates occur among patients aged 80 years and older [23]. Different age pattern was found in Egypt and Yemen since about 90% of the 30 Egyptian HCC patients were younger than 70 years and 70% of the Yemeni patients were below 60 years.

Beside viral hepatitis, the exposure to other risk factors for HCC was investigated during this study. An exclusive risk factor for the indirect development of HCC in Egypt was attributed to schistosomiasis and its parenteral therapy, which played a role in the transmission of HBV and HCV during mass-treatment campaigns [24].

History of PAT was found in 50% out of the Egyptian patients, 86.7% of them were positive for HCV. Interestingly, patients with negative PAT history showed 100% HCV seropositivity indicating that high incidence rates of HCV is continuing despite of the termination of the PAT campaigns. This is in part due to the large reservoir of infected individuals, which increases the potential for continued transmission.

It was suggested that smoking for long time in chronic liver patients is a risk factor to develop HCC among Egyptians. Cigarette smoking may promote the progression from hepatitis to cirrhosis and from cirrhosis to HCC [25]. In this study, 50% Egyptian and Yemeni patients were smokers. Beside cigarette smoking, khat chewing represents a common risk factor among Yemeni patients. Among our 30 Yemeni patients 76.6% were khat chewer. The mechanism by which khat induces hepatotoxicity is unclear.

Seropositivity for the HBs-Ag is one of the most important risk factors for HCC. In the present study, 36.7% out of the 30 Yemeni HCC patients were HBs-Ag positive. On the other hand, none of the 30 Egyptian HCC patients showed HBs-Ag positivity. Interestingly, 4 HCC Yemeni patients (13.3%) were HBe-Ag positive. HBe-Ag seropositivity beyond 40 years of age is relatively uncommon and is associated with a higher risk of cirrhosis and HCC [26].

Our results are consistent with previous studies carried in Yemen reporting that 30%- 55.5% of HCC patients had HBV marker reactivity [27, 28]. Absence of HBs-Ag among Egyptian patients comes in concordance with the reported decline in HBV infection prevalence in Egypt over the last few decades [5]. This decrease in prevalence suggests lower contribution of HBV infection in development of HCC among Egyptians.

However, the use of HBs-Ag as a sole marker of HBV infection among HCC cases carries a lot of fallacies. Previous large-scale epidemiological studies in Japan showed that serum anti-HBc was positive in as many as 50% of patients positive for HCV and negative for HBs-Ag [29,30]. Additionally, Ikeda et al [31] showed that HCV-positive patients with cirrhosis who had anti-HBc in sera had a much higher risk for HCC than those without anti-HBc.

Anti-HBc is considered the most sensitive marker for present or past HBV infection. In the present study, anti-HBc was present in 60% of the 30 Egyptian HCC patients. Out of the 18 anti-HBc positive, 16 were anti-HCV positive. Previous Egyptian studies carried during the last 2 decades reported similar results with an Anti-HBc seropositivity ranging from 62-84% [32, 33].

The observation that anti-HBc-positivity in presence or absence of HBV-DNA was associated with severe liver disease compared to anti-HBc-negativity supports the view that isolated serum anti-HBc is a marker of clinical significance [34].

Testing chronic hepatitis patients for HBV genome appears to be an important tool for identification of those who need to be more carefully monitored for early diagnosis of HCC [35]. In the present study, we used three PCR procedures for detecting HBV-DNA. Two real time PCR namely SYBR Green (amplifying *s*, *c* and *x* genes), and TaqMan probe technique (a commercial Artus kit targeting a 134 base pairs) and a conventional nested PCR with primers amplifying the *pol* gene encoding region.

No HBV-DNA could be detected in all anti-HBc-negative Egyptian and Yemeni HCC patients by TaqMan probe technique as well as conventional nested PCR. HBV-DNA was not detected as well in all Egyptian patients showing positive anti-HBc using both techniques. On the other hand, amplification of *pol* gene by nested PCR was successful in the 11 HBs-Ag positive Yemeni patients while only 7 were positive by Taqman probe technique.

Using SYBR green technique S and Core genes were detected in the 11 HBs-Ag positive Yemeni patients while *x* gene was detected in 9 cases. On the other hand, HBV-DNA was detected in all of the 18 anti-HBc positive Egyptian patients. Detection of x gene was the dominant finding with 15 (83.3%) followed by s gene in 14 (77.7%) and core gene in 10(55.5%) of the cases.

HBV genotypes were associated with differences in their clinical significance. Development of chronic infection occurs frequently after acute infection with genotypes A and D than with the other genotypes. Advanced liver disease and development of HCC is associated with chronic infections with genotypes C and D in contrast to genotypes A and B [36]. In the present study, 10 (90.9%) out of the 11 HBV isolates from Yemeni patients were genotype D while 1 (9.1%) was genotype E.

HCV is an important risk factor for HCC in developed countries. In Egypt chronic HCV infection is exceedingly common with the highest prevalence in the world. Several studies [5-7] have tackled the attributable risk of HCV infection among Egyptian patients with HCC, HCV was shown to be one of the major risk factors with prevalence ranging between 67-90% among HCC Egyptian patients. In the current study, 28 (93.3%) out of 30 Egyptian patients were anti-HCV positive. HCV RNA was detected in 26 patients out of them using real time Taqman probe PCR assay.

On the other hand, much lower prevalence (about 30%) of HCV infection has been reported among HCC patients in Yemen. In the present study, only 5 (16.7%) out of the 30 Yemeni HCC patients were anti HCV positive. HCV-RNA was detected in 4 patients out of them.

Among the six major genotypes of HCV, genotypes 1-4 accused for about 90% of HCV-infected cases in western countries [37]. HCV-4 is the most prevalent one in the Middle East with a prevalence up to 90% in Egypt, and is considered the most important cause of chronic hepatitis, liver cirrhosis, HCC, and liver transplantation in the country [38].

HCV-4 is a highly heterogeneous genotype, to date, 20 subtypes have been identified with 4a and 4d being the most common [16, 17].

Direct nucleotide sequencing of the 5'UTR did not resolve completely all existing HCV-4 subtypes. It has been found by many studies that phylogenetic analysis of the NS5B region is the method of choice for HCV subtype determination. However, Failure in sequencing the NS5B gene has been reported by some investigators due to primer-target mismatch within the highly variable NS5B sequence [39].

In the present study, out of the 26 HCV RNA positive Egyptian HCC cases only 23 could be sequenced. Nine isolates were genotyped using NS5B gene. 8 were HCV-4 of which 7 were subgenotyped as 4a and one as 4a/4o while only one isolate belonged to genotype 2c.

The remaining 14 isolates were genotyped using 5'UTR region. Thirteen cases were found to be HCV-4 and one isolate was subgenotyped as 1g. Sequencing of 5'UTR region was less discriminatory than NS5B gene in HCV-4 subgenotyping as only 4 could be subgenotyped as 4a, 8as 4a/4c and 1 as 4g/4o.

By sequencing both regions in our 23 isolates, 11 were subgenotyped as HCV-4a while 10 (43.4%) were of HCV-4 but could not be further subgenotyped.

On the other hand, 3 out 5 HCV-RNA positive Yemeni patients were genotyped by sequencing 5` UTR. Three patients showed different genotypes; 4, 1/1b and 4/1g.

In a study done in Egypt, Ray et al [40] showed no association between subtypes of genotype 4 and the development of HCC although HCV-4a was slightly more frequently encountered in HCC. On the other hand, Abdel-Hamid et al [17] reported that HCV-4o was over represented (21.4%) among HCC cases suggesting an association between this subtype and HCC development. Our findings agree with the previous studies since 21 out of the 23 HCV isolates were found to be HCV-4.

From the current study HCC appears to be significantly associated with HBV and HCV chronic infections in Yemen and Egypt respectively. In Egypt, occult HBV co-infection might increase the risk of HCC development in HCV patients.

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