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Genetic Variability of Hepatitis C Virus in South Egypt and Its Possible Clinical Implication

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Egypt is one of the countries with very high rates of hepatitis C virus (HCV) related morbidity and mortality. However, little is known about geographical and clinical differences in genetic variability of HCV in Egypt. Using direct sequencing and phylogenetic analysis of partial core/E1 and NS5B regions of the HCV genome, HCV genotype/subtype was determined in 129 HCV-infected patients residing in three governates in south Egypt: Assuit, Sohag, and Qena. According to clinical stage of infection, patients were categorized into four groups: asymptomatic carriers, n = 16; chronic hepatitis C patients, n = 36; liver cirrhosis, n = 54; and hepatocellular carcinoma (HCC), n = 23. Genotype 4a was detected in 80.6%, whereas 1g, 4l, 4n, 4o, 4f, and 4m were identified in 7.7%, 4.7%, 3.9%, 1.6%, 0.8%, and 0.8% of cases, respectively. The prevalence of 4a differed regionally; from 88.5% (in Sohag) to 64% (in Assuit, $P = 0.002$). Genotypes 4l and 4n had a higher prevalence in Assuit (12.8%, 10.3%) than Sohag (0%, 0%; $P \leq 0.011$). Difference in clinical features of determined genotypes/subtypes was observed; more carriers of non-4a variants (4l and 4n, 4f, or 4m) had chronic hepatitis compared to carriers of 4a (53.3% vs. 23.1%, $P = 0.025$), while more patients with 4a had liver cirrhosis (45.2% vs. 13.3%, $P = 0.023$). Two HCV-4o strains were isolated in this study, both from patients with HCC. In conclusion, geographical diversity of HCV was revealed in this study in southern Egypt. A further case–control study is required to confirm the trends of differential pathogenicity of HCV subtypes, indicated by this study. ***J. Med. Virol. 81:1015–1023, 2009.*** © 2009 Wiley-Liss, Inc.

KEY WORDS: HCV; Egypt; hepatocellular carcinoma; genotype 4o; epidemiology

INTRODUCTION

Hepatitis C virus (HCV) is a positive single stranded enveloped RNA virus. The HCV genome consists of >9,500 bp [Choo et al., 1991]. Infection with hepatitis C is associated closely with chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [Saito et al., 1990; Purcell, 1997].

Based upon phylogenetic analysis of genomic regions and the complete genome of HCV, six genotypes (HCV genotype types 1–6) have been described and subclassified into numerous subtypes (e.g., HCV subtype 1a, 1b) [Simmonds et al., 2005]. Molecular epidemiological studies have indicated a geographical restriction for some HCV genotypes (e.g., genotype 4 to the Middle East, genotype 5a to South Africa, and genotype 6 to Southeast Asia) [Simmonds et al., 1993] while others are distributed globally, for example, 1a, 1b, 2a, 3a [Smith

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et al., 1997]. HCV genotype is an important predictor of response to interferon-alpha and ribavirin combination therapy [Hnatyszyn, 2005]. The route of transmission and serological reactivity are other factors which may differ among different HCV genotypes [Dhaliwal et al., 1996]. However, it is unclear, whether or not HCV genotypes differ in their pathogenicity [Zein, 2000].

One hundred seventy million people worldwide estimated to be infected with HCV are at a risk of developing progressive liver disease and death due to infection with HCV [WHO, 1999]. According to different reports, the prevalence of HCV in Egypt ranges from 11% to 14% of the general population, with 5–7 million people with active infection (have detectable HCV RNA) [Abdel-Wahab et al., 1994; Arthur et al., 1997; Abdel-Aziz et al., 2000]. By these estimates, Egypt is considered to be the country with the highest prevalence rate of HCV in the world with a majority of genotype 4 [Frank et al., 2000].

Few data are available regarding the molecular epidemiology of HCV in south Egypt. The aim of this study was to extend the mid-core and NS5B database for HCV strains isolated from HCV-infected patients residing in south Egypt with respect to the clinical and virological characteristics of the isolates examined.

PATIENTS AND METHODS

Patients

Serum samples were collected from August to October 2007 from 151 consecutive chronic liver disease patients seen in affiliated hospitals of Sohag University Hospital and South Egypt Cancer Institute. Among the 151 patients with hepatitis, 30 were assigned into asymptomatic carrier group, 86 into a liver cirrhosis group, and 35 into an HCC group. Anti-HCV was detected in a total of 126/151 (83.4%); 25/30 (83.3%) in the asymptomatic carrier group, 68/86 (79.1%) in the liver cirrhosis group, and 33/35 (94.3%) in the HCC group. In addition to the specimens collected consecutively, sera were collected also from 41 patients with diagnosed chronic hepatitis C. The clinical classification of the patients infected with HCV was based upon (1) measurement of serum alanine aminotransferase (ALT), (2) ultrasound examination, and (3) detection of a serological tumor marker (alpha fetoprotein for the diagnosis of HCC). The asymptomatic carrier group include patients with anti-HCV with normal liver enzymes for more than 6 months with minimal or no symptoms. Patients with liver cirrhosis were diagnosed clinically by the presence of splenomegaly, ascites, and other peripheral signs of portal hypertension together with ultrasonographic findings such as a shrunken, coarse texture liver with enlarged portal and splenic veins. A total of 167 anti-HCV-positive samples belonged to patients from three governates in south Egypt, 93 samples from the Sohag governate (467 km from Cairo), 45 samples from the Assuit governate (375 km south of Cairo), and 29 samples from the Qena governate (600 km south of Cairo).

Serological Methods

Serum samples were examined for anti-HCV, hepatitis B surface antigen, anti-HBc, and anti-HBs by chemiluminescence enzyme immunoassay using commercial assay kits (Fujirebio, Inc., Tokyo, Japan). Hepatitis C core antigen (HCVcAg) was measured using enzyme immunoassay (Fujirebio, Inc.) [Aoyagi et al., 1999].

Detection of the HCV RNA

Viral RNA extraction was carried out with a Sepa-Gean RV-RN Nucleic acid extracting kit (Sanko Junyaku Co. Ltd., Tokyo, Japan) following the manufacturer's protocol. The extracted RNA was reverse transcribed into cDNA using SuperScript II RNAse H Reverse Transriptase (Invitrogen Corp., Carlsbad, CA) and random hexamer primers (Takara Shuzo, Co. Ltd., Tokyo, Japan) as described previously [Ohno et al., 1997]. Confirmation of HCV RNA was performed by amplifying partial genome of the 5-non-coding region according to the protocol described previously [Takeuchi et al., 1999].

Sequencing and Phylogenetic Analysis

For genotyping the HCV isolates, PCR was used to amplify parts of both the structural (core/E1) and non-structural (NS5B) coding regions of the HCV [Tanaka et al., 2002]. The sequencing reaction of the amplified products was performed with the Prism Big Dye (Pekrin-Elmer Applied Biosystems, Foster City, CA) in an ABI 3100 DNA automated sequencer according to the manufacturer's protocol. Sequences were aligned with the CLUSAL X software program [Thompson et al., 1994]. The phylogenetic trees were constructed by neighbor joining method with Tamura-Nei distance correction model using online tools in the HCV database [Shin-I et al., 2008]. Bootstrap values were determined on 1,000 resampling tests in the HCV database. The sequences of other HCV isolates used for the phylogenetic analysis were retrieved from the DDBJ/EMBL/GenBank sequence database and are indicated by their accession number in the phylogenetic tree. The nucleotide sequence data reported in this article will appear in the DDBJ/EMBL/GenBank sequence databases with accession numbers: AB470005–AB470069, AB470243–AB470255, and AB470103–AB470215. Statistical analysis was performed with Fisher's exact probability test and an independent *t*-test for continuous variables using the SPSS software package (SPSS, Chicago, IL). *P*-values (two-tailed) less than 0.05 were considered statistically significant.

RESULTS

Demographic and Clinical Characteristics of the Patients With Chronic Liver Disease

Figure 1 summarizes the detected rates of hepatitis markers and coinfection patterns among the clinical

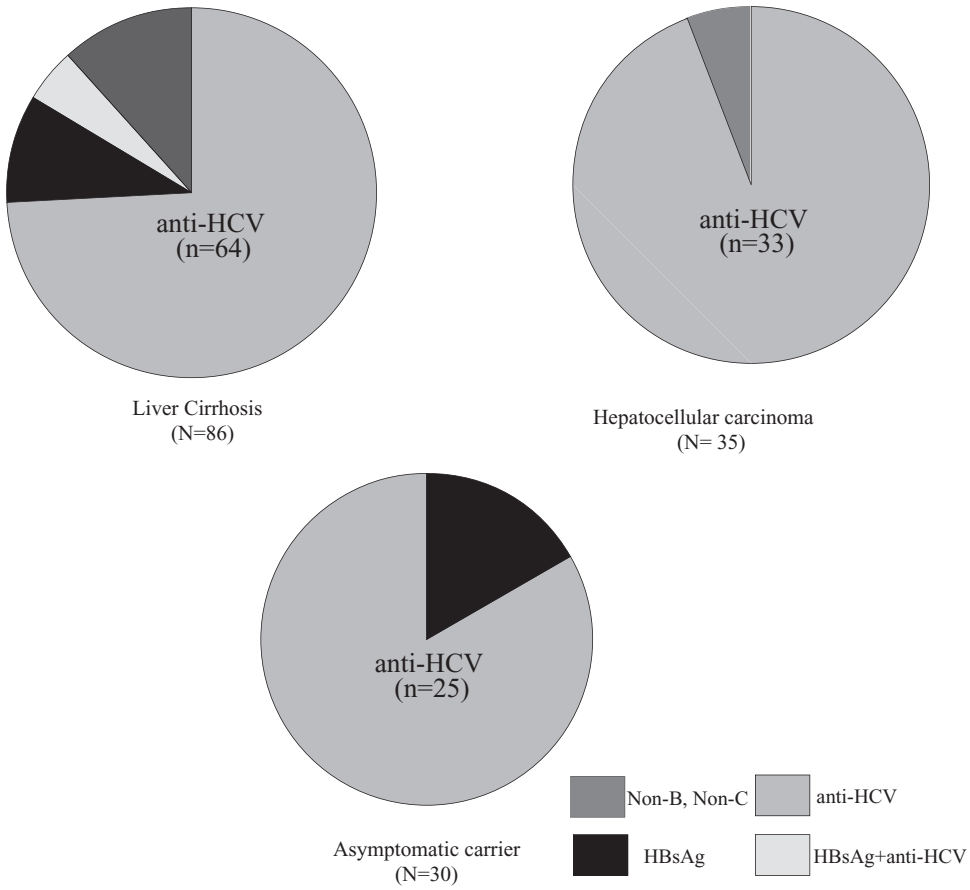

Fig. 1. Incidence of hepatitis markers among patients with progressive liver diseases.

groups studied; asymptomatic carriers (n = 30), liver cirrhosis (n = 86), and HCC (n = 35). Anti-HCV and HBsAg were detected in 25/30 (83.3%) and 5/30 (16.7%) of the asymptomatic carriers, respectively. In the liver cirrhosis group, anti-HCV was detected in 64/86 (74.4%), HBsAg was detected in 8/86 (9.3%), and simultaneous presence of both markers was detected in 4/86 (4.7%). Ten patients (11.6%) in the liver cirrhosis group were negative serologically for both anti-HCV and HBsAg and were designated as non-B hepatitis and non-C hepatitis, respectively. In the HCC group, anti-HCV was detected in 33/35 (94.3%), whereas the remaining two patients (5.7%) were negative for both anti-HCV and HBsAg and were designated as non-B hepatitis and non-C hepatitis.

Table I summarizes the baseline features of the 167 patients infected with HCV subdivided into four clinical groups: 25 asymptomatic carriers, 41 chronic hepatitis, 68 liver cirrhosis, and 33 patients with HCC. Patients in the HCC group were older than patients in

TABLE I. Description of the Patients Infected With HCV Stratified by Their Clinical Characteristics

	Total (n = 167)	Asymptomatic carrier (n = 25)	Chronic hepatitis (n = 41)	Liver cirrhosis (n = 68)	HCC (n = 33)	*P* value
Age[a]	47.5 ± 11.8	29.8 ± 7.0*	43.4 ± 6.7	52.5 ± 7.8	59.0 ± 8.6*	<0.006
Gender (male)	116 (69.4)	16 (64)	32 (78)	41 (60.2)	27 (81.8)	NS
ALT (IU/L)[a]	42.7 ± 28.5	28.4 ± 16.2*	42.3 ± 17.1	42.8 ± 29.6	70.8 ± 42.1*	<0.005
AST (IU/L)[a]	64.9 ± 46.0	29.6 ± 9.8*	63.5 ± 34.2	75.4 ± 53.8	86.1 ± 47.2*	<0.0001
HCVcAg (fmol/L)[b]	434.2 (0.1–19,654)	463 (0.1–12,549.8)	1,162.5 (5.0–19,654)	409.6 (0.1–13,219.2)	741.6 (50.7–7,703.8)	NS
HCV RNA[c]	139 (83.2)	19 (76)	38 (92.7)	56 (82.4)	26 (78.8)	NS
HBsAg[c]	5 (3.0)	0	1 (2.4)	4 (5.9)	0	NS
Anti-HBs[c]	33 (19.8)	6 (24)	9 (22)	10 (24.4)	8 (24.2)	NS
Anti-HBc[c]	97 (58.1)	12 (48)	20 (48.8)	49 (72.1)	24 (72.7)	0.023

[a]Mean + SD.
[b]Median (range).
[c]Positive number (%).
*$P < 0.05$.

the other clinical groups. Similarly, patients with liver cirrhosis were significantly older when compared to asymptomatic carriers and patients with chronic hepatitis. Male predominance was observed in all groups with a maximum in the HCC group (81.8%) and minimum in the liver cirrhosis group (60.2%, $P = 0.041$). Ninety-seven (58.1%) patients infected with HCV were also positive for anti-HBc. The prevalence of anti-HBc was significantly higher in the HCC and liver cirrhosis groups with 72% when compared to chronic hepatitis 48.8% and asymptomatic carriers 58.1% ($P < 0.05$, Table I). Anti-HBs was detected in 19.8% (33/167) of patients infected with HCV with no significant difference found between the studied groups.

Detected HCV viremic cases tended toward a higher rate in the chronic hepatitis group 92.7% (38/41) compared to the asymptomatic carrier group (92.7% vs. 76%, $P = 0.072$), and a similar trend was observed in the HCVcAg level when the clinical groups were compared. To investigate the association between viral markers and progression of liver disease, 38 asymptomatic carrier and chronic hepatitis cases were compared to 38 age-sex-matched liver cirrhosis and patients with HCC (Table II). The prevalence of anti-HBc was the only significant difference between the two groups, with a higher rate in patients with progressive liver diseases ($P = 0.038$). HCV genotypes were determined in 65/76 of the age-sex-matched groups. Although no significant difference in the prevalence of HCV genotypes was found between the non-progressive and progressive liver disease groups, 4m and 4o were detected only in the latter group, and 1g prevalence was higher also in the latter, that is, patients with progressive liver disease.

Phylogenetic Analysis of the Core/E1 and NS5B Regions and Determination of the HCV Genotype/Subtype

Among the 167 anti-HCV-positive cases, HCV RNA positivity was examined by 5′UTR-targeted PCR (detection limit 1,000 copies/ml); in total 139 (83.2%) cases were positive and subjected to determination of HCV genotype by amplification, direct sequencing, and phylogenetic analysis based on the NS5B and/or the E1 regions. The HCV genotype was determined in a total of 129 (92.8%) of the 139 HCV RNA-positive cases. In 75/129 (58.1%), HCV phylogenetic analyses of the nucleotide sequences were available in both the core/E1 and NS5B regions simultaneously. In these 75 patients, no discrepancy was observed regarding the genotyping results as obtained by the phylogenetic analysis of both regions (E1 and NS5B), excluding possibility of presence of recombinant strains within these cases (Table III).

In the NS5B phylogeny, 89.7% (70/78) of samples were clustered with genotype 4 whereas 10.3% (10/78) were clustered with genotype 1g. Bootstrap values for all subtypes within genotype 4 was more than 75%, being lowest in the subtype 4a (BV = 79%). Eight samples were clustered with genotype 1g strains of Egyptian origin with BV of 100% (Fig. 2A). In the core/E1 phylogeny, BV was 99% for genotype 4a; 100% for genotypes 4n, 4l, 4o, and 4m; and 99% for genotype 1g (Fig. 2B). Inspection of the NS5B and core/E1 trees indicated no specific clustering by geographic regions in Egypt.

HCV Genotypes/Subtypes Among Different Governates in South Egypt

A total of 129 HCV samples which were genotyped were used to analyze the geographical distribution of HCV variants. Seventy-eight isolates belonged to the population studied in the Sohag governate, 39 isolates were from the Assuit governate population, and 12 isolates belonged to patients from the Qena governate. In south Egypt, the predominant genotype was HCV-4a (104/129, 80.6%), followed by genotype 1g (7.8%), genotype 4l (4.7%), then 4n (4.1%), and 4o (1.6%). Genotypes 4m and 4f were rarely found and were detected in 0.8% for each genotype. HCV genotype 4a was the predominant genotype in the Sohag governate and was detected in 88.5% (69/78) of the population studied, followed by genotype 1g (6.4%). Sporadic cases of infection with subtypes of genotype 4 (other than genotype 4a) were also observed including genotype 4o (2.6%), genotype 4f (1.3%), and genotype 4m (1.3%).

In the Assuit governate, HCV genotype 4a was observed in 64% (25/39), that is less frequently than in

TABLE II. Comparison Between Non-progressive and Progressive Liver Disease in Age and Gender-Matched Groups

	Total (n = 76)	Non-progressive liver disease (n = 38)	Progressive liver disease (n = 38)	*P*-value
Age[a]	45.6 ± 4.8	44.9 ± 5.4	46.3 ± 4.1	Matched
Gender (male)[b]	50 (65.8)	29 (76.3)	21 (55.3)	Matched
Asymptomatic carrier/chronic hepatitis/liver cirrhosis/HCC	6/32/30/8	6/32/0/0	0/0/30/8	
Anti-HBc[b]	40 (52.6)	15 (39.5)	25 (65.8)	0.038
Schistosoma Ab[b]	57 (80.3)	34 (89.5)	23/33 (70)	NS
HCV-RNA[b]	69 (90.8)	36 (94.7)	33 (86.8)	NS
HCV genotype[b]				
4a	50/65 (76.9)	26/35 (74.3)	24/30 (80)	NS
4n	4/65 (6.2)	4/35 (11.4)	0	NS
4l	4/65 (6.2)	4/35 (11.4)	0	NS
4m	1/65 (1.5)	0	1/30 (3.3)	NS
4o	2/65 (3.1)	0	2/30 (6.6)	NS
1g	4/65 (6.2)	1/35 (2.9)	3/30 (10)	NS

[a]Mean + SD.
[b]N (%).

TABLE III. Genotyping Results as Determined by Sequence and Phylogenetic Analysis of the E1 and NS5B Regions

	Classification based on NS5B								
Classification based on E1	1g	4a	4n	4l	4o	4m	4f	ND	Total
1g	8							2	10
4a		59						32	91
4n			2					2	4
4l				4				1	5
4o					1			1	2
4m						1			1
4f								1	1
ND		13	1	1					15
Total	8	72	3	5	1	1		39	129

the Sohag governate, genotype 1g found in 12.8%, genotype 4l in 10.3%, and genotype 4n in 12.8%. Only two genotypes were detected in patients from the Qena governate and they were genotype 4a (10/12, 83.3%) and genotype 4l (2/12, 16.7%) (Fig. 3). A significant difference in HCV genotypes distribution was observed between patients from the Sohag and Assuit governates, where incidence of infection with HCV genotype 4a was higher in the Sohag governate ($P = 0.002$). Infection with genotypes 4l and 4n was significantly higher in the population studied from the Assuit governate compared to the Sohag governate ($P = 0.003$, 0.011 respectively).

Clinical and Virological Characteristics of the Determinant HCV Genotypes/Subtypes

Clinical and virological characteristics were compared between patients infected with different variants of HCV; 104 cases infected with genotype 4a, 15 cases infected with genotype non-4a (including six cases with 4l, five cases with 4n, two cases with 4o, and one case with 4f, one with 4m), and 10 cases infected with HCV genotype 1g (Table IV). The mean age was similar between patients infected with different HCV genotypes. More patients among those infected with HCV-4a had liver cirrhosis than among patients infected with non-4a. More patients among those infected with (non-HCV-4a) had chronic hepatitis than among patients infected with HCV-4a. Interestingly, two cases were found to be infected with genotype 4o and both cases were patients with HCC. A significantly higher level of HCVcAg was observed in patients infected with HCV genotype 4a (median, range; 939.5, 24.7–13,219.2 fmol/L), compared to patients infected with genotype 1g strains (median, range; 203.1, 5.0–924.4 fmol/L) ($P < 0.0001$).

DISCUSSION

Genotype analysis of HCV within a defined population is an important issue in the study of the evolution of HCV infection in different geographical regions besides its importance for the development of an effective vaccine [Pybus et al., 2001; Cantaloube et al., 2005]. In addition, evidence supporting the differential pathogenicity of HCV subtypes has emerged in many studies. To the best of our knowledge, this is the first study concerned with the genetic diversity of HCV, and the clinical and virological characteristics of HCV infection in south Egypt.

Genotype 4 strains belonged to different subtypes including 4l, 4n, 4o, with solitary isolates of 4f and 4m together with the predominant genotype 4a in the population studied from south Egypt. Studies on the epidemic history of HCV in Egypt have indicated an exponential spread of the infection, occurring from the 1940s through 1980s; a period coinciding with mass campaigns of parenteral treatment for schistosomiasis [Tanaka et al., 2004, 2006]. This explosive epidemiological spread of HCV was also responsible for the simultaneous dissemination of multiple lineages of genotypes 4 and 1 [Pybus et al., 2003]. Genovese et al. [2005] indicated also the unexpected diversity of genotype 4 in a population studied in Alexandria in north-central Egypt compared to another study [Simmonds, 2004; Genovese et al., 2005]. However, the second most frequent subtype in the Alexendria study population was 4m (11% of cases), while that in the present study in patients from south Egypt was 4l (5% of HCV genotype 4), and genotype 4m was the less observed genotype in this cohort study. Genotype 1 was less frequent and only HCV genotype 1g was detected and observed in a considerable prevalence of the genotyped samples examined from this cohort. In a previous study where 68 blood donor specimens were selected from geographically distinct governates and analyzed phylogenetically genotype 1g was detected in 5/68 (7.4%), three of these five patients were from south Egypt [Ray et al., 2000].

The HCV prevalence throughout Egypt is associated directly with the amount of intravenous tartar emetic used to control schistosomiasis in the period, 1950–1980. The lowest rates were observed in Cairo and Alexendria (<8%), the highest in rural areas of the Nile Delta [Lower Egypt (>15%)], and intermediate prevalence (8–16%) in rural areas along the Nile south of Cairo (Middle and Upper Egypt) [Abdel-Aziz et al., 2000; Nafeh et al., 2000] which is the geographical area representing the samples collected in the current study. Geographical differences in the distribution of the HCV subtype was observed also between the Assuit and Sohag governates as represented by the presence of a high proportion of HCV subtypes 1g, 4l, and 4n in the former governates. The difference in the variability between the two governates might be explained by the fact that the Assuit governate, which is the largest town

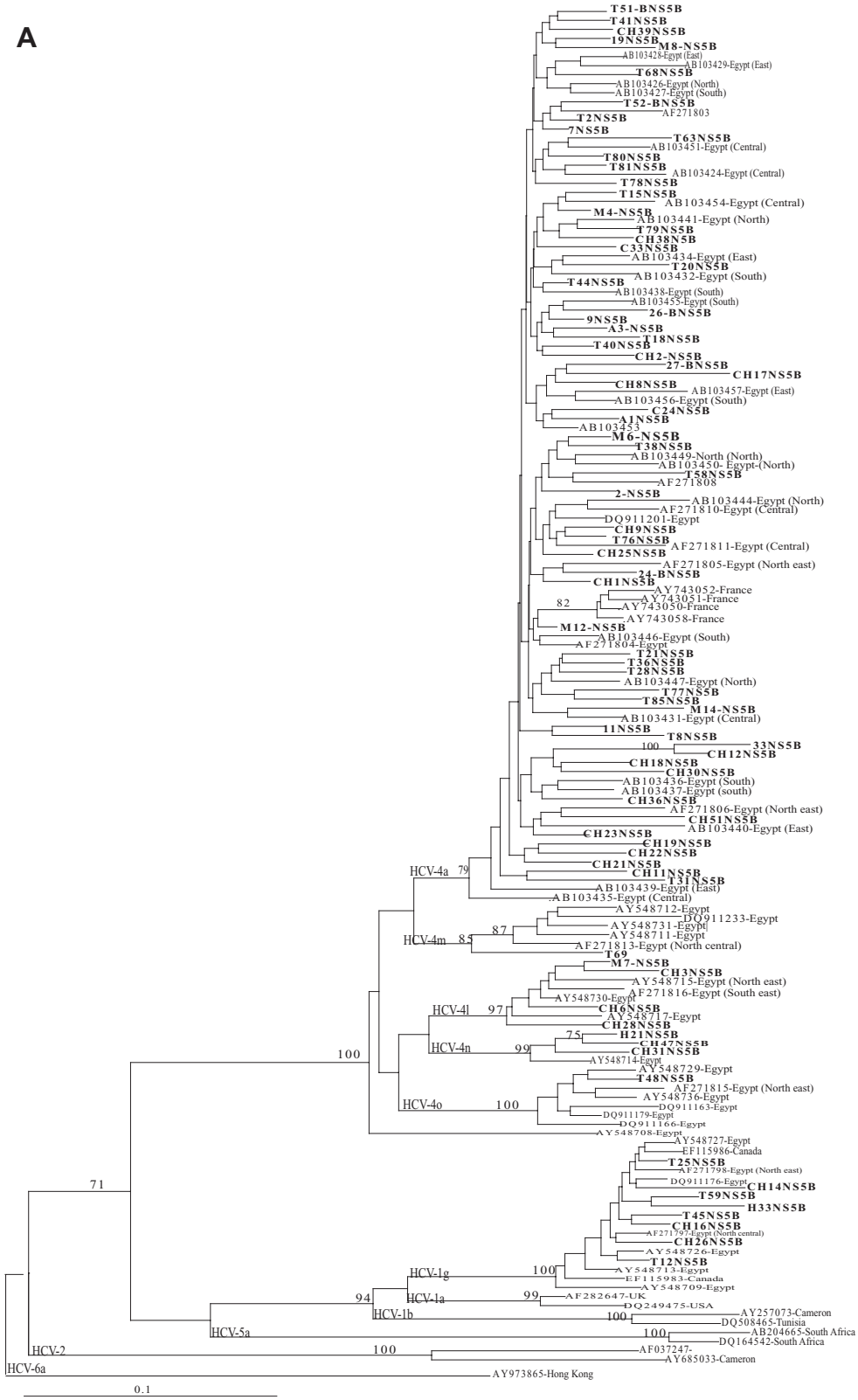

Fig. 2. Phylogenetic tree of the HCV (**A**) partial NS5B region sequences and (**B**) partial core/E1 region sequences isolated from patients infected with HCV in south Egypt (are indicated in bold) and a panel of reference strains retrieved from DDBJ/EMBL/GenBank identified by their accession number. The origins of reference strains are indicated. Data regarding the regional origin of HCV reference sequences of Egyptian origin are also indicated in parentheses when available in the GenBank database. Boot strap values are indicated in the tree root.

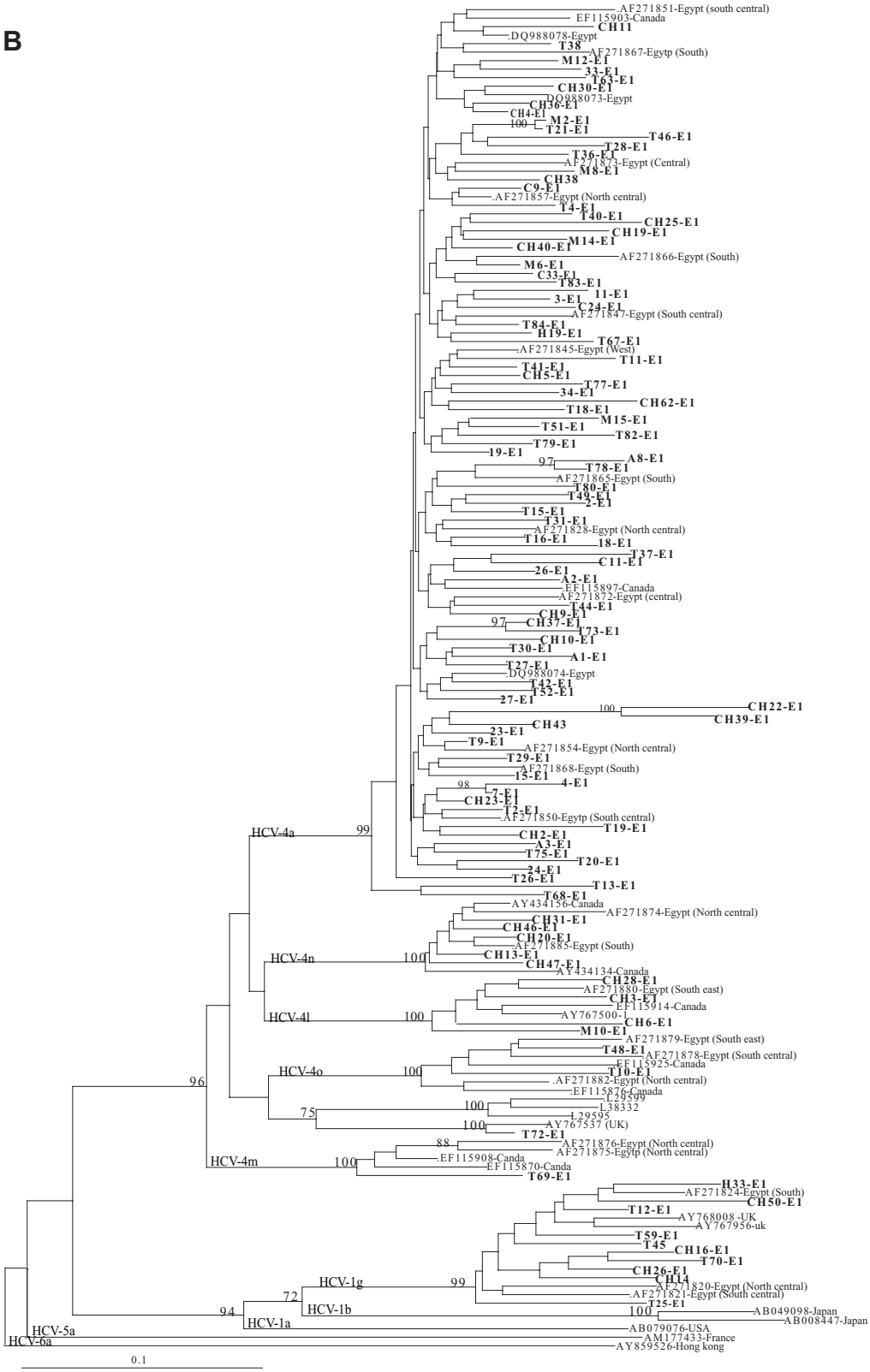

Fig. 2. (*Continued*)

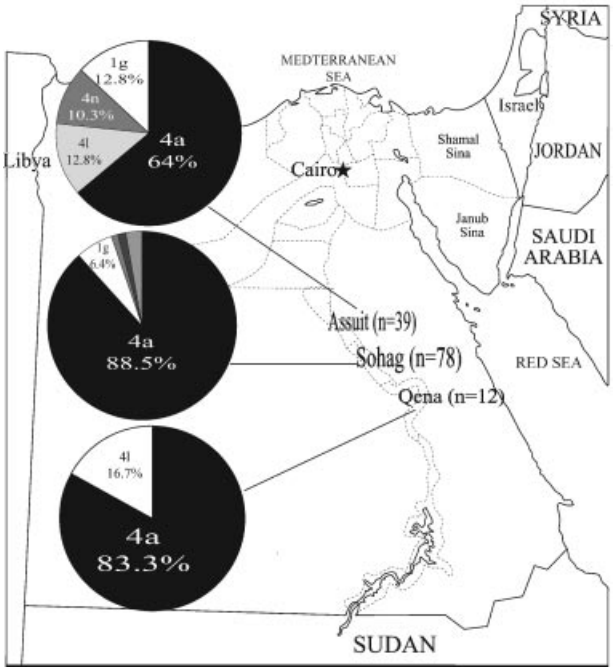

Fig. 3. Distribution of HCV genotypes in the population studied from different governates in south Egypt.

in southern Egypt, lies geographically in the Middle Egypt, while the Sohag governate is in the Upper Egypt. In Middle and Lower Egypt, intravenous tartar emetic was used more extensively and for several years longer to treat Schistosomiasis than it was in Upper Egypt [Arthur et al., 1997; Frank et al., 2000]. The high level of viral heterogeneity may be related to the high level of exposure to reinfection [Argentini et al., 2000].

Examining the association between HCV subtypes and liver diseases, a higher incidence of cirrhosis was observed in patients with HCV-4a (45.2%) than HCV-non-4a (13.3%), whereas HCV genotypes 4l and 4n, 4f, and 4m (non-4a) were more prevalent in patients with chronic hepatitis. Genotype 4o circulates in regions geographically distant from each other in Egypt [Ray et al., 2000]. In this study, genotype 4o was detected in 2/129 (1.6%). Interestingly both cases with genotype 4o were included in the HCC group. Abdel-Hamid et al. [2007] was the first to report the novel association between HCC and cluster subtype 4o as indicated by the significantly higher frequency of HCC in patients infected with subtype 4o than those infected with other subtypes. This novel association is not explained by the older age as the mean age in the two groups HCC with 4o versus HCC with the non-4o subtype was not different [Abdel-Hamid et al., 2007]. The data associating the HCV genotype with clinical and histological characteristics within HCV-infected patients are conflicting. The present study revealed a significant association between infection with HCV genotype 4n/4l and chronic hepatitis. Definite evidence for a differential pathogenicity of HCV genotypes has been limited by several factors, including the naturally long duration of HCV-related liver disease and the cohort effects in the circulation of viral types. However, a recent case–control study established a definite association between chronic infection with HCV genotype 1b and the high risk of cirrhosis [Osella et al., 2001]. Another report also indicated that all patients who developed cirrhosis in <10 years were infected with genotype 1b [Kurbanov et al., 2003].

Measurement of HCVcAg is specific, sensitive, and suitable for the detection of viremia in HCV-infected subjects. In the present study, there was a significantly lower level of HCVcAg in patients infected with genotype 1g compared to those infected with genotype 4a. The same result was also observed even after adjusting the compared clinical groups. In a previous report, a lower level of HCVcAg was observed in specimens with genotype 4 compared to genotype 1 [Agha et al., 2004]. A possible explanation for this discrepancy between the present study and previous study was the difference in the studied groups, as the previous study included only blood donors with genotype 1b when the HCVcAg level was compared. On the other hand, the mean level of HCVcAg in genotype 4 was similar to that in the previous study [Agha et al., 2004].

In conclusions, genetic diversity in HCV was detected in the present cohort study in south Egypt. Increasing evidence of differences in the clinical and virological

TABLE IV. Clinical Characteristics of the Patients Infected With HCV Stratified by the HCV Genotypes

		Genotype 4			
	Total (n = 129)	4a (n = 104)	Non-4a (n = 15)	1g (n = 10)	*P*-value
Age[a]	49.1 ± 11.2	49.1 ± 11.4	49.2 ± 10.7	48.4 ± 9.9	NS
Gender (male)[b]	91 (70.5)	70 (67.3)	12 (80)	9 (90)	NS
ALT (IU/L)[a]	43.4 ± 28.9	40.8 ± 27.8	60.1 ± 30.8	43.0 ± 31.0	0.049[d]
AST (IU/L)[a]	68.5 ± 48.3	62.1 ± 43.3	105 ± 64.3	73.6 ± 46	0.035[d]
HCV-cAg (fmol/L)[c]	897.2 (5.0–19,654)	939.5 (24.7–13,219.2)	2,354.8 (59.4–19,654.2)	203.1 (5.0–924.4)	<0.0001[e]
Asymptomatic carrier[b]	16 (12.4)	16 (15.4)	0	0	NS
Chronic hepatitis[b]	36 (27.9)	24 (23.1)	8 (53.3)	4 (40)	0.025[d]
Liver cirrhosis[b]	54 (41.9)	47 (45.2)	2 (13.3)	5 (50)	0.023[d]
HCC[b]	23 (17.8)	17 (16.3)	5 (33.3)	1 (10)	NS

[a]Mean + SD.
[b]N (%).
[c]Median (range).
[d]Patients infected with HCV genotype 4a versus patients infected with HCV-4 non-subtype 4a.
[e]Patients infected with HCV genotype 4a versus patients infected with 1g.

characteristics of the isolated strains between different genotypes/subtypes has been introduced in the present study. The data highlight the need for further studies exploring the HCC mortality burden within different districts in Egypt; a country with the highest HCV prevalence in the world. There is also a need for a case–control study to investigate the trends in the association of HCC development with infection by HCV genotype 4o.

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