Egypt. J. Lab. Med. Vol: (13) No. (1) Feb., 2001. 21-34.

HCV GENOTYPES IN EGYPTIAN PATIENTS WITH CHRONIC HEPATITIS AND HEPATOCELLULAR CARCINOMA

By

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ABSTRACT

To study HCV genotype in Egyptian patients and its relation to disease type and complications, sixty patients were studied in three groups, twenty in group I with no clinical or chemical findings. Twenty in group II with chronic active hepatitis as proved histopathologically. Twenty in group III with hepato-cellular carcinoma as proved histopathologically also. All cases were subjected to ALT, AST, ALP, GGT, AFP and auto-antibodies (ANA and ASMA) serum levels and HCV genotyping. The results of the study showed that genotype 4a is the most prevalent in all groups (being 35.7%, 61.6% and 48% in groups I, II and III respectively). From the pathological point of view, genotype 4a is the most pathological type followed by 4c & d (being 0%, 11.5% and 28% in groups I, II and III respectively). The least pathological genotypes in the study groups were 1b (being 3.6%, 7.7% and 8% in group I, II and III respectively) followed by 2a (being 4% in group III). The work concluded the importance of HCV genotyping for proper evaluation of clinical status of HCV patients.

INTRODUCTION

Hepatitis C virus (HCV) causes acute and often chronic hepatitis[21]. It is the causative agent of most post-transfusion non-A, non-B hepatitis cases. HCV infection is now recognized to be a major risk factor for hepatocellular carcinoma (HCC), evidenced by finding both antibody to HCV (anti-HCV) and HCV-RNA in serum of a substantial proportion of patients with HCC around the world and by the progression of liver disease to cirrhosis and HCC in individual patients infected with HCV[8].

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Like other members of the togaviridae (pestiviruses, Flaviviruses), HCV are, small enveloped viruses, sensitive to chloroform and containing a single-stranded, positive sense RNA genome[22]. Hepatitis C virus (HCV), like other RNA viruses display a high genetic variability, which segregates into phylogenetically distinct variants [35]. On the basis of variations in nucleotide sequence, at least six genotypes, and several subtypes have been identified[21].

The prevalence of different genotypes of hepatitis C virus may vary between geographic areas and it is possible that various genotypes have different pathogenic characteristics [32].

The capacity of variation in incorporation enables HCV to escape from the host's immune system. This demonstrated by the genetic drift observed in the envelope E1 and E2 region during the course of a chronic infection. Thus, in vivo, HCV exists as a population of slightly different viruses, representing a collective identity which known as the quasispecies[10]. HCV genotype titer and quasispecies determine the success of treatment[26].

As early as the availability of diagnostic tool for HCV detection was established, the characterization of Egyptians thought to be at high risk for the disease, was described. In Europe, the magnitude of the problem is examplified by HCV mean carrier rates in general population of 2% or less in North Europe and UK, 0.5-1% in Western Europe, 1-1.5% in Southern Europe and over 2% in Eastern Europe[6].

This is different in Egypt. The incidence of seropositivity to HCV antibodies among Egyptians in different reports varied widely. Saeed et al. (1991)[31], reported their observation about the high incidence of HCV antibody seropositivity (24.3%) among Egyptian blood donors, during the safety screening of the blood units in Riyadh Military Hospital in Saudi Arabia which did not represent the Egyptian population. However, Kabil et al.(1995)[14], in a large survey included 40,000 subjects found only 9% HCV positive, others found different but, near incidences like 8.7%[13] and 9.2%[24].

However, in patients with chronic hepatitis C, the influence of the genetic heterogeneity of the HCV on the progression of liver disease and on the responsiveness to interferon therapy is a matter of controversy[19]. These observations have raised the issue of a potential clinical role of HCV types in determining long-term liver disease outcome.

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The aim of the study was to, analyze the possible role of HCV genotype in the chosen groups in Egypt, and to investigate whether the HCV type influence the clinical presentation or not. This is for better aetiological diagnosis of different HCV infection expression which will help in the improvement of the prognosis of the disease on the long run.

PATIENTS AND METHODS

This work included sixty patients attending the Internal Medical and Hepatology Clinics at Benha University Hospital and Tanta Cancer Institute. They were classified into 3 groups:-

Group I: asymptomatic HCV patients (age ranging from 20-42 yrs). This group was collected from those persons investigating their sera for the absence of HCV Ab and HBs Ag for travelling abroad.

Group II: chronic active hepatitis (CAH) (age ranging from 36-57 yrs).

Group III: hepato cellular carcinoma (HCC) (age ranging from 49-73 yrs).

All cases were HBs Ag negative, HCV Ab positive and HCV RNA positive.

For each patient the following were done at Immunology and Molecular Biology Units, Clinical Pathology Department Benha University Hospital:-

(1) Full clinical history especially for previous operations and blood transfusion. Clinical examination with special stress on liver, spleen, lymph node enlargement.

(2) Serum levels of ALT (Alanine aminotransferase)and AST (Aspartate aminotransferase) were done by a colorimetric method of Reitman and Frankel[29], ALP (Alkaline phosphatase) was done by Kind and King method[17] and GGT (Gamma glutamyl transferase) was done by a kinetic method of Szasz et al[37].

(3) PT (Prothrombin time) was done by quick method[27].

(4) Auto-antibodies :ASMA (Anti-smooth muscles antibodies) and ANA (Antinuclear antibodies) were done using IIF Method in liver-Kidney Microsomal (LKM) ready-slide preparation of Binding Site Company[40].

(5) Serum level of AFP (Alpha fetoprotein) was done on Immulyte system, a method described by Rose[30].

(6) HBs Ag and HCV Ab by ELISA of Murex[41].

(7) HCV RNA PCR.

(8) Histopathology: liver biopsy for patients in group II and III.

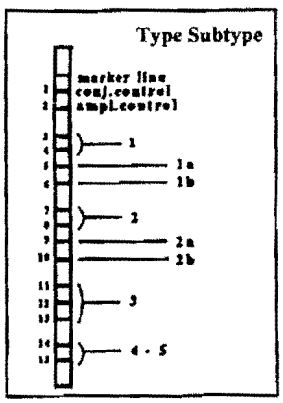
(9) HCV RNA Genotyping: is a sensitive amplification protocol used for the highly conserved 5`UR with sets of nested, universal primers.

Principle of the procedure:

The INNO-LiPA HCV is based on the reverse-hybridization principle[2]. Specific oligonucleotide-probes immobilized as parallel lines on membrane strips are hybridized with amplified sample material. During amplification biotinylated primers are incorporated in the amplified DNA fragments. After hybridization, streptavidin labelled with alkaline phosphatase is added and becomes bound to any biotinylated hybrid previously formed. Incubation with NBT/BCIP chromogen results in a purple/brown precipitate. Using the INNOLiPA HCV test strips, the five major genotypes and 6 subtypes are detected simultaneously. The LiPA strip contains a test control line and 14 parallel DNA probe lines. Line 1 is a control line for the color development reaction and line 2 contains universal probes hybridizing to amplified products of any HCV type.

Table (1): Reactivity of the different types in relation with the applied probes.

Type	Obligatory reactivity with the lines on the strip	Common reactivity with other probes
Positive control lines	Line 1 and line 2.	
1	Line 3 and/or line4.	
1a	Line 3 and/or line 4, line 5.	
1b	Line 3 and/or line 4, line 6.	
2	Line 7 and/or line 8.	Line 5.
2a	Line 7 and/or line 8, line 9.	Line 5.
2b	Line 7 and/or line 8, line 10.	
3a	Line 11 and/or line 12 and/or line 13.	
3b	Line 11 and line 14 and line 15	
4a	Line 14 and line 15 or line 15 only.	Line 5 or line 6
5a	Line 14 only	Line 5 or line 6



Interpretation of results:

The results are interpreted by comparing the pattern of the purple/brown lines on the INNO-LiPA HCV strips with the interpretation table (INNO-LiPA HCV reactivity pattern interpretation table). The plastic transparent reading chart aids in the positioning of the lines before interpretation. A reaction was interpreted as positive when the signal was clearly stronger than the corresponding signal on the negative control strip.

The reaction on line 2 is used to check if indeed HCV DNA (5`UR) has been added during hybridization, while the reaction on the control line 1 is used to check the addition of reactive conjugate solution during the detection procedure. Both line 1 and line 2 should always be positive if 5`UR HCV DNA product is present.

RESULTS

The results are summarized in the following tables.

Table (2): Showing the mean age in years in the three study groups.

	Mean age (yrs)	Mean age in males	Mean age in females
Group I	30.2 ± 5 (20 cases)	30.6 (15 cases)	29 (5 cases)
Group II	45.8 ± 5.6 (20 cases)	43.9 (13 cases)	42.7 (7 cases)
Group III	58 ± 5.8 (20 cases)	58.7 (12 cases)	56.7 (8 cases)

Table 2 shows that mean age is always higher in males than females, the mean age increases as we go from group I through II and III.

Table (3):

Liver function tests	Group I		Group II		Group III		Pvalue		
	Mean	SD	Mean	SD	Mean	SD	Group I & II	Group II & III	Group I & III
ALT (U/L)	8.2	2.0	32.3	8.2	55.2	9.1	<0.001	<0.001	<0.001
AST (U/L)	7.2	2.1	29.9	8.1	53	9.3	<0.001	<0.001	<0.001
ALP (U/L)	86.9	26.2	160.6	16.3	217.4	48	<0.001	<0.001	<0.001
GGT (U/L)	25.6	7.3	104.7	17	189.4	33.2	<0.001	<0.001	<0.001
AFP (ng/ml)	4.8	2.6	5.2	2.9	334.5	300	>0.1	<0.0001	<0.0001
PT (second)	11.8	0.7	14	2.6	15.9	1.1	>0.01	<0.001	<0.001

Comparison between liver function tests in the studied groups
Reference interval of:

- **ALT: up to 12 U/L.**
- **AST: up to 12 U/L.**
- **ALP: 60-170 U/L in males and females.**
- **GGT: 9-52 in males 5-32 in females.**
- **AFP: <15 ng/ml**
- **Control for PT is 12.5 second.**