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RESEARCH ARTICLE

Distribution of Hepatitis C Virus (HCV) Genotype among Iraqi Hemodialysis Patients

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Abstract

Objectives: This study was established to assess the prevalence of HCV genotype among Iraqi hemodialysis patients

Patients and methods: This case- control study was conducted at the Teaching laboratories of Baghdad Medical city in the period from August 2012 to July 2013. Genotyping was attempted on 100 HCV-infected Iraqi patients from different centers in the country. These included 55 males and 45 females with mean age 55.2 years . Testing for anti-HCV was done using the available commercial kits of the third generation enzyme immunoassay (Foresight-USA). According to the manufacture instructions, a sample was considered positive if the optical density value was equal to or greater than that of a strong positive reaction control multiply by 0.2 (cut-off). The positive samples were re-tested in duplicate. Positive sera were then subjected to a confirmatory test using a PCR technique (Amplicor HCV test; Hoffman-La Roche, Nutly, NJ, USA). HCV genotyping was performed on PCR HCV RNA-positive samples using a commercial line probe assay (Abbott-USA)

Results:. HCV genotype 4 is found to be the predominant genotype among HCV-infected Iraqi patients (56%) followed by 1b (23%) and 1a (12%) and genotype 3 (9%).

Conclusions: The predominance of HCV genotype 4 infection in Iraqi hemodialysis patients which is similar to the Arab countries in the Middle East, this will affect the duration and response to treatment.

Introduction

A variety of epidemiological data provides evidence for the occurrence of nosocomial transmission of hepatitis C virus (HCV) infection to hemodialysis (HD) patients. The most important factor implicated in HCV transmission between patients treated in the same dialysis unit is cross-contamination from supplies and surfaces as a result of failure of staff to follow infection control procedures. Parts of the HCV genome are highly variable and lend themselves to fingerprinting of each isolate using nucleic acid testing (NAT) and sequencing. This approach has permitted investigation of possible transmission routes within HD units.

A systematic review of molecular virology papers revealed transmission of HCV via internal fluid pathways of the dialysis machines in a minority of reports only[(1)].

Hepatitis C is divided into six distinct genotypes throughout the world with multiple subtypes in each genotype class. A genotype is a classification of a virus based on the genetic material in the RNA (Ribonucleic acid) strands of the virus. Generally, patients are only infected with one genotype, but each genotype is actually a mixture of closely-related viruses called quasi-species. These quasi-species have the ability to mutate very quickly and become immune to current treatments, which explains why chronic Hepatitis C is so difficult to treat[(2)].

Outside of the United States, many other countries worldwide face significant HCV infection rates. Despite aggressive programs toward education, care, and treatment over the last 10 years, Egypt faces the largest burden of

HCV infection in the world with a 10% prevalence of chronic hepatitis C infection among persons aged 15–59 years, predominantly genotype 4[(3)].

Most patients with acute and chronic infection are asymptomatic. Patients and health care providers may detect no indications of the conditions for long periods; however, chronic hepatitis C infection and chronic active hepatitis are slowly progressive diseases and result in severe morbidity in 20-30% of infected persons[(4)].

looked at 11 WHO-based regions in 2006 and estimated that globally 27% of cirrhosis was attributable to HCV and 25% of hepatocelluer carcinoma(HCC) was attributable to HCV. In many countries and populations, only a small number of patients with known infection actually receive treatment and yet successful treatment has been shown to have a significant impact on outcomes[(5,6)].

The combination of peginterferon and ribavirin has been the standard of care for patients with chronic hepatitis C regardless of strain of the virus (genotype 1,2,3 ,4,5,or 6). This regimen results in rate of sustained virologic response of 70% to 80% among patients with HVC genotype 2 or 3 infection and rate of 45 to 70% among with anyof other genotypes[(7,8)].

Patients and methods

The study involved one hundred patients with anti-HCV positive, they undergoing regular hemodialysis for renal failure from five dialysis centers in Medical City Hospital, Al-Karma Teaching Hospital, Al-Kadhmyia Teaching Hospital -Baghdad and Al-Basra Teaching Hospital. This case- control study was conducted at the Teaching laboratories of Baghdad Medical city in the period from August 2012 to July 2013.

Clinical data were gathered from each patient including demographic information and laboratory values such as aminotransferase, albumin, and total bilirubin levels. No liver biopsy specimens were available from any of these patients. Since the exact time of infection with HCV was not known.

Testing for anti-HCV was repeated in all recruited anti-HCV-positive patients using a third-generation immunoassay that allows the detection of antibodies to the NS3, NS4 and NS5 core antigens of the virus (Foresight-USA); and, if this assays gave a positive result, a PCR technique was performed. Detection of HCV RNA was attempted on a 200-µl sample of each serum found positive for anti-HCV, using a commercial, PCR-based test (Amplicor HCV test; Hoffman–La Roche, Nutley, NJ, USA). The manufacturer's instructions were followed and the internal control supplied by the manufacturer was added to each specimen, as an extraction and amplification control.

HCV genotyping was performed using a commercial line probe assay (Abbot-USA), according to the manufacturer's instructions. In this assay, a PCR product obtained by amplification of the 5′ non-translated region of the HCV genome was labelled with biotin, reverse-hybridized with 21 different probes, fixed on a nitrocellulose membrane and then revealed using a streptavidin–phosphatase conjugate; the hybridization pattern produced not only reveals the presence of any of the six major HCV genotypes, but also allows for identification of the subtype.

Statistical analysis

The χ^2 test was used to determine the variation in genotype distribution between the different groups. Statistical significance was considered as $P<0{\cdot}05$. All statistical tests were performed using SPSS statistical software package version 11 (SPSS Inc., Chicago, IL, USA).

Results

Of one hundred patients with anti HCV positive that were screened, Hepatitis C virus RNA was detected in only76 samples (76%), no serologic confirmatory test such as an immunoblot assay was used.

The 76 patients with HCV RNA-positive patients included 46 males and 30 females. Ages ranged between 12 and 68 years (mean =45 years)as in table-1. Twenty five had diabetic nephropathy, twenty had history of hypertension, ten had a history of renal calculi requiring surgical intervention, Eight patients had a history of acute glomerulonephritis, fifth patients had vasculitis, three had recurrent pyelonephritis, , and the remaining had chronic renal failure without a clear etiology. The majority (70%) of these patients had received at least one transfusion of blood or blood products prior to HCV diagnosis. five had a surgical procedure performed at some time prior to HCV diagnosis, and other had no risk factor for HCV acquisition.

Table 1: The characteristics of the patients with anti HCV + in hemodialysis

Patients	characteristics
No. of patients(anti HCV +)	100
HCV-RNA +	76
SEX—male	46
Female	30

Age (years)	12-68	
History of blood transfusion	70%	
ALT(alanine aminotransferase) normal≤ 20 U L	Normal	68
	Elevated	32
AST(aspartate aminotransferase) Normal ≤ 20U L	Normal	69
	Elevated	31
ALP(alkaline phosphtase) Normal ≤ 92U L	Normal	74
	Elevated	26
TSP(total serum protein)	Normal	68
	Decrease	32
Serum Albumin Normal (36-52 g/ L	Normal	44
	Decrease	56
Total bilirubin Normal (5-17)µmol/L	Normal	83
	Elevated	17
Treatment with interferon	positive	10
	negatives	90

HCV genotype distribution in 76 HCV-positive Iraqi patients is shown in table-2, HCV genotype 4 was the predominant genotype (56%) followed by genotype 1 include 1b (23%) and 1a (12%) and genotype 3 (9%). Differences in genotype distribution were statistically significant, (table-2). There were no differences in the mean of aminotransferase level, albumin, total bilirubin, or the estimated length of infection among patients infected with different HCV genotypes (1a, 1b, 3, and 4).

Table2: The genotype distribution among Iraqi HCV infection patients

Genotype	No. of patients	%	P- value
1 a	10	12%	0.001
1b	18	23%	
3	5	9%	
4	43	56%	
Total No.	76	100%	

Table3: Age±SD for each genotypes and subtypes

Genotype	Age range (mean ± SD)
1 a	18±2
1b	50±12
3	20.2±9.2
4	43.7±7.0

Discussion

To our knowledge, this is the first study to report on the genotyping of HCV in Iraqi hemodialysis patients with positive HCV.

Our results show that HCV genotype 4 is the predominant genotype (56%) followed by 1b (23%) and 1a (12%), while genotype 3 was detected in only (9 %). However, the predominance of HCV genotype 4 in the Iraqi hemodialysis patients is in agreement with other reports on genotyping of HCV isolates in different Middle Eastern countries such as in Egypt(up to 80%)[9].

On the other hand our results shows that, patients with genotype 1b were older than those infected with other genotypes, this results come in compatible with those reported by Zein*et al*.[10] who found that patients infected with HCV genotype 1b were older than those infected with other genotypes and may have been infected for a longer period. Unfortunately we are unable to comment on genotype-clinical outcome relationship as liver biopsy was not performed on all our patients. Moreover, the date of initial exposure to HCV was unknown by us in most cases. The

issue of the pathogenicity of different genotypes/subtypes remains controversial and long-term prospective studies in various population groups are required.

Most of the patients in our study not receive interferon therapy duo to comorbidities such as heart failure, diabetes mellitus or low live expectances duo to malignancy, or duo to cost or side effect of interferon therapy ,that compatible with Nahumetal[11].

Our patients response poorly to treatment and most of them die before clearance of the virus which compatible with other study by Halfon[12](patients infected with hepatitis c virus genotype 4(HCV-4)respond to interferon alpha(IFN) as poorly as those infected with genotype 1.

Genotype 2 not available in Iraqi hemodialysis patients similar to other study in Saudi Arabia which also predominant genotype 4 (62%) of HCV infection, followed by genotype 1 (24%), genotype 3 (9%), and genotype 5 (0.3%) which suggest same route of transmission of virus[13,14].

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