The diagnostic value of Hepatitis C genotyping by Real time-PCR in Iraqi patients

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Abstract

In this study, fifty patients diagnosed with hepatitis C infection (samples) were collected from March 2011 to January 2012,the genotyping for Iraqi patient were observed by used Real time-PCR as following genotype 1 form 46%, type 4 form 40%, type 3 form 8% and type 2 form 6%, This study showed that the most prevalent genotype in Iraqi patients type 1 then type 4 forming together 86% rather than other types and because these types don't well respond to treatment the dose and time of the therapy are increased in these patients.

Keywords: Real-Time PCR, Hepatitis C Genotypes, Hepatitis C virus.

القيمة التشخيصية للتنميط الجيني لالتهاب الكبد الوبائي نوع (ج) بطريقة Real time-PCR في المرضى العراقيين

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الخلاصة

الدراسة شملت جمع خمسون عينه من مرضى مشخصين بالتهاب الكبد الفايروسي نوع ج خلال الفترة من اذار 2011 الى كانون الثاني 2012, وتم ملاحظة التنميط الجيني للمرضى نوع ج باستخدام تقنية (Real-time PCR) وكان النمط الاكثر شيوعا هو النمط 1 وبنسبة 46٪، ثم النمط 4 وبنسبة 40٪، يليه النمط 3 وبنسبة 8٪ واخيرا النمط 2 وبنسبة 6٪, وأظهرت الدراسة أن التركيب الوراثي الأكثر انتشارا للمرضى العراقيين هو النمط 1 يليه النمط 4 ويشكلان معا 86٪ بدلا من الأنواع الأخرى، ولأن هذه الأنواع لا تستجيب جيدا للعلاج تطلب الامر زيادة الجرعة ومدة العلاج واعتماد العلاج المركب في هؤلاء المرضى.

الكلمات المفتاحية: التهاب الكبد الفايروسي نوع ج ، انماط التهاب الكبد الوبائي نوع ج.

Introduction

Hepatitis C virus: is a member of flaviviridiae family of viruses that are single-strand positive sense RNA viruses with no DNA stage. The nomenclature of hepatitis A, B, C, etc… follows the chronological of discovery (1). In 1989 by Michael Houghton's laboratory at Chiron in conjunction with Daniel Bradley's laboratories at the Centers for Disease Control and Prevention, and it was the first virus identified, and it was the first virus identified solely by using molecular methods (2) .

The used of PCR was concedard a revolutionary method in the diagnosis and research because of it's accuracy compared to any of the currently used methods specially in the field of microbiology and organisms which are difficult to cultivate and in other fields such as genetics and others (11) .

The development of Real-Time PCR in 1996 make it more easy and safe in that field and substitute the use of gel electrophoresis with the use of fluorescent signals the points to the target more easily and accurately obtained in our study which shows the fluorescent dye that was linked to the cloned DNA obtained from viral RNA which increase until passing the cycle threshold of amplification of DNA (13) .

Hepatitis C virus infection approximately 3% of the world's population, or 175 million to 200 million persons, with an estimated 3-4 million new infection each year, Prevalence is higher in some countries in Africa and Asia (4). So it is one of the most common chronic blood born infections in the world (9).

The genome of hepatitis C virus: is highly mutable. Mutations are not randomly distributed along the genome, but are most pronounced within hypervariable region (17). This region maps at a surface loop of the E2 protein containing a B-cell epitope that undergoes antigenic evolution overtime (3). Hepatitis C virus classified into eleven major genotypes designated 1-11, many subtypes designated a, b, c, etc.. (12). based on the genomic heterogeneity, a genotype is described as genetic heterogeneity among different hepatitis C virus isolates, the non coding region at either end of the genome 5'-UTR and 3'-UTR;UTR untranslated region are more conserved and suitable for virus detection by PCR (polymerase chain reaction), the genes coding for envelop E1 and E2 glycoprotein are the most variable. Amino acid

changes alter the antigenic properties of the proteins, thus allowing the virus to escape neutralizing antibodies (17) .

Genotypes 1-4 have a worldwide distribution. Type 1 is the most common, accounting for about 60% of global infection. They predominate in Northern Europe and Northern America, and in Southern and Eastern Europe and Japan (18). Type 2 is less common than type 1, while type 3 is endemic in south-east Asia and is variably distributed in different countries (19). Genotype 4 principally founded in Middle East, Egypt, and central Africa. The determination of infecting genotype is important for the prediction of response to antiviral treatment: genotype 1 and 4 are generally associated with poor response to interferon alone, whereas genotype 2 and 3 are associated with more favorable response. At patient with subtype 1b the disease progress to chronic condition in 90% (8).

Aim of the study

To recognize the prevalence of Hepatitis C virus genotypes in Iraqi patients.

Materials and Methods

Patients and Controls:

This study include fifty patients diagnosed with hepatitis C virus by serological method which is Enzyme Linked Immunosorbent Assay (ELISA) and confirmed by Real-time PCR for quantitative detection of the severity of infection, the age range of the patients were 20-55 years and 20 healthy control with similar age range, the blood sample of the patients were collected from March 2011 to January 2012.

Blood sample collection and preparation:

The blood samples were collected in EDTA tubes the plasma separated after centrifuging 1600 rpm for 20 min. and kept in deep freezing -20^{o}C until the work time.

The patients samples were collected from the Gastroenterology and Liver diseases teaching hospital, each of them was diagnosed serologically by ELISA method and molecularly;viral load by real-time PCR having hepatitis C virus the reason of collecting hepatitis C virus with high viral load to avoid limitation of the genotyping

procedure which should be over 1000 IU/ml to be detected by the fluorescent detection according to the kit of PCR used (7) .
The work done in Al Razy medical laboratory – PCR unit.

Statistical Analysis:
Statistical Package for Social Sciences (SPSS) version 15 was used for data entry and analysis. Results were expressed in simple statistical terms . Finding P value less than 0.05 was considered significant.

Results

General aspects and clinical manifestations:

By assessing the results obtained from patient and control groups; the following results observed: The mean age for males was 42.7 and females was 41.1 years old; 34% of patients 40-49 years of age, 32% which represents 30-39, 20% over 50 years old, 14%for the age range 20-29 years old and male to female ratio was 1:1; shown in (table,1).

Table,1: Distribution of cases by age and gender.

Age of the patient	Male No. (%)	Female No. (%)	Total No. (%)
20 – 29	5 (20)	2 (8)	7 (14)
30 – 39	7 (28)	9 (36)	16 (32)
40 – 49	11 (44)	6 (24)	17 (34)
50-55	2 (8)	8 (32)	10 (20)
Total	25 (100)	25 (100)	50 (100)

The presenting sign and symptoms of the patients differ but the majority of the patients 60% have no presenting sign and symptoms and were discovered accidently during routine screening for hepatitis C virus Ab before operation while others, with jaundice 14%, and on

routine dialysis for chronic renal failure ,and patient who had general discomfort with no specific sign and symptoms 12% then people with thalassaemia who are on regular blood transfusion 2% as shown in the (table,2).

Table (2): Distribution of patients by presenting symptom at onset of the disease.

Presenting Symptoms	Male No. (%)	Female no. (%)	Total No. (%)
No complaints	**14 (56)**	**16 (64)**	**30 (60)**
Jaundice	**4 (16)**	**3 (12)**	**7 (14)**
On dialysis	**2 (8)**	**4 (16)**	**6 (12)**
Thalassaemia	**1 (4)**	**0 (0)**	**1 (2)**
General incomfort	**4 (16)**	**2 (8)**	**6 (12)**
Total	**25 (100)**	**25 (100)**	**50 (100)**

Hepatitis C genotyping:

This study showed that the most prevalent genotype was type 1 with 46% followed by type 4 which is 40% while the lowest frequency was observed for type 3 which is 8% and type 2 which is 6% as shown in (table,3).

Table,3: Hepatitis C genotyping in patients and healthy controls

HCV Genotypes By Real time PCR method	The groups		Positive		Negative		Total		P
			No.	%	No.	%	No.	%	
	Hepatitis C patients	1	23	46	0	0	23	46	0.0034
		2	3	6	0	0	3	6	0.072
		3	4	8	0	0	4	8	0.065
		4	20	40	0	0	20	40	0.0049
		Total	50	100	0	0	50	100	
	Healthy control	All types	0	0	20	100	20	100	

Note: significant probability means $P < 0.05$

Discussion

The minimum age of presentation observed by this study was 20 years of age while the maximum 55 years The mean age for males was 42.7 and females was 41.1 years old, of course hepatitis C virus could affect any age from neonate through vertical infection from infected mother to old ages, this result could differs if extended study done or if the target was other hospitals such as gynaecology or paediatric hospitals intended. These results slightly similar to those observed in Brazil where the median age was 46 years (20) .

The genotype observed in Iraqi patients points to the prevalence of the presence of the resistant or difficult to treat types in Iraq these are the most prevalent type is one with 46% followed by type 4 with 40 % these type are the most difficult to treat with extended course of treatment and combined therapeutic protocol of interferon and ribavirin antiviral drug these results slightly similar to those observed in Brazil where typr1 form 64% of infection (20), and in USA where type 1 predominate over 65% (15) and in Japan over 80 % of type 1 (16), same in Iran over 61% with type 3 (7) England and Wales type 1 forms 48 % (10) and in Greece 47% type 1(5), in Egypt type 4 forms over 80% (16),while in Australia type 1 forms 55 % (6), and lastly in India type 1 is the most prevalent forming 61% (14) .

Conclusion

1. The detection of genotype is one of the most important steps in assessing the therapeutic course of the patient with the viral load and both done by PCR methods.
2. The most prevalent genotype observed was type one followed by type four with small percentages of other relatively rare genotypes.
3. These observation means that the most difficult to treat genotypes are the most prevalent in Iraqi patients more combined regime of treatment expected with prolonged course time and of course higher expenses on it.

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