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International Journal of Current Research
Vol. 7, Issue, 04, pp.14523-14525, April, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

DETECTION OF HEPATITIS C VIRUS RNA USING RT-NESTED PCR, IN LIBYAN PATIENTS

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ARTICLE INFO

Article History:
Received 10th January, 2015
Received in revised form
05th February, 2015
Accepted 22nd March, 2015
Published online 28th April, 2015

Key words:

RT-nested PCR,
HCV,
Libya.

ABSTRACT

Background: The detection of hepatitis C virus (HCV) RNA is essential to diagnose acute and chronic hepatitis C infection, therapy administration and follow up of treatment, and for the HCV genotype characterization.
Materials and Methods: In current study fifty HCV infected patients were included; blood samples were collected from each and RT-nested PCR for HCV RNA detection were done.
Results: out of 50 patients 29 were males (58.0 %) and 21 were females (42.0%). Their age range was from 5 to 67 years, and they were distributed into four groups 1. 1-20 years, 2. 21-40 years, 3. 41-60 years, and 4. >61 years), three of them were in group 1 (6.0%), 30 in group two (60.0%), 14 in group three (28.0%), and 3 in group four (6.0%).
All samples were subjected to Qualitative HCV RT-nested PCR, and all of it were positive (100.0%).
Conclusion: Molecular diagnosis of HCV is important for HCV treatment and genotype detection, in current study HCV RT-nested PCR was successfully applied for HCV detection in Libya.

INTRODUCTION

Hepatitis C virus (HCV) is the leading cause of chronic hepatitis worldwide and its subtypes/genotypes are clinically important for clinical management and vaccine development.
Since identified as non-A non-B hepatitis, by Choo and coworkers in the late 1980s (Choo *et al*., 1989; Wasley and Alter 2000), hepatitis C virus (HCV) continues to be a major disease burden on the world with an estimated over 150 million people in the world, and a majority of them will develop chronic infection such as cirrhosis or hepatocellular carcinoma, (Hanafiah *et al*., 2013; WHO 2014) the prevalence rate of HCV in North Africa and Arabian countries was between 1.4% and 2.1% in certain countries such Libya, Tunis and Saudi Arabia, though it was the highest in Egypt as it reached up to 19.3% (Pybus *et al*., 2003; Daw *et al*., 2002). HCV infection is a common cause of chronic viral hepatitis which often leads to end-stage liver disease and/or hepatocellular carcinoma (McOmish *et al*., 1993). Progression to chronic disease occurs in the majority of HCV-infected persons, and infection with the virus has become the main indication for liver transplantation (Lauer and Walker 2001). Hepatitis C virus (HCV) is a member of Flaviviridae family (James *et al*., 2005; Rho *et al*., 2008). The virus is positive-sense, single strand RNA of a proximately 9.6 Kb which codes for a polyprotein with a single open reading frame (ORF) of 3008-3033 amino acid (Bartenschlager *et al*., 2004; Tanaka *et al*., 1995). Different HCV isolates from around the world show substantial nucleotide sequence variability throughout the viral genome (Choo *et al*., 1991). Based on the identification of these genomic differences, HCV has been classified into multiple strains. It is thought that genetic heterogeneity of HCV may account for some of the differences in disease outcome and response to treatment observed in HCV-infected persons.

There are six variants of the virus (HCV genotypes 1, 2, 3, 4, 5, and 6), with 15 recorded subtypes that vary in prevalence across different regions of the world. A variety of methods are used to diagnose hepatitis C, including HCV antibody test, HCV viral load test, HCV genotype test and liver biopsy. Rapid, inexpensive, sensitive, and robust analytical methods are therefore essential for effective diagnosis and monitoring of disease treatment (Uliana *et al*., 2014).

HCV infection has low cure rates and causes serious adverse effects in some patients, with chronic infections including cirrhosis and hepatocellular carcinoma (Castello *et al*., 2010). Accurate and sensitive diagnosis in the early stages of HCV infection is important (Schnuriger *et al*., 2006). Serological and virological tests have become essential in the management of HCV infection in order to diagnose infection, plan treatment, and assess the virological response to antiviral therapy. Virological tools include anti-HCV antibody detection,

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serological determination of the HCV genotype, and molecular assays that detect and quantify HCV RNA and determine the HCV genotype (Castello *et al*., 2010).

Detection of anti-HCV antibodies is unable to distinguish between a current or past infection, because people will retain anti-HCV antibodies for life once they have been exposed to HCV (Pei *et al*., 2013). In addition, the testing for anti-HCV antibodies might provide false negative results, because it takes 45-68 day to develop anti-HCV antibodies following HCV infection (Hofmann *et al*., 2005). Only patients with detectable HCV RNA should be considered for pegylated interferon-alpha and ribavirin therapy, and the HCV genotype should be systematically characterized before treatment, as it determines the type and duration of treatment, the dose of ribavirin, and the virological monitoring procedure. HCV RNA monitoring during therapy is used to tailor the duration of treatment in HCV genotype 1 infection, and molecular assays are used to establish the end of treatment and, most importantly, the sustained virological response (Chevaliez *et al*., 2006).

MATERIALS AND METHODS

Fifty HCV infected patients diagnosed by ELISA attending Saint James Medical Laboratory, Tripoli, Libya were involved in this study. EDTA whole blood samples were collected and plasma were obtained and stored at -80^{o}C.

Extraction of HCV RNA

RNA extraction was performed using the SV total RNA isolation kit as described by the manufacturer (Promega USA). Briefly, a total of 175 µl of RNA lysis buffer containing β-mercaptoethanol (BME) was added into a 1.5 ml microcentrifuge tube and 175µl of plasma was added. This was mixed by inversion for 3-4 min and 350µl RNA Dilution Buffer (RDA) was added, mixed by inversion for 3-4 min, and heated in Dry heat block, at 65ºC for 5 min. The mixture was centrifuged at 14,000 rpm for 10 min and the cleared lysate was transferred to a new microcentrifuge tube. A total of 200µl of ethanol (96%) was added to the sample, and mixed by pipetting. The mixture was transferred to spin basket assembly and centrifuged for 1 min and elute was discarded. A total of 600µl of RNA Wash Solution (RWA) was added to the mixture and centrifuged at 14,000 rpm for 1 min and elute discarded, another 250µl RWA was added to the mixture and centrifuged at 14,000 rpm for 2 min and the elute was discarded. The spin basket was then transferred to the elution tube, where 100µl nuclease free water was added and centrifuged at 14,000 rpm for 1 min. The RNA was stored -80ºC until further processing.

Qualitative HCV RT-nested PCR

All samples were tested for HCV using RT-nested PCR, two set of primer (MK1, MK2 and MK3, MK4) targeting gene for core protein were used, the component of the first round PCR consists of 20µl of (1X) Access Quick master mix, 0.2µM of MK1 and MK2 oligonucleotide primers, AMV reverse transcriptase, 5µl of RNA template, and nuclease free water. The mixture was placed in the thermocycler (Palm cycler, Corbett Research, Australia) then subjected to cycling profile consisted of reverse transcription at 42 ^{o}C for 45 min, then an initial denaturation step at 94 °C (2 min), followed by 30 cycles of 94 °C for 40 s, 45 °C for 30 s, 72 °C for 40 s, and a final extension step at 72 °C for 5 min.

In the second round a 23µl reaction containing (1X) Go Taq green mix, 0.2µM of MK3 and MK4 oligonucleotide primers and nuclease free water was prepared and 2µl of the first round product was added. The reaction was subjected to 30 cycles of denaturation at 94^{o}C for 40 sec, annealing at 55^{o}C for 30 sec, and elongation at 72 ^{o}C for 40 sec then final extension step at 72 °C for 5 min.

PCR products were analyzed by 3% agarose gel electrophoresis and a band of 276 bp were indicate positive result.

RESULTS

Fifty HCV patients were included in this study, out of them 29 were males (58.0 %) and 21 were females (42.0%). Their age range was from 5 to 67 years, and they were distributed into four groups 1. 1-20 years, 2. 21-40 years, 3. 41-60 years, and 4. >61 years), three of them were in group 1 (6.0%), 30 in group two (60.0%), 14 in group three (28.0%), and 3 in group four (6.0%) Table 1.

All samples were subjected to Qualitative HCV RT-nested PCR, and all of it were positive (100.0%).

Table 1. Distribution of age group and sex within HCV RT-nested PCR positive samples

	Male	Female	Total
1- 20 year	1	2	3
21- 40 year	22	8	30
41- 60 year	6	8	14
More than 61 year	0	3	3
Total	29	21	50

DISCUSSION

HCV infection is one of the most important *Flaviviridae* infections with significant clinical problems throughout the world in humans and it is responsible for the second most common cause of viral hepatitis (Leiveven and Pegasys 2004). The viral genome of HCV is RNA and the virus genetically is very unstable and mutates rapidly. This means that the virus can quickly become resistant to anti-viral agents making treatment more difficult. In addition, with rapid mutation making an effective vaccine will also be a challenge. Data obtained from different parts of the world have focused on the increasing interest of HCV genotyping by mass screening as it is useful for the solution of epidemiological questions and development of vaccines against HCV. Furthermore, it has been shown to be beneficial to facilitate therapeutic decisions and strategies (McHutchison *et al*., 1999) Anti-HCV antibody testing and HCV RNA testing are used to diagnose acute and chronic hepatitis C. Only patients with detectable HCV RNA should be considered for pegylated interferon-alpha and ribavirin therapy, and the HCV genotype should be systematically characterized before treatment, as it determines the type and duration of treatment, the dose of

ribavirin, and the virological monitoring procedure. HCV RNA monitoring during therapy is used to tailor the duration of treatment in HCV genotype 1 infection, and molecular assays are used to establish the end of treatment and, most importantly, the sustained virological response (Chevaliez *et al*., 2006).

In current study nested RT-PCR for HCV were used to diagnose serologically diagnosed HCV patients (ELISA), the test was successfully established and its result were 100% agreed the ELISA result.

Conclusion

Molecular diagnosis of HCV is important for HCV treatment and genotype detection, in current study HCV RT-nested PCR was successfully applied for HCV RNA detection in Libya.

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