Original Article

Detection and Quantification of HCV-RNA by RT-PCR among Anti-HCV Positive Patients

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Abstract

Introduction: The most common contemporary strategy to diagnose chronic hepatitis C virus (HCV) infection consists of initial screening with an HCV enzyme immunoassay (EIA) antibody test followed by supplemental testing of positive screening tests with a quantitative HCV RNA assay to confirm the positive EIA and to determine whether they have active or resolved hepatitis C infection.

Objectives: To detect and quantify HCV-RNA by real-time polymerase chain reaction (real-time PCR) among anti-HCV positive patients and to identify the socio demographic factors among these patients.

Materials and Methods: This was a descriptive type of cross-sectional study which was conducted in Combined Military Hospital and Armed Forces Institute of Pathology, Dhaka cantonment. A total of 108 anti-HCV positive patients by enzyme-linked immunosorbent assay (ELISA), who were clinically suspected and advised for anti-HCV test, were selected randomly for the study and subjected to do HCV-RNA analysis during the period of October 2016 to September 2017.

Results: Out of 108 anti-HCV positive patients by ELISA, HCV-RNA was detected in 72 (66.7%) cases with mean value of HCV RNA quantification was 2013323.95±2695207.41 (IU/ ml). Majority of anti-HCV positive patients (29.6%) belonged to 51-60 years age group with male predominance (58.33%). It was observed that 43.52% patients came from middle income group family, 31.48% came from poor and 25.0% came from high income group family. Risk factor for HCV infected population was found maximum in dialysis patients (47.37%), followed by blood transfusion (13.89%), Injecting drug User (IDUs) (12.04%), surgery & intervention (9.26%) and sexual transmission (1.85%). Mean alanine aminotransferase (ALT) was found 67.30±44.99 U/L among HCV-RNA detected patients ($p < 0.05$).

Conclusion: The quantification of HCV RNA by RT-PCR will be helpful to rationalize the treatment, enhance antiviral responses and mitigate mortalities of HCV infected patients.

Key-words: ELISA, HCV-RNA, Real time PCR

Introduction

Hepatitis C virus (HCV) infection is a serious global health problem that affects 180 million people worldwide. It is estimated that three to four million people are infected with HCV every year[1]. The hepatitis C virus is an RNA virus that belongs to the family flaviviridae[2]. HCV replicates in the cytoplasm of hepatocytes, but is not directly cytopathic. Persistent infection appears to rely on rapid production of virus and continuous cell-to-cell spread, along with a lack of vigorous T-cell immune response to HCV antigens. The HCV turnover rate can be quite high with replication ranging between 1010 to 1012 virions per day, and a predicted viral half-life of 2 to 3 hours[3]. The rapid viral replication and lack of error proofreading by the viral RNA polymerase are reasons why the HCV RNA genome mutates frequently[4]. The transmission of HCV is primarily through exposure of infected blood. Risks for transmission include blood transfusion, intravenous drug use, high risk sexual activity, solid organ transplantation from an infected donor, occupational exposure, hemodialysis, household exposure and birth to an infected mother[5]. The aim of the present study was to find out HCV-RNA among anti- HCV positive (ELISA) patients by real time reverse transcriptase polymerase chain reaction (RT-PCR) method.

Materials and Methods

This is a descriptive type of cross-sectional study which was conducted in Combined Military Hospital (CMH) and Armed Forces Institute of Pathology (AFIP), Dhaka cantonment through the period from October 2016 to September 2017. Study population was anti-HCV antibody positive cases by ELISA of both sex and all age groups. A total of 108 anti-HCV positive cases, who were clinically suspected and advised for anti-HCV test, were selected randomly for the study. Relevant history was taken from the patients. Patients who were under treatment of HCV infection and other liver diseases, who were suffering from hepatitis B virus (HBV), human immunodeficiency virus (HIV) infection along with HCV infection and pregnant women, were excluded. Face to face interview was done with the patients and a structural questionnaire was used to collect data. Ethical clearance was taken from Directorate General of Medical Services. With the written consent of the patients HCV-RNA was extracted first and then HCV-RNA was detected and quantified by real time reverse transcriptase polymerase chain reaction (PCR) among anti-HCV positive patients.

Nucleic acid extraction: RNA was extracted from 200μl of plasma with nucleic acid extraction kit and stored at -20 till used for PCR.

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Real time reverse transcriptase polymerase chain reaction (RT-PCR) for detection and quantification of HCV-RNA: The artus HCV RG RT-PCR Kit constitutes a ready-to-use system for the detection of HCV RNA using polymerase chain reaction (PCR) on Rotor-Gene Q Instruments. The Hepatitis C Virus RG Master A and B contain reagents and enzymes for the reverse transcription and specific amplification of a 240bp region of the HCV genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green of the Rotor-Gene Q. In addition, the artus HCV RG RT-PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an internal control (IC) in fluorescence channel Cycling Orange of the Rotor-Gene Q. The detection limit of the analytical HCV RT-PCR is not reduced. External positive controls are also supplied, which allowed the determination of the amount of viral RNA.

Results

Age distribution of the anti-HCV positive patients was observed that out of 108 patients, majority 32(29.6%) patients belonged to age 51-60 years and lowest number 6(5.6%) was at the age of ≤20 years. Sex distribution was observed that 63(58.33%) patients were male and 45(41.67%) were female. Male to female ratio was 1.4:1. In economic status of the HCV infected patients observed that majority 47(43.52%) patients were from middle income group family, 34(31.48%) came from poor and 27(25.0%) from high income group family (Table-I). Figure-1 shows that risk factor for HCV infected population was found maximum in dialysis patients (45.37%), followed by blood transfusion (13.89%), intravenous drug users (IDUs-12.04%), surgery and intervention (9.26%) and sexual transmission (1.85%).

In the present study, among 108 anti-HCV antibody positive patients, 72(66.7%) were HCV-RNA positive by RT-PCR and the mean value of HCV-RNA quantification was 2013323.95±2695207.41 (IU/ml), minimum was 75 (IU/ml) and maximum was 9962885.00 (IU/ml). In the present study, mean ALT was 67.30±44.99 U/L among HCV-RNA positive patients and 36.72±34.76 U/L whose HCV-RNA was not detected or absent. That was statistically significant ($p<0.05$). Here, Out of 72 HCV-RNA positive cases, 31(43.05%) received dialysis.

Table-I: Socio-demographic characteristics of the study population (n=108)

Characteristics		Frequency	Percentage
Age in years	≤ 20 years	06	05.6
	21-30 years	14	13.0
	31-40 years	12	11.1
	41-50 years	27	25.0
	51-60 years	32	29.6
	> 60 years	17	15.7
Sex	Male	63	58.3
	Female	45	41.7
Economic status	Poor income (<10,000TK)	34	31.48
	Middle income (10,000-40,000TK)	47	43.52
	High income (>40,000TK)	27	25.00

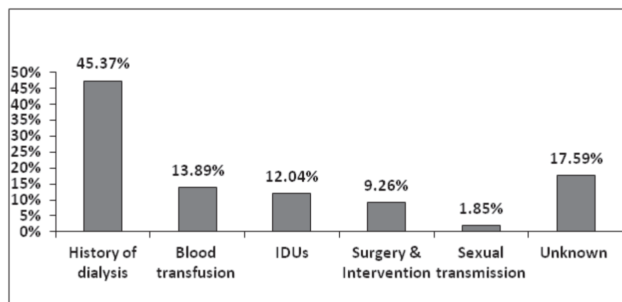

Figure-1: Risk factors of the HCV transmission among study population (n=108)

Discussion

Enzyme linked immunosorbent assay test allowing the detection of anti-HCV antibodies has false positive results and require the development of more sensitive and specific assays to confirm the results. PCR, allowing the hepatitis C virus diagnosis by showing directly HCV RNA sequences, offers a complementary approach to immunoserological tests[6]. Quantification of hepatitis C virus (HCV) RNA is useful in clinical practice. Indeed, monitoring of the fall in HCV RNA levels is presently used to decide whether to continue or stop therapy for patients. New directions in HCV therapy suggest that future treatments will be tailored to the individual patient and that HCV RNA load monitoring during therapy will be a major treatment-tailoring tool. Thus, HCV RNA quantification assays need to be sensitive enough to detect HCV RNA reductions during therapy and also accurate in both the higher range (baseline viral load in untreated patients) and the lower range (patients on therapy) of HCV RNA levels. This can help to clarify the mechanisms underlying antiviral treatment efficacy and failure and derive recommendations on the basis of viral load monitoring for routine patient care. Thus, accurate HCV RNA quantification is absolutely crucial for hepatitis C therapy[7].

In the present study, 108 patients were found anti-HCV positive by ELISA who were clinically suspected and advised for anti-HCV test by clinicians. Similar observation was found by Al-Mahtab et al. in their study[8]. It was observed that out of 108 patients, majority 32(29.6%) patients belonged to age 51-60 years and lowest 6(5.6%) numbers belonged to age ≤ 20 years. Sixty three (58.33%) patients were male and 45(41.67%) were female. Male to female ratio was 1.4 : 1. In a study of Al-Mahtab et al[8] found that anti-HCV was positive in the age group between 17-50 years and was male predominated 66% (6/9) over female 33%.

In this study, risk factor for HCV infected population was found maximum in dialysis patients (45.37%), followed by blood transfusion (13.89%), IDUs (12.04%), surgery and intervention (9.26%) and sexual transmission (1.85%). Blood and its components (plasma, serum, albumin etc) are the main source of HCV infection. In recent years, an increasing role has been assigned to hospital transmitted infections (nosocomial)[9]. Rahman et al in his study found 27.3% of the hemodialysis patients were positive for anti HCV[10]. In present study, 72(66.7%) were HCV-RNA positive tested by real time PCR among 108 ELISA positive cases. Gao et al[9] reported that the result-agreement rate of ELISA and real time PCR methods were 78.38%. It was observed in the

present study that the mean ALT was 67.30±44.99 U/L among HCV-RNA detected patients and 36.72±34.76 U/L whose HCV-RNA was not detected or absent.

Out of 72 HCV-RNA positive cases, 31(43.05%) received dialysis. A study carried out by Rahman study it was observed that HCV RNA was detected in 25 (75.7%) out of total 33 Anti HCV antibody positive patients who have received dialysis. Hepatitis C virus (HCV) infection remains frequent in patient receiving long-term dialysis both in developed and less-developed countries. The natural history of HCV infection in dialysis patients remains incompletely understood. HCV plays a detrimental effect on survival in the dialysis population but remains unknown whether the elevated mortality risk because of HCV infection is only attributable to an increase in liver disease-related deal[10].

Conclusion

The 2002 NIH consensus conference[11] gave recommendations for the use of molecular tests, in addition to enzyme-linked immunosorbent assays, for diagnosis. These recommendations concern the decision to treat, optimal treatment schedules, and assessment of the viral response to antiviral therapy. For all of these indications, a real-time quantitative PCR assay is very important for clinical and therapeutic management of chronic HCV infection without additional costs.

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