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Research Article

STUDY ON PREVALENCE OF HEPATITIS C VIRUS INFECTION AND ITS GENOTYPE DISTRIBUTION AMONGST CHRONIC RENAL FAILURE PATIENTS ON MAINTENANCE HEMODIALYSIS: A SINGLE CENTER EXPERIENCE

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Abstract- Background: Hepatitis c virus (HCV) infection is a very common infection in chronic renal failure (CRF) patients on maintenance hemodialysis (MHD). Genotype detection is crucial for management of chronic hepatitis c patients, prediction of prognosis, epidemiological study and also for vaccine preparation. Based on the sequence divergence, till date HCV strains are divided into 7 main genotypes and multiple subtypes (67 confirmed, 20 provisional). The study was aimed to find out single centre prevalence and distribution of HCV genotypes in CRF patients on MHD. Methods: Genotyping was performed by nested reverse transcriptase PCR. Isolation of HCV RNA, reverse transcription and nucleic acid amplification of 5' UR was carried out. Biotinylated oligonucleotide primers were used to generate amplified product and reversely hybridized to type-specific probes on nitro-cellulose strips. Conjugate and substrate were added post-hybridization to observe generated bands which were then matched with control bands. The genotypes studied were 1a to1c, 2a to 2d, 3a to 3f, 4a to 4k, 5a and 6a. Results: Out of 2550, 210 patients (12.14%) (Male: 166, Females: 44) were HCV positive. Genotype 1 was found in 179 (85.2%) and genotype 3 in 31(14.8%) patients. Amongst, genotype 1, subtype 1a, 1b, 1ba and undetermined comprised 71.4%, 24.6%, 0.6% and 3.4% respectively. Amongst, genotype 3, subtypes 3a, 3b and undetermined comprised 70.9%, 9.7% and 19.4% respectively. No other genotypes were found. Conclusion: HCV infection was found in 12.14 % CRF patients on MHD with genotype 1 (85.2%) being predominant followed by genotype 3 (14.8%) in our study.

Keywords- *Hepatitis C Virus, Genotypes, HCV Subtypes, Nested Reverse Transcriptase PCR, Chronic Renal Failure, Haemodialysis*

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Introduction

Hepatitis C virus (HCV) infection is a major public health problem, with an estimated global prevalence of 3% occurring in about 180 million carriers and approximately 4 million people have been newly infected annually [1]. HCV infection is a major health problem among dialysis patients in developing countries. Dialysis patients remain a high- risk group for HCV infection [2]. Moreover, prevalence of HCV is high in patients with chronic kidney disease (CKD) under maintenance hemodialysis and also Kidney transplant recipients. In India, Prevalence of HCV in dialysis population varies from 4.3-46% [3]. This high prevalence may be due to various factors like multiple blood transfusions, long term maintenance hemodialysis and patients with low immunity undergoing hemodialysis in multiple centers. HCV is an enveloped, positive single-stranded RNA virus containing a single large open reading frame [3]. HCV virus belongs to genus Hepcivirus in the family Flaviviridae which was the first virus detected by molecular techniques in 1989. HCV genome has both highly conserved and highly variable regions. The 5' non-coding region (5'UTR), core (C), envelope 1 (E1) and non-structural protein 5B (NS5B) sites of the HCV genome are relatively well-conserved regions and used as the basis for classification. In contrast, the envelope 2 (E2) glycoprotein site of HCV is the more variable region into the genome [4]. HCV genome has high genetic heterogeneity due to the lack of proof-reading capability of the RNA-dependent RNA polymerase and high replicative activity of the virus [5, 6]. The polyprotein precursor is co-translationally processed by host signal peptidases to yield the structural proteins like core[c], envelope [E1 and E2] and the non-structural proteins like NS1, NS2, NS3, NS4A, NS4B, NS5A, and NS5B [4]. Based on the sequence divergence, till date, HCV strains are divided into seven main genotypes and multiple subtypes, 67 confirmed and 20 provisional subtypes [3]. Genotype 1 (GT1), genotype 2 (GT2) and genotype 3 (GT3), particularly subtypes 1a, 1b, 2a and 3a, are extensively distributed globally. HCV infection mainly produces hepatic manifestation like cirrhosis and liver cancers as well as extra-hepatic manifestation like cryoglobulinemia, lymphoproliferative disorders and chronic kidney disease, occurring largely in the form of mixed cryoglobulinemia and secondary glomerular disease. It is established that the presence of HCV infection shows higher rates of mortality as compared to HCV-negative subjects on dialysis or undergoing kidney transplant [3]. High rate of genotype diversity, chronicity of infection and lack of effective vaccine is a challenge for the clinicians to treat HCV infected patients successfully. Moreover, in recent trend selection of new antiviral agent and duration of treatment and prognosis of patients are decided on the basis of HCV genotype and its subtypes. All these factors have led to the necessity of the study of HCV prevalence with its genotypic variation in CKD patient so as to make the treatment effective. Therefore, our study was aimed to establish prevalence of HCV infection with genotypic variation in CKD patients who are on maintenance HD.

Materials and Methods

This was a prospective single center study carried out from Sept'17to May'19 All the patients with CKD who were HCV RNA positive and were on maintenance hemodialysis were included in the study. All patients were above 12 years of age and from both the genders. Patients under treatment for HCV infection were excluded from the study. The study population was explained about nature of the study and informed consent was taken before the study. IEC approval was obtained. Blood samples 7 ml of all the recruited patients were collected by venipuncture in ethylenediaminetetraacetic acid (EDTA) vacutainer under aseptic condition, centrifuged and separated plasma was analyzed for genotyping study by Nested Reverse Transcriptase PCR method [7]. Detailed history was taken to evaluate the associated risk factors in enrolled patients. RNA was extracted using Qiagen extraction protocol (QIAamp Viral RNA Mini Kit, Qiagen GmbH, Hilden, Germany).The RNA pellet was reverse-transcribed to complementary DNA (cDNA) and polymerase chain reaction (PCR) was used in the amplification of the 5' noncoding region of the viral genome that is very well protected for genotyping; cDNA and PCR reaction was performed by a one-step reverse transcriptase PCR (RT-PCR) using HCV PM BIO kit (AB Analitica, Italy). To determine the amplified PCR products, agarose gel electrophoresis was applied. Isolation of HCV RNA, reverse transcription and nucleic acid amplification of 5' UR was carried out. Biotinylated oligonucleotide primers were used to generate amplified product and reversely hybridized to type-specific probes on nitro-cellulose strips. Conjugate and substrate were added post-hybridization to observe the generated bands which were then matched with control bands. Second round of amplification was carried out if reported negative in the first round. Then different genotypes like 1a to1c, 2a to 2d, and 3a to 3f, 4a to 4 k, 5a and 6a were evaluated. Statistical Analysis was carried in the form of percent prevalence and percent genotyping and subtyping. We also tried to identify influence of risk factors like Blood transfusion, Duration of Dialysis (Dialysis Vintage) and number of centre visited for dialysis.

Results

Demographic characteristics: Out of 2550 patients, 210 (12.14%) patients (179 males, 31 females) with mean age, 36.2 years(range:12- 65 years) were enrolled in the study. Genotype 1 was found in 179 (85.2%) and genotype 3 in 31(14.8%) patients. Amongst, genotype 1, subtype 1a, 1b, 1ab and undetermined comprised 71.4%, 24.6%, 0.6% and 3.4% respectively. Amongst, genotype 3, subtypes 3a, 3b and undetermined comprised 70.9%, 9.7% and 19.4% respectively. No other genotypes were found [Fig-1].

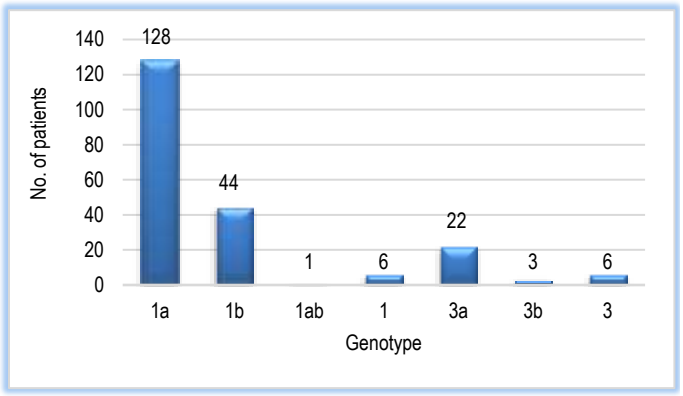

Fig-1 Distribution of HCV genotypes in HCV RNA +ve samples

High HCV prevalence rate was found in patients having blood transfusion, with dialysis vintage of >2 years and who visited more than one dialysis centre for their maintenance haemodialysis therapy [Fig. 2]. 134 (63.8%) underwent blood transfusions, 151 (71.9%) patients were on dialysis since >2 years, and 88 (69.5%) patients were visited more than 1 dialysis center in the past.

Discussion

It is well known that hemodialysis patients are at high risk for development of hepatitis C infection. However, the data on the prevalence of anti HCV among Indian hemodialysis patients is scanty. Salunkhe *et al* [8] in 1992 reported 45%, Chadher *et al* [9] in 1993 reported 12.1%, Sumathi *et al* [10] in 1993 reported 37.5%, Agarwal *et al* [11] in 1999 reported 42%, and Jaiswal *et al* [12] in a study from 1992-2000 reported prevalence of 30%. The prevalence of HCV infection among the hemodialysis patients at our institute is 12.14%.

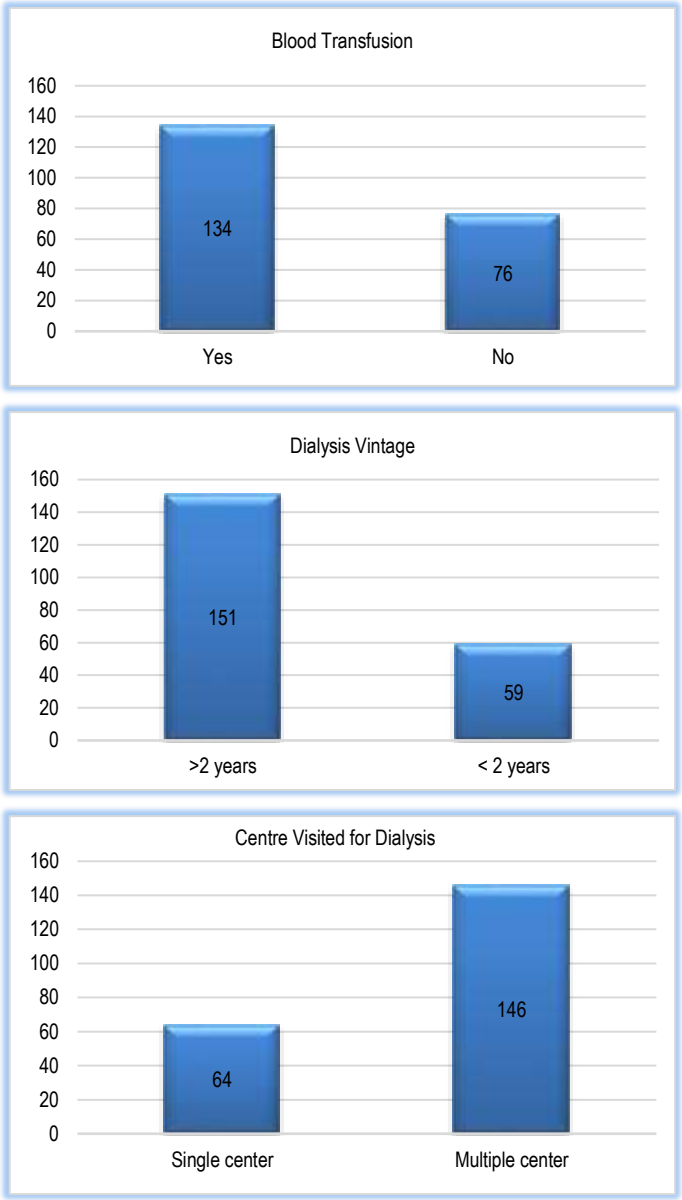

Fig-2 Correlation of HCV infection with associated risk factors

The distribution of HCV genotypes and sub-genotypes varies according to geographic variation in different parts of the world. Nowadays, it is very crucial to know the genotypes and its subtypes to determine clinical status, to decide effective therapy and prognosis of the patient. Yabaji *et al* showed Genotypes 1 and 3 have a major worldwide distribution accounting for 60-70% of infections which is correlating well with our study which showed genotype 1a most prevalent genotype [13]. Other study of Frank C *et al*. published, in Africa and the Middle East, the most prevalent genotype is 4, in South Africa and Asia Genotypes 5 and 6 are most common and Genotype 1a is most prevalent in the United States and Northern Europe, whereas in our study genotype 1a is the most commonly found subtype followed by genotype 1b [14]. One Indian study of Ashish *et al* Showed genotype 3 predominates in the north, east and west India, whereas genotype 1 is commoner in south India [15; Fig. 3]. This finding is further supported by study of Das *et al*. in one of the leading virology laboratories in India [16]. When we compared our result with international study of Perez RM *et al*., it was found that in Netherlands, France, Morocco, Mexico and Turkey, the predominant genotype was 1b among patients on HD whereas in our study it was genotype 1a [17].

Petruzziello *et al* from the United States found subtype 1a was the most frequent among dialysis patients which is correlating well with our finding while other study onItalian HD patients, subtypes 2a and 3a predominated which was differing from our finding. Moreover, smaragdimarinaki *et al* showed that dialysis patients are more susceptible to mixed genotype infections due to multiple exposures in the dialysis environment. On analyzing our data, we also found one mixed infection with 1ab subtypes. Mixed infections are not often identified due to their short duration and to the lack of sensitivity of the molecular techniques [18]. On analysis of independent risk factors, we found increased high prevalence of HCV infection in patient who had undergone multiple blood transfusions, this finding was not matching with the study of Agarwal SK *et al.* which showed multiple BT is not an independent risk factor for HCV positivity [12]. Moreover, our study showed high prevalence of HCV in patients who were on dialysis for more than 2 years and findings were correlating well with the study of Bdour *et al* which also reported > 48 months of duration of hemodialysis is a significant risk factor for HCV sero-positivity. We also found the patient with HCV positivity have visited multiple centers for their MHD which might be one of the independent risk factors for HCV sero-positivity.

Conclusion

In our study there was male preponderance of HCV positivity (79.04% males vs. 20.96% females).Majority of patients (53.33%) were in the age group of 20-40 years. About 70% of HCV +ve patients belonged to rural areas. HCV infection was found in 12.14% CRF patients on MHD with genotype 1 (85.2%) being predominant followed by genotype 3 (14.8%). Amongst, genotype 1, subtype 1a, 1b, 1ab and undetermined comprised 71.4%, 24.6%, 0.6% and 3.4% respectively. Amongst, genotype 3, subtypes 3a, 3b and undetermined comprised 70.9%, 9.7% and 19.4% respectively. No other genotypes were found. High prevalence rate was associated with blood transfusions, dialysis vintage of >2 years and visit to >1 dialysis centre for maintenance haemodialysis therapy. Patients on haemodialysis treatment are at high risk for HCV infection. In spite of various measures adopted to prevent nosocomial infections.

Application of research: This study will help to know the prevalence of Hepatitis C virus infection in chronic kidney disease patients on hemodialysis in Gujarat. Through this study we will be able to know genotype prevalence of Hepatitis C virus in our patient population.

Research Category: Medical Microbiology

Abbreviations: CKD- Chronic Kidney disease, CRF- Chronic renal failure
EDTA - Ethylenediaminetetraacetic acid, GT- Genotype, HD- Hemodialysis
HCV- Hepatitis c virus, MHD- Maintenance hemodialysis
NS5B- Non-structural protein 5B, PCR- Polymerase chain reaction
RNA- Ribonucleic acid, 5'UTR - 5' non-coding region
RT-PCR- Reverse transcriptase polymerase chain reaction

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Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Institute of Kidney Diseases and Research (IKDRC-ITS), Ahmedabad, 380016

Conflict of Interest: None declared

Ethical approval: The study was approved by our institutional review board and study number was IKRDC-ITS-LAB-10/02/2017. Consent was obtained from all participants.
Ethical Committee Approval Number: IKDRCITS- LAB-10-02-2017

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