Original Article

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Microbiology Section

Hepatitis C Virus (HCV) Infection among Seronegative Patients undergoing Haemodialysis in a Remotely Located Tertiary Care Hospital of Northern India: Value of HCV-RNA and Genotypes

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ABSTRACT

Background: Haemodialysis (HD) patients are at an increased risk of Hepatitis C virus (HCV) infection, which is significantly associated with increased morbidity and mortality.

Aim: The aim of this study was to find the prevalence of HCV infection in anti-HCV antibody negative haemodialysis patients by Real-time PCR (RT-PCR) and value of HCV-RNA among seronegative patients undergoing haemodialysis in a remotely located tertiary care hospital.

Materials and Methods: A total of 100 chronic renal failure patients on haemodialysis were studied. All the patients were screened for anti-HCV antibodies by ELISA test and for HCV-RNA by RT-PCR.

Results: The overall prevalence of HCV infection was 32%. Antibody positivity was 30% and HCV-RNA by RT-PCR was detected in 20%. HCV-RNA in seronegative patients was detected in 2.8%.

Conclusion: Serological assays (30%) are quite reliable for detecting HCV infection in patients undergoing haemodialysis in our tertiary care hospital. Only a small proportion of them (2.8%) require the documentation of viral genome for current infection.

Keywords: Anti-HCV antibodies, ELISA, RT-PCR

INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health problem, with an estimated global prevalence of 3% [1]. Worldwide, there are about 180 million carriers and 3-4 million new infections annually [2]. Patients on haemodialysis (HD) are at increased risk of HCV infection. This could be due to a failure to identify carriers or because of lack of truly effective biosafety measures implementation in the dialysis units [3]. ELISA test for anti-HCV antibodies which is usually employed to screen HCV infection, fail to assess the real magnitude of HCV infection in patients on haemodialysis (HD), as the window period of HCV infection in these immunocompromised patients can be longer [4]. Identification of viral genome is helpful to document active infection in such patients. HCV-RNA detection by PCR is widely accepted as a gold standard test for the diagnosis of current HCV infection [5]. HCV genotyping is important in the study of epidemiology, pathogenesis and response to antiviral therapy of HCV infection [6].

Our's is a remotely located tertiary care hospital which caters to patients of almost 14,981Km^2 area of Malwa region of Punjab, mostly of rural background who visit this tertiary care hospital only in chronic stage of the disease.

Therefore, the present prospective study was undertaken to determine the prevalence of HCV infection in patients undergoing haemodialysis in our hospital by detecting anti-HCV antibodies and HCV-RNA. This would help to assess importance of detection of HCV-RNA by RT-PCR in patients undergoing haemodialysis.

MATERIALS AND METHODS

A total of 100 consecutive patients of chronic renal failure from March 2012-June 2013 undergoing haemodialysis for the first time in the haemodialysis unit of our hospital were included in the study after taking their written consent and permission from Instititutional Research and Ethical Committee. Patients on maintenance dialysis in our hospital were excluded. All patients were interviewed regarding the risk factors of HCV infection (history of transfusion of blood /blood products, number of dialysis at other centers, frequency of therapeutic injections/intravenous fluids, surgical interventions (major/minor), dental treatment, shaving by communal barber, intravenous drug abuse (IVDA), ear/nose piercing, tattooing, accupuncture, sexual history and household exposure to HCV).

Single predialysis blood sample was collected from each patient after taking all aseptic and universal precautions. All the samples were screened for anti-HCV antibodies by 3rd generation HCV Microlisa (J. Mitra & Co. Pvt. Ltd.) according to the manufacturer's instructions. The plasma samples were stored at -70^{o}C and tested for HCV-RNA by Real time PCR using HCV REALTM (Sacace Biotechnologies). Genotyping was done by Linear array HCV –genotyping kit(Roche Diagnostic, Mannheim, Germany) from an accredited laboratory. All the patients undergoing haemodialysis in our tertiary care hospital were screened for HCV infection by detecting anti-HCV antibodies by 3rd generation HCV Microlisa (J.Mitra & Co. Pvt. Ltd.) and one dedicated machine is used for HCV reactive patients and used material is discarded separately.

STATISTICAL ANALYSIS

All the collected data were entered and analysed by SPSS (16.0) version. There is substantial agreement between the two tests. Kappa statistic was 0.61 (substantial agreement).

RESULTS

Screening of 100 HD patients for HCV infection showed that 30 (30%) were positive for anti-HCV antibodies by third generation ELISA test. PCR detected HCV-RNA in 20 (20%). The combined figure of the two tests revealed that in all, 32(32%) was having HCV infection. Of these 32, 18(56.25%) were positive by both the tests (ELISA+PCR) and 12 (37.5%) by ELISA for anti-HCV antibodies

Test	Positive	%age
ELISA only	12	37.5
RT-PCR only	2	6.25
ELISA +RT-PCR	18	56.25
Total	32	100

[Table/Fig-1]: Positivity of HCV infection by ELISA (anti-HCV antibodies) and RT-PCR (HCV-RNA) undergoing haemodialysis

Risk factor	Positive	%age
Previous Dialysis at other centers	20	62.5
Blood/blood products	18	56.2
Therapeutic injection(unsafe)	15	46.8
Shaving(at road side barber shops)	10	31.5
Major/Minor Surgical Procedures	7	21.8

[Table/Fig-2]: Various Risk Factors studied in 32 Patients having HCV infection

alone. HCV-RNA (PCR) alone detected HCV infection in 2(6.25%) patients [Table/Fig-1]. The overall agreement between the two methods of detection was substantial by Kappa agreement value. (Kappa value 0.61).

Major risk factors observed in 32 patients having HCV infection were number of dialysis at other centers (62.5%), history of transfusion of blood/blood products (56.2%), therapeutic injections/intravenous fluids (46.8%), Shaving at community barbers 31.5% and major/minor surgical procedures was (21.8%) [Table/Fig-2]. The most common genotype in 20 patients who had shown HCV viraemia was genotype 3 in 14 (70%) followed by genotype 1 in 5 (25%) and genotype 4 in 1 (5%).

DISCUSSION

There is variability in HCV testing practices in dialysis centres. In Northern India, most of the recent published data on HCV prevalence in haemodialysis patients is based on detection of anti-HCV antibodies by third generation ELISA test [7-9]. Only a limited number of studies have used PCR for detecting infection among which most have used PCR only for confirmation of infection in cases of positive samples for anti-HCV antibodies and not as a screening test in all the studied patients [10]. We observed the prevalence of 32% by detecting both anti-HCV antibodies and HCV-RNA, which corroborates the findings of Jaiswal et al., (30%) from central India [9]. Anti-HCV antibodies alone were detected in 30% in our study which is higher than that of Jasuja et al., (21.8%) from New Delhi, but falls within the range of 3-45% reported from different regions of India [8-11]. There were 70 seronegative (negative for anti-HCV antibodies) patients in our study and HCV-RNA by PCR was detected in 2 (2.8%) of them. The rate of HCV antibody negative viraemia by PCR in HD patients has been reported to be between 0-12% globally [12]. Various studies from neighbouring countries and our country have also reported HCV-RNA detection in range of 5-7% in HCV seronegative patients [8,13-15] [Table/Fig-3]. From all these studies, we have reported the lowest (2.8%) detection of HCV-RNA in HCV seronegative patients. It could be because most of our patients had already visited number of private clinics in smaller towns and by the time they reached our tertiary care hospital, were in chronic stage of disease, so easily diagnosed by detecting anti-HCV antibodies.

The most common risk factor observed in our patient population was the history of dialysis at other centres (62.5%). This is similar to other studies from India [9,16]. History of transfusion of blood/blood products (56.29%), therapeutic injections/intravenous fluids (46.8%) and shaving at community barbers (31.5%) were other important risk factors observed in the present study and are consistent with the findings of Mujtaba et al., [17]. Many patients believe that injectables/intravenous fluids act faster and relieve the symptoms more quickly than oral drugs. A high frequency of injection use, most of which are administered under unsterile conditions, put the patients on risk of acquiring HCV infection. The commonest HCV genotype observed in 20 patients of the present study having HCV Viraemia was 3 (70%) followed by 1 (25%) which is similar to other studies from Northern India [18,19]. However, an important finding of our study was the presence of genotype 4 which has been reported from Pakistan but not from Northern India [17]. This could be because of proximity of the area of the present study to that of Pakistan, a neighbouring country. As the response to therapy differs in different genotypes and upto 80% of the genotypes 2 and 3 can be cured with standard treatment, most of our patients could be cured of HCV infection as they carry genotype 3 [20].

Thus, we can conclude that HCV infection is a major problem in our haemodialysis patients. Although serological assays are reliable, easily available and have relatively low cost in detecting exposure to HCV in HD patients, they definitely fail to detect active infection especially so in this immunocompromised patient population. However, we observed that by the time patients reach our remotely located tertiary care centre, a significant proportion of them already have ongoing HCV infection which could be detected by testing for anti-HCV antibodies. This is also evident from the prevalence of 2.8% (HCV-RNA detected by RT-PCR) observed in anti-HCV antibody negative patients of our study. The value (2.8%) is much less than that reported by other studies from Northern India [8,21]. Kappa value (0.61) also shows that the overall agreement between the two HCV infection detection methods (anti-HCVantibodies and HCV-RNA) is substantial in our study. Although, HCV-RNA detection by PCR is the only sensitive, reliable and a gold standard test for HCV infection, it may not be possible to adopt it as a screening test due to its high cost, high technical skill requirement. However, the best way to control the transmission of HCV infection in HD units is strict adherence to universal precautions, sterilization and proper maintenance of dialysis machines and proper disposal of used material. There is no substitute for these measures and their implementation in the dialysis unit remains the cornerstone of decreasing the prevalence of HCV infection in HD patients.

Country	Author	Year of study	HCV seropositivity +/total	EIA generation	HCV-RNA +/total	Method	HCV-RNA(+) in HCVs eronegative patients
Italy	Enricio silini et al., [14]	1993	36/77(46.7%)	2nd generation	29/77(37.6%)	RT-PCR	7/41(17%)
Japan	Iwasaki et al., [13]	2000	34/142(23.9%)	1st or 2nd generation	38/142(26.8%)	RT-PCR	4/108(3.7%)
Saudi Arabia	Hussian et al., [15]	2007	34/180(18.9%)	3rd generation	39/180(21.6%)	RT-PCR	5/146(3.4%)
India	Jasuja et al., [8]	2010	26/119(21.8%)	3rd generation	33/119(27.7%)	RT-PCR	7/93(7.52%)
India	Present study	2013	30/100(30%)	3rd generation	20/100(20%)	RT-PCR	2/70(2.8%)

[Table/Fig-3]: Hepatitis C prevalence among Asian haemodialysis patients.

LIMITATION

HCV core antigen testing by ELISA was not performed which is another method for detection of current HCV infection.

CONCLUSION

Although serological assays are reliable, easily available and have relatively low cost in detecting exposure to HCV in HD patients, they definitely fail to detect active infection especially so in this immunocompromised patient population. However, we observed that by the time patients reach our remotely located tertiary care centre, a significant proportion of them already have ongoing HCV infection which could be detected by testing for antiHCV antibodies. Although, HCV-RNA detection by PCR is the only sensitive, reliable and a gold standard test for HCV infection, it may not be possible to adopt it as a screening test due to its high cost, high technical skill requirement.

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