



DETECTION OF HEPATITIS C VIRUS AMONG HEMODIALYSIS PATIENTS AT A TERTIARY CARE HOSPITAL, GUNTUR

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ABSTRACT

INTRODUCTION: Hepatitis C infection is one of the public health concerns. It is a pathogen responsible for a significant proportion of liver disease in various regions of India. In India, approximately 6-12 million people are chronically infected with Hepatitis C virus.

AIMS & OBJECTIVES: 1. To evaluate the prevalence of Hepatitis C Virus (HCV) infection in the End Stage Renal Disease (ESRD) patients attending the hemodialysis (HD).

2. To assess the risk factors associated with dialysis in relation to HCV.

MATERIALS AND METHODS: All 200 samples were assayed for parameters of HCV (anti-HCV antibodies) with rapid immunochromatography (ICT), ELISA (anti-HCV antibodies).

RESULTS: Out of 200 cases in the present study 29 (14.5%) cases were diagnosed as seropositive by ICT and 3rd generation ELISA.

CONCLUSION: Stringent adherence to universal precautions, isolation of hemodialysis machines, ideal nursing practices and proper sterilization of equipments and periodical viral parameters monitoring are essential in reducing HCV transmission.

KEYWORDS :

INTRODUCTION:

Hepatitis C infection is one of the public health concerns¹. It is a contagious liver disease that results from infection with the Hepatitis C virus, a hepatotropic RNA virus that belongs to the genus Hepacivirus of family Flaviviridae².

Hepatitis C infection is one of the public health concerns¹. The prevalence of HCV among hemodialysis patients is generally much higher than the general population⁸. HCV infection was evidently more frequent at dialysis centers with higher anti-HCV prevalence^{9,10}.

In India, approximately 6-12 million people are chronically infected with Hepatitis C virus^{10,11}. Hepatitis C Virus (HCV) is an emerging infection in India whose long term implications will be felt in the decades to come. It is a pathogen responsible for a significant proportion of liver disease in various regions of India¹².

The main sources of HCV infection include injection drug abuse, chronic dialysis, organ transplantation, blood, and its product transfusion, occupational exposure, unprotected sexual contact, and vertical transmission it is recommended that HD patients should be monitored in order to determine the full risk factors for HCV contamination observed in this study.¹

In dialysis patients, HCV infection is common. This is one of the most important causes of liver disease in patients on renal replacement therapy. Chronic kidney disease (CKD) is found in approximately 10 % of the general population. CKD is defined by a reduction in glomerular filtration rate for three months or more and/or proteinuria, anaemia and hypertension¹⁴.

Dialysis is commonly used as renal replacement therapy for end-stage renal disease (ESRD) patients. About 2.1 million patients are estimated to be in need of dialysis and are expected to increase annually by 7% worldwide¹⁵.

End-Stage Renal Disease patients who are attending hemodialysis for a long time are at high risk of infection because the process of hemodialysis requires repeated vascular access for prolonged periods.

Such patients are susceptible to person-to-person transmission of Hepatitis C Virus via the contaminated devices resulting in the generation of an HCV reservoir that disseminates the infection¹⁶.

Hepatitis C Virus can survive and remain potentially infective on the surface of equipment through splashes of blood that may not be visible to the naked eye¹⁷.

The diagnosis of HCV infection is based mainly on two categories of laboratory tests. They include serologic assays to detect HCV antibodies and tests to quantify HCV RNA. Both assays are done to minimize false-positive and false-negative results. The most sensitive tool to detect HCV infection is an antibody detection test.

The present study was conducted to evaluate the prevalence of Hepatitis C Virus infection in End-Stage Renal Disease patients attending hemodialysis by assessing the anti-HCV antibodies.

AIMS & OBJECTIVES

1. To evaluate the prevalence of Hepatitis C Virus (HCV) infection in the End Stage Renal Disease (ESRD) patients attending the hemodialysis (HD).
2. To assess the risk factors associated with dialysis in relation to HCV.

MATERIALS AND METHODS

The present study was conducted at the Department of Microbiology, Guntur Medical College, Guntur in collaboration with the Department of Nephrology from January 2018 to June 2019 to detect the prevalence of anti-HCV antibody by rapid immunochromatographic test (ICT), 3rd generation Enzyme linked immunosorbent assay (ELISA).

Study design: Prospective study.

Study period: 1 and ½ year from January 2018 to June 2019.

Study location: This study was performed in the Department of Microbiology in collaboration with the Department of Nephrology of Guntur Medical College/Government General Hospital, Guntur, Andhra Pradesh.

Study group: Chronic renal failure patients on Hemodialysis (HD).

Sample size: 200

Selection criteria:

Inclusion criteria:

1. Patients within the age group of 15 to 65 years.
2. Chronic renal failure patients on Hemodialysis (HD).

Exclusion criteria:

1. Age less than 15 years and more than 65 years.
2. Patients who tested HCV positive at the start of Hemodialysis were excluded.
3. Patients infected with Human Immuno Deficiency (HIV) virus.

Ethical clearance:

Ethical committee clearance was obtained from the college ethical committee

before the commencement of the study.

Consent

Informed consent was obtained from the patients who participated in the study.

Collection Of Samples

About 5 mL of whole blood was collected with a sterile syringe under aseptic conditions from hemodialysis patients and transferred into a clot activator vacuutainer (red color) and allowed the blood to clot by leaving it undisturbed at room temperature (RT) for 15 minutes and the sample was centrifuged at 2000 rpm (rotations per minute) for 5 minutes in Electra Lab Centrifuge (Electra BL). Fresh samples were used for detection of anti HCV antibodies by ICT AND ELISA and stored in two aliquots and frozen at -80°C.

The presence of heparin at the start of dialysis gives false positive results and the patients HCV RNA level in the blood decreases during hemodialysis due to dilution, adsorption of HCV virion particle to the dialysate membrane and its destruction. (Digdem Ozer et al, 2015, WJH).

Methods:

All 200 samples were assayed for parameters of HCV (anti-HCV antibodies) with rapid immunochromatography (ICT), ELISA (anti-HCV antibodies).

Rapid Immunochromatographic Technique (ICT)

Immunochromatography relies on the migration of liquid across the surface of a nitrocellulose membrane. Using recombinant HCV antigen prepared against HCV antibodies target that has been incorporated onto the strip of nitrocellulose.

All the 200 samples were tested for anti-HCV antibodies by Rapid ICT. Rapid Diagnostic Test The HCV CARD TEST was a one step, rapid, qualitative test for the detection of antibodies generated against proteins that are encoded by conserved sequence of core, NS3, NS4, NS5 parts of the HCV genome in the blood sample.

Procedure:

All the kit components and specimen were brought to room temperature prior to testing. The test device was removed from the foil pouch and placed on a flat, dry surface. With a 100 µL disposable specimen applicator provided, it is dipped into the serum containing vial and 2 drops (60 µL) were added into the sample well on the test strip. 3 drops of buffer was added into the sample well. Results were interpreted within 20 minutes.

Interpretation:

1.Negative result:

The presence of one color band 'C' control line within the result window was taken as negative.

2.Positive result:

The presence of two color bands (first band -test line and C-control line) in the result window was taken as positive.

THIRD GENERATION ELISA (Enzyme Linked Immunosorbent Assay)

Enzyme linked immunosorbent assay (ELISA) is a biochemical technique in which antigen or antibody are detected by binding of an enzyme coupled to either antibody or antibody specific for antigen.

The format of ELISA was developed in 1960's independently at the same time by Peter Pearlmann and Eva Engval of Stockholm University.

Third generation enzyme linked immunosorbent assay (ELISA) is a heterogenous assay for the detection of antibodies against Hepatitis C virus in the human serum. The third generation ELISA's are more sensitive and detects seroconversions earlier as it includes recombinant and synthetic antigens in the assay which increases the sensitivity and specificity to more than 99.5%. The end product in the ELISA is the development of the color which can be quantified using a spectrophotometer.

Principle:

Third generation ELISA test is a two-step solid phase enzyme

immunoassay for the qualitative detection of anti-HCV antibodies present in the human serum by utilizing the mixture of recombinant proteins of HCV i.e., core, NS3, NS4 and NS5 antigens.

Procedure:

- 1) All the reagents and specimens were brought to the room temperature before use.
- 2) Micro-titre plate with required number of strips were taken and pouch closed immediately.
- 3) Data sheet was prepared including the specimen identification number and controls.
- 4) 1 well for Blank, 3 wells for Negative control (NC) and 1 well for Positive control (PC) were put up
- 5) 100 µL of sample diluent was added to all wells
- 6) 10 µL of sample diluent was added to blank.
- 7) 10 µL of Controls and samples were added in the wells and covered with strip sealers for 45 min at 37°C and washed with wash buffer (1:20) for six cycles
- 8) 50 µL of assay buffer and 50 µL conjugate were added to each well, left it for 15 minutes at
- 9) 20–30 °C in dark
- 10) Finally 100 µL of stop solution was added to each well.
- 11) The plate was read at 450 nm within 15 minutes after adding the stop solution

Assay Run Criteria:

- 1) Blank value < 0.1
- 2) Individual absorbance value of Negative control < 0.1
- 3) Individual absorbance value of Positive control > 0.5

Calculation of cut-off value (COV):

Calculate the mean of the measured absorbance values (O.D) for the anti-HCV negative controls (NCX). $COV = 0.3 + NCX$

Assay Interpretation:

- 1) Samples with absorbance value < cut-off value is non-reactive
- 2) Samples with absorbance value > cut-off value is reactive.

RESULTS

The present study included 200 patients with End Stage Renal Disease (ESRD) attending hemodialysis from January 2018 to June 2019 at dialysis center in a tertiary care hospital.

Table 1: Results of the present study

| Total number of cases | Number of positives | Number of Negatives |
|-----------------------|---------------------|---------------------|
| 200 | 29 (14.5%) | 171 (85.5%) |

Among the 200 patients with ESRD, 29 (14.5%) cases were confirmed as Hepatitis C infection positive and 171 (85.5%) were confirmed as Hepatitis C infection negative.

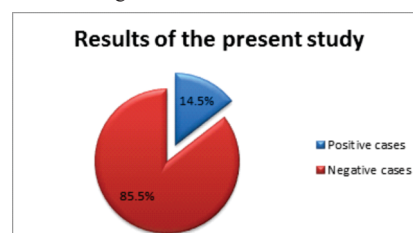


Table 2: Age wise distribution of Hepatitis C virus infected cases

| Age in years | No. of samples tested | Positive cases |
|--------------|-----------------------|----------------|
| 15-25 | 05(2.5%) | - |
| 26-35 | 26(13%) | 04(2%) |
| 36-45 | 49(24.5%) | 11(5.5%) |
| 46-55 | 68(34%) | 09(4.5%) |
| 56-65 | 53(26.5%) | 05(2.5%) |
| Total | 200(100%) | 29(14.5%) |

The age group of patients in this study ranged from 15-65 years. Mean age was 47.7 years

The lowest age positive for Hepatitis C infection positive is 22 years. The Highest age being 65 years.

Maximum number of cases 11(5.5%) were seen between the age group of 36-45 years of which 9(4.5%) were positive.

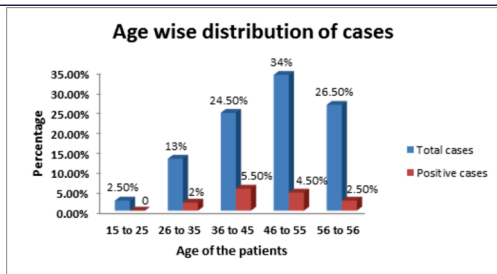


Table 3: Gender-wise distribution of Hepatitis C virus infection positive cases

| GENDER | TOTAL NUMBER OF CASES | POSITIVE CASES |
|---------|-----------------------|----------------|
| MALES | 152(76%) | 21(10.5%) |
| FEMALES | 48(24%) | 8(4%) |
| TOTAL | 200(100%) | 29(14.5%) |

Among the 200 cases in the study group 152(76%) were males of which 21(10.5%) were positive. Females were 38 (14%) of which 9(4.5%) were positive. The male to female ratio is 2:1

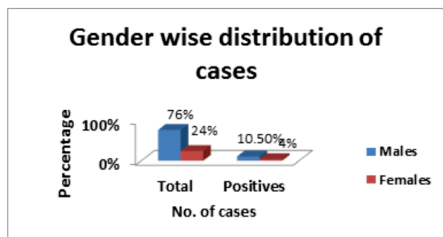


Table 5: History of Blood Transfusion during dialysis:

| No of blood transfusions | No of Seropositive cases | No. of Seronegative cases | Total |
|--------------------------|--------------------------|---------------------------|------------|
| 1-5 | 9 (31%) | 50 (29%) | 59 (29%) |
| 6-10 | 10 (34%) | 33 (19%) | 43 (22%) |
| >10 | 3 (11%) | 05 (3%) | 08 (4%) |
| None | 7 (24%) | 83 (49%) | 90 (45%) |
| Total | 29 (100%) | 171 (100%) | 200 (100%) |

Out of 29 anti-HCV antibody seropositive patients, 9 (31%) patients underwent blood transfusions in a range of 1 to 5 times. 10 (34%) patients underwent blood transfusions in a range of 6 to 10 times. And only 3 (10%) of patients underwent blood transfusion for > 10 times during dialysis. 7 (24%) members were never transfused blood.

Table 6: Duration of dialysis:

| Duration of hemodialysis | Seropositive cases | Seronegative cases | Total |
|--------------------------|--------------------|--------------------|-------------|
| 1 year | No | 05 (3%) | 05 (2.5%) |
| >1-2 years | 03 (11%) | 134 (78%) | 137 (68.5%) |
| >2 years | 26 (89%) | 32 (19%) | 58 (29%) |
| Total | 29 (100%) | 171 (100%) | 200 (100%) |

Out of 29 anti-HCV seropositive cases, only only 3 (11%) patients were attending dialysis since 2 years. The rest 26 (89%) patients were undergoing dialysis for more than 2 years.

Table 7: No of centers patient underwent hemodialysis:

| No. of centers | Anti-HCV positive | Seronegative | Total |
|----------------|-------------------|--------------|------------|
| 1 | 7 (24%) | 157 (91%) | 164 (82%) |
| >1 | 22 (76%) | 14 (9%) | 36 (18%) |
| Total | 29 (100%) | 171 (100%) | 200 (100%) |

It was observed that in 29 seropositive cases, 7 (24%) were undergoing dialysis in one center only. 22 (76%) of cases underwent hemodialysis in 2 centers.

Table 8: Risk factors for HCV infection in hemodialysis patients:

| | Seropositive | Seronegative | Total | P value | |
|----------------------|--------------|--------------|-----------|-----------|----------|
| Duration of dialysis | Upto 2 years | 03 (11%) | 139 (81%) | 142 (71%) | <0.0001 |
| | >2 years | 26 (89%) | 32 (19%) | 58 (29%) | |
| No. of centers | 1 | 7 (24%) | 157 (91%) | 164 (82%) | <0.00001 |

| | | | |
|----|----------|---------|----------|
| >1 | 22 (76%) | 14 (9%) | 36 (18%) |
|----|----------|---------|----------|

Among the risk factors for HCV infection in HD patients duration of dialysis and different dialysis centers had p value <0.05 which are extremely significant

Results of diagnostic assays:

The results of rapid immunochromatographic tests (ICT), 3rd generation Enzyme Linked Immuno Sorbent Assay (ELISA) for the detection of anti-HCV antibodies.

RESULTS OF DIAGNOSTIC ASSAYS:

Table 9

| Diagnostic test | Positive cases | Percentage of positivity |
|-----------------|----------------|--------------------------|
| Rapid ICT | 29/200 | 14.5% |
| ELISA | 29/200 | 14.5% |

Of the 200 End Stage Renal Disease patients screened for the presence of anti-HCV antibodies, 29/200 (14.5%) were positive by both the ImmunoChromatographic Technique (ICT) and 3rd generation Enzyme Linked Immuno Sorbent Assay (ELISA).

DISCUSSION

Hepatitis C virus infection is a major problem in the patients attending hemodialysis especially in the developing countries. It is one of the major public health concerns in our country.

During last the last decades several diagnostic techniques like immunochromatographic (ICT) assay and Enzyme Linked Immuno Sorbent Assays (ELISA) have been developed and evaluated widely that yield prompt and accurate diagnosis of the seropositivity of the patients with HCV infection.

In accordance to this, the present study has been designed to detect the presence of anti-HCV antibodies by ICT and ELISA. Out of 200 cases in the present study 29 (14.5%) cases were diagnosed as seropositive by ICT and ELISA.

Table 10: Prevalence of HCV seropositive cases:

| S.No | Name of the author, year | Common age groups |
|------|---|-------------------|
| 1. | Surender K ¹⁸ et al, 2011 | 12.4% |
| 2. | Kranthi Kosaraju ⁵ et al, 2013 | 1.11% |
| 3. | Rashmi K ¹⁹ et al, 2017 | 8.4% |
| 4. | Col Partha ²⁰ et al, 2018 | 18.8% |
| 5. | Amrit Dhar ²¹ et al, 2019 | 31.4% |
| 6. | Present study | 14.5% |

This table also showed prevalence of seropositive HCV cases by different authors. The prevalence of seropositive HCV cases from different Indian studies ranged from 1.11% by Kranthi Kosaraju⁵ et al, 2013 in Karnataka to 31% by Amrit dhar et al²¹, at Jammu and Kashmir in 2018. The prevalence of present study is 14.5% which is nearly correlating with the study conducted by Surender¹⁸ K et al, 2011 in Coimbatore which is 12.4% and Col Partha²⁰ et al, 2018 in Pune is 18.8%.

This variation could be because of the duration of HD and receiving HD at more than one center and independent factors significantly associated with the prevalence of HCV infection in patients attending hemodialysis.

Geographical location, socio economic factors and health care procedure related to adoption of universal precaution policy ,dialysis unit hygiene, isolation of HD machine ,proper sterilization of equipments and dialyser reuse are the factors which influence the prevalence of HCV.

The reason for higher prevalence in HD is mainly due to prolonged vascular access, increased frequency of blood transfusions and hospitalization, multiple invasive procedures ,nosocomial transmission via contaminated dialysis equipments and their comorbid condition.

Table 11: Age wise distribution of HCV seropositive cases:

| S.No | Name of the author, year | Common age groups |
|------|---|-------------------|
| 1 | Kranthi Kosaraju ⁵ et al, 2013 | 30-40 |
| 3 | Arora et al ²² , 2016 | 41-50 |
| 5 | Amrit Dhar ²¹ et al, 2019 | 35-55 |
| 6 | Present study | 36-45 |

The table showing age-wise distribution of HCV seropositive cases by different authors.

The present study correlated with Amrit Dhar²¹ et al, 2019 in Jammu and Kashmir 35-55. It showed maximum number of seropositive cases 11 (38%) belonged to the age group of 36-45 years.

The present study included the age groups ranging from lower age group of 15 years to higher age group of 65 years. The lowest age group in the seropositive HCV patient is 29 years and the highest age group being 65 years. The present study showed 3:1 which nearly correlated with the study conducted by Jain Parveesh et al in 2016 and Rashmi K¹⁹ et al, 2011 with 3:1 as males to females ratio.

The present study showed male predominance of 72% among the seropositive patients. The higher predominance of seropositivity in males may be attributed to the increased incidence of chronic kidney disease, diabetes mellitus and hypertension. (Col partha et al, 2018).

The present study showed 65% of seropositive patients were attending dialysis for upto 2 years and 35% in patients attending >2 years which is nearly correlating with the study conducted by SK Agarwal in 2009 showed that 51% were attending for upto 2 years and 49% for > 2 years.

In a study conducted by Surendra K¹⁸ et al in the year 2011 stated that the long duration of dialysis was a significant risk factor for acquiring the HCV infection.

The present study also found a significant association between anti-HCV antibody seropositivity and patients who had dialysis at more than one center where it showed 72% which is correlating with the study of Surendra K¹⁸ et al in the year 2011 (69%).

Among the risk factors for HCV infection in HD patients duration of dialysis had p value <0.05 which are extremely significant which correlates with the study of Ali kargar et al, 2016 and Kasawar Darahasa et al, 2018.

The present study showed that 76% of the seropositive patients underwent blood transfusion. It is nearly correlating with the study conducted by Moreira et al 125 and Al Jamal et al 139 in 2005 and 2009 from Brazil and Jordan respectively in hemodialysis patients which showed 75.8% of HCV positive patients.

In the present study, among the risk factors for HCV infection in HD patients the blood transfusions had p value <0.05 which played a significant role in the development of anti-HCV positive antibodies which correlated with the study of Omar Salem Khattab et al, 2000 Egypt in which the p value showed <0.05.

CONCLUSION

- The current study determines HCV infection prevalence in chronic kidney disease patients on dialysis.
- Rapid Immuno chromatographic test is easy to perform and interpret in low resource settings. This test is less sensitive and needs further confirmation.
- ELISA test is considered as a diagnostic tool for anti-HCV detection because of its ease of use, cost effectiveness and low variability. False negative ELISA is due to poor immunological reactivity in these patients.
- The best way for screening dialysis patients is to combine immune-enzymatic method and molecular method for synergistic effect in detecting HCV and this further helps in preventing HCV transmission.
- Stringent adherence to universal precautions, isolation of hemodialysis machines, ideal nursing practices and proper sterilization of equipments and periodical viral parameters monitoring are essential in reducing HCV transmission.

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