UNDETECTED HEPATITIS-C VIRUS INFECTION IN HAEMODIALYSIS PATIENTS: IMPORTANCE OF PCR HCV RNA AND ELIZA METHOD

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ABSTRACT

Background: Haemodialysis (HD) is one of the most common type of renal replacement therapy (RRT) used for end stage kidney disease (ESKD). Hepatitis-C is the most frequent infection in haemodialysis. Early detection of this viral infection can lead to early treatment, lesser morbidity and mortality. PCR test is being used instead of Elisa in centres where HCV infection is more prevalent as PCR for HCV RNA is considered more reliable than Eliza in haemodialysis patients.

Objective: To assess the accuracy of Eliza and PCR method for the detection of Hepatitis-C viral infection in haemodialysis patients.

Methods: A cross sectional study was conducted at Dialysis Centre of DHQ hospital Sheikhupura, Punjab, Pakistan in January 2018. All patients who were on maintenance haemodialysis for more than six months were included in the study. Patients with acute kidney disease and on dialysis less than six months were excluded. All patients were tested by enzymes linked immune-sorbent essay (ELIZA) and polymerase chain reaction (PCR) method for HCV status.

Results: Out of 152 haemodialysis patients tested, 94 (61.84%) cases were positive by both ELIZA and PCR methods while 6 (3.94%) cases with negative ELIZA were PCR positive. Number of dialysis, blood transfusions and serology were important risk factors with significant p value. (<0.05)

Conclusion: In centres where Hepatitis-C is more prevalent, PCR for HCV RNA should be preferred over ELIZA method for early detection of HCV viral infection.

Keywords: Hepatitis-C, haemodialysis, nephrologist, PCR.

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INTRODUCTION

End stage renal disease is a major health problem in Pakistan and the burden of disease is increasing with every passing day[1].About 71.1 million people in world are infected with hepatitis C[2]. Due to its major effects on mortality and morbidity it is an issue of global concern now[3-7]. Among hepatitis c untreated patients, a major number develop cirrhosis while few of them develop hepatocellular carcinoma[8]. Every 20th Pakistani has been exposed to hepatitis c infection with an overall prevalence of 5 % with a major role of healthcare workers in its transmission[9].

Hepatitis c is very common in dialysis patients due to many reasons like poor implication of infection control strategies in dialysis centres[10], poor screening in blood banks before transfusion, repetitive intravenous interventions and immunocompromised status of patients[11].According to a recent study done in Pakistan, prevalence of hepatitis c among haemodialysis patients ranges from 16.4% to 68.0% which is significantly higher than general population i.e. 5%[9]. Chronic liver disease (CLD) is of one of the top three causes of mortality in ESRD patients and hepatitis c is one of the most important causes of CLD.

Accurate and in time diagnosis will help in reducing mortality rate in ESRD patients and will reduce the horizontal transmission of virus in dialysis centres as well[12-13]. Commonly used diagnostic tests for detection

of hepatitis C are measurement of antibody level by ELIZA or PCR for viral RNA.

Antibodies take some time to develop after infection i-e window period or may not develop to same level in immunocompromised conditions like dialysis[14] ,making ELIZA less reliable to diagnose a patient with active infection of hepatitis C[15]. It has been studied in some parts of the world but no local data is available so the rationale of our study is to find out the most accurate method of diagnosing hepatitis c in ESRD patients of our population to ensure early diagnosis and treatment.

METHODS

A cross sectional study was conducted at Dialysis centre of DHQ hospital, Sheikhupura, Punjab, Pakistan in January 2018. All the patients who were on haemodialysis for more than six months were included in the study. A total of 152 patients, from 16 to 75 years old patients were in our study. Patients with Acute kidney injury (AKI), patients with ESKD who came to centre for short time period (less than six months) and Patients with age <15 or >75 were excluded from study. All patients were on maintenance dialysis, two sessions a week, each session of four hours. There was no isolation for HCV positive or negative patients. Dedicated machines were reserved for seropositive patients on same floor, where area was separated by a small brick wall. One dialyzer was being reused three times for dialysis and then discarded. Chemical disinfection of dialyzer was done after each dialysis. All patients fulfilling the study criteria were enrolled in the study. Demographic data of the patient's (age, gender, occupation and marital state) was collected by direct interview. Viral status, duration on HD, history of blood transfusion(s), attending more than one HD centre and type of vascular access for HD (arterio-venous fistula, double lumen catheter, permanent catheter) number of blood transfusions per month was taken from record and noted in preformed Performa. Blood samples were collected pre-dialysis. Four millilitres (ml) of blood for ELIZA and six millilitres (ml) of blood for PCR in separate serum vial were stored. All samples were centrifuged and then frozen in the centre before sending to laboratory for third generation ELIZA and real time PCR for HCV.

RESULTS

Table I shows the baseline characteristics of the studied population. Results showed 51.1% males were more than 50-years old, and 59.8% females were more than 50-years old, 36.1% males and 51.5% female were illiterate, 65.4% male and 96.9% females were unemployed, 60.2% male and 42.3% female were rural. Out of 152 haemodialysis patients 94 (61.84%) cases were positive by both ELIZA and PCR methods while 6 (3.94%) cases were negative by ELIZA but positive by PCR.

Table II gives the results of PCR association with clinical parameters. It was observed that total number of dialysis and blood transfusion required had significant association with PCR. 56% PCR positive cases had more than 100 number of dialysis and 6% had more than 4-times blood transfusion per month. 94 cases were PCR positive with positive serology and 19 cases were found PCR negative with negative serology as shown in figure 1.
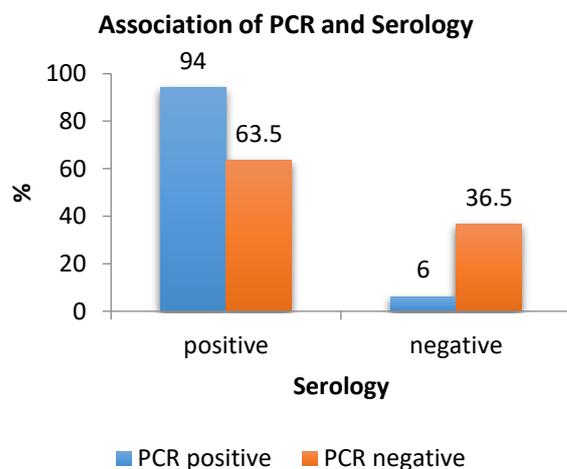
Figure 1:

Association of PCR and Serology

Table I: Baseline Characteristics of Studied Samples (n=152)

Characteristics		Male (n=88)		Female (n=64)	
		N	%	N	%
Age group	16-30	11	12.5	09	14.1
	30-50	32	36.3	17	26.6
	>50	45	51.2	38	59.3
Education	Literate	56	63.6	31	48.4
	Illiterate	32	36.4	33	51.6
Employment status	Employed	30	34.0	02	3.1
	Unemployed	58	66.0	62	96.9
Address	Urban	35	39.8	37	57.8
	Rural	53	60.2	27	42.2

Table II: Association of PCR with Clinical Variables

Clinical Variables		Positive (n=100)		Negative(n=52)		P value
		N	%	n	%	
Cause of ESKD	DM	12	12.0	1	1.9	
	HTN	38	38.0	22	42.3	
	Stone disease	2	2.0	1	1.9	
	chronic GN	6	6.0	1	1.9	0.45
	cystic disease	2	2.0	2	3.8	
	both DM and HTN	37	37.0	23	44.2	
	Other	3	3.0	2	3.8	
Dialysis starts via	AVF	22	22.0	6	11.5	
	double lumen catheter	78	78.0	46	88.5	0.10
Total number of dialysis	<50	12	12.0	16	30.8	
	50-100	32	32.0	12	23.1	0.006*
	>100	56	56.0	24	46.2	
need for transfusion	Yes	79	79.0	35	67.3	
	No	21	21.0	17	32.7	0.28
blood transfusion required	<2 times per month	67	67.0	24	46.2	
	2-4 times per month	27	27.0	15	28.8	<0.01*

DISCUSSION

Hepatitis C in Pakistan is common in general population. And is even more common in CKD due to multiple risk factors like frequent hospitalizations and need of repetitive blood transfusions. Which ultimately contributes to increased frequency of hepatitis c in end stage renal disease patients on haemodialysis ,ranging from 23.7%-56.6% [10,16-17].

Untreated hepatitis c increases the risk of death in dialysis patients[18]. And poses a risk of spreading it to other patients as well as healthcare workers if infection control measures are not taken efficiently. Risk of acquiring hepatitis c infection is directly related to duration of dialysis .It has been proven in many studies[19-23].Our study results also endorsed the similar idea. In our study we divided the patients in three categories on the basis of duration of dialysis and results showed that 12% patients having dialysis less than 50 ,32 % patients having 50 -100 dialysis and 56 % having more than 100 dialysis got HCV infection respectively. This suggests that many patients acquire infection from dialysis centre via horizontal transmission.

Horizontal transmission of hepatitis c is an important issue to date in Pakistani dialysis centres as compared to many parts of the world where it is negligible now because of timely diagnosis and management of hepatitis c as well as due to proper infection control measures.

Commonly used test to diagnose hepatitis c is either ELIZA or PCR. With ELIZA there is a possibility of false negative results in immunocompromised patients like on dialysis or recently infected patients and false positive results in previously treated patients making PCR for HCV RNA more reliable. In our study out of 152 haemodialysis patients, 94 % cases were positive by both ELIZA and PCR methods while 6 % cases were ELIZA negative but PCR positive. Similarly in another study done in 112 haemodialysis patients 78 patients were tested negative for HCV antibodies by ELIZA. but in 22 (28.2%) patients out of 78,HCV RNA was detected by PCR[24]. So, we suggest screening of Dialysis patients with PCR before enrolment in dialysis centres and then at regular intervals afterwards.

In Pakistani population blood transfusion is another important source of hepatitis c infection because of poor screening in many blood banks[25] .Haemodialysis patient needs multiple blood transfusions as compared to general population making it one important factor responsible for hepatitis c infection in them[19].

In our study we found that blood transfusions clearly increases the risk of hepatitis c .out of 100 positive cases 67% had >2 transfusions per month,27 % had 2-4 transfusions per month and 6 % had more than 4 transfusions per month .another study also showed that number of blood transfusion is directly related to hepatitis c infection in dialysis patients(10)it has been proven in many other studies as well.(19,23). Management of anaemia with adequate dialysis and adequate dose of iron and erythropoietin will reduce the need of blood transfusion indirectly reducing the possibility of viral transmission.

CONCLUSION

In centres where hepatitis-c is more prevalent, PCR for HCV RNA should be preferred over ELIZA method for early detection of HCV viral infection.

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ETHICAL APPROVAL
The study was approved from Institutional Review Board of DHQ Hospital, Sheikhupura, Pakistan, in its meeting held on 10/09/2017.

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AUTHOURS CONTRIBUTIONS
YH: Concepts, Design, Literature Review
RA: Manuscript Writing, Data analysis
NM: Manuscript Writing, Statistical Analysis
ZF: Manuscript Review
SN: Data Collection
AM: Manuscript Review