ORIGINAL ARTICLE

HEPATITIS C VIRUS DIAGNOSIS AMONG MULTI-TRANSFUSED BETA THALASSEMIA MAJOR PATIENTS

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ABSTRACT

Background: Hepatitis C virus (HCV) infection is major health problem. The objectives of the study was to diagnose HCV infection in multi-transfused beta thalassemia major patients by using ICT, ELISA and real time PCR.

Material & Methods: This cross sectional study was conducted in Department of Pathology, Bacha Khan Medical Complex, Mardan from April 2013 to January, 2015. Sample size was 44. Sampling technique was purposive. Inclusion criteria was multi-transfused patients of beta-thalassemia major. Those patients who were on HCV therapy were excluded. Demographic variables were gender and age groups. Research variable was presence of HCV. The study was approved from the departmental ethical committee. Informed written consent was taken from patients. All the patients were subjected to HCV detection using ICT, ELISA and real time PCR. All the variables being categorical were analyzed through count and percentages. The data was analyzed by chi-square test. The correlation between different techniques was calculated by Cohen's K coefficient test.

Results: Out of 44 cases, 52.2% were males and 47.7% were females. ICT diagnosed HCV in79.5% subjects, ELISA in 63.6% subjects and on real time PCR, HCV was detected in 43.1% subjects. Cohen's Correlation was done. These Kappa values confirmed a weak correlation between any of two HCV diagnostic techniques. Significant difference was detected among male patients as compared to female patients and among age group of 15-17 years by the three diagnostic techniques.

Conclusion: It is better to employ a coupled diagnostic strategy for the diagnosis of HCV infection among the multi-transfused beta thalassemic major patients than using a single technique.

KEY WORDS: Hepatitis; beta-Thalassemia; Hepatitis C virus.

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INTRODUCTION

Hepatitis C virus (HCV) infection continues to be a major health problem globally. HCV infection is often asymptomatic and causes an undiagnosed infection which leads to a persistent infection followed by liver failure and cirrhosis. HCV is a causative agent of chronic infection too that leads to hepatocellular carcinoma (HCC).[1,2] According to World Health Organization (WHO) and the Viral Hepatitis Prevention Board (VHPB), almost 170 million people were having HCV infection globally in 2011, leading to the death of 350000 to 500000 people per year.[3] It is estimated that 3.3% population of the world is infected with HCV. WHO reported that two-thirds of developing countries have not proper arrangements for screening of blood of donor samples for the identification of HCV however, regular donor blood test and screening procedure decreased the transmission of HCV to great extent.[4]

HCV infection is predominantly prevalent in transfusion-dependent patients. Although, most acute cases of HCV occur in young ones, it can infect individuals of all ages. Sharing of medical

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equipments such as non sterilized needles and syringes may also increase the chances of getting HCV infection. Patients suffering from thalassemia and organ transplant are also considered at a high risk.[5,6] Beta thalassemia major is a group of inherited recessive blood disorders characterized by abnormal formation of hemoglobin, known as hemoglobinopathies.The imbalance condition leads to red blood cells destruction in bone marrow which is followed by hemolysis and severe anemia. Such patients need frequent blood transfusion to sustain their life.[7,8] HCV is the most important cause of morbidity and mortality among thalassemic patients due to receiving multiple blood transfusions.[9] Systemic testing procedure and suitable donor selection programs have decreased transmission of HCV through transfusion of blood products, still there are many countries where standards of blood products managements do not satisfactorily protect chronically transfused patients especially thalassemia from this problem.[10] Diagnosis of HCV among beta thalassemic patients is an ultimate strategy to control HCV infection. Moreover, diagnosis of HCV genotypes plays an important role in epidemiology, clinical course and the effects of antiviral therapy. Serological and molecular assays are widely in practice. Serological assays identify specific antibody to HCV while molecular assays detect viral nucleic acid.[11] Among serological assays, Enzyme Linked Immunosorbant Assay (ELISA) is more sensitive diagnostic technique as compared to Immunochromatographic Technique (ICT) in detecting anti-HCV.[12] Among molecular assays, polymerase chain reaction (PCR), real time PCR and reverse transcriptase polymerase chain reaction (RT-PCR) are highly sensitive assays for detection of HCV RNA. Detection of HCV by PCR is better to control HCV infection.[13,14] Limited data is available on the HCV infection in multi-transfused beta thalassemia major patients. The objectives of the study was to diagnose HCV infection in multi-transfused beta thalassemia major patients by using ICT, ELISA and real time PCR.

MATERIAL AND METHODS

This cross sectional study was conducted in Department of Pathology, Bacha Khan Medical Complex, Mardan from April 2013 to January, 2015. Sample size was 44. Sampling technique was purposive. Inclusion criteria was multi-transfused patients of beta thalassemia major. Those patients who were on HCV therapy were excluded. Demographic variables were gender and age groups having attributes of upto 2 years, 3-5, 6-8, 9-11, 12-14 and 15-17 years. Research variable was presence of HCV having attribute of yes and no. The study was approved from the departmental ethical committee. Informed written consent was taken from patients. Blood samples were screened through hemoglobin electrophoresis.

All the patients were subjected to HCV detection using ICT, ELISA and real time PCR. Blood samples were serologically processed for ICT as per manufacturer instructions (Accurate Rapid Device, USA). Briefly blood sample were added to the device and incubated for 20 minutes at room temperature. Results were interpreted as per vendor recommendations. ELISA Biocan kit was used for HCV diagnosis in serum samples using vendor protocol. Briefly, 10μl serum sample and 100μl sample diluents were added to the wells and incubated at 37^{o}C for 30 minutes. After washing, 100μl of conjugate was added to each well except blank one and incubated for 20 minutes at 37^{o}C followed by washing. 50μl of substrate solution A and B was added to each well and incubated at 37^{o}C for 10 minutes. The reaction was stopped by adding stop solution and change of colour was interpreted as positive for anti-HCV antibodies. HCV RNA was first converted in cDNA and then amplified by using Analytika Jena quantification kit (Germany). The limit of detection and the linear range of the kit was 68 IU/ml and > 8 logs respectively for the real- time PCR assay. Briefly for PCR amplification, 1 μL of template cDNA was added to 25 μL of the master mixture having 1.5 μL of DNTP mixture (0.2 mM of each), 2.5 μL of PCR buffer, 0.5 μL of Taq polymerase, 0.5 μL of each primer stock solution (50 pmol/μL), 0.5 μL of $MgCl_2$, and the remaining 18 μL volume was filled by nuclease free water (Fermentas, USA). After amplification reaction the product was resolved on agarose gel and visualized under a UV transilluminator. All the variables being categorical were analyzed through count and percentages. The data was analyzed by chi-square test. The correlation between different techniques was calculated by Cohen's K coefficient test using online tool (Graph pad quick calcus software).

RESULTS

Out of 44 cases, 23 (52.2%) were males and 21 (47.7%) were females. Six patients ware aged upto 2 years, 8 from 3-5, 15 from 6-8, 5 from 9-11, 6 from 12- 14 and 4 from 15-17 years. ICT diagnosed HCV in 35 (79.5%) subjects; ELISA in 28 (63.6%) subjects and on real time PCR, HCV was detected in 19 (43.1%) subjects (Table 1).

Cohen's Correlation was done. These Kappa values confirmed a weak correlation between any of two HCV diagnostic techniques (Table 2).

Significant difference was detected among male patients as compared to female patients by the three diagnostic techniques (Table 3).

The highest detection of HCV was found among age group of 15-17 years, whereas HCV was detected low in the age group of 12-14 according to ICT and PCR whereas ELISA showed less detection in age group 3-5 (Table 4).

Table 1: Detection of HCV on available diagnostic methods among beta thalassemia major patients (n=44).

Techniques	HCV Positive	HCV Negative	χ2	P value
ICT	35 (79.5%)	9 (20.4%)	8.5	0.03
ELISA	28 (63.6%)	16 (36.3%)	0.64	0.799
PCR	19 (43.1%)	25 (56.8%)	10.06	0.0015

Table 2: Cohen's correlation between different techniques for the detection of HCV among beta thalassemia major patients (n=44).

ICT Vs ELISA				Cohen's kappa coefficient	Strength of agreement
ELISA	ICT				
	Positive	Negative			
	Positive	22	6	Kappa = −0.030 SE of kappa = 0.137 95% CI: −0.299 to −0.240	Poor
	Negative	13	3		
ELISA Vs PCR					
PCR	ELISA				
	Positive	Negative			
	Positive	14	5	Kappa = 0.167 SE of kappa = 0.135 95% CI: −0.097 to −0.432	Poor
	Negative	14	11		
PCR Vs ICT					
ICT	PCR				
	Positive	Negative			
	Positive	17	18	Kappa = 0.133 SE of kappa = 0.106 95% CI: −0.074 to −0.340	Poor
	Negative	2	6		

[1]Kappa value<0.20= strength of agreement "Poor"; Kappa value 0.21 −0.40 = strength of agreement "Fair"; Kappa value 0.41−0.60= strength of agreement "Moderate"; Kappa value 0.61−0.80= strength of agreement "Good"; Kappa value 0.81−1.00= strength of agreement "Very good"

Table 3: Gender wise detection of HCV among beta thalassemia major patients (n=44).

Technique	Male (n=23)		Female (n=21)	
	Positive	Negative	Positive	Negative
ICT	18 (78.26%)	5 (21.73%)	17 (80.95%)	4 (19.04%)
ELISA	15 (65.21%)	8 (34.78%)	13 (61.90%)	8 (38.09%)
PCR	12 (52.17%)	11 (47.82%)	7 (33.33%)	14 (66.66%)

Table 4: Age group wise detection of HCV among beta thalassemia major patients (n=44).

Age group	Count	ICT	ELISA	PCR
Upto 2	06	6 (100%)	4 (66.6%)	3 (50%)
3-5	08	6 (75%)	3 (37.5%)	3 (37.5%)
6-8	15	10 (66.66%)	9 (60%)	5 (33.33%)
9-11	05	5 (100%)	4 (80%)	2 (40%)
12-14	06	4 (66.66%)	4 (66.66%)	2 (33.33%)
15-17	04	4 (100%)	4 (100%)	4 (100%)

DISCUSSION

Thalassemia is an inherited genetic disorder and is the major disorder of health concern in Pakistan. There are estimated one lac thalassemia major patients while 5000-9000 new cases of thalassemia major are added every year.[15,16] Conventional treatment of patients suffering from thalassemia is a regular blood transfusion improving their overall survival but may carry a definite risk of acquiring blood borne infection particularly hepatitis C viral infection.

In the present study among 44 beta thalassemic patients, 35 (79.5%) were positive for HCV by ICT which is in line with a study reported by Waqar-ul-Huda *et al* in 2013, presenting 73.07% detection rate of HCV among 26 patients.[17] Though ICT is a rapid and simple test, however the higher false positivity rate is also reported. Therefore ICT should not be employed as sole diagnostic test for HCV but may serve the purpose of initial screening only in high HCV burden area of Pakistan.

All the samples were further processed through ELISA for HCV-antibody detection. Among 44 samples, the observed presence of anti-HCV was 28 (63.6%). Similar findings were documented by Ain Q et al in 2011. According to them, HCV detection was 65% among thalassemic patients in Faisalabad, Pakistan.[18] The prevalence of HCV in patients of thalassemia in Iran and India were 63.8% and 16.7% respectively. A slightly different data was reported from Rawalpindi Region of Pakistan, the HCV prevalence in thalassemic patients was 60% while it has been reported from Karachi, Pakistan that the prevalence of HCV infection in thalassemia children was 20.5%.[19]

For a more specific detection of HCV among thalassemic patients, RT-PCR detected HCV in 19 (43.1%) samples. Similarly Muhammad R et al documented 40% HCV detection through PCR.[20] It is worth mentioning that ICT and ELISA could not differentiate between HCV disease and resolved infection, while RT-PCR is required to confirm active HCV infection. Comparison of all the techniques showed that the detection rate was less specific on ICT as compared to ELISA, but more specific on PCR.

CONCLUSION

It is better to employ a coupled diagnostic strategy for the diagnosis of HCV infection among the multi-transfused beta thalassemic major patients than using a single technique.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.

AUTHORS' CONTRIBUTION

Conception and Design:	FB, SFS, SSM, HR, AM
Data collection, analysis & interpretation:	FB, SFS, SSM, BR, HR, AM
Manuscript writing:	FB, HR