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RISING BURDEN OF HEPATITIS C VIRUS (HCV) IN PAKISTAN: A CROSS SECTIONAL STUDY ON MULTITRANSFUSED BETA THALASSEMIA TYPE MAJOR PATIENTS

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Abstract

Background: Hepatitis C virus (HCV) is a serious health issue which infected 3.3% of world's population. The major transmission route of this virus is blood and blood products. Thalaseemic patients are dependent upon blood transfusions throughout their life and are at high risk of getting HCV infections.

Objectives: The aim of the study was to estimate the presence of hepatitis C virus in multitransfused thalaseemic population of capital twin cities of Pakistan.

Patients and Methods: The study was conducted from November 2021 to April 2022 and 262 multitransfused beta thalaseemic from the capital twin cities of Pakistan were enrolled for analysis. The presence of hepatitis C virus, alanine amino transferase (ALT) level, serum creatinine, hepatomegaly, splenomegaly and splenectomy were analyzed.

Results: The overall prevalence of Hepatitis C virus was 55.73% observed with 100% of patients greater than 20 years of age have HCV infection. The average ALT in HCV positive thalaseemic patients was observed 98U/L and average creatinine values were 0.39 mg/dl. 82.20% of HCV positive thalaseemic patients have hepatomegaly with average increase of 4.33 cm in liver size. Splenomegaly was observed in 67.12% of HCV positive thalaseemic patients with average increase of 4.46 cm in spleen size. Splenectomy was observed in 15.75% of cases.

Conclusion: The presence of HCV is very high in thalaseemic population of Pakistan. The chance of getting HCV infections also increases with the increase in age of thalaseemic patient. Raised ALT and hepatomegaly was observed in majority of HCV positive thalaseemic patients. There is a strong need to properly screen the blood before transfusions to decrease the future load of HCV from Pakistan.

Keywords Hepatitis C virus, Thalaseemic patients, Transfusions, Hepatomegaly, splenectomy.

INTRODUCTION

In 1975 Hepatitis C Virus (HCV) was initially recognized as Non-A, non-B viral hepatitis (NANBH). The newly discovered flavi-like virus was responsible for transfusion-associated hepatitis (1-3). It belongs to family Flaviviridae and is single stranded RNA virus with 55–65 nm size (4). The World Health Organization (WHO) has compared Hepatitis C Virus to a "viral time bomb" with an estimated global prevalence of 3.3% of world's population (i.e. about 200 million people receive HCV infection). 130 million of them are chronic carriers and are at risk of developing liver cirrhosis or/ and liver cancer. 2.3 -4.7 million people get new HCV infection annually, 70% of them would develop chronic hepatitis. WHO statistical analysis (2008) revealed liver cirrhosis as 18th most common cause of mortality and it is estimated to achieve 13th position by 2030 (5-6). WHO has declared South East Asia a high risk area for HCV with prevalence rate of 2.15% (7). Liver cirrhosis develops in 20% of HCV infected patients after 10-20 years, while liver cancer usually appears after 20-40 years post infection (8).

Blood transfusions, intravenous administration of drug needle stick injuries, perinatal transmission, hemophilia, hemodialysis and transplants are considered as most common risk factors for HCV transmission. While some of the uncommon risk factors for HCV transmission includes intranasal cocaine use, body piercing, tattoos, shared shaving equipments, sexual activity, erosion of boxers knuckles and transmission from health care worker to patients (9).

In Pakistan HCV infection is increasingly recognized as major health care problem. Several studies have revealed prevalence estimates of HCV infection in adults and children between the range of 2.4-6.5% and 0.44-1.6% respectively (10-13). Health care professionals are also among high risk population with HCV prevalence of 4-5.6%, which is 20 fold greater than the global prevalence (14, 15). According to WHO, 1.2 to 1.5 million transfusions are carried out each year in Pakistan

but unfortunately the country suffers 40% shortage of blood and blood components. For a nation to reach its basic blood requirements, at least 1% of population should donate blood. It is estimated that average donation rate is only 0.37% and 0.75% in developing and transitional countries, compared to 3% in developed countries. The average number of blood donations per 1000 persons in low-income countries is 10 times lower as compared to the blood donations from developed countries. Inadequacy of blood supply usually drags the health providers to switch towards replacement or paid blood donations, in low income countries. It has been reported that in Pakistan; 75% (sometimes 90% in northern areas), 15% and 10% of blood donations are offered by replacement donors, professional donors and unpaid voluntary donors respectively (16-20). In a study conducted on "well-selected blood donors" it was revealed that the overall frequency of HCV antibodies was 1.87% (1.83% and 2% amongst first time and second time donors respectively) (21). In 2010, a survey report by WHO (conducted in 173 countries) stated that only 47% of total 93 million blood donations in developing countries were screened under quality assurance procedures. Lack of centralized blood transfusion service, properly trained and educated staff, continuous supply of electricity, low efficiency of blood transfusion services and operations, less political support and commitment, inappropriate resources and infrastructure, crude low sensitivity kits with cheap prices and commercialization of blood screening setups are the major challenges associated with sustainability of blood safety in Pakistan (22).

Thalassaemia is presumed to be most common fatal genetic disorder of Pakistan, where 5000-9000 children are born with β-thalassaemia each year. The approximate carrier rate of this autosomal recessive disorder is 5-7% with nearly 9.8 million carriers (23). The proportion of marrying to cousins and relatives is higher in Pakistan as compared to rest of the North African Muslim countries (24). There exist a strong relationship between consanguineous marriages and occurrence of thalassemia. On average thalassaemia patients receive 25 blood transfusions per year. Such high rate of blood transfusions may cause development of transfusion transmitted infections (TTIs). Previous studies have reported prevalence of HCV in multi-transfused thalassaemia population of Pakistan within the range of 34.8 to 60%; perhaps these figures are significantly alarming among rest of Asian countries (25-29). Low educational standards, poor health status and non implementation of international blood safety measures are the major cause of disseminating TTIs (such as HBV, HCV and HIV) via multiple blood transfusions. This article describes seroprevalence of Hepatitis C virus in thalassaemia patients of capital twin cities (Islamabad & Rawalpindi) of Pakistan. Relationships among HCV positivity and various study variables were analyzed and statistically validated. This study provides valuable justifications for implementation of blood safety standards at different public health sectors of rural and urban areas of Pakistan.

MATERIALS AND METHODS

Study Settings and Data Collection

The cross sectional study was conducted during the period of November 2021 to April 2022 among 262 multi transfused β-thalassaemia patients enrolled from Islamabad, Rawalpindi and its suburb areas, on the basis of random sampling technique. The study was approved by ethical committee of Atta ur Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST). Complete Physical and clinical examination of each participant was carried out after acquisition of patient demographic history data and informed written consent. All subjects were subjected to clinical examination for hepatomegaly, splenomegaly and splenectomy by the assistance of ultrasound technique in accordance to standard operating procedures. For all participants, pre-transfusion hemoglobin count, alanine transferase (ALT) count and serum creatinine level were identified via assistance of spectrophotometer. Sterile syringes were used to

obtain venous blood, which was further kept in vacutainer (EDTA) tubes. For invitro diagnostic analysis, blood samples were brought to Viral Hepatitis Lab of ASAB, NUST. The sera were separated into two aliquots and frozen at -80°C for screening of HCV positive samples.

Anti HCV antibody third generation test was performed for the initial screening of HCV positive β-thalassaemia patients, in accordance to guidelines provided by Accurate Diagnostics USA. The test device was significantly reliable due to 99% correlation with leading commercial HCV EIA test kit. The relative sensitivity, specificity and accuracy for HCV screening was >99.8% (98.7%-100.0%), 99.9% (99.8%-100.0%) and 99.9% (99.7%-100.0%) respectively (with 95% confidence Interval). The viral RNA confirmation was done by HCV qualitative PCR analysis.

Statistical analysis was conducted on study variables via assistance of Statistical Package for Social Sciences (SPSS version 17). χ^2 test were applied to identify relationships between HCV positivity and study variables. Significance value was evaluated at 0.05 levels with 95% confidence level.

RESULTS

Prevalence of Hepatitis C Virus in beta-Thalassemia Patients:

Among 262 enrolled beta thalassemia patients, 146 individuals were found HCV positive with percentage prevalence rate of 55.73%. The prevalence of HCV in 157 male and 105 female participants were 54.77% and 57.14% respectively.

Among patients of 1-10 years of age, HCV prevalence was found 48.47%. The HCV prevalence was found 68.42% in patients of 11-20 years of age; while among patients of more than 20 years of age prevalence rate was found 100%. Convert this data in bar graph

Hepatomegaly in HCV infected beta-Thalassemia Patients:

Increase in liver size (Hepatomegaly) was observed in 74.8% of cases. Among all patients with Hepatomegaly, HCV was detected with prevalence rate of 61.202%. Among all HCV infected beta thalassemia patients, hepatomegaly was observed in 82.20% cases. The average increase in liver size among all HCV infected individuals was approximately 4.33 cm. Among most of HCV infected thalassemia patients 4cm increase in liver size was observed. The figure 1 shows hepatomegaly in hepatitis C infected thalassemia patients.

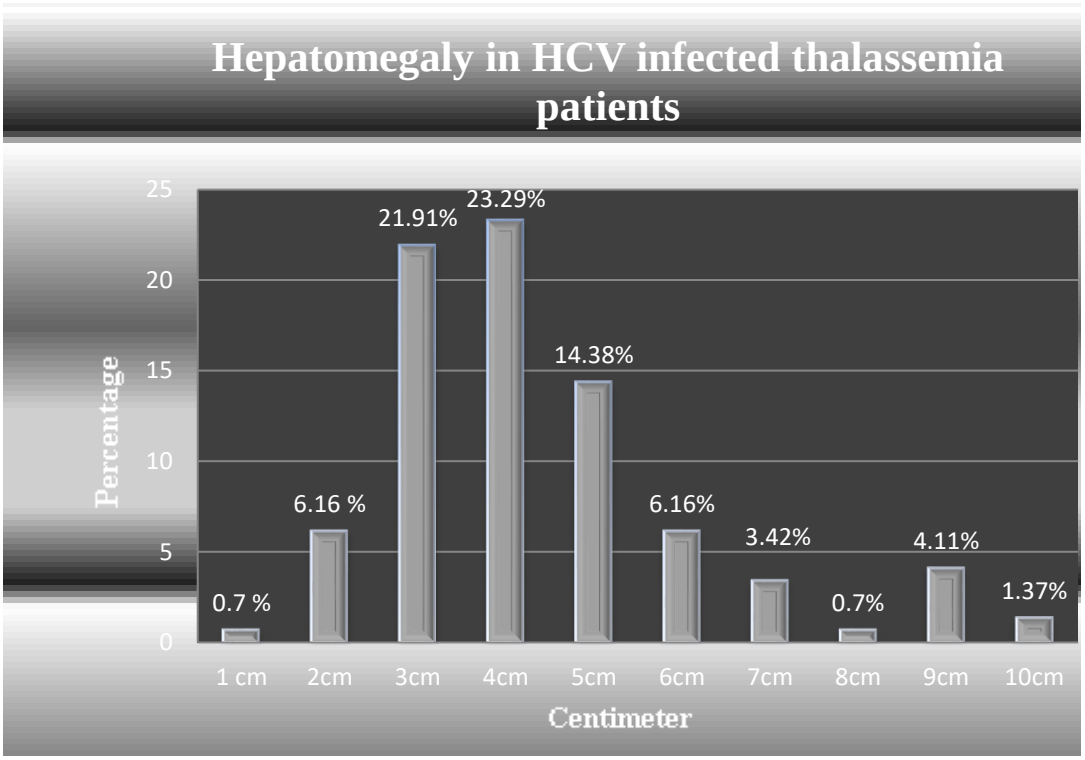

Figure 1: The figure represents percentage occurrence of increase in liver size in HCV infected thalassemia patients. The X axis shows increase in liver size in centimeters, while Y axis shows percentage occurrence.

Splenomegaly in HCV infected beta-Thalassemia Patients:

Increase in spleen size (Splenomegaly) was observed in 64.9% of cases. Among HCV infected beta thalassemia patients, splenomegaly was found in 67.12% of subjects. Among these patients, splenectomy was observed in 15.75% of cases. While among all HCV infected beta thalassemia patients, only 17.12% possessed normal spleen. The average increase in spleen size was 4.46cm in all HCV patients and most of these patients had 2-3cm increase in spleen size. Figure 2 represents increase in spleen size among HCV infected patients.

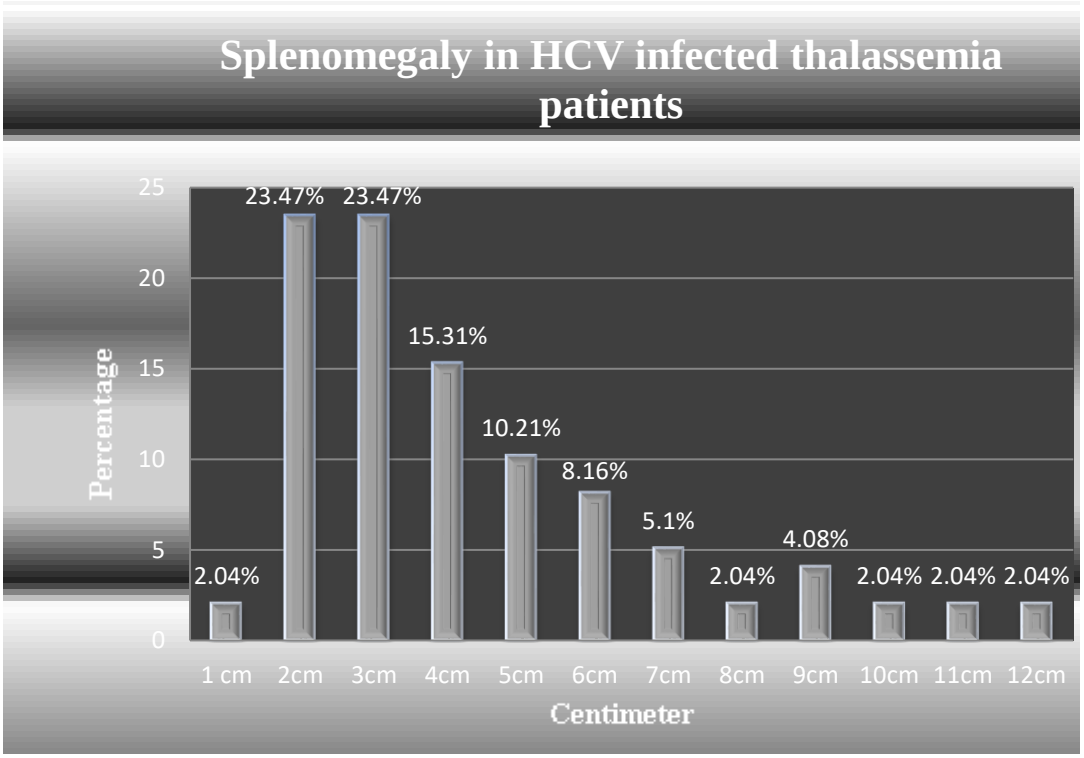

Figure 2: The figure represents percentage occurrence and splenomegaly among HCV infected thalassemia patients. The X axis shows increase in spleen size in centimeters, while Y axis shows percentage occurrence.

Alanine Transaminase Level and Serum Creatinine Count in HCV infected beta thalassemia patients:

Among males and females, the normal ranges of ALT and serum creatinine are 10-40U/L, 10-32U/L and 0.7-1.4mg/dl, 0.6-1.1md/dl respectively. Among all HCV positive beta thalassemia patients, the average ALT level was observed 98U/L; while among HCV negative individuals, the average ALT level was observed 71.5U/L. Similarly among all HCV positive patients, average serum creatinine level was found 0.39mg/dl; while among HCV negative individuals, the average serum creatinine count was observed 0.37 mg/dl. The table I shows statistically validated relationships among selected variables.

Table 1: Statistical Analysis for Relationship Studies among Different Study Variables.

Relationship Between Variables	Pearson Chi-Square test value	P-value (Confidence level)
Relationship Between Hepatomegaly and HCV Positive Thalassemia Patients	26.719	0.021
Relationship Between Splenomegaly with HCV Positive Thalassemia Patients	21.129	0.33
Relationship Between HCV Positive Individuals and ALT Level	1.79 E2	0
Relationship Between HCV Positive Individuals and Creatinine Level	16.65	0.002

DISCUSSION

Pakistan is a developing country with increased socioeconomic burden of infectious diseases. Majority of people have high prevalence of anemia, communicable diseases, malnutrition, thalassaemia, obstetrical emergencies, traumatic injuries and road traffic accidents. In such circumstances, the demand for blood supply increases, and blood transfusion becomes life saving. The quality of transfusion practice, at various blood establishment setups around the country, has become a question mark due to extreme commercialism. Most of the blood transfusion services are confined to hospitals and majority of blood donations are offered by replacement blood donors and paid professional donors. Replacement blood donors are family members or friends, who donate blood for saving the lives of their loved ones, without involvement of any monetary benefit. These blood donations are not considered safe because they donate blood under family peer or circumstances pressure and thus may hide their illnesses or high risk behavior. For a thalassemia patient nearly twenty five blood transfusions are required per year. Such a huge number of blood transfusions usually drag thalassaemia patients at risk of acquiring blood

borne pathogens. (short down this para, write summary of this para and decrease length)

In Pakistani setup, marriages are not only the union of two individuals; instead it is intermingling of two families, where people care about their loved ones. At time of need of blood, patients and their family members mostly rely on their own family members (replacement blood donors) instead of volunteer blood donors. In Pakistani setup consanguineous marriages are highly frequent in both rural and urban areas. Most of senior family heads prefer availability of individual for marriage from close relatives. In Pakistani society, once the young couples get committed to their relationship as fianc (boy) or fiance (the girl), they become unable to change their opinion of marriage due to fear of family disputes. In order to prevent family disputes and clashes, consanguineous marriages are accepted by couples. Rejection of marriage is serious taboo for a woman as it can destroy her social life. In some circumstances it may prevent her from ever getting married. Premarital screening resulting into unpleasant outcomes could challenge the reputation of their parents. Although Islamic teachings promote healthy marriages and concept of counseling, yet unfortunately some individuals misinterpret their religion and create serious hurdles in success of premarital screening. The average treatment cost per thalassaemia patient varies from 4500 Pakistani Rupees to Rs 7500 (30). Wall street journal 18-08-2005 reported average monthly income of Pakistanis to be 41$ which is equal to Rs 2542. Management of extra expenditures on such patients is very difficult task for the family. It has been reported that 75% of thalassaemia patients belonged to uneducated and poor families (31). (short down this para, write summary of this para and decrease length)

According to various epidemiological studies, it is likely that HCV infects larger proportion of transfusion dependent beta-thalassemia major patients. Up to 80% of adult thalassaemia patients are infected by HCV in world but, depending upon regions, considerable variations also exist (22,32). In Egypt, the prevalence of HCV infection is highest i.e. 22% of population. Center for Disease Control and Prevention has reported prevalence of anti-HCV in 1.8% of US population, with 8000-10,000 deaths per year (33). In Europe and South East Asia, the prevalence of HCV is 1.03% and 2.13% respectively (34). According to a study conducted in Pakistan, the percentage prevalence of HCV in general adult population, pediatric population, young population applying for recruitment, injecting drug users and multitransfused population was 4.95%, 1.72%, 3.64%, 57% and 48.67% respectively (35). It is a well known fact that thalassaemic patients acquire Hepatitis C through administration of unscreened blood collected during donor window period. In thalassaemic patients, seroprevalence of HCV infection varies in different parts of world. In Iran, India, Italy and Malaysia, the prevalence of HCV in thalassaemic patients was reported as 63.8%, 16.7%, 47% and 23.8% respectively (36-38). According to a study conducted at Rawalpindi region of Pakistan, the HCV prevalence in thalassaemia patients was 60% (27). It has been reported from Karachi that the prevalence rate of HCV infection in thalassemia children was 20.5% (39). Some of the other studies depicting percentage prevalence of HCV in different thalassemia populations of Pakistan have been summarized in Table II.

Table II: HCV in Thalassemia patients of Pakistan: A systemic review of available data.

Author	Year	Population Size	Population Type	Region	Prevalence	References
Bhatti et al.	1995	35	Thalassemia Patients	Rawalpindi	60%	[27]
Moatter et al.	1999	100	Thalassemia Patients	Karachi	35%	[40]
Muhammad J.	2003	80	Thalassemia Patients	Peshawar	36.20%	[41]
Akhtar et al.	2004	256	Thalassemia Patients	Karachi	34.80%	[25]
Younus et al.	2004	75	Thalassemia Patients	Islamabad	42%	[42]
Burki et al.	2005	180	Thalassemia Patients	Islamabad	41.70%	[43]
Shah et al.	2005	250	Thalassemia Patients	Peshawar	57%	[44]
Hussain et al.	2008	180	Thalassemia Patients	Islamabad & Peshawar	41.70%	[45]

It is evident from previous studies that with the passage of time, the prevalence of HCV in thalassemia patients is gradually increasing. According to Hussain et al (2008), the seroprevalence of HCV in thalassemia patients of Islamabad and Peshawar was 41.7%; but our study states that, the prevalence of HCV in thalassemia patients of capital twin cities (Islamabad and Rawalpindi) of Pakistan is 55.73% (45). Our study shows that prevalence of HCV is higher among females as compared to males. There exists an overall increasing trend of HCV prevalence in thalassemia patients of different age groups. The data shows that among beta thalassemia patients, as the age increases from 1-10 years of age to more than 20 years of age, the prevalence of HCV significantly increased from 48.47% to 100%. This data clearly states that among multitransfused beta thalassemia patients as the age increases, the chances of acquiring HCV also increases. Majority of enrolled participants (75%) had hepatomegaly with increased prevalence of viral hepatitis (61.2%). Similarly majority of the beta thalassemia patients (65%) had splenomegaly with increased prevalence of HCV (67.1%).

Among HCV infected beta thalassemia patients, maximum increase in liver and spleen size was observed 10cm and 12 cm respectively. The graphical analysis shows that the number of individuals with hepatomegaly of 1cm and 10cm were least (i.e. 0.7% and 1.37% respectively). Majority of individuals with hepatomegaly had 3-4cm increase in liver size. But among patients groups of significantly increased liver size, the proportion of individuals was considerably low; which depicted increased risk of death. Similarly graphical analysis of HCV infected beta thalassemia patients with splenomegaly suggested that, the number of individuals with splenomegaly of 1cm and 12cm were least (i.e. 2.04% for each case respectively). This analysis showed that majority of patients with splenomegaly had 2-3cm increase in spleen size. But among patients groups of significantly increased spleen size, the proportion of individuals was considerably low; which depicted increased death risk among patients. This data also suggests that as liver and spleen size increases in HCV infected beta thalassemia patients (with passage of time), there is an increased risk of death. Increased ALT level is associated with diseased or unhealthy state of liver. The ALT count

in HCV infected beta thalassemia patients was significantly higher as compared to HCV negative beta thalassemia patients. But the serum creatinine level was approximately similar in both HCV positive and HCV negative thalassemia patients. The relationships between HCV positive thalassemia patients and hepatomegaly, ALT and Creatinine level were found highly significant.

CONCLUSION

Hepatitis C Virus is rapidly propagating in beta thalassemia type major populations of Pakistan due to fragmented, disorganized and decentralized hospital based blood transfusion setups. In Pakistani societies, consanguineous marriages are highly frequent and have substantial association with increased prevalence of thalassemia. Raised ALT & creatinine values, hepatomegaly and splenomegaly were observed in majority of thalaseemic patients. The chance of getting HCV infection increases with the increase in age of thalaseemic patients. Proper blood screening before transfusion is highly recommended to decrease the future burden of HCV from Pakistan.

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