

Estimation of Hepatitis B Virus, Hepatitis C Virus, and Different Clinical Parameters in the Thalassemic Population of Capital Twin Cities of Pakistan

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ABSTRACT: Hepatitis B and C are serious public health problems worldwide. Thalassemia patients are dependent on blood transfusions throughout their life and are at high risk of viral infections. The aim of this study was to estimate the prevalence of hepatitis B/C infections and different clinical parameters in multitransfused thalassemia population. In this study, 262 multitransfused β -thalassemia patients were enrolled from the capital twin cities of Pakistan. The presence of hepatitis B virus (HBV)/hepatitis C virus (HCV), alanine aminotransferase (ALT) level, serum creatinine, serum ferritin, hepatomegaly, splenomegaly, and splenectomy were analyzed. The overall prevalence of HBV and HCV was 3.08% and 55.73%, respectively, with 100% of patients older than 20 years had HCV infection. The ALT levels among HBV- and HCV-positive thalassemia patients were 92.62 ± 41.57 U/L and 98 ± 63.65 U/L, respectively; creatinine values observed were 0.4 ± 0.35 mg/dL (for HBV) and 0.39 ± 0.24 mg/dL (for HCV), while serum ferritin levels were 6865.87 ± 1649.13 ng/dL (for HBV) and 5445.95 ± 3059.28 ng/dL (for HCV). A total of 74.8% and 82.20% of HBV- and HCV-positive patients had hepatomegaly with an average increase in liver size of 4.17 and 4.33 cm, respectively. Splenomegaly was observed in 64.9% and 67.12% of HBV- and HCV-positive patients with an average increase in spleen size of 4 and 4.46 cm, respectively. Splenectomy was observed among 14.50% and 15.75% of HBV- and HCV-infected thalassemia patients. There is a strong need to properly screen blood before transfusions to reduce the future load of viral hepatitis from Pakistan.

KEYWORDS: hepatitis B virus, hepatitis C virus, thalassemic patients, transfusions, hepatomegaly, splenomegaly, splenectomy

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Introduction

Viral hepatitis B and C are life-threatening agents responsible for transfusion transmitted infections (TTIs) worldwide. Hepatitis B virus (HBV) infection is considered as the 10th leading cause of mortality, and it is estimated that one-third of world's population has serological evidence of HBV.^{1–3} Both HBV and hepatitis C virus (HCV) have infected 530 million of the 6 billion world's population.^{4–6} The World Health Organization (WHO) has compared HCV to a “viral time bomb” with an estimated global prevalence of 3.3% of the world's population. In all, 130 million of them are chronic carriers and are at risk of developing liver cirrhosis or/and liver cancer.⁷ Currently, no vaccine is available for HCV.⁸ Worldwide, on average, HBV infection causes the death of one patient every 30–45 seconds. The Centers for Disease Control and Prevention, USA, estimated HBV to be 10 and 100 times more infectious than HCV and human immunodeficiency virus (HIV), respectively.^{4–6}

Pakistan is a developing country of 190 million people with the huge burden of infectious diseases. In Pakistan, viral infections are increasing day by day.^{3,9–11} It is reported that the prevalence of HBV was 4.6% in the general population of Pakistan,³ while the prevalence of HCV was 4.9% in the general population of Pakistan.¹² Pakistan has started an expanded HBV vaccination program for children, but still there is no vaccine available for HCV.^{8,13}

WHO has recommended the screening of HBV, HCV, HIV, and syphilis for all blood donations by highly sensitive and specific methods. Each country should have a national program for blood screening, and there should be a regulatory mechanism for oversight of the activities of blood transfusion services, including blood screening.¹⁴

From 2001 to 2011, interferon and ribavirin remained the standard of care for the patients living with HCV. Recently, a couple of direct-acting antiviral drugs are approved by FDA; these combinations showed a very good response rate with

minimal adverse effects.^{15,16} Oral nucleoside inhibitors such as entecavir and tenofovir are also showing good results in patients with hepatitis B infection with a low resistance rate.¹⁷

The proportion of marrying cousins and relatives is higher in Pakistan as compared to the rest of the North African Muslim countries. There exists a strong relationship between consanguineous marriages and occurrence of thalassemia.¹⁸ Thalassemia is presumed to be the most common fatal genetic disorder of Pakistan, where 5000–9000 children are born with β -thalassemia each year. The approximate carrier rate of this autosomal recessive disorder is 5%–7% with ~9.8 million carriers.¹³ Thalassemia patients receive multiple blood transfusions per year, which may cause the development of TTIs. This article describes seroprevalence of HBV/HCV and different clinical parameters in thalassemia patients of the capital twin cities (Islamabad and Rawalpindi) of Pakistan.

Materials and Methods

A cross-sectional study was performed during the period of November 2011 to April 2012 among 262 multitransfused β -thalassemia type major patients from the capital twin cities of Pakistan. The study was approved by the ethical committee of Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Pakistan. Patients or the guardians of minor patients gave their written, informed consent to participate in the study, which was conducted in accordance with the principles of the Declaration of Helsinki. Among all patients, β -thalassemia type major was considered as inclusion criteria, whereas patients with α -thalassemia type minor/major or patients having β -thalassemia type minor were excluded.

Demographics and information regarding age at diagnosis of thalassemia, age at first blood transfusion, history of thalassemia in family, number of blood transfusions per month, alanine aminotransferase (ALT) count, serum creatinine, ferritin levels, hepatomegaly, splenomegaly, splenectomy, presence of HBV, presence of HCV, chelation therapy, and use of deferoxamine were included in this study.

HBV detection was done by using accurate diagnostic kit, USA. The sensitivity, specificity, and accuracy of the accurate diagnostic kit were >99%, 96.7%, and 98.3%, respectively. The presence of HCV was also detected by accurate diagnostic kit with relative sensitivity, specificity, and accuracy of >99.8%, 99.9%, and 99.9%, respectively. The presence of viral hepatitis was also confirmed from the enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) reports in the patient files.

The normal reference range value for ALT was 7–40 U/L, serum creatinine was 0.5–1.2 mg/dL, and ferritin was 11–336 ng/mL. Normal liver span is 6–12 cm. Thus, 12 cm was selected as cutoff value for determining hepatomegaly.¹⁹ Normal adult spleen size is 9–12 cm; therefore, 12 cm was considered as cutoff value for determining splenomegaly.²⁰

Statistical analysis was conducted on study variables via the assistance of Statistical Package for Social Sciences

(version 17). The mean and standard deviation values of different parameters were calculated. χ^2 tests were applied to identify relationships between viruses and study variables. Significance value was evaluated at 0.05 levels with 95% confidence level.

Results

Among the 262 enrolled participants, 59.92% were male and 40.08% were female. Patients ranged from 1 to 28 years, while the mean age of participants was 9.26 years. The average age of diagnosis of β -thalassemia disease was 7.7 months. A total of 60.68% had a family history of thalassemia. The average age of patients at the first blood transfusion was 10 months. The average rate of transfusions per month was 2.08 (ie, patients received 25 blood transfusions per year). Most of the patients (38.17%) received two blood transfusions each month. Average hemoglobin count prior to blood transfusion was 5–12 g/dL. 32.06% patients had an average hemoglobin count of 8 g/dL; 38.55% patients received chelation therapy and used deferoxamine. Among 262 β -thalassemia patients, 8 individuals were found HBV positive with percentage prevalence of 3.05% and 146 people were HCV positive with the prevalence rate of 55.73%.

Normal liver span is 6–12 cm. Thus, 12 cm was selected as cutoff value for determining hepatomegaly.¹⁹ Hepatomegaly was found in 74.8% of the total patients, while all HBV-positive patients and 82.2% HCV-positive patients had hepatomegaly. The average increase in liver size was 6 and 4.3 cm in HBV and HCV patients, respectively. Figure 1 shows the presence of hepatomegaly in HBV/HCV-infected β -thalassemia patients.

Normal adult spleen size is 9–12 cm; therefore, 12 cm was considered as cutoff value for determining splenomegaly.²⁰ Splenomegaly was found in 64.9% of all thalassemia patients, while splenectomy was observed among 14.50% of patients. Among HBV-infected β -thalassemia patients, no individual had normal spleen. Splenomegaly was found in 75% of cases, while splenectomy was observed in 25% of cases. Among all HBV-infected patients, most of the patients had a 4-cm increase in spleen size, while, on average, increase in spleen size was 3.16 cm. Among HCV-infected β -thalassemia

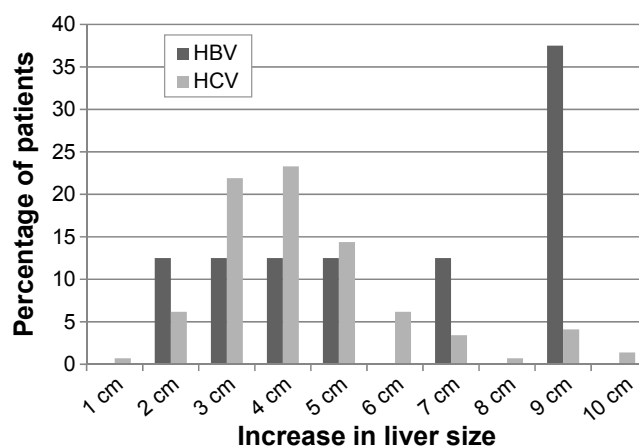


Figure 1. Hepatomegaly in HBV/HCV-infected thalassemia patients.

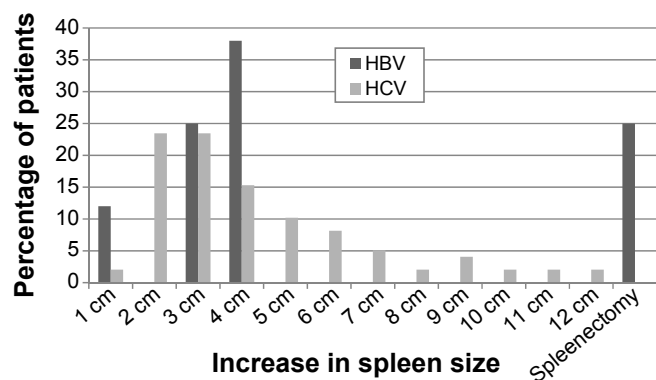


Figure 2. Splenomegaly in HBV/HCV-infected thalassemia patients.

patients, splenomegaly was noted in 67.12% of cases. Average splenomegaly in HCV-infected patients was 4.46 cm. Splenectomy was observed in 15.75% of HCV-infected patients. Figure 2 indicates the presence of splenomegaly in HBV/HCV-infected β -thalassemia patients.

Normal values of ALT, serum creatinine, and ferritin are 10–40 U/L, 0.7–1.4 mg/dL, and 12–300 ng/mL (in males) or 2–150 ng/mL (in females), respectively. Among HBV/HCV-infected individuals, on average, serum ALT, serum creatinine, and ferritin levels were determined as listed in Table 1. For patients with HBV and HCV coinfection, average ALT level was found to be 103.2 U/L. Among such patients, mean serum creatinine was observed as 0.4 mg/dL and mean ferritin level was found to be 7320.002 ng/dL.

The statistical analysis was performed for the mean and standard deviation values of different study variables, including age, age of diagnosis, family history of thalassemia, age at first transfusion, transfusions per month, hemoglobin count prior to transfusion, amount of blood received, liver size, spleen size, ALT, creatinine, and ferritin, as listed in Table 2.

Different relationships among different study variables were statistically evaluated as listed in Table 3. When the P -value is <0.05 , then the relationship is considered as highly significant. Among all aforementioned relationships, most relationships were found highly significant with a P -value of 0.000.

Table 1. Levels of ALT, creatinine, and ferritin in HBV- and HCV-infected thalassemia patients.

	ALT	CREATININE	FERRITIN
HBV positive individuals	92.62 $\pm$ 41.57 U/L	0.4 $\pm$ 0.35 mg/dL	6865.87 $\pm$ 1649.13 ng/dL
HBV negative individuals	86 $\pm$ 65.28 U/L	0.37 $\pm$ 0.31 mg/dL	4963.62 $\pm$ 2749.33 ng/dL
HCV positive individuals	98 $\pm$ 63.65 U/L	0.39 $\pm$ 0.24 mg/dL	5445.95 $\pm$ 3059.28 ng/dL
HCV negative individuals	71.5 $\pm$ 63.23 U/L	0.37 $\pm$ 0.32 mg/dL	4360.72 $\pm$ 2140.77 ng/dL

Table 2. Analysis of different clinical parameters.

AGE	AGE AT DIAGNOSIS	FAMILY HISTORY OF THALASSEMIA	AGE AT 1ST TRANSFUSION IN MONTHS	TRANSFUSION PER MONTH	Hb PRIOR TO TRANSFUSION	LIVER SIZE	SPLEEN SIZE	ALT	CREATININE	FERRITIN
9.26 $\pm$ 5.18	7.68 $\pm$ 8.03	1.39 $\pm$ 0.49	9.98 $\pm$ 15.71	2.08 $\pm$ 0.74	8.87 $\pm$ 1.35	3.37 $\pm$ 2.1	2.80 $\pm$ 2.52	86.42 $\pm$ 64.7	0.37 $\pm$ 0.08	4.96 E3 $\pm$ 2.74 E3

**Table 3.** Statistical analysis for relationship studies among different study variables.

RELATIONSHIP BETWEEN VARIABLES	PEARSON CHI-SQUARE TEST VALUE	P-VALUE (CONFIDENCE LEVEL)
Relationship between age of diagnosis and age at first blood transfusion	2.37 E3	0
Relationship between number of blood transfusions and increase in liver size	2.53 E2	0
Relationship between number of blood transfusions and increase in spleen size	2.29 E2	0
Relationship between hepatomegaly with age	6.21 E2	0
Relationship between hepatomegaly and hbv positive thalassemia patients	34.443	0.002
Relationship between hepatomegaly and hcv positive thalassemia patients	26.719	0.021
Relationship between splenomegaly with age	7.42 E2	0
Relationship between splenomegaly with hbv positive thalassemia patients	14.487	0.755
Relationship between splenomegaly with hcv positive thalassemia patients	21.129	0.33
Relationship between age and amount of blood received	1.5410	0
Relationship between age and alt level	3.68 E3	0
Relationship between age and creatinine level	1.37 E2	0.001
Relationship between age and ferritin level	5.61 E3	0
Relationship between HBV positive individuals and alt level	2.10 E2	0
Relationship between HBV positive individuals and creatinine level	5.406	0.248
Relationship between HBV positive individuals and ferritin level	2.61 E2	0
Relationship between HCV positive individuals and ALT level	1.79 E2	0
Relationship between HCV positive individuals and creatinine level	16.65	0.002
Relationship between HCV positive individuals and ferritin level	2.53 E2	0

Discussion

In Pakistan, 7–9 million people are living with HBV with an approximate carrier rate of 3%–5%.²¹ Several seroprevalence studies have been conducted on blood transfusion populations from Peshawar, Rawalpindi, Abbottabad, Multan, Bahawalpur, Quetta, and Karachi, which depicted HBV prevalence rates of 2.51%, 1.9%, 3.3%, 1.55%, 4.93%, 2.69%, and 4.90%, respectively.^{22–29} The prevalence of HCV in the 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.³⁰ Prevalence of HCV in multitransfused thalassemia population of Pakistan is within the range of 34.8%–60%;^{31–38} perhaps these figures are significantly alarming among rest of the Asian countries. Shah et al (from India) reported that majority of blood transfusions were provided once per month to young patients of 0–5 years, but when the age of patients increased up to 20–25 years, the number of blood transfusions were increased up to four times per month.³⁹

Our study has also identified a similar trend among β -thalassemia patients of different age groups from the capital twin cities of Pakistan. Farooqi et al reported 2.28% prevalence of HBV in thalassemia patients, while our study

identified a prevalence rate of 3.05%.⁴⁰ This study shows that the prevalence of HCV is 55.73% in the thalassemia population of the capital twin cities of Pakistan; these results are slightly higher than our systematic review, which showed that the prevalence of HCV was 48.67% in multitransfused population of Pakistan.¹² We also confirmed the viral hepatitis status from the patient files. Most of the patients had ELISA or PCR reports in their files. Other important parameters associated with HBV and HCV prevalence in the thalassemia population of Pakistan have also been investigated. Our study has identified prevalence of HBV- and HCV-associated study variables such as age, sex, age at diagnosis of β -thalassemia type major, history of β -thalassemia in family, age at first blood transfusion, number of blood transfusions per month, pretransfusion hemoglobin count, quantity of blood provided, type of transfusion acquired (whole blood/blood cells), type of thalassemia (minor/major), hepatomegaly, splenomegaly, splenectomy, ALT count, serum creatinine level, serum ferritin level, chelation therapy, and use of deferoxamine. Up to date, no scientific study has been conducted in Pakistan depicting such valuable information regarding the aforementioned study variables and their association with HBV and HCV.

Depending on our study, on average, 25 blood transfusions are carried out for thalassemia patients. Our study has also identified that as the number of blood transfusions increased, increase in liver and spleen sizes was observed. Among HBV-positive patients, hepatomegaly and splenomegaly were found in 100% and 75% of cases, respectively. Thalassemia patients with normal liver and spleen were also investigated. However, these cases were observed only in younger children. As the age of patients was increased, the chances of patients with normal liver and spleen were decreased. Among HBV-infected patients, hepatomegaly was more frequently observed as compared to HCV-infected patients. Among HBV-infected patients, 62.5% individuals had 2 cm (12.5%), 3 cm (12.5%), 4 cm (12.5%), 5 cm (12.5%), and 7 cm (12.5%) increase in liver size, while 37.5% individuals had up to 9 cm increase in liver size. Maximum increase in liver size (ie, 10 cm) was observed in HCV-infected individuals as shown in Figure 1.

Our study demonstrated that the HCV-infected thalassemia patients had more chances of splenomegaly as compared with HBV-infected patients. But interestingly among HBV-infected patients, more severe cases of splenomegaly were observed, with most of the individuals who had splenectomy. More than 37% of HBV-infected persons had a 4-cm increase in spleen size. As compared to HBV-infected patients, a few HCV-infected patients had splenectomy. And most of the HCV-infected patients had splenomegaly. Maximum increase in spleen size, ie, 12 cm, was observed in HCV-infected persons.

Among all HBV- and HCV-infected thalassemia patients, the level of ALT was significantly higher than the normal range. On the other hand, the level of creatinine was very low as compared to the normal level. The level of ferritin in serum was found to be significantly high, which depicts that there was an increased proportion of iron in these patients. It is a well-known fact that when there is an increased accumulation of iron in the heart, it becomes much larger and beating becomes irregular. Later on, if the iron keeps on accumulating, it becomes unable to pump the blood. This is the main cause of death in iron overload thalassemia patients. The main advantage of using Desferal is that it protects the heart from iron. Intensive use of Desferal treatment can prevent serious heart problems in thalassemia patients.⁴¹ In thalassemia patients, the liver problems are usually caused due to iron overload or viral infection or both. Among HBV/HCV-infected thalassemia patients, the condition of the liver becomes worse with the passage of time. At the initial stage of viral infection, liver damage upregulates the average ALT level among patients, which provides a clue of viral hepatitis. Our study reported that the average ALT levels among HBV and HCV patients were 92.62 and 98 U/L, respectively, which is significantly higher than the normal range.

Our study showed high prevalence of HBV/HCV in β -thalassemia patients. There is a strong need to provide properly screened blood to multitransfused populations. In Pakistani societies, consanguineous marriages are very frequent

and have substantial association with increased prevalence of thalassemia. Policy makers should provide wider opportunities for the dissemination of awareness about risk factors associated with viral transmission among high-risk populations. Our study proposes the importance of nonfragmented, organized, and nonhospital-based nationally centralized hygienic blood transfusion setups. It provides valuable justifications for implementation of blood-safety laws in various public health sectors of Pakistan.

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Author Contributions

Conceived and designed the experiments: YW, US, UW, MA. Analyzed the data: US, YW, MA, UW, SA, MSA. Wrote the first draft of the manuscript: US. Contributed to the writing of the manuscript: YW, MA. Agree with manuscript results and conclusions: US, YW, MA, UW, SA, MSA. Jointly developed the structure and arguments for the paper: YW, US, UW, MA. Made critical revisions and approved final version: US, YW, MA, UW, SA, MSA. All authors reviewed and approved of the final manuscript.

REFERENCES

1. De-Franchis R, Hadengue A, Lau G, et al. EASL International Consensus Conference on hepatitis B consensus statement. *J Hepatol*. 2003;39:S3–S25.
2. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat*. 2004;11: 97–107.
3. Waheed Y, Rahat TB, Safi SZ, Qadri I. Epidemiological patterns and risk factors associated with hepatitis B virus in Pakistani population. *Asian Biomed*. 2010;4:547–554.
4. Hwang LY, Krammer JR, Troisi C, et al. Relationship of cosmetic procedures and drug use to hepatitis C and hepatitis B virus infections in a low-risk population. *Hepatology*. 2006;44:341–351.
5. World Health Organization. Hepatitis C WHO Fact sheet 164; 2000. Available at: <http://www.who.int/media/centre/factsheets/fs164/en/>
6. National Foundation for Infectious Diseases. 2012. Available at: <http://www.nfid.org/pdf/factsheets/hepbadult.pdf>
7. World Health Organization. World Health Statistics Geneva; 2009. Available at: http://www.who.int/gho/publications/world_health_statistics/EN_WHS08_Full.pdf
8. Waheed Y, Saeed U, Anjum S, Afzal MS, Ashraf M. Development of global consensus sequence and analysis of highly conserved domains of HCV NS5B protein. *Hepat Mon*. 2012;12:e6142.
9. Noorali S, Hakim ST, McLean D, Kazmi SU, Bagasra O. Prevalence of hepatitis B virus genotype D in females in Karachi, Pakistan. *J Infect Devel Count*. 2008;2:373–378.
10. Hakim ST, Kazmi SU, Bagasra O. Seroprevalence of hepatitis B and C genotypes among young apparently healthy females of Karachi-Pakistan. *Libyan J Med*. 2008;3:66–70.
11. Saeed U, Waheed Y, Manzoor S, Ashraf M. Identification of novel silent HIV propagation routes in Pakistan. *World J Virol*. 2013;2:136–138.
12. Waheed Y, Shafi T, Safi SZ, Qadri I. Hepatitis C virus in Pakistan. A systematic review of prevalence, genotypes and risk factors. *World J Gastroenterol*. 2009;15: 5647–5653.
13. Ahmed S, Saleem M, Modell B, Petrou M. Screening extended families for genetic hemoglobin disorders in Pakistan. *N Engl J Med*. 2010;347:1162–1168.



14. World Health Organization. Screening donated blood for transmission transmissible infections: recommendations; 2009. Available at: <http://www.who.int/bloodsafety/ScreeningTTI.pdf>
15. Waheed Y. Effect of interferon plus ribavirin therapy on hepatitis C virus genotype 3 patients from Pakistan: treatment response, side effects and future prospects. *Asia Pacific J Tropical Medicine*. 2015;8:85–89.
16. Waheed Y. Ledipasvir and sofosbuvir: interferon free therapy for hepatitis C virus genotype 1 infection. *World J Virol*. 2015;4:33.
17. Croagh CMN, Bell SJ, Desmond PV. Therapies for chronic hepatitis B: review of indications, outcomes and new tools for tailoring treatments to patients. *Antivir Antiretrovir*. 2014;6:2.
18. Khattak I, Khattak ST, Khan J. Heterozygous beta thalassemia in parent of children with beta thalassemia major. *Gomal J Med Sci*. 2006;4:52–56.
19. Weber JR, Kelley JH. *Health Assessment in Nursing*. 5th ed. Netherlands: Wolters Kluwer; 2013:491.
20. Torigian DA, Kitazono MT. *NETTER's Correlative Imaging: Abdominal and Pelvic Anatomy*. Amsterdam: Elsevier; 2013:56.
21. Ali M, Idrees M, Ali L, et al. Hepatitis B virus in Pakistan: a systematic review of prevalence, risk factors, awareness status and genotypes. *Virol J*. 2011;8:102.
22. Fayyaz M, Khan MA, Qazi MA, Chaudhary GMD, Ahmed G. Hepatitis B, C and HIV: sero-prevalence of infection in blood donors. *Profess Med J*. 2006;13:632–636.
23. Mujeeb SA, Mehmood K. Prevalence of HBV, HCV and HIV infections among family blood donors. *Ann Saudi Med*. 1996;16:702–703.
24. Asif N, Khokhar N, Ilahi F. Seroprevalence of HBV, HCV and HIV infection among voluntary non remunerated and replacement donors in northern Pakistan. *Pak J Med Sci*. 2004;20:24–28.
25. Ahmad J, Taj AS, Rahim A, Shah A, Rehman M. Frequency of hepatitis B and C in healthy blood donors of NWFP: a single centre experience. *J Post Med Inst*. 2004;18:343–352.
26. Khattak MF, Salamat N, Bhatti FA, Qureshi TZ. Seroprevalence of hepatitis B, C and HIV in blood donors in northern Pakistan. *J Pak Med Assoc*. 2002;52:398–402.
27. Ahmad F, Shah SH, Tariq M, Khan JA. Prevalence of hepatitis B carrier and HIV in healthy donors at Ayub teaching hospital. *Pak J Med*. 2000;39:91–92.
28. Jehangir W, Ali F, Shah Nawaz U, Iqbal T, Qureshi HJ. Prevalence of hepatitis B, C and HIV in blood donors of south Punjab. *Esculapio*. 2006;2:6–7.
29. Khan ZA, Aslam MI, Ali S. The frequency of hepatitis B and C among volunteer blood donors in Balochistan. *Hep Mon*. 2007;7:73–76.
30. Luby SP, Qamruddin K, Shah AA, et al. The relationship between therapeutic injections and high prevalence of hepatitis C infection in Hafizabad, Pakistan. *Epidemiol Infect*. 1997;119:349–356.
31. Bhatti FA, Amin M, Saleem M. Prevalence of antibody to hepatitis C virus in Pakistani thalassaemics by particle agglutination test utilizing C 200 and C 22-3 viral antigen coated particles. *J Pak Med Assoc*. 1995;45:269–271.
32. Moatter T, Adil S, Haroon S, Azeemuddin S, Hassan F, Khurshid M. Prevalence of hepatitis G virus in Pakistani children with transfusion dependent beta-thalassemia major. *Indian J Pathol Microbiol*. 1999;42:475–482.
33. Muhammad J, Hussain M, Khan MA. Frequency of Hepatitis B and Hepatitis C infection in thalassemic children. *Pak Pediatr J*. 2003;27:161–164.
34. Akhtar S, Moatter T. Hepatitis C virus infection in polytransfused thalassemic children in Pakistan. *Ind Pediatr*. 2004;41:1072–1073.
35. Younas M, Hassan K, Ikram N, Naseem L, Zaheer HA, Khan MF. Hepatitis C virus seropositivity in repeatedly transfused thalassemia major patients. *Int J Pathol*. 2004;2:20–23.
36. Burki MFK, Hassan M, Hussain H, Nisar YB, Krishan J. Prevalence of anti-hepatitis C antibodies in multiply transfused beta thalassemia major patients. *Ann Pak Inst Med Sci*. 2005;1:150–153.
37. Shah SMA, Khan MT, Zahour U, Ashfaq NY. Prevalence of hepatitis B and hepatitis C virus infection in multitransfused thalassaemia major patients in North West Frontier Province. *Pak J Med Sci*. 2005;21:281–284.
38. Hussain H, Iqbal R, Khan MH, Iftikhar B, Aziz S, Burki FK. Prevalence of hepatitis C in beta thalassaemia major. *Gomal J Med Sci*. 2008;6:87–90.
39. Shah N, Mishra A, Chauhan D, Vora C, Shah NR. Study on effectiveness of transfusion program in thalassemia major patients receiving multiple blood transfusions in Western India. *Asian J Transfus Sci*. 2010;4:94–98.
40. Farooqi JI, Farooqi RJ, Khan N. Mussarat: frequency of hepatitis B and C in selected groups of population in NWFP, Pakistan. *JPMI*. 2007;21:165–168.
41. Thalassaemic Free Pakistan, Section 4, Thalassaemic Associated with Abnormal Hemoglobin. Available at: <http://thalassaemic.com.pk/kb/section4.html>