

## SEROPREVALENCE OF HIV, HBV, AND HCV AMONG MULTI-TRANSFUSED BETA THALASSEMIA MAJOR PATIENTS IN A TERTIARY CARE HOSPITAL

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### Abstract

**Background:** Beta thalassemia major is a hereditary hematological disorder characterized by severe chronic anemia that necessitates lifelong, regular blood transfusions for survival. Although transfusion therapy is life-saving, it exposes patients to a significant risk of transfusion-transmitted infections (TTIs), including hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). This risk is further amplified in developing countries due to variability in donor screening practices.

**Objective:** The present study aimed to determine the seroprevalence of HBV, HCV, and HIV among multi-transfused patients with beta thalassemia major in a tertiary care hospital. Additionally, the study assessed the association of these infections with demographic characteristics and transfusion history.

**Methodology:** A cross-sectional study design was employed involving patients diagnosed with transfusion-dependent beta thalassemia major. Blood samples were collected and tested for HBsAg, anti-HCV antibodies, and HIV antibodies using standard serological methods. A structured questionnaire was used to obtain data on patient demographics, transfusion frequency,

and clinical history. The collected data were analyzed to determine prevalence rates and to evaluate variations in infection distribution.

**Results:** The study found that transfusion-transmitted viral infections remain a significant health burden among multi-transfused thalassemia patients. HCV was identified as the most prevalent infection, followed by HBV, while HIV positivity was rare. A higher prevalence of infections was observed in older patients and in those who had received a greater number of blood transfusions, indicating a positive correlation between cumulative transfusion exposure and infection risk.

**Conclusion:** Despite advancements in blood safety measures, transfusion-transmitted infections continue to pose a serious threat to patients with beta thalassemia major. The findings highlight the urgent need for improved donor screening strategies, including the implementation of nucleic acid testing (NAT), alongside strengthened preventive policies to reduce infection transmission and improve long-term patient outcomes.

## INTRODUCTION

Thalassemia is a hereditary hemoglobin disorder characterized by defective synthesis of globin chains, leading to chronic hemolytic anemia.  $\beta$ -thalassemia major is the most severe form and is inherited in an autosomal recessive pattern, resulting in reduced or absent  $\beta$ -globin production and ineffective erythropoiesis. This condition causes severe anemia requiring lifelong regular blood transfusions for survival. Pathophysiologically, imbalance between alpha and  $\beta$  globin chains leads to hemolysis, bone marrow expansion, and complications such as bone deformities and organ dysfunction. In severe cases, iron overload and chronic anemia contribute significantly to morbidity and mortality. [1]

A major complication of repeated transfusion therapy in  $\beta$ -thalassemia major is the risk of transfusion-transmitted infections (TTIs), particularly hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) [2]. These infections are primarily transmitted through infected blood products and remain a major cause of morbidity in transfusion-dependent patients. HCV is especially prevalent among multi-transfused patients due to its historical transmission before effective screening practices, while HBV remains endemic in resource-limited

settings despite vaccination programs. HIV transmission through transfusion has decreased significantly due to improved screening, but risk persists in low-resource healthcare systems [3]. In developing countries such as Pakistan, TTIs remain a significant public health issue among  $\beta$ -thalassemia patients due to high transfusion frequency and variable blood screening quality. HCV and HBV are reported to be highly prevalent, whereas HIV remains comparatively rare. The burden of transfusion-related infections contributes substantially to long-term complications, alongside iron overload and organ damage caused by repeated transfusions. Additionally, thalassemia patients suffer from multiple systemic complications including splenomegaly, endocrine dysfunction, bone deformities, psychosocial stress, and reduced quality of life [4]. Diagnosis and management of thalassemia involve hematological tests such as complete blood count (CBC), hemoglobin electrophoresis, and high-performance liquid chromatography (HPLC), along with molecular genetic testing for confirmation. Preventive strategies include carrier screening, genetic counseling, and prenatal diagnosis to reduce disease burden [5]. Advances in blood safety, including ELISA and nucleic acid amplification testing (NAT), have improved detection of infectious agents in donor blood, although limitations persist in many regions. Treatment includes regular transfusions, iron chelation, splenectomy in selected cases, and emerging gene therapy approaches. Despite these advances, transfusion safety remains a critical concern, particularly in resource-limited settings, necessitating further research into the prevalence of TTIs in multi-transfused  $\beta$ -thalassemia patients [6].

### Literature Review

Biswas et al. (2016) conducted a large study involving 1711 multi-transfused thalassemia major patients in Eastern India to evaluate the seroprevalence of transfusion-transmitted infections (TTIs) and associated socioeconomic factors. The study reported that HCV was the most common infection (18.70%), followed by HIV (3.74%) and HBV (3.33%). Most patients belonged to low socioeconomic backgrounds, and awareness regarding TTIs was very limited. The study highlighted that inadequate awareness, poor socioeconomic conditions, and insufficient donor

screening significantly contributed to the high burden of TTIs among thalassemia patients, emphasizing the need for strict blood screening and public health education [40].

Gugnani et al. (2019) assessed the prevalence of TTIs among 126 multi-transfused  $\beta$ -thalassemia patients in North India and found an overall seroreactivity rate of 14.28%. HCV was the most prevalent infection (13.4%), while HBV was rare (0.79%) and no HIV cases were detected. The study also demonstrated a strong association between infection rates, age, and number of transfusions, with higher prevalence in patients receiving more than 250 transfusions. The authors recommended strict pre-transfusion screening and early HBV vaccination to reduce infection risk among transfusion-dependent patients [41].

Mishra et al. (2020) evaluated 196  $\beta$ -thalassemia patients using both ELISA and nucleic acid testing (NAT) and found a high seroprevalence of HCV (51.1%), followed by HIV (3.1%) and HBV (1.5%). Molecular testing revealed even higher infection detection rates, including HCV RNA in 33.7% and HBV DNA in 2.5% of patients, indicating missed infections by conventional screening methods. The study also identified co-infections in several patients, highlighting the importance of NAT-based screening for improving transfusion safety and early detection of viral infections [43].

Mahmood et al. (2022) conducted a study in Azad Jammu and Kashmir, Pakistan, on 224 multi-transfused thalassemia patients and reported a high prevalence of HCV (28.1%) and HBV (5.36%), while no significant HIV cases were detected. The study found a higher infection rate in older patients and those receiving frequent transfusions. It emphasized that despite existing screening methods, transfusion-transmitted infections remain a major concern in Pakistan and stressed the need for improved donor screening, vaccination programs, and enhanced transfusion safety protocols.[49].

### Material and Methods

The present study was a cross-sectional descriptive study designed to evaluate seroprevalence and related variables in  $\beta$ -thalassemia major patients. It was conducted over a period of four months after approval of the synopsis. The study aimed to assess transfusion-transmitted infections and

associated factors in a defined patient population receiving regular transfusions. The cross-sectional design allowed data collection at a single point in time, enabling estimation of prevalence and comparison across different demographic and clinical variables. This design is appropriate for identifying associations between transfusion history and infection status without manipulating study variables, ensuring a practical and ethically feasible approach in a clinical setting.

The study was conducted at Sundas Foundation Diagnostic Laboratory and Thalassemia Center, along with Children Hospital Lahore. These institutions provide specialized care and regular transfusion services to thalassemia patients, making them suitable settings for data collection. The study population included patients diagnosed with  $\beta$ -thalassemia major who attended these centers for routine transfusions and follow-up care. A total sample size of 277 was determined using a standard proportion formula with  $Z = 1.96$ ,  $P = 0.5$ , and margin of error 0.05, followed by finite population correction based on an estimated population of 1000. Simple random sampling was applied to ensure equal selection probability.

Patients included in the study were selected based on specific inclusion and exclusion criteria. Inclusion criteria comprised confirmed diagnosis of  $\beta$ -thalassemia major, age between 1 and 35 years, and requirement of frequent blood transfusions. Patients with pre-existing hepatitis B or hepatitis C infection prior to transfusion were excluded to avoid confounding results. Additionally, individuals with other chronic conditions such as active liver disease or endocrine disorders unrelated to thalassemia, as well as those with acute illnesses interfering with data collection, were excluded. Ethical approval was obtained, informed consent was taken, confidentiality was ensured, and participants were informed about voluntary withdrawal rights.

Data collection involved obtaining 5 ml venous blood samples under aseptic conditions for screening hepatitis B virus, hepatitis C virus, and HIV. Serum was separated using centrifugation and analyzed through ELISA for HBsAg, anti-HCV, and anti-HIV antibodies. In some cases, rapid immunochromatographic test kits were used for preliminary screening, followed by ELISA confirmation to improve diagnostic accuracy. Patients also underwent routine transfusions from screened blood units obtained from institutional blood banks. Data were collected using structured

questionnaires and clinical examinations, and statistical analysis was performed using SPSS version 24, applying descriptive statistics and chi-square tests where appropriate.

## Results

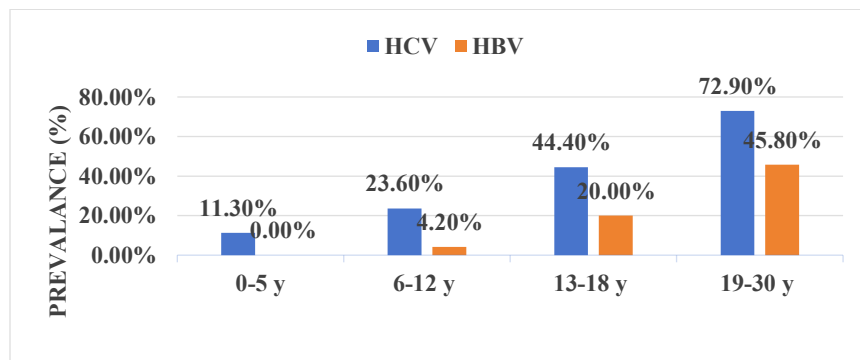
The study included a total of 277 patients diagnosed with  $\beta$ -thalassemia major. The age of participants ranged from 1 to 35 years, with a mean age of 13.9 years, and the majority belonged to the pediatric age group. The study population consisted of 160 males (57.8%) and 117 females (42.2%). Overall screening results indicated a considerable burden of transfusion-transmitted infections among participants. A gender-based comparative analysis revealed variation in infection distribution between males and females, particularly for hepatitis B virus (HBV) and hepatitis C virus (HCV).

### Gender based Distribution

Gender	HCV Positive(%)	HBV positive (%)
Male (N=160)	37.50%	21.90%
Female (N=117)	47.90%	17.90%

Gender-wise analysis showed that females had a higher prevalence of HCV infection (47.9%) compared to males (37.5%). In contrast, males demonstrated a slightly higher HBV positivity rate (21.9%) than females (17.9%). These findings suggest a differential pattern of viral exposure between genders, although both groups showed substantial infection rates. The results indicate that transfusion-related viral infections remain a major concern in both male and female thalassemia patients, highlighting the need for consistent preventive strategies across all demographic groups. Age-wise distribution demonstrated a strong association between increasing age and higher infection rates. HCV prevalence was lowest in children aged 0–5 years (11.3%) and progressively increased with age, reaching 72.9% in the 19–30 years group. Similarly, HBV infection was absent in the 0–5 years age group but increased significantly to 45.8% in the 19–30 years group. These findings indicate that prolonged exposure to repeated blood transfusions over time contributes significantly to higher infection rates in older patients.

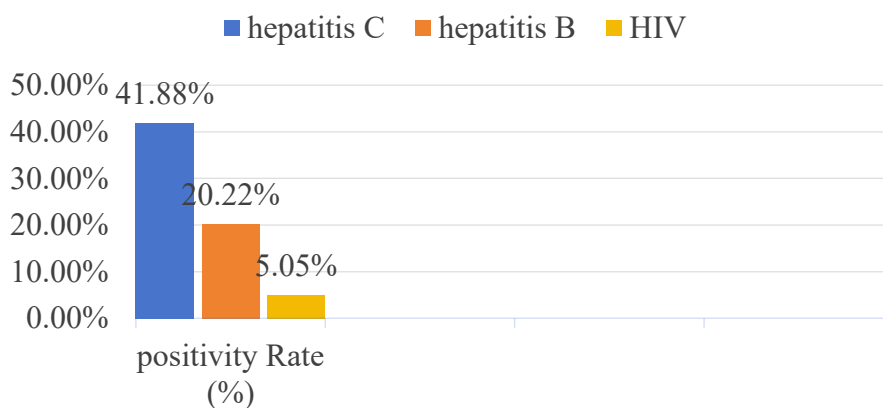
Viral positivity by age category



Prevalence analysis showed that HCV was the most common infection, affecting 41.8% (n=116) of patients, followed by HBV at 20.2% (n=56), and HIV at 5.0% (n=14). These results confirm that HCV represents the dominant transfusion-transmitted infection in the study population. The presence of HBV and HIV, although lower, still indicates a significant public health concern. The high overall burden of viral infections emphasizes the need for improved screening and preventive measures in transfusion-dependent patients.

Prevalence of Transfusion Transmitted Infections (N=277)

Prevalence of transfusion transmission infections



The findings highlight a significant prevalence of transfusion-transmitted infections among  $\beta$ -thalassemia major patients, with increasing infection rates observed in older age groups. The results strongly suggest the need for advanced blood screening methods such as nucleic acid testing

(NAT), strict donor selection, and universal HBV vaccination from early childhood. Regular monitoring, early diagnosis, and timely antiviral management are essential to prevent long-term complications such as liver cirrhosis and hepatocellular carcinoma in this vulnerable population.

### Discussion

The present study assessed the prevalence of transfusion-transmitted infections (TTIs)—Hepatitis C virus (HCV), Hepatitis B virus (HBV), and Human Immunodeficiency Virus (HIV)—among 277 patients with  $\beta$ -thalassemia major. The findings demonstrated a substantial combined burden of viral infections, with HCV identified as the most prevalent pathogen (approximately 41.8%), followed by HBV (20.2%) and HIV (5.0%). This pattern is consistent with several regional and international studies, which also report HCV as the leading transfusion-related infection among thalassemia patients due to the absence of an effective vaccine and the risk of transmission during the serological window period. Compared to HBV, which has a preventive vaccine, HCV continues to represent a persistent challenge in transfusion-dependent populations.

The age-wise analysis in this study showed a clear increasing trend in infection prevalence with advancing age, particularly for HCV and HBV. HCV positivity increased markedly from 11.3% in children under 5 years to 72.9% in the 19–30-year age group, indicating cumulative exposure to multiple blood transfusions over time. Similar age-related trends have been reported in previous studies, where longer transfusion history was strongly associated with higher infection risk. This supports the concept that both duration and frequency of transfusions are key determinants in the acquisition of TTIs in thalassemia patients.

Gender-based findings revealed only minor variations in infection distribution, with slightly higher HCV prevalence among females and marginally higher HBV prevalence among males. However, these differences were not statistically significant, aligning with previous literature suggesting that gender is not a major independent risk factor for transfusion-transmitted infections. Instead, the overall infection burden is more strongly influenced by cumulative transfusion exposure, quality of donor screening, and adherence to infection control protocols rather than patient sex.

Overall, the study highlights persistent gaps in transfusion safety despite improvements in screening practices. The detection of HIV cases, although relatively low, indicates potential limitations in current serological screening methods, particularly during the window period. The findings strongly support the implementation of more sensitive diagnostic techniques such as nucleic acid testing (NAT), alongside strict donor selection criteria, universal HBV vaccination, and regular monitoring of transfusion-dependent patients. Strengthening these preventive strategies is essential to reduce long-term complications, improve patient outcomes, and minimize the burden of TTIs in  $\beta$ -thalassemia major populations.

### Conclusion

Despite significant improvements in blood transfusion services and screening technologies, transfusion-transmitted infections (TTIs) such as Hepatitis C virus (HCV), Hepatitis B virus (HBV), and Human Immunodeficiency Virus (HIV) continue to pose a serious health burden for patients with  $\beta$ -thalassemia major. The findings of this study highlight that the most important determinant of infection risk is cumulative blood exposure rather than disease subtype, with a clear association between increasing age, longer transfusion history, and higher likelihood of seropositivity. This pattern reflects repeated exposure to donor blood during viral window periods, where infections may not be detected by conventional screening methods. The persistence of TTIs also indicates gaps in current transfusion safety systems, including inconsistent implementation of screening protocols and suboptimal vaccination coverage, particularly for HBV. To address these challenges, a comprehensive preventive approach is required, including the adoption of highly sensitive nucleic acid testing (NAT), universal HBV immunization with booster monitoring, and strict adherence to donor selection and blood bank quality standards, ultimately improving patient survival and quality of life.

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