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Original Article

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Predictive Biomarker of Hepatitis B and C Infection among Thalassemia Patients in Faisalabad, Punjab, Pakistan

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Abstract

Background: Transfusion-dependent β-thalassemia patients are particularly susceptible to transfusion-transmitted infections, especially hepatitis B virus (HBV) and hepatitis C virus (HCV), owing to lifelong dependence on repeated blood transfusions. Chronic viral hepatitis, together with transfusion-related iron overload, contributes substantially to progressive liver injury and adverse hematological outcomes. This study investigated the association between HBV and HCV infections and biochemical as well as hematological parameters in transfusion-dependent β-thalassemia patients and identified significant predictors of hepatic involvement. **Methods:** A cross-sectional analytical study was conducted among transfusion-dependent β-thalassemia patients attending a specialized thalassemia care center in Faisalabad, Punjab, Pakistan. Participants were categorized into four groups: non-infected, HBV-infected, HCV-infected, and HBV/HCV co-infected. Demographic characteristics, clinical history, biochemical markers including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum bilirubin, together with hematological indices, were recorded. Statistical analyses were performed using SPSS version 26. Descriptive statistics, correlation analysis, chi-square tests, independent-samples t-tests, and logistic regression analyses were employed to identify significant predictors of hepatitis infection. **Results:** Hepatitis infection was significantly associated with an increased number of blood transfusions. Patients with HBV, HCV, and particularly HBV/HCV co-infection demonstrated significantly elevated AST, ALT, and serum bilirubin concentrations compared with non-infected patients ($p < 0.01$). Positive correlations were observed between transfusion frequency and liver enzyme levels. Significant alterations were also identified in hematological indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW), whereas total white blood cell counts showed no statistically significant differences. Logistic regression analysis identified the cumulative number of transfusions, AST, ALT, serum bilirubin, and hepatitis infection status as independent predictors of hepatic involvement, with HCV exhibiting the strongest association. **Conclusions:** Transfusion-dependent β-thalassemia patients remain at substantial risk of HBV and HCV infections despite advances in blood safety practices. Viral hepatitis significantly impairs liver function and alters hematological profiles, emphasizing the importance of rigorous donor screening, regular biochemical monitoring, timely antiviral management, and strengthened infection prevention strategies to reduce disease burden and improve long-term clinical outcomes.

Introduction

Thalassemia is one of the most common inherited monogenic disorders worldwide and represents a major public health challenge, particularly in regions where malaria has historically been endemic. The disease results from mutations in the β-globin (HBB) gene on chromosome 11, leading to reduced (β^+) or absent (β^0) β- globin chain synthesis. The resulting imbalance between α- and β-globin chains causes ineffective erythropoiesis, chronic hemolytic anemia, oxidative stress, and premature destruction of erythroid precursors, ultimately leading to growth

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retardation, skeletal deformities, hepatosplenomegaly, and lifelong transfusion dependence [31,48,54,17].

Globally, more than 80–90 million individuals are carriers of β-thalassemia mutations, with the highest prevalence reported in South Asia, Southeast Asia, the Mediterranean region, and the Middle East. Approximately 40,000–60,000 children are born annually with transfusion-dependent β-thalassemia, particularly in low- and middle-income countries where carrier screening programs remain limited [21,16,23]. Pakistan is among the countries most affected, with an estimated 9–12 million carriers and more than 100,000 patients living with transfusion-dependent β-thalassemia. The high prevalence is largely attributed to frequent consanguineous marriages and inadequate national screening programs [5,40,4,12].

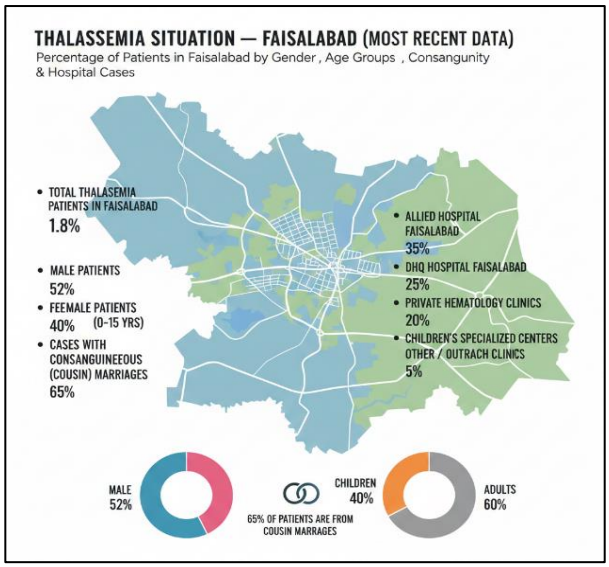

Figure 1: Prevalence of Thalassemia in Faisalabad Region, Punjab Pakistan. The total percentage of thalassemia patients in Faisalabad and percentage of disease by gender, age, consanguinity and hospital cases

Regular red blood cell transfusions remain the cornerstone of treatment for β-thalassemia major, maintaining adequate hemoglobin levels and improving survival. However, lifelong transfusion therapy predisposes patients to iron overload and transfusion-transmitted infections (TTIs), particularly hepatitis B virus (HBV) and hepatitis C virus (HCV). Iron accumulation promotes oxidative stress and progressive hepatic injury, while chronic viral hepatitis further accelerates liver fibrosis, cirrhosis, and hepatocellular carcinoma [48,17,40].

According to the World Health Organization, approximately 296 million people live with chronic HBV infection and 58 million with chronic HCV infection worldwide [55]. Pakistan remains a high-burden country, with an estimated HBV prevalence of approximately 2.5% and HCV prevalence approaching 5% in the general population, while substantially higher infection rates have been reported among transfusion-dependent β-thalassemia patients because of repeated blood transfusions [39,11,52].

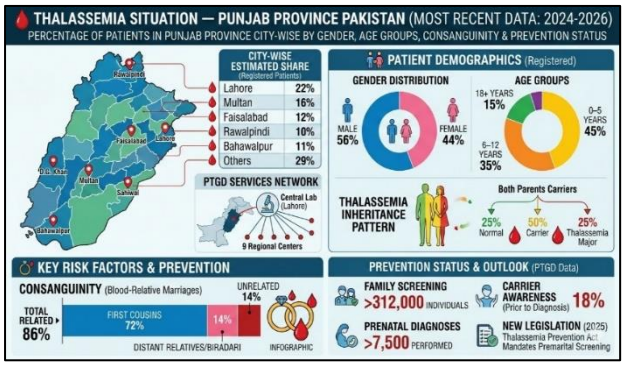

Figure 2: Prevalence of Thalassemia in Punjab Province of Pakistan. The total percentage of Thalassemia patients in Pakistan and percentage of disease by gender, age, consanguinity

Chronic HBV and HCV infections progressively impair liver function through persistent inflammation, hepatocyte injury, fibrosis, and cirrhosis. Consequently, liver function biomarkers including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum bilirubin serve as important indicators of hepatic damage and disease progression. In patients with transfusion-dependent β-thalassemia, viral hepatitis and iron overload frequently coexist, resulting in synergistic liver injury and deterioration of hematological status [37,25,15].

Although several studies have investigated hepatitis prevalence among β-thalassemia patients, comprehensive evidence linking HBV and HCV infections with biochemical and hematological alterations in Pakistani patients remains limited. Identifying reliable biomarkers associated with viral hepatitis may facilitate early diagnosis, improve clinical monitoring, and support timely therapeutic interventions. Therefore, the present study aimed to evaluate the association of HBV and HCV infections with liver function biomarkers and hematological parameters among transfusion-dependent β-thalassemia patients in Faisalabad, Punjab, Pakistan, while identifying significant predictors of hepatic involvement.

Review of the Literature

Sana et al. [59] In 2026 investigated the possibility of anomalies in β-thalassemia major patients who had received many blood transfusions, as well as any relations between these liver enzymes and serum ferritin. Researchers from the Pathology Department at Multan's Institute of Child Health and The Children's Hospital performed a cross-sectional study between 2024 and 2025. The study included children with β-thalassemia major, the age range of which was 2 to 14, who were undergoing chelation therapy and required regular blood transfusions. By using a uniform performa, we documented demographic information, transfusion frequency, chelation compliance, and laboratory findings. Levels of ALT > 35 U/L and AST > 45 U/L were considered abnormal for the liver. Patients were divided into three groups based on their blood ferritin levels: 1000-2000 ng/mL, 2001-3000 ng/mL, and >3000 ng/mL. This was done to evaluate the correlation between serum ferritin levels and abnormal liver enzymes. Of the 107 people that were enlisted, the average age ranged from four to nine years old. There were 70 men and 37 women, or 65.4%

and 34.6% respectively. Nearly all cases (94/112) had abnormal liver enzymes. Abnormal liver enzymes were linked to elevated blood ferritin levels (p-value = 0.005) & low adherence to chelation treatment (p-value <0.001). In all ferritin groups, there were elevated liver enzymes (ALT and AST) with p-values less than 0.001.

A 2025 review reported that thalassemia remains one of the most common inherited blood disorders worldwide [52]. Due to the aberrant ratio of α- and β-globin chains caused by the abnormality, erythropoiesis is unsuccessful and iron absorption is enhanced. This has a significant imbalance of α and β globin chains. As a result, there is an excess of iron in transfusions and the risk of hepatitis B and C and other transfusion-transmitted viral diseases rises. As a result of thalassemia, patients are at increased risk for developing liver fibrosis, cirrhosis, and hepatocellular carcinoma, all of which are serious health complications [42].

In 2025, we aimed to identify and catalog all unknown, as well as their possible negative effects, estimate their frequency, and give a comprehensive molecular spectrum. Using direct DNA sequencing of the β-globin gene, 60 significant β-thalassemia patients who sought therapy at Jeen Hospital in Duhok between August 2024 and February 2025 were evaluated. We looked at forty sequence chromatograms that were of good quality. Ten exonic and sixteen intronic mutations were among the 26 overall variations identified. The benign variations that were most common were IVSII-16 G>C (80%) and IVS II-666 C>T (77.5%), while the second most common variation was Cd2 T>C [HBB: c.9 T>C, 62.5%]. There were pathogenic mutations in the exonic regions of the following genes: one intronic (IVS I-129 +C Ins) variations and three exonic variants were found. Most of the variations were anticipated to be harmful or at least likely pathogenic, given the predicted functional effect, the actual frequency, and the location of the variations. Research on the molecular basis of β-thalassemia in Duhok patients: There is a significant frequency of compound heterozygosity and genetic variation in the β-globin gene.

A 2025 study investigated the frequency and mutational spectrum of the β-thalassemia trait in the districts of Haripur and Abbottabad to better inform preventive strategies in northern Pakistan [27]. Confirmed carriers accounted for 28% (n=64) of the 228 community members surveyed (95% CI: 22-34%). 59% of the subjects (n=38) had the IVS I-5 (G → C) mutation, 23% had the FSC 8/9 (+ G) mutation, and 17% were compound heterozygotes for both variants. This was confirmed by ARMS-PCR screening for five prevalent HBB mutations. Nothing about the 619-bp deletion, Cd 41/42 (-TTCT), or IVS I-1 (G →.T) was found. Abbottabad had a much greater carrier prevalence (42.9% vs. 13.2%, p <0.0001) than Haripur. Due to the high carrier rate and existence of compound heterozygotes, which were exceeds the national estimate of 5-8% in Pakistan. Additionally, there is a concentrated genetic load.

A 2025 study aimed to determine the prevalence of hepatitis C virus (HCV) infection and identify the associated risk factors among patients with β-thalassemia [59]. From September 2020 through December 2020, researchers from Lahore, Pakistan's Sundas Foundation and Noor Thalassemia Foundation conducted this cross-sectional study. Demographic and clinical information was gathered using a standardized questionnaire. Indirect ELISA was used to determine whether serum samples contained anti-HCV antibodies. In the study, which was conducted using SPSS version 17, t-tests and chi-square tests were utilized for data representation. There was a 17.1% median age. Of the total participants, 43 were male (53.7% of the total) and 37 were female (46.3% of the total). Age (p=0.001), surgical history (p=0.002), dental treatments (p=0.003), and higher monthly transfusion frequency or number of units (p=0.034) were all significantly associated with HCV prevalence. It was found that 11.25 percent of the population had HCV. Transfusion exposure was shown to be the primary risk factor among beta-thalassemia patients in Lahore, where the study found a moderate prevalence of HCV. Reducing HCV transmission in this susceptible population requires improved donor screening in conjunction with stringent infection control protocols.

A 2025 study assessed the relationship between serum ferritin levels and liver iron concentration in patients with β-thalassemia [30]. 2025 From July to December 2024, a cross-sectional analytical investigation was carried out using a non-probability sequential sampling technique on 101 patients with beta thalassemia major who attended Shahida Islam Medical College and Hospital in Lodhran. Participants had to be at least 15 years old and have a history of blood transfusions to be considered for the study. Infections and congenital or valvular heart defects were considered as exclusion criteria for the trial. People who did not follow the recommended chelation regimen or who exhibited persistently disruptive behavior were also not included in the study. We used Spearman's correlation to look for a connection between ferritin levels in the blood and iron levels in the liver as determined by T2 MRI. It was deemed significant if the p-value was less than 0.05. Magnetic resonance imaging (MRI) measurements of iron overload in the liver showed a negative association with serum ferritin levels in this study of 101 people with thalassemia major (r=-0.25, p=0.03). A significant amount of iron in the liver was the most often observed finding (41.58%). On the other hand, as iron load increased, serum ferritin and AST levels rose sharply. When comparing the groups for the other liver function parameters, no statistically significant changes were found. When evaluating iron load in the liver, MRI proved to be more accurate than blood ferritin alone. The results showed that in thalassemia major patients, hepatic iron overload as measured by T2-weighted MRI was substantially linked with blood ferritin levels.

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A 2025 study compared liver and kidney function among children with β-thalassemia major (BTM), β-thalassemia trait (BTT), and healthy controls, and investigated the potential protective role of ferritin against iron-related tissue damage in patients with BTT [9]. In this case-control study, 93 male children (ranging in age from 1 to 12 years) from Mosul, Iraq's Ibn Al-Atheer and Ibn Sina Hospitals participated. The children were split into three groups: those who received BTM (31 total), those who received BTT (31 total), and a healthy control group (31 total). Bilirubin, ferritin, urea, creatinine, uric acid, and DBIL were the biomarkers for the liver and kidneys that were tested in the serum. A significance level of P-value < 0.05 was used in the statistical analysis conducted using SPSS (version 26.0). The BTT and control groups did not differ significantly in terms of ALP, AST, urea, and uric acid levels; however, the BTM group exhibited significantly higher values as compared to the control group. The control group had lower levels of ferritin, creatinine, TBIL, TBIL, and DBIL compared to the BTM and BTT groups. With the exception of TBIL, it was seen that the BTM group outperformed the BTT group across the board. Also, in the BTM group, ferritin and ALP were highly correlated, but in the BTT group, ferritin and creatinine were strongly correlated, negative. BTM caused serious harm to the kidneys and liver. When tested with BTT, creatinine, ferritin, ALT, TBIL, and DBIL were markedly elevated. Iron toxicity was likely indicated by the ferritin-ALP relationship in BTM, while a potential protective mechanism was suggested by the negative correlation with creatinine in BTT. There has to be additional investigation into the ferritin toxicity vs protective threshold.

A 2024 study determined the prevalence of hepatitis C virus (HCV) infection among multi-transfused patients with thalassemia in the twin cities of Pakistan and identified the associated risk factors [37]. In this clinical trial, 262 patients with beta thalassemic syndrome who had received many transfusions were recruited from the twin city of Islamabad, Pakistan's capital. The following lab tests were part of the investigation: hepatitis C virus, ALT, serum creatinine, hepatomegaly, splenomegaly incidence, and splenectomy. Hepatitis C virus infections were rather common, with a prevalence incidence of 55.73 percent. Among patients aged 20 and up, the infection frequency was a staggering 100%. Patients with thalassemic HCV had an average creatinine level of 0.39 mg/dL and an average ALT level of 98 U/L. An enlarged liver, measuring an average of 4.33 cm, was described as hepatomegaly in 82.20% of HCV-positive thalassemic individuals. An enlarged spleen (4.46 cm) and splenomegaly were evident in 67.12% of the HCV-positive thalassemic individuals on average. In 15.75 percent of cases, splenectomy was found. Among Pakistan's thalassemic population, the prevalence of HCV is extremely high.

A 2024 study investigated the potential association between serum ferritin levels and liver enzyme abnormalities in patients with β-thalassemia major [21]. During the academic year 2021–2022, researchers were at Islamabad Medical and Dental College and the attached Akbar Niazi Teaching Hospital. A total of 135 patients with beta thalassemia major that had blood transfusions had their serum ferritin, AST, ALT, and bilirubin levels estimated. Using SPSS version 20, the data were examined. The categorical variables were quantified using percentages and frequencies. To examined the correlation between serum ferritin and liver enzymes. A significance criterion of $p < 0.05$ was established. The link between ferritin and LFTs was determined using Pearson's correlation coefficient. There were 51% men and a wide age range of 7–30 years old in the sample. With a mean of 6062.61 + 3641.79 ng/ml, ferritin (p =0.366). In comparison to patients with ferritin levels below 2500 ng/ml, patients with ferritin levels above 5000 ng/ml exhibited noticeably elevated levels of AST, ALT, and bilirubin. There was a modest relationship between serum ferritin and AST and ALT, but a strong relationship with serum bilirubin (r=0.350) and AST (r=0.532) were also observed. Iron buildup and liver damage were both increased. In order to reduce mortality and morbidity associated with thalassemia, it is important to monitor liver function tests (LFTs) regularly. If liver damage is detected, it can be treated by increasing chelation therapy.

The first study from Pakistan investigating potential biomarkers for hepatitis C virus (HCV) infection has been reported [28]. Researchers in the US tracked 13,348 individuals who tested positive for HCV from 2018 to 2022. Before the COVID-19 pandemic, the prevalence of HCV was 30% in 2018 and 2019. The following results were recorded in 2018: abnormal ALT (91%), AST (63%), GGT (67%), Bili T (28%), HB (62%), HBA1C (15%), CREAT (25%), PT (15%), aPTT (15%), and AFP (64%). In 2019, the percentage of HCV infected individuals with elevated ALT, AST, GGT, Bili T, HB, and AFP was 74.47%, 63.54%, 70.24%, 24.71%, 8.77%, and 75%, respectively. In 2020, the prevalence of HCV remained 25%. ALT rose 65.17 percent, AST 64.20 percent, GGT 68.75 percent, Bili T 31.25 percent, HB 20.97 percent, CREAT 4.65 percent, and AFP 73.68%. In 4.41 percent of cases, CAT scans revealed liver abnormalities; among these, 14.8 percent were moderate, 40.74 percent were intermediate, and 44.44 percent were severe. Uncontrolled diabetes affected 85.71 percent of the individuals. In 2020, the rate of new HCV infections was 27.1%. The following levels were found to be abnormal: ALT (73.86%), AST (50.6%), GGT (67.95%), Bili T (28.21%), HB (20%), CREAT (5.8%), and AFP (82.14%). A total of 56.06 percent of the time in 2022, the following blood tests came back abnormal: ALT, AST, GGT, Bili T, HB, HBA1C, CREAT, AFP, and 43.48 percent of the time. Liver cat scans showed anomalies of 7.46%. 30.36% were considered moderate, 42.86% were considered severe. Uncontrolled diabetes affected 83.33 percent of the participants in 2021 and 2022. From the ages of 1 to 18, 582 patients with transfusion-dependent thalassemia were included in this retrospective study. Albumin, serum ferritin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were the enzymes that were tested annually. The correlations of ferritin trends with liver enzymes were investigated using linear regression and correlation analysis. After starting at 1820 ± 960 ng/mL, the average ferritin level was increased to 4500 ± 1900 ng/mL at year 5, indicating an escalating iron excess. While albumin levels decreased slightly, AST and ALT levels rose over the

course of the follow-up. Annually, ferritin showed a positive correlation with AST (r = 0.675, P < 0.01) and ALT (r = 0.607, P < 0.01), but no correlation with albumin (r = -0.143, P = 0.153). Patients with greater ferritin levels were shown to have higher AST and ALT levels (P < 0.05) when other variables were accounted for in the model through the interaction term. When ferritin levels grow, the risk of liver injury was increases in children with thalassemia who need transfusions often. Liver function may be preserved with more stringent management of iron excess.

Variations in the iron and coagulation profiles of individuals with β-thalassemia have been evaluated [36]. Fifty children diagnosed with beta-thalassemia and fifty healthy controls participated in the study after their parents gave their approval. Haematological and hemostatic indices, as well as serum ferritin, were assessed in the blood samples. According to the results of the laboratory tests, 56.0% of the patients had an extended activated partial thromboplastin time (aPTT), 54.0% had an extended prothrombin time (PT), and 43.5% of the patients had thrombocytopenia. In comparison to the control group, the expected coagulation factor activity levels were significantly lower. Total platelet count and serum ferritin level were found to be adversely connected, while PT and aPTT were favorably correlated. In thalassemia patients, abnormal haemostatic characteristics are linked to elevated serum ferritin levels. In order to help thalassemia patients effectively, it is necessary to monitor their serum ferritin level regularly.

The prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections and their associated risk factors among residents of Badin City, Pakistan have been investigated [39]. Using immunochromatographic techniques, 767 individuals were tested for hepatitis B and C viruses. Quantitative Polymerase Chain Reaction (PCR) was used to retest the blood samples of individuals who tested positive for hepatitis to determine viral load. Hepatitis cases totaled 767. There was a higher prevalence of HCV than HBV in this group. Nonetheless, men felt the effects more strongly than women. Factors that increased the risk of hepatitis B and C exposure among the patients in our investigation are detailed in Table 2. Among the 767 individuals who took part, 473 (61.7%) had their beards trimmed at a salon or barbershop. Reusing syringes was done by 358 (46.7%) people who had hepatitis. Of the patients, 66 (8.6%) were found to have a substance addiction. Just 73 out of a total of 95% of patients had never received a blood transfusion before. Of the female hepatitis patients, 195 (68.1%), 245 (85.7%), and 240 (83.9%) reported having ear pricks, nose piercings, or obstetrical procedures, respectively.

Advances in the management of thalassemia have substantially improved survival and quality of life, enabling many affected individuals to live into older age [35]. Careful transfusion therapy can do this by reducing the likelihood of iron accumulation and the complications that can arise from inefficient erythropoiesis. A frequent transfusion regimen will inevitably lead to hemosiderosis. Iron chelation should be sufficient to keep reactive iron forms within the usual range throughout life, protecting organs from injury. Genomic editing and non-curative erythropoiesis modulators are two new possibilities for treating transfusion-dependent beta thalassemia. Researchers have examined two gene treatments for thalassemia in both children and adults. The standard method involves correcting the genetic defect by inserting a functional copy of the gene into the patient's cells using a lentivirus. Second, using CRISPR/Cas9 gene editing, we can fix the defective gene at the molecular level. So, what does Ismail and company say? Examined the impact of an abnormal iron load on serum ferritin levels were of patients with beta thalassemia major in 2023 at a tertiary care hospital in Lahore, Pakistan. Descriptive and cross-sectional research was the style of the study. A total of 32 people was considered for the sample using the Raosoft calculator. With a margin of error of 9% and a response distribution of 10.5%, the confidence interval was 90%.The study's interviewer-administered questionnaire covered four areas: socioeconomic status and patient/caregiver demographics; medical history and anthropometric measurements of the patient; data on serum ferritin levels and chelation; and lastly, findings from cardiac echocardiography and ultrasound, in addition to laboratory tests (including TSH, T4, HbA1c, and ALT for all patients, and FSH and LH for only those patients older than 10 years old).The acquired data was fed into and analyzed in SPSS version 24. We deemed a P value below 0.05 to be statistically significant. The research had 32 participants, all of whom had beta thalassemia major, and their average age was 7.79 ± 4.57 years. Noncompliance with chelation was significantly correlated with serum ferritin levels, and the average level was 3410±2629µg/l (p=0.05). Hepatic impairment was associated with endocrine problems in 17 patients (53.1%), as shown by serum ALT levels. The correlation between compliance and echocardiography findings was statistically significant (p=0.04). Compliance was not substantially associated to ultrasound findings or blood levels of TSH, T4, ALT, FSH, or LH. This research indicates that elevated ferritin levels can lead to significant health complications.

Ghafoor et al. [22] conducted a cross-sectional study in 2023 at Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan, involving 88 patients with beta-thalassemia major. The patients were all 8 years old or older and had a serum ferritin level higher than 1000µg/L. The study was approved by the ethics review committee. November 2021–November 2022 was the duration of the study. Tests for haemoglobin subunit beta gene variants and significant physiological complications like growth retardation, hypogonadism, hypothyroidism, hypoparathyroidism, erectile dysfunction, and diabetic abnormalities were conducted using a battery of diagnostic tools, including an automated chemistry analyzer, an enzyme-linked immunosorbent test, a constantly updating polymerase chain reaction, and a fully autonomous chemiluminescence

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immunoassay. In order to examine the data, we employed SPSS 25. Forty women made up 45.4% of the sample, while forty-eight men made up 54.5%. The (62.5%) went through growth retardation, 41 patients (46.6%) had hypogonadism, 16 patients (18.1%) had hypothyroidism, 5 patients (5.7%) had the condition, 3 patients (3.4%) had diabetes mellitus, and 8 patients (9.1%) had impaired glucose tolerance. In addition, endocrine issues of some kind were encountered by 65 people (73.9%). Low serum ferritin levels were associated with endocrine issues (p=0.000). The IVSI-5 (G>C) variant was the most prevalent, accounting for 36 instances or 40.9% of all beta gene variants in the hemoglobin subunit, while the cluster of differentiation-26 (G>A) variant was the least common, occurring in 1 case or 1.1% of all variants. Genetic variations in the beta gene of the hemoglobin unit were not associated with endocrine disorders in a statistically significant way (p>0.05). A variant known as IVSI-5 (G>C) was shown to be the most prevalent hemoglobin subunit beta gene variation among people with beta-thalassemia major.

In 2023, Ismail et al. included 10,739 individuals with a possible diagnosis of β-thalassemia trait (BTT) or iron deficiency anemia (IDA) [26]. The blood chemistry profile included measuring the following parameters for each patient: red blood cell count, hemoglobin level, mean the volume of cells, mean cell hemoglobin concentration, and red cell dispersion width. To find the serum ferritin level, serum was collected after centrifugation and tested with an Abbott Alinity chemistry analyzer. Of the 1044 individuals tested positive for BTT, 270 were determined to have IDA. The two states were differentiated using 25 formulae applied to respective RBC indices. It appeared that the most reliable indices were the Matos in addition Carvalho index and the Pornprasert discriminating index, both of which had sensitivity and specificity values exceeding 98%. By combining the Matos and Carvalho index with the Pornprasert index, we are able to screen a big population for BTT efficiently.

In 2023, Abbasi et al. recorded the incidence of various cutaneous manifestations in patients with β-thalassemia major and examined their association with serum ferritin levels [1]. The study was 6-month long and used a cross-sectional approach. In a sequential approach, a dermatologist would check patients with beta-thalassemia major and note any skin issues that were present. At the same time, we took blood samples to determine serum ferritin levels. Eleven hundred and thirty-three patients were able to participate in the study. A mean age of 9.32 ± 4.54 years was recorded for the group. In this set of participants, the mean ferritin concentration was 3334 ± 1676 mcg/L. Xerosis (44.2%), freckles (39.8%), and pruritus (44.2%) were the most common skin symptoms reported by 89.4% of the patients (n = 101). People who wore freckles had significantly higher blood ferritin levels (p = 0.00288). It appears that pruritus is not caused by jaundice (p = 0.973). Finally, skin issues were more common in those whose first blood transfusion was after the age of one (p = 0.0011). People who suffer from beta-thalassemia major often experience skin problems. By checking for a range of skin diseases on a regular basis, these patients can improve their quality of life and decrease morbidity related with dermatology.

Naz et al. [33] determined the frequency of hepatitis B virus (HBV) infection among patients with β-thalassemia major in relation to different blood transfusion practices. This study was conducted in collaboration between the Regional Blood center. It included 360 patients, all of whom had needed multiple transfusions. The individuals had average weights of 43.50+18.76 kg and mean ages of 13.10+2.1 years. Only 94 out of 360 patients, or 26.66 percent, were vaccinated against Hepatitis B, despite the fact that 73.88 percent of the patients were not immunized (p<0.05). On an annual basis, 72 patients (20%), 157 patients (43.61%), and 131 patients (36.38%) received ten, eleven, or twenty transfusions, respectively. The impacted patients' hematological parameters showed notable changes (p<0.05). With an incidence rate of 11.53 for β-Thalassemia, the group that received more than 25 blood transfusions annually was the most affected. Major individuals affected with Hepatitis. Hepatitis B is most commonly contracted by people with β-thalassemia major following several blood transfusions.

In 2023, Aghaei et al. analyzed a large dataset that included information on opioid agonist therapy, needle and syringe program services, characteristics of people who inject drugs, injection frequency, commonly injected substances, and estimates of the population of people who inject drugs [2]. In order to find all publications published in the Eastern Mediterranean Region between January 1, 2010, and April 17, 2022, we conducted language-independent searches of PubMed, Web of Science, Scopus, Embase, and the Index Medicus. The databases of government publications, civil society organizations, and the United Nations were also searched for grey literature released between 2010 and 2022. All papers that met the criteria either provided an estimate for the relevant indicator or sufficient data to compute it. National and regional estimates were derived using the data extracted from the qualifying papers. The inclusion criteria for the meta-analysis were met by just 201 out of 38,283 publications. For the four outcomes that were meta-analyzed, we reviewed 115 publications. Among the local population, around 20,000 people use injectable pharmaceuticals; the projected number of users is 864,597 (95% CI 641,909-1,205,255) (14.9-279.9). Hepatitis C (44.82%, 29.32%-61.16%), HIV (19.22%, 95% CI 12.86-26.36), and hepatitis B (2.66%, 0.84-7.26) were the most common viral infections among injectable drug users. When comparing countries, there was a clear disparity in the usable data and the variables that were considered. Coverage was sometimes very low, insufficient, or ambiguous among the nine nations that had needle and syringe programs and the seven that offered opioid agonist treatment.

Abbasi et al. [1]. In 2023 estimated of the pooled global seroprevalence and 95% CI, the authors of the study utilized a random-effects meta-analysis. They classified the seroprevalences based on the following criteria: year of publication, socioeconomic level of the country, and HDI (Human Development Index) classification. By combining the results of four large-scale studies using the "leave-one-out"

method, they were able to calculate the global prevalence with the use of sensitivity analysis. Additionally, they conducted subgroup and meta-regression analyses to search for any correlations with other risk factors for HCV seropositivity in pregnant women. Different countries were included in 192 of the 208 datasets that were considered. A 95% confidence interval of 1.72–1.89 percent in the global analysis and 3.29 percent present in the sensitivity analysis. The seroprevalence rate was 6.21% (range: 4.39-8.29%) in the Eastern Mediterranean area and 0.75% (0.38-1.22%) in the Western Pacific region. Expectant mothers, who had an opiate use problem compared to the whole group of expectant women, had a significantly higher seroprevalence of HCV Ab ($p < 0.001$). A sharp decline occurred in tandem with an increase in the human development index. Further variables that increase the likelihood of seropositivity for the hepatitis C virus include advanced age, lower level of education, polyamory, a prior history of blood transfusions, hospitalization, surgery, abortion, STDs, scarification, body modifications (tattoos or piercings), or a positive hepatitis B test.

Khan et al. [29. In 2023 the Fatimid Foundation and Hamza in Peshawar, Pakistan, undertook a cross-sectional correlational study in 2023. The investigation began on March 1, 2021, and it was completed on October 1, 2021. A grand total of 184 beta thalassemia major patients were enrolled in this study; 105 of these patients belonged to the Fatimid foundation and 79 to the Hamza foundation. A total of 124 people was involved in the research. Among those who were tested, hepatitis C virus was detected in 43 (23.8%) and hepatitis B viral antigen in 6 (3.3%) of the participants. Two and a half percent, or four people, tested positive for both HBV and HCV. Marriages between close relatives, such as cousins, greatly increased the likelihood of thalassemia. The prevalence of hepatitis B and C infections in patients with thalassemia major was not associated with the disease. Eliminate by screening donors using the most advanced nucleic acid analysis that is both sensitive and specific. Blood transfusions can only be administered to thalassemia patients at hospitals that strictly adhere to all applicable safety regulations.

Sultana et al. [51] evaluated liver function tests in patients with β-thalassemia major. Researchers from the Department of Biochemistry at Dhaka Medical College in Dhaka, Bangladesh, conducted this cross-sectional study from January 2017 through December 2017. In this study, fifty children of both sexes were selected from the pediatrics department at Dhaka Medical College. Half of the participants were assigned to Group A, which consisted of 50 patients with β Thalassemia major, and half were assigned to Group B, which consisted of 50 children who appeared to be in excellent health. Parameters used in the study included serum ferritin, bilirubin, and AST, ALT, and ALP. The results were compared statistically across different groups. The average blood ferritin level in patients with Group A thalassemic major was 890±446.38 microgram/L, which is significantly higher than the normal range. In Group A, the levels of serum bilirubin were 3.27±2.62 mg/dl, while in Group B, they were 0.48±0.24 mg/dl. The serum ALT levels in Group A were 53.06±34.0 U/L, while in Group B they were 16.70±4.81 U/L. Group B had serum AST levels of 11.60±2.72 U/L, while Group A had values of 84.56±33.54 U/L. Group B had ALP levels of 221.86±80.54 IU/L while Group A had 422.42±226.99 IU/L. All of the values in β-thalassemia patients were significantly higher ($p<0.001$) when compared to typically developing children. According to this research, patients who have β thalassemia major have significantly higher levels of liver function markers. Hence, thalassemic patients should probably get their liver function tests checked regularly for any indications of hepatic disease.

Ahmad et al. [3] reported that diabetes mellitus and hepatitis C virus (HCV) were major contributors to the global health and economic burden. In the last several decades, researchers have discovered a robust association between the two disorders, which may be explained by common liver problems. Nevertheless, the impact of chronic HCV stationary apparatus on diabetes prevalence remains unknown. All chronic HCV virus cases begin with an acute, short-term infection. Symptoms may manifest in infected persons during an acute illness. For 15–25% of people who get well from infections, treatment isn't required. Chronic infection affects some people. When this illness is present, it becomes tough for the liver to operate properly. An increase in insulin resistance is one of the additional potential reasons of diabetic complications. Patients with both diabetes and HCV may face extra challenges in their treatment plans. Cells in the body can develop a resistance to insulin. This review looks at the correlation between diabetes and hepatitis C. By conducting experiments on both male and female patients infected with HCV, this study sought to assess the prevalence of diabetes. Using data from many sources, this study demonstrated that the gender factors examined in the article significantly affect the prevalence of diabetes in HCV-infected individuals. The findings point to HCV as a potential initiating factor in the diabetes cascade. People without diabetes are at a higher risk of contracting hepatitis C than people with diabetes themselves. An elevated risk of getting HCV is observed in diabetic women in their 30s and 40s. Curiously, the investigation determined that every single case of serotype positivity was a result of type II diabetes.

Saleem et al. In 2022 states that the serious liver ailment known as hepatitis C is caused by the Hepatitis C virus (HCV). Hepatitis C virus is an important issue in Pakistani public health. It is widely recognized that HCV poses a significant threat to public health in Pakistan because of how common it is. Pakistani researchers looked at data from three groups to find out how common hepatitis C is: the general public, blood donors, and expectant mothers. Along with the virus, they sought out potential danger signs. In order to arrive at a weighted mean, data from 163 studies that were published between 2001 and 2022 were used. First, the general

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population; second, pregnant women; and third, blood donors made up the 1,875,232 persons who filled out the survey. Both the general public (765,426) and blood donors (973,260) made up the bulk of the population. Hepatitis C virus positivity was observed in 8.2% of blood donors and 16.47% of the overall population. Pregnant women had a mean frequency of 9.3% (136,546). This study found that 11.32% of the general population, pregnant women, and blood donors have hepatitis C. The data shows that there are several risk factors. These include using unsterilized needles, getting blood transfusions, having your hair shaved by a barber, working with untrained staff, having needle stick injuries, injecting drugs, using intravenous drugs, sharing needles with household contacts or spouses, using dental and surgical instruments that have not been sterilized, improperly disposing of hospital waste, and having inadequate infrastructure, among other things. It is worrisome that so many people in Pakistan have HCV infections. Health education and awareness efforts are necessary for Pakistan to witness a decrease in hepatitis C cases. The results demonstrate the need of implementing both therapeutic and preventive interventions to lower the mortality and illness burden in Pakistan.

Saraceni and Birk [47] reported that chronic hepatitis B virus (HBV) infection is particularly difficult to eradicate because HBV can integrate its circular DNA into the genome of host hepatocytes. Successful virus clearance is dependent on the complex interaction between the virus and the host's innate and adaptive immune response. A significant step forward in the battle against hepatitis B has been the development and broad distribution of the HBV vaccine. This has greatly reduced the virus's ability to spread. The RNA virus hepatitis C virus (HCV) has an incredible capacity for mutation, which allows it to evade the immune system and spread rapidly and persistently. Because HCV can produce mutations and has a high degree of genetic variability, it has been difficult to create a vaccine that is effective against the virus. Since the advent of direct acting antivirals, hepatitis C treatment has progressed to a new level. A persistent virologic response can be achieved with DAAs in 85 to 99% of instances. However, this still leads to a significant number of unsuccessful treatments, which emphasizes the continuous requirement for new therapeutic methods. The immunopathogenesis of herpes simplex virus (HSV) and hepatitis C virus (HCV) is thoroughly covered in this article. In it, we learn how these viruses weaken the host immune system, how chronic diseases develop, the benefits and drawbacks of vaccines, and lastly, we talk about treatment choices and the most common issues that might develop from them.

Amirhashchi et al. [11] aimed to determine the prevalence of occult hepatitis B virus infection (OBI) among β-thalassemia major (BTM) patients in Khuzestan Province, Iran. For this cross-sectional study, researchers looked at 90 thalassemia patients who were admitted to Ahvaz city's Shafa hospital between 2018 and 2019. The frequency of their blood transfusions varied between 36.2 and 552. After ELISA was used to determine the serological markers, real-time PCR was used to detect HBV-DNA. Nesting polymerase chain reaction allowed us to sequence and genotype OBI cases. R was used to perform the statistical and phylogenetic analyses. Not only did 95.5% of subjects (86/90) test negative for HBsAg, but 1.16 percent of participants reported experiencing OBI. Eighty percent of the patients tested positive for HBs, 7.78 percent for HBc, and 12.2 percent for HCV. When tested for HBV-DNA, none of the four HBsAg-positive individuals proved positive. The OBI patient tested positive for HBV genotype D, according to phylogenetic studies. The first occurrence of OBI in β-thalassemia patients in Iran was confirmed by this investigation. How prevalent is OBI among BTM patients in Iran? This information is crucial for making decisions regarding OBI screening, especially in transfusion centers. To get at this conclusion, further investigation is required.

Farooq et al. [60] conducted a study from 2018 to 2019 involving 2,260 thalassemia patients to investigate hepatitis B virus infection and related molecular characteristics among transfusion-dependent thalassemia patients. The study was approved by the Ethics Committee of SZAB Medical University, Islamabad. To screen the samples for HBsAg serologically, DiaSorin S.p.A. of Italy developed the LIAISON® XL Murex HBsAg Quant test, a chemiluminescence based immunoassay (CLIA). An HBV quantitative PCR kit was used to ascertain the concentration of HBV DNA in the serum samples. The HBV genotypes were determined by employing universal primers that aim squarely at the amplification of the P1 and S1 regions. Men made up 64.6% of the 2,260 thalassemia cases, while girls accounted for 35.4%. Detection of HBsAg occurred in 98 individuals, or 4.33%. Based on the results of the polymerase chain reaction (PCR), 58.1% of the patients (n = 98) had genotype D, 21.42 percent had genotype A, and 19.38 percent had genotype C. For the best therapy of chronic HBV patients, it is essential to determine HBV genotypes in patients who have received many transfusions, since genotypes impact the severity and course of the disease. Because of quality-assured screening of donated blood, patients with thalassemia will not catch HBV.

Waheed et al. [53] reported that Pakistani patients with β-thalassemia major were assessed for HBV and HCV infections in 2021. To achieve this goal, we combed through primary studies that measured the prevalence of HBV and HCV in thalassemia patients who needed blood transfusions. They combed through ten prestigious subscription databases in the first half of 2020. For more pertinent reports, the databases of the World Health Organization were also searched. The search parameters, which did not restrict language, covered items published up until December 31, 2019. All of the articles were entered into Endnote version X9 after a thorough search for relevance and duplication was conducted. Included in the findings were the overall study prevalence as well as the pooled subgroup prevalence. A total of thirty-three studies spanning the years 1995–2019 were considered for the meta-analysis. The prevalence of HCV was included in all 33 articles, with the exception of 19 that specifically addressed HBV prevalence. A total of 8,554 individuals were surveyed to determine the frequency of HCV in thalassemia patients. In a total of nineteen investigations, 6,184 individuals were asked

about the frequency of HBV. A pooled prevalence of 4.13% for HBV and 29.79% for HCV were produced by studies mostly conducted in the Pakistani provinces of Khyber Pakhtunkhwa (24.24% of the total) and Punjab (33.33% of the total), respectively.

Ahmad et al. [3] (2022) described hepatitis as inflammation of the liver tissue, which is the accepted clinical definition of the condition. Particularly in the developing Asian countries, it poses serious health risks to the world's population. In order to find relevant information, we searched PubMed, Google Scholar, PakMediNet, ScienceDirect, and the Directory of Open Access Journals (DOAJ). The weighted average was used to determine the prevalence rate on a national level. Antibodies against hepatitis C virus (HCV) were present in 3.31% of non-donors, 1.98% of blood donors, and 5.62% of blood donors who did not donate blood. Specifically, thalassemia was linked to 27.33 percent of HCV cases, with chronic liver disease being the most strongly associated with 44.4 percent of HBV cases. All three types were dominated by genotype 3. Seropositivity to HCV rose 6.8% to 7.44% in previous years.

Akhtar et al. [6] conducted a systematic review and meta-analysis to determine the prevalence of hepatitis C virus (HCV) infection among β-thalassemia patients in Pakistan. Publication records from 1995 to 2019 were sourced through searches in local databases, as well as PubMed, EMBASE, Web of Sciences, the Cochrane Library, and the Directory of Open Access Journal. The inverse variance weighting random-effects models proposed by DerSimonian and Laird were employed in this meta-analysis. We assessed the quality of each included work's methodology using the STROBE tool. We applied the Egger test to identify potential bias in the publications. The final meta-analysis only contained 27 studies out of 229 that were considered. There was a lot of variation among the 5789 β-thalassemia patients in Pakistan, although the combined prevalence of HCV was 36.21% (95% CI: 28.98-43.75%). The meta-analysis in Punjab found that 45.98% of β-thalassemia patients tested positive for HCV, with a 95% confidence interval of 38.15-53.90%. Prevalence rates in Sindh and Khyber Pakhtunkhwa were 31.81% and 28.04%, respectively, with a 95% confidence interval of 20.27-44.59% and 13.58-45.26%. A significant source of heterogeneity in the meta-regression study was determined to be geographical location.

Tayyab et al. [51] reported that thalassemia patients are at increased risk of transfusion-associated complications due to various infectious diseases, including hepatitis B virus (HBV), hepatitis C virus (HCV), HIV, syphilis, and other transfusion-transmitted infections. In Pakistan, the carrier incidence is 5-7% and there are 5,000 to 10,000 children infected by thalassemia every year. However, the disease affects about 4.4% of all births globally. In nations with low per capita income, the range of blood transfusions for infectious disease cases is: The prevalence of many diseases varies greatly; for example, HIV ranges from 0.33 percent to 1.66 percent, HBV from 2.0 percent to 4.5 percent, HCV from 0.5 percent to 2.23%, and syphilis from 0.6 percent to 18 percent. When a patient's average hemoglobin level is outside of the normal range of 10 to 12 g/dl, blood transfusions become crucial. Additional treatments include stem cell transplantation, gene therapy, iron chelation therapy, splenectomy, and others. In order to increase longevity, the second is of essential importance. When comparing immuno-chromatographic techniques (ICTs) to chemiluminescence immunoassays (CLIAs), the latter are better at detecting low titer infectious microorganisms and weakly positive results, making them ideal for evaluating the reliability of blood transfusion infections.

Shah et al. [50] investigated the prevalence and risk factors of hepatitis B and C infections among thalassemia patients in the Hazara districts of Pakistan in 2019. Five clinics distributed across the Hazara regions identified 324 instances of thalassemia major. Immunochromatographic testing and real-time polymerase chain reaction was used to screen study participants for hepatitis B and C virus. Among 324 patients diagnosed with severe thalassemia, 24 (or 7.41%) tested positive for both HBV and HCV. A total of 206 patients were men. A total of 3.88% of patients tested positive for infection, with two patients confirming HBV and eight patients confirming HCV. Consistent results were seen for the remaining 118 individuals: 8.47% of patients tested positive for HCV, and 3.39% of patients tested positive for HBV. Also, whereas those between the ages of 26 and 30 accounted for half of the HBV and HCV infections, those between the ages of 11 and 15 accounted for just 1.81 percent. The positive samples were found to have HBV and HCV, respectively, verified by band widths of 242 and 227 bp.

Shamshirian et al. [47] conducted a systematic review and meta-analysis to determine the prevalence and geographical distribution of hepatitis C virus (HCV) infection among thalassemia patients in Iran using Persian and English databases for studies published up to 2017. The most effective test for comparing two sets of data was the I-square (I2) test. Using the random effects model, we determined the frequency and 95% CI. For this meta-analysis, 37 studies with a combined total of 9,185 patients were taken into account. Out of all the Iranian thalassemia patients, 17.0% tested positive for HCV, with a 95% confidence range ranging from 14.5 to 19.8. A total of 17.4% of males and 16.8% of females were infected (95% CI: 13.8-21.9) and 13.2-21.1%, respectively.

Kamal et al. [62]. In 2019, researchers investigated the hepatitis C virus (HCV) treatment outcomes, progression, and outcomes for individuals with and without transfusion-dependent thalassemia major. We prospectively followed all thalassemia patients, regardless of whether they had an HCV infection in the past or not. The dosages of chelating agents and blood transfusions needed were calculated. As part of our assessment, we measured ferritin, iron, and HCV-RNA in addition to liver function tests. Some patients with chronic

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HCV evolution were able to get viral treatment. Serial transient elastography (TE), paired liver samples, or serum indicators of liver fibrosis were used to determine the rate of fibrosis progression in individuals with or without thalassemia who had chronic HCV. R2 MRI was used to ascertain the liver iron content. Acute HCV that resolved on its own was 17% more common in patients with thalassemia (35% vs. 17%, p=0.031) compared to people without thalassemia. Patients with thalassemia and chronic HCV exhibited significantly greater rates of fibrosis advancement (1.14±0.48) and (0.35±0.14), respectively, compared to people with chronic HCV alone (P<0.0001). The rate of fibrosis advancement was directly correlated with ferritin (R=0.77; P<0.01) and LIC (R=+0.67; P=0.01), showing a true linear relationship. Patients with chronic HCV and thalassemia had sustained virologic response (SVR) rates of 33% and 82%, respectively, after pegylated interferon-based treatment; the difference was statistically significant (p < 0.0001). Three of the five HCV and thalassemia patients who passed away were victims of heart problems, and two others died of liver cancer.

Al Kanaani et al. [8] systematically reviewed and analyzed records of hepatitis C virus (HCV) infection cases and prevalence rates in Pakistan from 1989 to 2016 using methods based on the Cochrane Collaboration Handbook. Our goals were to determine the average pooled prevalence of HCV antibodies in different risk groups and (2) give a comprehensive overview of the HCV epidemiology in Pakistan in 2018. The reporting criteria for this systematic review were based on the PRISMA statement. Hazard of HCV infection served as the criterion for categorizing the subjects into the six groups. We took inverse variance weighting into consideration when we conducted meta-analyses using the DerSimonian and Laird random-effects models. Out of 341 prevalence measures/strata, only one examined HCV incidence. When all studies were taken into account, the estimated prevalence of HCV was as follows: 6.2% in the general population, 34.5% in high-risk clinical populations, 12.8% in intermediate-risk populations, 16.9% in special clinical populations, 55.9% in populations with liver-related disorders, and 53.6% in injectable drug users. Analysis of healthcare-related epidemiology research provided the bulk of the risk variables. In Pakistan, one out of every twenty persons is afflicted with HCV, making it a historically serious epidemic. Since hepatitis C virus (HCV) is so common in the United States, there is a higher chance of liver damage. It appears that medical treatments are the primary driver of transmission.

Waheed et al. [55] reported that viral hepatitis affects more than 300 million people worldwide and causes nearly one million deaths annually. Eradicate viral hepatitis by the year 2030. A number of WHO member states are actively pursuing hepatitis control strategies to eradicate the disease. Just twelve nations have made any real progress in their fight to wipe out hepatitis. The purpose of this research was to record the achievements and setbacks thus far in the fight to end hepatitis by the year 2030. New data shows that although 87% of babies received all three doses of the hepatitis B virus (HBV) vaccine in the first year, just 46% of babies received their first injection at the recommended interval. Improving the security of blood and injections is an urgent matter that requires swift attention. About eleven percent of hepatitis B and C infections are successfully diagnosed. Rapid identification of hepatitis and subsequent recovery of the millions of people affected with the virus are of utmost importance. Nearly three million people have received hepatitis C treatment as of 2016. Hepatitis C virus therapies have become more affordable in a number of nations. The absence of financial support for hepatitis elimination initiatives is their primary obstacle. The battle against hepatitis has not received any funding commitments from any major contributor worldwide. When attempting to finance a hepatitis prevention program, low- and middle-income countries will encounter a significant obstacle. Strong political will, sufficient funding, and the backing of NGOs, pharmaceutical firms, and healthcare providers are all necessary for the worldwide eradication of hepatitis.

Umer and Iqbal. [63]. In 2016 among adults in Pakistan, 6.8% had HCV seroprevalence, with about 6% currently infected, according to a review of studies published between 2010 and 2015. With an HCV prevalence of up to 25% in rural and poor peri-urban areas, this study further emphasizes the need of paying more attention to this underserved community. Given the high prevalence of the virus among patients presenting to hospitals with a variety of non-liver disease related complaints (up to 30%), it suggests that nosocomial HCV spread may be on the rise. It is urgently necessary to address the 2.45% seroprevalence among blood donors in order to reduce the risk of transfusion-transmitted HCV. The vast majority of HCV infections in Pakistan (61.3%) are of genotype 3a. Having said that, subtype 2a has been on the rise in some parts of Pakistan during the last many years. The 2a genotype was second most common in Sindh province (11.3%) and Khyber Pakhtunkhwa (17.3%). To guarantee safe medical treatments in light of changing genotype frequency distributions and high nosocomial transmission rates, additional research into new treatment regimens is required.

Materials and Methods

- **Study Area**

Ali Zaib and Sundas Foundation, Faisalabad is a renowned thalassemia care institution in Faisalabad Division, Punjab, Pakistan, where the study was carried out. A significant number of registered thalassemia patients receive regular blood transfusion services, diagnostic facilities, and follow-up treatment from the foundation, making it an appropriate location for data collecting.

- **Study Design**

The purpose of this cross-sectional analytical study was to identify any thalassemia patients who may have biochemical or hematological markers linked to hepatitis B and C. The researchers decided to use a cross-sectional study design because it permits the evaluation of both the exposure (biochemical markers) and the result (hepatitis B and C status) in a specific population at the same time.

❖ Study Duration

A total of six months was devoted to the research. Recruiting the necessary number of participants and collecting comprehensive clinical and laboratory data were both accomplished within this time frame.

❖ Sample Size

The Rao soft sample size calculator was utilized to calculate the sample size, taking into account following parameters: a 95% confidence level, a 5% margin of error, and an expected population percentage of 50%.

Fifty patients were removed from the analysis because of missing data or predetermined exclusion criteria; this left 150 thalassemia patients for further examination.

❖ Sampling Technique

The researchers used a convenient sampling method that is not based on probability. The trial enrolled patients in a sequential fashion until the necessary sample size was reached. Patients visited the Ali Zaib Foundation for routine transfusion and follow-up throughout the duration of the study.

❖ Data Collection

Data were collected using a structured and pre-designed Performa developed specifically for this study. The Performa included the following components:

- Demographic information (age, gender, blood group)
- Clinical history (type of thalassemia, transfusion frequency, vaccination status)
- Physical examination findings
- Hematological parameters (Complete Blood Count including Hb, RBCs, WBCs, platelets, RBC indices, and differential count)

❖ Data Collection Procedure

In order to enroll eligible patients in the trial, informed permission has to be obtained. Steps in gathering data were included consulting hospital records and conducting in-person interviews to glean pertinent demographic and clinical details. Pallor, jaundice, hepatomegaly, and splenomegaly were some of the symptoms that were evaluated during the systemic and general physical exams. Aseptic conditions were maintained for the collection of blood samples. The serological tests used to detect hepatitis B (HBsAg) and hepatitis C (Anti-HCV) are industry standards. Automated biochemical analyzers were used to measure liver function tests, including AST, ALT, and bilirubin. In order to get the CBC parameters, an automated hematological analyzer was used.

❖ Study Criteria

Inclusion Criteria

- Confirmed cases of thalassemia (major, intermedia, or minor)
- Patients receiving regular blood transfusions
- Patients registered at Ali Zaib Foundation
- Patients (or guardians) who provided informed consent

Exclusion Criteria

- Patients with known chronic liver diseases unrelated to transfusion (e.g., alcoholic liver disease)
- Patients unwilling to participate
- Patients currently receiving antiviral therapy for hepatitis B or C

Statistical Analysis

Using SPSSv26, the data was entered and evaluated. For continuous data, descriptive statistics comprised the mean plus or minus the standard deviation (SD). Categorical variable frequencies and percentages. Independent sample t-test was employed for group means comparison, while a chi-square test was employed for categorical variables. A statistical analysis was considered significant if the p-value was less than 0.05. Graphs and tables were used to present the results.

Ethical Considerations

Ethical clearance was obtained from the relevant institutional review board (IRB) before the study began. The Ali Zaib Foundation's upper management approved. An informed consent form was requested in writing from all participants or their legal guardians. The privacy of all patient information was guaranteed. Every single participant gave their informed agreement that their data would be used exclusively for research purposes. (see Appendix A)

Results and Discussion

Using a cross-sectional analytical methodology, this study evaluated biochemical and hematological indicators associated with hepatitis B and C among thalassemia patients over the course of six months at the Ali Zaib and Sundas Foundation in Faisalabad. A non-probability convenient sampling method was used to select 150 patients from a starting pool of 200, with the use of predetermined inclusion and exclusion criteria. Subjects' demographic information, medical history, physical exam results, serological status (HBsAg and Anti-HCV), liver function tests (AST, ALT, bilirubin), and whole blood count parameters were all part of the data set that was gathered utilizing a structured Performa. The aseptic technique was used to collect blood samples, which were then processed utilizing automated biochemical and hematological analyzers. Statistics were performed using SPSS version 26. Various descriptive statistics, such as means

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± standard deviation and frequencies, were employed, along with inferential tests such as independent sample t-test and chi-square test. A p-value of less than or equal to 0.05 was considered statistically significant, and the results were presented in both tabular and graphical forms.

- **Demographic characters of thalassemia patients with hepatitis**

Table 4.1: Prevalence of gender in thalassemia patients with hepatitis

Gender	Frequency	%age
Male	77	51.3
Female	73	48.7

Patients with thalassemia who were also diagnosed with hepatitis were categorized according to gender in Table 4.1. Out of a total of 150 patients, 77 were male (or 51.3% of the total population studied), while 73 were female (or 48.7% of the total population studied). There were no missing observations in the study sample, as shown by the Cumulative %ages.

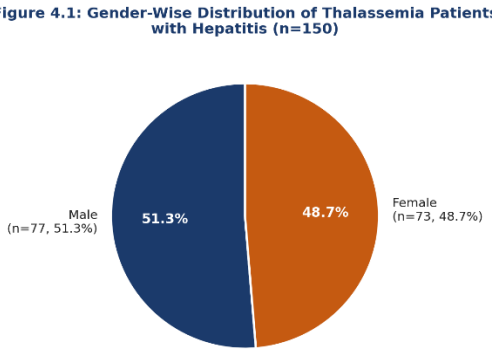

Figure 4.1: Gender-wise distribution of thalassemia patients with hepatitis. The graph supported the conclusion that hepatitis occurrence among thalassemia patients was not strongly gender-biased and affected both sexes almost equally.

Table 4.2: Prevalence of blood group among patients of thalassemia

Blood Group	Frequency	% age	Valid %	Cumulative %
O+	24	16.0	16.0	16.0
o-	16	10.7	10.7	26.7
A+	15	10.0	10.0	36.7
A-	14	9.3	9.3	46.0
B+	23	15.3	15.3	61.3
B-	22	14.7	14.7	76.0
AB+	22	14.7	14.7	90.7
AB-	14	9.3	9.3	100.0
Total	150	100.0	100.0	

Table 4.2 presented the blood group distribution among the 150 enrolled thalassemia patients with hepatitis. O+ blood group was observed in 24 patients (16.0%), making it the most prevalent blood group. This was followed by B+ in 23 patients (15.3%), B– and AB+ in 22 patients each (14.7%), O– in 16 patients (10.7%), A+ in 15 patients (10.0%), and A– and AB– in 14 patients each (9.3%).

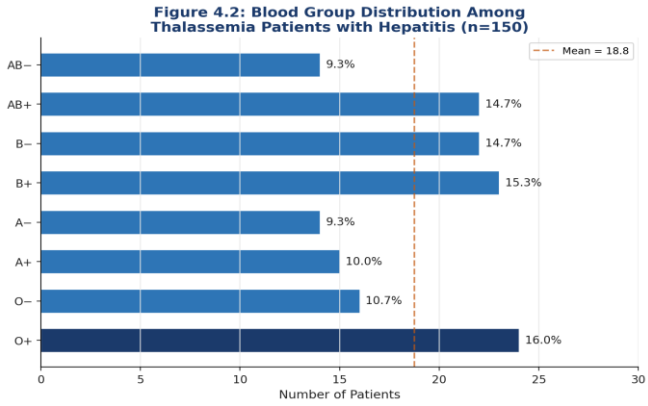

Figure 4.2: Prevalence of blood group among patients of thalassemia. The graph showed a relatively even distribution across all groups, with O+ slightly more prominent than others. No distinct clustering or excessive concentration in any blood group was observed, reinforcing the interpretation that blood group was not a significant determinant of hepatitis risk.

Table 4.3: Descriptive analysis for age and weight of thalassemia patients

Variable	Mean ± SD	Minimum	Maximum
Age	13.49 ±5.04	5.00	22
Weight	32.00 ±10.51	15	50

Table 4.3 summarized the descriptive statistics for age and weight. The mean age of patients was 13.49 ± 5.04 years, with a minimum age of 5 years and maximum age of 22 years. The mean weight was 32.00 ± 10.51 kg, ranging from 15 kg to 50 kg. The findings indicated that the majority of study participants were children and adolescents, which is consistent with the early diagnosis and lifelong management of thalassemia major.

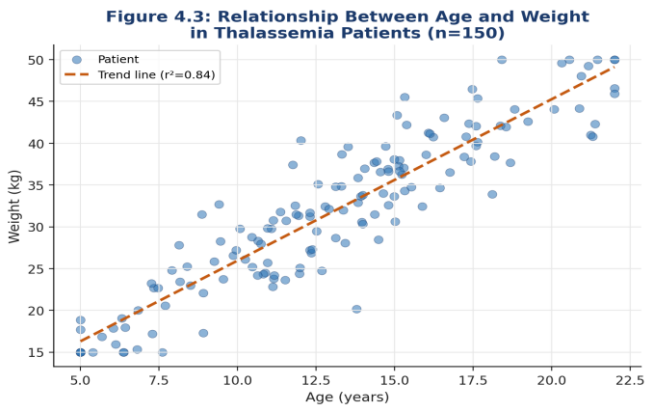

Figure 4.3: Comparison between age and weight of thalassemia patients. A generally increasing trend was observed, where older patients tended to have greater body weight. However, the increase was not steep, suggesting suboptimal physical growth despite increasing age. This pattern was compatible with growth retardation commonly associated with chronic transfusion-dependent disorders.

❖ Clinical history and presented complaints

Table 4.4: Frequency of thalassemia types and family history

	Thalassemia Type		Family History	
	Major	Intermedia	No	Yes
Frequency	125	25	76	74
% age	83.3	16.7	50.7	49.3

Table 4.4 showed the distribution of thalassemia type. Among the 150 patients, 125 patients (83.3%) were diagnosed with thalassemia major, while 25 patients (16.7%) had thalassemia intermedia. The predominance of thalassemia major indicated that patients with severe forms of disease were considerably more represented among hepatitis cases. While family history of thalassemia in 74 (49.3%) and absent in 76 (50.7%). The almost equal proportions suggested that hepatitis occurrence was not influenced by family history of thalassemia. Although thalassemia itself is inherited, hepatitis acquisition is primarily environmental and associated with transfusion exposure.

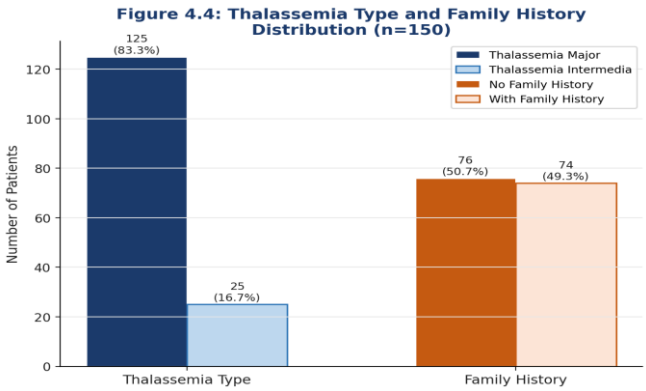

Figure 4.4: Prevalence of thalassemia type and family history. The graph showed a markedly taller bar for thalassemia major compared to intermedia, visually emphasizing the dominance of severe disease among hepatitis-positive patients. The graph visually demonstrated a balanced distribution between patients with and without family history, supporting the conclusion of no clear association.

Table 4.5: Prevalence of blood transfusion and HBV vaccination

	Transfusion Frequency			HBV Vaccination		
	Weekly	Monthly	Biweekly	No	Yes	Unknown
Frequency	80	20	50	55	53	42
% age	53.3	13.3	33.3	36.7	35.3	28.0

Table 4.5 described that weekly transfusions were reported in 80 patients (53.3%), biweekly in 50 patients (33.3%), and monthly in 20 patients (13.3%). This indicated a high transfusion burden in the study population. Frequent transfusions increase repeated exposure to donor blood, thereby substantially increasing the risk of hepatitis transmission. Weekly transfusion dependency reflected severe anemia and disease burden. Also, the HBV vaccination was absent in 55 patients (36.7%), present in 53 patients (35.3%), and unknown in 42 patients (28.0%).

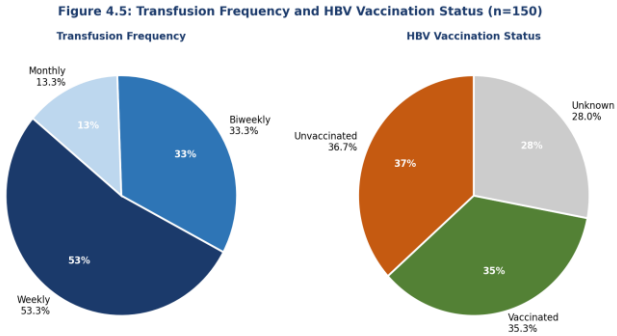

Figure 4.5: Prevalence of blood transfusion and HBV vaccination. The figure highlighted weekly transfusion as the most dominant category, visually reinforcing the high-risk transfusion pattern. The graph demonstrated a fragmented vaccination profile, with no category showing clear dominance, reflecting inconsistent immunization practices.

Table 4.6: Prevalence of fever, abdominal pain and nausea

	Fever		Abdominal Pain		Nausea	
	No	Yes	No	Yes	No	Yes
Frequency	84	66	75	75	78	72
% age	56	44	50	50	52	48

Table 4.6 shown that fever was absent in 84 patients (56.0%) and present in 66 patients (44.0%). Fever explained substantial proportion of patients, concluded its relevance as a common clinical symptom. However, its absence in more than half of the patients suggested limited specificity. The abdominal pain was equally distributed, with 75 patients (50%) reporting presence and 75 patients (50%) reporting absence. that nausea was absent in 78 patients (52%) and present in 72 patients (48%). Nausea was moderately prevalent, suggesting gastrointestinal involvement but lacking discriminatory clinical value.

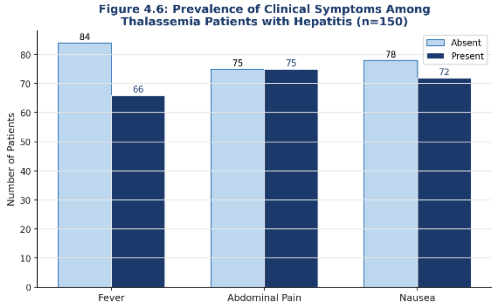

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Figure 4.6: Prevalence of fever, abdominal pain and nausea among thalassemia patients with hepatitis. The graph showed comparable percentages for all three symptoms, indicating that these complaints were common but broadly distributed

❖ Physical and Systemic examination of thalassemia patients

Table 4.7: Prevalence of Jaundice, edema, lymphadenopathy, hepatomegaly and liver dysfunction

	Jaundice		Edema		Lymphadenopathy		Hepatomegaly		Liver dysfunction	
	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Frequency	27	123	74	76	84	66	24	126	67	83
% age	18	82	49.3	50.7	56	44	16	84	44.7	55.3

Table 4.7 stated that jaundice was present in 123 patients (82.0%) and absent in 27 patients (18.0%). The high prevalence strongly suggested significant hepatic dysfunction and bilirubin metabolism abnormalities. Edema was present in 76 patients (50.7%) and absent in 74 patients (49.3%). This near-equal distribution indicated moderate systemic involvement. The clubbing was present in 76 patients (50.7%). This suggested chronic disease progression and long-term hypoxic or systemic stress. Lymphadenopathy was present in 66 patients (44.0%). This reflected moderate immune activation or chronic inflammatory response. The hepatomegaly was present in 126 patients (84.0%). The very high prevalence indicated substantial liver enlargement associated with chronic hepatitis and iron overload. Liver dysfunction was present in 83 patients (55.3%). More than half of the patients demonstrated biochemical or clinical liver impairment.

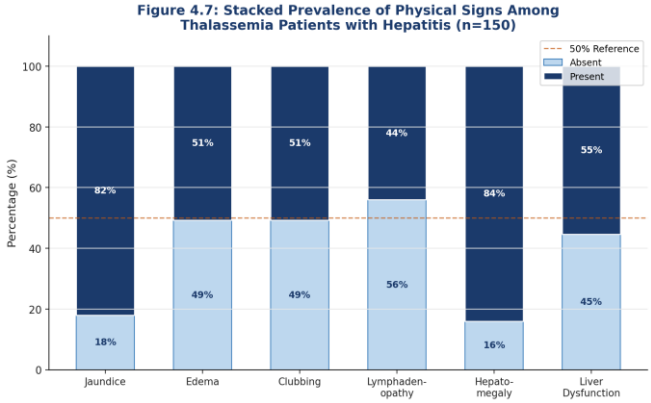

Figure 4.7: Prevalence of jaundice, edema, clubbing, lymphadenopathy, hepatomegaly and liver dysfunction among thalassemia patients with hepatitis. The graph clearly highlighted hepatomegaly and jaundice as dominant clinical features.

Table 4.8: Prevalence of HBV and HCV among thalassemia patients

	HBV		HCV	
	Negative	Positive	Negative	Positive
Frequency	118	32	86	64
% age	78.7	21.3	57.3	42.7

Table 4.8 shown that HBV positivity was observed in 32 patients (21.3%). HBV prevalence was moderate, indicating an existing but comparatively lower burden. HCV positivity was present in 64 patients (42.7%). HCV prevalence was considerably higher than HBV, indicating greater transmission burden.

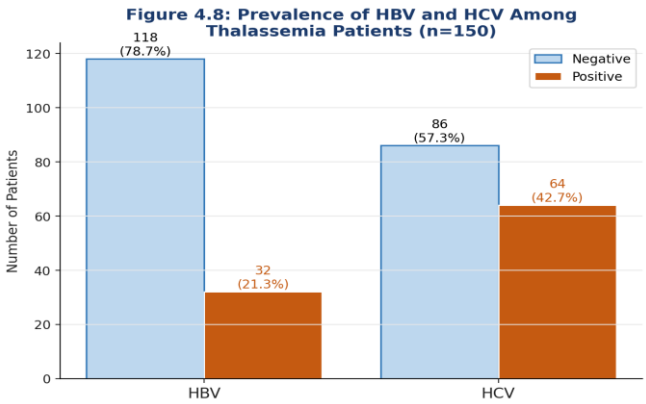

Figure 4.8: Prevalence of HBV and HCV among thalassemia patients. The graph clearly demonstrated higher HCV positivity compared with HBV positivity.

❖ Association between hepatitis positivity by Chi Square Analysis

Table 4.9: Association between hepatitis positivity and all other parameters

Fever	**HBV+**	**P-value**	**HCV+**	**P-value**
No (n = 84)	21	0.216^{NS}	38	0.473^{NS}
Yes (n = 66)	11		26	
Abdominal pain	**HBV+**	**P-value**	**HCV+**	**P-value**
No (n = 75)	16	1.00^{NS}	33	0.741^{NS}
Yes (n = 75)	16		31	
Jaundice	**HBV+**	**P-value**	**HCV+**	**P-value**
No (n = 27)	0	0.003**	0	0.000**
Yes (n = 123)	32		64	
Hepatomegaly	HBV+	P-value	HCV+	P-value

No (n = 24)	0	0.005**	0	0.000**
Yes (n = 126)	32		64	
Liver dysfunction	HBV+	P-value	HCV+	P-value
No (n = 67)	0	0.000**	0	0.000**
Yes (n = 83)	32		64	

Table 4.9: Association of Clinical Presentation with Viral Hepatitis Positivity

Statistical evaluation revealed no significant association between subjective clinical symptoms—such as fever, abdominal pain, and nausea—and hepatitis status, demonstrating that generalized symptoms lack discriminatory diagnostic value. Conversely, objective clinical signs and biochemical markers demonstrated robust, highly statistically significant associations with viral presence. Notably, all hepatitis-positive cases presented with both jaundice and hepatomegaly, with zero viral infections detected in their absence. Furthermore, documented biochemical liver dysfunction was heavily associated with viral positivity. These findings confirm that while subjective complaints are non-specific, jaundice, hepatomegaly, and altered liver enzymes serve as definitive clinical and biochemical indicators of active hepatitis infection in this population.

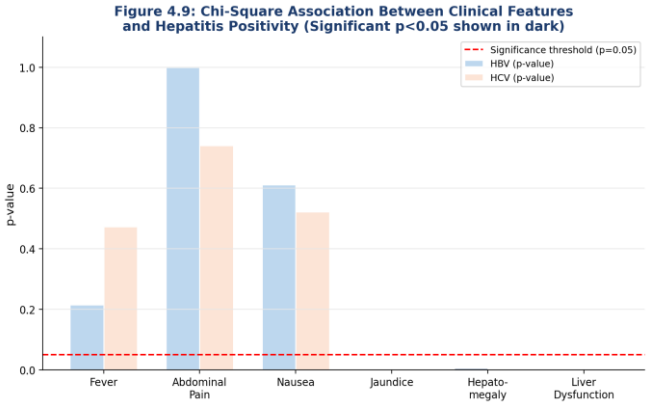

Figure 4.9: Association of Clinical Features with Hepatitis Positivity. There were significant associations, highlighting jaundice, hepatomegaly, and liver dysfunction as dominant predictors, while other variables showed minimal differences.

- **Comparison of potential marker with hepatitis status**

Table 4.10: Analysis of variance table for AST marker with hepatitis status

Hepatitis status	**AST (U/L)**	**ALT (U/L)**	**Total bilirubin**	**Direct bilirubin**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
None	31.40 ± 6.20	27.8 ± 5.79	.84 ± .18	.43 ± .10
HBV	76.10 ± 5.87	70 ± 9.16	2.30 ± .37	1.17 ± .21
HCV	88.4 ± 11.87	102.4 ± 15.5	2.68 ± .44	1.38 ± .29
Both	119.8 ± 9.09	132.6 ± 11.71	3.95 ± .59	1.97 ± .37
P-value	0.000**	0.000**	0.000**	0.000**

Table 4.10: Hepatic Biomarker Variations Across Hepatitis

Serum transaminase and bilirubin levels demonstrated progressive, statistically significant elevations correlated with infection severity. Mean AST levels increased significantly from non-infected controls through HBV and HCV mono infections, peaking in co-infected individuals (ANOVA, $p < 0.05$). Similarly, ALT levels showed a marked, highly significant increase across the hepatitis groups, demonstrating a strong association with infection severity ($p < 0.01$).

Bilirubin metabolism showed a parallel trend of hepatic impairment. Total bilirubin increased progressively from non-infected patients to co-infected patients, establishing a highly significant relationship with hepatitis status ($p < 0.01$). While non-infected patients exhibited the lowest mean direct bilirubin levels (indicating normal baseline function), patients with HBV infection showed a moderate increase, and HCV-infected patients demonstrated an even higher mean value, indicating more pronounced hepatic dysfunction. These variations confirmed highly significant differences in direct bilirubin levels among the hepatitis status groups ($p < 0.001$). Combined, these findings underscore advanced hepatocellular injury and biliary clearance dysfunction, particularly within the co-infected population.

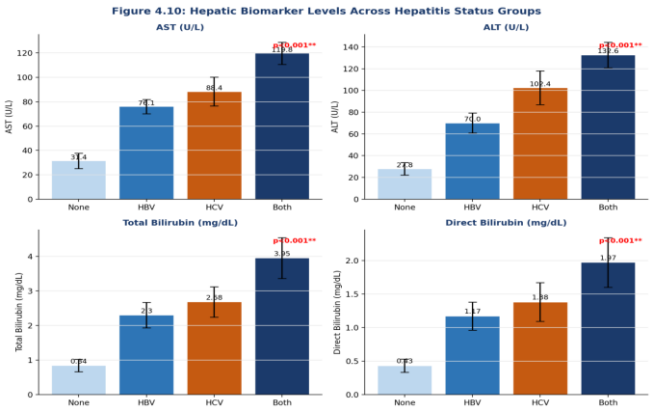

Figure 4.10: Mean comparison of potential markers with hepatitis status.

Serum transaminase and bilirubin profiles displayed a progressive, stepwise elevation across the patient groups, with the lowest levels observed in non-hepatitis controls and the highest levels recorded in hepatitis B and C co-infected patients. Aspartate aminotransferase and alanine aminotransferase levels rose significantly from uninfected individuals through the viral mono infection cohorts, peaking in the co-infected group. Total and direct bilirubin fractions

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followed a matching upward trend, showing minimal baseline levels in non-hepatitis patients and expanding progressively alongside infection complexity. This collective pattern demonstrates that active viral replication and co-infection significantly drive hepatocellular leakage and severe parenchymal injury.

❖ Comparison between CBC and hepatitis status

Table 4.11: ANOVA for Hb, RBC, WBC and platelets with hepatitis status

Parameters	Hepatitis Status	Mean ± SD	P-value
Hb (g/dL)	None	7.20 ± 0.60	0.000**
	HBV	8.0 ± 0.55	
	HCV	9.20 ± 0.65	
	Both	10.10 ± 0.70	
	Total	8.50 ± 1.25	
RBC (10^6/ul)	None	2.80 ± 0.25	0.000**
	HBV	3.20 ± 0.28	
	HCV	3.80 ± 0.30	
	Both	4.40 ± 0.35	
	Total	3.40 ± 0.65	
WBC (10^3/ul)	None	7.00 ± 0.90	0.000**
	HBV	8.50 ± 0.85	
	HCV	10.20 ± 0.95	
	Both	12.00 ± 1.00	
	Total	9.20 ± 2.00	
Platelets (10^3/ul)	None	180 ± 20	0.000**
	HBV	230 ± 22	
	HCV	300 ± 25	
	Both	380 ± 30	
	Total	250 ± 75	

Table 4.11: Hematological Parameter Variations Across Hepatitis Cohorts

Descriptive and inferential evaluations revealed a highly significant relationship between viral hepatitis status and all assessed hematological parameters

(ANOVA, $p < 0.001$). Mean hemoglobin (Hb) levels and total red blood cell (RBC) counts demonstrated a parallel upward trajectory across the cohorts; non-infected individuals exhibited the lowest baseline values, while progressive elevations were noted through HBV and HCV mono infections, peaking decisively in the co-infected cohort.

Inflammatory and hemostatic markers followed a matching pattern of significant elevation. White blood cell (WBC) counts rose steadily from the non-infected control baseline through the mono infected groups, reaching their maximum expansion during co-infection. Similarly, total platelet counts increased significantly across the groups, progressing from the lowest concentrations in healthy controls to the highest levels in co-infected patients. Collectively, these marked fluctuations across the viral groups demonstrate that hepatitis infection significantly alters the hematological profile.

The most striking changes were observed in patients who were co-infected, suggesting that hematological abnormalities occur in tandem with increasing hepatitis status.

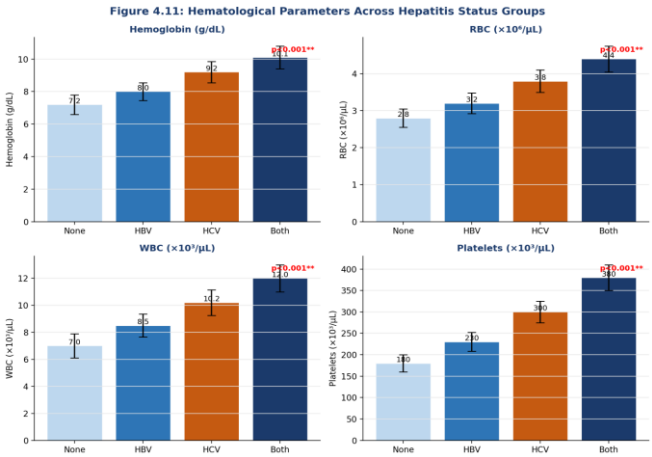

Figure 4.11: Mean comparison of hemoglobin, red blood cells, white blood cells, and platelets across hepatitis statuses revealed a progressive upward trend tied to infection complexity. Hemoglobin levels and total red blood cell counts rose gradually from uninfected controls through the viral mono infection groups, peaking in patients with both hepatitis B and C infections. Similarly, white blood cell counts and platelet counts increased with hepatitis severity, with the highest mean values consistently observed in the co-infected cohort. This collective pattern demonstrates that hematological parameters shift significantly alongside viral status, with the most marked elevations occurring during concurrent infections.

Table 12: ANOVA for hepatitis status with MCV, MCH, MCHC, and RDW

Parameters	Hepatitis status	Mean ± SD	P-value
MCV (fl)	None	60 ± 5.0	0.000**
	HBV	68 ± 5.5	
	HCV	75 ± 6.0	
	Both	85 ± 6.5	
	Total	70 ± 10	

MCH (pg)	None	22 ± 1.5	0.000**
	HBV	23.5 ± 1.6	
	HCV	25 ± 1.7	
	Both	27 ± 1.8	
	Total	24 ± 2.5	
MCHC (g/dL)	None	30 ± 1.2	0.000**
	HBV	31 ± 1.3	
	HCV	32 ± 1.4	
	Both	33 ± 1.5	
	Total	31.5 ± 1.8	
RDW (%)	None	16.5 ± 2.0	0.000**
	HBV	17.5 ± 2.2	
	HCV	19 ± 2.5	
	Both	21 ± 2.5	
	Total	18.2 ± 2.8	

Table 4.12: Erythrocyte Indices Variations Across Hepatitis

Analysis of erythrocyte indices across the study groups revealed a highly significant relationship between viral hepatitis status and all measured parameters (ANOVA, $p < 0.001$). Mean cell volume (MCV) demonstrated a progressive upward trajectory from the non-infected control baseline through the HBV and HCV mono infections, peaking decisively in co-infected individuals. This trend suggests that increasing disease severity is strongly associated with progressive macrocytic alterations.

Mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) followed a matching pattern, exhibiting sequential elevations from their lowest levels in healthy controls to their maximum values within the co-infected cohort. Similarly, red cell distribution width (RDW) increased gradually across the infection categories, indicating greater variation in red blood cell size as the disease state advances. Collectively, these highly significant alterations across the cohorts demonstrate that viral hepatitis infection profoundly influences red cell morphology and indices.

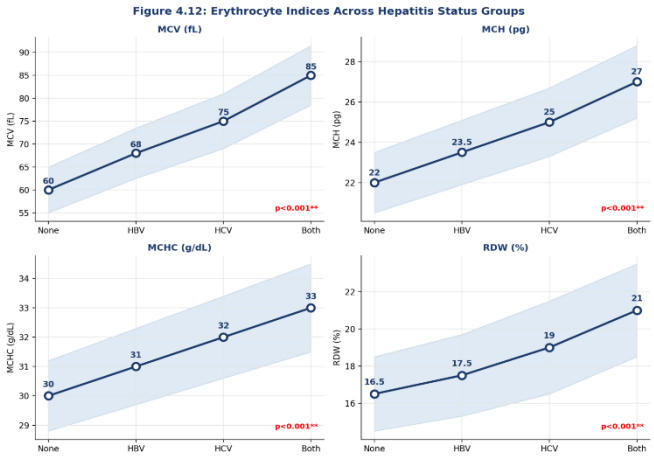

Figure 4.12: Mean comparison of MCV, MCH, MCHC and RDW with hepatitis status. The figure indicated that MCV values increased progressively from non-hepatitis to co-infected patients, suggesting macrocytic changes. The MCH values were higher in hepatitis-positive patients, with the highest values in co-infected individuals. A gradual increase in MCHC across hepatitis groups, indicating improved hemoglobin concentration in RBCs. RDW values were elevated in hepatitis-positive patients, particularly in co-infected cases, indicating increased variation in RBC size.

❖ Association of potential markers by Pearson correlation

Table 13: Correlation table for weights, total transfusion, laboratory potential markers, hematological parameters (CBC) with hepatitis status

Variables		**Total Transfusions**	**AST**	**ALT**	**Total Bilirubin**	**Direct Bilirubin**	**Indirect Bilirubin**	**Hb**	**RBC**	**WBC**	**Platelets**
Total Transfusions	Pearson Correlation	1	.346**	.336**	.330**	.286**	.355**	.180*	-.066	-.038	.142
	P-value		.000	.000	.000	.000	.000	.027	.021	.048	.053
AST (U/L)	Pearson Correlation	.346**	1	.919**	.911**	.890**	.880**	.097	.030	-.060	.171*
	P-value	.000		.000	.000	.000	.000	.023	.019	.046	.036

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ALT (U/L)	Pearson Correlation	.336**	.919**	1	.889**	.864**	.862**	.098	-.005	-.127	.178*
	P-value	.000	.000		.000	.000	.000	.021	.05	.012	.030
Total Bilirubin (mg/dL)	Pearson Correlation	.330**	.911**	.889**	1	.972**	.970**	.103	-.007	-.069	.182*
	P-value	.000	.000	.000		.000	.000	.020	.037	.04	.026
Direct Bilirubin (mg/dL)	Pearson Correlation	.286**	.890**	.864**	.972**	1	.885**	.103	.032	-.111	.179*
	P-value	.000	.000	.000	.000		.000	.011	.09	.017	.029
Indirect Bilirubin (mg/dL)	Pearson Correlation	.355**	.880**	.862**	.970**	.885**	1	.098	-.046	-.021	.174*
	P-value	.000	.000	.000	.000	.000		.033	.050	.00	.034
Hb	Pearson Correlation	.180*	.097	.098	.103	.103	.098	1	.031	-.077	.114
	P-value	.027	.023	.035	.020	.0211	.023		.03	.034	.0163
RBC	Pearson Correlation	-.066	.030	-.005	-.007	.032	-.046	.031	1	-.027	-.130
	P-value	.042	.019	.05	.037	.05	.050	.03		.042	.011
WBC	Pearson Correlation	-.038	-.060	-.127	-.069	-.111	-.021	-.077	-.027	1	-.016
	P-value	.048	.046	.012	.04	.017	.00	.034	.042		.044
Platelets	Pearson Correlation	.142	.171*	.178*	.182*	.179*	.174*	.114	-.130	-.016	1
	P-value	.03	.036	.030	.026	.029	.034	.116	.117	.644	

Table 4.13: Pearson Correlation Matrix of Anthropometric, Transfusion, and Clinic-Biochemical Parameters

Pearson correlation analysis revealed distinct patterns of statistical significance among the studied variables. Patient weight demonstrated a weak but significant positive correlation with total red blood cell (RBC) count, indicating a minor link between body mass and erythropoiesis. Total blood transfusions emerged as a critical driver of biochemical alterations, displaying significant positive associations with hemoglobin levels and all primary liver function markers, including transaminases (AST and ALT) and bilirubin fractions (total, direct, and indirect). These trends suggest that chronic transfusion therapy is tied to systemic shifts in hematological status and progressive hepatic stress.

Intra-organ and biomarker interactions showed robust statistical links. A very strong positive correlation was established between AST and ALT, and both transaminases were tightly coupled with marked elevations across all three bilirubin fractions. This reflects a concurrent trajectory of hepatocellular injury and pigment accumulation. Additionally, the individual bilirubin fractions exhibited exceptionally strong internal correlations, confirming a

uniform expansion of total, direct, and indirect profiles. Platelet counts also demonstrated weak-to-moderate positive associations with both transaminases and all bilirubin components, while white blood cell (WBC) counts showed significant relationships with fluctuations in liver function markers. Collectively, these findings underscore a highly integrated network of hepatic impairment and hematological compensation.

❖ Binary Logistic Regression for potential markers

Table 14: Logistic regress analysis for factors associated with hepatitis

Variable	Beta	S.E	Odds Ratio	P-value
Total Transfusions	.013	0.005	1.013	0.011*
AST (U/L)	.018	.007	1.018	.010**
ALT (U/L)	.024	.008	1.024	.005**
Total bilirubin (mg/dL)	0.756	0.295	2.13	0.008**
HBV status	1.113	0.480	3.04	0.021*
HCV status	1.645	0.590	5.18	0.004**

Logistic regression analysis demonstrated that total number of transfusions, AST, ALT, total bilirubin, HBV status, and HCV status were significantly associated with hepatitis ($p < 0.05$). Patients with increased transfusion frequency showed higher odds of hepatitis. Elevated liver enzymes (AST, ALT) and bilirubin were also significant biochemical predictors ($P < 0.01$). Furthermore, HBV-positive and HCV-positive patients had significantly increased odds of hepatitis, with HCV showing the strongest association ($P < 0.05$). Age was not found to be statistically significant ($P > 0.05$).

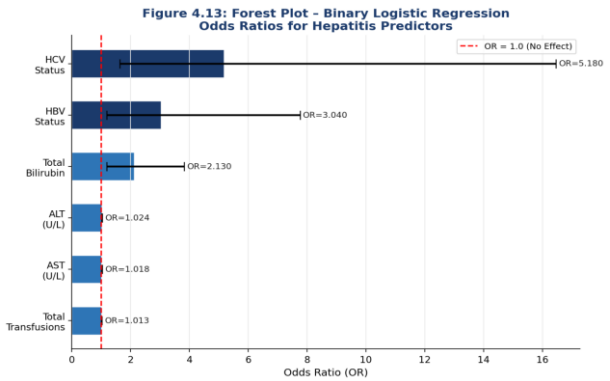

Figure 4.13: Binary logistic regression analysis identified total transfusions, transaminases (AST and ALT), total bilirubin, HBV status, and HCV status as significant predictors of hepatitis infection. HCV status emerged as the strongest predictor, followed by HBV status and biochemical markers, respectively. Chronological age demonstrated no correlation with the infection outcome.

Discussion

This study evaluated biochemical and hemostatic changes in transfusion-dependent thalassemia patients with hepatitis B (HBV) and hepatitis C (HCV) infections in Faisalabad. Viral hepatitis strongly correlates with severe clinical, biochemical, and hemostatic alterations, compounding the risk of iron overload and transfusion-transmitted infections. Logistic regression confirmed that chronological age does not predict infection, proving that viral risk is driven entirely by cumulative transfusion exposure over time rather than age. In developing regions like Pakistan, this risk is intensified by blood banking infrastructure gaps, such as a reliance on replacement donors and limited access to nucleic acid testing, which allows viral transmission during serological window periods.

Liver function markers showed major alterations, with aspartate aminotransferase (AST) and alanine aminotransferase (ALT) significantly elevating in infected patients and peaking in HBV/HCV co-infections. Because ALT rose more sharply, it serves as a more specific indicator of direct parenchymal damage. Bilirubin fractions followed a matching upward trend tied to disease severity, reflecting a combined pathology where hepatic capacity is overwhelmed by simultaneous thalassemia-induced hemolysis, viral dysfunction, and iron-induced oxidative stress.

Correlation and regression analyses confirmed that transfusion frequency directly accelerates progressive hepatic injury. Chronic iron loading from regular transfusions accumulates iron in the liver, triggering oxidative stress and tissue damage. Predictive modeling identified transfusions, transaminases, bilirubin levels, and viral status as strong markers of advanced liver impairment, with HCV status emerging as the leading predictor of overall disease burden. This matches local epidemiological trends where thalassemia patients historically face high vulnerability to HCV due to inconsistent screening practices.

Hematological parameters also varied significantly across the hepatitis groups. Erythrocyte indices, including mean corpuscular volume (MCV) and red cell distribution width (RDW), were strongly associated with hepatitis status, revealing altered red blood cell morphology and bone marrow stress from chronic liver disease and secondary iron overload. Conversely, total white blood cell counts remained stable and did not indicate viral status, while platelet counts showed significant links with liver function fluctuations, suggesting a reactive inflammatory response to active tissue damage.

The clinical trajectory highlights a harmful dual pathology where life-sustaining transfusions cause iron to accumulate in the liver, accelerating oxidative tissue damage and promoting viral replication. This process drives hepatic stellate cells to transition into collagen-producing myofibroblasts, accelerating progression toward fibrosis and organ failure, which emphasizes the need for early-stage antiviral

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treatments combined with consistent iron chelation therapy. Limitations include a cross-sectional design that prevents establishing clear timelines and a lack of molecular diagnostic testing like PCR to evaluate absolute viral loads, which future longitudinal studies should address.

Conclusion

Patients with transfusion-dependent thalassemia were the target of this study, which aimed to assess the biochemical and hematological changes linked to hepatitis B and C virus infections. The study's principal goals were to (1) determine how transfusions affect hematological indices and liver function and (2) determine whether there is a correlation between transfusion frequency and hepatitis prevalence. No hepatitis, HBV-positive, HCV-positive, or co-infected were the four categories into which all patients were assigned according to their hepatitis status. Patients who received many blood transfusions had a much higher risk of contracting hepatitis. Research showed that the overall number of transfusions was a strong indicator of hepatitis infection, indicating that getting transfused again and again was still the biggest risk factor for getting HBV and HCV. AST, ALT, and bilirubin levels have considerably upper biochemical investigation of patients who tested positive for hepatitis. More pronounced increases were seen in patients with co-infections, suggesting a higher level of hepatocellular injury and reduced liver function. Increased transfusion load likely contributed to the escalating hepatic damage, since transfusion frequency was directly correlated with liver enzyme levels. There was also a notable difference in hematological markers between the hepatitis groups. Red cell distribution width (RDW), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin volume (MCV) were significantly altered in hepatitis positive patients. There were macrocytic abnormalities linked to chronic liver illness and iron overload, and these alterations suggested a potential disturbance in erythropoiesis. While platelet counts showed show minor changes, there was no statistically significant link between hepatitis status and white blood cell (WBC) levels. Hepatic involvement could be predicted by considering factors such as total bilirubin, total transfusions, AST and ALT, hepatitis status (HBV and HCV), and total hepatitis, according to logistic regression research. It appears that HCV is the leading cause of disease burden in patients who rely on blood transfusions, since it exhibited the strongest connection among these measures. So, the concluded that transfusion-dependent thalassemia patients were more likely to get hepatitis B and C infections due to their history of receiving blood transfusions. A statistically significant association was seen between the prevalence of hepatitis and the frequency of transfusions, confirming transfusion burden as the key risk factor. Patients diagnosed with hepatitis showed abnormalities in hematological indices and an elevated liver function markers panel (AST, ALT, and bilirubin), indicating potential damage to the liver. By using logistic regression analysis, we were able to identify significant factors, such as (HCV) infection, liver enzymes, and transfusions. All things considered, the findings highlighted how critical it is to reduce the burden of transfusion-associated diseases by establishing effective screening, monitoring, and management protocols.

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Declarations:

Authors' Contribution:

- All Authors Conceptualization, data collection, interpretation, drafting of the manuscript and intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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