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PROSPECTIVE EVALUATION OF HBV, HCV, HIV AND SYPHILIS PREVALENCE IN B-THALASSEMIA MAJOR PATIENTS BEFORE AND AFTER BLOOD TRANSFUSIONS.

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Abstract

Patients with β-thalassemia (major) frequently require blood transfusions, which puts them at increased risk of transfusion-related infections (TTIs). The transfusion transmitted infections are HCV, HBV, HIV and Syphilis. The main victims of TTIs are β-thalassemia (major) patients because they require regular blood transfusions which are very necessary for the survival of patients. The major complications are HCV, HBV, HIV and Syphilis as their causative agents are transferred consciously or unconsciously to the recipients. To check the infection rates in β-thalassemia (major) patients before and after blood transfusion is the main objective of this study. The other objective is to check the reliability of Immunochromatography Technique (ICT) and Chemiluminescence Immunoassay (CLIA) in detecting these infections. A total of 421 β-thalassemia (major) patients were selected initially but 21 patients were reactive with HCV so they excluded from study. The remaining 400 β-thalassemia (major) patients were selected for the study. Blood samples were collected from patients before blood transfusion and then after 3 months of 1st transfusions and then 6 months of 2nd blood transfusions and results were analyzed. The serum/plasma was separated by centrifugation and stored in Ultra-low at -70°C until further processing. The results showed an increased in the prevalence of infections among patients over time. Initially, all β-thalassemia (major) patients were

free of infections. After the three months of 1st blood transfusions 3.5% of infections were detected in β-thalassemia (major) patients using Immunochromatography Technique (ICT) and 4% detected using Chemiluminescence Immunoassay (CLIA). The prevalence of Hepatitis C was 3.5% which is greater than other TTIs. The overall infection rates were increased after 6 months of blood transfusions. The prevalence of HCV increased by 4% and Hepatitis B (HBV) 0.50%. HIV was detected only in case of ICT technique but the CLIA did not confirm it. The specific analysis of age indicated a high infections rate of Hepatitis C was found in 11-20 years age group compared to other age groups. These results indicated that transfusions transmitted infections were increased with multiple transfusions. The study concluded that β-thalassemia patients are at high risk of transmitted transfusion infections. Hepatitis C is more prevalent infections in β-thalassemia patients. Infection rates were increased from 4% to 4.5 with successive transfusions. Two techniques ICT and CLIA were used for the detection of TTIs. Both techniques were effective for detecting TTIs but CLIA is more reliable than ICT due to capturing weak positive infectious agents which were not detected with ICT.
Chi square analysis revealed that there is no significant association between blood transfusions with transmitted transfusion infections. Future studies should be focused on the development of more advance and sensitive techniques for the detection of transmitted transfusion infections in β-thalassemia patients. Proper infections control practices and awareness about thalassemia will be helpful to minimize the infections rates.

Key Words: Transfusion transmitted infections (TTIs), β-thalassemia (major), Chemiluminescence Immunosorbents Assay (CLIA), Immunochromatography technique (ICT).

Introduction

β-thalassemia are diverse group of hereditary Hemoglobinopathies that are inherited by autosomal recessive inheritance and have abnormalities in β-globin chain of hemoglobin (1). When there is imbalance between α and non- α, globin chain is produced in unequal amount in homozygous and heterozygous form, there is ineffective erythropoiesis and as a result normal production of hemoglobin A is reduced (2). Thalassemia is a hereditary blood disorder that causes hemoglobin, the primary component of red blood cells and the oxygen transporter to be below normal, making it a major global health concern (3). This is abnormal condition which passed from parents to their children and effect the production of hemoglobin. The nature and severity of thalassemia vary, some children show signs from birth, while others develop symptoms within their first two years of life. The major cause of thalassemia is aberrant structure of hemoglobin. Anemia causes the destruction of erythrocytes as a result more RBCs produced and cause many complications like splenic enlargement, low bone density and heart diseases (4). Thalassemia is a single gene illness which cause by group of inherited conditions that in decreased or complete absence of β-globin chain formation, which lower the level of hemoglobin below normal range. Due to the lack of globin chain synthesis less O2 is bonded and transferred throughout the body which is caused by hemoglobin. Hemoglobin composed of four polypeptide chain α, β, γ, and δ. B-thalassemia occur due to defect in β-chain (5).
Thalassemia comes in three varieties. Thalassemia minor is a heterozygous condition that results in a 20% reduction in the synthesis of polypeptides (6). The additional HbA2 produced makes up for this decrease. The patient has simple anemia and is asymptomatic due to a single chain problem (7). Patients with intermediate thalassemia, a condition that is between minor and severe types, can lead regular lives but only occasionally need blood transfusions while they are ill. Thalassemia major is a homozygous type which totally stopped the β chain production so it has both mutant alleles (8). The generation of more HbF makes up for this decline. Because of their severe anemia, patients need regular blood transfusions to maintain a normal lifestyle. The majority of infected infants do not always survive birth. It was discovered that the ß-globin gene, which is located on chromosome 11, may result from more than 200 different mutations. Different mutations causing ß-chain malfunction might happen in different ways. The prediction of infection severity is examined by molecular DNA

techniques that analyze mutations in thalassemia patients, particularly in fetus in the earliest stages of pregnancy (prenatal analysis) (9).
People with long-term blood disorders like beta thalassemia major need regular blood transfusions. The most serious transfusion-related complications are transfusion-transmitted infections (TTIs), which include a variety of bacterial, viral, and parasitic infections. These include hepatitis C, hepatitis B, HIV and Syphilis (10). Pakistan boasts one of the highest global rates of thalassemia major patients who are dependent on transfusions. The main causes of this are high birth rates, consanguineous marital customs, a high frequency of hemoglobin β-subunit gene mutations, and an expanding population (11). Between 5,000 and 9,000 new instances of thalassemia major are thought to be diagnosed nationwide each year. β-thalassemia has historically been associated with significant rates of morbidity and mortality. But there have been major clinical and scientific advances in the treatment of TM, such as the creation of iron chelation therapy, novel gene therapies, bone marrow transplantation, safe blood transfusion practices based on evidence, and a decrease in transfusion-transmitted infections (12).

To boost hemoglobin levels, stop ineffective erythropoiesis, improve oxygen supply to tissues, encourage tissue growth, and improve general health, a regular transfusion program was initiated in the 1960s (13). One of the most crucial therapeutic methods in contemporary medicine is blood transfusion. On the other hand, there is a chance that the recipient will experience severe adverse effects. It has long been known that blood transfusions can spread bacterial infections. Blood-borne viruses including the human immunodeficiency virus (HIV), HBV, HCV and others remain the most common cause of morbidity and death globally (14). As a result, the World Health Organization (WHO) recommends that all blood be tested for HIV, syphilis, HBV, and HCV. In Pakistan, β-thalassemia major (TM) is a common inherited hemoglobinopathy. With an estimated 100,000 current cases, it has one of the highest prevalence rates of transfusion-dependent TM patients globally (15). Patients with thalassemia have an alarmingly higher infections rate, particularly HCV than the general population.

The purpose of this study was to ascertain the prevalence of infections caused by transfusions in individuals with β-thalassemia, both before to and following blood transfusions. For that purpose, blood samples of β-thalassemia patients were collected from renown thalassemia center of Lahore for detection of Transfusion transmitted infections.

Methodology

Study design

A survey was conducted on β-thalassemia patients from October 2023 to May 2024 at Sundas Foundation Lahore. The clinical histories of patients were taken and the complications they had during blood transfusions were noted. There were 400 significant participants included in our study. The purpose of the study was to find out how common HBV, HCV, HIV and syphilis were in individuals with β-thalassemia (major) patients both before and after blood transfusions.

Exclusion criteria: At the beginning of study, 421 patients with β-thalassemia (major) were selected. There were 21 patients were detected with HCV so they excluded from the study.

Sample Collection: Blood samples were obtained from patients with β-thalassemia (major) across various age groups. These samples were gathered from various Thalassemia centers in Lahore, such as the Noor Thalassemia Foundation and the Sundas Foundation Lahore. Until further processing, the drawn blood samples were kept in Ultra-low in Sundas Foundation Lahore at a temperature of -70 C* until further processing.

Screening of blood samples through Immune-Chromatography Technique (ICT) before blood transfusions.

The collected blood samples were screened for the Anti-HCV, Hepatitis B Virus, HIV and Syphilis by ICT Technique before blood transfusions. This test is based on lateral flow chromatographic immune assay. This cassette device consists of conjugate pad and nitrocellulose membrane containing a test line. When specimen dispensed at sample well, the specimen moves across capillary action. If antibody is present in its specimen, it will bind to its conjugate.

Screening for Hepatitis C: The screening of all blood samples was performed to detect the presence of antibodies in serum or plasma of specimen. For this purpose, commercially available Acu-check device (Anti-HCV device, made in USA) used with according to the given instructions (Gene Biotech, USA).

Screening for Hepatitis B: A quick chromatographic immunoassay for the qualitative identification of hepatitis B surface antigen in serum or plasma is the HBsAg one step hepatitis B surface antigen test device.

Screening for HIV: The HIV ½ Ultra rapid test device (serum/plasma) is a rapid test to qualitatively detect the presence of antibodies to HIV 1 and HIV 2 in plasma or specimen. The test utilizes gold conjugate and multiple recombinant HIV proteins to selectively detect antibodies to the HIV ½ in serum or plasma.

Principle: The HIV ½ immunoassay, which uses a twofold antigen system and is membrane-based and qualitative, is used to detect antibodies to HIV ½ in serum or plasma. Recombinant HIV antigen is pre-coated on the membrane. The test strip's colloidal gold particle coating reacts with serum or plasma specimens during testing. After that, the mixture migrates upward on the membrane chromatographically through capillary action, reacting with the HIV antigen to produce a colored line that denotes a positive result and an absence of it that denotes a negative result. Every time a specimen is put in the proper amount and membrane wicking has taken place; a colorful line always emerges at the control line region.

Screening for Syphilis: The syphilis is a rapid immunoassay for the qualitative detection of antibodies (IgG and IgM) to treponema pallidum in serum or plasma to aid in diagnosis of syphilis.

Principle: The syphilis is a lateral flow chromatographic immunoassay based on the double antigen-sandwich technique. In this test, syphilis recombinant antigen is immobilized in the test line region of the strip in the test device. After specimen is added to the specimen well of the device, it reacts with syphilis recombinant antigen coated particles in the test. The chromatographic mixture is then migrating upward through capillary action, reacting with the syphilis antigen to produce a colored line that indicates a positive result or its absence, which signals a negative result. A colored line always appears at the control line region, indicating that the correct volume of specimen has been added and membrane wicking has occurred.

Screening of all samples through Chemiluminescence Immunoassay before blood transfusions. (CLIA Method)

Chemiluminescence immunoassay (CLIA) is an assay that combine chemiluminescence technique with immunochemical reactions. Similar with other labeled immunoassays (RIA, FIA, ELISA), CLIA utilize chemical probes which could generate light emission through chemical reaction to label the antibody (Kricka, 2003). Enzyme-linked immunosorbent assays (ELISAs) are among the most extensively used types of IA and are safer and easier than the RIA. ELISA could be based on colorimetric, fluorescence or chemiluminescence (CL) detection (Zhao, Sun, & Chu, 2009). To perform the comparative study all blood samples performed on chemiluminescence immunoassay ARCHITECT (i1000SR). The chemiluminescence immunoassay is used for the detection of HCV, HBsAg, HIV and Syphilis in beta thalassemia major patients. The CLIA is suitable for the bulk of the sample and represent high magnitude of sensitivity. It detects larger markers of the infections and has a advantage due to least interference emission, wide dynamic range and rapid analytical signals.

Principle of Chemiluminescence Immunoassay: When complementary antigen and antibody are present, then the paratope of antibody bind with the epitope of antigen results into antigen-antibody complex. Estimation of antigen-antibody complex by applying labeled antibodies. It engrosses the stationary solid particles coated prior used for concern antigen or antibody. After incubation when antigen-antibody complex is formed then substrate is added which result is light production that is proportional to magnitude of complexes present. Result of light intensity is measured in the units of relative light units (RLU). Its major advantage is its sensitivity with ability to not affected by the background signals as well as its simplicity of design and operation.

Screening of all β-thalassemia patient's samples through ICT and CLIA after 1st blood transfusions (post-3 months).

Blood samples were collected again after 3 months of 1st blood transfusions. These collected samples were tested for HCV, HBV, HIV and Syphilis through ICT and CLIA techniques and results were analyzed. The results indicated that Transmitted transfusions infections were increase as the number of blood transfusions increase. The TTIs for HCV, HBV, HIV and Syphilis were 85%, 7.14%, 7.14% and 0% through ICT technique respectively and 87% for HCV 12% for HBV through CLIA technique. HIV and Syphilis was found to be 0% through CLIA technique.

Screening of all β-thalassemia patient's samples through ICT and CLIA after 2nd blood transfusions (post-6 months).

Blood samples were collected for the third time after 6 months of 2nd blood transfusions. These collected samples were tested for HCV, HBV, HIV and Syphilis through ICT and CLIA techniques and results were analyzed. The results indicated that Transmitted transfusions infections were increase as the number of blood transfusions increase. The TTIs for HCV, HBV, HIV and Syphilis were 83%, 11%, 5.56% and 0% through ICT technique respectively and 88% for HCV 11% for HBV through CLIA technique. HIV and Syphilis was found to be 0% through CLIA technique.

The statistical analysis revealed that there are no significant associations between TTIs with number of transfusions, age and gender. The P value for TTIs with multiple transfusions found 0.43 which is greater than significant (P=0.05) level. So, we can say P value is not significant.

Result

Prevalence of β-thalassemia (major) patients

Our research investigation included 400 patients with β-thalassemia (major). Blood samples were collected at Sundas Foundation, a thalassemia center of Lahore. Out of these 400 patients, 251 (63%) patients were male and 149 (37%) patients were female. All thalassemia patients were categorized into three age groups as shown in (Table 1).

Table 1: Prevalence of β-thalassemia major patients in different age groups

Patients age group	Total No. Of Patients	Male Patients	Female Patients
Age group 1 (1-10 years)	100	68 (68%)	32 (32%)
Age group 2 (11-20 years)	200	123 (61%)	77 (39%)
Age group 3 (21-30 years)	100	60 (60%)	40 (40%)

Prevalence of infections among β-thalassemia (major) patients before blood transfusions (ICT Method).

There were 421 β-thalassemia (major) patients selected under study but 21 patients were excluded from study because they did not fulfill the criteria. They selected only those patients (400) who were free from infections.

Prevalence of infections among β-thalassemia (major) patients before blood transfusions (CLIA Method).

There was a total of 400 β-thalassemia major patients selected. All blood samples were screened through a chemiluminescence immunosorbent assay (CLIA) technique to detect the transmitted transfusion infection. All samples were non-reactive as they were free of infections.

Prevalence of infection among β-thalassemia (major) patients after 1ST transfusion
(Post 3 month)

Immunochromatography technique (ICT): There were 400 patients with β-thalassemia (major) screened through immunochromatography technique (ICT) for the detection of transmitted transfusion infections after 3 months of blood transfusion. Infection percentage detected through ICT method after first transfusion. Most prevalent infection was Hepatitis C (85%) followed by Hepatitis B (7%) and HIV (7%). No Syphilis was detected through ICT.

Chemiluminescence Immunosorbent Assay (CLIA)

There were 400 patients with β-thalassemia (major) screened through Chemiluminescence immunosorbent assay technique (CLIA) for the detection of transmitted transfusion infections after 3 months of blood transfusion. Infection percentage detected through CLIA method after first transfusion is presented in the Pie chart (Figure 1). Most prevalent infection was Hepatitis C (87%) followed by Hepatitis B (12.5%). No HIV and Syphilis was detected through CLIA method.

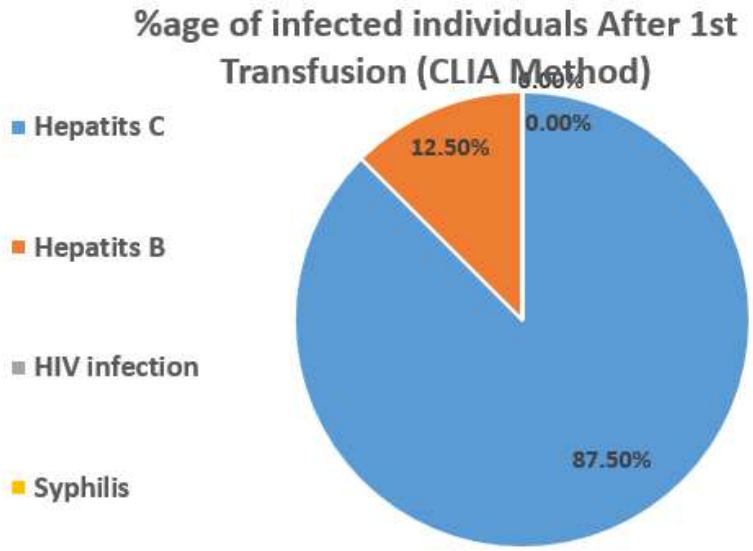

Figure 1: Pie chart of infection detected through CLIA method after first transfusion. Most prevalence infection Hepatitis C followed by Hepatitis B, HIV and Syphilis.

Comparison of Immunochromatography technique (ICT) and Chemiluminescence immunosorbent assay technique (CLIA) after 1st transfusions.

All 400 samples were examined through ICT and then CLIA. The summary of prevalence of 4 infections examined through ICT and CLIA are presented in (Table 2) for comparison. CLIA detected a higher percentage of Hepatitis C (3.5%) and Hepatitis B (0.50%) than ICT (3.00% and 0.25% respectively). However, CLIA detected no HIV in the samples while ICT detected 0.25%. No Syphilis was detected either through ICT or CLIA. This comparison indicated that there is a difference in the detection of infection in both the techniques where overall CLIA detected higher percentage of the infections compared to ICT.

Table No 2: A comparison of TTIs in β-thalassemia major patients screened through ICT and CLIA methods after 1st transfusions.

Infectious Disease	Immunochromatography Method		Chemiluminescence Immunoassay Method	
	No. Of Infected Individuals	% Of Infected Individuals	No. Of Infected Individuals	% Of Infected Individuals
Hepatitis C	12	3.00%	14	3.50%
Hepatitis B	1	0.25%	2	0.50%
HIV	1	0.25%	0	0.00%
Syphilis	0	0.00%	0	0.00%

Prevalence of infection among β-thalassemia (major) patients after 2nd transfusion.
Post 6 month
Immunochromatography technique (ICT)

There were 400 patients with significant β-thalassemia were tested using the immunochromatography technology (ICT) for the detection of transmitted transfusion infections after 6 months of blood transfusion. Infection percentage detected through ICT method after second transfusion is presented in the Pie chart (Figure 2). Most prevalent infection was Hepatitis C (83%) followed by Hepatitis B (11.11%) and HIV (5.5%). No Syphilis was detected through ICT.

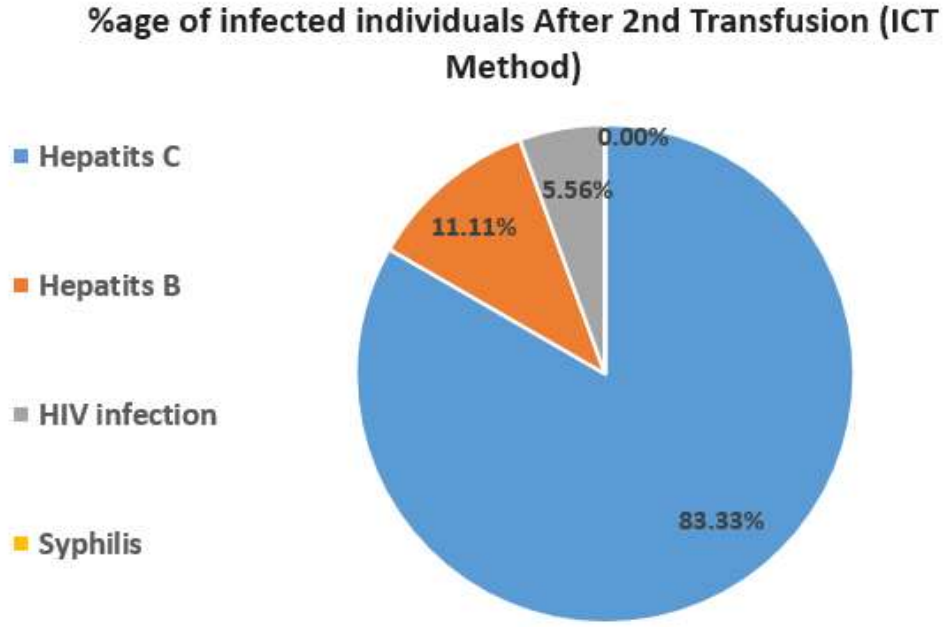

Figure 2: Pie chart of infections detected through ICT method after second transfusion. Most prevalent infection Hepatitis C followed by Hepatitis B, HIV and Syphilis.

Chemiluminescence Immunosorbent Assay (CLIA)

There were 400 β-thalassemia (major) patients were screened through Chemiluminescence immunosorbent assay technique (CLIA) for the detection of transmitted transfusion infections after 6 months of blood transfusion. Infection percentage detected through CLIA method after second transfusion is presented in the Pie chart (Figure 3). Most prevalent infection was Hepatitis C (89%) followed by Hepatitis B (11%). No HIV and Syphilis was detected through CLIA. The below Figures 8 and 9 were taken from Abbot Architect i1000 SR machine. These figures indicated the infection of HCV and HBV respectively.

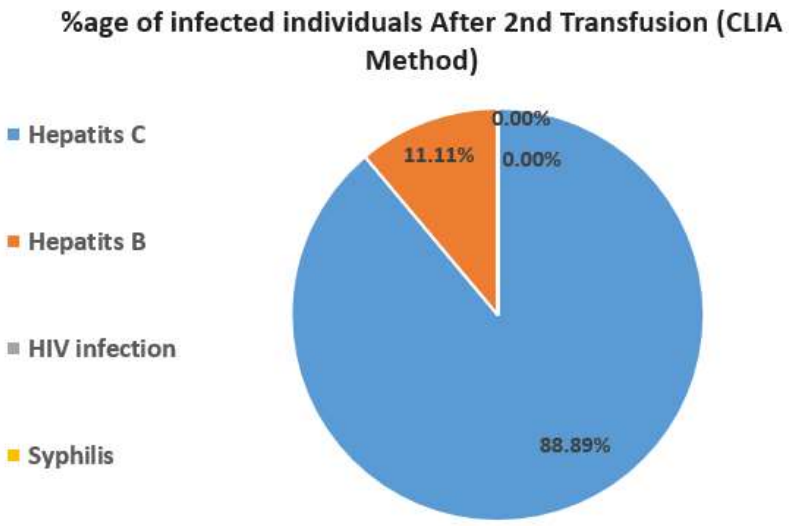

Figure 3: Pie chart of infection detected through CLIA method after second transfusion. Most prevalent infection Hepatitis C followed by Hepatitis B and HIV and Syphilis.

Comparison of Immunochromatography technique (ICT) and Chemiluminescence immunosorbent assay technique (CLIA) after 2nd transfusions.

All 400 samples were examined through ICT and then CLIA. The summary of prevalence of 4 infections examined through ICT and CLIA are presented in (Table 3) for comparison. CLIA detected a higher percentage of Hepatitis C (4%) than ICT (3.00%) but lower percentage of Hepatitis B (0.50%) than ICT (0.75%). However, CLIA detected no HIV in the samples while ICT detected 0.25%. No Syphilis was detected either through ICT or CLIA. This comparison indicated that there is a difference in the detection of infection in both the techniques where overall CLIA detected higher percentage of the infections compared to ICT.

Table No. 3: A comparison of TTIs in β-thalassemia major patients screened through ICT and CLIA methods after 2nd transfusions.

Infectious Disease	Immunochromatography Method		Chemiluminescence Immunoassay Method	
	No. Of Infected Individuals	% Of Infected Individuals	No. Of Infected Individuals	% Of Infected Individuals
Hepatitis C	15	3.75%	16	4.00%
Hepatitis B	2	0.50%	2	0.50%
HIV	1	0.25%	0	0.00%
Syphilis	0	0.00%	0	0.00%

Prevalence of infections in different age group of β-thalassemia (major) patients screened through ICT method

400 blood samples of patients were screened through ICT method and categorized in 3 groups. First group included the patients from age 1 to 10 years, group 2 included 11 to 20 years of age, group 3 included 21 to 30 years of age as given in Figure 4. The percentage of various infectious diseases calculated for different age groups. In case of group 1 the percentage of Hepatitis C was found to be 0.75%, Hepatitis B as 0.25%, HIV and Syphilis was detected as 0%. In case of group 2 the percentage of Hepatitis C was found to be 1.75%, hepatitis B as 0.25%, HIV and Syphilis as 0%. In case of group 3 the percentage of Hepatitis C was found to be 0.50%, HIV as 0.25%, Hepatitis B and Syphilis as 0% as shown in (Figure 4).

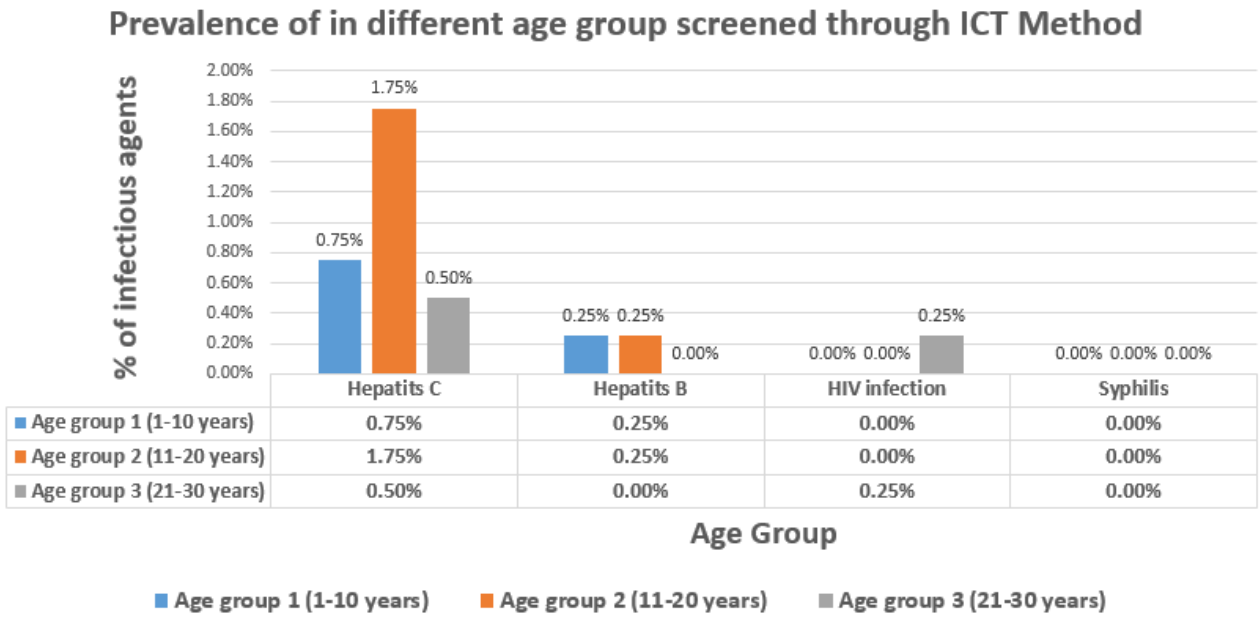

Figure 4: Prevalence of Transmitted Transfusion infections in different age group of β-thalassemia major patients screened through ICT method

Prevalence of TTIs in different age group of β-thalassemia major patients screened through CLIA method

400 blood samples of patients were screened through CLIA method and categorized into 3 groups. The first group included the patients from age 1 to 10 years, group 2 included 11 to 20 years of age, group 3 included 21 to 30 years of age as given in (Table 5). The percentage of various infectious diseases calculated for different age groups. In case of group 1 the percentage of Hepatitis C was found to be 1%, Hepatitis B, HIV and Syphilis was detected as 0%. In case of group 2 the percentage of Hepatitis C was found to be 2%, Hepatitis B as 0.50%, HIV and Syphilis as 0%. In case of group 3 the percentage of Hepatitis C was found to be 0.50%, hepatitis B as 0%, HIV and Syphilis also detected as 0% as shown in (Figure 5).

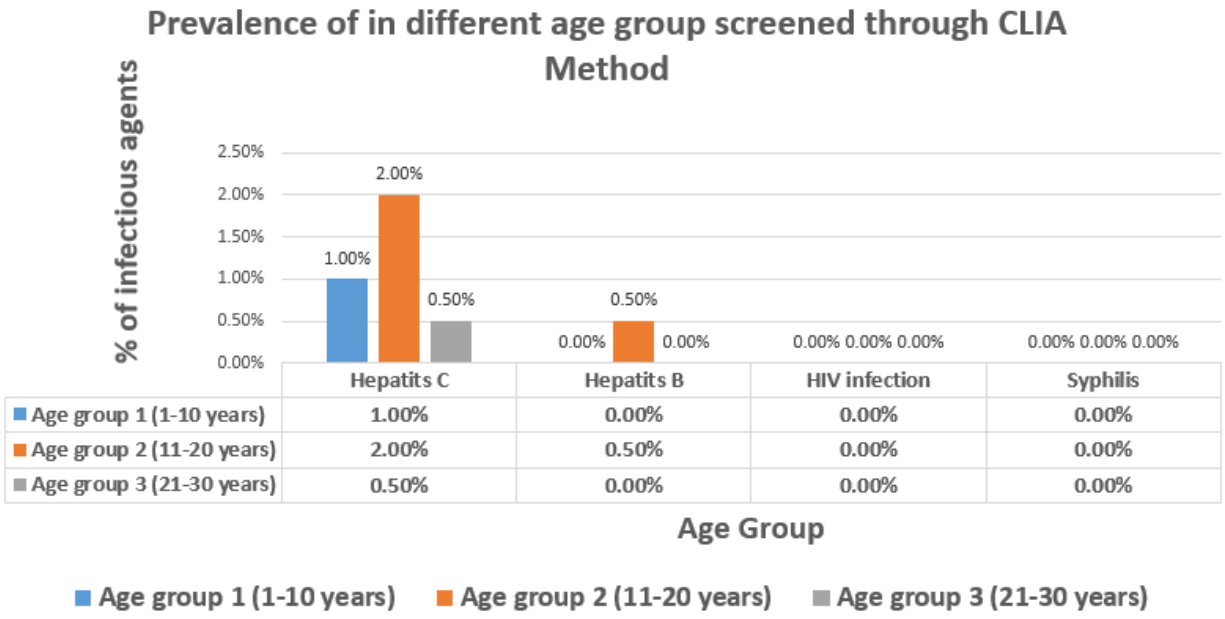

Figure 5: Prevalence of Transmitted Transfusion infections in different age group of β-thalassemia major patients screened through CLIA method

Prevalence of TTIs among male and female β-thalassemia major patients screened through ICT method

There was total 400 β-thalassemia major patients were screened through ICT method (Table 4). The prevalence of Hepatitis C in male was 3.98% while in female it was 1.34%. In case of Hepatitis B it was 0.40% and female 0%. In case of HIV infection, the prevalence was 0.40% in male and 0% in female. Prevalence of syphilis was 0% in both male and female.

Table 4. Prevalence of TTIs among male and female β-thalassemia major patients screened through ICT method

Table No. 4: Prevalence of TTIs through ICT method

Infectious Disease	Male	Female
Prevalence Of Hepatitis C	3.98%	1.34%
Prevalence Of Hepatitis B	0.40%	0.00%
Prevalence Of HIV	0.40%	0.00%
Prevalence Of Syphilis	0.00%	0.00%

Prevalence of TTIs screened through CLIA method

There was total 400 β-thalassemia major patients were screened through ICT method (Table 5). The prevalence of Hepatitis C in male was 4.38% while in female it was 2.01%. In case of Hepatitis B, it was 0.80% and female 0%. In case of HIV infection, the prevalence in both male

Table 5. Prevalence of TTIs through CLIA method

Infectious Disease	Male	Female
Prevalence Of Hepatitis C	4.38%	2.01%
Prevalence Of Hepatitis B	0.80%	0.00%
Prevalence Of HIV	0.00%	0.00%
Prevalence Of Syphilis	0.00%	0.00%

Statistical Analysis

IBM SPSS version 21 was used in this study to analyze the data statistically. The statistical analysis was done using chi-square test through IBM SPSS (21).

Anti-HCV association with Gender

The Chi-Square test results indicate that there is no significant association between the age with Anti-HCV infection in the study. The P-value is of 0.776, suggesting that the differences between observed and expected frequencies are not statistically significant as shown in (Table No 6).

Table No 6. Anti-HCV association with gender

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.081	1	.776		
Continuity Correction	.000	1	1.000		
Likelihood Ratio	.082	1	.774		
Fisher's Exact Test				1.000	.517
N of Valid Cases	400				

HBV association with Gender

The Chi-Square test results suggest that there is no significant association between the gender with hepatitis B being analyzed. The P value is 0.440 indicating that the differences between observed and expected frequencies are not statistically significant (Table 7).

Table No. 7.HBV association with gender

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.595	1	.440		
Continuity Correction	.000	1	1.000		
Likelihood Ratio	.934	1	.334		
Fisher's Exact Test				1.000	.628
N of Valid Cases	400				

HIV association with Gender

The Chi-Square test results indicate that there is no significant association between the HIV and patients' gender being analyzed. With a P-value of 0.194 and a Pearson Chi-Square test score of 1.689, it is possible that the variations between the observed and anticipated frequencies are not statistically significant. (Table No.8).

Table No.8 HIV association with gender

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.689	1	.194		
Continuity Correction	.070	1	.792		
Likelihood Ratio	1.979	1	.159		
Fisher's Exact Test				.373	.373
N of Valid Cases	400				

HCV association with Age

The Chi-Square test results indicate that there is no significant association between the HCV with age being analyzed. The Pearson Chi-Square test yields a p-value of 0.283, and the Likelihood Ratio test shows a p-value of 0.429, suggests that the observed differences in frequencies are not statistically significant (Table No 9)

Table No 9. HCV association with age

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	31.798	28	.283
Likelihood Ratio	28.678	28	.429
N of Valid Cases	400		

HBV association with Age

The Chi-Square test results show no significant association between the HBV infection with age. The Pearson Chi-Square test has a p-value of 0.991, and the Likelihood Ratio test has a p-value of 1.000, both indicating that there is no statistically significant relationship (Table No 10).

Table No.10. HBV association with age

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13.319	28	.991
Likelihood Ratio	5.352	28	1.000
N of Valid Cases	400		

HIV association with Age

The Chi-Square test results indicate that there is no significant association between the HIV with age. The Pearson Chi-Square test has a p-value of 0.753, and the Likelihood Ratio test shows a p-value of 1.000, both of which are well above the common significance level of 0.05. This suggests that any observed differences are not statistically significant (Table No.11)

Table No 11. HIV association with age

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	22.586	28	.753
Likelihood Ratio	6.374	28	1.000
No of Valid Cases	400		

Transfusions Association with HCV and HBV

P value of HCV and HBV is 0.43 and 0.93 respectively as shown in (Table No 12 and 13) which is greater than typical significance levels (e.g., 0.05) suggest that there is no significant evidence of a relation between transfusions and TTIs in the provided data between 3 months and 6 months after blood transfusions. So, the null hypothesis cannot be rejected.

Table No 12. Transfusion-HCV Association

Chi-Square Tests

	Value	Df	Asymp.Sig. (2-Sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.605	1	.437		
Continuity Correction	.001	1	.976		
Likelihood Ratio	.470	1	.493		
Fisher's Exact Test				.392	.392
No of Valid Cases	400				

Table No 13. Transfusion-HBV Association

Chi-Square Tests

	Value	Df	Asymp.Sig. (2-Sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.008	1	.931		
Continuity Correction	.000	1	1.000		
Likelihood Ratio	.015	1	.902		
Fisher's Exact Test				1.000	.992
No of Valid Cases	400				

Transfusion with HIV and Syphilis

There is no statistics computed in case of HIV and Syphilis because both values are constant.

Discussion

The blood disorder like Thalassemia is the most prevalent genetic illness worldwide (16). The basic cause of thalassemia is Genetic defect in the manufacturing of hemoglobin. In β-thalassemia (major) patients the prevalence of TTIs (transmitted transfusion infection) increased as a result of multiple transfusions (17). In the current research study prevalence of Transmitted transfusion infections was checked in β-thalassemia patients. A total of 400 β-thalassemia patients were included which were taken from different thalassemia center of Lahore like Sundas Foundation and Noor Thalassemia Foundation Lahore. The blood transfused in 400 β-thalassemia (major) patients. The overall results showed that the transmitted transfusion infections increased as a result of multiple transfusions. Hepatitis C is most prevalent in β-thalassemia patients than HBV, HIV and Syphilis (18).

There were 420 samples taken in which 400 samples were analyzed to be infection free. These selected samples constituted major male proportion (63%) and (37%) were female. The male β-thalassemia patients have more transmitted transfusions infections than female patients because female have two X chromosomes (XX) which provide an extra copy of genes involved in immune functions and inflammation while male have one X and one Y chromosomes (XY) which may increase their susceptibility to infections due to absence of second X chromosome, leaving them with only one copy of immune-related gene (19). The selected individuals were divided into three groups. The thalassemia patients with age 1 to 10 kept in group 1, while 11 to 20 were included in 2nd group and the last group was 21 to 30 years of age (20). These group of patients having 100 samples in first group, 200 samples in second group and the last group having 100 samples. In group 1 (1-10 years) a total of 100 patients were included in which male and female patients were 68 (68%) and 32 (32%) respectively (21). Similarly in group 2 (11-20 years), a total 200 patients were included out of which male patients were 123 (61%) and female were 77 (39%). In third age group 3 (21-30 years), a total of 100 patient were included, out of which 60 (60%) patients were male and female patients were 40 (40%). These patients were examined before and after blood transfusions. The blood samples were collected before blood transfusions and then after blood transfusions. The samples were taken after 3 months of first blood transfusions and then after 6 months of second blood transfusions (22).

The results were obtained from 400 β-thalassemia (major) patients screened through immunochromatography (ICT) method after blood transfusions for the detection of TTIs indicated the rate of TTIs as (4%) among beta thalassemia major patients (23). The remaining 96% patients were free of infections. In infected patients (4%) were infected with hepatitis C infection, (0.5%) were infected with hepatitis B, (0.2%) with HIV and No patients was infected with Syphilis (24). The percentage of various infectious diseases were calculated for different age group. In case of group 1, the percentage of Hepatitis C was found to 0.75 %, Hepatitis B as 0.25 %, HIV and Syphilis as 0 %. In case of group 2, the percentage of Hepatitis C was 1.75%, hepatitis B 0.25%, HIV and Syphilis were 0%. In case of group 3, the percentage of Hepatitis C was 0.50%, Hepatitis B as 0%, HIV was 0.25% and Syphilis also 0% (25).

Our study indicated that the highest patients were suffering with hepatitis C, hepatitis B and HIV. The prevalence of HIV and Syphilis was lowest among all the infectious diseases (26). The blood samples of beta-thalassemia patients were screened through ICT and CLIA methods. Iron chelation therapy and lifelong red blood cell transfusions are necessary for the treatment of β-thalassemia major, a hereditary hemoglobinopathy that can lead to problems from iron excess (27). Historically, the Mediterranean, Middle East, and Southeast Asia have been home to a higher prevalence of β-thalassemia. However, migration is the main reason why the frequency of β-thalassemia is rising in other areas, such as Northern Europe and North America. Out of 6 billion people, 530 million have been infected by HBV and HCV combined. According to the most recent study, among patients with multi-transfusion thalassemia, HCV was the most common TTI. The efficacious HBV vaccination is probably the reason why thalassemic individuals have a lower prevalence of HBV infections than HCV. HBV vaccination is quite efficient (80–100%) in reducing the incidence of HBV infection in those who receive it. It is also investigated by another researcher on 262 multi-transfused β-thalassemia patients were selected from Pakistan capital cities. He concluded that the prevalence of Hepatitis C was high in all transmitted transfusion infections. Hepatitis C and B was 55.7%, 3.08% respectively (28).

The prevalence of Hepatitis C is common in the general population and, consequently, among blood donors in many nations. The number of transfusions raises the prevalence rate of seropositivity (29). There are three primary forms of thalassemia: Thalassemia minor occurs when one of the beta globin chain genes is defective and the other is normal. Thalassemia intermedia is the word used to describe a condition that manifests later in life (in older children and adults) and involves both genes. When the problem appears in infancy and both genes are implicated, it is referred to as Thalassemia major, a more severe form of the disease (30). Many nations, including Iran, Greece, Italy, and Cyprus, have

previously effectively tackled thalassemia through educational efforts that raise public awareness of the condition and emphasize preventive methods for its elimination. In Pakistan, prenatal diagnosis and carrier detection facilities have been around for more than ten years, but due to a lack of public awareness and information, their use is still restricted (31). It will take time to understand the severity of the illness, and actionable efforts should be done to lower its prevalence. It is crucial to educate the individuals about the illness, its repercussions, and preventative measures.

The statistical analysis was performed to check the relationship between blood transfusions, age and gender with infections. It was noted that the infection rates appeared to increase with the number of blood transfusions (32). This observation suggested a possible link between the frequency of transfusions and higher infection rates. A statistical analysis was conducted. The primary objective was to determine if there was a significant association between the number of transfusions and the occurrence of infections (33). The results of the statistical tests showed that the P value was not statistically significant. This means that the evidence was insufficient to establish a meaningful or statistically reliable association between the number of blood transfusions and the incidence of transmitted infections. This analysis also indicated that age and gender did not have a significant association with infections. Finally, we concluded that the statistical analysis did not support a significant link between transfusions and infections (34). The relationship of gender and age with infections was checked by a researcher. He concluded that the P value was not significant ($P > 0.05$). So, it is statistically proved that there is no significant association between age, gender of β-thalassemia patients with infections.

It is investigated that prevalence of HCV is high respectively in multi-transfused β-thalassemia patients than HBV and HIV infections. Same type of study conducted by a researcher. He described that hepatitis C is most common transmitted transfusion infections in multi-transfused patients followed by HBV and HIV (35). Another study was performed by a researcher in west Bengal India. They conducted a study on 359 β-thalassemia patients. The final results of his study showed that hepatitis is the most prevalent infection among β-thalassemia (major) patients.

Conclusion

In study performed on β-thalassemia (major) patients, it has been observed that multiple blood transfusions can lead to transfusion-transmitted infections, including Hepatitis C Virus (HCV), Hepatitis B Virus (HBV), HIV, and some bacterial infections such as syphilis. However, statistical analyses have revealed no significant association between the frequency of transfusions and the incidence of these infections. The P value was found high for Hepatitis C ($P=0.47$) and Hepatitis B ($P=0.93$) which is statistically not significant. Meanwhile, there are many other factors involved which can transmit infections in β-thalassemia patients. These factors are inadequate screening practices, Contaminated injections or medication used during iron chelation therapy, inadequate infections control practices, use of reusable equipment's, immunocompromised status, Poor personal hygiene, unprotected sexual contact, use of drugs. These are possible factors which can transmitted infections in β-thalassemia patients. Two types of techniques including Immuno-chromatographic technique (ICT) and chemiluminescence immunosorbent assay (CLIA) were used for the screening of blood samples before and after blood transfusions. Chemiluminescence immunoassay (CLIA) was found to be more reliable comparatively due to capturing weak positive and low titer infectious agents which are not detected during their window period with Immunochromatographic technique (ICT).

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