A COMPARATIVE STUDY OF CHEMILUMINISCENCE IMMUNOASSAY (CLIA) AND IMMUNOCROMATOGRAPHIC TECHNIQUE (ICT) FOR β-THALASSEMIA (MAJOR) PATIENTS IN LOCAL POPULATION

Muhammad Tayyab¹, Saima Irum², Saima Naz³, Muhammad Zaid⁴, Adnan Ahmad Khan⁵, Hafiz Zeshan Wadood*¹

¹Department of Biology, Lahore Garrison University, Sector C, Phase VI, DHA Lahore Pakistan.
²Department of Transfusion Medicine & Pathology Azra Naheed Medical College, 17-Km, Raiwind Road Lahore, Pakistan.
³Government Sadiq College Women University, Bahawalpur, Pakistan
⁴Department of Life Sciences, University of Management and Technology, Lahore.
⁵Department of Transfusion Medicine, Institute of Blood Transfusion Service Punjab, Lahore.

Correspondence: dr.zeshanwadood@lgu.edu.pk, shan_wadood@yahoo.com

ABSTRACT

The sustainability of beta thalassemia (major) patients is dependent on regular blood transfusion so they are at a greater risk of acquiring transfusion transmitted infections (TTIs). The objective of this research project was to compare the efficiency of two blood screening techniques such as chemiluminescence immunoassay (CLIA) and immunochromatographic technique (ICT) for the detection of transfusion transmitted infections in β-thalassemia (major) Patients in local population. For this purpose, a total of 250 blood samples were collected and screened through CLIA and ICT. Results showed that 30% of the samples were positive for different infectious diseases like hepatitis B or C virus, Human immunodeficiency virus (HIV) infections, infections with syphilis or malarial agents, while 70% samples were free from any kind of infections. Among all the infectious diseases the incidence of hepatitis C virus (HCV) was the highest i.e., 23.6% and 26.4% incidence of HCV was recorded through ICT and CLIA respectively. The second highest incidence was of hepatitis B virus followed by the other infectious diseases. The results showed that CLIA was more sensitive as compared to ICT in detecting the infections in blood. All blood banks and transfusion centers need to implement authentic screening of blood to counter the TTIs.

Keywords: Blood Transfusion; Chemiluminescence immunoassay; transfusion transmitted infections; beta thalassemia (major); HCV.

Highlights

➢ Regular blood transfusion is mandatory for the survival of beta thalassemia (major) patients.
➢ The efficiency of two blood screening techniques, chemiluminescence immunoassay (CLIA) and immunochromatographic technique (ICT) was studied for the detection of transfusion transmitted infections.
Comparison of CLIA and ICT for blood screening before transfusion

➢ Incidence of hepatitis C virus was found to be high.

1. INTRODUCTION

Thalassemia, being an autosomal recessive disorder exhibits a unique characteristic of severe hemolysis in its victims. It is the most common blood disorder recognized globally with some serious impacts like higher death rates along with economical and emotional stress on the families of affected individuals (Angastiniotis and Lobitz, 2019; Riaz et al., 2022). The root cause of this deadly disease is the different types of mutations that can alter the amino acid sequence of hemoglobin molecules that results in partial or complete absence of globin chains (Shang and Xu, 2017). Thalassemia can be divided into two main types depending upon the hemoglobin chain defected. In alpha thalassemia (α-thalassemia), the mutation occurs in the gene responsible for the synthesis of alpha globin chain whereas, in beta thalassemia (β-thalassemia) mutation occurs in the gene responsible for the synthesis of beta globin chain (Surani et al., 2018). Beta thalassemia is more deadly and dangerous form of thalassemia which is characterized by breakdown of red blood cells. Depending upon the severity of the disease, β-thalassemia is divided into three types such as, β-thalassemia major (transfusion-dependent thalassemia), moderate β-thalassemia intermedia and asymptomatic β-thalassemia trait (Dumaidi et al., 2018). The therapies for the treatment of thalassemia patients include iron chelation, folic acid supplements and regular blood transfusion. The cornerstone therapy is one of the best methods for a better lifespan of the thalassemia patients which includes adequate blood transfusion along with iron chelation (Riaz et al., 2022). The major source for the sustainability of the lives of beta thalassemia major patients is the regular blood transfusion, but this practice can put the lives of patients in danger if the blood will not be properly screened before transfusion. These complications which are known as TTIs might include infections with hepatitis B or C virus, HIV infections, infections with syphilis or malarial agents (Mittal et al., 2017). Beta thalassemia major patients are totally dependent on blood transfusion therapy to keep a healthy life as compared to expensive chelation therapy, this type of expensive treatment poses a lot of financial and psychological stress on the patients and associated families (Tayyab, 2022). In developing countries like Pakistan, the lives of beta thalassemia major patients are at greater risk for TTIs due to unreliable screening methods for the blood before its transfusion. TTIs are the main cause of morbidity and mortality in β-thalassemia major patients (Borgna-Pignatti et al., 2004; Ladis et al., 2005). In western world, the life expectancy of thalassemia patients has improved due to better blood transfusion facilities, whereas, in Pakistan, the situation is still pathetic due to lacked organized transfusion services which is the major cause of TTIs in Pakistan (Ehsan et al., 2020). TTIs are the second major cause of deaths in thalassemia major patients in Pakistan and the prevalence of TTIs is significantly higher in thalassemia major patients as compared to the general population (Gower et al., 2014).

The aim of this study is to compare the efficiency of CLIA and ICT for the detection of TTIs in β-thalassemia (major) Patients in local population of Lahore, Punjab. For this purpose, the incidence of TTIs in β-thalassemia (major) patients was checked in local population of Lahore region by using these two techniques.

2. MATERIALS AND METHODS

Study Design
Comparison of CLIA and ICT for blood screening before transfusion

This research study was conducted at the Institute of Blood Transfusion Services Punjab, Lahore to check the incidence of transfusion transmitted infections (TTIs) in β-thalassemia (major) patients from September 2020 to April 2021. A total of 250 β-thalassemia (major) patients were included in this study.

Sample collection

The blood samples were collected from β-thalassemia (major) patients of different age groups and from the different Thalassemia centers working in Lahore region. The demographic data like; patient’s family history of disease, prior blood transfusion history, financial status or profession of the patient and any surgical treatment done previously or in recent times was also collected along with the sample collection. The collected blood samples were stored at the storage facility of the laboratory of institute of blood transfusion services Punjab, Lahore. Both the plasma and serum were extracted from the collected blood samples, as the plasma is required for chemiluminescence Immunoassay (CLIA) and serum is mandatory for Immuno-chromatography technique (ICT).

Screening of blood samples through Immuno-Chromatography Technique for the detection of infectious diseases

All the collected serum samples were screened for Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human immunodeficiency Virus (HIV), syphilis and malaria through Immuno-chromatography technique. It is based on the lateral flow chromatographic immune assay. The cassette device includes conjugate pad with nitrocellulose membrane having a marked test line over it. When specimen is dispensed at sample well, it flows across through capillary action. If antibody of any disease is present in the specimen, then it will bind to its conjugate (Pruett et al., 2015; Waheed et al., 2019).

Screening of blood samples through Chemiluminescence Immunoassay for various infectious diseases

All the collected blood plasma samples were screened through CLIA for the detection of infectious agents present in samples. The main principle behind CLIA is the formation of antigen-antibody complex or immune complex which is then detected by the use of labeled antibodies. In CLIA method, Antigen or antibody of interest is coated on a solid substrate which is then reacted with the plasma samples (containing antigen or antibody). A chemiluminescent labeled antibody is then added to this Ag-Ab complex which results in the emission of light as a result of a chemical reaction. The intensity of this emitted light is measured in terms of relative light units (RLU). This CLIA method is more sensitive and accurate as compared to others methods of this type like enzyme linked immunosorbent assay (ELISA). The Chemiluminescence immunoassays and enzymes were commonly used assays for the detection of TTIs in the donated blood samples collected from centers. The CLIA was a better choice for blood samples collected in bulk and represent the high magnitude of sensitivity with noticeable ability to detect the larger markers of infections. It has a considerable advantage due to lowest level of interference emission and wide dynamic range (Kiani et al., 2016; Cinquanta et al., 2017).

Statistical analysis

In this research work, IBM SPSS (25) was used for the statistical analysis of the results.

3. RESULTS
Comparison of CLIA and ICT for blood screening before transfusion

**Overall incidence of $\beta$-thalassemia (major) patients**

A total of 250 $\beta$-thalassemia (major) patients were included in the current research study from different thalassemia centers of Lahore region. Out of these 250 patients, 150 (60%) were male while 100 (40%) were females. These patients were divided into three age groups. The 1\textsuperscript{st} age group was from 1 to 10 years old, 2\textsuperscript{nd} group was from 11 to 20 years old and the 3\textsuperscript{rd} group was from 21 to 30 years old. The percentage of male and female patients within a particular age group was also noted and it was found that in all age groups, more males were affected with this disease (Figure 1). In 1\textsuperscript{st} age group; total patients were 100 with 58% males and 42% females, similarly, in 2\textsuperscript{nd} age group number of patients was 100 with 62% males and 38% females. The 3\textsuperscript{rd} age group, included 50 patients with 60% males 40% females (Figure 1).

![Figure 1: Incidence of male and female $\beta$-thalassemia (major) patients in different age groups.](image)

**Incidence of Transfusion Transmitted Infections (TTIs) among $\beta$-thalassemia (major) patients screened through ICT and CLIA**

The collected samples of $\beta$-thalassemia (major) patients were screened through ICT and CLIA techniques for the detection of TTIs. Among all the patients screened through ICT method, 30% were found to be infected with different TTIs while remaining 70% patients were found to be free of any kind of infections in their blood samples (Table 1). Among the infected patients, 23.6% were infected with hepatitis C, 3.2% were infected with hepatitis B, 1.6% with HIV, 0.4% with Syphilis and 1.2% was infected with malaria (Figure 2). On the other hand, the screening results of CLIA indicated the rate of TTIs as 34.4% among the $\beta$-thalassemia (major) patients while the remaining 65.6% patients were found to be free of any kind of infections in their blood samples (Table 1). Among the infected patients, 26.4% were infected with hepatitis C, 4.8% with hepatitis B, 3.2% were infected with HIV (Figure 2). Syphilis and Malaria were not performed through CLIA.
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Table 1: Incidence of Transfusion Transmitted Infections among β-thalassemia major patients screened through ICT and CLIA.

<table>
<thead>
<tr>
<th>Infectious Diseases</th>
<th>Percentage of infected individuals screened through ICT</th>
<th>Percentage of infected individuals screened through CLIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C</td>
<td>23.6%</td>
<td>26.4%</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>3.2%</td>
<td>4.8%</td>
</tr>
<tr>
<td>HIV infection</td>
<td>1.6%</td>
<td>3.2%</td>
</tr>
<tr>
<td>Syphilis</td>
<td>0.4%</td>
<td>-</td>
</tr>
<tr>
<td>Malaria</td>
<td>1.2%</td>
<td>-</td>
</tr>
<tr>
<td>Without any TTIs</td>
<td>70%</td>
<td>65.6%</td>
</tr>
</tbody>
</table>

Figure 2: Incidence of Transfusion Transmitted Infections among β-thalassemia (major) patients screened through ICT and CLIA.

**Incidences of TTIs in different age groups of β-thalassemia (major) patients screened through ICT and CLIA**

The percentage of various infectious diseases was calculated for different age groups screened through ICT and CLIA. In case of age group 1, the percentages of Hepatitis C, Hepatitis B, HIV Infection, syphilis and malaria were found to be 22%, 04%, 02%, 01% and 01%, respectively for ICT screening. Similarly, for age group 2, 24%, 02%, 01%, 0% and 02% rate was observed for Hepatitis C, Hepatitis B, HIV Infection, syphilis and malaria respectively for ICT screening. In case of age group 3, a percentage of 26%, 04%, 02%, and 0% was observed for Hepatitis C, Hepatitis B, HIV Infection, syphilis and malaria respectively for ICT screening. In case of CLIA screening the results were different as given below. In case of group 1, the percentage of Hepatitis C was found to be 25%, Hepatitis B as 06% and HIV infection was found to be 03%. In the case of group 2, the percentage of Hepatitis C was found to be 28%, Hepatitis B as 03% and HIV infection was found to be 02%. In third group, the percentage of Hepatitis C was found to be 26%, Hepatitis B as 06% and HIV infection was found to be 04% (Figure 3).
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Figure 3: Incidence of TTIs in different age groups of β-thalassemia (major) patients screened through ICT and CLIA.

Incidence of various TTIs among male and female β-thalassemia (major) patients screened through ICT and CLIA

Among all the β-thalassemia (major) patients screened through ICT method, 14% males and 9.6% females acquired hepatitis C, 2.4% males and 0.8% females acquired hepatitis B, 0.4% males and 1.2% females acquired HIV infection, 0.4% females acquired syphilis, 0.8% males and 0.4% females acquired malaria infection during blood transfusion. On the other hand, patients screened through CLIA method, 15.2% males and 11.2% females acquired hepatitis C 2.8% males and 2% females acquired hepatitis B, 1.2% males and 1.6% females acquired HIV infection during blood transfusion (Figure 4).

Incidence of various TTIs among the male and female β-thalassemia (major) patients screened through ICT and CLIA in all age groups

Among all the patients of age group 1 screened through ICT method, 13% males and 9% females were found infected with hepatitis C, 3% males and 1% females were infected with hepatitis B, HIV infection was found in 0% males and 1% females, 0.4% females were found infected with syphilis, and malarial infection was found in 1% males and 1% females. On the other hand, the same patients when screened through CLIA method gave different percentages of infections such as, 14% males and 11% females were found infected with hepatitis C, 4% males and 2% females were infected with hepatitis B and 1% males and 2% females were having HIV infection. In case of age group 2 patients screened through ICT method, 16% males and 8% females were found positive for hepatitis C, 2% males and 0% females were positive for hepatitis B, 0% males and 1% females were infected with HIV infection, 0.4% females were positive for syphilis, and for malarial infections, 1% males and 0% females were positive. The same patients when screened through CLIA method, 18% males and 10% females were having
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hepatitis C, 2% males and 1% females were positive for hepatitis B, whereas, 1% males and 1% females were found positive for HIV infection.

Among all the patients of age group 3 screened through ICT method, 14% males and 12% females were suffering from hepatitis C infection, 2% males and 2% females were positive for hepatitis B infection, 2% males and 0% females were infected with HIV, and for syphilis and malaria, no male and female were found positive. On the other hand, the patients screened through CLIA method, 14% males and 12% females were found infected with hepatitis C, 4% males and 2% females were positive for hepatitis B infection, whereas, 2% males and 2% females were positive for HIV infection (Figure 5).

Statistical analysis

In this research work, IBM SPSS (25) was used for the statistical analysis of the results. Statistical analysis was done using chi-square test through IBM SPSS (25). The P-value was found to be 0.003 which is considered as highly significant one. Thus, the null hypothesis was rejected and alternative hypothesis was accepted because there exist a relationship between the Transfusion Transmitted Infections (TTIs) in the case of β-thalassemia (major) patients.
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Figure 5: Incidence of various TTIs among the male and female β-thalassemia (major) patients screened through ICT and CLIA in all age groups.

4. DISCUSSION

Beta thalassemia (major), also known as ‘transfusion-dependent thalassemia’ is the most severe kind of thalassemia which results due to mutation in both genes for the beta chains of hemoglobin molecule (Al-Moshary et al., 2019; Riaz et al., 2022). The survival of these patients is only dependent on the regular transfusion of blood. These patients are at a great risk of acquiring the agents for various deadly infectious diseases (HCV, HBV, HIV, syphilis and malaria disease) while blood transfusion because of poor blood screening procedures (Al-Moshary et al., 2019; Kapure, 2020). The current study is designed to make a comparison between two blood screening techniques, ICT and CLIA to check their sensitivity for proper screening of blood before its transfusion to beta thalassemia (major) patients. In the current research study, a total of 250 β-thalassemia (major) patients were included from different thalassemia centers of the provincial capital, Lahore. Out of all the individuals, 60% were males and 40% were females. The selected individuals were divided into three different age groups, first age group included patients with age from 1 to 10 years, the second group included patients with age from 11 to 20 years and the third group included patients with age from 21 to 30 years.

The overall incidence of β-thalassemia (major) shows that more males (60%) were affected with the disease compared to the females (40%) among 250 patients. In this study, the incidence of male β-thalassemia (major) patients was higher in all age groups (Figure 1). Beta thalassemia is an autosomal recessive disorder so it can affect both males and females equally as it does not involve sex chromosomes, so the incidence of more males having β-thalassemia (major) in this study is just a coincidence. The results obtained from a total of 250 samples of β-thalassemia (major) patients screened through ICT and CLIA techniques, 30% and 34.4% were positive for any kind of infections respectively. Remaining patients were clear from any kind of infectious diseases. Out of
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this infected percentage of patients, the highest rate of infection was found to be of HCV in both techniques. HCV was found in 23.6% of patients screened through ICT and in 26.4% of patients screened through CLIA. This highest percentage of HCV is in accordance with different other studies conducted locally and in different other regions, which have reported 21% to 30% or even 42% of HCV cases in β-thalassemia (major) patients (Al-Sheyyab et al., 2001; Jaiswal et al., 2001; Younus et al., 2004; Kiani et al., 2016; Al-Moshary et al., 2019; Ali et al., 2023).

Another reason for this highest incidence of HCV in β-thalassemia (major) patients is Pakistan has the second highest burden of HCV patient’s worldwide (Khan et al., 2020). The second highest incidence, i.e., 3.2% through ICT and 4.8% through CLIA screening, was found to be of hepatitis B virus infection. These findings are being supported by other studies in which a percentage from 1.5% to 3% or even 6.4% has been reported for the incidence of HBV as a TTI in thalassemia patients (Mirmomen et al., 2006; Al-Moshary et al., 2019; Premawardhana et al., 2019). The reason for this variation in the incidence of HBV as a TTI might be due to the fact that the prevalence of HBV is different in different parts of the world. The incidence of HIV was found to be 1.6% and 3.2% through ICT and CLIA screening respectively. Compared to the previous studies in Pakistan which have reported 0.3 to 0.7% rate of HIV infections (Kiani et al., 2016; Al-Moshary et al., 2019; Ehsan et al., 2020), this percentage seems to be quite high. These findings suggest that HIV is spreading in the country at a higher rate like other infectious diseases. Pakistan is the second largest country in South Asia after India and Nepal for HIV epidemic and the recent years have witnessed a significant increase in HIV infections throughout the country despite a lot of efforts (Marfani et al., 2022).

Overall, the percentage of HCV was highest in the patients screened through both the techniques but the CLIA was found to be the most sensitive and accurate method for the screening the blood as it screened the blood for those infections like malaria and syphilis which could not be screened through ICT. Other researchers have also found CLIA as the best technique for blood screening for viral markers which cannot be detected by other techniques (Tiwari et al., 2023).

5. CONCLUSION

The results of the current study suggest that the rate of TTIs is considerably high among β-thalassemia major patients. The infectious disease with the highest percentage was HCV followed by HBV and HIV. Out of the two types of techniques used for the screening of blood, CLIA was found to be more reliable comparatively due to capturing weak positive and low titer infectious agents. CLIA is most appropriate and trust worthy one for blood screening purpose to capture weak positive and low titer infectious agents which are not detected (during their window period) with ICT. The thalassemia centers should consider the reliable screening methods before the blood transfusion and for that CLIA can be one of the best methods to screen the blood before transfusion to patients.

Conflict of Interest

The authors declare no competing interests.

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Service Punjab, Lahore.

Authors Contribution
Muhammad Tayyab, Saima Irum and Adnan Ahmad Khan performed the experimental part of the project i.e., collection of blood samples and their screening through ICT and CLIA. Saima Naz and Muhammad Zaid analyzed the data for results. Hafiz Zeshan Wadood supervised the overall project.

6. REFERENCES


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