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Research

Detection of Alloantibodies Using Gel Card Technique and Its Correlation with Liver Function Tests in Transfusion-Dependent Thalassemia Patients

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Abstract:

Background: Thalassemia is a hereditary hemoglobin disorder characterized by chronic anemia that requires regular blood transfusion therapy. Repeated transfusions increase the risk of red blood cell alloimmunization and iron overload, both of which may contribute to clinical complications, particularly hepatic dysfunction. **Aim:** This study aimed to detect alloantibodies using the Gel Card technique and to evaluate their correlation with liver function parameters in transfusion-dependent thalassemia patients. **Methods:** A descriptive cross-sectional laboratory-based study was conducted on 100 patients diagnosed with thalassemia in Wasit province. Venous blood samples were collected and divided for biochemical and immunohematological analysis. Serum levels of aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), and alkaline phosphatase (ALP) were measured using the Roche cobas c111 automated analyzer. Alloantibody screening and cross-matching were performed using the Gel Card (column agglutination) technique. **Results:** The results showed elevated mean levels of liver enzymes (AST: 42.76 ± 15.28 U/L, ALT: 48.92 ± 17.64 U/L, ALP: 185.34 ± 52.11 U/L). Higher Gel Card reactivity (3+ and 4+) was more frequently observed among females compared to males. Statistical analysis revealed highly significant associations between Gel score and liver enzyme levels ($p < 0.001$). Correlation analysis demonstrated moderate positive correlations between Gel score and AST ($r = 0.36$), ALT ($r = 0.42$), and ALP ($r = 0.31$). Linear regression analysis indicated that Gel score significantly predicted ALT levels ($R^2 = 0.17$, $p < 0.001$). **Conclusion:** transfusion-dependent thalassemia patients showed significant liver enzyme elevation and detectable alloantibody formation. A significant association was observed between alloimmunization and liver function abnormalities, likely reflecting cumulative transfusion exposure and iron overload.

KEYWORDS: Alloantibodies, Thalassemia, Gel Card Technique, Liver Function Tests.

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INTRODUCTION

Thalassemia is a group of inherited hemoglobin disorders characterized by reduced or absent synthesis of one or more globin chains, leading to ineffective erythropoiesis and chronic anemia. Patients with severe forms, particularly β-thalassemia major, require lifelong regular blood transfusions to maintain adequate hemoglobin levels and prevent complications associated with anemia. Although transfusion therapy significantly improves survival and quality of life, it is associated with several immunological and metabolic complications [1].

One of the most important transfusion-related complications is alloimmunization, which occurs when the recipient's immune system produces antibodies against foreign red blood cell antigens introduced through transfusion. The presence of alloantibodies can complicate cross-matching, delay transfusion therapy, and increase the risk of hemolytic transfusion reactions. Therefore, accurate detection of alloantibodies is essential for ensuring transfusion safety in thalassemia patients [2,3]

Modern immunohematological techniques have improved the detection of clinically significant antibodies. Among these methods, the gel card technique has gained widespread acceptance because of its high sensitivity, reproducibility, and ability to standardize testing procedures. Compared with traditional tube methods, gel technology reduces subjective interpretation and enhances the identification of weak antibodies [4].

In addition to immunological complications, transfusion-dependent thalassemia patients are prone to iron overload due to repeated blood transfusions. Excess iron accumulates primarily in the liver, leading to hepatocellular injury, fibrosis, and potentially cirrhosis if left untreated. Liver function tests (LFTs), including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin levels, are commonly used to monitor hepatic status in these patients. Investigating the relationship between alloantibody formation and liver function is clinically relevant because both conditions may reflect cumulative transfusion exposure. Understanding this correlation may contribute to improved monitoring strategies and patient management. Therefore, this study focuses on detecting alloantibodies using the gel card technique and evaluating their association with liver function parameters in patients with thalassemia [5, 6].

The present study was conducted to detect alloantibodies using the Gel Card technique and to evaluate their correlation with liver function parameters in transfusion-dependent thalassemia patients.

MATERIALS AND METHODS

1. Study Design

This study was designed as a descriptive cross-sectional laboratory-based investigation of transfusion-dependent thalassemia patients attending the thalassemia center in Wasit Province. All laboratory analyses were performed under standardized conditions using automated equipment to ensure accuracy and reproducibility of results. A total of 100 patients diagnosed with thalassemia were enrolled in this study. Patients were selected based on confirmed clinical diagnosis and history of repeated blood transfusions.

2. Sample Collection

Approximately 5 mL of venous blood was collected from each patient using sterile disposable syringes under aseptic conditions. The collected blood was divided into two portions: a plain tube (gel tube) used for serum separation to measure liver enzymes (GOT, GPT, ALP), and an EDTA tube used for immunohematological testing, including Gel Card cross-matching for alloantibody detection. After collection, samples were labeled clearly with patient identification codes to prevent mix-ups.

3. Sample analysis

Blood in plain tubes was allowed to clot at room temperature for approximately 5–10 minutes. The samples were then centrifuged at 3000 rpm for 10 minutes to obtain clear serum. EDTA samples were gently mixed to prevent clot formation and processed according to immunohematology testing protocols. While the measurement of liver enzyme serum levels of GOT (AST), GPT (ALT), and ALP was done using the automated chemistry analyzer Roche cobas c111. The analyzer operates using

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spectrophotometric methods and provides reliable quantitative results. [7].

4-Detection of Alloantibodies Using Gel Card Cross-Match

Alloantibody screening was performed for all patients using the Gel Card technique, a sensitive and widely accepted method in transfusion medicine.

Principle of Gel Card Method

The Gel Card contains microtubes filled with a dextran-acrylamide gel that acts as a filtration medium. During centrifugation:

Agglutinated red blood cells remain trapped in the upper portion of the gel.

Non-agglutinated cells pass through the gel and form a pellet at the bottom.

This method enhances the detection of clinically significant antibodies and reduces subjective interpretation compared to conventional tube testing.

Cross-Matching Procedure

Patient plasma was added to the microtube containing reagent red cells. The Gel Card was incubated at 37°C to allow antigen–antibody reactions. Cards were centrifuged using a specialized gel centrifuge. Results were interpreted based on the distribution of red cells within the gel column. [8].

5. Statistical analysis

Descriptive statistics, association tests, correlation analysis, and linear regression analysis were performed using SPSS and GraphPad Prism software.

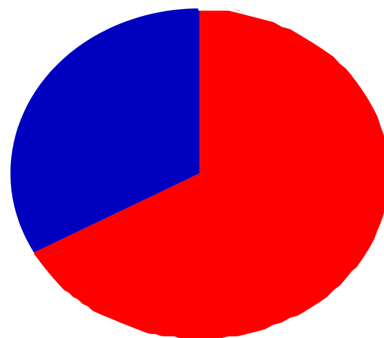
Results and Discussion

1-Demographic and biochemical characteristics of the study population.

In this study, 100 patients were evaluated, of whom 67 were male and 33 were female Table 1 and Figure 1 shows the descriptive statistics for age, liver enzymes, and gel score in the study population.

Table 1: Descriptive Statistics for Age, liver enzymes, and gel score in the study population

Variable	Mean	Std. Deviation	Std. Error
Age (years)	18.45	4.32	0.43
GOT (U/L)	42.76	15.28	1.53
GPT (U/L)	48.92	17.64	1.76
ALP (U/L)	185.34	52.11	5.21
Gel Score	1.36	1.12	0.11



67.00% Male
33.00% Female

Figure 1: Showing Distribution of Sex (Male and Female).

The mean age of the patients included in this study was 18.45 ± 4.32 years, which is comparable to the age distribution commonly reported among transfusion-dependent β-thalassemia patients. Previous studies have indicated that advances in transfusion protocols and iron chelation therapy have markedly improved survival rates allowing many patients to reach adolescence and early adulthood [9, 10].

The elevated mean serum levels of AST (42.76 U/L), ALT (48.92 U/L), and ALP (185.34 U/L) observed in the present study are in agreement with previous reports demonstrating hepatic involvement among multi-transfused thalassemia patients. Several studies

have shown a significant association between elevated liver enzymes and increased serum ferritin levels, reflecting iron overload–induced hepatic injury. Furthermore, persistent elevation of transaminases has been documented even in hepatitis-negative patients suggesting that iron overload represents the primary etiological factor contributing to liver dysfunction in β-thalassemia patients [11, 12, 13].

2. Association between sex and gel card score.

Table 2 and Figure 2 show the distribution indicates variation in gel card reactivity between males and females.

Table 2: Association between Sex and Gel Card Score.

Sex	Negative	1+	2+	3+	4+
Male	25	10	8	5	2
Female	15	12	10	8	5

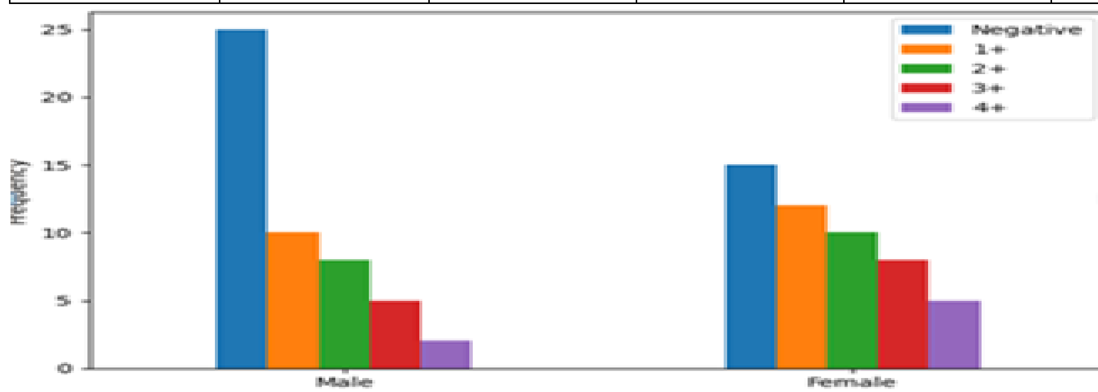


Figure 2: Distribution of Gel Card Score by Sex

The present study demonstrated higher Gel Card reactivity grades (3+ and 4+) among female patients, suggesting that sex-related immunological differences may contribute to increased alloantibody formation. Previous studies have indicated that females may exhibit stronger immune responsiveness than males, which could enhance antibody production following repeated blood transfusions [14, 15]. Furthermore, [16]. emphasized that the risk of alloimmunization is influenced by both immune responsiveness and the degree of antigenic exposure, factors that may vary among individuals.

3. Association between Gel Score and Liver Enzymes

Table 3 shows the association between liver function enzymes and gel card score.

Table 3: Association between liver function enzyme and gel card score.

Comparison	Mean Diff.	95% CI	Significant	Summary	P Value
Gel Score vs GOT	-48.70	-61.52 to -35.88	Yes	***	<0.001
Gel Score vs GPT	-40.39	-53.21 to -27.57	Yes	***	<0.001
Gel Score vs ALP	-192.8	-205.6 to -180.0	Yes	***	<0.001

Highly significant associations ($p < 0.001$) were observed between Gel Score and all measured liver enzymes (AST, ALT, and ALP). These findings suggest that patients with stronger alloimmune responses may have undergone greater cumulative transfusion exposure, which could contribute to increased hepatic burden and subsequent liver dysfunction. Previous studies have demonstrated a positive correlation between serum ferritin and ALT levels in multi-transfused children, indicating that chronic iron overload induces oxidative stress and hepatocellular injury, ultimately resulting in elevated liver enzyme levels [17, 18].

4. Correlation Analysis

Table 4 shows the correlation between gel score and liver enzymes. Gel score showed a moderate positive correlation with GOT (r = 0.36), GPT (r = 0.42), and ALP (r = 0.31). All correlations were statistically significant at $p < 0.05$.

Table 4: Correlation between gel score and liver enzymes.

Variable	Gel Score	GOT	GPT	ALP
Gel Score	1	0.36*	0.42*	0.31*
GOT	0.36*	1	0.58*	0.47*
GPT	0.42*	0.58*	1	0.52*
ALP	0.31*	0.47*	0.52*	1

***Correlation is significant at $p < 0.05$ level**

Table 5 shows the linear regression analysis (Gel Score Predicting GPT). Linear regression analysis was performed to evaluate the predictive effect of Gel score on GPT levels. The regression model showed that Gel score significantly predicted GPT levels ($p < 0.001$). The model explained 17% of the variance in GPT levels ($R^2 = 0.17$).

Table 5: Linear Regression Analysis (Gel Score Predicting GPT)

Model	Std. Error	t	p-value
Constant	3.21	3.87	0.001
Gel Score	1.48	3.79	0.000

$R^2 = 0.17$

The moderate positive correlations between Gel Score and GPT (r = 0.42), GOT (r = 0.36), and ALP (r = 0.31) indicate a biologically plausible relationship between alloimmunization and hepatic dysfunction [19]. Previous studies have described transfusion burden as a major factor linking immune complications with progressive organ damage. Linear regression analysis showed that gel score explained 17% of the variance in GPT ($R^2 = 0.17$) indicating that although alloimmunization contributes to liver enzyme alterations, additional factors such as iron overload severity, and adherence to iron chelation therapy may also play important roles[18, 20, 21].

Conclusion:

Transfusion-dependent thalassemia patients showed significant liver enzyme elevation and detectable alloantibody formation. A significant association was observed between alloimmunization and liver function abnormalities, likely reflecting cumulative transfusion exposure and iron overload. The study emphasizes the need for RBC antigen typing before the first transfusion and the issue of antigen-matched blood. Early institution of transfusion therapy after diagnosis is another means of decreasing alloimmunization.

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