Effect of Implementing a Blood Group Matching Program for Rh (D, C, c, E, e) and Kell on Biochemical and Hematological Parameters in Beta Thalassemia Patients (βT)

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Abstract
Background: βT is a genetic disease of the chromosomes. As long as normal RBCs are present, the normal red blood cells do not last long and do not function properly. Pure group patients (extended Rh + Kell matching) had lower biochemical, hematological, and alloimmunization complications, while Traditional group patients (ABO + RhD only) showed higher rates of these risk particulary immune activation. Therefore, extended matching remains the safer strategy.
Material and method: A total of 180 samples were collected from patients with beta-thalassemia at Ibn Al-Baladi Specialized Center for Genetic and Hematological Diseases. The samples were divided into three groups: 60 patients who received extended Rh (D, C, c, E, e) and Kell-matched blood, 60 patients who received routine ABO and RhD-matched blood, and 60 healthy individuals as the control group. Serum samples were processed for biochemical analysis (urea, creatinine, TSB, ALT, AST, iron, and ferritin), while whole blood was used for CBC analysis.
Results: TSB levels were higher in the Traditional group; blood parameters (RBC, HGB, and iron) showed greater deterioration, and outcomes were closer to the control group in several markers (ALT, TSB) and fewer alloimmunization complications.
Conclusion: Extended antigen matching can improve long-term patient outcomes by enhancing hematological and biochemical stability.

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Keywords: βT, Matching Program, Rh (D, C, c, E, e), Kell, Effect

Introduction

Thalassemia (βT) is the most common single-gene hematologic disorder in the world. This disorder has been predestined; more than 60.000 children are born each year with βTand more than 80 million are carriers of this disease [1]. βT is caused by a decrease or absence of ß-globin chain production [2]. These patients do not make enough hemoglobin in their red blood cells(RBCs). These RBCs do not last long and non_ function as normal RBCs. βT patients have anemia as a result of a decrease in the amount of RBCs due to continuous hemolysis [3].

There are 44 blood group systems comprising 354 red cell antigens (Ag). These are genetically controlled by 49 genes [5]. Compatibility testing in transfusion practices has generally been based on the presence of the RhD Ag, which is not sufficient for prevention of alloantibodies production, where many antibodies are formed against other non-D Rh antigens [6]. The Rh blood group system (54 Ag) is the second-most clinically important blood group system after the ABO system [7]. There are five major Ags which are connected with immunogenic and complex protein structures with many polymorphisms and clinically important alleles, namely D, C, c, E and e [8]. In addition, the Kell blood group system (30 Ag) is the third most important blood group system in transfusion medicine. Kell Ags are well-expressed on the surface of fetal RBCs and erythroid precursors [9]. It

is polymorphic and immunogenic during mismatched transfusion [10,11]. However, in the recent past, thanks to the advanced technologies in use in RBC, it has become possible for the implementation of extended antigen-matching at the multi-antigen level [11]. There is rising interest in transfusion medicine and obstetric field regarding these developments [12]. Implementation of genotyping improved transfusion practice and minimized biochemical complications including iron overload and liver dysfunction [13]. Findings: In 250 cases of thalassemia, partial phenotyping matching (RhD, C, c, E, e, Kell) led to a decrease in alloantibody production (0% versus 15.7%) and transfusion reactions (3.3% versus 35.7%) [14]. Extended matching reduced alloimmunization and helped to stabilize the red cell indices (MCV, MCH), thereby increasing transfusion effectiveness [13]. Extended matching for Rh + Kell resulted in increased hemoglobin levels and fewer decreases in hematocrit compared to traditional matching [15].

The current study aims to evaluate the effect of implementing the matching program on Rh (D, C, c, E, e) and Kell antigens in blood transfusion to thalassemia patients through a study of the biochemical and hematological changes to these patients.

Material and Methods

Samples and Collection Place

Samples of beta thalassemia patients were collected from Ibn Al Baladi Specialized for Genetic and Hematological Diseases. The number of samples reached 180, which were divided into:

- 60 samples for the group of pure patients who received blood with expanded Rh (D, C, c, E, e) and Kell blood group matching
- 60 samples for the traditional group who received blood according to routine blood group matching (RhD+ABO)
- 60 samples for the healthy control group.

Collection Method

- Serum Sample Collection for Biochemical Analysis by serum separator tube (SST),
- allow the sample to clot at room temperature for 15–30 minutes.
- Centrifuge at 3000 rpm for 10 minutes to separate
- Store serum in a sterile, tightly sealed tube.-20 °C
- Serum is used for biochemical tests (Urea, Creatinine, Total serum Bilirubin (TSB), Aspartate aminotransferase (ALT), Alanine aminotransferase (AST), Iron, and Ferritin).
- Whole Blood Collection for hematological tests: Complete Blood Count (CBC)
- All these tests were carried out based on reagents from Beckman Coulter Company, Australia

Operation

- Beckman Coulter analyzers (Australia) operate on the principle of spectrophotometry (light absorption measurement).
- Complete Blood Count (CBC) on Beckman Coulter hematology analyzers (Australia), which rely on electrical impedance and flow cytometry principles.

Statistical Analysis

All data were analyzed using SPSS, with one-way ANOVA followed by Duncan's test. All graphs were generated using the GraphPad Prism program (V.8).

The Results

Biochemical Results

The renal functions for urea and creatinine were observed; there were no significant differences in the concentration of Blood urea between control, pure, and traditional groups. A similar result was seen with serum creatinine levels, as no significant changes were detected between the studied groups (Table 1).

Table 1: The serum level of renal function tests

Parameter	Control	Pure	Traditional	P-value	Significance
S. Urea (mmol/L)	4.26 ± 0.31	4.72 ± 0.47	4.38 ± 0.17	0.7	NS
S. Creatinine (µmol/L)	37.84 ± 2.28	38.10 ± 1.01	42.19 ± 0.76	0.1	NS

Abbreviation: NS = Non-significant differences.

Liver Function Tests

As shown in Table 2, the results of the liver function tests revealed significant findings, where the concentration of TSB significantly increased (P<0.001) in the traditional group as compared to the control. In contrast, no effect in its concentration was found in the pure group in comparison to the control group. On the other hand, a significant decrease was seen in TSB levels between pure and traditional groups (Table 2).

A significant elevation in ALT levels was observed in the pure group compared with healthy individuals (control) (P<0.003). Also, the same significant level was seen in the traditional group than control (P<0.003). Here, it must be mentioned that ALT Con. in the pure group (64.62±7.86) was closer to control than that of the Traditional group (73.36±10.10) (Table 2). While there were no significant decreases in pure than traditional groups in its concentration. The results of AST revealed that there is a significant increase in its level in pure and traditional groups in comparison to the control group (p=0.02) (Table 2). In contrast, There are non significant decreasing effect was detected in AST levels among the pure than the traditional groups.

Table 2: The serum level of Liver functions tests

Parameter	Control	Pure	Traditional	P-value	Significance
TSB (µmol/L)	19.00 ± 2.28a	23.11 ± 1.74a	36.74 ± 2.94b	0.001	**
ALT (U/L)	24.96 ± 1.72a	64.62 ± 7.86b	73.36 ± 10.10b	0.003	**
AST (U/L)	25.00 ± 1.42a	50.49 ± 5.34b	73.70 ± 11.83b	0.020	*

Values are expressed as mean ± SD.
Different superscript letters (a, b) within the same row indicate significant differences between groups, while similar letters indicate non-significant differences.
$p < 0.05$ (significant).
** = $p < 0.01$ (highly significant).

Iron and Ferritin

The concentration of Iron significantly decreased ($p<0.05$) in pure groups as compared to control group (Table 3). While, Its concentration increased in Traditional group than control. Moreover, the concentration of iron significantly declined in pure group in comparison to traditional group.

Interestingly, a significant elevation was also observed in the concentration of ferritin in pure and traditional groups ($p<0.001$) as compared to control group. Furthermore, a significant differences was seen in its level between pure and traditional groups as it was increased in traditional group in comparison to pure group (Table3).

Table 3: The serum level of Iron and Ferritin

Parameter	Control	Pure	Traditional	P-value	Significance
Iron (µmol/L)	14.15 ± 1.64c	8.49 ± 0.66a	20.52 ± 1.40b	<0.001	**
Ferritin (ng/mL)	86.17 ± 5.58a	1598.60 ± 148.26b	4644.24 ± 223.38c	<0.001	**

Values are expressed as mean ± SD.
Different superscript letters (a, b, c) within the same row indicate significant differences between groups, while similar letters indicate non-significant differences.
** = $p < 0.01$ (highly significant)

Hematological Tests

The Total and Agranulocytes WBC Counts

The WBC count did not affect between all the studied groups, although it was slightly increased in traditional group, while the statistical analysis revealed that there is no significant differences between control, pure and traditional groups in WBC count (Table 4). Whereas, the percentage of lymphocytes significantly increased ($p=0.001$) in pure and traditional groups in comparison to healthy individuals (control). Although no significant effect was seen in lymphocyte count between pure and traditional groups (Table 4). Furthermore, monocyte percentage slightly increased in pure and traditional groups, this increase did not match the significant effect as compared to the control group (Table 4).

Table 4: The Total and Agranulocytes WBC Counts

Parameter	Control	Pure	Traditional	P-value	Significance
WBC count ($\times 10^3/\mu L$)	8.32 ± 0.94	9.69 ± 0.49	11.44 ± 3.26	0.5	NS
Lymphocytes (%)	30.18 ± 2.87a	43.60 ± 1.88b	42.67 ± 2.76b	0.001	**
Monocyte (%)	6.85 ± 0.62	8.56 ± 0.52	8.16 ± 0.58	0.1	NS

Values are expressed as mean ± SD.
Different superscript letters (a, b) within the same row indicate significant differences between groups, while similar letters indicate non-significant differences.
** = $p < 0.01$ (highly significant).
NS = Non-significant differences.

The Granulocyte Wbc Counts (Neutrophils, Eosinophils, And Basophils)

As shown in Table 5, the percentage of neutrophil non significant decreased in pure groups as compared to control groups, whereas no significant effect was found between pure and tradition groups (Table 5).

In addition, no significant impact was detected in eosinophil percentage between control, pure and traditional groups. In contrast, the percentage of basophil significantly elevated ($p=0.045$) in the pure and traditional groups in comparison to control group; however no significant difference was seen in basophil percentage between pure and traditional groups (Table 5).

Table 5: The granulocyte WBC counts (neutrophils, eosinophils, and basophils)

Parameter	Control	Pure	Traditional	P-value	Significance
Neutrophils (%)	44.28 ± 1.78a	46.71 ± 2.39a	59.55 ± 3.42b	<0.001	**
Eosinophils (%)	3.21 ± 1.38	3.19 ± 0.33	2.11 ± 0.51	0.45	NS
Basophils (%)	0.271 ± 0.021a	0.353 ± 0.022b	0.349 ± 0.021b	0.045	*

Values are expressed as mean ± SD.
Different superscript letters (a, b) within the same row indicate significant differences between groups, while similar letters indicate non-significant differences.
$p < 0.05$ (significant).
** = $p < 0.01$ (highly significant).
NS = Non-significant differences

RBC count, HGB concentration, MCV, Platelets

The count of RBC significantly declined ($p<0.001$) in pure and traditional groups as compared to the control group. Moreover, RBC count also significantly decreased in the traditional group in comparison to the pure group (Table 6).

The concentration of hemoglobin (HGB) was also decreased in pure and traditional groups with a significant difference ($p<0.001$) as compared to the control group. Furthermore, HGB concentration was decreased more in the traditional group compared to the pure group (Table 6).

Mean Corpuscular Volume (MCV) concentration also was decreased significantly ($p=0.001$) in both pure and traditional groups in comparison to healthy individuals (control). Whereas no significant difference was seen in its concentration between pure and traditional groups (Table 6).

The statistical analysis revealed a significant increase ($p=0.007$) in the platelet count in pure and traditional groups as compared to the control group, while no significant effect was detected between pure and traditional groups (Table 6).

Table 6: The counts of RBC, HGB concentration, MCV and platelets counts.

Parameter	Control	Pure	Traditional	P-value	Significance
RBC count ($\times 10^6/\mu L$)	5.18 ± 0.22c	3.61 ± 0.14b	2.59 ± 0.19a	<0.001	**
HGB (g/dL)	14.41 ± 0.58c	9.26 ± 0.27b	6.90 ± 0.41a	<0.001	**
MCV (fL)	86.22 ± 1.47b	78.43 ± 1.20a	79.98 ± 1.43a	0.001	**
Platelets ($\times 10^3/\mu L$)	272.7 ± 14.3a	410.3 ± 21.7b	436.7 ± 59.0b	0.007	**

Values are expressed as mean ± SD.

Different superscript letters (a, b, c) within the same row indicate significant differences between groups, while similar letters indicate non-significant differences.

** = $p < 0.01$ (highly significant).

Discussion

The integration of Rh and Kell matching into transfusion protocols represents a crucial advancement in the management of beta thalassemia, especially in regions with high disease prevalence. Such programs contribute to better clinical stability, reduced morbidity, and improved quality of life for affected patients [16]. The pure group showed a slightly higher mean (4.72 mmol/L); the difference was statistically non-significant ($p = 0.7$). The traditional group had a slightly higher mean (42.19 µmol/L) compared to the control (37.84 µmol/L), but again, the difference was not statistically significant ($p = 0.1$). Urea is considered less specific than creatinine for renal function assessment because it is affected by extrarenal factors. Urea levels are influenced by protein intake, catabolism, and hydration status [17]. While creatinine clearance or estimated GFR (eGFR) would provide more sensitive measures [18]. Therefore, the current study measured serum creatinine across groups. The stable results support the conclusion of renal safety in our patients, given the lack of significant differences between the pure and traditional.

The traditional group showed a significant increase in TSB compared to the control, indicating higher hemolytic stress and impaired bilirubin clearance (19). In contrast, the pure group did not differ significantly from the control, suggesting that extended Rh and Kell matching reduced alloimmune-mediated hemolysis (10). This aligns with evidence that alloantibodies against non-D Rh antigens and Kell antigens are highly immunogenic and contribute to post-transfusion hyperbilirubinemia (19,11). ALT

Both pure and traditional groups exhibited significant elevations compared to control, reflecting hepatocellular stress than control (4). However, ALT levels in the pure group were closer to control than those in the traditional group. This suggests that extended antigen matching of non-D Rh antigens and Kell antigens mitigates hepatic injury associated with transfusion (10,11). AST levels were significantly elevated in both transfused groups compared to the control (12). No significant decrease was observed in pure than traditional groups, indicating that while extended matching reduces the excess of AST (11,12).

The pure group showed a significant lowering of circulating iron compared to the control. This suggests that extended Rh and Kell matching reduces ineffective erythropoiesis and hemolysis (20). Reduced alloimmunization and transfusion reactions: A cross-sectional study in Punjab found that patients transfused with extended Rh and Kell matched blood had significantly fewer alloantibodies (0% vs 15.7%) and lower autoantibody rates (5% vs 37%), directly reducing iron overload complications (16) In contrast, An Iranian cohort reported that Anti-K (38.2%) and Anti-D (23.5%) were the most frequent alloantibodies in thalassemia patients, underscoring the risk of iron overload and transfusion inefficiency when extended matching is not applied (21). Ferritin concentration in both transfused groups was significantly elevated compared to the control. However, ferritin was much higher in the traditional group (4644 ng/mL) than in the Pure group (1598 ng/mL). This indicates that extended antigen matching reduces transfusional iron overload (Ferritin), a critical complication in thalassemia (22).

Our results represent immune activation in the transfusion-dependent patients. The percentages of monocytes in the two transfusion groups were slightly higher than those in the control group. This slight elevation is due to immune activation caused by continuous transfusions that are well-matched (22). Previous research indicated that the Rh and Kell matching transfusions greatly decrease the formation of alloantibodies; hence, they minimize immune activation in comparison to ABO-only matching (14). Despite continuous transfusions, transfusion-dependent thalassemia patients have elevated lymphocyte activity as a result of chronic antigen exposure and iron overload in the case of ABO + RhD-only matching.

In the traditional group, there was a higher percentage of neutrophils (59.55%) without a statistically significant difference compared to pure. Furthermore, the basophils were significantly elevated in the pure and Traditional group compared to the control. The presence of elevated basophils shows that the person has chronic immune stimulation due to recurrent transfusions. Significantly, there was no difference between pure and Traditional groups. This is consistent with the recommendations provided by the International Collaboration for Transfusion Medicine Guidelines (ICTMG, 2025), which highly recommend the ABO, RhD, RhCcEe, and Kell matching (pure group) for thalassemia patients to

prevent alloimmunization and delayed hemolytic transfusion reactions (23). Furthermore, another single center study demonstrated that β-thalassemia patients, who received extended Rh- and Kell-matched transfusions, had less alloimmunization and immune complications compared to the patients with ABO only matched transfusions (24). An updated systematic review (2024) showed that alloimmunization prevalence varied from 0–50% in limited matching compared to 0–24% in extended matching (pure group), indicating the protective effect of extended antigen matching (25).

Another author mentions the possible dangers of using only ABO + RhD, which have immune complications, endothelial injury, and problems with hemostasis (26).There was a significant fall in the RBC count in the traditional group with higher reduction compared to the pure group. The result from the RBC count is in agreement with Gupta and Kumar, who noted that transfusion methods (ABO and RhD matching only) in thalassemia patients usually cause some changes in hematological parameters due to chronic use of red cells [14]. There was a more marked reduction in HGB in the traditional group when compared to the control group. Hussein *et al*. have shown that extended antigen matching (RhCcEe+Kell) maintains the stability of the hemoglobin since there is less alloimmunization, and the results obtained in this study confirm those findings due to the stability of the hematological parameters (Hb, Hct).[27].

MCV levels were statistically different in both groups compared to healthy patients, but no statistical difference was found between pure and traditional groups. This indicates that variations in MCV are mainly due to disease pathology and not to transfusion matching protocols [14]. Platelet levels showed a statistically higher increase in both groups compared to the control group, while no statistically different level was found between pure and traditional groups. This confirms ICTMG 2025 guideline results, which state that neither protocol is risky for hematology and that extended matching mainly helps in preventing alloimmunization [23].

Conclusion

This study clearly shows that extended antigen matching is a major breakthrough in the treatment of thalassemia requiring transfusions, especially in areas where the disease is highly prevalent. This practice will help achieve better health results in patients since it will contribute to more stable blood parameters, lower bilirubin content, and a lesser amount of iron in the body.

Conflict of Interest: There are no interferences in the current study.

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