Iraqi Journal of Cancer and Medical Genetics

Assessment of complement regulatory proteins CD35 and CD55 in β-thalassemia patients

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Abstract:

Background

Reduced or absent production of Beta globin chain causing a hemolytic anemia known as beta thalassemia which is an inherited anemia of variable severity results from imbalances of globin chains, ineffective erythropoiesis and hemolysis. Red Blood cells are protected from lysis caused by complement via the complement regulatory proteins CD35 and CD55.

Aim of the study

To evaluate CD35 and CD5 in beta thalassemia cases and correlate the serum level of CD35 and CD55 with clinical and hematological parameters.

Patients and methods

A case control study , included 60 patients who were a known case of β-thalassemia aged between 2_30 years and control group of 20 participants who were healthy and age and sex matched with case group. All hematological parameters were done by automated hematological analyzer.Serum level of CD35 and CD55wereestimated in both patients and control subjects by ELISA technique.

Results

In this study, Mean RBC, Hb, PCV, MCH, and MCV were significantly lower in thalassemia cases. Serum level of CD35 was significantly lower ,while CD55was significantly higher in patients with thalassemia .There were significant weak positive correlations between CD35and both of RBC and PCV level.

Conclusion

A low level of CD35 in β thalassemia patients can accelerate the RBC lysis by permitting complement deposition on them .CD35 level showed positive correlation with RBC and PCV and negative correlation with RDW. CD55 is high in thalassemia but with no significant correlation with any of thalassemic patients' characteristics.

Keywords: *Thalassemia, CR1 (CD35),DAF (CD55).*

Introduction:

Thalassemia is an inherited chronic haemolytic anemia caused by reduced or no production of the hemoglobin chains. The two main categories of thalassemia are alpha thalassemia which is due to alpha chain defect and beta thalassemia as a result of beta globin chain defect (1)(2). In Beta thalassemia ,the imbalances between alpha and beta globin chains production lead to excess α-globin tetramer formation that interaction with the red cell membrane causing haemolysis and bone marrow erythroid hyperplasia (3)(4) with ineffective erythropoiesis, .In addition to these causes of hemolysis another mechanisms have been suggested in some studies to be additional factors to worsen the hemolysis in β-thalassaemia (5).

The clinical severity of β-thalassaemia are widely variable ranging from the severe cases which are "transfusion-dependent" to mild asymptomatic cases "thalassaemia trait". The intermediate severity between them is known as "thalassaemia intermedia" (6).

The complement system . in addition to its role in innate immune responses it has a role in hemolysis .the complement system is regulated by proteins , one of them is CD35

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which has a negative regulation effect on complement cascade by processing and removing complement opsonized immune complexes , also mediates immune adherence and phagocytosis, and clearing the immune complexes and pathogens coated with C3b and C4b (7). The other protein is CD55 which protect cells from lysis by preventing the C3/C5 convertase production. Defect or low level of these proteins may be additional cause of hemolysis in thalssemia cases and may affect the severity of anemia and the clinical outcome. (8,9).

Aim of the study:

* To evaluate the serum CD55 and CD35 in β- thalassemia case.

* To Correlate the serum level of CD35 and CD55 with clinical and hematological parameters.

Patients and methods:

A case control study , included 60 patients who were a known case of β-thalassemia (12 patients who were non transfusion dependent thalassemia and 48 patients who were transfusion dependent thalassemia) aged between 2_30 years and control group of 20 participants who were healthy and age and sex matched with case group. All hematological parameters were done by automated hematological analyzer.Serum level of CD35 and CD55wereestimated in both patients and control subjects by ELISA technique. The samples were collected from AL-Karama Thalassemia Center/ Baghdad ,in 2019.

The serum collected and stored at -80˚C, and then used for measuring serum CD35 andCD55 level by enzyme linked immunosorbent assay (ELISA) using the Elabscience CD35and CD55 Immunoassay kits.

The basic hematological parameters and indices were done by automated device "Cell-DYN, RUBY Abbott Diagnostics, USA ,Automated Haematology Analyser".

Investigation results of previous complete blood count, blood film, virology screen, renal function test, liver function test,serum ferritin, reports of abdominal ultrasound and echocardiogram(were obtained from the files of the patients).

Inclusion criteria

Patients with transfusion dependent and non transfusion dependent beta thalassemia.

Exclusion criteria

Patients with other form of haemolytic anemia, other hemoglobinopathies and patients receiving blood transfusion in less than 3 weeks.

Results:

Hematological characteristics of patients and control subjects are listed in table (1).The mean RBC,PCV.Hb , MCV and MCH were significantly lower in patients group. There was no significant difference in mean MCHC ,while the mean RDW was significantly higher in patients group. The serum level of CD35and CD55 are shown in table (2)and figures (1) and (2). The level of CD35 was lower in patients with thalassemia in comparison with control group. There was also highly significant difference in mean CD55 between patients and control group (P = 0.001), with a higher level in patients group. The level of CD35 and CD55 in patients with transfusion dependent thalassemia(TDT) and non transfusion dependent thalassemia (NTDT)are listed in table (3).In which no significant difference between two groups. The correlations of CD35 and CD55levels to patient's characteristics in thalassemia group are shown in table(4). The CD35 was positively correlated to female gender in highly significant ($r = 0.508$; $P< 0.001$). It was also positively correlated with RBC and PCV and negatively correlated to RDW. On the other hand, CD55was not significantly correlated to any of thalassemia patients' characteristics.

Discussion:

In the current study, the median level of CD35 was significantly lower while CD55 was higher in patients group.

A difference observed in a study conducted by Kurtoğllu and colleagues,in which the results obtained showed that overall (mean ± SD) percentage of both CD55-positive RBCs and CD35-positive RBCs among β-thalassemia major patients was significantly lower than healthy controls , this indicates their role in the aetiology and pathogenesis of hemolysis and the complications of thalassemia (10).

Another study by Obaid et al study ,also found that CD55 expression was lower in β-thalassemia patients than healthy controls (11).

The difference observed in the above mentioned results can have attributed to the sample size participated in each study and to the method of measuring the level of CrPs, as in the current study, method used was ELISA to measure serum levels of CD35 and CD55, while in other studies depended on assessment the expression of these proteins on RBC membrane by flow-cytometry.

Low serum level of CD35 may be due to the low mean corpuscular volume in this study, or due to loss of CD35 by exocytosis and proteolysis of red cells membrane vesicles in ageing RBCs, and by the effect of increased α-globin tetramers on red cells membrane .That is why low serum level of CD35 subject red cells to lysis by the effect of complement because they lost the complement activation control and efficient immune complex clearance (11,12,13)

The CD55 is expressed by RBCs as a protecter protein that help cells to escape from by stander hemolysis , so rapid pathological turned over of red cells and proteolysis may results in high serum CD55 levels.

In the present study, mean RBC, mean PCV, mean Hb, mean MCV, mean MCH were significantly lower in thalassemia, while no significant difference in mean MCHC and higher RDW .Comparable Results observed in a study conducted by Karim and colleagues in 2016, (14). other study conducted by FilizSimsek and colleagues in their study, in which additionally found higher RDW and reticulocyte counts in patient group (15). Results observed in Abbassy et al study , in which 60 thalassemic patients enrolled, results obtained showed lower

Mean of Hb, mean of RBC and mean of MCV in thalassemic patients than control group, while mean of RBC and platelets were higher(16).The changes observed in blood indices in thalassemic patients can explained by fact that decrease in the hemoglobin level is accompanied by a decrease in the number of erythrocytes because of haemolysis and diminished values of their specific indices (MCV, MCH, HCT).

In this study, CD35 was positively correlated to female gender in highly significant rate. It was also positive correlated to RBC and PCV and negatively correlated to RDW .On the other hand, CD55 was not significantly correlated to any of thalassemia patients' characteristics.

In comparison to other studies, a different results observed in an Egyptian study conducted in 2014 by Obaid and colleagues,the results obtained showed that CD55 was not correlated with any complete blood count parameters apart from a negative correlation with platelet count (11).

Conclusions:

A low level of CD35 in β thalassemia patients accelerates the RBC lysis by permitting complement deposition on them .CD35 level showed positive correlation with RBC and PCV and negative correlation with RDW. CD55is high in thalassemia but with no significant correlation with any of thalassemic patients' characteristics.

Recommendations

Further studies on larger sample size.Study the expression of CD35and CD55 on the red blood cells in β thalassemia patients by flow cytometry technique.

Table (1): Hematological characteristics of patients and control subjects

Characteristic	Thalassemia group n = 60	Control group n = 20	† P
RBC $X10^{12}$/L	±0.60 3.01	±0.38 4.70	0.001 > HS
% PCV	±4.57 24.50	±3.59 38.80	0.001 > HS
Hb g/dl	±1.63 8.14	±1.21 12.74	0.001 > HS
MCV fl	±5.11 78.94	±6.54 82.99	0.018 S
MCH pg	±1.94 25.01	±1.95 26.92	0.001 > HS
MCHC g/dl	±1.92 33.38	±1.30 32.87	0.270 NS
% RDW	±5.81 22.29	±4.45 15.65	0.001 > HS
Serum ferritin	±1617.73 2432.10	----	----

n: number of cases; data were presented as mean ± standard deviation; †: independent samples t-test; NS: not significant at P > 0.05; HS: highly significant at P ≤ 0.01 ,t; NS: not significant at P > 0.05

Table (2): The serum level of CR1 (CD35) and DAF (CD55) in patients and control groups.

Characteristic	Thalassemia group n = 60	Control group n = 20	P
CD35 ng/mL			
Median (IQR)	1.04 (1.99)	7.79 (12.35)	< 0.001 £
Range	0.15 -13.20	0.64 -19.10	HS
CD55 pg/mL			
Mean ±SD	695.35 ±171.44	544.32 ±159.69	0.001 †
Range	307.56 - 1204.85	293.63 -902.78	HS

n: number of cases; SD: standard deviation; IQR: inter-quartile range; †: independent samples t-test; £: Mann Whitney U test; HS: highly significant at P ≤ 0.01

Table (3): The level of CR1 (CD35) and DAF9 (CD55) in patients with transfusion dependent thalassemia(TDT) and non transfusion dependent thalassemia (NTDT)

Characteristic	TDT *n* = 48	NTDT *n* = 12	*P*
CR1(CD35)			
Median (IQR)	0.92 (1.66)	1.39 (2.35)	0.365 £
Range	0.18 -9.04	0.15 -13.20	NS
DAF9 (CD55)			
Mean ±SD	677.59 ±149.02	699.78 ±177.75	0.692 †
Range	320.64 -879.29	307.56 -1204.85	NS

n: number of cases; SD: standard deviation; IQR: inter-quartile range; †: independent samples t-test; £: Mann Whitney U tes

Table (4): The correlations of CD35 and CD55 levels to Patient's characteristics in thalassemia group

Characteristic	CD35		CD55	
	r	P	R	P
Age	0.162	0.218	0.229	0.078
Gender	0.508	**0.001>	0.011	0.935
Thalassemia type	-0.118	0.370	0.036	0.784
RBC *10^12/L	0.258	*0.047	0.039	0.767
PCV	0.266	*0.040	0.014	0.916
Hb g/dl	0.251	0.053	0.029	0.828
Serum ferritin	0.217	0.096	-0.148	0.258
Hepatosplenomegaly	-0.231	0.075	-0.083	0.526
Other disease	-0.066	0.619	0.112	0.393
MCV fl	-0.179	0.172	0.106	0.421
MCH pg	-0.181	0.167	0.018	0.894

MCHC g/dl	0.057	0.668	-0.053	0.688
% RDW	-0.278	*0.032	0.059	0.653
Family history of thalassemia	0.020	0.879	0.192	0.141

*r: Spearman correlation coefficient; *: Significant at P ≤ 0.05; ** Highly significant at P ≤ 0.01*

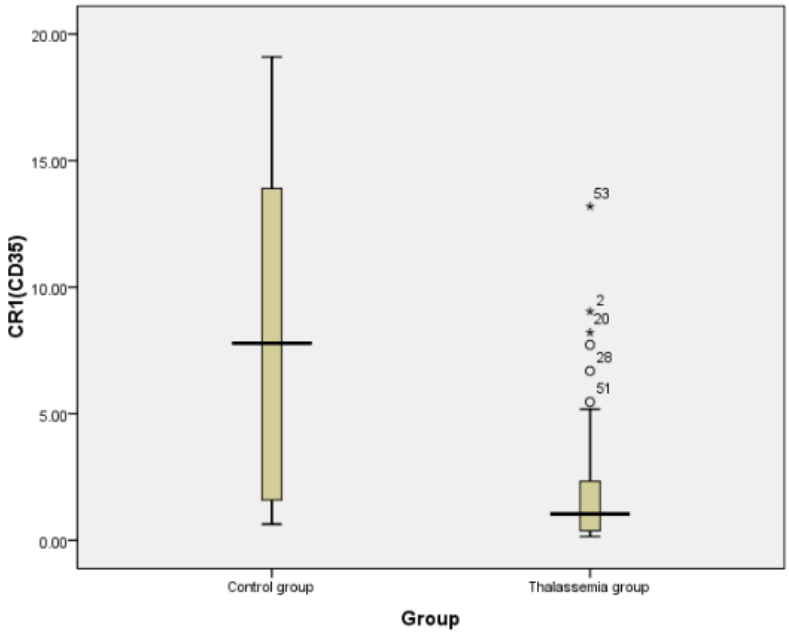

Figure 1. Box plot showing median level of CD35 in patients with thalassemia and control group.

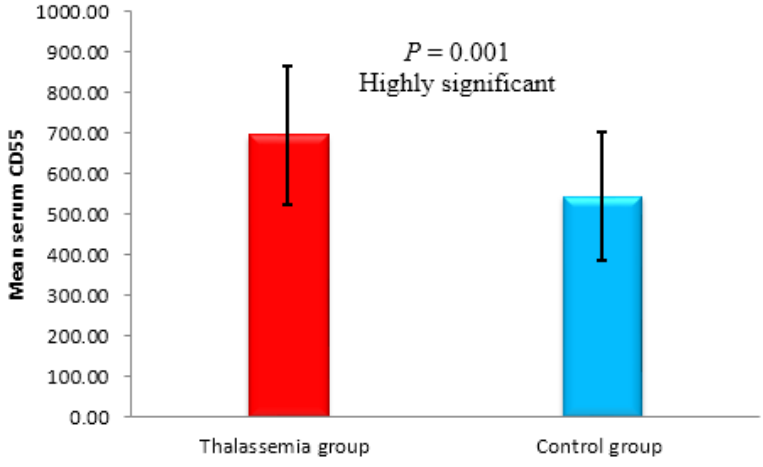

Figure (2): Bar chart showing mean level of CD55 in patients with thalassemia and control group.

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