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The expression of CD177 and it`s relation to serum soluble transferrin receptors (sTfR-1) in β thalassemia patients

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Abstract:

Background: Ineffective erythropoiesis has a well-known role in the pathophysiology of β-thalassemia syndromes. CD177 expression was studied in diverse hematological, clinical, and immunological disorders (like myeloproliferative neoplasms MPN, transfusion-related acute lung injury TRALI; neonatal alloimmune neutropenia).

Aims of the Study:

1. To assess significance of CD 177 as a marker of increased erythropoietic activity in patients with β thalassemia major and minor.
2. to correlate CD177 expression with serum transferrin receptors (sTfR -1) and absolute neutrophils count.

Patients and Methods: this cross sectional study performed on 25 patients with β thalassemia major, 25 patients with β thalassemia minor, along with 25 healthy control, all were selected from Babylon Maternity and Children Teaching Hospital,

Investigations included complete blood counts, hemoglobin analysis (by high performance liquid chromatography HPLC ,CRP, ESR, serum ferritin, flowcytometric immunophenotyping for CD177 expression and percentage, mean fluorescence intensity (MFI) expression on gated neutrophil and serum sTfR-1 levels were measured by enzyme immunoassay.

Results: The mean expression of CD177% was higher in β thalassemia major than minor and control, but it did not reach level of significance (p=0.22).

CD177 Mean fluorescence intensity (MFI)had no significant difference (p=0.74) among β thalassemia major , minor and control.

Mean levels of sTfR-1 in thalassemia major, minor and control group were significantly differing (p<0.0001).

Within β thalassemia major, there was a negative significant correlation between Hb level and serum transferrin receptor-1 concentration (p=0.007), ` a significant positive correlation between serum ferritin and sTfR-1(p=0.03).

Conclusions: CD177 expression (percentage and MFI) as a marker of erythropoietic activity was insignificantly elevated in β thalassemia patients ,while sTfR-1 levels showed statistically significant difference in relation with erythropoietic activityin thalassemia patients.

Keywords: *CD177, sTfR, thalassemia.*

Introduction:

Beta-thalassemia syndromes are diverse groups of inherited blood disorders characterized by decreased or absent beta globin chain subunits of the hemoglobin (Hb)tetramers along with relative excess of α-chains.(1)

The clinical syndromes related with thalassemia derived from the com¬bined effects of inadequate Hb production with consequent hypochromic microcytic anemia and unbalanced accumulation of free α-globin subunits, unpaired α-chains aggregates precipitate to make inclusion bodies, which initiate oxidative membrane damage within the RBC, ending in apoptosis and destruction of immature developing erythroblasts in the bone marrow. Ineffective erythropoiesis, in addition to hemolytic anemia , that reduce the survival of the few RBCs produced. (1)(2)

CD177 is a 58- 65-kDa glycoprotein, which harbors the human neutrophil antigen 2 (HNA-2; neutrophil antigen B1 or NB1).It is a glycosylphosphatidylinositol GPI-anchored glycoprotein, belongs to the leucocyte antigen 6 superfamily .The gene coding for CD177 is confined to chromosome 19q13.2 and has at least two alleles, NB1 and polycythemia rubra vera PRV-1.(3)

CD177 expression in humans varies greatly among different ethnic groups, two neutrophil subpopulations can be rec

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ognized depending on CD177 expression. HNA-2-positive individuals with a frequency exceeds 90%, while the HNA-negative phenotype varies between 0.0% and 2.0%. The mean size of the HNA-2–positive subpopulation of neutrophils is 45% to 65%.(4)

CD177 expression can be increased by administration of granulocytes stimulating factor (G-CSF, increases to nearly 90%), polycythaemia vera (PV), vasculitis and patients with neutrophilia secondary to acute infections, concerning both the percentage of positive cells and number of molecules per cell.(3)

Soluble transferrin receptors (TfR-1) is soluble truncated monomer of TfR produced by proteolysis. The bulk of sTfR-1 originates largely from erythroblasts . sTfR-1 concentration has been demonstrated to be proportional to the mass of erythriod and hence, erythropoietic activity.(5)

Aims of Study

1. To assess significance of CD 177 as a marker of increased erythropoietic activity in patients with β thalassemia major and minor
2. to correlate CD177 expression with serum transferrin receptors (sTfR -1) and absolute neutrophils count.

Patients and Methods:

During the period extending from January 2019 to July 2019, a cross sectional study of a convenient sample was conducted from three groups:

Group I consisted of 25 patients diagnosed as having homozygous beta thalassemia major; 13 male and 12 females aged 5-28 years who were registered in Babylon thalassemia center ,at Babylon Maternity and Children Teaching Hospital, as a regular blood transfusion dependent patients, diagnosed on the bases of clinical features with laboratory evaluation including evaluation of peripheral blood counts and film, hemoglobin analysis by electrophoretic or chromatographic techniques from early years of life by reviewing patients` medical records and on regular red cell transfusions nearly every 4 weeks. Samples were collected at the day of patients' visit, just before transfusion.

Group II 25 samples of β thalassemia minor, with 11 males and 14 females aged between 4-27 years were obtained from family members of thalassemia major patients who did not receive any blood transfusion and diagnosed depending on complete blood counts and HbA2 level > 3.5-7% .(6)(7)

Group III consisted of 25 age and sex matched control samples were collected from normal persons (with normal total blood counts, serum ferritin within normal ranges, CRP lower than detection limit and ESR within normal reference limits).

A verbal consent from each patient and control, was obtained for accepting to take PB samples. The data was collected, by interviewing the patients and looking over their medical records, including: name, age, gender, symptoms and physical signs , age of diagnosis, age at first blood transfusion, drugs history, chelation therapy, and history of splenectomy.

A total venous blood sample of 5 ml was obtained from each patient and control included in this study by venipuncture under aseptic technique. The total venous blood sample divided into smaller samples as follows:

- 2.5 ml in K3-EDTA tube were used for complete blood counts(CBC) (Cell-DYN, RUBY Abbott Diagnostic, USA), erythrocytes sedimentation rates (ESR), immunophenotyping for CD177 on gated neutrophil by flowcytometry (PE Mouse Anti-Human CD177 MEM-166 BD Biosciences (USA) using eight colour BD FASCantoTMII, Becton Dickinson and Company, United States), gating of neutrophils was done depending on SSC and CD45. The analysis of CD177 on neutrophils involved both percent (%) of CD177 positive cell and mean fluorescence intensity (MFI) in (arbitrary units of FI a.f.u.) described as geometric mean using FCS Express7 software.(8)(9)
- Hb variant analysis by D-10Dual HbA2/F/A1c Program BIO-RAD Laboratories (USA).
- 2.5 ml were put in a gel tube which was placed in water bath at 37°C for one hour and then centrifuged for 10 minutes at 3000rpm, to separate the serum and divided into small aliquots; which were used for measurement of serum ferritin level and CRP immediately and the remaining was stored at -80 °C until assay of (serum transferrin receptors sTfRs by ELISA technique using Human sTfR1(Soluble Transferrin Receptor 1) ELISA Kit Elabscience Biotechnology Inc (USA) following the manufacturer's instructions.

Statistical analysis:

Statistical analysis was performed with the SPSS 23 statistical software program. Univariate data were reviewed using standard descriptive statistics, tabulation of categorical variables and histograms of numerical variables. The Mann–Whitney test and Kruskal-Wallis test (a rank-based nonparametric test equivalent to ANOVA) had been used to verify if there was statistically significant differendes between two or more groups of an independent variables on a continuous or ordinal dependent variable. Spearman`s correlation had been used to measure association between two continuous variables or when at least one variable was ordered. Exact tests were used to calculate the P value. In all statistical analyses, a p value < 0.05 was counted significant.

Results:

The mean age of patients in this study was (13.48 ± 7.0 years) and(14.0 ±7.84 years) in thalassemia major and minor groups respectively, and (12.68±7.55 years) for controls. Patient's gender was selected randomly, in thalassemia major M:F(male:female) ratio was 1.08:1 and in thalassemia minor M:F ratio was 0.9:1.

In thalassemia major patients , the frequency of skeletal changes included elongation of the skull with frontal and parietal bossing, enlargement of the maxilla, protruding molar eminences, with depressed nasal bridge was 17(68%)patients and 8(32%) patients were with no changes.

Mean serum ferritin levels were (2001.12±853.05 µg/L, 71.49 ± 21.99 µg/L, 43.81±19.95 µg/L) in β thalassemia major, minor and control respectively with a significant difference among the three groups (p=0.001),Figure 1.

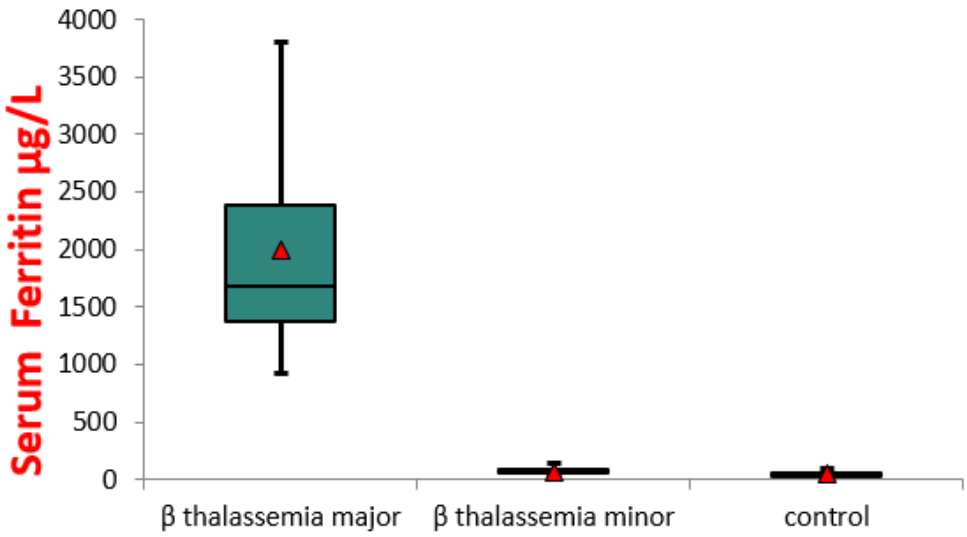

Figure 1. Boxplot of serum ferritin level in study groups, In each group the centeral line denotes the median and upper and lower lines denote the 75th and 25th percentiles, respectively; the caps show the range, (▲) denotes the mean.

There was a high significant increase in s-TfR concentrations in β-thalassemia groups and control (p<0.0001), table 1.

Ranges and mean percent of CD177 expression on gated neutrophils were (68.08-98.53%, 56.66-93.5%, 53.54-78.41%) and (84.30 ± 9.23% ,77.31 ± 7.86% , 69.75± 8.65%) in β thalassemia major, minor and control respectively . The percent of positive neutrophils for CD177 was insignificantly differing among the studied groups (P=0.22) as shown in (figure 2 and table 1).

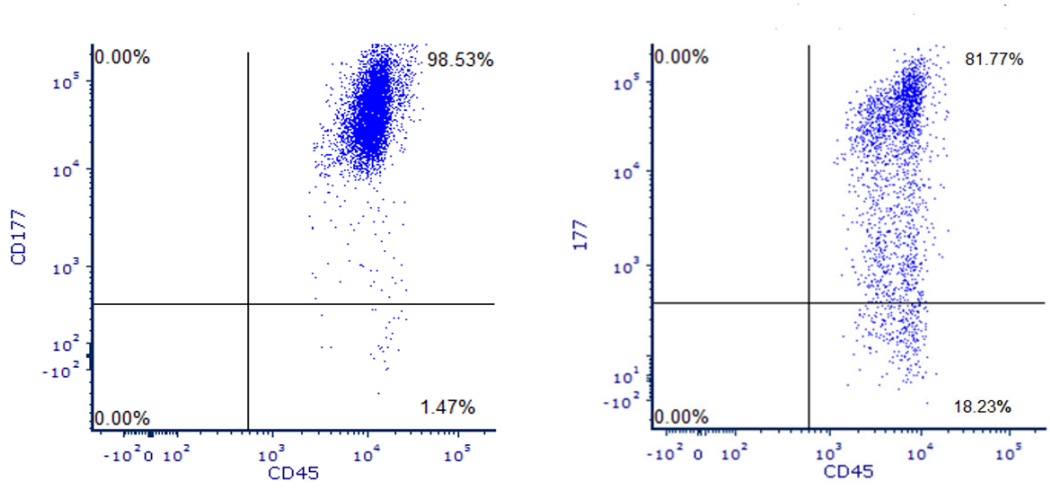

Figure 2. An example of flow cytometric dot plot of CD177% expression in whole blood sample of a β thalassemia major (left) and a patient with β thalassemia minor patient (right).

Mean Fluorescence Intensity MFI expressed as Geometric Mean was higher in patients than in control but it did not reach the level of significance (p=0.74), (figure 3 and table 1).

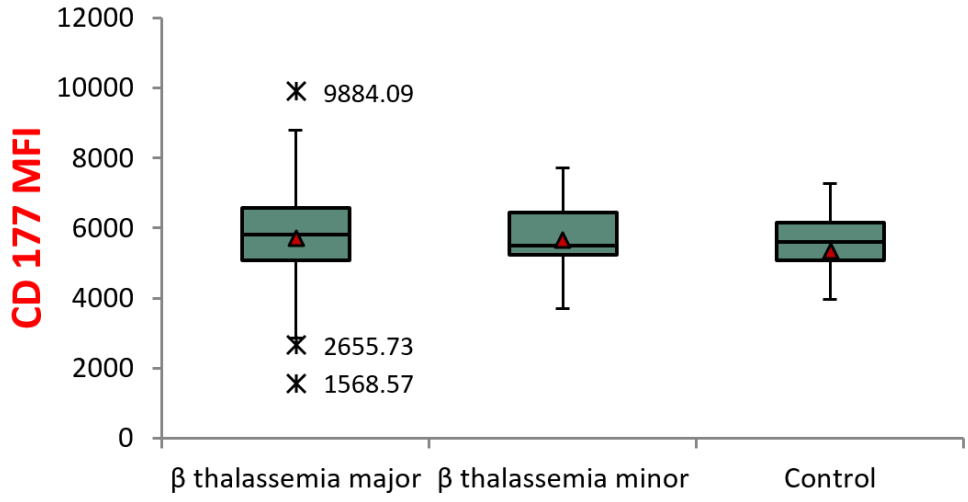

Figure 3. CD177 MFI in the three groups. In each group the center line represents the median and the upper and lower lines represent the 75th and 25th percentiles, respectively; (▲) represents the mean.

Table 1. Comparison of sTfR-1, CD177 percent and MFI (geometric mean) on positive neutrophils among the study groups .

Mean ± SD	Beta-Thalassemia major, n=25	Beta-Thalassemia minor, n=25	Control, n=25	P
sTfR-1 (ng/mL)	1279.9 ± 478.9	627.6 ± 243.8	281.3 ± 75.6	0.0001*
CD177 positive neutrophils (%)	84.30 ±9.23	77.31 ±7.86	69.75 ±8.56	0.22
MFI (geometric mean) in a.f.u.	5711.44 ± 1793.2	5663.16± 1073.93	5341.47± 895.80	0.74

Kruskal-Wallis test

Comparison between patients with skeletal changes and those without in the β thalassemia major group showed that difference between patients with skeletal changes and those without was significant regarding sTfR-1 was significant (p=0.04) (figure 4), but not with regards to CD177% and MFI (p= 0.47 and 0.54 respectively).

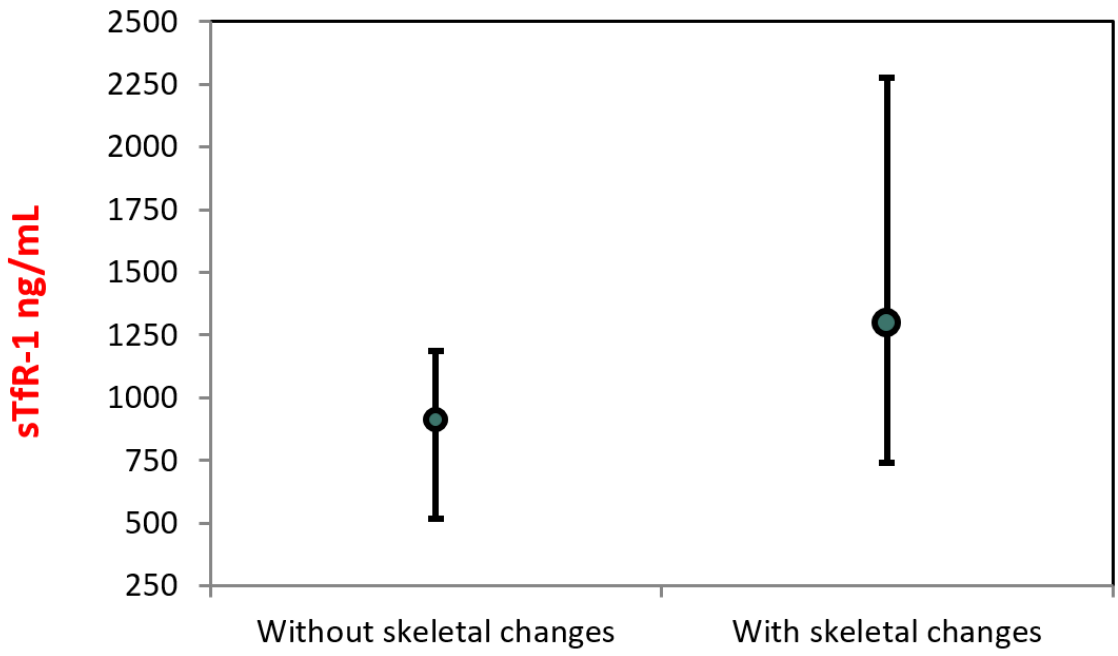

Figure 4. sTfR-1 in patient without skeletal changes and those with skeletal changes , the (●) represents the mean ,the caps represent the range.

No significant correlation was found in CD177(percentage and MFI) on gated neutrophils and s-TfR1(ng/mL) in β thalassemia minor and major patients, (table 2).

Table 2. Correlation between CD177 percentage and MFI on gated neutrophils and sTfR1(ng/mL) in β thalassemia groups.

	sTfR-1		
		r	p
β thalassemia minor, n=25	CD177%	-0.036	0.864
	CD177 (MFI)	-0.228	0.274
β thalassemia major, n=25	,CD177%	-0.167	0.425
	CD177 (MFI)	0.316	0.124

Spearman،s rho

There was a significant negative correlation between Hb level and sTfR-1 concentrations in β thalassemia major group(p=0.007), while in thalassemia minor group the correlation was insignificant (p=0.313). In β thalassemia major, a significant positive correlation between serum ferritin and sTfR-1 was found (p=0.036) (figure 5), while it was insignificant in β thalassemia minor patients.

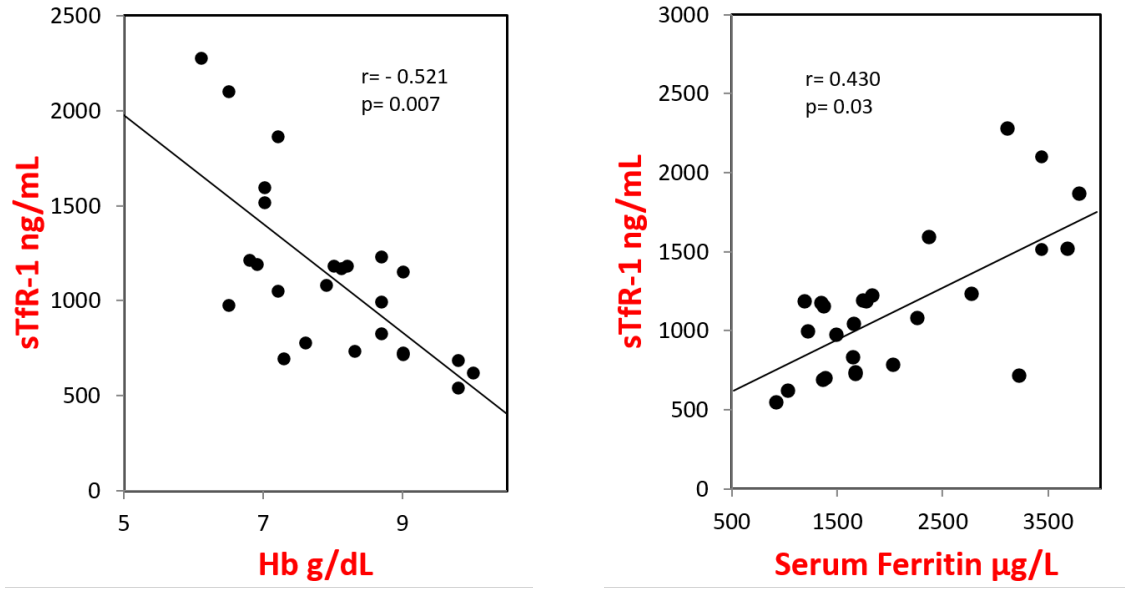

Figure 5. Correlations between the Hb level and serum ferrtin with sTfR-1 concentration in β thalassemia major.

There were insignificant correlations in CD177(percentage and MFI) on gated neutrophils with sTfR1or absolute neutrophils count in each of β thalassemia major and minor patients,(Figure 6).

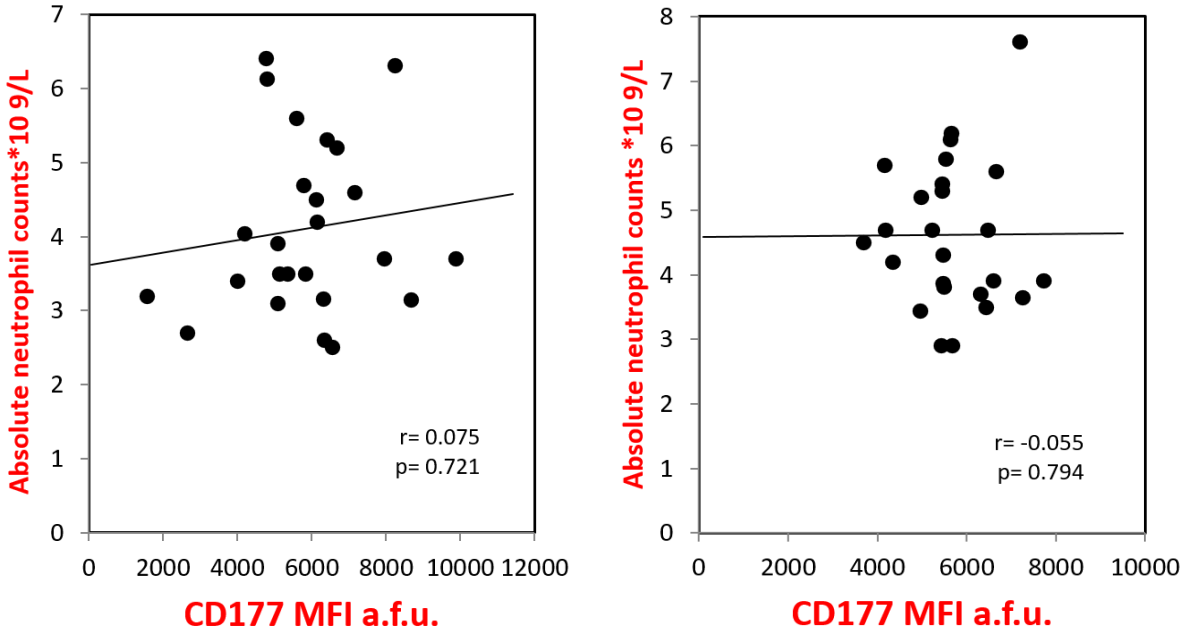

Figure 6. Correlation between CD177 MFI and absolute neutrophil counts in β thalassemia major (left) and minor patients (right).

Discussion:

β-Thalassemia is an inherited diverse hemoglobin disorder characterized by the reduced or absent synthesis of β-globin chain which produces globin chain imbalance. Ineffective erythropoiesis is the hallmark of the disease which results in progressive marrow expansion and extra medullary hematopoiesis(EMH).(10)

CD177 mRNA levels have not been noticed to increase in JAK-2–associated disorders including polycythemia vera, essential thrombocythemia but also CD177 protein expression studies showed that the percentage of CD177+ granulocytes is increased in a number of conditions and physiologic states, including in umbilical cord blood, pregnancy, anti-neutrophil cytoplasmic antibody (ANCA)–associated systemic vasculitis, β-thalassemia, and after G-CSF therapy.(11)

Serum ferritin levels revealed a significant statistical difference among β thalassemia major, minor and control, (p=0.001), with agreement to other studies.(10)(12-16) In patients with β thalassemia major, the molecular pathophysiology implicates supression of hepcidin; the major molecule inhibiting iron absorption and recycling, with increased intestinal absorption of iron, regular transfusion therapy; and propably shortage of supply and poor adherence to chelation therapy in our thalassemia patients.

Serum transferrin receptor concentrations showed statistically significant difference among patients groups and control, between β thalassemia major and control , between β thalassemia minor and control (p=0.0001), similar figures were found by Demir A et al(17), Guimaraes et al.(18), Jalali MT et al.(19), Tancabelic et al.(20) Montaser et al.(21) and Zoi et al.(22) . The erythriod marrow is the key source of soluble transferrin receptors and increased levels are encountered in erythroid hyperplasia.(23) Since both groups of patients showed Hb levels below normal; despite RBC transfusion in β thalassemia major ; this could elicit erythropoietin(EPO) secretion. The EPO levels probably signify the message conducted to increase erythropoiesis, which implicate the presence of great amounts of TfR on the erythriod cells and the production of great amounts of the soluble receptor in an effort to compensate for the diminished Hb concentration. (23)

Also patients with β thalassemia minor show increased sTfR due to mild associated ineffective erythropoiesis.(24)

CD177% expression showed insignificant difference among the three groups (p= 0.22), which was consistent with findings of Montaser et al.(21) and Abd El-Wahed MR et al.(25)

MFI of CD177 on gated neutrophils showed statistically insignificant difference among the three groups (p=0.74) which disagrees with results obtained by Abd El-Wahed MR et al.(25), Montaser et al.(21) who described the MFI as a ratio, and Zoi et al.(22) who used real-time quantitative polymerase chain reaction (RQ-PCR) to quantify CD177 mRNA expression, so plausible reason might simply be the smaller sample size of this study and the different methodology in expressing MFI.

An insignificant correlation between CD177 (percentage and MFI) on gated neutrophils and sTfR-1(ng/ml) in each β thalassemia groups, was encountered by Abd El-Wahed MR et al.(25) in contrast to results obtained by Montaser et al.(21) and Zoi et al.(22)

Comparison between patients with bone deformities and those without in the thalassemia major group as regards CD177 (MFI and percentage) showed a non-significant difference, agreeing with Abd El-Wahed MR et al.(25) while sTfR-1 showed significantly higher levels in β thalassemia major patients with bony deformities that could be explained by augmented ineffective erythropoiesis with expansion of the bone marrow that causes distortion and fragility of the bones. (26)(27)

There was a negative correlation between serum transferrin receptor-1 concentrations and Hb level in each of β thalassemia groups but was significant only in β thalassemia major group with same figures obtained by Montaser et al.(21) Papassotiriou I et al.(28), Ren ZM et al.(29) , and Porter J B et al.(30) , which supported by the concept that sTfR-1 concentrations imitate the total erythroid mass, that increases with increasing anemia.

sTfR-1 showed statistically significant positive correlation with serum ferritin in patients with β thalassemia major (p=0.03) which was consistent with findings of El-Rashidi et al.(31), Tanno T et al(32), and linked to the fact that iron regulation in humans with symptomatic thalassemia syndromes is mediated by the erythroblast secretion of growth differentiation factor(GDF15). The high degree of ineffective erythropoiesis caused by globin chain imbalances, anemia, hemolysis and tissue hypoxia, which trigger the release of EPO, leading to marked erythriod proliferation accompanied by raised sTfR levels, with simultaneous elevation of GDF15 (the main hepcidin inhibitor), and the low hepcidin lets increased iron absorption, which take part in iron overload.(31)(33-35)

There was statistically insignificant correlation between CD177 (percentage and MFI) and absolute neutrophils count in each of β thalassemia group patient agreeing with the results of Montaser et al.(21)

Conclusions

In this study, CD177 expression (percentage and MFI) as a marker of erythropoietic activity was insignificantly elevated in β thalassemia patients ,while sTfR-1 levels showed statistically significant difference.

No correlation was found between CD177expression (percentage and MFI) and sTfR-1 or absolute neutrophils count in β thalassemia patients. Within β thalassemia major, sTfR-1 revealed a significant negative correlation with Hb levels and a significant positive correlation with serum ferritin.

Recommendations

Further larger studies may be needed to evaluate the role of CD177 in detecting the erythropoietic activity in thalassemia patients and whether it`s linking with sTfR-1 is significant or not, and correlating CD177 expression with serum erythropoietin (Epo) in thalassemia patients as an associated marker of expanded erythropoiesis.

REFERENCES:

1. Chapin J, Giardina PJ. Thalassemia Syndromes. In Hoffman, R., Benz, E. J., Silberstein, L. E., et al. (Eds.), Hematology: Basic Principles and Practice. Philadelphia: Elsevier; 2018. p. 546–70.
2. Tulchinsky TH. Eliminating Beta Thalassemia Major and Other Congenital Blood Disorders. Case Studies in Public Health.2018,:423–42.
3. Bauer S, Abdgawad M, Gunnarsson L, Segelmark M, Tapper H, Hellmark T. Proteinase 3 and CD177 are expressed on the plasma membrane of the same subset of neutrophils. J Leukoc Biol. 2007;81(2):458–64.
4. Wang E, Adams S, Stroncek DF, Marincola FM. Human Leukocyte Antigen and Human Neutrophil Antigen Systems. Seventh Ed. Hematology: Basic Principles and Practice. Elsevier Inc.; 2018.ch(113):1721-1737.
5. Thambiah S, George E, Aini UN, Sathar J, Zarida H. Serum Soluble Transferrin Receptor Concentration as a Biomarker of Erythropoietic Activity : Surrogate Marker of Adequate Transfusion in Adult Beta-Thalassaemia Intermedia Patients. Mal J Med Hea Sci. 2013;9(June):3–12.
6. Thein SL, Rees D. Haemoglobin and the inherited disorders of globin synthesis. In: Postgraduate Haematology. John Wiley & Sons;2016.p.72-97.
7. Bain BJ, Wild BJ. Investigation of Variant Haemoglobins and Thalassaemias. In: Dacie and Lewis Practical Haematology E-Book. Elsevier Health Sciences;2016 (Aug):ch(14):282-311.
8. Radbruch, A. Immunofluorescence : Basic Considerations. In: Flow cytometry and cell sorting. Berlin/Heidelberg: Springer.2000;ch(3):34-46.
9. Bonnevie J, Hammerbeck C, Goetz C. The Language of Flow Cytometry And Experimental Setup. In: Flow Cytometry Basics for the Non-Expert. Springer ,2018.
10. Oikonomidou PR, Rivella S. What can we learn from ineffective erythropoiesis in thalassemia?. Blood reviews. 2018 Mar 1;32(2):130-43.
11. Meyerson HJ, Osei E, Schweitzer K, Blidaru G, Edinger A, Balog A. CD177 Expression on Neutrophils In Search of a Clonal Assay for Myeloid Neoplasia by Flow Cytometry. Am J Clin Pathol. 2013;658–69.
12. Al sharifi LM, Haider AS, Hussain FA. Hemotological and Coagulatory Alteration in Splenectomized Versus Non-Splenectomized Transfusion Dependant B-Thalassemic Major Patients. Medical Journal of Babylon. 2015;12(2):551-60.
13. Alathari RA, Mahdi WT. Investigating Some of Blood Parameters in Women with β-Thalassemia. J Phys Conf Ser. 2019;1294(6).
14. Marbut SM, Zeidan MM P. The effect of Iron overload on PCV, hemoglobin & serum ferritin of thalassaemia major male patients Departments of Biology, College of Science, Tikrit University. Al-Kufa Uni J Bio. 2016;8(3):444–51.
15. Hanoon BD, AL-Mudalal SS. Evaluation of Interleukin 8, Interleukin 2 Receptor and Serum Ferritin in 60 Patients with Beta-Thalassemia Major: Relationship to Splenectomy. Iraqi Acad Sci J.2018;17(1):6-11.
16. Karim F, Ismail M, Hasan AM, Shekhar HU. Hematological and biochemical status of Beta thalassemia major patients in Bangladesh: A comparative analysis. Int J Hematol Stem Cell Res. 2016;10(1):224–9
17. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Serum transferrin receptor levels in beta-thalassemia trait. J Trop Pediatr. 2004;50(6):369–71.
18. Guimarães JS, Cominal JG, Silva-Pinto AC, Olbina G, Ginzburg YZ, Nandi V, et al. Altered erythropoiesis and iron metabolism in carriers of thalassemia. Eur J Haema. 2015;94(6):511–8
19. Jalali MT, Mohseni A, Keikhaei B, Latifi M. Evaluation of diagnostic efficacy of serum sTfR assay in iron-deficiency anemia and beta-thalassemia trait in Shafa Hospital, Ahvaz, Iran 2010. Eur Rev Med Pharmacol Sci. 2012;16(10):1441–5.
20. Tancabelic J, Sheth S, Paik M, Piomelli S. Serum transferrin receptor as a marker of erythropoiesis suppression in patients on chronic transfusion. Am J Hematol. 1999;60(2):121–5.
21. Montaser LM, El□Rashidi FH, Essa E S, Azab S M. Analysis of CD177 neutrophil expression in β□thalassemia patients. Apmis.2011;119(10):674-80.
22. Zoi K, Terpos E, Zoi C, Loukopoulos D. Increased CD177 (PRV1) expression in thalassaemia and the underlying erythropoietic activity. Br J Haematol. 2008;141(1):100–4.
23. Cazzola M, Beguin Y, Bergamaschi G, Guarnone R, Cerani P, Barella S, et al. Soluble transferrin receptor as a potential determinant of iron loading in congenital anaemias due to ineffective erythropoiesis. Br J Haematol. 1999;106(3):752–5
24. Nobili B, Perrotta S, Matarese SMR, Conte ML, Del Giudice EM. Evaluation of body iron status in Italian carriers of beta-thalassemia trait. Nutr Res. 2001;21(1–2):55–60.
25. Abd el-Wahed MR, Gawish HH, Ghobashy A A, Ismail N E. CD177 expression in β-thalassemia patients in Zagazig University. Egyp J Haem. 2018;(43)1: 25-31.
26. Wonke B. Bone disease in β-thalassemia major. Br J Haematol. 1998;103:897-901.
27. Mahachoklertwattana P, Pootrakul P, Chuansumrit A, Choubtum L, Sriphrapradang A, Sirisriro R, Rajatanavin R. Association between bone mineral density and erythropoiesis in Thai children and adolescents with thalassemia syndromes. J Bone Mine Meta. .2006;24(2):146-152.
28. Papassotiriou I, Flevari P, Poziopoulos C, Zaliou S, Tsaousis V, Larissi K, Voskaridou E. Non Invasive Evaluation of Bone Marrow Activity in Patients with Sickle Cell Disease: Correlation with Disease Features, Genotype, Markers of Erythropoiesis, Iron Metabolism and Hydroxyurea Treatment. Blood. 2019 ;134: 4821
29. Ren ZM, Huang LL, Huang BX, Li CG, Chen YS. [Serum level of soluble transferrin receptor in children with hemoglobin H disease]. Zhongguo Dang dai er ke za zhi = Chinese Journal of Contemporary Pediatrics. 2019 Sep;21(9):894-897.
30. Porter J B, Cappellini MD, Kattamis A, Viprakasit V, Musallam KM, Zhu Z, TaherA. T. Iron overload across the spectrum of non□transfusion□dependent thalassaemias: role of erythropoiesis, splenectomy and transfusions. Bri J Haem ;2017,176(2): 288-299.
31. El-Rashidi FH, El-nemr FM, Ragab SM, Fathallah SMKE, Rakha RI. The role of soluble transferrin receptor in iron overload in children with chronic hemolytic anemia. 2013;132–7.
32. Tanno T, Bhanu N V, Oneal PA, Goh S, Staker P, Lee YT, et al. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. 2007;13(9).
33. Huang Y, Lei Y, Liu R, Liu J, Yang G, Xiang Z, Liang Y, Lai Y. Imbalance of erythropoiesis and iron metabolism in patients with thalassemia. Int J Med Sci 2019; 16(2):302-10.
34. Kattamis A, Papassotiriou I, Palaiologou D, Apostolakou F, Galani A, LadisV, Papanikolaou G. The effects of erythropoetic activity and iron burden on hepcidin expression in patients with thalassemia major. haematologica, ;2006, 91(6):809-12.
35. Cazzola M, Stefano PD, Ponchio L, Locatelli F, Beguin Y, Dessì C, Galanello R. Relationship between transfusion regimen and suppression of erythropoiesis in β□thalassaemia major. Bri J Of Haem; 1995,89(3): 473-78.