ORIGINAL ARTICLE

Effects of Transfusion and Splenectomy on Globin Chain Expression in NTDT HbE/β-thalassaemia

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ABSTRACT

Introduction: Majority of HbE/β-thalassaemia patients resembles the phenotype of non-transfusion dependent thalassaemia (NTDT). Current management strategies are highly diverse, and the objective of this study is to examine the effects of different treatments on multiple parameters in NTDT HbE/β-thalassaemia to further streamline the management of this disorder. **Methods**: In this cross-sectional study, we analysed the correlation between different treatment strategies with variable parameters including haematological parameter and globin gene expression. Statistical analyses were carried out using non-parametric tests such as Kruskal-Wallis and Mann-Whitney tests. **Results:** A total of 29 HbE/β-thalassaemia patients were included in the study. Data showed statistically significant differences were observed in the MCV, MCHC levels, reticulocyte count and log α/β fold change between the groups. Further analysis showed higher log α/β fold change in the transfusion only group compared to the non-treated group. Red blood cell count was found to be lower in transfused and splenectomised group compared to transfusion only. Significantly higher MCV level and reticulocyte count was seen in transfusion and splenectomised group compared to both non-treated and transfusion only groups and higher MCH level in the transfusion and splenectomised group compared to transfusion only group. **Conclusion:** In general, regardless of single or double combined therapies, HbE/β-thalassaemia showed variable changes in laboratory parameters to the therapies received particularly splenectomy.

Keywords: HbE/β-thalassaemia, transfusion, splenectomy, log α/β expression, globin gene expression.

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INTRODUCTION

HbE/β-thalassaemia is characterised by a compound heterozygosity of β-thalassaemia and structural variant HbE with a wide clinical phenotype spectrum extending from mild to severe. Almost half of HbE/β-thalassaemia patients manifest clinical symptoms similar to β-thalassaemia major while the rest have a similar appearance to β-thalassaemia intermedia or non-transfusion dependent thalassaemia (NTDT) (1). The management strategies for HbE/β-thalassaemia are highly diverse and encompass close observation, intermittent transfusion, splenectomy and HbF-inducing agents (2, 3). Unlike thalassaemia major, progress of thalassaemia intermedia management including HbE/β-thalassaemia and prevention of complications later in life has been slow until recently (4).

The conventional treatment for HbE/β-thalassaemia, if necessary, is blood transfusion to provide sufficient oxygen for the body's physiological need and almost always accompanied by iron chelation to improve their quality of life and survival rate (5-7). NTDT individuals may only need intermittent transfusion particularly during periods of stress for example pregnancy, infection and surgery. Growth failure or severe anaemia with splenomegaly are indicators for the need of higher transfusion frequency (8). Moreover, to compensate for the inadequacy of functional erythrocytes from the bone marrow, extramedullary erythropoiesis may cause complications such as hypersplenism and splenomegaly

(9).

A normal spleen serves as a site to destroy abnormal red blood cells and a spleen's size increases due to increased burden especially in haematological diseases. All NTDT patients will undergo spleen enlargement over time regardless of transfusion frequency (1). However, hypersplenism is counteractive as it leads to accelerated red cell destruction, even the transfused ones, and therefore increases transfusion requirements (10). If the spleen size continues to expand in either non-transfused or occasionally transfused individuals, splenectomy is normally indicated to reverse the process and decrease dependency on transfusion therapy (7). Nevertheless, splenectomy for an NTDT patient is a complex decision as the post-splenectomy complication risks are high (1). Thus far, there have been limited reports on the effects of these treatment options, especially splenectomy in NTDT HbE/β-thalassaemia. We would like to evaluate the effects of transfusion therapy and splenectomy in NTDT HbE/β-thalassaemia to facilitate further improvements to the current treatment and management strategy of this disorder. Therefore, we investigated the effects of transfusion and splenectomy on multiple parameters particularly red cell parameters and globin gene expression in NTDT HbE/β-thalassaemia patients as the underlying ineffective erythropoiesis and subsequently anaemia severity in these patients is influenced by globin chain imbalance ratio.

MATERIALS AND METHODS

Study subjects

This cross-sectional study recruited NTDT HbE/β-thalassaemia intermedia patients attending the Thalassaemia Clinic in Hospital Ampang, Selangor, Malaysia. Informative treatment histories were collected retrospectively, including transfusion history and splenectomy history. These individuals have never been transfused or 3 months has lapsed since their last transfusion with no record of hydroxyurea therapy and without co-inheritance of α-thalassaemia. The study cohort was divided into different groups according to the types of treatment or non-treatment received as described in Table I (no treatment (N), transfusion only (T), and transfusion and splenectomy (TS)). Iron chelation therapy was not included in our analysis as it is not used to improve the haemoglobin level nor to reduce extramedullary haematopoiesis. It has no logical bearing on the outcomes of the laboratory parameters that were analysed. However, it is important in understanding the development of events. Ethical approvals were obtained from Medical Research Ethics Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM/FPSK/PADS/T7-MJKEtikaPer/F01(LECT_JUN(08)10) and the Medical Research and Ethics Committee, Ministry of Health Malaysia (KMM/NIHSEC/08/0804/P09-341). Informed consent was acquired from participating patients before blood was taken and all data were anonymised and known by the principal investigators only.

Blood analysis

Sysmex® XE-5000 (Sysmex®, Kobe, Japan) was used for full blood count analysis and VARIANT II β-Thalassemia Short Program (Bio-Rad Laboratories, Hercules, CA) for determination of abnormal haemoglobins, HbA% and HbF%. Tina-quant®Ferritin assay was utilised for plasma ferritin measurement (Cobas, Roche Diagnostics GmbH, Mannheim, Germany).

Expression studies

Whole blood was centrifuged to enrich the reticulocytes according to Lai et al. (2006). RNA was extracted from the reticulocytes and reversed transcribed according to the manufacturer's protocol (QIAGEN® GmbH, Hilden, Germany)(11). PrimeTimeTM Mini qPCR Assay (IDT®, Singapore and Applied Biosystems) was used to determine the α-, β-, γ-globin gene, α-haemoglobin stabilising protein (AHSP) and GAPDH expressions. In the analysis, one healthy individual without HbE/β-thalassaemia was selected as the calibrator sample and GAPDH was used as the housekeeping gene. GAPDH was chosen as the least variable housekeeping gene among 5 housekeeping genes (succinyl dehydrogenase A (SdhA), b2M, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), HBS1L and hypoxanthine phosphoribosyltransferase (HPRT)). GAPDH had the lowest Ct values, indicating a high level of expression and repeated measurements of GAPDH expression in different biological samples showed that it had the lowest standard deviation and standard error value among all 5 genes (data not shown). AHSP was chosen as one of the parameters studied as previously our group found that AHSP could be a secondary compensatory mechanism in HbE/β-thalassaemia (12). The derivative AHSP/α-globin expression was investigated as AHSP is the nascent α-globin chain molecular chaperone and any changes in the expression ratio could modify the phenotype of the disorder (13). As the underlying cause of ineffective erythropoiesis in thalassaemia is due to the globin chain imbalance ratio, the log α/β expression was analysed to see if any of the treatments given alter the imbalanced ratio in HbE/β-thalassaemia. Log excess alpha (α - (β + γ)) considers any free α-globin chain expression with the assumption that all β- and γ-chains have paired with available α-globin chains. The limitation of this calculation is the assumption that all mRNA expression is fully translated into protein chains.

Statistical analysis

Variables such as age, haematological data (red blood cell indices, HbA and HbF%), plasma ferritin level, AHSP, globin gene and its derivatives (AHSP/α, α/β and excess alpha) expression studies and beta genotypes were statistically correlated to transfusion and splenectomy history. The analyses were performed using SPSS 22.0. Various analyses were done according to

the therapy(ies) received. Due to low sample size, non-parametric Mann-Whitney test was used for comparing mean values between two groups while groups of three or more used Kruskal-Wallis test. Groups with sample size less than 5 were discontinued from further analysis.

RESULTS

A total of 29 NTDT HbE/β-thalassaemia patients with variable β-mutations were included in this study (Table I). The variables data according to treatment therapy groups can be found in Table II. Kruskal-Wallis statistical analysis on the 3 cohort groups correlated to age, haematological data and gene expression data showed significant difference in MCV level (p=0.009), MCHC level (p=0.044), reticulocyte count (p=0.002) and log α/β fold change (p=0.029) (Table III). On the other hand, we used the Mann-Whitney analysis for comparing between 2 groups; N vs T, N vs TS and T vs TS. There was significant statistical difference between the groups of N vs T in log α/β fold change (p=0.024). In the comparison between N group vs TS group, significant statistical differences were found in RBC count (p=0.010), MCV level (p=0.016) and reticulocyte count (p=0.006). Statistically significant difference between T group vs TS group were found in haematocrit level (p=0.047), MCV level (p=0.005), MCH level (p=0.018), MCHC level (p=0.035) and reticulocyte count (p=0.001). Correlations to beta genotypes were not carried out as after categorising the genotypes according to treatment groups, the numbers were too small for proper statistical analyses and has the potential to lead to a type I error due to chance alone (Table I).

Table I: Summary of patients of different treatment therapy groups according to beta genotypes

Types of treatment received	Beta Genotypes	Sample size (n)
No treatment at all (n=6)	HbE/IVSI-5	3
	HbE/CD41/42	2
	HbE/CD19	1
Transfusion only (n=15)	HbE/IVSI-1	8
	HbE/IVSI-5	6
	HbE/IVSII-654	1
Transfusion and splenectomy (n=6)	HbE/IVSI-5	2
	HbE/IVSII-654	2
	HbE/CD41/42	1
	HbE/IVSII-1	1
Splenectomy only (n=2)	HbE/IVSI-5	1
	HbE/CD35/36	1

DISCUSSION

The clinical phenotype of HbE/β-thalassaemia is vastly varied, spanning from non-transfusion dependent to transfusion dependency resembling β-thalassaemia major clinical manifestations. Among HbE/β-thalassaemia individuals, 15% of patients in Southeast Asia have mild phenotype (Hb levels between 9-12 g/dL), while the majority of them are moderately severe (Hb levels between 6-7 g/dL) and almost half of them are treated as β-thalassaemia major individuals (Hb levels between 4-5 g/dL) (14-16).

This study demonstrated statistically significant

Table II: Summary of age, red cell parameters, gene expressions and their derivatives among the different treatment groups.

Parameter	No treatment (N), Median (Range) n = 6	Transfusion only (T), Median (Range) n = 15	Transfusion and splenectomy (TS), Median (Range) n = 6
Age, year	31.5 (19-50)	30 (19-51)	28 (18-46)
RBC, x10^6/ml	3.85 (3.55-4.52)	3.79 (2.19-5.55)	3.42 (3.07-3.72)
Hb, g/dL	7.85 (6.7-8.5)	6.7 (4.1-8.8)	7.7 (6.5-8.4)
Hct, %	25.0 (21.4-27.2)	22.0 (13.1-28.0)	26.2 (24.0-28.1)
MCV, fL	63.4 (55.6-68.7)	59.0 (50.5-84.8)	77.8 (65.9-81.8)
MCH, pg	19.95 (16.7-22.0)	18.2 (14.6-26.1)	21.9 (18.5-26.1)
MCHC, g/dL	31.6 (27.5-32.3)	30.4 (28.9-32.5)	28.2 (27.1-31.9)
RDW, %	31.3 (24.3-35.9)	33.5 (26.1-37.4)	30.7 (26.3-36.8)
Reticulocyte count, x10^6/μL	0.098 (0.076-0.114)	0.084 (0.017-0.145)	0.304 (0.182-0.461)
HbF, %	27.6 (11.3-45.4)	25.1 (7.7-49.1)	27.0 (17.4-46.9)
HbA, %	4.5 (1.4-29.3)	4.1 (1.1-23.0)	4.95 (1.3-37.8)
Plasma ferritin, ng/dL^	623.1 (335.1-772.9)	648.6 (232.4-922.8)	794.5 (341.3-964.3)
Log AHSP expression, fold change	-0.060[(-0.253)-0.136]	0.087 [(-0.801)-0.467]	-0.315 [(-0.443)-0.668]
Log α expression, fold change	0.015 [(-0.290)-0.950]	0.105 [(-0.740)-0.54]	-0.099 [(-0.510)-0.060]
Log β expression, fold change	-0.525 [(-1.010)-0.480]	-0.512 [(-1.500)-(-0.110)]	-0.695 [(-1.230)-(-0.520)]
Log γ expression$^{\#}$, fold change	1.88 (1.43-2.81)	1.81 (0.91-2.34)	1.95 (1.26-2.34)
Log α/β, fold change	0.48 (0.40-1.00)	0.66 (0.60-0.80)	0.59 (0.50-0.70)
Log AHSP/α expression, fold change	-0.044 [(-0.820)-0.090]	-0.096 [(-0.740)-0.610]	-0.035 [(-0.440)-0.620]
Log excess α expression {Log [α – (β+γ)]} , fold change	1.05 (0.38-1.93)	1.12 (0.32-1.60)	0.87 (0.53-1.03)

Abbreviation: SD, standard deviation; RBC, red blood cell; Hb, haemoglobin; Hct, haematocrit; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; RDW, red cell distribution width; ^8 samples had plasma ferritin level beyond the detection limit; AHSP, α-haemoglobin stabilising protein.$^{\#}$ γ expression was calculated using a healthy individual as the calibrator sample. $^{\aleph}$The globin gene expressions were calculated using a healthy individual as the calibrator sample.

Table III: Statistical analyses results (*p* value) using non-parametric Mann-Whitney test and Kruskal-Wallis test correlating selected variables to different treatment therapies

Parameters	Mann-Whitney test analyses			Kruskal-Wallis test analysis
	N vs T	N vs TS	T vs TS	N vs T vs TS
Age, year	0.876	0.873	0.640	0.904
RBC, x10^6/ml	0.640	0.010*	0.243	0.142
Hb, g/dL	0.086	0.574	0.197	0.153
Hct, %	0.311	0.297	0.047*	0.111
MCV, fL	0.276	0.016*	0.005*	0.009*
MCH, pg	0.392	0.200	0.018*	0.056
MCHC, g/dL	0.119	0.091	0.035*	0.044*
RDW, %	0.640	1.000	0.586	0.817
Reticulocyte count, x10^6/μL	0.350	0.006*	0.001*	0.002*
HbF, %	0.938	0.631	0.815	0.940
HbA, %	0.585	0.748	0.459	0.703
Plasma ferritin, ng/dL^	0.482	0.302	0.586	0.564
Log AHSP expression, fold change	0.586	0.200	0.276	0.417
Log **α** expression, fold change	0.697	0.200	0.073	0.183
Log β expression, fold change	0.815	0.262	0.161	0.340
Log γ expression#, fold change	0.436	0.522	0.876	0.711
Log α/β, fold change	0.024*	0.150	0.080	0.029*
Log AHSP/α expression, fold change	0.876	0.631	0.876	0.936
Log excess α expression {Log [α – (β+γ)]}, fold change	0.586	0.262	0.073	0.189

Abbreviation: N, no treatment, T, transfusion only, TS, transfusion and splenectomy; RBC, red blood cell; Hb, haemoglobin; Hct, haematocrit; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; RDW, red cell distribution width; ^ 8 samples had plasma ferritin level beyond the detection limit; AHSP, α-haemoglobin stabilising protein; # **γ** expression was calculated using a healthy individual as the calibrator sample; $^{\text{x}}$The globin gene expressions were calculated using a HbE/beta-thalassaemia individual as the calibrator sample; * statistically significant.

differences in MCV, MCHC level, reticulocyte count and log α/β fold change between the three groups with a near-significant level of MCH level. We observed a higher MCV level and reticulocyte count in TS group but with a lower MCHC level. Higher log α/β fold change is seen in transfusion only group. We took a closer look by comparing between two groups using non-parametric Mann-Whitney test.

The significantly higher α/β ratio seen in transfusion only patients compared to those not receiving any treatment could be attributed to the increasing extramedullary haematopoiesis to make up for the lack of functional red cells, however, these added non-functional red cells leads to high α-globin expression (8). Transfused patients in our study generally had lower haemoglobin level thus their propensity to have extramedullary erythropoiesis is higher than those who do not need any treatment. Extramedullary erythropoiesis is commonly seen in β-thalassaemia with an overactive spleen, which subsequently results in an enlarged spleen (17).

Next, the red blood cell count between the transfused and splenectomised group compared to non-treated group is significantly different. We believe that the TS group has a more severe phenotype, hence the double treatment, compared to N group due to higher ineffective erythropoiesis in the patients. Higher ineffective erythropoiesis would result in increased premature red blood cell deaths in the bone marrow leading to lower red blood cell output into the peripheral blood (6).

From our study, we observed significantly higher MCV level and reticulocyte count in TS group compared to both N and T groups and higher MCH level in the TS group compared to the T group. These results suggest that splenectomy plays the contributing factor. Tripatara et al. (2012) demonstrated a statistical difference only in MCV level between splenectomised and non-splenectomised HbE/β-thalassaemia patients in Thailand while Kalpravidh et al. (2013) showed a 5-fold increment in the reticulocyte count of splenectomised patients compared to non-splenectomised HbE/β-thalassaemia patients, significantly lower MCV level and near significant lower level of MCH in non-splenectomised patients, $p<0.001$ and $p=0.062$, respectively, which is similar to our study (16, 19). This phenomenon has been attributed to the role of the spleen in reticulocyte pooling for maturation before release in the peripheral blood circulation thus the lack of spleen caused these parameters to increase. We also found a significantly higher haematocrit percentage in TS group compared to T group and significant lower MCHC level ($p=0.051$) which is similar to studies by Kalpravidh et al. (2013) and De Haan et al. (1988) (19-20). Kalpravidh et al. found 3-fold more platelets and 10-fold higher WBC levels (we did not cover these parameters in our study) but similar non-significant haemoglobin concentration difference (19). These factors and the larger reticulocyte size and count could contribute to the haematocrit and MCHC

levels. However, the Malaysian National Thalassaemia Programme uses MCH level as an indicator as MCH is measured while MCV and MCHC are computed values (23).

Thus, our study has shown that splenectomy does improve the haematological parameters in HbE/β-thalassaemia. However, splenectomy has a higher risk of thrombotic complications and post-splenectomy sepsis thus splenectomy is recommended only in children older than 6 years old or extremely necessary i.e. in those who require 200 – 250 ml/kg of packed red blood cells per year to maintain a haemoglobin level of 10 g/dL or has a drop in their haemoglobin level by 0.5 g/week (10,18).

CONCLUSION

In general, regardless of single or double combined therapies, variability was observed in laboratory parameters of HbE/β-thalassaemia individuals. As current management strategies are highly diverse, intermittent transfusion therapy is recommended for NTDT patients as there have been suggestions that health-related quality of life of NTDT patients is worse than transfusion dependent patients and transfusion therapy should be as prophylaxis (1, 24). Most benefits could be seen in splenectomised individuals with higher MCV, MCH, HCT levels, and reticulocyte count. However, post-splenectomy complications should be carefully considered before splenectomy is performed.

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REFERENCES

1. Viprakasit V, Tyan P, Rodmai S, Taher AT. Identification and key management of non-transfusion-dependent thalassaemia patients not a rare but potentially under-recognised condition. Orphanet Journal of Rare Diseases. 2014;9:131.
2. Porter JB, Cappellini MD, Kattamis A, Viprakasit V, Musallam KM, Zhu Z and Taher AT. Iron overload across the spectrum of non-transfusion-dependent thalassaemias: role of erythropoiesis, splenectomy and transfusions. British Journal of Haematology. 2017;176:88-299. https://doi.org/10.1111/bjh.14373
3. Taher AT, Radwan A, Viprakasit V. When to consider transfusion therapy for patients with non-transfusion-dependent thalassaemia. The International Journal of Transfusion Medicine. 2015;108:1-10.
4. Taher A, Hershko C and Cappellini MD. Iron overload in thalassaemia intermedia: reassessment of iron chelation strategies. British Journal of Haematology. 2009;147:634-640. https://doi.org/10.1111/j.1365-2141.2009.07848.x
5. Angelucci E, Baronciani D. Allogeneic stem cell transplantation for thalassaemia major. Haematologica. 2008;93(12):1780-84.
6. Tanno T, Miller JL. Iron Loading and Overloading due to Ineffective Erythropoiesis. Advances in Hematology. 2010;2010:358283.
7. Borgna-Pignatti C. Modern treatment of thalassaemia intermedia. British Journal of Haematology. 2007;138(3):291-304.
8. Sharma A, Easow Mathew M, Puri L. Splenectomy for people with thalassaemia major or intermedia. Cochrane Database Systematic Reviews. 2019;9(9):CD010517. doi:10.1002/14651858.CD010517.pub3.
9. Rachmilewitz EA, Giardina PJ. How I treat thalassaemia. Blood. 2011;118(13):3479-88.
10. Al-Salem AH. Splenectomy for children with thalassaemia: total or partial splenectomy, open or laparoscopic splenectomy. Journal of Pediatric Haematology/Oncology. 2016;38:1-4.
11. Lai MI, Jiang J, Silver N, Best S, Menzel S, Mijovic A, et al. α-Haemoglobin stabilising protein is a quantitative trait that modifies the phenotype of β-thalassaemia. British Journal of Haematology. 2006;133:675-682.
12. Lim WF, Muniandi L, George E, Sathar J, Teh LK, Gan GG, Lai MI. α-Haemoglobin stabilising protein expression is influenced by mean cell haemoglobin and HbF levels in HbE/β-thalassaemia individuals. Blood Cells Molecules and Diseases. 2012;48(1):17-21. doi: 10.1016/j.bcmd.2011.10.002.
13. Hirsch RE, Sibmooh N, Fucharoen S, Friedman JM. HbE/β-Thalassemia and Oxidative Stress: The Key to Pathophysiological Mechanisms and Novel Therapeutics. Antioxidants & Redox Signaling. 2017;26(14):794-813. doi: 10.1089/ars.2016.6806.
14. Galanello R, Origa R. Beta-thalassaemia. Orphanet Journal of Rare Diseases. 2010;5:11.
15. Olivieri NF. Treatment strategies for haemoglobin E beta-thalassaemia. Blood Reviews. 2012;26 Suppl 1:S28-30.
16. Tripatara A, Srichana N, Lamool P, Amnuaykan S, Hongart P, Jetsrisuparb A. Relationship between plasma ferritin level and siderocyte number in splenectomized beta-thalassaemia/HbE Patients. Anemia. 2012;2012:890471.
17. Premawardhena A, Fisher CA, Olivieri NF, de Silva S, Arambepola M, Perera W, et al. Haemoglobin E beta thalassaemia in Sri Lanka. Lancet. 2005;366(9495):1467-1470.
18. Taher AT, Musallam KM, Cappellini MD, Weatherall DJ. Optimal management of

β-thalassaemia intermedia. British Journal of Haematology 2011;152:512-523.
19. Kalpravidh RW, Tangjaidee T, Hatairaktham S, Charoensakdi R, Panichkul N, Siritanaratkul N, et al. Glutathione Redox System in β-thalassaemia/HbE patients. The Scientific World Journal. 2013;543973.
20. de Haan LD, Werre JM, Ruben AM, Huls HA, de Gier J, Staal GE. Alterations in size, shape and osmotic behaviour of red cells after splenectomy: a study of their age dependence. British Journal of Haematology. 1988;69(1):71-80.
21. Taher AT, Porter JB, Viprakasit V, Kattamis A, Chuncharunee S, Sutcharitchan P, et al. Deferasirox effectively reduces iron overload in non-transfusion-dependent thalassaemia (NTDT) patients: 1-year extension results from the THALASSA study. Annals of Hematology. 2013;92(11):1485-1493.
22. Cappellini MD, Robbiolo L, Bottasso BM, Coppola R, Fiorelli G, Mannucci PM. Venous thromboembolism and hypercoagulability in splenectomized patients with thalassaemia intermedia. British Journal of Haematology. 2000;111:467-473.
23. Bahagian Pembangunan Kesihatan Keluarga. (BPKK). Modul Kaunseling Talasemia Di Peringkat Kesihatan Primer. 3rd ed: Kementerian Kesihatan Malaysia; 2016.
24. Sleiman J, Tarhini A, Bou-Fakhredin R, Saliba AN, Cappellini MD, Taher AT. Non-Transfusion-Dependent Thalassemia: An Update on Complications and Management. International Journal of Molecular Sciences. 2018;19(1):182. https://doi.org/10.3390/ijms19010182.