Research Article

Prevalence and Risk Factors for Complications in Patients with Nontransfusion Dependent Alpha- and Beta-Thalassemia

Poramed Winichakoon,1 Adisak Tantiworawit,1 Thanawat Rattanathammethe,1 Sasinee Hantrakool,1 Chatree Chai-Adisaksopha,1 Ekarat Rattarittamrong,1 Lalita Norasetthada,1 and Pimlak Charoenkwan2

1Division of Hematology, Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, 110 Intravaros Road, A. Muang, Chiang Mai 50200, Thailand
2Division of Hematology and Oncology, Department of Pediatrics, Faculty of Medicine, Chiang Mai University, 110 Intravaros Road, A. Muang, Chiang Mai 50200, Thailand

Correspondence should be addressed to Adisak Tantiworawit; atantiwo@yahoo.com

Received 31 May 2015; Accepted 5 November 2015

Academic Editor: Aurelio Maggio

Copyright © 2015 Poramed Winichakoon et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Nontransfusion dependent thalassemia (NTDT) is a milder form of thalassemia that does not require regular transfusion. It is associated with many complications, which differ from that found in transfusion-dependent thalassemia (TDT). Currently available information is mostly derived from beta-NTDT; consequently, more data is needed to describe complications found in the alpha-NTDT form of this disease. Methods. We retrospectively reviewed the medical records of NTDT patients from January 2012 to December 2013. Complications related to thalassemia were reviewed and compared. Results. One hundred patients included 60 females with a median age of 38 years. The majority (54 patients) had alpha-thalassemia. Overall, 83 patients had one or more complications. The three most common complications were cholelithiasis (35%), abnormal liver function (29%), and extramedullary hematopoiesis (EMH) (25%). EMH, cardiomyopathy, cholelithiasis, and pulmonary hypertension were more commonly seen in beta-thalassemia. Osteoporosis was the only complication that was more common in alpha-thalassemia. The risk factors significantly related to EMH were beta-thalassemia type and hemoglobin <8 g/dL. The risk factors related to osteoporosis were female gender and age >40 years. Iron overload (ferritin >800 ng/mL) was the only risk factor for abnormal liver function. Conclusion. The prevalence of alpha-NTDT complications was lower and different from beta-thalassemia.

1. Introduction

Thalassemia is a well-known inherited hematologic disorder caused by a decrease or an absence of globin production [1]. Patients with thalassemia suffer from chronic hemolytic anemia and its sequelae. Thalassemia originates from varying genetic abnormalities that result in different clinical presentation. Nontransfusion dependent thalassemia (NTDT) or thalassemia intermedia (TI) is a milder form of thalassemia which does not require regular blood transfusion for survival. This group of thalassemia patients was recognized earlier as a TI but no consensus on diagnostic criteria has been reached due to high clinical variations ranging from asymptomatic to multiorgan involvement [2–9]. The terminology has been changed from TI to NTDT [10]. Generally patients with NTDT can maintain hemoglobin levels at 6–10 g/dL with occasional blood transfusions that may be required with fever, infection, or pregnancy [3, 4, 7, 8, 10]. Complications of NTDT result from chronic hemolysis and tissue hypoxia, causing iron overload and problems in many organ systems [5, 6, 8, 11–20]. According to the largest observational study on thalassemia intermedia (OPTIMAL CARE study; n = 584 TI patients), the three most common complications were osteoporosis, extramedullary hematopoiesis (EMH), and hypogonadism, respectively [8]. Several complications that are associated with thalassemia intermedia are less frequently seen in thalassemia major, including EMH, leg ulcers, gallstones, and thrombophilia [8].
One of the most serious complications in NTDT is pulmonary hypertension which can be found in 11–50% of patients and leads to heart failure; the most common cause of death in NTDT patients [3, 4, 6, 8, 11, 13, 14, 16]. In our region, the proportion of patients classified by thalassemia type is changing due to advances in prenatal diagnoses and early detection. Higher numbers of NTDT patients are diagnosed and more fetuses with severe thalassemia are terminated.

Many previous studies aim to establish predictive factors for thalassemia complications and report that mechanisms for complications in thalassemia are multifactorial [3, 6, 8, 12, 15, 21–27]. In our region, the prevalence of alpha-thalassemia is greater than that of beta-thalassemia which is different from the prevalence found in other regions [25, 26, 28–30]. The lack of studies and clear guidelines in this group can present a significant clinical challenge. This study aims to elucidate the prevalence of complications and identify predictive factors affecting complication of both alpha- and beta-NTDT patients.

### 2. Material and Method

We retrospectively reviewed medical records of NTDT patients who attended the Chiang Mai University Hospital Adult Hematology Clinic for the two-year period from January 1, 2012, to December 31, 2013.

#### 2.1. Population

The NTDT patients, age 15 years or older, were included in the study. NTDT is defined by thalassemia disease that does not require regular transfusion for survival [10]. However, the definition of transfusion varies among studies. We used the criteria of less than an average of three transfusions per year for the purpose of the study. The patient needed to visit the clinic at least once in order to be enrolled.

The diagnosis for the type of NTDT patients was made by hemoglobin analysis using a high-performance liquid column chromatography (HPLC) method. The molecular confirmation of α^+-thalassemia (Southeast Asian or Thai deletion) and HbCS was done for cases with HbH disease and HbH/CS disease. Molecular diagnosis of beta-globin mutations was done in cases with beta NTDT when the results from hemoglobin analysis by HPLC method showed abnormal hemoglobin peak other than HbE.

#### 2.2. Data Collection

From January 1, 2012, to December 31, 2013, medical records of NTDT patients who met the inclusion criteria were reviewed. Data collected from medical records included demographic characteristics and diagnosis obtained by hemoglobin analysis. Also, findings from physical examination, laboratory investigations, and records of complications were recorded. The definition of conditions and complications in this study are shown in Table 1 [8].
2.3. Complications. Complications of NTDT patients were retrospectively collected from the medical record.

All NTDT patients had regular evaluation and investigations for these complications: three-monthly liver function tests and serum ferritin, annual tests for endocrine function which included fasting plasma glucose, thyroid function test, and hormonal assays for hypogonadism. Hepatitis B and C virus test were also done annually. Chest radiograph and echocardiogram were obtained for suspected cases of cardiomyopathy. Spine radiograph and bone mass densitometry were conducted in suspected osteoporosis cases.

For other complications such as extramedullary hematopoiesis (EMH), pulmonary hypertension (PHT), thrombosis, cardiomyopathy, cholelithiasis, pseudoxanthoma elasticum (PXE), leg ulcers, and osteoporosis (OP), the information was obtained retrospectively from medical records. Investigations for complications listed in Table 1 were conducted for putative cases where risk factors were present.

2.4. Statistical Analysis. Data were entered into database, cross-checked, and analyzed using SPSS statistics software. Descriptive results of categorical and continuous variables were expressed as mean (±SD) or median (range in continuous variables) depending on their distribution or as percentages of the group from which they were derived (categorical variables). The Chi-square test or Fisher exact test was used to compare categorical variables and Student’s t-test was used to compare between continuous variables as appropriate. Variables that were significantly related to complications or with $p$ values less than 0.05 in the univariate analysis were entered into the multivariate analyses. Multivariate logistic regression analysis was used to identify independent risk factors for complications. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for all associations that emerged. A $p$ value less than 0.05 was considered as statistically significant.

3. Results

3.1. Patient Characteristics. During the study period, 250 thalassemia patients attended our clinic. Of these, 100 NTDT patients who matched our inclusion criteria were included in this study, 60 patients (60%) were female. Table 2 summarized patient demographics, underlying diseases and conditions, and clinical characteristics. The median age was 38 years (range 19–78 years). More than half of patients (54%) were diagnosed with alpha-thalassemia. The mean ferritin level was 1,563.46 ng/mL while 76% and 44% of patients had ferritin levels more than 800 and 2,500 ng/mL, respectively. Chronic hepatitis B infection (27%) was the most common comorbid condition.

3.2. Complications and Treatment Outcomes. Figure 1 summarizes patient treatments and the outcomes. Fifty-five of 100 patients (55%) received iron chelation treatment for iron overload, and 33 of these patients (33%) underwent a splenectomy. Overall, complications occurred in 83% of the study population. The three most common complications were cholelithiasis (35%), abnormal liver function (29%), and EMH (25%). Other complications included osteoporosis (17%), abnormal plasma glucose (16%), pulmonary hypertension (14%), hypothyroidism (13%), cardiomyopathy (11%), thrombosis (4%), hypogonadism (7%), and leg ulcers (2%), respectively.

| Parameter                              | Frequency, number (%) |
|----------------------------------------|-----------------------|
| Gender                                 |                       |
| Male                                   | 40 (40%)              |
| Female                                 | 60 (60%)              |
| Age                                    |                       |
| <40 years                              | 54 (54%)              |
| ≥40 years                              | 46 (46%)              |
| Region                                 |                       |
| Northern Thailand                      | 97 (97%)              |
| Other                                  | 3 (3%)                |
| Comorbidities                          |                       |
| Cerebrovascular disease                 | 2 (2%)                |
| Chronic lung disease                    | 4 (4%)                |
| Chronic kidney disease                  | 11 (11%)              |
| Cirrhosis                              | 9 (9%)                |
| Diabetes mellitus                      | 11 (11%)              |
| Dyslipidemia                           | 2 (2%)                |
| Endocrine disease                      | 16 (16%)              |
| Eye-ENT disease                        | 4 (4%)                |
| Gynecologic disease                    | 4 (4%)                |
| Heart disease                          | 8 (8%)                |
| Hypertension                           | 7 (7%)                |
| HBV infection                          | 27 (27%)              |
| HCV infection                          | 12 (12%)              |
| Malignancy                             | 4 (4%)                |
| Seizure                                | 4 (4%)                |
| Personal history                       |                       |
| Alcohol drinking                       | 14 (14%)              |
| Herb use                               | 1 (1%)                |
| Smoking                                | 4 (4%)                |
| Thalassemia type                       |                       |
| Alpha-thalassemia                      | 54 (54%)              |
| Hemoglobin H                           | 38 (38%)              |
| Hemoglobin H/CS                        | 16 (16%)              |
| Beta-thalassemia                       | 46 (46%)              |
| Beta-thalassemia and HbE disease       | 36 (36%)              |
| Beta-thalassemia intermedia            | 10 (10%)              |
| Hemoglobin                             |                       |
| Mean hemoglobin level                  | 7.8 g/dL              |
| Platelet                               |                       |
| Mean platelet count                    | 330,900/mm$^3$        |
| Serum ferritin, ng/mL                  |                       |
| Mean ferritin                          | 1,563.46 ng/mL        |
| Maximum ferritin > 800 ng/mL           | 76 (76%)              |

Hemoglobin H/CS: hemoglobin H with Hb constant spring.
Anemia

Table 3: Treatment, outcome, and complications in study population.

| Parameter                  | Frequency | α-Thal (%) | β-Thal (%) |
|----------------------------|-----------|------------|------------|
| **Treatment**              |           | N = 100 (%)| N = 54 (%) | N = 46 (%) |
| Antiplatelet               | 26%       | 3 (5.6)    | 23 (50)    |
| Iron chelation             | 55%       | 21 (38.9)  | 34 (73.9)  |
| Splenectomy                | 33%       | 8 (14.8)   | 25 (54.3)  |
| **Complications**          |           |            |            |
| Abnormal plasma glucose    | 16%       | 9 (16.6)   | 7 (15.2)   |
| Abnormal liver function    | 29%       | 15 (27.7)  | 14 (30.4)  |
| Cardiomyopathy             | 11%       | 3 (5.5)    | 8 (17.4)   |
| Cholelithiasis             | 35%       | 15 (27.7)  | 20 (43.5)  |
| Cholecystectomy            | 25%       | 10 (18.5)  | 15 (32.6)  |
| EMH                        | 25%       | 3 (5.6)    | 22 (47.8)  |
| Hypothyroidism             | 13%       | 6 (11.1)   | 7 (15.2)   |
| Hypogonadism               | 7%        | 2 (3.7)    | 5 (10.9)   |
| Leg ulcers                 | 2%        | 1 (1.8)    | 1 (2.2)    |
| Osteoporosis               | 17%       | 11 (20.4)  | 6 (13.0)   |
| PHT                        | 14%       | 3 (5.6)    | 11 (23.9)  |
| PXE                        | None      | None       | None       |
| Thrombosis                 | 4%        | 2 (3.7)    | 2 (4.3)    |
| Overall complications      | 83%       | 43 (79.6)  | 40 (87)    |

EMH: extramedullary hematopoiesis, PHT: pulmonary hypertension, PXE: pseudoxanthoma elasticum, α-Thal: alpha-thalassemia, and β-Thal: beta-thalassemia.

3.3. Complications in Alpha and Beta-NTDT. The differences of complications classified by type of thalassemia were summarized in Table 3. The most common complications were similar between alpha- and beta-thalassemia groups: cholelithiasis and abnormal liver function test. However, the prevalence of cardiomyopathy, cholelithiasis, and pulmonary hypertension was higher in beta-thalassemia but the differences were not statistically significant. Osteoporosis was the only complication that was more commonly seen in alpha-thalassemia.

Though not statistically significant, beta-thalassemia patients tended to have higher clinical severity and required further treatment more frequently than those with alpha-thalassemia. The mean ferritin level for the beta-thalassemia group (1,971 ng/mL) was higher than the alpha-thalassemia group (1,202 ng/mL). Seventy-four percent of beta-thalassemia patients received iron chelation as compared to 39% in alpha-thalassemia patients. Splenectomy was performed in 54.3% of beta-thalassemia and only 14.8% of alpha-thalassemia patients.

3.4. Risk Factors Affecting Complications. Results from the univariate analysis of significant risk factors for each complication were shown in Table 4. The following factors were significant in the model: extramedullary hematopoiesis, female gender (p = 0.05), beta-thalassemia (p = 0.031), hemoglobin level below 8 g/dL (p = 0.003), platelets above 400,000/mm³ (p = 0.025), maximum ferritin more than...
Table 4: Significant risk factors affecting complications from univariate analysis.

| Complication                          | Significant variables                  | p value | 95% CI     | Odd ratio |
|---------------------------------------|----------------------------------------|---------|------------|-----------|
| Extramedullary hematopoiesis          | Gender (female)                        | 0.050   |            |           |
|                                       | Thalassemia type (beta)                | 0.031   |            |           |
|                                       | Hemoglobin < 8 g/dL                    | 0.003   |            |           |
|                                       | Platelets > 400,000 per cumm.          | 0.025   |            |           |
|                                       | Maximum ferritin > 800 ng/mL           | 0.004   |            |           |
|                                       | Splenectomy                            | 0.019   |            |           |
|                                       | Iron chelation                         | 0.001   |            |           |
| Pulmonary hypertension                | None                                   |         | —          |           |
| Heart failure                         | Splenectomy                            | 0.035   |            |           |
| Cholelithiasis                        | None                                   |         | —          |           |
| Abnormal LFT (ALT >50 U/L)            | Iron chelation                         | 0.007   | 1.033–13.9 | 3.79      |
|                                       | Maximum ferritin > 800 ng/mL           | 0.041   | 1.514–38.6 | 7.64      |
|                                       | HCV infection*                         | 0.143   |            |           |
| Osteoporosis                          | Gender (female)                        | 0.006   |            |           |
|                                       | Age > 40 years                         | 0.003   |            |           |
| Abnormal plasma glucose               | None                                   |         | —          |           |
| Hypothyroidism                        | None                                   |         | —          |           |
| Hypogonadism                          | Splenectomy                            | 0.016   |            |           |

* p value is not significant.

Table 5: Significant risk factors affecting complications from multivariate analysis.

| Complication                          | Significant variables                  | p value | 95% CI     | Odd ratio |
|---------------------------------------|----------------------------------------|---------|------------|-----------|
| Extramedullary hematopoiesis          | Thalassemia type (beta)                | 0.031   | 1.173–27.9 | 5.72      |
|                                       | Hemoglobin < 8 g/dL                    | 0.007   | 1.736–31.2 | 7.37      |
| Osteoporosis                          | Gender (female)                        | 0.014   | 1.514–38.6 | 7.64      |
|                                       | Age > 40 years                         | 0.017   | 1.313–16.5 | 4.66      |
| Abnormal liver function               | Maximum ferritin > 800 ng/mL           | 0.035   | 1.033–13.9 | 3.79      |

800 ng/mL (p = 0.004), iron chelation (p = 0.001), and splenectomy (p = 0.019). Splenectomy was also associated with heart failure (p = 0.035) and hypogonadism (p = 0.016). The significant risk factors affecting abnormal liver function tests were a maximum ferritin more than 800 ng/mL (p = 0.041) and iron chelation (p = 0.007). There was no statistically significant difference for the relationship between HCV infection and abnormal liver function. Female gender (p = 0.006) and age over 40 years (p = 0.003) were significant factors for osteoporosis. No significant risk factors were found in pulmonary hypertension, cholelithiasis, abnormal plasma glucose, and hypothyroidism.

From multivariate analysis, significant risk factors affecting complications in EMH were beta-thalassemia with an odds ratio 5.7 (95% CI 1.2–27.9, p = 0.03) and hemoglobin level below 8 g/dL with an odds ratio 7.4 (95% CI 1.7–31.3, p = 0.007). Significant risk factors affecting complications in osteoporosis were female gender with an odds ratio 7.4 (95% CI 1.5–38.6, p = 0.014) and age more than 40 years with an odds ratio 4.6 (95% CI 1.3–16.5, p = 0.017). Iron overload (ferritin > 800 ng/mL) was the only risk factor for abnormal liver function with an odds ratio of 3.7 (95% CI 1.0–13.9, p = 0.035) (Table 5).

4. Discussion

NTDT is thought to be a less severe form of thalassemia since regular transfusions are not required. However, several studies revealed that many complications occur in patients with this form of thalassemia [8, 11, 14, 19]. We compare prevalence and complications between alpha-NTDT and beta-NTDT and identify putative risk factors affecting complications in this group of patients.

Eighty-three percent of the study population (83 patients) experienced NTDT-related complications. Cholelithiasis (35%), abnormal liver function (29%), and EMH (25%) were the three most common complications found in this study. These results were similar to that found in a study of 37 NTDT patients in Lebanon [6] where common complications were cholelithiasis, pulmonary hypertension, leg ulcer, and EMH. Another study from Taher et al. [8] found that osteoporosis, EMH, hypogonadism, and cholelithiasis were the most common complications in NTDT. These findings indicate that complications from NTDT are quite different from TDT related complications which are mainly cardiomyopathy, endocrinopathy, and abnormal liver function [6].

Differences in the prevalence of complications across NTDT studies can be explained by the various complication
definitions used, different in population numbers and type of NTDT (alpha or beta type). Our study had a higher portion of patients with alpha-NTDT which was different from previous studies [6, 8]. This study was done only in adult patients who tended to have more complications.

Another reason that can explain the high prevalence of EMH, cholelithiasis, and iron overload is that our study site is a referral center where most patients within the region with these complications were referred for further treatment.

The lower prevalence of thrombosis in our study may be due to a low incidence of thrombosis for the general Thai population when compared with other countries [31]. Moreover, thrombosis in thalassemia patients was largely due to a low incidence of thrombosis for the general population when compared with other countries [31].

The prevalence of complications in alpha-NTDT was lower than and descriptively different from beta-NTDT. The prevalence of complications in alpha-NTDT was lower than and descriptively different from beta-NTDT.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Poramed Winichakoon was responsible for collecting the data, analyzing the data, interpreting the data, and writing the paper in part; Adisak Tantiworawit was responsible for conceiving and designing the study, obtaining funding and/or ethics approval, analyzing the data, interpreting the data, and writing the paper in whole; Thanawat Rattanathammetee, Ekarat Rattarittamrong, Lalita Norasetthada, Chatree Chai-Adisaksopha, Sasinee Hantrakool, and Pimlak Charoenkwan were responsible for revising the paper.

Acknowledgments

The authors thank Elizabeth Matovinovic (Faculty of Medicine, Research Administration, Chiang Mai University) for revising the paper. This work was supported by a grant from Chiang Mai University research fund.

References

[1] P. J. Giardina, “Thalassemia syndromes,” in Hematology: Basic Principles and Practice, R. Hoffman, E. J. Benz, and S. S. Shattil, Eds., Elsevier Churchill Livingstone, Philadelphia, Pa, USA, 5th edition, 2008.

[2] C. Camaschella and M. D. Cappellini, “Thalassemia intermedia,” Haematologica, vol. 80, no. 1, pp. 58–68, 1995.

[3] M. D. Cappellini, K. M. Musallam, and A. T. Taher, “Insight onto the pathophysiology and clinical complications of thalassemia intermedia,” Hemoglobin, vol. 33, supplement 1, pp. S145–S159, 2009.

[4] F. El Rassi, M. D. Cappellini, A. Inati, and A. Taher, “Beta-thalassemia intermedia: an overview,” Pediatric Annals, vol. 37, no. 5, pp. 322–328, 2008.

[5] K. M. Musallam, A. T. Taher, and E. A. Rachmilewitz, “β-thalassemia intermedia: a clinical perspective,” Cold Spring Harbor Perspectives in Medicine, vol. 2, no. 7, Article ID a013482, 2012.

[6] A. Taher, H. Ismael, and M. D. Cappellini, “Thalassemia intermedia: revisited,” Blood Cells, Molecules, and Diseases, vol. 37, no. 1, pp. 12–20, 2006.

[7] A. T. Taher, K. M. Musallam, and M. D. Cappellini, “Thalassemia intermedia: an update,” Mediterranean Journal of Hematology and Infectious Diseases, vol. 1, no. 1, Article ID e2009004, 2009.
[8] A. T. Taher, K. M. Musallam, M. Karimi et al., "Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study," Blood, vol. 115, no. 10, pp. 1886–1892, 2010.

[9] J. E. Maakaron, M. D. Cappellini, and A. T. Taher, "An update on thalassemia intermedia," Journal of Medical Libanais, vol. 61, no. 3, pp. 175–182, 2013.

[10] D. J. Weatherall, "The definition and epidemiology of non-transfusion-dependent thalassemia," Blood Reviews, vol. 26, supplement 1, pp. S3–S6, 2012.

[11] C. Borgna-Pignatti, M. Marsella, and N. Zanforlin, "The natural history of thalassemia intermedia," Annals of the New York Academy of Sciences, vol. 1202, pp. 214–220, 2010.

[12] H. Ismaeel, A. H. E. Cha fic, F. E. Rassi et al., "Relation between iron-overload indices, cardiac echo-Doppler, and biochemical markers in thalassemia intermedia," American Journal of Cardiology, vol. 102, no. 3, pp. 363–367, 2008.

[13] M. Karimi, K. M. Musallam, M. D. Cappellini et al., "Risk factors for pulmonary hypertension in patients with β thalassemia intermedia," European Journal of Internal Medicine, vol. 22, no. 6, pp. 607–610, 2011.

[14] B. N. Matta, K. M. Musallam, J. E. Maakaron, S. Koussa, and A. T. Taher, "A killer revealed: 10-year experience with beta-thalassemia intermedia," Hematology, vol. 19, no. 4, pp. 196–198, 2014.

[15] K. M. Musallam, M. D. Cappellini, and A. T. Taher, "Iron overload in β-thalassemia intermedia: an emerging concern," Current Opinion in Hematology, vol. 20, no. 3, pp. 187–192, 2013.

[16] A. Taher, F. El Rassi, H. Ismaeel, and A. Inati, "Complications of β-thalassemia intermedia: a 12-year Lebanese experience," American Journal of Hematology, vol. 83, no. 7, pp. 605–606, 2008.

[17] A. Taher, C. Hershko, and M. D. Cappellini, "Iron overload in thalassemia intermedia: reassessment of iron chelation strategies," British Journal of Haematology, vol. 147, no. 5, pp. 634–640, 2009.

[18] A. T. Taher, K. M. Musallam, and A. Inati, "The hypercoagulable state in thalassemia intermedia," Hemoglobin, vol. 33, supplement 1, pp. S160–S169, 2009.

[19] J. E. Maakaron, "Complications and management of thalassemia intermedia," Journal of Applied Hematology, vol. 3, no. 4, pp. 143–146, 2012.

[20] M. D. Cappellini, K. M. Musallam, E. Poggiali, and A. T. Taher, "Hypercoagulability in non-transfusion-dependent thalassemia," Blood Reviews, vol. 26, no. 1, pp. S20–S23, 2012.

[21] C. Camaschella, U. Mazza, A. Roetto et al., "Genetic interactions in thalassemia intermedia: Analysis of β-mutations, α-genotype, γ-promoters, and β-LCR hypersensitive sites 2 and 4 in Italian patients," American Journal of Hematology, vol. 48, no. 2, pp. 82–87, 1995.

[22] C. Camaschella, G. Saglio, A. Serra et al., "Molecular characterization of thalassemia intermedia in Italy," Birth Defects Original Article Series, vol. 23, no. 5, pp. 111–116, 1987.

[23] F. X. Kleber, L. Niemoller, and W. Doering, "Impact of converting enzyme inhibition on progression of chronic heart failure: results of the Munich Mild Heart Failure Trial," British Heart Journal, vol. 67, no. 4, pp. 289–296, 1992.

[24] K. M. Musallam, M. D. Cappellini, J. C. Wood, and A. T. Taher, "Iron overload in non-transfusion-dependent thalassemia: a clinical perspective," Blood Reviews, vol. 26, no. 1, pp. S16–S19, 2012.

[25] L. Nuntakarn, S. Fucharoen, G. Fucharoen, K. Sanchaisuriya, A. Jetsrisuparb, and S. Wiangnon, "Molecular, hematological and clinical aspects of thalassemia major and thalassemia intermedia associated with Hb E-β-thalassemia in Northeast Thailand," Blood Cells, Molecules, and Diseases, vol. 42, no. 1, pp. 32–35, 2009.

[26] I. C. Verma, M. Kleanthous, R. Saxena et al., "Multicenter study of the molecular basis of thalassemia intermedia in different ethnic populations," Hemoglobin, vol. 31, no. 4, pp. 439–452, 2007.

[27] K. M. Musallam, A. Beydoun, R. Hourani et al., "Brain magnetic resonance angiography in splenectomized adults with β-thalassemia intermedia," European Journal of Haematology, vol. 87, no. 6, pp. 539–546, 2011.

[28] G. Fucharoen, H. Srivorakun, S. Singsanan, and S. Fucharoen, "Presumptive diagnosis of common haemoglobinopathies in Southeast Asia using a capillary electrophoresis system," International Journal of Laboratory Hematology, vol. 33, no. 4, pp. 424–433, 2011.

[29] S. Fucharoen, Thalassemia: From Molecular Biology to Clinical Medicine, Mahidol University, Bangkok, Thailand, 2007.

[30] S. Fucharoen and P. Winichagoon, "Haemoglobinopathies in Southeast Asia," Indian Journal of Medical Research, vol. 134, no. 10, pp. 498–506, 2011.

[31] P. Angchaisukiri, V. Atichartakarn, K. Aryurachai et al., "Risk factors of venous thromboembolism in Thai patients," International Journal of Hematology, vol. 86, no. 5, pp. 397–402, 2007.

[32] A. T. Taher, K. M. Musallam, M. Karimi et al., "Splenectomy and thrombosis: the case of thalassemia intermedia," Journal of Thrombosis and Haemostasis, vol. 8, no. 10, pp. 2152–2158, 2010.

[33] E. B. Fung, P. R. Harmatz, P. D. K. Lee et al., "Increased prevalence of iron-overload associated endocrinopathy in thalassaemia versus sickle-cell disease," British Journal of Haematology, vol. 135, no. 4, pp. 574–582, 2006.

[34] K. M. Musallam, M. D. Cappellini, and A. T. Taher, "Evaluation of the 5mg/g liver iron concentration threshold and its association with morbidity in patients with β-thalassemia intermedia," Blood Cells, Molecules, and Diseases, vol. 51, no. 1, pp. 35–38, 2013.