PEDIATRIC SCIENCES JOURNAL
The Official Journal of the Pediatric Department, Faculty of Medicine, Cairo University, Egypt

Original Article

Thalassemia …Who is to be screened ?

Niveen Salama[1], Omnia Y. Abd El Dayem[2], Doaa Shaltout[1], Mariam Saad Nassim[1]*
[1] Department of Pediatrics, Faculty of Medicine, Cairo University, Egypt.
[2] Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt.
* Correspondence: nasimmariam@cu.edu.eg
Received: 25/9/2023; Accepted: 10/11/2023; Published online: 20/11/2023

Abstract:
Background: Thalassemia carrier rate in Egypt is as high as 9-10%. The first step to eradicate thalassemia is to define population at risk.
Aim of the work: to define the high risk population to be screened for thalassemia.
Subjects and Methods: This observational cross-sectional study included children aged between 1 and 16 years presenting with microcytic anemia to Cairo university Children Hospitals from the period between March and September 2022. Children with hemoglobin <10mg/dL and mean red blood corpuscle volume (MCV) <70fl were included in the study. Complete blood count, serum ferritin, and hemoglobin electrophoresis were done.
Results: Out of a total of 180 patients, 120 patients (66.7%) had iron deficiency anemia, 2 patients (1.1%) had alpha thalassemia, and 14 patients (7.7%) had beta thalassemia, 14 (7.7%) patients had thalassemia trait, 3 (1.67%) had sickle thalassemia, 7 (3.88%) had combined iron deficiency anemia (IDA) and thalassemia, and 20 (11.1%) required further investigations. Patients were divided according to MCV into 3 groups MCV<60, 60-65, >65. There was no significant difference in the diagnosis between the three groups.
Conclusion: 22 % of children with MCV < 70 fl were found to have an inherited hemolytic anemia. Children with MCV <70 should be screened for thalassemia.

Level of Evidence of Study: IV (*1*)**.**
Keywords: Thalassemia; prevention; microcytic anemia; iron deficiency; screening.
Abbreviations: HB: hemoglobin; Hb A1: Adult hemoglobin; HbA2: Hemoglobin A2; HbF: Fetal Hemoglobin F; HbS: Sickle Hemoglobin; HCT: hematocrit; IDA: Iron deficiency anemia; MCHC: mean corpuscular hemoglobin concentration; MCV: mean red corpuscular volume, RDW: red cell distribution width; SF: serum ferritin

Introduction

Thalassemia is the commonest form of inherited anemia worldwide. It is transmitted in an autosomal recessive fashion. It occurs when there is unbalanced production of alpha and beta chains of hemoglobin leading to precipitation of either chains within erythroid cells, ineffective erythropoiesis in bone marrow and excessive hemolysis peripherally (*2*). Clinically, initially the patient may complain of jaundice, and pallor requiring regular long life blood transfusions starting early in life. Later, the patient starts to accumulate an iron burden from repeated blood transfusions and will need iron chelating agents for life. Thalassemia exerts a financial and social burden on the government in supplying the medications, securing the blood and screening it. There are other expenses to be covered that include laboratory tests, medical consultation costs, prevention costs and indirect expenses like travel costs (*3*). Families may face employment problems, and other siblings may suffer from negligence. From here comes the importance of screening for thalassemia especially in countries that lie within the thalassemic belt. Iran, for instance, has implemented one of the successful thalassemia prevention programs in 1997 over a number of stages. First, in an attempt not to stigmatize women, men were first tested and those who were identified as thalassemia carriers were asked to test their female partners to be (*4*). The most convenient method for diagnosis of thalassemia carriers is detection of hemoglobin A2 (HBA2) where HBA2 more than 3.5% indicates thalassemia trait (*5*). The carrier's status can be confirmed by hemoglobin electrophoresis (*6*) and/or high performance liquid chromatography. Next, abortion for affected fetuses at early weeks of pregnancy was religiously legalized (*7*). Eventually, some prenatal diagnosis centers were founded (*8*). As a result of this obligatory prevention program, the number of newborns with thalassemia major in Iran declined from 1087 cases in 1989 to 239 in 2009 giving a success rate approaching 80 % (*9*). The costs of screening are incomparable to the costs of following up and treating patients with thalassemia.

In Egypt, there is a high carrier rate of thalassemia of 9-10% (*10*, *11*). Screening of thalassemia carriers is the first step towards the eradication of thalassemia. The aim of this study is to define the high risk population to be screened for thalassemia.

Subjects and Methods

This is an observational cross-sectional study conducted on 180 children aged between 1 and 16 years presenting with microcytic anemia to Cairo University Children Hospitals, Egypt from the period between March and September 2022. The study was approved by the Research Ethics Committee, Faculty of Medicine, Cairo University, Egypt (MS-685-2021). Care takers of recruited children consented to the trial. The study complied with the Declaration of Helsinki for trials (*12*).

Participants

Children with hemoglobin (Hb)<10mg/dL and mean red blood corpuscle volume (MCV) <70fl were included in the study. Patients with current infections and history of blood transfusion in the preceding 3 months and patients on iron therapy were excluded. The sample size for our study was calculated using Medcalc 19 program (MedCalc Software, Ostend, Belgium) as follows: by setting alpha error of 5%, 95% confidence level and 80% power sample. The sample size was calculated from prevalence haemoglobinopathies in children with microcytic anemia (*13*) (18.9%), by equations mentioned by Machin and co-workers in 2009 (*14*).

Methods

All patients included in the study were subjected to a detailed history taking and a thorough physical examination and the following investigations: Complete blood count, serum ferritin, and hemoglobin electrophoresis were done. Five milliliters of blood were collected from each patient into three vacutainer tubes. The first two vacutainer tubes containing sterile EDTA for complete blood picture and hemoglobin electrophoresis. The third plain sterile vacutainer tube for ferritin quantification by ELISA assay.

Patients were then diagnosed according to serum ferritin and Hb electrophoresis results into:

- Iron deficiency anemia (IDA): Serum ferritin less than 7ng/dl (*15*).
- Hemolytic anemia: absent adult hemoglobin (HbA), elevated Hb A2 and fetal hemoglobin (HbF) levels (*16*) or abnormal hemoglobin.
- IDA deficiency anemia and hemolytic anemia: Serum ferritin levels less than 7ng/dL and elevated HbF levels.
- Others group: do not fulfill any of the above criteria.

The hemolytic anemia group in turn was divided into the following groups: Thalassemia trait group: Hb A2 >3.5% (*5*), non-transfusion dependent thalassemia group, alpha thalassemia: as diagnosed by molecular studies or by the presence of HbH (*17*), and sickle thalassemia group: presence of sickle hemoglobin (HbS) >60%, HbA 20-30%, HbA2 >3.5%, HbF <20% (*18*).

Statistical Analysis

Data were statistically described in terms of mean ± standard deviation (± SD), median, range, or frequencies (number of cases) and percentages when appropriate. Numerical data were tested for the normal assumption using Kolmogorov Smirnov test. Comparison of numerical variables between the study groups was done using Kruskal Wallis test. For comparing categorical data, Chi-square (x^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Accuracy was represented using the terms sensitivity, and specificity. Two-sided p values less than 0.05 was considered statistically significant. IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows was used for all statistical analyses.

Results

The mean age of the studied 180 patients was 4.06 ± 3.318 years with a range of 1-15 years. The study group comprised 116 (64.4%) males and 64 (35.6%) females. Those living in rural areas were 104 (57.8%) and 76 (42.2%) were living in urban areas. Family history of blood disease was positive among only 18 (10%) patients. The laboratory parameters of the studied cohort is shown in Table 1. The diagnosis of type of anemia among the studied 180 children with MCV <70fl is presented in Figure 1.

Table 1. Laboratory findings of the studied cohort.

	Number	Mean (±S.D)	Range
Hb (g/dL)	180	8.806 (1.2257)	3.0-9.9
MCV (fl)	180	60.763(6.4605)	43.1-70.0
MCHC (g%)	180	32.211 (2.8950)	18.2-41.0
HCT (%)	180	27.794 (4.0585)	10.9-36.6
RDW (%)	180	24.504(10.5783)	12.3-73.4
Mentzer (fl/10 6 /µl)	180	13.715 (3.9238)	7.9-42.7
SF (ng/dL)	180	31.494 (84.1615)	1.2-740.2
Hb F (%)	34	18.474 (20.7427)	0.4-84.1
Hb A1 (%)	180	92.42 (15.815)	6-99
Hb A2 (%)	180	2.80 (0.971)	0-7
Hb S (%)	3	70.73 (5.518)	67-77

Hb: Hemoglobin; Hb A1: Adult hemoglobin; HbA2: Hemoglobin A2; HbF: Fetal Hemoglobin F; HbS: Hemoglobin S; HCT: hematocrit; MCHC: mean corpuscular hemoglobin concentration; MCV: mean red corpuscular volume, RDW: red cell distribution width; SF: serum ferritin.

Table 2. The diagnostic yield to thalassemia according to the MCV

	MCV <60 (n=75)	**MCV 60-65 (n=48)**	**MCV 65-70 (n=57)**	**P value**
Gender				
Male	50	27	41	0.234
Female	25	21	16	
Age				
Mean ± SD	4.1±3.16	4.26±3.61	3.99±3.31	0.959
Range	(1-13)	(1-13)	(1-15)	
Residence				
Rural	45	26	33	
Urban	30	22	24	0.815
Diagnosis				
Iron deficiency	44	36	40	
Alpha thalassemia	2	0	0	
Beta thalassemia	7	3	4	
Thalassemia trait	10	2	2	0.277
Sickle thalassemia	0	1	2	
IDA + thalassemia	4	0	3	
Others	8	6	6	
Hemoglobin				
Mean ± SD	8.44 ± 1.13	8.92 ± 1.4	9.19 ± 1.07	0.02
Range	5.1 - 9.9	3 – 9.9	4.3 – 9.9	
Mean Corpuscular Volume				
Mean ± SD	54.3 ± 3.63	62.16 ± 1.35	68.09 ± 1.44	<0.001
Range	43.3 – 59.9	60-65	65.4 – 70	
Mean Corpuscular Hemoglobin Concentration				
Mean ± SD	32.19 ± 3.71	32.1 ± 2.52	32.32 ± 1.79	0.930
Range	18.2 – 41	23.4 – 36.3	28.7 – 36	
Hematocrit				
Mean ± SD	26.38 ± 3.34	28.3 ± 4.42	29.23 ± 4.07	<0.001
Range	16- 34.6	12.8 – 33.9	10.9 – 36.6	
Red Cell Diameter Width				
Mean ± SD	22.29 ± 5.49	23.87 ± 9.61	27.94 ± 14.88	0.008
Range	12.4 – 44	12.3 – 52.3	12.7 – 73.4	
Hemoglobin A				
Mean ± SD	94.09 ± 8.97	93.89 ± 13.54	89 ± 22.84	0.717
Range Hb	42.6 – 98.5	14.4 - 98	5.6 - 98	
Hemoglobin A2				
Mean ± SD	3.06 ± 1.29	2.55 ± 0.66	2.65 ± 0.53	0.006
Range	0.8 – 7.4	0.00 – 5.6	2.0 – 4.7	
Hemoglobin F				
Mean ± SD	11.39 ± 14.58	13.4 ± 13.7	34.06 ± 26.27	0.016
Range	0.4 – 53.9	0.7 - 40	4.1 – 84.1	

The yield of diagnosis of thalassemia according to the MCV; MCV<60 fl, MCV 60-65 fl, MCV >65 fl is shown in Table 2. A total of 40 (22.2%) children with MCV < 70 fl were found to have a hemolytic anemia.

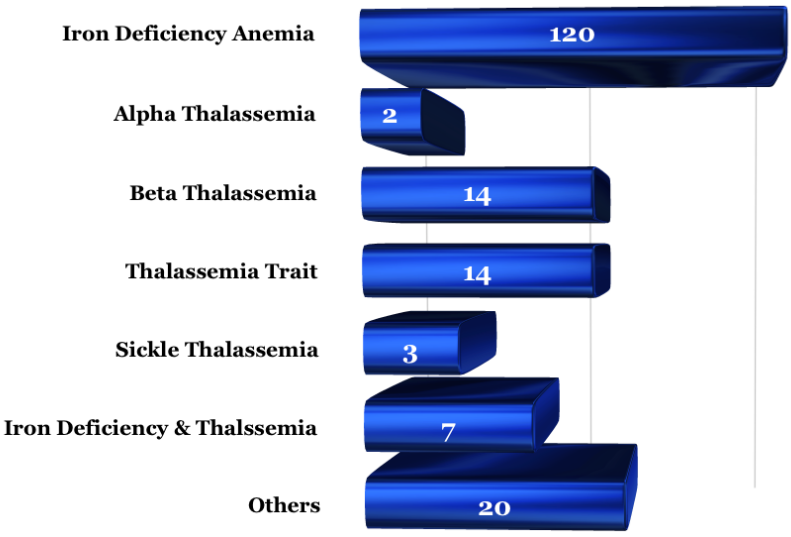

Figure 1. Diagnosis of type of anemia among our studied cohort with MCV< 70fl.

Discussion

Iron deficiency anemia was present among 67% of our studied cohort. Generally, iron deficiency anemia is the most common cause of microcytic anemia (*19*). Different studies reported lower prevalence of IDA among children with microcytosis ranging from 17 – 26.6%. It was concluded, that the prevalence of IDA in children across Egypt was around 33% (*20*).

The prevalence of thalassemia trait among our patients was around 7.7%. This is in accordance to the reported percentages among the Egyptian population as reported in one study (*21*). However, this is less than that reported by El-Shanshory et al., who stated that 35.8% of the study population were thalassemia carriers. El Shanshoury recruited relatives of thalassemia major patients, which explains the high prevalence rate. It has been estimated that around 3% of the world population are thalassemia carriers but Egypt lies within the thalassemic belt with a high carrier rate (*10*).

Differentiation between thalassemia and iron deficiency anemia is of ultimate importance for a number of reasons. First of all, iron supplements used to correct iron deficiency anemia are not recommended in patients with thalassemia unless iron deficiency is reported (*22*). Second, whenever iron deficiency is diagnosed, further investigations are needed to identify the possible causes such as malabsorption, blood loss or inadequate intake which are unnecessary in the case of thalassemia. Lastly, proper identification of patients with thalassemia allows for an effective genetic counseling to prevent recurrence of the condition in one's family (*23*).

By comparing patients with different MCV (Table 2) we found no significant difference in the diagnosis between the three groups implying that persons with MCV below 70 should be screened for thalassemia. Screening for thalassemia first took place in Italy, in the 1970's (*24*). Several countries implemented the national screening program for thalassemia since then including Bahrain (1985), Turkey (1995) and Iran (1997). The Iranian experience was successful as there was a twenty nine fold decrease in thalassemia cases over a thirty year period (*25*).

The low socioeconomic status of the majority of our patients can be a hurdle to requesting further diagnostic investigations. On the other hand, by giving iron therapy to all patients with low MCV can lead to unnecessary increase in iron stores that in severe cases may require iron chelation which is costly itself. Hence the implementation of national screening and preventive measures is mandatory as Egypt lies within the thalassemic belt with high prevalence of consanguinity. It could be started by inexpensive methods as raising awareness about the disease and its prevention techniques among the Egyptian population. Thalassemia could be also

taught to school children and be a part of their syllabus. Another approach that could be attempted is to implement mandatory screening program that is enforced by law where premarital candidates undergo screening for carrier states and consequences should be clearly emphasized if carrier couples decide to have children. This, however, could be faced by a number of challenges. Abortion for example is considered illegal in our country.

Conclusion

From our study we conclude that there is a high rate of thalassemia carriers in Egypt, MCV alone cannot conclude or exclude the presences of carriers and Hb electrophoresis is mandatory in any anemic patient with MCV less than 70, we also emphasize that iron therapy should be withheld in children with microcytic anemia unless iron deficiency had been confirmed by iron studies. In order to reduce the incidence of thalassemia, a premarital screening program with proper action taken must be implemented.

Author Contributions:

NS analyzed the data, provided guidance, reviewed, and approved the final manuscript. OYA performed all the laboratory work needed DS performed the literature search and collected the data. MSN analyzed the data and drafted the manuscript. All read and approved the final manuscript.

FUNDING

Authors declare there was no extramural funding provided for this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest in connection with the reported study. Authors declare veracity of information.

References

1. S. Tenny, M. Varacallo, *Evidence Based Medicine.* (StatPearls Publishing; Treasure Island (FL), 2020; https://www.ncbi.nlm.nih.gov/books/NBK470182/).
2. Y. Aydinok, Thalassemia. *Hematology* **17**, s28–s31 (2012).
3. F. Esmaeilzadeh, A. Azarkeivan, S. Emamgholipour, A. Akbari Sari, M. Yaseri, B. Ahmadi, M. Ghaffari, Economic Burden of Thalassemia Major in Iran, 2015. *J. Res. Health Sci.* **16**, 111–115 (2016).
4. H. Abolghasemi, A. Amid, S. Zeinali, M. H. Radfar, P. Eshghi, M. S. Rahiminejad, M. A. Ehsani, H. Najmabadi, M. T. Akbari, A. Afrasiabi, H. Akhavan-Niaki, H. Hoorfar, Thalassemia in Iran: Epidemiology, Prevention, and Management. *J. Pediatr. Hematol. Oncol.* **29**, 233–238 (2007).
5. R. Galanello, R. Origa, Beta-thalassemia. *Orphanet J. Rare Dis.* **5**, 11 (2010).
6. R. J. A. Trent, Diagnosis of the haemoglobinopathies. *Clin. Biochem. Rev.* **27**, 27–38 (2006).
7. B. S. Strauss, Genetic counseling for thalassemia in the Islamic Republic of Iran. *Perspect. Biol. Med.* **52**, 364–376 (2009).
8. H. Najmabadi, A. Ghamari, F. Sahebjam, R. Kariminejad, V. Hadavi, T. Khatibi, A. Samavat, E. Mehdipour, B. Modell, M. H. Kariminejad, Fourteen-year experience of prenatal diagnosis of thalassemia in Iran. *Community Genet.* **9**, 93–97 (2006).
9. M. Hadipour Dehshal, M. Tabrizi Namini, A. Ahmadvand, M. Manshadi, F. Sadeghian Varnosfaderani, H. Abolghasemi, Evaluation of the national prevention program in iran, 2007-2009: the accomplishments and challenges with reflections on the path ahead. *Hemoglobin* **38**, 179–187 (2014).
10. M. R. El-Shanshory, L. M. Sherief, H. M. Hassab, S. M. Ragab, S. Yahia, A. K. Mansour, A. S. Ahmed, S. H. Abdou, A. M. Helmy, M. M. Watany, A. M. Gad ALllah, M. A. Guindy, Z. I. Mourad, M. A. Soliman, R. M. El-Farahaty, F. El-Dahtory, A. Darwish, S. A. Elmabood, I. A. Kabbash, S. M. Saied, Prevalence of iron deficiency anemia and beta thalassemia carriers among relatives of beta thalassemia patients in Nile Delta region, Egypt: a multicenter study. *J. Egypt. Public Health Assoc.* **96**, 27 (2021).
11. A. El-Beshlawy, I. Youssry, Prevention of Hemoglobinopathies in Egypt. *Hemoglobin* **33**, S14–S20 (2009).

12. World Medical Association, WMA Declaration of Helsinki- Ethical Principles for Medical Research Involving Human Subjects (2013). https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/2013/.
13. S. A. Khan, S. Aaraj, S. N. F. Hussain, Frequency and types of haemoglobinopathies in children with microcytic anaemia. *JPMA J. Pak. Med. Assoc.* **71**, 78–80 (2021).
14. Machin D, Campbell MJ, Tan SB, Tan SH, *Smple Size Tables For Clinical Studies* (Wiley-Blackwell, Chichester, West Sussex, PO19 8SQ, UK, Third., 2009; https://download.e-bookshelf.de/download/0000/6005/91/L-X-0000600591-0001311942.XHTML/index.xhtml).
15. Pagana KD, Pagana TJ, Pagana TN, *Mosby's® Diagnostic and Laboratory Test Reference - Elsevier eBook on VitalSource* (Elsevier, ed. 15th, 2020).
16. D. Rund, E. Rachmilewitz, Beta-thalassemia. *N. Engl. J. Med.* **353**, 1135–1146 (2005).
17. S. Fucharoen, V. Viprakasit, Hb H disease: clinical course and disease modifiers. *Hematol. Am. Soc. Hematol. Educ. Program*, 26–34 (2009).
18. S. Uçucu, T. Karabıyık, F. Azik, Difficulties in the diagnosis of HbS/beta thalassemia: Really a mild disease? *J. Med. Biochem.* **41**, 32–39 (2022).
19. T. G. DeLoughery, Microcytic anemia. *N. Engl. J. Med.* **371**, 1324–1331 (2014).
20. El-Beshlawy A, Kaddah N, Moustafa A, Mouktar G, Youssry I., Screening for beta-thalassaemia carriers in Egypt: significance of the osmotic fragility test. **13**, 780–6 (2007).
21. El-Beshlawy A, Kaddah N, Ragab L, Hussein I, Mouktar G, Moustafa A, El-Raouf E, Hassaballa N, Gaafarand T, El-Sendiony H, "Thalassemic prevalence and status in Egypt." in *Proceedings of the Annual Meeting of the American Pediatric Society;* (San Francisco, CA, USA., 1999)vol. abstract 102.
22. T. Yermiahu, M. Ben-Shalom, A. Porath, H. Vardi, A. Boantza, D. Mazor, N. Meyerstein, Quantitative determinations of microcytic-hypochromic red blood cell population and glycerol permeability in iron-deficiency anemia and beta thalassemia minor. *Ann. Hematol.* **78**, 468–471 (1999).
23. J. F. Matos, L. M. S. Dusse, R. V. B. Stubbert, M. R. Ferreira, W. Coura-Vital, A. P. S. M. Fernandes, J. R. De Faria, K. B. G. Borges, M. D. G. Carvalho, Comparison of discriminative indices for iron deficiency anemia and ß thalassemia trait in a Brazilian population. *Hematology* **18**, 169–174 (2013).
24. E. Silvestroni, I. Bianco, B. Graziani, C. Carboni, S. U. D'Arca, First premarital screening of thalassaemia carriers in intermediate schools in Latium. *J. Med. Genet.* **15**, 202–207 (1978).
25. M. Zeinalian, R. F. Nobari, A. Moafi, M. Salehi, M. Hashemzadeh-Chaleshtori, Two decades of pre-marital screening for beta-thalassemia in central Iran. *J. Community Genet.* **4**, 517–522 (2013).