Original Paper

The Prevalence of Cases of B-Thalassemia Minor Among the Hematologically Suspected Cases in Kerbala.

Duha Maitham Hassan[1], Mohammed Shnain Ali[2*]

[1]Immam AL-Hussain medical city laboratory/Kerbala/Iraq.
[2]University of Kerbala/College of medicine/department of pathology/Kerbala/Iraq.

Abstract

Background: B-thalassemia minor is the symptomless carrier state of B-thalassemia that can be misdiagnosed as mild iron deficiency anemia (IDA).
Aim: This work aims to assess the prevalence of cases of B-thalassemia minor among the suspected cases and to compare the results of diagnosis depending on single confirmatory test (Hb electrophoresis) and three confirmatory tests (reticulocyte count, serum ferritin and Hb electrophoresis).
Materials and methods: In this study 50 cases of mild anemia (Hb ≥9-11.99 g/dl) with initial hematological suspicion of B-thalassemia minor were studied for detailed confirmatory diagnostic tests that include reticulocyte count, Hb electrophoresis for HbA2 estimation and S.Ferritin level.
Results: Results showed that 43/50 i.e 86% of suspected cases are confirmed by three tests (41/50 i.e (82%) have B-thalassemia minor, 2/50 i.e (4%) have B-thalassemia minor and IDA), 6/50 i.e (12%) have iron deficiency anemia (IDA), while 1/50 i.e (2%) have polycythemia (on venesections).
Discussion: Results showed that 86% of initially suspected cases are confirmed to have B-thalassemia minor and 4% of cases have B-thalassemia minor with IDA depending on three tests for confirmatory diagnosis which are reticulocytosis, HbA2 concentration and S.Ferritin level. Similar results were encountered by Cristina Passarello, Antonino Giambona, et al.
Conclusion: The prevalence of cases of B-thalassemia minor among the hematological suspected cases in Kerbala is 86%.
Key words: B-thalassemia minor, HbA2, S.Ferritin.

Introduction

B-thalassemia minor is usually symptomless disorder that is easily misdiagnosed as mild iron deficiency anemia when mild symptoms arise. The prevalence of B-thalassemia minor among the initially suspected cases by hematologists is unknown in Iraq-Kerbala city. Combination of three confirmatory tests (reticulocyte count, serum ferritin and Hb electrophoresis) might improve the diagnosis compared to single confirmatory test (Hb electrophoresis). B-thalassemia minor is a hereditary form of anemia in which there is low production of B-globin chains of hemoglobin. The disorder is inherited as autosomal recessive; however, autosomal dominant cases are also reported [(1)]. Patients are usually symptomless and they represent the carrier states of B-thalassemia which may be not diagnosed until there is a stressful life event like pregnancy [(2)]. Once anemia is clinically diagnosed, initial hematological investigations that include complete blood count and blood film are indicated. When such initial tests show suspicion of B-thalassemia minor, detailed hematological investigations are mandatory to confirm the diagnosis [(3)]. Cases of B-thalassemia minor

*For correspondence E-mail: mohammadshnain@yahoo.com

usually show high HbA2 (the normal value is 2.2-3.3% of the total Hb) with normal or slightly high HbF [4,5]. Results of HbA2 are commonly ranging between 4.5-6.5% in cases of B-thalassemia minor when there are typical mutations while the range is 3.1-4.5% when there is atypical mutation (in which even MCV might be normal)[6].Hematological investigations might also be not clear enough to separate clearly cases of B-thalassemia from other cases (IDA, B-thalassemia with IDA, B-thalassemia with normal HbA2, B-thalassemia with delta or alpha thalassemia) unless they are carefully studied by specialist in hematology since there are factors that increase HbA2 and include unstable hemoglobin types, hemoglobin E disease, Sickle cell trait and sickle cell anemia, KLF1 mutation, pseudoxanthoma elasticum, type1 congenital dyserythropoietic anemia, HIV infection, Ziduvodine antiviral treatment in HIV infection, some cases of megaloblastic anemia, hyperthyroidism, and others while factors that decrease levels of HbA2 include IDA, anemia of chronic disorders, sideroblastic anemia, lead poisoning, juvenile myelomonocytic leukemia, acquired Hb H disease, erythroleukemia, some aplastic anemias, hypothyroidism, delta and delta-beta thalassemias, alpha thalassemia (including HbH disease), Hb lepore, and others[7]. It is very important to mention that the accuracy of measurement of HbA2 is critical since there are very small differences between normal and abnormal levels [8,9]. Such accuracy depends on the type of method which is used to measure HbA2 which include microcolumn chromatography (MC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) [10]. There is a statistically significant difference among these methods and they may underestimate or overestimate HbA2 levels [11]. Reticulocytosis is generally seen in B-thalassemia minor; however it is also seen in other types of inherited and acquired types of anemia as seen in megaloblastic anemia, IDA with treatment, HbH disease and others [12]. It is very important to estimate serum ferritin level because low levels are generally causing lower HbA2 and need treatment with iron and re-evaluation of HbA2 for proper diagnosis of B-thalassemia minor[13]. So that we arranged for this study for proper confirmatory diagnosis of B-thalassemia minor.

Materials and methods

A cross sectional descriptive study was designed for a total number of 50 cases of patients with age range of 16-30 years with the following eligibility criteria: mild anemia (Hb ≥9-11.99 g/dl) and initial hematological suspicion of B-thalassemia minor which is defined as hypochromic microcytic blood film picture with mild to moderate anisopoikilocytosis that is diagnosed by hematologist from the results of blood film. Cases were studied for detailed confirmatory diagnostic tests that include reticulocyte count, Hb electrophoresis for HbA2 estimation and Serum ferritin level in addition to CBC. Patients with low S.Ferritin are further evaluated by another CBC, reticulocyte count, s.ferrtin, and Hb electrophoresis after one month of iron therapy (ferrous sulfate oral tablets 325 mg three times daily). The study was done at AL-Hussain medical city, AL-Hussain teaching medical hospital, hematology laboratory in Kerbala/Iraq during the period from 3/January/2016 to 6/May/2016 after taking consent of each patient. Each suspected case is already having 3 cc EDTA venous blood sample and blood film report with or without CBC report. Hematological tests that are done for the patients include CBC (Hb, RBC count, MCV, MCH, MCHC, RDW, WBC count, differential count, and platelet count) by automated machine (sysmex, 5 differential count) in which the normal Hb level is 12.0-16.0 g/dl, normal RBC count of 4.06-5.30 X 10^6/µl. Reticulocyte count is done by manual

staining using the ordinary reticulocyte stain[14] with a normal range of 0.2-2% (non corrected count) while serum ferritin levels were estimated using fully automated biochemistry machine (Cobus e 411) with normal value of 13-150 ng/ml for males and 30-400 ng/ml for females. Venous blood samples (2 cc plain tube venous blood samples) were already present for each patient and quite enough for S.Ferritin estimation. Intranet is available in the laboratory and each patient has clinical information and results of all investigations so that clinical and laboratory notes are easily accessed for each patient. HbA2 is estimated by Hb electrophoresis using ULTRA2 machine with normal value of 2.2-3.3% of the total Hb in the presence of normal serum ferritin level. The study is descriptive and analyzed by mean, SD and paired T-test for the significance which is defined when P value below or equal to 0.05%.

Results

The results of confirmatory diagnostic tests and the final results of diagnosis of all cases are shown in table 1 and 2, respectively.
Results showed that 50/50 i.e 100% of patients have both mild anemia with a mean Hb of 9.9 g/dl, range 9.0-11.2 g/dl and hypochromic microcytic blood picture with moderate anisopoikilocytosis which is favoring the initial diagnosis of thalassemia minor, 43/50 i.e 86% of suspected cases are confirmed to have thalassemia minor by the combined three tests. 46/50 i.e 92% of patients have high RBC count with a mean of 5.6 X 10^6/µl, range 5.33-7.1 X 10^6/µl, 4/50 i.e 8% have normal RBC count with a mean value of 4.9 X 10^6/ µl. 40/50 i.e 80% have reticulocytosis with a mean non corrected reticulocyte count of 3.8%, range of 2.2%-6.2%, mean corrected reticulocyte count of 2.9%, 10/50 i.e 20% have normal reticulocyte count with a mean of 1.6% and range of 0.6-2% for non-corrected count, 9/50 i.e 18% have low serum ferritin levels with a mean of 8 ng/ml [6/50 have IDA, 2/50 have B-thalassemia minor and IDA, 1/50 have polycythemia on frequent venesection], range 1.7-28 ng/ml, 31/50 i.e 62% have normal values of serum ferritin with a mean of 88 ng/ml, range 33-108 ng/ml, 6/50 i.e (12%) have iron deficiency anemia (IDA), 2/50 i.e (4%) have B-thalassemia minor and IDA while 1/50 i.e (2%) have polycythemia (available clinical data from intranet of hematology unit in the laboratory) on frequent venesections that causes anemia.
The total number of patients with low S.Ferritin is 9/50 (18%). One of nine patients with low S.Ferritin is found to be polycythemic by clinical data available in intranet and he is not treated by iron tablets. The other 8 patients received iron therapy for one month (details available in materials and methods) and CBC, S.Ferritin and reticulocyte count were repeated and revealed the following results: 2/8 of patients showed normal S.Ferritin (mean 53 µg/L), reticulocytosis (mean non corrected values 2.8%), and high HbA2 (mean 4.3%) and diagnosed to have thalassemia minor while 6/8 of patients normal S.Ferritin (mean 34 µg/L), reticulocytosis (mean non corrected values 3.2%), and normal HbA2 (mean 2.8%) and diagnosed to have iron deficiency anemia.

Discussion

Precise interpretation of hematological results will greatly increase the rate of diagnosis of B-thalassemia minor [11]. Results showed that 86% of initially suspected cases are confirmed to have B-thalassemia minor and 4% of cases have B-thalassemia minor with IDA depending on careful evaluation of confirmatory diagnostic tests mainly HbA2 concentration and S.Ferritin level.

Table 1. The final results of confirmatory diagnostic tests for 50 cases.

	confirmatory diagnosis using three tests (reticulocytes, S.Ferritin and Hb electrophoresis)	confirmatory diagnosis using one test (Hb electrophoresis)	P value
B-thalassemia minor	41, 82%	41, 82%	Not significant P≥ 0.05
Iron deficiency anemia (IDA)	6, 12%	*	
B-thal. minor with IDA	2, 4%	1, 2%	Significant P≤ 0.05
Polycythemia	*	*	

*The test is not suitable for the diagnosis of item

Table 2. The mean values of confirmatory diagnostic tests for 50 cases.

Number of cases out of 50	*S.Ferritin µg/L	HbA2	Corrected reticulocyte count	confirmatory diagnosis
41	64± 23.2	5.3± 1.2	5.5% ± 1.3	B-thalassemia minor
6	4.3± 1.7	2.1±0.4	2.6% ± 0.8	Iron deficiency anemia
2	12± 0.9	3.9± 0.6	3.75% ± 0.3	B-thal. Minor with IDA
1	10	2.9	0.5%	Polycythemia

*The normal s. ferritin is 30-400ng/ml for females and 13-150 ng/ml for males.

Similar results were encountered by Cristina Passarello, Antonino Giambona, et al[15] who concluded that HbA2 level is reduced by IDA which is reflected by low S.Ferritin level; however, the reduction is statistically not significant with clear cut off value of 30 µg/L. Cases of IDA were 12% with 100% initial suspicion of B-thalassemia minor that required a confirmation or exclusion by Hb electrophoresis and S.Ferritin level. Results showed low S.Ferritin in 100% of cases of IDA and normal HbA2; however, the interesting case was that of B-thalassemia minor with IDA which showed a maximal upper normal HbA2 (3.3%). Such findings were consistent with the study of Mandan N, Sikka M, et al [16] which showed that iron deficiency causes normal or low HbA2 levels in cases of B-thalassemia minor. Many physicians in Iraq request HbA2 estimation as confirmatory test for a suspected case of thalassemia minor. This is not scientific because serum ferritin and reticulocyte count can reflect the important results. On one hand reticulocyte count might be high and might show positive HbH which gives a diagnosis of alpha thalassemia which is not encountered in this study which may be due to low number of patients. In such case, HbA2 is normal and the physician will not reach the diagnosis if he/she depends on HbA2 estimation alone as confirmatory test for thalassemia minor. On the other hand S.Ferritin might be low, reflecting iron deficiency anemia alone or iron deficiency anemia with thalassemia minor. In such case HbA2 is also normal or even low and thalassemia minor is hidden. Iron therapy for such patients for one month and repetition of HbA2 estimation will lead to the diagnosis of thalassemia minor if HbA2 is raised above normal values as seen in 2 out of 50 cases in this study (4% of cases). In such cases the physician will also misdiagnose cases of thalassemia minor if he/she depends on HbA2 estimation as single confirmatory diagnostic test for thalassemia minor. All three tests are mandatory for the confirmatory diagnosis of thalassemia minor and the idea that S.Ferritin is not recommended for the confirmatory diagnosis of thalassemia minor is not scientific.

Conclusion

The prevalence of cases of B-thalassemia minor among the hematologically suspected cases in Kerbala is 86%.
Detailed evaluation with careful interpretation of three hematological parameters in suspected cases of B-thalassemia minor who have mild anemia is highly essential to improve the rate of diagnosis of B-thalassemia minor with special attention for cases with low serum ferritin that need iron therapy and another evaluation of HbA2 for accurate diagnosis and hence to detect the other symptomless family members (parents and siblings).

References

1. Victor Hoffbrand, S. Mitchell Lewis and Edward G.D Tuddenham: Postgraduate hematology, fourth edition; Wiley Blackwell; 1999; USA; chapter 6; page 101.
2. Peters M, Heijboer H, Smiers F, et al; Diagnosis and management of thalassaemia. BMJ. 2012 Jan 25; 344.
3. Green JP, Foerster J, Rodgers CM. Wintrobe's clinical hematology Vol 1.
4. Giordano PC Editorial: Measurment of HbA2. Int J Lab Hematol. 2012; 34: 335.
5. Colah RB, Surve R, sawant P, et al: HPLC studies in hemoglobinopathies. Indian J med. 2007; 74:657-62.
6. Mosca A, Paleari R, Ivaldi G, Galanello R, Giordano PC: the role of hemoglobin A2 testing in the diagnosis of thalassemias and related haemoglobinopathies. J ClinPathol; 2009, 62:13–17.
7. Bain BJ, Wild BJ, Stephens AD, et al: Variant hemoglobins: a guide to identification. First eddition, west Sussex, Uk; Wiley-Blackwell; 2010.
8. Shokrani M, Terrell F, Turner EA, et al:Chromatographic measurement of HbA2 in blood samples that contain sickle Hb. Ann Clin Lab Sci. 2000; 30:191-4.
9. Madan N, Sharma S, Sood SK, Colah R, Bhatia LHM: Frequency of β-thalassemia trait and other hemoglobinopathies in northern and western India. Indian J Hum Genet; 2010, 16:16–21.
10. Keren DF, Hedstrom D, Gulbranson R, Ou CN, Bak R. Comparison of Sebia capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies. Am J ClinPathol 2008; 130:824-31.
11. Giambona A, Passarello C, Renda D, Maggio A. The significance of the hemoglobin A2 value in screening for hemoglobinopathies. ClinBiochem 2009; 42:1786-96.
12. McPherson RA, Pincus MR. et al. eds. Henry's clinical diagnosis and management by laboratory methods. 21st ed. W.B. Saunders Company, 2006:521.
13. Clarke GM and Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. ClinChem 2000; 46:1284-90.
14. V. Dacie and S. M. Lewis: Practical hematology, eleventh edition; Elsevier 2011; China; chapter 5, page 49-82.
15. Passarello C, Giambona A, Cannata M, Vinciguerra M, Renda D, Maggio A. Iron deficiency does not compromise the diagnosis of high HbA2 β thalassemia trait. Haematologica. 2012 Mar 1; 97:472-3.
16. Madan N, Sikka M, Sharma S, Rusia U. Phenotypic expression of hemoglobin A2 in beta-thalassemia trait with iron deficiency. Ann Hematol. 1998; 77:93-6.