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Screening for Beta Thalassemia in Damietta

AMAL EL-BESHLAWY, M.D.*; MONA EL-GHAMRAWY, M.D.*; AZZA MOUSTAFA, M.D.**; NADIA MOSALEM, M.D.** and FATMA EL-RIES, M.Sc.***

The Departments of Pediatrics, New Children Hospital, Cairo University, Clinical Pathology, Cairo University** and Pediatrics, Damietta General Hospital.****

ABSTRACT

Introduction: Beta thalassemia is not only an important public health problem in Egypt, but also a socioeconomic one. A carrier rate of 9-10.2% was estimated in different geographical areas of Egypt.

Objective: To detect ß-thalassemia carriers among a random sample of population in Damietta Governorate, to help in the prevention of further births of ß-thalassemia patients. Increasing awareness about the disease was our second objective.

Patients and Methods: One thousand and four random subjects in Damietta were included in this study. Their mean age was 18.6±13.4 years. All studied population was subjected to a complete blood picture and high performance liquid chromatography (HPLC), using Variant 11 Rad, was done for those with evidence of microcytosis (MCV<80fl).

Results: In our study, 633 subjects (63.05%) had microcytosis, for whom HPLC was performed. Among our studied population (n=1004), 46 subjects (4.58%) had HbF>1%, 26 subjects (2.59%) had HbA2 >3.5% and 6 subjects (0.6%) had both increased HbF and HbA2.

Conclusion: The carrier rate of ß-thalassemia in Damietta was found to be 7.77%. This highlights the need for immediate implementation of a national preventive program all over Egypt. Increasing population awareness and premarital screening remain the best ways to reduce the disease incidence.

Key Words: *Beta-thalassemia – Carrier – HPLC.*

INTRODUCTION

Thalassemias are common autosomal recessive disorders especially in populations of Mediterranean, Middle Eastern and Far Eastern descent **[1]**. Beta thalassemia has an estimated frequency of 3-10% in certain regions **[2]**. In Egypt, it is the most common chronic hemolytic anemia (85.1%). The Hematology Clinic Study Group at New Children Hospital of Cairo University reported a carrier rate varying between 6 and 10%. Moreover, 1000 children affected with thalassemia are expected out of 1.5 million live births per year in Egypt **[3]**.

The determination of prevalence of beta thalassemia in endemic areas is important in order to develop programs for their control and management **[4]**.

Several tests have been proposed for detection of beta thalassemia variants including accurate measurement of mean corpuscular volume (MCV), mean cell hemoglobin (MCH), osmotic fragility, estimation of HbA2, HbF and identification of Hb variants **[5,6]**.

Therefore, the aim of this study was to detect ß-thalassemia carriers in a random sample of population in Damietta Governorate for prevention of further births of ß-thalassemia patients with its physical, social and financial burden. Increasing awareness about the disease was our second objective.

PATIENTS AND METHODS

Patients:

One thousand and four subjects from Damietta Governorate were randomly selected to be recruited in this study in a two months' interval. All subjects were above one year of age and were apparently healthy. Their age ranged between 1.1 and 76.0 years with a mean of 18.6± 13.4 years.

Laboratory investigations were done for all our subjects. These included a complete blood picture (by Advia 120 automated cell counter). Red cell indices included hemoglobin concen-

tration (gm/dl), mean corpuscular volume (MCV) (fl), mean cell hemoglobin (MCH) (pg), mean cell hemoglobin concentration (MCHC) (g/dl) and red cell distribution width (RDW) (%). High performance liquid chromatography (HPLC), using Variant 11 Rad, was done for those with evidence of microcytosis (MCV <80fl). Our cutoff points for diagnosis of ß-thalassemia carriers were the presence of microcytosis (MCV <80 fl) associated with elevated levels of HbA2 (>3.5%) and/or elevated levels of HbF (>1%).

Sample preparation:

Whole blood specimens were collected in a vacuum collection tube containing EDTA. The 16mm sample tubes were loaded into the Variant 11 sample racks in random order and placed on the VARIANT11 Sampling Station conveyer belt. Special rack inserts were used for 13mm tubes and special adapters for 10 mm pediatric tubes.

Procedure:

The VARIANT 11 ß-thalassemia Short Program is intended for separation and area percent determinations of HbA2 and HbF as an aid in the identification of abnormal hemoglobins in whole blood, using ion-exchange HPLC. The VARIANT 11 Clinical Data Management (CDM) software performed reduction of raw data collected from each analysis. One-level calibration was used for adjustment of the calculated HbA2/F values. To aid in the interpretation of results, windows (e.g. ranges) had been established for the most frequently occurring hemoglobins based on their characteristic retention times.

Statistical methods:

All numerical data were expressed in the form of mean and standard deviation.

RESULTS

Statistical analysis of data of all our studied subjects is shown in Table (1). Fig. (1) shows the scatter distribution of MCV and HbA2 among our subjects. Applying our cutoff value for microcytosis to our studied population, 633 subjects (63.05%) had microcytosis, for whom HPLC was performed with their data shown in Table (2).

Among our studied population, 78 subjects (7.77%) were defined as beta thalassemia carriers by fulfilling our diagnostic cutoff values of microcytosis associated with elevated HbA2 and/or elevated HbF levels. The statistical data of this carrier group is shown in Table (3), forty six subjects (4.58%) had HbF >1%, 26 subjects (2.59%) had HbA2 >3.5% and 6 subjects (0.6%) had both increased HbF and HbA2 (Fig. 2).

Among our studied population, 195 subjects (19.42%) had borderline HbA2 levels (≥3 and ≤3.5%).

Table (1): Descriptive statistical data of studied subjects (n=1004).

Variables	Min	Max	Mean	SD
Age (years)	1.1	76.0	18.6	13.4
Hb (g/dl)	7.26	17.80	12.74	1.49
MCV (fl)	50.60	93.70	76.81	6.90
MCH (pg)	14.60	37.90	26.44	2.68
MCHC (g/dl)	26.40	38.10	34.37	1.40
RDW (%)	12.00	29.60	15.05	1.57

Table (2): Descriptive statistical data of subjects with MCV <80 (n=633).

Variables	Min	Max	Mean	SD
Age (years)	1.1	65.0	15.1	12.3
Hb (g/dl)	7.26	16.40	12.29	1.33
MCV (fl)	50.60	79.90	72.92	5.56
MCH (pg)	14.60	29.70	25.10	2.38
MCHC (g/dl)	26.40	38.10	34.37	1.53
RDW (%)	12.90	29.60	15.46	1.71
HbF (%)	0.00	7.60	0.46	0.73
HbA2 (%)	0.40	5.50	2.80	0.55

Table (3): Descriptive statistical data of carriers according to cut off point MCV <80 and HbF>1 and/or HbA2 >3.5 (carrier rate) (n=78).

Variables	Min	Max	Mean	SD
Age (years)	1.1	45.0	9.4	10.8
Hb (g/dl)	9.30	15.90	12.06	1.09
MCV (fl)	54.70	79.90	70.85	6.45
MCH (pg)	18.40	28.50	24.43	2.61
MCHC (g/dl)	29.50	37.40	34.45	1.46
RDW (%)	13.30	21.10	15.89	1.74
HbF (%)	0.00	7.60	1.58	1.50
HbA2 (%)	0.60	5.50	3.28	0.92

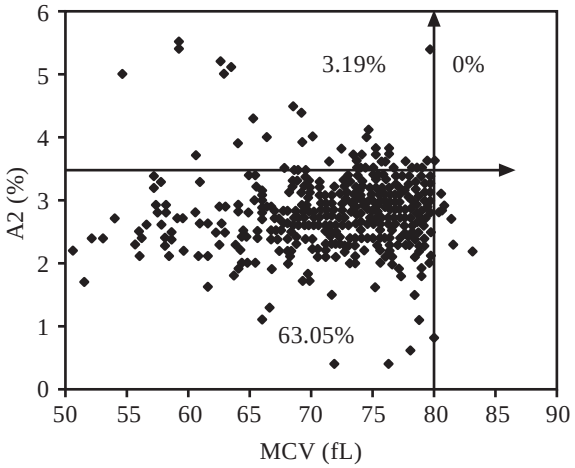

Fig. (1): Scatter distribution of MCV and A2 of 1004 subjects included in the study.

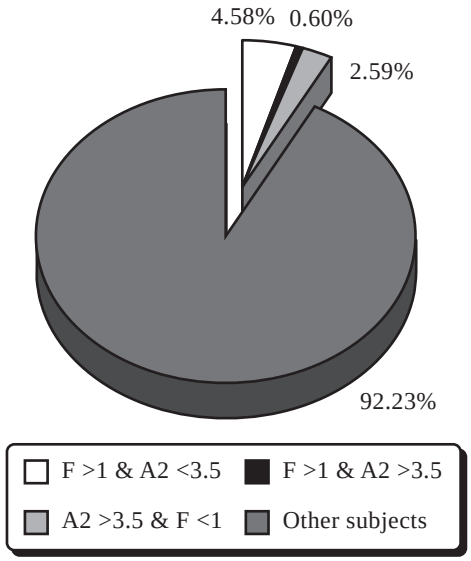

Fig. (2): Distribution of carriers among studied patients.

DISCUSSION

Beta thalassemia is not only an important public health problem but also a socioeconomic one. There are 270 million people who are carriers of globin gene mutations worldwide. Up to a half million infants are born annually with severe hemoglobinopathies **[7]**. The approach to deal with thalassemic problem is to prevent and control births of new cases, which requires an accurate identification of carriers **[8]**. Screening for beta thalassemia is extremely difficult, mainly because of the heterogeneity of beta thalassemias and the absence of a single pathognomonic finding to cover all beta thalassemia variants **[9]**.

In our study, the carrier rate found in Damietta (7.77%) was close to that reported by Tongsong et al. in 2000 (7.97%) **[10]**. On the other hand, a carrier rate of ≥9% was reported from different geographical areas of Egypt **[11]**, whereas a prevalence of beta thalassemia trait of 2.9% was reported in Bahrain **[12]** and of 1.84% in Gaziantep in Turkey **[13]**. This shows the regional difference in carrier rate of beta thalassemia as well as the need for standardized as well as accurate diagnostic tools.

Although, elevated HbA2 is a characteristic feature for beta thalassemia trait **[14,15]**, some individuals with beta thalassemia trait have normal indices **[16]** and HbA2 levels may be normal in the rare 'silent' beta thalassemia trait, so that an increased HbA2 can not be used as the sole discriminant for beta thalassemia trait **[17]**. In our study, 32 subjects (3.19%) showed both microcytosis and elevated HbA2 (HbA2 >3.5%) with a mean HbA2 value of 4.15% (ranging from 3.6 to 5.5%). This is slightly lower than that reported by other authors **[18]**. However, iron deficiency with its known reducing effect on HbA2 level may account for this difference. It was suggested that iron deficiency must be corrected before making any diagnostic or therapeutic decisions based on HbA2 **[19]**. Among our studied population, 195 subjects (19.42%) had HbA2 levels ≥3 and ≤3.5% which needs further investigations.

On the other hand, 52 subjects (5.18%) of our population were diagnosed as carriers by having microcytosis as well as elevated HbF (>1%), six of them had elevated HbA2 as well. This is in agreement with results of El-Beshlawy et al. (1992) **[18]** who reported elevated HbF in 17.5% of their carriers ranging from 2-7.4%. In another study, raised HbF was shown in 32 cases out of 69 cases of beta thalassemia heterozygote but no case showed raised HbF without the raise of HbA2 **[10]**.

The MCV is a key diagnostic indicator especially with all automated hematology analyzers now providing a measure of MCV that is both precise and accurate. Thalassemia carriers do not have significant anemia, but invariably have microcytosis (MCV < 80 fl) and hypochromia (MCH < 27 pg) **[20]**. It was reported that microcytosis was a consistent feature in all heterozygous beta thalassemia in several studies **[10,18]**. This was shown in our study, where the

mean MCV of beta thalassemia carriers was 70.85 fl±6.45 (ranging from 54.7 to 79.9 fl), in spite of their mean Hb level being 12.06g/dl± 1.09 (with a range of 9.3-15.9 g/dl). Among our subjects, 633 (63.05%) had microcytosis which highlights the importance of differentiating between different causes of microcytosis, especially iron deficiency being common in our community.

A reduced MCH <27pg was found among beta thalassemia carriers in previous studies [18,21,22]. This was also shown in our study where the mean MCH among carriers was 24.43 pg±2.61 (range 18.4 to 28.5 pg). However, the MCH can not be used as a discriminant factor due to the common association of iron deficiency which may significantly affect the MCH **[23]**.

Traditionally, electrophoresis has been the method of choice for identification and quantification of Hb variants. However, it is slow, labor-intensive and inaccurate in identification of low-concentration Hb variants (e.g. HbA2) or in the detection of fast Hb variants (HbH, Hb Barts) **[24]**. In our study, high performance liquid chromatography (HPLC) was used for quantification of HbA2 and HbF as it has relatively high sensitivity or specificity **[25]**. The simplicity of sample preparation, superior resolution of the method and accurate quantitation of Hb concentrations, combined with complete automation, make this an ideal methodology for routine diagnosis of Hb disorders in a clinical laboratory **[25]**.

The reliability of HbA2 measurement by HPLC without any false positive or false negative results is of great advantage **[26]**. However, HbA2 and HbF concentrations obtained should be interpreted together with other variables such as erythrocyte indices, iron studies or family studies in some individuals.

In conclusion, Beta thalassemia remains an important health problem in Egypt, including Damietta Governorate, with a high carrier rate which increases the possibility of a high birth rate of thalassemic patients. This necessitates the immediate implementation of a national preventive program all over Egypt. Increasing population awareness and premarital screening remain the best ways to reduce the disease incidence with potentially significant financial saving and social and health benefits.

REFERENCES

1- Cao A, Saba L, Galanello R, Rosatelli MC. Molecular diagnosis and carrier screening for beta thalassemia. JAMA. 1997, 15: 1273-1277.

2- Wu G, Hua L, Zhu J, Mo QH, Xu XM. Rapid, accurate genotyping of beta-thalassemia mutations using a novel multiplex primer extension/denaturing high performance liquid chromatography assay. Br J Hematol. 2003, 122 (2): 311-316.

3- El-Beshlawy A, Kaddah N, Ragab L, Hussein L, Mouktar G, Moustafa A, El-Raouf E, Hassabballa N, Gaafar T, El-Sendiony H. Thalassemia prevalence and status in Egypt. Pediatric Research. 1999, 16 (5).

4- Masmas TN, Garly ML, Lisse IM, Rodriques A, Petersen PT, Birgens H. Inherited hemoglobin disorders in Guinea-Bissau, West Africa: a population study. Hemoglobin. 2006, 30 (3): 355-364.

5- Katamis C, Efremov G, Pootrakul S. Effectiveness of one tube osmotic fragility screening in detecting ß thalassemia trait. Journal of Medical Genetics. 1981, 18: 226.

6- Bento C, Relvas L, Vazao H, Campos J, Rebelo U, Ribeiro ML. The use of capillary blood samples in a large scale screening approach for the detection of beta-thalassemia and hemoglobin variants. Hematologica. 2006, 91 (11): 1565.

7- Weatherall DJ. The global problem of genetic disease. Ann Hum Biol. 2005, 32: 117-122.

8- Winichagoon P, Thitivichianlert A, Lebnak T, Piankijagum A, Fuchareon S. Screening for the carriers of thalassemias and abnormal hemoglobins at the community level. Southeast Asian J Trop Med Public Health. 2002, 33 Suppl 2: 145-150.

9- Weatherall DJ, Clegg JB. The thalassemia syndromes. Third edition Oxford: Blackwell Scientific Publications. 1981.

10- Tongsong T, Wanapiak C, Sirivatanapa P, Sanguansermsri T, Sirichotiyakul S, Piyamongkol W,Chanprapaph P. Prenatal control of severe thalassemia. Chiang Mai Strategy. Prenat Diagn Mar. 2000, 20 (3): 229-234.

11- El-Beshlawy A, Kaddah N, Moustafa A, Mouktar G, Youssry I. Screening for beta-thalassaemia carriers in Egypt: significance of the osmotic fragility test. East Mediterr Health J Jul-Aug. 2007, 13 (4): 780-6.

12- Al-Arrayed S, Hafadh N, Amin S, Al-Mukhareq H, Sanad H. Student screening for inherited blood disorders in Bahrain. East Mediterr Health J May. 2003, 9 (3): 344-352.

13- Gurbak M, Sivasli E, Coskun Y, Bozkurt AI, Ergin A. Prevalence and hematological characteristics of beta-thalassemia trait in Gaziantep urban area, Turkey. Pediatr Hematol Oncol Jul-Aug. 2006, 23 (5): 419-25.

14- Benz EI, Schwartz E. Thalassemia Syndromes, in Miller and Baehner; 6th ed, Blood Diseases of Infancy and Childhood. The C.V. Mosby Company. 1990, 428.

15- Samperi P, Mancuso GR, Dibenedetto SP, Di Cataldo A, Ragusa R, Schiliro G. High performance liquid chromatography (HPLC): a simple method to quantify HbC, O-Arab, Agenogi and F. Clin Lab Haematol. 1990, 13: 169-175.

16- Steinberg MH, Adams III JG. Hemoglobin A2: Origin, evolution and aftermath. Blood. 1991, 78: 2165-2177.

17- Tan GB, Aw TC, Dunstan RA, Lee SH. Evaluation of high performance liquid chromatography for routine estimation of hemoglobin A2 and F. J Clin Pathol. 1993, 46 (9): 852-856.

18- El-Beshlawy A, Omran S, Ragab L, Risk S, EL-Tagui M, Sobh H. Osmotic fragility as a screening test for detection of beta thalassemia trait in Egypt. JAC Dec. 1992, vol 3, No. 4: 287-292.

19- El-Agouza I, Abu Shahla A, Sirdah M. The effect of iron deficiency anemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Clin Lab Haematol Jun. 2003, 25 (3): 203.

20- David HK Chui, Melody J Cunningham, Hong-yuan Luo, Lawrence C Wolfe, Ellis J Neufeld, Martin H Steinberg. Screening and counseling for thalassemia. Blood Feb. 2006, 15, 107 (4): 1735-1737.

21- Weatherall DJ. The thalassemias in hematology, 4th ed, Williams W.J. et al. New York, Mc-Graw Hill, 1990, 510.

22- Roberta A, Pagon. Gene Reviews. Editor-in –chief: Suzanne B. Cassidy; Thomas C. Bird; Mary Beth Dinulos; Gerald L. Feldman; Richard J.H. Smith; Cynthia R. Dolan; Associate editors; Seattle (WA): University of Washington. 2005.

23- El- Beshlawy A, Kaddah N, Omran N, Moustafa A, El-Aiady A, Abd El-Azeem K. Detection of thalassemia carrier among Egyptians. Proc EMS, Ann Sci Cong Feb. 1993.

24- Clarke G, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemia: review and update. Clin Chem. 2000, 46: 1284-1290.

25- Ou CN, Rognerud CL. Diagnosis of hemoglobinopathies: electrophoresis vs. HPLC. Clin Chim Acta Nov. 2001, 313 (1-2): 187-194.

26- Bravo-Urqiola M, Arends A, Montilla S, Velasquez D, Garcia G, Alvarez M, Guevara J, Castillo O. Advantages in the use of high performance liquid chromatography technique for screening hemoglobinopathies in Venezuela. Invest Clin Dec. 2004, 45 (4): 309-315.