Prevalence of beta thalassemia minor, iron deficiency and glucose-6-phosphate dehydrogenase deficiency in Iranian boy's primary schools in Yasuj

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Abstract: The prevalence of thalassemia and iron deficiency in Iran varies greatly from area to area. **Aims:** determination of prevalence of beta thalassemia minor, iron deficiency and glucose 6 phosphate dehydrogenase (G6PD) in yasuj iran. **Materials and Methods:** Two hundred eighty boy's primary schools were selected. Two ml of blood in EDTA tubes for hematologicals parameters and 4 ml in non anticoagulated tubes for serum iron (SI), total iron binding capacity (TIBC) and serum ferritin (SF) was collected from all participants by antecubital vein puncture. HbA_2 was estimated. **Result:** MCV≥80fl in 80% and MCV below 80 fl in 20 % of all participants was reported .MCH ≤25 pg, and RBC count ≥ $6 \times 10^6/\mu L$ were found in 10% and 6 % of participants respectively. Out of all participant 38 (13%) was reported with MCV ≤ 80 fl. Hemoglobin A2 more than 3.5 percent was reported in 17 (6%) and hemoglobin A2 less than 3.5 percent was 21 (7%) of participants. From 280 participants 17 students (6%) were diagnosed as beta thalassemia minor. Out of 280 participants, 39 (13.92%) subjects had SF below 12 ng/ml and 241(86.08%) subject had SF more than 12 ng/ml .The prevalence of G6PD deficiency was reported 12.7%. **Conclusion:** out of 280 students screened 39 (13.92%) were found to be anaemic, while 17(6%) students were positive for BTM and only 35 (12.5%) students were positive for G6PD deficiency.

Key words: Beta Thalassemia Minor, Iron Deficiency, Glucose-6-Phosphate Dehydrogenase Deficiency, Yasuj

Introduction

Beta-thalassemia is a globin chain disorder that transmitted by heredity and describes by a genetic deficiency in the synthesis of beta globin chains. Clinically depend to beta globin chains defects β-thalassaemias are divided into β- thalassaemia major and β-thalassaemia minor (BTM). Hemoglobin synthesis is defects in the thalassemiasv. In beta thalassemia major there is loss of β chain synthesis, while in minor thalassemia there is a decrease in the synthesis of β globin chains was occurs. [2]. In β-thalassemia major unfavorable effects was occurs on production of red blood cells and may cause a decrease in the level of the globin chain and hemoglobin concentration, resultant microcytosis and hypochromic formed. Beta thalassemia major (Cooley's Anemia) present almost 3-8 months after birth. The patients for survival need individual therapeutic services and regular blood transfusion. Beta thalassemia minor is the heterozygous disease, in which only one abnormal beta gene is inherited from the parent. In beta thalassemia minor, hypochromia and microcytosis with or without anemia in red blood cell morphologhy may be seen. These finding can mimic iron deficiency states. Although, thalassemia major is worldwide disease but it's genes distribution is more prevalent in the Mediterranean area, Africa, Middle East, India,

Southeast of Asia, Greece, Italy, Cyprus and Iran where 3-5% of the people are carriers for mutant genes of thalassemia(2). The prevalence of thalassemia (2.5-15%) in Iran varies greatly from area to area, and the highest rate found around the Caspian Sea and the Persian Gulf. There is about more than 15,000 patients recorded with thalassemia major in Iran .However, cases of α-thalassemia are very rare. Determining of BTM prevalence is a main way of prevention of β-thalassaemia major in the following generations (3). Investigators have more interest to differentiate beta thalassemia minor from iron deficiency anemia as an hypochromic and microcytic anemia. Iron deficiency considered the most common nutritional deficiency that widespread among children. It can produce anemia by low concentration of iron in the body. A classic differential diagnosis between beta thalassemia minor and iron deficiency anemia is managed on hemoglobin electrophoresis, serum iron levels, and ferritin concentrations (4, 5).Glucose-6-phosphate dehydrogenase deficiency (G6PD) is one of the most frequent enzyme deficiencies that affected with 400 million people in the world. G6PD characterized by wide biochemical abnormality such as anemia and icter. G6PD is X-linked disease with mother-to-son transmission with high prevalence in Iran (6). Due to the significance of thalassemia disorders and

inherited in a recessive autosomal model this study was managed. The aim of present study was prevented and eliminated the new cases of beta thalassemia major by low-cost screening procedures before marriage program. In according to high prevalence of beta thalassemia in Iran present screening program was cost effective.

Material and Methods

A total of two hundred eighty students 9-11 ages were collected in 11 schools included in the analytical cross-sectional study that selected from a list of all primary schools using a systematic sampling method. The schools were more or less randomly selected, but an attempt was made to include most of the local population from different areas in the cities. After providing the necessary permission from the Education Departments an informative write-up on thalassemia and anemia was sent to the parents and obtaining their written informed consent, blood collection was organized during school hours. They were screened for beta-thalassemia, iron deficiency and G6PD using complete blood count (CBC) and hemoglobin electrophoresis with EDTA anti-coagulated whole blood samples and serum ferritin levels, serum iron was determined. Two ml of blood in EDTA tubes for hematological parameters and 4 ml in non-anti-coagulated tubes for iron deficiency parameters. Serum iron (SI), and total iron binding capacity (TIBC) were determined by a calorimetric method and serum ferritin (SF) was estimated by radio immunoassay method within 24 hours from sampling. Transferrin saturation for all samples was determined (7). Routine hematological tests such as red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin and Concentration (MCHC) were measured immediately after blood sampling by automated cell counter (Sysmex KX21; Sysmex, Kobe, Japan). Analysis of hemoglobin variants was carried out (8). HbA_2 was estimated in participants with MCV < 80 fl. Hemoglobin electrophoresis was determined on cellulose acetate gel in Tris-Borate-EDTA buffer, pH 8.6. In alkaline buffer in electrophoresis hemoglobins were separated. HbA_2 was estimated following elution after electrophoresis. Hemoglobin F was estimated by Singer's alkali denaturation procedure (8).The diagnosis of BTM was defined by MCH ≤27 pg, MCV ≤ 80 fl, HbA2 ≥ 3.5%, normal serum ferritin and transferrin saturation and hypochrom microcytic anemia (9, 10). Samples which including borderline HbA2 content (3.2-3.5%),

the results were rechecked by exchange cation chromatography technique (Helena kit, France). Individuals without anemia and ferritin level < 12 μg/L or transferring saturation < 15% and HbA2 < 3.5% were considered to have ID.Testing for iron deficiency must analyze the patient's red cell status and iron status. Samples with HbA2 ≥ 3.5% coincident with ID considered having ID plus β-thalassemia minor.Individuals without disease and have normal hemoglobin electrophoresis, CBC, ferritin, iron, TIBC concentration was regarded as normal group (11, 12). For screening of G6PD activity, a rapid fluorescent spot test detects the generation of NADPH from NADP was applied (8). Inadequate G6PD activity fails to fluoresce under ultraviolet light.

Statistical Analysis

All data were express as means ± standard deviation (n=3). SPSS ver. 21 was used for calculation of descriptive parameters.

Results

The study demonstrated that Out of 280 school children screened; 120 (42.8%) were of the age group age 9-10 years, 140(50%) 10-11 years and 20(7.2%) 11-12years. MCV≥80fl in 80% and MCV below 80fl in 20 % of all participants was reported .MCH ≤25 pg, and RBC count ≥ 6× 10^6/μL were found in 10% and 6 % of participants respectively. Out of all participant 38 (13%) was reported with MCV ≤ 80 fl. Hemoglobin A2 more than 3.5 percent was reported in 17 (6%) and hemoglobin A2 less than 3.5 percent was 21 (7%) of participants. The mean and SD of hemoglobin in all participants 14.08 ± 1gr / dl, Hct = 43.57 ± 2.98, MCV = 82.6 ± 7 Fl, MCH = 26.4 ± 2.5 pg and MCHC= 32.3±1 g / dl were reported (Table 1).The mean and SD of hemoglobin = 12. 1 ± 1gr / dl, Hct = 40.4 ± 2.3, MCV = 64.3 ± 5.53 fl, MCH = 19.3 ± 2.55 pg and A2 hemoglobine = 5.6 ±.92 % were reported in thalassemic students (Table 2). From 280 participants 17 students (6%) were diagnosed as beta thalassemia minor. According to recent finding, no thalassemia minor had been reported with MCV more than 80 fl.The mean serum (SI) iron and serum ferritin (SF) were 105±33.2 μg/dL and 19.2± 12.5 ng /ml respectively. From 56 participants with microcytic hypochromic pattern, 39 students (13.92%) had anemia (Table 3).Out of 280 participants, 39 (13.92%) subjects had SF below12 μg/ml and 241(86.08%) subject had SF more than 12 μg/ml (Table 3).The prevalence of G6PD deficiency was reported 12.7%.

Table 1. Hematological parameters (mean ± SD) in boy's primary schools in Yasuj, Iran

	Hct	Hb	RBC	MCH	MCV	MCHC	HbA2	Hb F
	%	g/dL	10^6/μL	pg	fl	%	%	%
Normal	43.57±3	14.08±1	5.26± 0.4	26.4±2.5	82.6 ±7	32.3 ±1	2.2± 0.15	0.4 ±0.04
ID	31±4.2 *	10.1±1.2 *	4.6±0.4	15.1±2.5 *	61.2±6.4*	29.8±	2± 0.2	0.5±0.04
BTM	40.4±2.3	12.1±1	6± 0.6±	19.3±2.55	64.3±5.5*	30.5±	5.6± 0. 92*	1.08±0.06*
ID+BTM	33± 1.4 *	11.1±0.45	5.1±0.38	18.3±2.1	62.3±4.4*	31.4±3.5	4.2±0.21*	0.8 ±0.05*

Hematocrit (Hct), hemoglobin (Hb), Red blood cell (RBC) count, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), Fetal (F), ID=Iron deficiency, BTM, beta thalassemia minor.*significant at P< 0.05 compare to normal

Table 2. Hematological parameters (mean ± SD) in boy's primary schools with beta thalassemia minor in Yasuj

	Platelet*10^3/μl	RDW %	WBC*10^3/μl
Normal	261±25.1	14.1±1.2	6.8±1.1
ID	245± 27.1	19.3±1.7	6.1± 1.2
BTM	253± 19.4	16.1±1.4	6.5± 1.5
ID+BTM	245± 22.1	17.2±1.1	6.3± 1.7

ID=Iron deficiency, BTM, beta thalassemia minor, Red cell distribution width (RDW), white Blood Cell (WBC).

Table 3. Hematological parameters (mean ± SD) in boy's primary schools in Yasuj, Iran

	SI	TIBC	SF
	μg/dL	μg/dL	μg/L
Normal	82 ±13.5	356 ± 25.1	39 ± 11.8
ID	27 ±1.9*	398 ± 23.4	5.9 ± 1.5**
BTM	90 ± 12.7	325 ± 32.5	125 ± 18.4**
ID+BTM	65 ± 14.1	382 ± 25.8	21 ± 4.5*

Serum iron (SI), total iron binding capacity (TIBC), serum ferritin (SF), ID=Iron deficiency, BTM, beta thalassemia minor, .*significant at P< 0.05 compare to normal. .**significant at P< 0.01 compare to normal

Discussion

The incidence of BTM was 6% in present study and the most of participants who had BTM were asymptomatic therefore, screening for detection of BTM in target population is essential program (10). The mean of SF in present work was 19.2 ± 12.5 ug/dl. 39 (13.92%) students had the low SF (SF <12 ug/mL) and a lot of the boys also had the low level of Hb and Hct. The prevalence of iron deficiency in recent study was 13.92% which comparable with study of Karimi with prevalence of 17.7 in Fars Province, southern Iran. The present prevalence is less than other developing countries. This difference is due to low iron content of diets since; iron deficiency is depended to iron level of foods (7). Thalassemia is one of the most common hereditary and global problems in the Mediterranean, Asia, Africa and Middle Eastern countries. According to shown data prevalence of BTM is high in the southern part of Iran. Yasuj is a city located near this region and, therefore, the authors was investigated the prevalence and hematological characteristics of the beta-thalassemia traits in primary school students in Yasuj. For prevention of beta thalassemia major in high-risk population different programs such as screening, genetics consultation and prenatal diagnosis can be useful. Iran with population of 75,000,000 million, surface area of 1,648,000 km^2 and birth rate of 1.6 percent located on thalassemia belt. Therefore, prevention of birth children with major thalassemia is very essential. However, in Iran screening of BTM is compulsory. According to present data carriers number of BTM and major beta thalassemic patients is 3-4 million and 18,000 respectively (2). According to one study in Italian primary school children based analysis of HB A_2 and MCV, BTM prevalence was reported 3.1% which lower than present study with 6 %. In another research in Italy birth of major thalassemia cases from 1 in 250 decreased to 1 in 4000 by used successful programs such as screening, genetics consultation and prenatal diagnosis (2). The prevalence of BTM in Pakistan population was reported 7.96% that higher than the present work. In beta thalassemia minor mostly MCV and MCH were low, Hb A_2 and RBC count are elevated. The normal value of MCV (80-100 fL) reveals a red cell with normal size 6 to 8 μm range. However, the MCH (27 to 31 pg) and MCHC (32% to 36 %) present data about red cell hemoglobinization. Normal MCH reveals that the mean weight of Hb in a given amount of red blood cells is in the correct range. Normal MCHC implies that the quantity of Hb per red blood cell is in the proper concentration (13,14).

The range of HbA$_2$ content in the literature for BTM was 5–10%, however in present study mean value of Hb A$_2$ was reported 5.6 %. Red cell count in BTM mostly is higher than normal due to erythrocytes produced are small and inadequately haemoglobinized. The mean erythrocyte of red blood cells in recent study was 6 ± 0.82 million /μl.

According to in literature, MCV and MCH values are predominantly low in MBT. In present study all BTM cases were low similar result with literature. In one research on β-thalassaemia carriers, the MCV was 61±6.8 fl, and MCH was 20.27±2.4 ρg whereas in present study, these parameters were 64.3±5.5 fl and 19.3±2.55 ρg . According to present study MCV and MCH parameters are sensitive screening tests for BTM (10). BTM prevalence in another study in Fars province, southern Iran near the research place (Yasuj, Iran) was estimated to have about 6.9% which quite comparable with present study(1). For evaluation of iron deficiency some laboratory tests such as serum iron, total iron binding capacity (TIBC) and ferritin are essential. The last one test is most sensitive indicators of iron stores. Serum ferritin is one of the most sensitive indicators of iron stores, with a normal value of 20 to 250 μg/L for men and 10 to 120μg/L for women (13, 14). Prevalence of iron deficiency was reported in India 59.9% and Madrid, Spain 0.94%, with significant difference with present study. In India with high population iron deficiency is one of the most anemia's due to food and nutrient with iron not well available for everybody. G6PD deficiency is a hemolytic anemia with prevalence 12.7 that comparable with northern Iran with prevalence of 10.1%. According to some researches incidence of G6PD deficiency in Mazandaran and Gilan provinces of Iran was reported 8.6% lower than present study. Difference in present finding (12.7) with Mazandaran and Gilan provinces with highest incidence in Iran (8.6) owing to participant which in present study only male but in Mazandaran and Gilan provinces both sexes were registered. Incidence of G6PD deficiency in female is very low (9). In present study, fava as clinical condition usually found in individuals with the G6PD deficient similar to Mediterranean region. Neonatal jaundice with high bilirubin was also most prevalent due to G6PD deficiency within 2 to 3 days after birth (15).

Conclusion

The study showed that out of 280 students screened 39 (13.92%) were found to be anaemic,

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while 17(6%) students were positive for BTM and only 35 (12.5%) students were positive for G6PD deficiency.

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