Prevalence of β-Thalassemia Carriers Among a Cohort of University Students in Hawler Province of Iraqi Kurdistan

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Abstract

A representative sample of a thousand volunteer university students was screened for evidence of thalassemia minor.Complete blood counts using automated blood cell analysers and blood smears were examined. Patients having anemia, abnormal red cell indices or morphological features of thalassemia minor like hypochromia, microcytosis, target cells erythrocytosis and family history of thalassemia were then investigated for determination of HbA2 & HbF levels. Estimation of hemoglobin A_2 was performed by micro-column chromatography while HbF was done using alkali denaturation. Seventy seven out of the thousand samples tested positive for thalassemia minor. They all showed a hemoglobin A_2 of more than 3.6 percent and higher, associated in most of the cases with mild anemia, erythrocytosis and hypochromic microcytic red cells. We reached to the conclusion that the prevalence of thalassemia minor in our community, represented at college students at fertile age, to be 7.7%. We hope that similar figures could be made available in the future for the rest of Kurdistan and the bigger Iraq so that a national figure could be presented to the world literature.

Key word : β-Thalassemia , hemoglobin A_2 , hemoglobin F

الخلاصة

في هذه الدراسه تم اخذ عينه مؤلفه من (١٠٠٠) طالب وطالبه جامعيه في محافظة اربيل وتم اجراء المسح لايجاد نسبة انتشار او تفشي حملة صفة فقر الدم البحري في هذه العينه . لقد تم اجراء فحص التحليل الكامل لمكونات الدم واجراء فحص فلم الدم وقد تم فرز الحالات للطلاب الذين يعانون من فقر الدم كذلك حين يكون حجم الكريات الحمراء او نسبة تشبعها بالخضاب اقل من الحد الطبيعي ، كذلك حين يكون عدد الكريات الحمراء اكثر من الحد الطبيعي , وخصوصاً حين يصاحب ذلك لدى الطالب تاريخ عائلي لفقر الدم ، لكل هذه الحالات اجري فحص تحديد نسبة خضاب الدم نوع (A2) ونوع (F) . لقد تبين ان (٧٧) طالباً من العينه المدروسة يحملون الصفة الوراثيه لفقر الدم البحري وجميعهم اظهروا نتائج خضاب الدم A2 اكثر من (٣.٦ %) يصاحبه في معظم الحالات فقر دم خفيف وازدياد عدد الكريات الحمراء مع قلة حجم الكريات وقلة تشبعها بالخضاب. نستنتج من هذا ان انتشار حملة صفة فقر الدم البحري في مجتمعنا ممثله بطلبة الجامعة حيث عمر الخصوبه هو (٧.٧%) ونأمل ان تتوفر دراسات وارقام مماثله لهذه الدراسه في المستقبل القريب لكافة انحاء كوردستان والعراق ومن ثم الحصول على ارقام وطنية تقدم للبحوث العالميه .

Introduction

The thalassemias are characterised by reduced synthesis of one or more of the globin chains that form the oxygen-carrying hemoglobin molecules found in red blood cells[18]. Hemoglobins are tetrameric molecules, with 2 α-like and 2 β-like globin polypeptide chains, each associated with a heme group[21]. In normal adult, HbA ($\alpha_2\beta_2$) accounts for around 97.5% of the hemoglobin in erythrocytes; there is another component called HbA_2 ($\alpha_2\delta_2$), which normally constitutes <2.5% of total hemoglobin. Fetal hemoglobin or HbF ($\alpha_2\gamma_2$) is the major hemoglobin synthesised before birth, but normal adult have< 1% Hb F [1].

β-thalassemia constitutes one of the most serious health problems worldwide, accounting for a major number of childhood deaths per year primarily in regions of the world endemic for malaria [19]. It is an autosomal recessive disorder characterized by microcytosis and hemolytic anemia. It results from a variety of molecular defects that reduce (β^+-thalassemia) or abolish (β^0-thalassemia) the synthesis of the β-globin chains of hemoglobin[2]. β-thalassemia mutations differ greatly in their phenotypic effects. These could range from the extremely mild mutations, which are both clinically and phenotypically silent[3,20], to those which are rare and produce

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Received : 16 / 12 / 2008
Accepted : 18/ 5 /2009

phenotype of thalassemia intermedia, even in the heterozygous state, due to the inheritance of a single copy of the abnormal gene[4]. Between these two extremes lie the majority of β-thalassemia mutations whose carriers are asymptomatic, whereas homozygotes and compound heterozygotes suffer from a transfusion-dependent anemia [5]. In Iraq, there is a definite need for a carrier screening program. It is really hard to reach a consensus regarding the time of screening due to lack of education and public awareness about the disease. The stigma attached to being a carrier for thalassemia gene usually creates reactions against blood testing for this public health problem. There have been reports for the incidence of thalassemia minor in different provinces and cities of Iraq with varying results, but usually ranging between "3.7% to 6.5%". It was reported as 6.5% in Mosul [6]; 4.6% in Basrah [7]; 4.4% in Baghdad [8] and 4.1% in Sulaimaniyah [9]. Our aim is to find the prevalence in Erbil city to complement data already available. Hopefully, in the future a national survey will utilize athe data available to provide a consensus figure for the prevalence of this extremely important genetic problem.

Subjects and Methods

One thousand university students were randomly involved in this study, including (368) females and (632) males, aged 18-49 years (S.D±5.4). Students were included in this study on a voluntary basis. Participants were requested to give information regarding personal and health data. After having all the requested permissions, sample collection started on December 12th 2007. Samples were collected in the colleges after giving a brief talk to the students regarding the nature of the disease and concentrating on the way to prevent it by population and premarital screening programmes. One thousand university students were screened. Samples collection ended on May 2008. A sample of 2 ml venous blood was obtained by venipuncture from each participant and collected into EDTA tube. A complete blood count (CBC) using electronic blood analyzer that was coulter counter model (ACT diff Beckman with eighteen parameters) including (Hb, WBC count, platelet count,packed cell volume(PCV),mean cell volume(MCV), mean cell hemoglobin(MCH),mean cell hemoglobin concentration(MCHC),red blood cell(RBC) count. Those with low red cell indices (MCV, MCH and MCHC) and high RBC count were further investigated for determination of hemoglobin A2(HbA_2) and hemoglobin F (HbF) levels. CBC was done within one hour of sample collection. For red cell morphology; freshly prepared, Leishmann stained blood films were used. any individual is considered as a carrier when in addition of having low red cell indices has an elevated HbA_2 (To more than 3.5%) with or without elevation of HbF. HbA_2 estimation was done chromatographically using commercial kit (Beta-Thal HbA_2 Quik Column) from (Helena Laboratories). Hemoglobin F estimation was done by alkali denaturation method[10].

Results

A total of one thousand students were studied, they included 632 males (63.2%) and 368 females (36.8%) correspond to a male: female ratio of 1.7:1. Ages of screened students ranged between 18-49 years with a mean age of 22.7 years (S.D±5.4). Seventy five percent of them were below 24 years. Figure 3 shows the age and sex distribution of studied students. Six hundred ninety eight students were from Erbil city, the rest were from Koya, Soran, Khabat, Dashti Hawler and Shaqlawa Fig 2 (All these towns related to Erbil) .

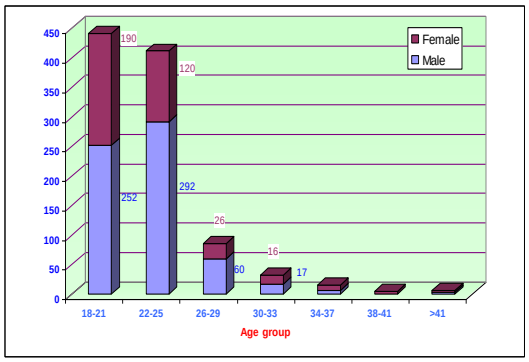

Figure 1: Age and gender distribution of studied students.

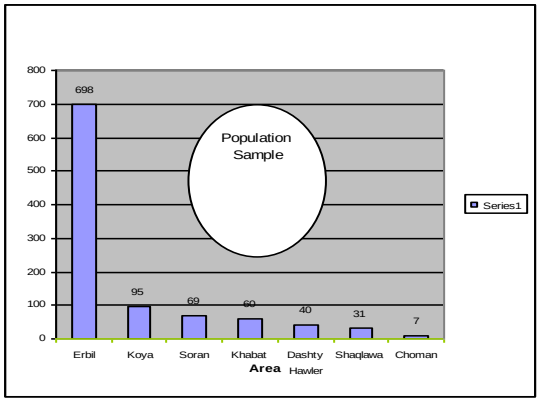

Figure 2: Residence of screened students.

ccording to the results of the CBC and hemoglobin fractions pattern; subjects were divided into three groups:

Group I: Individuals with normal CBC. This group included 857 students.

Group II: Individuals with low red cell indices and high HbA_2 levels with or with out elevation of HbF levels, these individuals were considered to be β-thalassemia carriers. This group included 77 students; this figure makes the frequency of β-thalassemia trait in this sample (7.7%).

Group ш: Individuals with low red cell indices but normal hemoglobin fractions, these were labeled as having anemia with low indices This group included 66 students. Figure 3 shows prevalence of β-thalassemia minor among the studied sample. The various haematological parameters of studied groups are summarized in table (1)

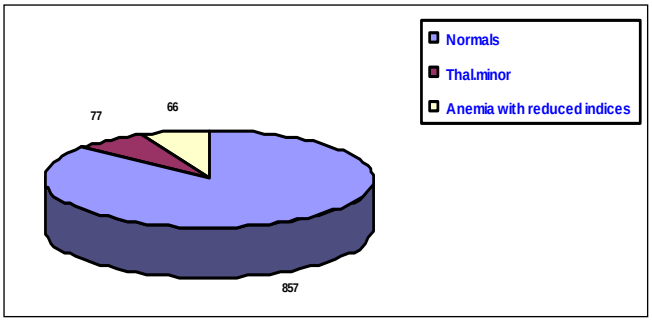

Figure 3 :Distribution of students in different groups

.**Table1: Statistical summary of hematological and clinical parameter of studied subjects**.

Variable	Group	Number	Mean	Standard Deviation	P Value
Age	Normal	857	22.7	3.7	0.615
	Tha. minor	77	23.1	3.5	
	H.ch.Anemia	66	22.9	4.2	
	Total	1000	22.7	3.8	
*Hb gm/dl	Normal	857	14.4	1.6	0.000
	Tha. minor	77	12.7	1.8	
	H.ch.Anemia	66	12.5	2.4	
	Total	1000	14.1	1.8	
Pcv%	Normal	857	42.7	5.6	0.000
	Tha. minor	77	39.9	5.2	
	H.ch.Anemia	66	38.7	6.7	
	Total	1000	42.2	5.8	
MCV fl	Normal	857	87.9	3.8	0.000
	Tha. minor	77	69.1	8.3	
	H.ch.Anemia	66	75.3	6.3	
	Total	1000	85.6	7.3	
MCH pcg	Normal	857	29.7	1.7	0.000
	Tha. minor	77	22.1	3.2	
	H.ch.Anemia	66	24.4	2.9	
	Total	1000	28.8	3.1	
MCHC g/dl	Normal	857	34.1	0.9	0.000
	Tha. minor	77	31.5	1.5	
	H.ch.Anemia	66	31.9	1.7	
	Total	1000	33.8	1.3	
RBC×10^6/ul	Normal	857	4.9	0.5	0.000
	Tha. minor	77	5.8	0.8	
	H.ch.Anemia	66	5.1	0.8	
	Total	**1000**	**5.0**	**0.6**	

*Hemoglobin F was elevated to more than 1% in 33% of carriers. No significant differences in total Hb, HbA_2 level and other red cell indices were noted between carriers with elevated HbF and those with normal HbF level.

Discussion

Among our studied subjects male predominated. Out of a thousand people we studied, 632 were male and 368 were female, with a male to female ratio of 1.7 to 1. 77 subjects were β-thalassemia carriers, giving it a prevalence arte of 7.7%. Of the carriers, 52 (8.2%) were male and 25 (6.8%) were females. The mean age of the normal subjects was 22.7 years, while mean age of the thalassemia carriers was 23.1 years, this result is not significant. The residence distribution of the studied students showed that 698 were from Erbil center, 95 subjects from Koya, 69 subjects from Soran, 60 from Khabat and the rest were from the other provinces of Hawler shown in figure 1. The bulk of carriers were from Erbil Center (56) which means that 72.7% of carriers were from Erbil center. The second populous city studied was Koya; there were 95 subjects tested and seven of them were carrier, that is (9%) of carriers are from this town. According to the number of subjects tested, the third town was Soran, with sixty nine person tested and one carrier = (1.29%) of all carriers. Then Khabat from which we have 60 subjects, eight of them were carriers (10.3%) of all the carriers. Then Shaqlawa from which we have 31 student and four of them (5.2%) were carriers. In Iraq, few studies has been done on this topic, in Baghdad a study have been done [8], on 500 pregnant ladies and it revealed a percentage of 4.4% β-thalassemia carriers. Another study was done in Basra and the prevalence was 4.6% [7], In Duhok it was 3.7% [6], Sulaimani was 4.14% [9], this study was done on couples as premarital test and in Mosul it was 6.5% [6], and this result is comparable to the relatively high prevalence of β-thalassemia minor as documented in this study from Erbil. Erbil region is a region located in the North of Iraq on (14.428) square kilometer with an estimated population of 1,392,093 (MoPDC/UNDP, 2005) mainly Kurdish muslims, this region includes seven main towns, these in turn includes hundreds of small towns and villages, Hawler province is about 400 meter above the sea level. Malaria was endemic throughout Iraq including Erbil. It would be expected to find thalassemia genes prevalent in Erbil and we are now in the control phase of eradication of malaria in Hawler. Regarding the countries near or neighbouring Iraq, the estimated prevalence of β-thalassemia minor was, in Qatar 28% [11], Saudia Arabia 3% [12], Lebanon 2 to 3% [13,14], Jordan 3-3.5% [15], prevalence in Turkey is ranging between 3.4 in East Anatolia to 11% in Western Thrace and Antalya [16,17], these results are comparable to our results. The mean difference between the means of MCV for the three groups were 88, 69 and 75 fl respectively; here there is an obvious difference of 19 fl between the MCV of the normal students in comparison with the carriers. The MCH values were 29.7, 22.1, 24.4 pcg respectively with a p value of less than 0.001which is highly significant difference. 7.6 pcg was the difference between the MCH values of the carriers and the normal students(Table 1). Mean cell hemoglobin concentration (MCHC) values were 34.1, 31.5 and 31.9 g/dl respectively; there is a significant difference of 2.6 g/dl between the normal subjects and carriers with a p value of < 0.001. Finally RBC counts were notably elevated among β-thalassemia carriers as compared to the normal. RBC counts were 4.9, 5.8, 5.0 $\times 10^{\wedge 6}$ /ul with 0.9 $\times 10^{\wedge 6}$/ul difference between carriers and normal subjects and p value was < 0.001(Tabe 1). During this study Complete Blood Count (CBC) was performed on a total of one thousand university students. It was the cornerstone to determine the subjects on whom HbA_2 had to be estimated. Reduced MCV or MCH values in the majority of heterozygous β-thalassemia has been used as a basis for population screening for these disorders [18,,22], and although cut-off values for the MCV and MCH of 80 fl and 27 pcg respectively may involve a relatively large number of confirmatory HbA_2 estimation it would detect virtually all affected cases. Hemoglobin A_2 estimation was done for 143 students with hypochromic microcytic parameters. Seventy seven of these were β-thalassemia carriers based on elevated HbA_2 levels. No association could be noticed between the severity of the anemia and the HbA_2 level. It was noted that increasing level of HbA_2 above 6% was negatively associated with MCV. , when MCV values were less than 72 fl, among 11 carriers with HbA_2 more than 6% we have 9 individual (81.8%) whom MCV values were less than 72 fl. Same thing when applied to MCH we have 81.8% of carriers having MCH values of (22 pcg) and less, no such relation could be found between HbA_2 level and RBC count.

Conclusions

1. This study revealed that the prevalence of thalassemia minor or thalassemia carrier state in our community is 7.7%.
2. Thalassemia carriers can be detected through clinical examination and complete blood count. The cardinal feature of thalassemia carrier state is elevated HbA_2 level .

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