Journal of Bioscience and Applied Research

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Hemoglobin A2 Cut off Values in Egyptian Cohort as a marker of β -Thalassemia carriers

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Abstract

Beta thalassemias (β-thalassemias) are a group of inherited blood disorders caused by reduced or absent synthesis of beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. The total annual incidence of symptomatic individuals is estimated 1 in 100.000 throughout the world. HbA2 determination plays a key role in screening programs for β-thalassemia since a small increase in this fraction is the most important marker of β-thalassemia heterozygous carriers. This study aims to detect the cut off value of Hb A2 in Egyptian individuals with β-thalassemia and microcytic hypochromic anemia, using automated HPLC.

The study was carried out on 112 male and female cases. Their ages ranged from 2 years to 35 years. They were 28 normal cases, 20 cases diagnosed as microcytic hypochromic anemia, 52 cases as β-thalassemia trait and 12 cases as β-thalassemia intermedia. Informed consents were obtained from their care givers. CBC was done to all groups and HbA2 levels were measured using HPLC Bio-Rad D-10 dual program (β-thal short program) for identification of types of Hb and determining the cut off value of Hb A2**.** The Hb A2 cut off value in microcytic hypochromic anemia cases was >2.2%, in β-thalassemia intermedia cases was >3.2% and in β-thalassemia trait cases was >3.6% which showed the best cut off value, and hence the cut off value of Hb A2 could be used as a factor that plays a key role in screening programs for β-thalassemia carriers.

Keywords: β-thalassmia carriers, HbA2 cut off.

1 Introduction

The thalassaemia syndromes are a heterogeneous group of genetic disorders characterized by a reduced rate of production of one or more of the globin chains of haemoglobin. They are usually caused by point mutations or deletions in, or close to, these globin genes which reduce or abolish expression of the affected gene (Bowden,2001).

Hemoglobin is the iron-containing protein in red blood cells that carries oxygen to cells throughout the body. Hemoglobin consists of four protein subunits, two subunits of beta-globin protein and two subunits of alpha-globin protein (Weatherall and Clegg, 1981). HbA2, composing of two α chains and two δ chains, is a minor component of the hemoglobin present in normal adult red blood cells, accounting for about 2.5% of the total hemoglobin in healthy individuals (Wethers et al.,1989). Three main forms of β-thalassemia have been described: thalassemia major, thalassemia intermedia and thalassemia minor or trait. Individuals with thalassemia major usually present within the first two years of life with severe anemia, requiring regular red blood cell (RBC) transfusions. Patients with thalassemia intermedia present later in life with moderate anemia and do not require regular transfusions. Thalassemia minor is clinically asymptomatic but some subjects may have moderate

anemia (Galanello, and Origa, 2010). Parents who carry β-thalassemia trait can have a child with B-thalassemia disease or Sickle cell disease ($S\beta^+$ Thalassemia or $S\beta^+$ Thalassemia disease). So, it is important to understand how beta thalassemia trait passed down, and how it could affect the health of children and grandchildren (St. Jude Children's Research Hospital, 2015). Beta thalassemia trait appears if there is one working copy of the beta globin gene and one non-working copy. The body produces less of the beta chains, but is still able to make plenty of hemoglobin A. β-thalassemia trait is not considered a disease which affects the health but causes the red blood cells to be smaller than usual. Sometimes, this is mistaken for iron-deficiency anemia. However, taking an iron supplement does not change the size of the red blood cells. A person with β-thalassemia trait would never develop β-thalassemia disease (Wethers et al., 1989). β- thalassemia is encountered in polymorphic frequencies in almost all Arab countries with carrier rates of 1– 11 % and a varying number of mutations. Incidence and prevalence rates of recessive genetic diseases, such as haemoglobinopathies could be influenced by the demographic and cultural characteristics of the population studied. The populations of many Arab countries are characterized by marriage at a young age, large family sizes and advanced maternal and paternal ages. Consanguineous marriage is customary in most- if not all- Arab communities and intra-familial unions currently account for 20–50 % of all marriages. First cousin unions are especially popular and constitute almost one quarter of all marriages in many Arab countries, particularly the paternal parallel subtype (Bittles, 2012; St. Jude Children's Research Hospital, 2015). The absence (βo) or reduction of (β+) beta chain production is the main cause of basic molecular defect in β-thalassemia, however, α chain synthesis proceeds at a normal rate. The first result of reduced β-chain production causes decreased production of the adult hemoglobin (HbA: α2 β2). A second consequence is imbalanced globin chain synthesis, in which α chain synthesis proceeds at a normal rate and hence there is an excess of α chain in the erythrocytes. The excess α chains are unstable and precipitate in the bone marrow red cell precursors, giving rise to a large intracellular inclusions that interfere with the red cell maturation, function and survival (Cao et al., 1998; Hamamy and Bittles 2008). Several procedures have been proposed for β-thalassemia carrier screening. The cheapest and simplest way to detect β- thalassemia could be based on MCV and MCH determination, followed by HbA2 quantitation for subjects showing microcytosis (low MCV) and reduced Hb content per red blood cell (low MCH). However, because with this procedure a considerable proportion of double heterozygotes for beta- and alpha-thalassemia might be missed (these are found in many populations, such as Sardinians, where both disorders are common), it could only be used in populations with a low frequency of α-thalassemia (Vrettou et al., 2003). The definition of the type of thalassemias in these carriers is solely recommended when they mate with a carrier of a typically high HbA2 β-thalassemia or an undetermined type of thalassemia. In those rare cases showing normal or low MCV-MCH, normal or reduced HbA2 levels, and high HbF, we suspect the presence of delta β-thalassemia, which should be differentiated from HPFH. This distinction is performed by globin chain synthesis analysis (normal in HPFH and unbalanced in delta beta-thalassemia) or beta-cluster gene analysis or both (Cao et al., 1998). The suspicion of interacting delta-thalassemia might arise when borderline HbA2 levels are found or when family studies show segregating delta-thalassemia (characterized by normal MCV-MCH and low HbA2) and Co inheritance of heterozygous beta-thalassemia and alpha-thalassemia might raise the MCV and the MCH, high enough to determine normal values at least in some of these double heterozygotes. This might occur as a result of either a deletion of two alpha globin structural genes or as a non deletion lesion affecting the major alpha globin gene (the two functional alpha genes, denominated as alpha l and alpha 2, have a relative expression of 1:3). Fortunately, these carriers might be easily identified for their high HbA2 levels (Rosatelli et al., 1984). Elevation of HbA2 is the most important feature in the detection of heterozygous β-thalassemia, but a substantial group of β-thalassemia heterozygotes might have normal HbA2. The first mechanism to account for the abnormally low HbA2 levels in a β-thalassemia carrier is the presence of a specific mild β-thalassemia mutation, such as the beta IVS I.6 mutation (Tamagnini et al., 1983).

This study aims to detect the cut off value of Hb A2 in Egyptian individuals with β-thalassemia and microcytic hypochromic anemia, using automated HPLC.

2 Materials and Methods

Patients and methods:

The study included 112 Egyptians, 50 males and 65 females. Their ages ranged from 2 to 55 years. They were 28 normal cases, 20 cases diagnosed as microcytic hypochromic anemia, 52 cases as β-thalassemia trait and 12 cases as β-thalassemia intermedia. Informed consents were obtained from their care givers. All microcytic hypochromic anemia cases, normal cases, β-thalassemia trait and β-thalassemia intermedia cases were subjected to the following:

(1) Complete history taking and a thorough clinical examination with a focus on the degree of consanguinity.

(2) Blood samples were collected on EDTA from each individual, for CBC analysis (Coulter Gen T-890 analyzer Beckman USA with manual reticulocyte count (brilliant cresyl blue stain), and for measurement of HbA2 levels using HPLC (BioRad D10;BioRad, France) with Bio-Rad D-10 dual program (β-thalassemia short program) for identification of types of Hb and determining the cut off value of Hb A2 (Jiffri et al., 2010).

Statistical methods:

Statistical Package for Social Sciences (SPSS) computer program (version 19 windows) was used for data analysis.

3 Results

This study was carried out on 112 Egyptian individuals (50) males and (65) females. Age ranged between 2 to 35 years. They were 28 normal cases, 20 cases diagnosed as microcytic hypochromic anemia, 52 cases as β-thalassemia trait and 12 cases as β-thalassemia intermedia. The 112 individuals were subjected to complete blood picture and HPLC for hemoglobin types identification (Hb: A, A2, F). HPLC analysis of Hb A2 levels showed the following: The mean value for the normal group (n= 28) was 2.65 ± 0.44, for the Microcytic hypochromic anemia group (n= 20) was 2.86 ± 0.46, for the Beta thalassemia Intermedia (n= 12) was 3.18 ± 1.20 and for the Beta thalassemia Trait (n= 52) was 4.93 ± 1.15. Comparing the different means of Hb A2 levels in the different groups showed highly statistical significant difference between the Beta thalassemia trait group and the other three groups with (p-value= 0.001) where the Hb A2 value was the highest among all groups (table 1, figure 1).

Table (1): Comparison between mean values of HbA2 in the four studied groups.

Variables	Normal (n= 28)	Microcytic hypochromic anemia (n= 20)	Beta thalassemia Intermedia (n= 12)	Beta thalassemia Trait (n= 52)	P value
Mean ± SD	2.65 ± 0.44	2.86 ± 0.46	3.18 ± 1.20	4.93 ± 1.15	0.001**
P value vs normal	---	0.169	0.299	0.001**	
P value vs microcytic	---	---	0.893	0.001**	
P value vs intermedia	---	---	---	0.001**	

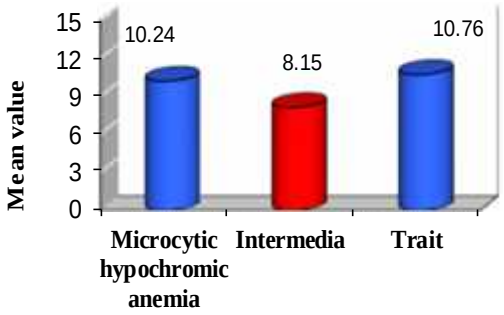

Figure (1). : Comparison between mean values of HbA2 in the four studied groups.

ROC curve analysis was done to identify HbA2 cut off values in the different groups stated above:
Comparing the 28 normal cases versus the 20 microcytic hypochromic anemia cases, the area under the ROC curve was 0.617. The cut off value was >2.2% providing 100% sensitivity, 21.43% specificity, 47.6% PPV and 100%NPV
Comparing the 28 normal cases versus the 12 β-thalassemia intermedia cases, the area under the ROC was 0.607. The Cut off value was >3.2 % providing 33.33% sensitivity, 92.86% specificity, 66.7% PPV and 76.5% NPV.
Comparing the 28 normal cases versus 52 β-thalassemia trait cases, the area under the ROC was 0.952 .The cut off was >3.6% providing 84.62% sensitivity, 100% specificity, 100% PPV of , 77.8% NPV (table 2, figures 2-4).

Table (2): Cut off value of HbA2 in the three studied groups using ROC curve.

Variables	Microcytic hypochromic anemia	Beta thalassemia Intermedia	Beta Thalassemia Triat
Area under the roc	0.617	0.607	0.952
Cut off	> 2.2	>3.2	> 3.6
Sensitivity	100	33.33	84.62
Specificity	21.43	92.86	100
Positive predictive value	47.6	66.7	100
Negative predictive value	100	76.5	77.8

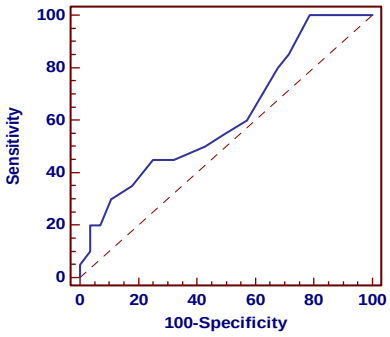

Figure (2). : ROC curve analysis of microcytic hypochromic anemia cases.

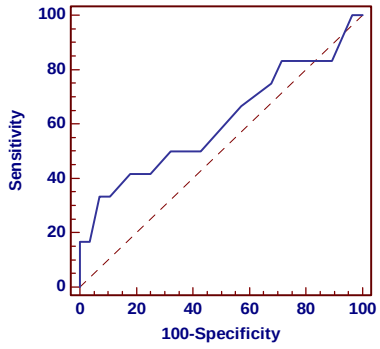

Figure (3). : ROC curve analysis of β-thalassemia intermedia cases.

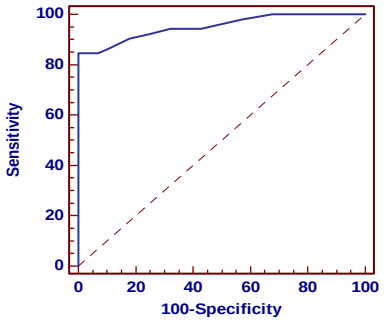

Figure (4). : ROC curve analysis of β-thalassemia trait cases.

4 Discussion

Hemoglobinopathies are considered as a major health problem in many areas worldwide (Weatherall and Clegg 1981). Thalassemias are heterogeneous group of genetic abnormalities with decreased or absent production of one or more of the globin chains. Beta thalassemia is a group of blood disorders with reduced or absent production of beta globin chains of hemoglobin (Wethers ,1989) Heterozygous states for β -thalassemia genes, known as β -thalassemia minor, are usually not associated with clinically significant disease. β –thalassemias are highly prevalent in the Mediterranean area, and in the Middle and Far East (Weatherall and Clegg 1981). Difficulties in screening for the thalassemia trait are related to the heterogeneity of β-thalassemia and the absence of a single abnormal test to cover all β-thalassemia variants. The increase in hemoglobin A2 (HbA2) level is the most significant parameter in the identification of β-thalassemia carriers (Serjeant et al,. 1975, Pembrey et al,. 1978, Pearson et al,. 1979). HbA2, composing of two α chains and two δ chains, is a minor component of the hemoglobin present in normal adult red blood cells, accounting for about 2.5% of the total hemoglobin in healthy individuals (Ou et al ., 2011). It is estimated that 1.5% of the world's population are β thalassemia carriers (Niazi et al, 2010). Egypt is located in an area where β -thalassemia is prevalent. It has been estimated that 1000 children out of the 1.5 million live births are born annually with β-thalassemia major (Hussein et al., 2007). But still no definite screening program has yet been developed. Since screening will continue to be the cornerstone of the strategies aimed at β-thalassemia control, attempts to develop effective and economic techniques for thalassemia screening have become very important, especially in the countries that have populations with high percentage of such diseases (Sachdev et al., 2010). The aim of screening is to identify couples at risk of producing a fetus with severe thalassemia (Mamtani et al., 2006). The aim of this study is to determine the cutoff points of HbA2 as a mean of screening of β-thalassemia carriers among a group of Egyptians presented with microcytic hypochromic anemia.The study was carried out on 112 male and female cases. Their ages ranged from 2 years to 35 years. They were 28 normal cases, 20 cases diagnosed as microcytic hypochromic anemia, 52 cases as β-thalassemia trait and 12 cases as β-thalassemia intermedia. CBC was done to all groups and HbA2 levels were measured using HPLC Bio-Rad D-10 dual program (β-thal short program) for identification of types of Hb and determining the cut off value of Hb A2 using ROC curve analysis. Comparing the different means of Hb A2 levels in the different groups showed highly statistical significant difference between the Beta thalassemia trait group and the other three groups with (p-value= 0.001) where the Hb A2 value was the highest among all groups. The cut off value for microcytic hypochromic anemia cases was >2.2% providing 100% sensitivity, 21.43% specificity, 47.6% PPV and 100% NPV. The cut off value for β-thalassemia intermedia cases was >3.2 % providing 33.33% sensitivity, 92.86% specificity, 66.7% PPV and 76.5% NPV. The cut off value for β-thalassemia trait cases was >3.6% providing 84.62% sensitivity, 100% specificity, 100% PPV and 77.8% NPV. A cut off of >4% HbA_2 was recommended for diagnosis of classical βTT by automated HPLC according to (George et al., 2001, Colah et al., 2007). However, the best cut off of HbA_2 according to our study was established at >3.6% for the beta thalassemia trait group. In a recent Egyptian study 2015, using HPLC, the Hb A_2 cut off value of >3.5% provided 100.0% sensitivity, 70.0% specificity, 75.0% positive predictive value (PPV), 100.0% negative predictive value (NPV) and accuracy of 70.0% to identify β-thal trait and at a cut-off of 4.0%, it provided 97.4% sensitivity, 72.7% specificity, 92.6% PPV, 88.8% NPV and a diagnostic accuracy of 92% (Abdel-Messih et al .,2015). Hb electrophoresis which measures various Hb bands is a well known established method of screening thalassaemia carriers. If Hb A2 is in between 3.5% and 7%, this is suggestive of thalassaemia carriers (Ahmed et al.,1996; Shamsi ,2004). A previous study in Jordan showed that the cut off value of Hb A2 was >3.3% in 52 thalassemia carriers. This study showed that the thalassemia trait is more prevalent in Jordan than previously reported, (Bashir et al, 1992; Sunna et al 1996; Mamtani et al, 2006), a finding which may be explained by the lower HbA 2 cutoff point used. The prevalence of thalassemia trait observed in this study is also higher than that reported from neighboring Saudi Arabia (Weatherall and Clegg, 1981, Nita et al 1989, Gharaibeh et al., 1998). Another recent study in Iran showed that that the cut off value of Hb A2 was ≥ 3.5% in thalassemia carriers as a standard diagnostic marker for b-thalassemia. Iran, Khuzestan in particular, being on the thalassemia belt, is a thalassemia hot zone (Akhavan et al., 2011; Alizadeh et al., 2013). In united arab emirates, a study showed that the mean HbA2 was 5.2 0.5%, with values ranging from 3.9% to 6.2% in 29 subjects with BTT (Denic et al .,2013). Another recent study in Malaysia showed that in classical β-thalassaemia trait, the cut off value of HbA2 was 4% and above. However, milder mutations can result in HbA2 values between 3.2 to 3.9% (Elizabeth, 2014). In a previous study in Italy among 410 subjects with borderline HbA2 values in β-thalassemia cariers, the HbA2 cut off value ranged from 3.1 to 3.9%. At the 3.5% HbA2 cut-off value, sensitivity and specificity were 77.81% and 67.90% respectively. They concluded that, borderline HbA2 levels are not a rare event in a population with a high prevalence of β-thalassemia Carriers, with the most severe genotypes associated with microcytosis. These data support the necessity to investigate these cases at a molecular level, particularly if the partner is a carrier of β-thalassemia (Giambona et al., 2008). Another study in Egypt showed that the thalassaemia carrier group had high levels of HbA2 (> 3.6%), the indeterminate group had borderline levels of HbA2 (range 3.3%–3.5%) (El-Beshlawy et al., 2007).

In conclusion, the Hb A2 cut off value in β-thalassemia trait cases was >3.6% which showed the best cut off value,

and hence the cut off value of Hb A2 could be used as a factor that plays a key role in screening programs for β-thalassemia carriers. HPLC could be a reliable and affordable primary screening tool for β-thalassemia trait at a Hb A2 level of ≥3.6%, while a larger number of samples together with further confirmatory molecular testing is needed.

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