Tikrit Journal of Pure Science 21 (6) 2016 *ISSN: 1813 – 1662 (Print)*
E-ISSN: 2415 – 1726 (On Line)

Study the Hemoglobin Electrophoresis Patterns in two Populations from Salah - addin Province - Iraq

Wajdi Sabeeh Sadeq
Dep. Of Biology , College of Science , Tikrit University , Tikrit , Iraq

Abstract
The early identification of some clinically significant hemoglobinopathies and precise differentiation of hemoglobin variants are important to provide early comprehensive medical care to prevent some serious complications, assess prognosis and offer genetic counseling.
This study examined the prevalence of α 2 - gene deletion in neonates in Samarra and Tikrit, Iraq. Screening study was carried out by examining fresh blood samples obtained from excised umbilical cord of 42 neonates born in neonates clinics in Samarra hospital and Tikrit hospital from October 2012 to March 2013. Hemoglobin electrophoresis were performed using Hellabio hemoglobin electrophoresis kit (He 10) and Hellabioscan system for detection of Hb bands and value % of fractions.
The results showed 40 cases out of 42 (95.24%) have normal hemoglobin components. 2 cases out of 42 (4.76%) there was no visible band of HbA2 in hemoglobin electrophoresis pattern. Considering the missing of HbA2 band the two cases may have the deletion of δ gene so each one of them may has the genotype δ^0/δ^0.
Key words: Thalassemia, HbA2, δ^0/δ^0 genotype, Helabio hemoglobin electrophoresis kit, Fetal hemoglobin variants.

Introduction
"Electrophoresis, a test based on the migration of electrically charged molecules under an applied electric field, occupies one of the most important places in the history of abnormal Hb detection" [1].
"Hemoglobin electrophoresis is a well established technique routinely used in clinical laboratories for screening samples for hemoglobin abnormalities" [1,4, 12].
The inherited disorders of hemoglobin are the commonest single-gene disorders in man. They fall into three overlapping groups : structural variants; thalassemias characterized by reduced rate of synthesis of one or more globin chains; and conditions in which fetal hemoglobin synthesis persists beyond the neonatal period, collectively known as hereditary persistence of fetal hemoglobin. Hemoglobin disorders are responsible for an extremely complex series of clinical phenotypes" [2].
"The thalassemias are among the most common genetic disorders worldwide, occurring more frequently in Mediterranean region, the Indian subcontinent, Southern Asia, and West Africa. "In developing countries, in which there is high mortality from infections and malnutrition in the first year of life, many of the hemoglobinopathies are unrecognized"[3].
The World Health Organization (WHO) has suggested that about 5% of the world population are carriers for different inherited disorders of hemoglobin [4]. WHO reports also state that about 370,000 severely affected homozygotes or compound heterozygotes of thalassemia are born every year.
"The thalassemias are heterogeneous group of disorders of hemoglobin synthesis resulting from the reduced rate of synthesis of one or more globin chains of hemoglobin. Decreased synthesis of α chain produces α thalassemia, while decreased synthesis of β chains produce β thalassemia" [5].
The key element in the diagnosis of a β-thalassaemia trait is the presence of non-iron deficient microcytic hypochromia anaemia. "The screening of thalassaemia trait in the areas with limited laboratory facilities is often done by NESTROFT test"[6]. "Despite its sensitivity and rapidity, in around one out of four cases of iron deficiency anemia, this test leads to a false positive result" [7].
The best approach to screen the thalassaemia traits is, therefore, determining the Hb A2 level. "The large majority of β-thalassaemia carriers present with a high Hb A2 level, and this often accompanied with a moderate increase in Hb F level" [8].
"Haemoglobin A2 (HbA2, α 2 δ 2) is a minor adult haemoglobin, comprising between 2. 0 % and
3. 2% of the total circulating haemoglobin in healthy adults" (2). At the molecular level, a single gene (HBD) encodes d-globin and is found in a cluster on chromosome 11 with the other genes encoding the b–like globins.
The aim of the current study is to shad some light on electrophoretic properties and frequencies of hemoglobin types in cases of live- borne babies of two cities in Salahaddin province-Iraq Samarra city and Tikrit City from October 2 to April 22- 2013.

Materials and Methods
Hellabio Hemoglobin Electrophoresis Kit (He10) and Hellabioscan system were used for identification of different species of hemoglobin molecules. Reagents included in the kit was: Agarose gel, Hemolyzing solution, Electrophoresis buffer, and staining solution. Samples of umbilical blood were collected by multiple visits to neonatal clinic in samarra and Tikrit hospitals from 2 October to 22 April 2013. Methodology was applied as mentioned in the kit.
The Hellabioscan, is constituted by the software system that can easily install in a common PC or laptop. It does not require maintenance and is not limited in the measurement of films with certain

definite dimensions. The possibilities of Hellabioscan are unlimited and it allows the user to work according to his work parameters.

The specimens of cord blood were taken after excising and primary processing were done in hospital neonatal clinic as following:-

1- 400 µl of cord blood were taken in a test tube, enough quantity of normal slain were added and centrifuged for 5 minutes. Then the supernatant discarded and the pellet maintained.

2- To prepare the hemolysates 40 µl of the pellet were transferred to 5ml centrifuge tube and 130 µl of lysing solution were added.

3- Hemolysates were transferred to Dijla Clinical Laboratory to complete samples processing as following:-

1- Discovering the agarose gel and the buffered blocks out of its packaging.

2- Applying 0.35 ml of dest. Water on the electrophoresis area of the system.

3- Placing the suitable sample template on the application zone. Rub the template with forefinger so that it contacts with the gel surface and no air bubbles exist.

4- Using a 5 µl pipette to apply 5 µl of the lysate across each corresponding slit and let them to absorb into the gel for 20 second.

5- Blotting the gel with a gel blotter strip on the sample application zone, gently remove both the sample template and gel blotter strip and discard them.

6- Applying the buffered agarose on the electrod carrier, gently apply the electrode carrier on the electrophoresis area.

7- Putting on the system, press start. Automatically the process of the electrophoresis begins with constant temperature and volts.

8- At the end of electrophoresis, put of the system and remove the electrode carrier and discard the buffered agarose blocks, dry the gel and leave it more than 2 hrs.

9- Apply the dried gel in the gel carrier and put it in the staining/ distaining bath. After drying the stained gel it will be ready to analyze by Hellabio Scan.

10- HellabioScan and software were used.

11- The data analyzed by using SSPS Ver.17.

Results and Discussion

Percentage of consanguineous marriages is 54.7 % and 45.2 % for non consanguineous marriages in the two populations. Table 1 shows gender of neonates and the status of parents in term of consanguinity.

Table (1) Numbers of neonates, gender and consanguinity

Gender	Cons		T
	r	nr	
Male	19	11	30
Female	4	8	12
T	23	19	42
	54.7 %	45.2%	

We found that 29 out of 42 have normal value of HbA2 (1.123 ± 0.157) and 7 of 42 have low value (0.200 ± 0.035) while we found 9 out of 42 have very low value of HbA2 fraction which only reached (0.038 ± 0.016). The second and third groups suggest abnormalities in HbA2 fraction of hemoglobin Table 2 shows values of HbA2 hemoglobin fraction in examined cord blood specimens of screened neonates in the current study.

Table (2) Numbers of neonates, values of HbA2 hemoglobin fraction as means ± standard error means.

group	No.	HbA2 M ± S.E
1	29	1.123±0.157
2	7	0.200±0.025
3	6	0.038± 0.016

Tables 3 and fig. 1, table 4 and fig. 2 shows fractions of hemoglobin types of 2 cases out of 42 screened neonates. We found that each of these two cases has the value 0.00 % of HbA2 and this may suggest missing of α 2 or δ gene.

Table (3) Fractions and values of hemoglobin case no. 20.

Fractions	Values (%)	NV (%)
HbA	0.59	
HbF	99.41	
HbA2	0.00	

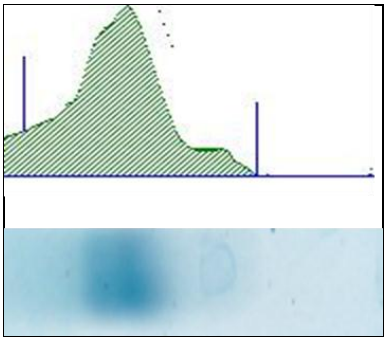

Fig. 1 Electrophoresis pattern of cord blood sample case No. 20 shows no HbA2

Table (4) Fractions and values of hemoglobin case no. 42

Fractions	Values (%)	NV (%)
HbA	2.67	
HbF	97.33	
HbA2	0.00	

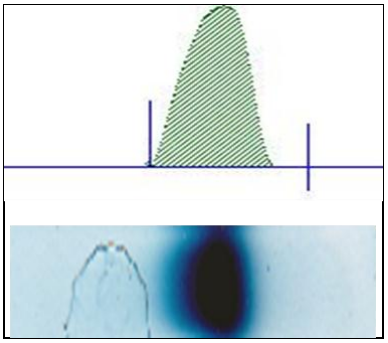

Fig. 2 Electrophoresis pattern of cord blood sample case No. 42 shows no HbA2

The results in table 5 show normal fractions and values of hemoglobin of cord blood sample while figure 3 shows normal electrophoresis pattern for the same sample.

Table (5) Fractions and values of hemoglobin case no. 34

Fractions	Values (%)	NV (%)
HbA	3.36	
HbF	96.64	
HbA2	1.91	

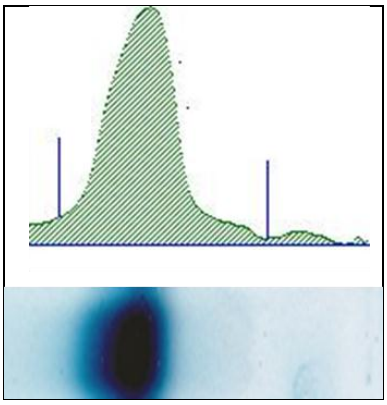

Fig. 3 Electrophoresis pattern of cord blood sample case No. 5 shows normal HbA2

The incidence of δ^0/δ^0 neonates within studied population is = 0.0476.
Gene frequency; q= 0.2182
P= 0.7818
The current study included 43 newborns, 0ne case with no information so it was excluded. 42 (29 male and 14 female) all were normal at birth. Out of 42 cord blood samples examined no Hb Bart's. Electrophoretic pattern of only 2 samples showed absence of HbA2..
The results in table 1 may suggest low level of consanguinity in the studied population.
The results in table 2 shows very low level of HbA2 fraction mean in 6 neonates which reached 0.038 ± 0.016 and low level in 7 neonates which reached 0.200 ± 0.157 and "this may suggest types of α + thal/ α + thal of various types and combinations"[9] .
Diagnosis of an α thalassaemia will be suspected by haematological indices showing some microcytosis and hypochromia, with a degree depending upon the type of thalassaemia. "The Hb A2 percentage is theoretically decreased in proportion with the number of defective α genes"[1].
"Alpha thalassemia (alpha-thal) is one of the most common hemoglobin (Hb) disorders in the world. Alpha-globin genes are located on chromosome 16. The majority of alpha-thal mutations are deletions, but point mutations are found as well" [10].
Fig.1 and Fig. 2 the electrophoresis pattern of the cord blood sample for the case number 1 and case number 34 which shows the missing of HbA_2 band, table. 1 and table 2 shows values % of HbA2 fractions. The value % of HbA_2 in each of the two cases were 0.00, this mean that each of neonates may has the genotype δ^0/δ^0 .
Fig 3 shows the normal electrophoresis pattern of cord blood sample for case No 34. The Hb A2 level may be modified by many factors. "The most frequent problem is the co-existence of an iron deficiency which may even normalize the Hb A2 level requiring a novel Hb assay after iron deficiency treatment" [1].
In β-thalassaemia carriers presenting with a normal Hb A2 level, the most frequent cause is a co-inherited δ-globin abnormality. "The thalassaemic δ alleles are not exceptional affecting at least more than one per cent of general population" [11]. "The most frequent δ thalassaemic variant is Hb Yialoussa [δ27 Ala>Ser] which is quite frequent in Mediterranean region"[12]. Other δ- and α-globin chain abnormalities lead to different type of Hb A2. In these cases one should add the two Hb A2 fractions to obtain the total Hb A2 level. "In some cases the abnormal Hb A2 fraction may only be visualized by using another analytical test. Conversely, falsely increased levels of Hb A2 may result from the co-existence of a variant with electrophoretic or chromatographic properties close to that of Hb A2" [13].
"HbA2 has no known physiological function, but measurement of HbA2 values is essential in screening programmes for thalassaemia" [2].
"Elevated HbA2 levels in the presence of hypochromic microcytic red blood cells is diagnostic of heterozygous b-thalassaemia, and results in part from increased transcriptional activity from both cis and trans HBD genes" [14].
As a rule, this situation has to be verified when a level of Hb A_2 higher than 8 per cent is observed. In the current study we noticed that the most Hb band occupied by the HbF as expected with our specimens. HbA was in normal value % for the all cases while HbA2 value % was zero for 2 cases .
"The HbA2 percentage is theoretically decreased in proportion with the number of defective
α genes" [15] .
"It is presently not known how HbA2 (and d-globin chain) expression changes during adult erythropoiesis, but it has been suggested that d-globin chain synthesis declines as maturation in erythroid progenitors progresses" [16].
Screening should consist of a complete blood count, as well as hemoglobin electrophoresis or hemoglobin high performance liquid chromatography. "This investigation should include quantitation of HbA2 and HbF" [17].
"Structurally, Hemoglobin, the carrier molecule of O2, is made up of heme-iron and four globin chains arranged in a matching pairs. HbF, HbA, and HbA2 are made up of α 2 γ 2, α 2 ß2 and α 2 δ 2 globin chain respectively" [18].
Electrophoresis on cellulose acetate medium at alkaline ph is a useful screening procedure for separating hemoglobin variants that are interacting

with thalassemia and the hemoglobin of the thalassemia syndromes such as HbS. H Barts, Constant Spring and Lepore . "Specimens that give results in the borderline or indeterminate range when quantitated by densitometry will usually give results that are clearly either normal or elevated when studied by chromatography" [19] .

Abnormal reference values can vary by laboratory, but the HbA2 value% appear within these ranges, results are usually associated with the conditions that follow in parentheses, 4-5.8% (thalassemia minor) and under 2% (Hb H disease)

"Hb Bart's in these cases were suggestive of heterozygous α thalassemia with single gene deletion (-α/αα)"[20]. The incidence of α thalassemia in South Western Maharashtra is 0.4% (i.e. 4 in 1000).

The current study suggests that using electrophoresis pattern of hemoglobin from umbilical cord blood may be important for early diagnosis of thalassemia.

References

[1] Henri Wajcman and Kamran Moradkhani (2011) Abnormal haemoglobins: detection and characterization. Indian J Med Res 134; pp 538-546.

[2] Weatherall DJ, Clegg TB, Higgs DR, Wood WG. The hemoglobinopathies. In : Scriver CR, Beaudet AL, Sly WS, Valle D (Eds.), (2001) The Metabolic and Molecular Bases of Inherited Diseases, 8th Edition. New York: McGraw Hill; 4571-4636.

[3] Shivashankara A.R, Jailkhanir, Kini A. (2008) Hemoglobinopathies in Dharwad, North Karnataka: A Hospital- Based Study. *Journal of Clinical and Diagnostic Research ; (2)593-599*

[4] Angastinosis M, Modell B. (1998) Global epidemiology of hemoglobin disorders. Proc Natl Acad Sci USA; 850: 251.

[5] Singh SP, Gupta SC.(2008) Effectiveness of red cell osmotic fragility test with varying degrees of saline concentration in detecting beta-thalassaemia trait. *Singapore Med J* ; *49* : 823-6.

[6] Bobhate SK, Gaikwad ST, Bhaledrao T. (2002) NESTROFF as a 6. screening test for detection of Beta-thalassaemia trait. *Indian J Pathol Microbiol*; *45* : 265-7.

[7] Louahabi A, Philippe M, Lali S, Wallemacq P, Maisin D. (2006) Evaluation of a new Sebia kit for analysis of haemoglobin fractions and variants on the Capillary system. *Clin Chem Lab Med 44* :340-5.

[8] Old JM. (2003) Screening and genetic diagnosis of hemoglobin disorders. *Blood Rev*; *17* : 43-53.

[9] Ida Bianco, Mria Pia Cappabianca, Enrica Foglietta, Mria Lerone, Giancarlo Deidda, LuigiI Morlupi, Paola Grisanti, Donatella Ponzini, Silvana Rinaldi, Bruno Graziani (1997) Silent Thalassemias: Genotypes and Phenotypes. *Haematologica 82:269-280*.

[10] Hadaviv, Taromchiah AH, Malekpour M, Gholamib, Law HY, Almadanin, Afroozan F, Sahebjame F, Pajouhp, Kariminejad R, Kariminejad MH, Azarkevivan A, Jafroodi M, Tamaddoni A, Puehringer H,Oberkanins C, Najmabadih. (2007) 0nElucidating the spectrum of alpha-thalassemia mutations in Iran. Haematologica; 92: 992-993.

[11] Lacerra G, Scarano C, Lagona LF, Testa R, Caruso DG, Medulla E, *et al*. (2010) Genotype-phenotype relationship of the δ-thalassaemia and Hb A(2) variants: observation of 52 genotypes. *Hemoglobin*; *34* : 407-23.

[12] Giambona A, Passarello C, Renda D, Maggio A. (2009) The significance of the haemoglobin A(2) value in screening for haemoglobinopathies. *Clin Biochem*; *42* : 1786-96.

[13] Vichinsky E. (2007) Hemoglobin E syndromes. 29. *Hematology Am Soc Hematol Educ Program;*. p. 79-83.

[14] Codrington, J.F., Li, H.-W., Kutlar, F., Gu, L.-H., Ramachandran, M. & Huisman, T.H.J. (1990) Observations on the levels of Hb A2 in patients with different b-thalassemia mutations and a d chain variant. Blood, 76, 1246‑1249.

[15] Rund D, Fucharoen S. (2008) Genetic modifiers in hemoglobinopathies. 31. *Curr Mol Med*; *8* : 600-8.

[16] Steinberg, M.H. & Nagel, R.L. (2009) Hemoglobins of the embryo, fetus, and adult. In: Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management, 2nd edn (ed. by M.H. Steinberg, B.G. Forget, D.R. Higgs & D.J. Weatherall), pp. 119‑135. Cambridge University Press, New York, USA.

[17] Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC) (2008) Carrier Screening for Thalassemia and Hemoglobinopathies in Canada. Clinical Practic Guid Lines No. 218. Wood WG. Hemoglobin synthesis during human fetal development. Br Med Bull, 1976; 32 (3): 282-287.

[19] The Virginia Sickle Cell Awareness Program: A Counseling Guide for Sickle Cell and Other Hemoglobin Variants. Division of Women's and Infant's Health, 109 Governor Street, Richmond, Virginia 23219. www.vahealth.org/sicklecell/

[20] Abdulrahaman A Momin, Mangesh P Bankar, Gouri M Bhoite (2012) The prevalence of α Thalassemia in South Western Maharashtra. *Biomedical Research 23 (1): 152-154*

Tikrit Journal of Pure Science 21 (6) 2016 *ISSN: 1813 – 1662 (Print)*
E-ISSN: 2415 – 1726 (On Line)

دراسة انماط الهجرة الكهربية للهيموغلوبين في الاطفال حديثي الولادة لمجموعتين سكانيتين في محافظة صلاح الدين– العراق

وجدي صبيح صادق

قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

يعد التعرف على بعض الاعتلالات المرضية والتمييز الدقيق لضروب الهيموغلوبين ذات الاهمية السريرية مهما لغرض توفير رعاية طبية شاملة لمنع التعقيدات الخطيرة, كما يساعد في تقديم المشورة الوراثية.

أجريت هذه الدراسة بغرض التحري عن انتشار طفرة الحذف للجين α 2 في حديثي الولادة في كل من مدينتي سامراء وتكريت– العراق.

تضمنت الدراسة فحص 42 نموذج من الدم الذي تم الحصول عليه من الحبل السري عند قصه في صالة الولادة في كل من مستشفى سامراء ومستشفى تكريت العام للفترة من الاول من تشرين اول 2012 لغاية نهاية اذار 2013, وتم اجراء الترحيل الكهربائي للهيموغلوبين باستخدام العدة التشخيصية Hemoglobin Electrophoresis on Agarose Gels ونظام Hellabioscan and software للتعرف على حزم الهيموغلوبين والنسبة المئوية لكل من مكونات الهيموغلوبين.

بينت النتائج ان 40 حالة من مجموع 42 بنسبة مئوية (95.24 %) كانت ذات مكونات طبيعية للهيموغلوبين, بينما اظهرت حالتين من مجموع 42 بنسبة مئوية (4.76 %) نمطا للهجرة الكهربائية خالي من حزمة HbA2. وبافتراض غياب HbA2 فان هاتين الحالتين قد تحمل طفرة حذف للجين α 2, او الجين δ ولذلك فان الطراز الوراثي لكل منهما قد يكون δ^0/δ^0.