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Patterns of Haemoglobinopathies Diagnosed by High Performance Liquid Chromatography in Karbala Population and Correlations between Different Hematological Parameters

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Abstract

Aim: To know the patterns of hemoglobinopathies and their prevalence in Kerbala society by using high performance liquid chromatography and to conclude if there is any correlations between different hematological parameters in Hemoglobinopathic patients. A total of 70 cases studied from 16th of February 2018 to 20th of June 2018 for hemoglobin variant analysis at the teaching labrotarioes of Al- Hussseini Teaching hospital which were studied for patterns of hemoglobinopathies. When the samples were received, Complete Blood Counts including: Hemoglobin concentration, Total White blood cells count (WBC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Serum ferritin, Total Iron Binding Capacity (TIBC), Hemoglobin H (HbH) preparation were done. Out of total 70 patients screened for patterns of hemoglobinopathies the result of the study revealed that 48 Patients which represent(68.57 %) of total patients had B-thalassemia trait which form the predominant percentage of patients with haemoglobinopathy, 11.85% of patients had sickle cell trait, 5.71% of them had B-Thalassemia Major, 4.29% had Hb S/B+Thalassemia Trait, 2.86% had HbS/Alpha thalassemia trait, 1.43% of them had Hb S homozygosity, 1.43% Alpha thalassemia trait, 1.43% had D-Los Anglos, 1.43% had HbS/B+ Thalassemia trait and 1.43% had HbC/B-Thalassemia trait.

Key Words: *Hemoglobinopathies, Hb electrophoresis, Hemoglobin Variants, retention time, hemoglobin A2.*

Introduction

Haemoglobinopathies are groups of inherited disorders which result either by an abnormal structure of hemoglobin as in sickle cell anemia or by decreased formation of one or more globin chains as in thalassemia [1] There is worldwide increment of the prevalence of inherited hemoglobinopathies such as thalassemia and sickle-cell disorders due to people's migration [2,3]. Hemoglobin abnormalities are present as carrier states in About 5% of the world's population. [4] The normal structure of hemoglobin in healthy individual consisted of tetramers of 4 globin chains of polypeptide, every one of them connected to haem protein . The predominant hemoglobin in normal adults is HbA (forming about 97%) and it has 2 α and 2 β chains. A remaining part of hemoglobin consists of approximately 2-3% HbA_2 which has two α and two δ chains, and a few portion of hemoglobin of fewer than 1% represents HbF, which has two α and two γ chains. In the neonatal period, the major haemoglobin is HbF (approximately 80-90%), with a variable amount of HbA (approximately 10-20%). The switch from γ chain synthesis (which is the predominant form of hemoglobin in the fetus and neonate) to β chain synthesis (which predominates in children and adults), together with the production of δ chains, is usually finished by about one year from time of baby delivery. So, at that period of life, the infant

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get full maturation of HbA2 and Hb F levels.[5] HPLC is used for identification, and estimation of both normal and many abnormal Hb categories.[6] HPLC provides the clear benefits and priorities in comparison to traditional Hb electrophoresis because of it's ability to detect and estimate the quantities of different types of disrupted Hbs in very accurate and perfect manner.[7] It has been proved that HPLC is very rapid, sensitive, specific, and reproducible replacing technique to conventional Hb electrophoresis.[(8)] It's important to mention that every disordered haemoglobin (variant of hemoglobin) can represented in HPLC in specific area and had specific imprecision of retention time that can differentiate it from other types of hemoglobin's. Retention time is the time that extended from the moment of the person's sample injection to the appearance of the summit(apex) of a haemoglobin high point(peak) . The established values at which the well-known disrupted hemoglobin's (variants) were noticed to be eluted and separated by applying the "Variant Beta- thalassemia's short scheme or program are termed as "Windows". During printing the chromatogram's paragraph, there is existence of the following components: all portions of the separated disordered haemoglobin (variants), retention times, the reagons of of hemoglobin's peaks and the levels (%) of various haemoglobin elements. If hemoglobin peaked area was separated at an imprecision of retention time that is not pre-identified, it will be named as an "unknown" area.[(9)] Several structural Hemoglobin disorders and the rarest hemoglobinopathies that can't be identified and diagnosed by Hb electrophoresis, they were detected by HPLC which is preferred in such acases and gave very accurate results as it's the ideal, sensitive and gold standard technique.[(10)] Beside HPLC, there are numerous hematological tests and tools could be used in the assessment and diagnosis of many hemoglobin abnormalities, as example for these tests: sickling test, alkaline and acid electrophoresis, isoelectric focusing (IEF), capillary electrophoresis and DNA testing /protein testing and analysis. On the other hand, it is mandatory to correlate the results of all above mentioned tests when applied with the peripheral blood smears findings of the patients as there is pathognomonic picture of blood film for many hemoglobin abnormalities and disorders.[(11)]

Methodology

This Descriptive Cross Sectional study analyzed 70 blood samples received from February 2018 to June 2018 in Teaching Laboratories In Al-Hussaini teaching hospital in Kerbala governorate / Iraq. Patients with the following eligibility criteria included in the current study: anemia with Hb level extending from 9 to 11.2 g/dl , generalized weakness, patients from both genders with age limits from 17 to 30 years old with suspected haemoglobinopathy depending on the bases of clinical and family history and the results of blood film for those patients which show accordingly: hypochromic microcytic red cells, anisopoikilocytosis, target cells, sickle cells in sickle cell disease confirmed by sickling test were included for study on High Performance Liquid Chromatography (HPLC). Beside above mentioned Criteria, Patients with a recent blood transfusion and inadequate sample did not included in the current study. About Five milliliters of patient's blood was collected in dipotassium ethylenediaminetetraacetic acid vacutainers and was run in Sysmex autoanalyzer for hemogram and red cell indices; The initial laboratory tests done for the patients were: Comple Blood Picture CBP (RBC count, Hb level, Packed Cells Volume (PCV), Mean Cells Volume (MCV), Mean Cells Hematocrit (MCH), Mean Cells Hematocrit Concentration (MCHC), Total and differential white blood cells counts and Platelets Count in which the normal RBC count is **4.06-5.30 X 10^6/µl** and normal Hb level is **12.0-16.0 g/dl**. The manual blood film was performed using the method of Leishman stain[**(12)**]. The samples were run on High Performance Liquid Chromatography instrument HPLC manufactured by Bio-Rad Laboratories. Based on retention time and proportion of Hb variants, different hemoglobinopathies were diagnosed and their prevalence was analyzed. The assigned windows of Manufacturer for Bio-Rad variant HPLC system was shown in Table 1. The presence of Hb H was proved by the usage of brilliant crystal blue staining for the visualization of Hb H inclusions by the light microscope. Another parameters tested in present study are: Serum Iron, Serum ferritin, Total Iron Binding Capacity (TIBC) which were evaluated for all patients in this study.

Results and Discusion

This descriptive cross sectional study was attempted to see the difference in the prevalence of various hemoglobinopathies in Kerbala Governorate in Iraq Country and to conclude if there is any significant correlations between different hematological parameters. Different types of Hemoglobinopathies including thalassemia's and other groups of hemoglobin disorders are distributed worldwide and are known to be autosomal recessive in inheritance with defects in Hemoglobin synthesis.[**(14)**] Thalassemia is a haemoglobinopathy

that result from defect in the quantity and amount of hemoglobin formed in which there is reduction in the level of hemoglobin synthesized resulting in a form of anemia .with RBCs have reduction in their size and their hemoglobinization (hypochromic microcytic) RBCs. There are many forms of thalassemia which are classified according to which type of globin chain are mutated and depleted for example there is α and β thalassemia due to depletion of α and β chains synthesis. Also there is further clinical sub classifications of thalassemia's in to thalassemia major and minor(homozygous and heterozygous) respectively which is based on number of genes affected , either one gene mutated or two genes affected in respective manner. Thus the patients presented in different clinical manifestation. The patients who are of homozygous in type are so ill in which only very limited number of them can still alive to the adulthood period of life and this fatality is due to the severity of anemia and due to cardiopulmonary deteriorations. Regarding thalassemia of heterozygous gene defect, also categorized as thalassemia trait and the clinical finding in such type is solely very mild anemia with hemoglobin concentration not getting below 9g/dL.[15] The current research conclude that the predominant number of Hemoglobinopathic cases were of Beta thalassemia trait (68.57%) and were common in the age limit of 20-30 years and this is in consistent with Buch et al study . The high incidence of thalassemia trait require antenatal screening tests and screening of marriageable age groups. This will help in the limitation and prevention of thalassemia major in the offspring's. This may be the cause for detecting less number of cases of thalassemia major in our study group. We think that antenatal screening or screening of higher secondary school children to detect hemoglobinopathies, counseling of the individuals with hemoglobinopathies will definitely help in drastically reducing the incidence of the thalassemia major and sickle cell disease. This study found that the incidence of B-Thalassemia Major was (5.71%), Similar research done by Patel et al in Gujarat had reported the incidence of thalassemia major (5.63%).[16] The Current study concludes that the incidence of remaining Hemoglobinopathies was as Follows: Sickle/B+-Thalassemia trait(2.86%), sickle cell trait (10%), Hemoglobin S homozygosity (1.43%), Hemoglobin S/alpha thalassemia trait(2.86%), Hemoglobin C/B-Thalassemia(1.43%), Hemoglobin D-Los Anglos (1.43%). There was no gender difference in the different hematological parameters in the sample and this agreed with *Bhokare SB et al*[16] The present study shows that there were significant differences regarding some hematological parameters between the patients of different hemoglobinopathies including: Hemoglobin A, Hemoglobin F and White Blood Cell Count and this agreed with Patel et al. [8] Also there were significant differences between Thalassemia trait patients and Those with Thalassemia Major regarding HbA, HbA2, HbF, Hemoglobin Level, Mean Cell Hemoglobin Concentration (MCHC), Hb H preparation and Serum Iron and this is in consistent with Colah et al.[17] And Patel et al[8] HPLC also helped us in detecting various heterozygous states. Though these abnormal variants have less clinical significance but they can produce severe disease when coexisted with other variants. This once again highlights the significance of mass screening of the population. Although these states can be detected by both HPLC and gel electrophoresis, while there is further advantage of using HPLC further sub-classifying these syndromes based on detection and estimation the amount of various Hemoglobin's depending on their retention time. Regarding Hb D Los Anglos it had identical electrophoretic finding and existence at alkaline pH, on the other hand the average of its retention time in chromatography is creative and had variable results in predictable manner. This differentiation is in fact quite significant especially in a double heterozygous state with Hb S, as Hb SD [18, 19]

Table 1: Manufacturer assigned windows for Bio-Rad variant high-performance liquid chromatography system[13]

Hematoligical Parameters	Gender	Mean	Standard-Deviation	Significance (P value)
HemoglobinA(HbA)	Male	82.868	22.347	0.141
	Female	73.390	30.023	
HemoglobinA2(HbA2)	Male	6.265	10.350	0.315
	Female	4.358	1.774	

Cont... **Table 1: Manufacturer assigned windows for Bio-Rad variant high-performance liquid chromatography system[13]**

HemoglobinF (HbF)	Male	3.619	6.406	0.263
	Female	6.461	13.735	
HemoglobinS(HbS)	Male	42.614	23.200	0.472
	Female	52.313	26.946	
HemoglobinC(HbC)	Male	64.657	24.654	0.255
	Female	73.700	34.543	
Retic Count	Male	3.322	1.980	0.745
	Female	3.955	2.617	
HemoglobinConcentration(Hb)	Male	9.422	1.482	0.831
	Female	9.542	1.608	
Hemotocrit (HCT)	Male	28.919	4.175	0.615
	Female	29.145	4.647	
Mean Cell Volume(MCV)	Male	63.738	8.186	0.198
	Female	64.794	9.286	
Mean Cell Hemoglobin(MCH)	Male	20.908	3.452	0.781
	Female	22.031	3.720	
	Female	73.672	36.566	

Table 2: The mean level of different haematlogical parameters of hemoglobinopathy patients and its relation to gender in Al Hussieni Teaching hospital in Kerbala in Iraq in 2018 (n=70)

Hb Type		**Summation of Squares**	**Means of Squares**	**P value**
HbA	Between Groups	40636.56	3386.38	<.0.001
	Within Groups	5898.831	107.251	
	Total	46535.39		
HbA2	Between Groups	207.63	17.302	0.994
	Within Groups	3804.839	69.179	
	Total	4012.47		
HbF	Between Groups	4295.342	330.411	<.0.001
	Within Groups	3360.108	60.002	
	Total	7655.45		
HbS	Between Groups	6579.804	822.476	0.155
	Within Groups	2083.393	347.232	
	Total	8663.197		
Hb. H. Preparation	Between Groups	2.871	0.221	.0.8888
	Within Groups	0	0	
	Total	2.871		
Retic. Count	Between Groups	299.899	23.069	<.0.001
	Within Groups	67.333	1.202	
	Total	367.232		
Hb level	Between Groups	42.571	3.275	0.134
	Within Groups	119.486	2.134	
	Total	162.058		

Table 3. The Differences within and between groups of Different Hematological Parameters and their significance among Patients of different patterns of Hemoglobinopathies.

	Hemoglobinopathies Types	frequency
1	B-Thalassemia Trait	68.57%
2	sickle cell trait	11.85%
3	B-Thalassemia Major	5.71%
4	Sickle/B+-Thalassemia trait	4.29%
5	Hemoglobin S/alpha thalassemia trait	2.86%
6	Hemoglobin S homozygousity	1.43%
7	Hemoglobin C/B-Thalassemia	1.43%
8	Hemoglobin D-Los Anglos	1.43%
9	alpha thalassemia trait	1.43%

Table 4. The distribution of different types of Hemoglobinopathies in patients in Al Hussieni Teaching hospital in Kerbala in Iraq in 2018 (n=70)

Hematological Parameters	**Disease**	**Mean**	**Std. Deviation**	**Significance**
HbA	B Thal trait	92.534	3.441	<0.001
	B-Thal Major	52.125	12.809	
HbA2	B Thal trait	6.440	9.084	<0.001
	B-Thal Major	5.125	1.315	
HbF	B Thal trait	2.373	3.239	0.002
	B-Thal Major	35.200	25.130	
Retic. Count	B Thal trait	2.450	0.858	0.377
	B-Thal Major	8.200	1.166	
Hb level	B Thal trait	9.581	1.499	0.002
	B-Thal Major	7.200	0.963	
Hb H preperaion	B Thal trait	29.394	4.351	0.0022
	B-Thal Major	22.275	2.680	
MCV	B Thal trait	62.235	7.162	0.004
	B-Thal Major	59.000	2.582	
MCH	B Thal trait	20.533	2.728	0.06
	B-Thal Major	19.500	3.109	

Table 4. The distribution of different types of Hemoglobinopathies in patients in Al Hussieni Teaching hospital in Kerbala in Iraq in 2018 (n=70)

MCHC	B Thal trait	26.863	3.123	0.001
	B-Thal Major	24.500	3.416	
WBC	B Thal trait	8.923	3.405	0.74
	B-Thal Major	6.900	2.255	

Conclusion

The current study conclude that B-Thalassemia Trait represent the higher frequency or incidence of hemoglobinopathies among Al-Hussaini teaching Hospital Patients followed by Sickle cell/B+Thalassemia trait disease. There is no gender difference in relation to different hematological parameters among Hemoglobinopathic patients. There in significant differences regarding some hematological parameters among Hemoglobinopathic patients including: HbA, HbF,MCV,MCH, MCHC. There is significant differences between some hematological parameters between Patients of Thalassemia Major and those of Thalassemia Trait including: HbA, HbA2,
HbF, HbH preparation, MCV,Hb Level, MCHC and Serum Iron. We conclude, HPLC is an ideal method for routine diagnosis of hemoglobinopathies.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Pathology, College of Medicine, University of Karbala, Karbala, Iraq and all experiments were carried out in accordance with approved guidelines.

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