

Analysis of hemoglobin electrophoresis results and physicians investigative practices in Saudi Arabia

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BACKGROUND AND OBJECTIVES: Riyadh and central province falls in a moderate prevalent zone of hemoglobinopathies in Saudi Arabia. However, it has been observed that the physicians working in Saudi Arabia invariably advise all cases of anemia for hemoglobin electrophoresis (HE). The present work was carried out to study the yield of the HE in Riyadh and the investigative practices of the physicians advising HE.

SETTINGS AND DESIGN: The study was carried out in the hospitals of King Saud University from 2009 to 2011 in order to assess the yield of HE in referred cases of clinical anemia.

MATERIALS AND METHODS: A total of 1073 cases divided in two groups of males and females had undergone complete blood count and red blood cell morphology. Cellulose acetate HE was performed and all the positive results were reconfirmed on the high performance liquid chromatography (HPLC). The results were analyzed for the type of hemoglobinopathies. For statistical analysis Statistical Package for Social Sciences 15 version (SPSS Inc., Chicago, IL, USA) was used.

RESULTS: A total of 405 males and 668 females blood samples were included in the present study. 116 (28.5%) males and 167 (25%) females showed an abnormal pattern on HE. The incidence of beta thalassemia trait was higher in females while sickle cell trait was predominantly seen in males. Red cell indices were reduced considerably in thalassemias, but were unaffected in sickle cell disorders, except those which had concurrent alpha trait. The total yield of HE was 26.6% which was much less than expected.

CONCLUSION: The physicians are advised to rule out iron deficiency and other common causes of anemia before investigating the cases for hemoglobinopathies, which employs time consuming and expensive tests of HE and HPLC.

Key words: Electrophoresis, hemoglobin, physician's practices, Saudi Arabia

Introduction

Normal hemoglobin (Hb) A has got two α and two β chains. α gene clusters are present on chromosome 16 while β genes are present on chromosome 11. Hereditary Hb disorders are caused by structural abnormalities as a result of decreased production of either chain due to the deletion or mutation.

Hemoglobinopathies have been reported from every part of the world.^[1-3] However, Mediterranean region, tropical and sub-tropical Africa, India,^[4,5] South East Asia and the middle East including Saudi Arabia^[6-8] are the regions from where the maximum number of cases are reported.

In Saudi Arabia sickle cell anemia and thalassemias are prevalent predominantly in the eastern region, western and southern regions.^[9-13] First cousin and other consanguineous marriages is the most important cause of higher prevalence of hemoglobinopathies in these regions.^[12]

Thalassemias were first reported from the Eastern region by Lehman *et al.* in 1963.^[14] Sickle cell heterozygotes and homozygotes are also more prevalent from the same region. Subsequently several studies on the prevalence and genetic structure of hemoglobinopathies have been carried out in the kingdom.^[6-8,15-20] The overall prevalence of hemoglobinopathies in Saudi Arabia is definitely higher than the Europe and Americas.^[1-3,21] Central region and Riyadh fall in the low prevalent zones for hemoglobinopathies of Saudi Arabia.^[6,22]

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In 2004 through a royal decree premarital screening of the couples for thalassemia and sickle cell anemia was made obligatory in Saudi Arabia.^[22] If prospective husband and wife came out to be the carriers of thalassemia or sickle cell or either of the two is homozygote for the disease the couple is referred to the counseling centers and is advised to abstain from marriage.

Neonatal screening for hemoglobinopathies is a part of a national program adopted by Saudi Arabia.^[9,22]

A scientific advisory committee for Hereditary Blood Disease Centers (HBDC) for every region of Saudi Arabia was formed in 2008.^[22]

These measures have considerably reduced the incidence of hemoglobinopathies in the Kingdom in the last few years.

The present study was designed to analyze retrospectively the results of hemoglobin electrophoresis (HE) carried out in Riyadh region and to assess the justification of HE advised by the physicians managing anemia.

Materials and Methods

The present study was carried out in the hematology laboratory of the college of health sciences and the hospitals of the King Saud University Riyadh, Saudi Arabia from 2009 to 2011.

A total of 405 males and 668 females (in all 1073) from adult and pediatric age groups presenting with clinical anemia were referred for HE. 2 ml blood sample from every patient was collected in an ethylenediaminetetraacetic acid vacutainer for estimation of hemoglobin (Hb), hematocrit (Hct), total red blood cell count, total leukocyte count, platelet count, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). The tests were performed on a Beckman Coulter (USA) hematology analyzer. $MCV < 78$ fl was considered microcytosis and $MCH < 27$ pg and $MCHC < 32$ g/dl were considered hypochromic. RBC morphology was studied on Wright's stained peripheral blood smears (PBS). Reticulocyte count was carried out on methylene blue stained smears.

Hemolysate was prepared from washed cells treated by cold distilled water, which was subjected to HE

at alkaline pH of 8.6 in cellulose acetate medium by exprime 72 methodology provided by Amplimedical corporation, Italy (Associate of Roche, USA). All Hb variants discovered on HE were reconfirmed on high performance liquid chromatography (HPLC). Hb F was also estimated by alkali denaturation technique of Betke *et al.*^[23]

Level of Hb A2 $> 3.5\%$ was considered the cut off for β thalassemia trait.

For α - and β -genotyping, genomic deoxyribonucleic acid was prepared from peripheral blood by the standard phenol chloroform extraction method.

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) version 15 software (SPSS Inc., Chicago, IL, USA) and an independent *t*-test was used for comparison of hematological parameters. Results are presented as mean values and standard deviation (SD). A $P < 0.05$ was considered statistically significant.

Results

1073 HE results were analyzed in the present study, out of which 668 were females and 405 males. The adults' age range was 14-61 years with a mean age of 30.97 ± 9.95 while in the children group of 577 the age range was 1-9 years with a mean of 4.44 ± 2.82 .

289 (71.5%) males and 501 (75%) females had normal AA pattern on HE. 116 (28.5%) males and 167 (25%) of females had shown abnormal Hb pattern.

The significant finding was the predominance of female carriers of β thalassemia trait 91 (13.6%) and of males 43 (10.3%) with sickle cell trait in the study population. The males were also ahead of females in all other types of hemoglobinopathies.

Four cases (0.9%) of Hb E heterozygotes were detected in males and only 2 (0.2%) in females. Out of these six cases, only two patients were Filipinos while the rest four were Saudis. The D variant heterozygotes were found exclusively in males.

The number and percentage of each type of hemoglobinopathy in both groups of males and females is given in Table 1.

The reticulocyte count was in the range of 3-4% in the positive cases. Hb and Hct were mildly low in cases of thalassemia and sickle cell trait while moderate to markedly low in cases of thalassemia major and sickle cell disease. The MCV and MCH were significantly low ($P < 0.001$) in cases of thalassemias presenting microcytic hypochromic picture on PBS, however, these values were within the normal limits in sickle cell disorders. The red cell count was increased in cases of thalassemias while it was not much affected in sickle cell disorders. The indices were lower in sickle cell α thalassemia trait ($P < 0.05$) [Table 2]. Two cases of Hb E heterozygote had low MCV and MCH.

Discussion

Extensive studies have been carried out in Saudi Arabia in the last two decades to study the prevalence and genotypes of different hereditary Hb disorders. Very high prevalence of sickle cell anemia, α and

β thalassemias and interaction of genes have been reported from the eastern region of Saudi Arabia.^[6-8,15-20,22]

In the present study, we had analyzed the results of cases of anemia, which had undergone HE. Out of 1073 samples screened only 286 (26.3%) showed an abnormal pattern demonstrating a lower yield compared with reports from eastern and western regions. The results of the premarital screening and other population based studies have placed central region and Riyadh in a moderate prevalence zone for hemoglobinopathies.^[6,22]

The results of the blood screening carried out in different HBDC of Saudi Arabia in 2007 showed prevalence of 4.2% carriers and 0.26% of sickle cell disease while 3.22% carriers and 0.07% homozygotes of thalassemia at the national level.

In Riyadh, 2.08% carriers of sickle cell and 2.01% of thalassemia and 0.15% of the disease of both sickle and thalassemia were reported.^[22] Sickle cell disorders

Table 1: Sex wise distribution of Hemoglobinopathies in the study population

Hemoglobinopathies	Distribution in sample populations					
	Males		Females		Total	
	Frequency		Frequency		Frequency	
	No	%	No	%	No	%
β -thalassemia trait (AA2)	41	10.1	91	13.6	132	12.3
β -thalassemia major (FA2/FAS)	5	1.2	4	0.5	9	0.8
Sickle cell trait (AS/AFS)	43	10.6	44	6.5	90	8.3
Sickle cell disease (SS/SFA)	10	2.4	11	1.6	21	1.9
Sickle cell α thalassemia trait ($-\alpha/\alpha\alpha$)	10	2.4	15	2.2	25	2.3
Hemoglobin E variant	4	0.9	2	0.2	6	0.5
Hemoglobin D variant	3	0.7	0	0	3	0.25
Abnormal pattern (total)	116	28.5	167	25.0	286	26.4
Normal pattern (AA)-(total)	289	71.5	501	75.0	787	73.6
Sample size	405	100.0	668	100.0	1073	100.0

AA2: (Hb. A and A2) FA2: (Hb.F and A2) FAS: (Hb.F, A and S) AS: (Hb.A and S) AFS: (Hb.A, F and S) SS: Hb.S, and S) SFA: (Hb.S, F and A)

Table 2: Hematological Profile of Hemoglobinopathies

Hemoglobinopathies	Hb (g/dl)		MCV (fl)		MCH (pg)		MCHC		TRBC	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
β -Thalassemia trait (AA2)	10.27±0.91	9.21±1.56	65.29±7.05	67.22±7.56	24.79±2.65	23.70±2.01	33.12±2.01	31.22±2.50	4.5±2.6	3.8±1.4
β -Thalassemia major (FA2/FAS)	6.43±0.64	6.01±0.77	72.21±7.56	74.21±8.11	21.31±1.99	21.21±2.09	31.22±3.99	30.45±4.16	4.6±2.5	4.3±1.5
Sickle cell trait (AS/AFS)	12.10±0.93	11.80±1.03	84.21±6.79	83.42±6.11	30.48±2.76	30.43±4.54	34.14±4.54	35.12±5.10	4.1±1.6	3.9±2.9
Sickle cell disease (SS/SFA)	9.23±0.65	8.99±0.11	82.43±7.11	82.55±7.81	27.14±2.89	26.43±2.11	31.56±4.76	32.62±4.14	3.9±1.8	3.8±2.1
Sickle cell α -thalassemia trait ($-\alpha/\alpha\alpha$)	10.32±0.89	9.93±0.91	70.23±7.99	70.23±7.99	21.94±2.10	20.83±2.99	30.24±3.78	30.12±4.16	4.4±1.2	4.1±2.1

Hb: hemoglobin, MCV: Mean Cell Volume, MCH: Mean Cell Hemoglobin, MCHC: Mean Cell Hemoglobin Concentration, TRBC: Total RBC Count in millions/ μ l.

AA2: (Hb. A and A2) FA2: (Hb.F and A2) FAS: (Hb.F, A and S) AS: (Hb.A and S) AFS: (Hb.A, F and S) SS: H b.S, and S) SFA: (Hb.S, F and A)

comprise 74% of all hemoglobinopathies in Saudi Arabia.^[22]

The statistically significant sex based difference in the incidence of hemoglobinopathies was observed in β thalassemia trait and sickle cell trait. The incidence of β thalassemia trait was higher in females while sickle cell trait was higher in males. In accordance with the previous studies from this region, we too have found a significant difference in the red cell indices of thalassemias and sickle cell disorders.^[7,16,17] Invariably the MCV, MCH and MCHC remain within normal limits in the cases of sickle cell carriers and disease while they are reduced in thalassemias. α gene interaction in cases of sickle cell anemia leads to reduction of MCV and MCH.^[16,24,25]

Since the present study is a hospital based analysis of the cases of clinical anemia referred for HE and not a general population based study, hence the number of positive cases are much higher compared with the prevalence reported earlier in the general population of Saudi Arabia. However, the higher percentage of positive results does reflect the higher prevalence of hemoglobinopathies in the general population. The findings correspond to the previous studies from Saudi Arabia and other Gulf states.^[26,27]

The Hb variant E and D, which are more prevalent in Southeast Asia^[28] are rarely found among Saudis. Incidentally we had detected four cases of Hb E in Saudis.^[18,29] However, our results show relatively higher incidence of these rare Hb variants. The red cell indices were lower only in two cases of Hb E heterozygotes. The number of E and D cases being very few has not been displayed in the Table 2.

The most common cause of microcytic hypochromic anemia is iron deficiency anemia (IDA). It is obligatory on physicians to first rule out IDA and then investigate for hemoglobinopathies,^[26,30] otherwise the overenthusiastic approach inflates the cost of treatment for the patient. In our study, only 26.6% cases of anemia yielded a positive result on HE.

The findings of our analysis could not be attributed to a particular ethnic group too since Riyadh is a metropolis of the cosmopolitan nature and here people from almost every region of Saudi Arabia reside.

Conclusion

Riyadh region is having a low incidence of hemoglobinopathies compared with the Eastern and Western regions of Saudi Arabia. It has been observed that physicians have a practice of advising HE invariably in every case of anemia before ruling out the common causes of anemia such as iron deficiency and chronic diseases, causing unnecessary burden on the laboratory resources and delay in initiating the proper management. The physicians are advised to follow the recommended protocol for investigating hemolytic anemia and must rule out IDA in cases of microcytic hypochromic blood picture before referring the patient for HE and HPLC.

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