Hemoglobin H disease in the Eastern region of Saudi Arabia

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ABSTRACT

Objective: α−thalassemia is frequently encountered in eastern Saudi Arabia. We wanted to find out laboratory based incidence and laboratory features of Hemoglobin H disease in the Dammam region.

Methods: We retrospectively analyzed the results of Hemoglobin electrophoresis carried out during the last 5 years in our laboratory. Hemoglobin electrophoresis was performed on cellulose acetate, pH 8.6 using Helena or Biomidi kits. Hemoglobin S was confirmed by sickle solubility test. Variant hemoglobin if present, was confirmed by citrate agar (pH 6.0) electrophoresis. Helena rapid electrophoresis system was used for plate densitometry. The diagnosis of Hemoglobin H disease was made on the basis of the presence of Hemoglobin H on electrophoresis supplemented by demonstration of Hemoglobin H inclusions in red blood cells.

Results: Fifteen thousand, four hundred and ninety two blood samples were analyzed by Hemoglobin electrophoresis. We found 100 cases of Hemoglobin H disease, only one case was non-Saudi. The age ranged between 45 days to 85 years. There were 51 females and 49 males. Children (less than 12 years) were 35 and of adults there were 65. There were 35 adult females and 30 adult males. The mean ± standard deviation of Hemoglobin H in children was 13.54 ± 7, in adult females the mean ± standard deviation of Hemoglobin H was 12 ± 5.4, and in adult males it was 11.99 ± 6.4. The Hemoglobin H inclusions seen in red blood cells ranged from 2.6-80 in children and 10-80 in adults. The sickle cell trait was co-existent in 7 cases. Hemoglobin Bart's along with Hemoglobin H was seen in 32 cases. Hemoglobin F was present, beyond first year of life in 34 cases. The Hemoglobin A_2 as measured by densitometry was significantly low in all of the 3 age groups as compared to corresponding controls. The complete blood count results were available for analysis in only 26 cases of Hb H disease. The mean ± SD values of Hb (g/dl), Hct (ratio), MCV (fl), MCH (pg) MCHC (g/dl), RDW-SD (fl) and RDW-CV (%) in these patients (all age groups together) were 8.15 ± 1, .278 ± .04, 59.4 ± 5.8, 17.65 ± 2.1, 29.4 ± 1.7, 37.8 ± 8.7 and 25.1 ± 4.6. The mean Hb, Hct, MCV, MCH and MCHC were significantly reduced in all 3 age groups as compared to corresponding controls. RBC counts and RDW-CV were elevated in Hb H disease compared to corresponding controls. The blood film showed typical red cell morphology.

Conclusion: Hb H disease is not infrequently encountered in the Dammam region. This condition should be kept in mind while evaluating patients for anemia. The genetic studies to determine the exact α−thalassemia determinants producing Hb H disease in eastern Saudi Arabia are needed.

Keywords: α-thalassemia, Hemoglobin H disease, Hemoglobin H, Hemoglobin H inclusions, Hemoglobin Bart's, red cell indices.

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The α−thalassemia is frequently seen in the eastern region of Saudi Arabia and prevalence of 28-60% has been reported in various reports.[1-4] It is reported that Hemoglobin H (Hb H) disease is less commonly seen in Africa and the Middle East as α^{o}–thalassemia(which is an important determinant to produce Hb H disease or Hb Bart's hydrops syndrome) is less frequently seen in these areas.[1,5,6] Homozygosity of non-deletional α−thalassemia mutants ($\alpha^{T\text{-}Saudi}\alpha/\alpha^{T\text{-}Saudi}\alpha$) producing Hb H disease is

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reported from Saudi Arabia and Bahrain.[2,5-7] In our prospective study of Hb electrophoresis and complete blood count (CBC) on 504 cord blood samples from Qatif area, we found Hb H disease as common (if not more) as sickle cell disease.[3] This prompted us to look into our records regarding the occurrence of Hb H disease in our day to day routine Hb electrophoresis.

Methods. We retrospectively analyzed the results of Hb electrophoresis for the last 5 years (June 1994 - June 1999). Our department receives ethylenediaminetetraacetic acid (EDTA) blood samples for Hb electrophoresis mostly from the hospitals and dispensaries of the Dammam region.

Results of Hb electrophoresis on cord blood for neonatal screen (Qatif and Dammam area) were not included in this study. We have analyzed 15,492 samples by cellulose acetate Hb electrophoresis (pH 8.6) during the above mentioned period. There are patients whose Hb electrophoresis may have been repeated. However, we believe that less than 3% of samples are repeat samples among these 15,492 samples. Hb S was confirmed by sickle solubility test or by citrate agar Hb electrophoresis (pH 6.0) which was also used to confirm other abnormal hemoglobins, if present. Hb electrophoresis kits from Helena Laboratories (Beamont, TX 77704, USA) and Biomidi (Parc de la Plaine, 35, av Marcel Dassault, F-31500 Toulouse France), or both were used. The scanner used to scan the plates was Rapid Electrophoresis (REP) Helena Laboratories, and print outs with percentage of various hemoglobins were obtained. Complete blood counts (CBC) were performed by Sysmex NE-8000, Toa Corporation, Japan.

The diagnosis of Hb H disease was made purely on the basis of presence of Hb H in cellulose acetate Hb electrophoresis. This was supplemented by demonstrating Hb H inclusions in RBC after their incubation with brilliant cresyl blue (10g/l) for 3 hours in all the cases. It is our practice to perform CBC and examine blood film while reporting on Hb electrophoresis in all the cases.

The normal controls used in this study were our laboratory controls but some of the controls used for CBC were prospectively studied. Therefore some of the normal controls used for CBC were not necessarily the same as used for Hb electrophoresis. All the controls used for CBC had normal Hb electrophoresis or had negative sickle solubility test

Table 1 - Clinical indications listed for hemoglobin electrophoresis in 22 cases.

Clinical indication	Number
Anemia	12
Jaundice	2
Splenomegaly	4
Skull bossing	1
Hepatomegaly	4
Screening:	
Sib with Hb H disease	1
Hemoglobinopathy in family	2
Osteomyelitis	1
Renal failure	1
Rheumatic heart disease	1
Child with Hb H disease	1
Joint pains	2
Pregnancy	1
Chest infections	2
Operative proceedures	4

Table 2 - Break up of cases.

Period of study (June 94 - June 99)	Number
Total No of blood samples for electrophoresis	15492
Cases with Hb H disease	100
No of Saudi patients	99
Age range 45 days - 85 years	
Sex: Males Females	49 51
Children (45 days - <12 years Children <1 year	35 3
Adults (12-85 years)	65
Adult females (12-50 years)	35
Adult males (12-85 years)	30
Hb H disease with sickle trait	7
Hb H disease with Constant Sprint (non-Saudi lady)	1
Presence of Hb F beyond one year of life	34
Presence of Hb Bart's along with Hb H	32
Presence of Hb Bart's along with Hb H & S	7
Presence of Hb Bart's, Hb F and Hb H	16
Presence of Hb Bart's, Hb F and Hb S along with Hb H	3

(if electrophoresis was not carried out). In all the control samples alpha and beta thalassemia trait was excluded on the basis of normal Hb and red cell indices, (which is not 100% reliable). The age range of control children was 1-12 years and had Hb of >11g/dl. The age range of control adult females was 15-65 years and all had Hb of >12 g/dl. The control male adults were 12-85 years of age and all had Hb of >13 g/dl.

Results. We found 100 cases of Hb H disease during these 5 years. The only one non-Saudi subject among these 100 cases was a Southeast Asian lady. In most of the cases, Hb electrophoresis was part of a routine investigation protocol for some other unrelated diseases or for the investigation of anemia. Though we did not analyze the clinical symptoms in these cases for want of adequate details, the indications written (single or combinations) for Hb electrophoresis in 22 cases are listed in Table 1. The break up of all the 100 cases are given in Table 2. The results of Hb electrophoresis for the patient and control groups are given in Table 3. As we preserve accessory reports like CBC only for a limited period of time, the CBC values were available to us in only 26 cases of Hb H disease, 8 children and 18 adults (13 females/5 males). The CBC results of these 26 cases along with corresponding controls are given in Table 4. The blood film findings recorded were severe anisopoikilocytosis, hypochromia, target cells, fragments, misshapen RBC in all and occasional nucleated RBC in 3 cases. Co-existent hereditary elliptocytosis was present in one patient. G6PD deficiency was recorded in 2 cases though all the cases were not screened for G6PD deficiency. The mean Hb values in female adults and children were

Table 3 - Hb electrophoresis results in Hb H disease.

Parameter + 1 SD (Range)	**Children Up to 12 years (35)**	**Adults 12-84 years (65)**	**Female Adult (35)**	**Male Adult (30)**	**Control***		
					Children (20)	**Female (20)**	**Male (40)**
HbA Percentage	79.34±8 (52.5-92)	80.9±6.5 (53.8-95)	79.7±7.4 (53.8-95)	82.2±5.5 (63-92.9)	97.1±5.3 (96.3-98.1)	97.2±0.3 (96.7-97.7)	96.9±0.4 (96-97.6)
HbA2 Percentage	1.75±0.7 (0-3.6)	1.85±0.7 (0-3.8)	1.84±0.69 (0-3.8)	1.87±0.69 (0-3.8)	2.9±0.5 (1.9-3.7)	2.8±0.3 (2.3-3.7)	3.1±0.4 (2-4)
HbH Percentage	13.54±7 (3.5-46)	12±5.9 (1.7-44.2)	12±5.4 (1.7-44.2)	11.99±6.4 (1.5-27.3)	-	-	-
HbBart's** Percentage	6.19±3 (2.4-12.5)	4.8±1.5 (2.3-10.5)	4.97±1.5 (2.8-10.5)	4.5±1.6 2.3-7	-	-	-
HbS** Percentage	17.4	20±1.3 (16.9-22)	19.8±1.5 (16.9-22)	20.5±0.7 (19.7/21.2)	-	-	-
HbF** Percentage	6.6±4 (1-6.8)	6.1±2 (3.5-22)	6.5±2.6 (3.5-21.9)	5.7±1.2 (3.6-10.8)	-	-	-
HbH inclu+ Percentage	41.5±24 (2.6-80)	43±16 (10-80)	39.3±15.5 (10-70)	52.3±14.5 (26-80)	-	-	-

*All the controls had normal Hb electrophoresis and possibly had no α-thalassemia trait on the basis of red cell indices.

**Hb Bart's, S & F in children/adults were present in 15/17, 1/6 and 12/22.

+Hb H inclusion result were available in 8 children and 22 adults

similar, and Hb values in adult males were higher than adult females (p=< .01). The overall mean ± SD values in 26 cases of Hb H disease were Hb (g/dl) 8.15 ± 1, Hct (ratio) 0.278 ± 0.04, MCV (fl) 59.4 ± 5.8, MCH (pg) 17.65 ± 2.1, MCHC (g/dl) 29.4 ± 1.7, RDW-SD (fl) 37.8 ± 8.7 and RDW-CV (%) 25.1 ± 4.6. The results of Hb H inclusion screen were available in only 30 cases (8 children and 22 adults). The leukocyte and platelet counts were not significantly different than control population.

Discussion. Hb H disease appears a frequently encountered disease in the Dammam region. We found 100 (99 Saudi) cases of Hb H disease among 15,492 samples of blood subjected for Hb electrophoresis. This gives a laboratory-based incidence of 1 in 155. We encountered about 1,267 cases of sickle cell disease (SCD) in the same period. This data indicates that sickle cell disease is almost 13 times more common than Hb H disease. However, this may be over estimate, as not all cases of Hb H disease report to hospital like sickle cell disease. It is expected that population based incidence of Hb H disease in the Qatif area will be much higher. Our earlier study showed that around 1 in 85 births in the Qatif area is born with Hb H disease. Some cases of Hb H disease have been reported from eastern and western Saudi Arabia.[3,8] It

Table 4 - Complete blood count results in Hb H disease.

Parameter + 1 SD (Range)	**Children Up to 12 years (8)**	**Adults 12-84 years (18)**	**Female Adult (13)**	**Male Adult (5)**	**Control***		
					Children (20)	**Female (20)**	**Male (40)**
Hb g/dl	7.86±1 (5.2-9.3)	8.27±1.04 (5.7-11.1)	7.88±0.86 (5.7-11.1)	9.3±0.8 (7.6-10.6)	12.7±0.7 (11-13.9)	12.97±0.7 (12.1-13.4)	15.5±0.9 (13-18)
RBC $x10^{12}$/l	4.83±1 (3.39-6.01)	4.69±0.6 (3.38-5.68)	4.4±0.5 (3.38-5.22)	5.4±0.3 (4.67-5.74)	4.6±0.23) (3.92-5.15)	4.68±0.3) (4.14-5.53)	5.37±0.4) (4.46-6.31)
Hct Ratio	.276±.03 (.175-.341)	.279±.04 (.195-.359)	.263±0.3 (.195-.327)	.319±.04 (.255-.347)	.353±.02 (.295-.388)	.38±.02 (.342-.408)	.441±.04 (.391-.504)
MCV fl	58.16±8 (47.4-82.6)	59.89±4.6 (51.7-86.3)	60.4±5.4 (51.7-86.3)	58.5±3.4 (54-63.3)	77±4.6 (68.9-87.2)	81±3.5 (75-90)	82.4±3.5 (75.4-94.3)
MCH pg	16.72±2.9 (14.2-27.4)	18.06±1.7 (16.1-29.3)	18.4±2.15 (16.1-29.3)	17.1±0.6 (16.3-18.5)	27.7±1.8 (23.9-31.8)	27.8±1.3 (24.2-31.2)	29±1.49 (25.7-33.3)
MCHCg/dl	28.67±2.3 (22-33.2)	29.7±1.4 (24.8-33.9)	29.9±1.4 (24.8-33.9)	29.2±1.43 (27-31)	36±0.7 (34.7-36.8)	34.2±0.8 (32.2-35.8)	35.2±0.7 (33.3-37.3)
RDW SD fl	36.68±6.9 (26.5-48.2)	38.61±10 (26.1-52.9)	37.3±9.6 (23-48)	43.2±9 (34.2-52.3)	35.8±0.6 (35-37.1)	41.3±2.5 (37-45.5)	40.8±2.4 (36-64.2)
RDW CV %	21.5±6 (13.1-27.8)	26.2±4.3 (12.2-30.9)	25±5.4 (12.2-31)	27.3±2.8 (24-31)	14±1.3 (11.5-17.8)	13.8±0.8 (9.5-13.5)	13.5±0.7 (12.4-15.9)
Platelets $x10^9$/l	357±81 (225-594)	349.5±151 (156-805)	356±138 (156-805)	332±179 (166-780)	342±61 (225-547)	287.1±66 (141-410)	272.7±50 (170-443)
WBC $x10^9$/l	8.54±3.4 (4.87-18.7)	5.85±1.4 (4.01-14.3)	5.5±1 (4.01-6.18)	6.9±2.9 (4.53-14.3)	6.7±1.4 (4-11.93)	6.4±1.2 (5.24-10)	7.08±1.5 (3.5-10.2)

*All the controls had normal Hb electrophoresis or negative sickle solubility test.

has been shown that Hb H disease in eastern region of Saudi Arabia and Bahrain (where people may belong to a common ethnic background) is a result of homozygosity of non-deletion α-thalassemia (poly A signal mutation).[5,6,9,10] In Bahrain Hb H disease has been exclusively shown to be due to Saudi type poly A signal mutation.[6] The genotype studies in α-thalassemic families from western Saudi Arabia have also shown that Hb H disease in Saudi Arabia is also mostly due to interaction with non-deletional mutants.[8]

The mean Hb, Hct, MCV, MCH, and MCHC are reduced markedly in our patients as compared to our controls (p value ranging from < .01 to < .0001). In the adult Hb H disease patients the mean Hb values are lower than as reported in literature including those where Hb H disease is due to non-deletional α−thalassemia mutants.[5] It may be that Saudi type Hb H disease is more severe. It is reported that non-deletional α-thalassemia determinants give rise to a severe reduction in α−chain synthesis than deletion α-thalassemia.[5,8] In some cases it may be explained on the basis of co-existence of sickle cell trait in our patients, which in itself produces mild reduction in Hb and mild microcytosis.[3] However, the range of blood Hb and Hb H in our cases is very wide. Therefore a variable clinical course (mild to severe) is expected in our patients. This is also supported by the fact that in many cases Hb electrophoresis was asked for some routine and screening purposes (Table 1). Keeping in view these facts it is difficult to draw any definite conclusions regarding genetic background (deletional vs non-deletional α-thalassemia) in our cases of HbH disease. Possibly both types of Hb H disease are present. The incidence of α^{o} thalassemia has been reported as 2.2% in eastern Saudi Arabia (Al-Hafof) which is higher than in any other region in Saudi Arabia.[4] Therefore some cases of Hb H may be due to inheritance of α^{o}thalassemia. Hb H disease reported from western Saudi Arabia is also mild and has no gross bony changes.[8]

RBC count in Hb H disease (in relation to their Hb) is high and is almost identical to RBC count in control population (who have normal Hb). RDW-CV (%) is elevated in Hb H disease patients as compared to corresponding controls. However, when RDW is expressed as SD (fl), the results are inconsistent, there is no significant difference between the values in children and male adult patients with those of corresponding controls. However, the values are significantly reduced in female adult patients as compared to control females (p<.001). Iron deficiency can affect red cell indices. We did not have any information regarding the iron status of these patients. It is unlikely that any of our patients had co-existent iron deficiency as Hb H disease is associated with hemolytic anemia since birth. Iron overload is uncommon but iron and ferritin levels are higher in Hb H disease and iron therapy is contraindicated.[9-11] Typical red cell morphology was present in Hb H disease. The presence of Hb F in 31 cases (>1 years of age) may be due to extremely stressful hemopoiesis in these patients. Hb H is functionally a useless pigment and a hemolytic substance.[11] In some cases, co-existent G6PD deficiency may accentuate the process of hemolysis. Whether there is delayed switch over from γ-chains to β-chains in Hb H disease is not known. The presence of Hb Bart's in 32 cases of Hb H disease may be also related to severity of disease and persistence of γ-chains. The other significant finding is low Hb A_2 in Hb H disease patients than corresponding controls (p=< .0001). This is due to non-availability of α-chains to bind with δ-chains. Somewhat conflicting results of Hb A_2 levels have been reported in thalassemia trait (severe form) from Saudi Arabia[12,13] but we are not aware of any large-scale reports of Hb A_2 estimation in Hb H disease from Saudi Arabia. As mentioned earlier, it is unlikely that our patients had co-existent iron deficiency, which could have affected their Hb A_2 levels. Reduced levels of Hb A_2 like ours are reported in the literature.[2,8-10] There was no case where one could suspect that presence of Hb H could be due to some acquired cause. No patient had any myeloprolofretive disorder. Our routine practice of examining CBC and blood film (and at times G6PD screening/quantitation, Hb A_2/F quantitation, and family studies) in all cases at the time of reporting on electrophoresis is extremely helpful in assessing various diseases which may affect the interpretation of electrophoresis findings.

From this study, which is largest study of Hb H disease from Saudi Arabia, it is concluded that Hb H disease is not an uncommon condition in Saudi Arabia[4] especially in the Dammam region. Based on the levels of hemoglobin and Hb H, the disease appears to run a mild course in some and severe in others. It is suggested that the disease may not be only due to non-deletion α-thalassemia as reported from Bahrain but also due to inheritance of α^{o}-thalassemia. The genetic studies in large number of cases with Hb H disease in eastern Saudi Arabia needs to be undertaken at the earliest and genetic counseling may have to be undertaken. Furthermore, the use of more sensitive techniques like isoelectric focussing and polymerase chain reaction will be useful for easy and quick diagnosis of Hb H disease.

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