The Turkish Journal of Pediatrics 28:67 - 72, 1986

HOMOZYGOSITY FOR HEMOGLOBIN O-ARAB*
(α_2 $\beta_2$121 Glu → Lys)
Hb O-Arab Disease

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Key words : hemoglobinopathy, hemoglobin O-Arab

Cases of simple heterozygosity for Hb O-Arab and double heterozygosity for Hb O-Arab with Hb S, Hb C and β -thalassemia have been reported from various countries[1-12]. Homozygosity for Hb O-Arab, however, has only been reported previously in the case of two individuals with mild hemolytic anemia and splenomegaly[1,2].

We recently had the opportunity to examine a family in whom two siblings were homozygous for Hb O-Arab. A detailed hematological study including *in vitro* hemoglobin synthesis analysis of these patients is presented and we believe that the presentation of these new cases could be helpful in the definition of the Hb O-Arab disease.

Case Report

An 18-year-old male was referred to Hacettepe University Children's Hospital for evaluation of recurrent jaundice and splenomegaly. Past history revealed that he had had two episodes of jaundice in the past, one five, and the other three years previously. On each occasion the symptoms had continued for 7-10 days. He had been well until two weeks before admittance when he had a slight fever which lasted for a few days. Two days after the onset of the illness the jaundice became apparent. Physical examination revealed a well-developed male with slight jaundice. The spleen was palpable 9 cm below the costal margin. The rest of the

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TABLE I

Summary of the Hematological Data

	Age, Sex	Hb g/dl	RBC 10^{12}/L	PCV L/L	MCV F1	Ret %	HbF AD	HbA_2 HPLC	HbO-Arab*	RBC survival in days	In vitro Hb synthesis** 20	60	120	β^A/β^0
Propositus	18/M	12.5	4.3	0.36	87	0.8	1.2	–	(98.8)	21	0.88	0.9	0.98	–
Affected sister	15/F	11.8	4.6	0.36	75	0.6	1.2	2.9	97 (98.8)	–	0.78	0.86	0.99	–
Mother	38/F	13.5	4.1	0.35	85	0.2	0.7	2.55	34.1 (42)	–	–	–	0.90	1.64
Father	42/M	15.0	4.9	0.45	80	–	0.8	2.8	30.3 (39.5)	–	–	–	0.88	1.68
Previously reported patient[3]	5/F	7.0	2.5	–	–	–	2.0	–	–	–	–	–		
Previously reported patient[11]	18/F***	11.3–12.0	3.9–4.05	–	–	0.9–4.0	1.2–1.6	–	(100)	–		–		

* The first figure refers to the value obtained by HPLC and the figure in parentheses refers to the value obtained by column chromatography

** Incubation time in minutes

*** Patient underwent splenectomy at the age of 14 years

Hb: Hemoglobin, RBC: Red blood cell, PCV: Packed cell volume, MCV: Mean corpuscular volume, Ret: Reticulocyte, AD: Alkali denaturation, HPLC: High pressure liquid chromatography

physical findings were within normal limits. The family history revealed that the parents were cousins. The younger brother and two sisters were apparently healthy. However on physical examination the 15-year-old sister was found to have splenomegaly (the spleen was palpable 8 cm below the costal margin). She was otherwise healthy and had never had jaundice. The family had been living in a town in western Anatolia for many generations and denied any connection with immigrants from Thrace or from other regions of the old Ottoman Empire.

The hematological findings along with the findings of the previously reported patients are given in Table I. The peripheral blood smear revealed slight anisocytosis and some target cells (Figure 1). The serum bilirubin level was 3.5

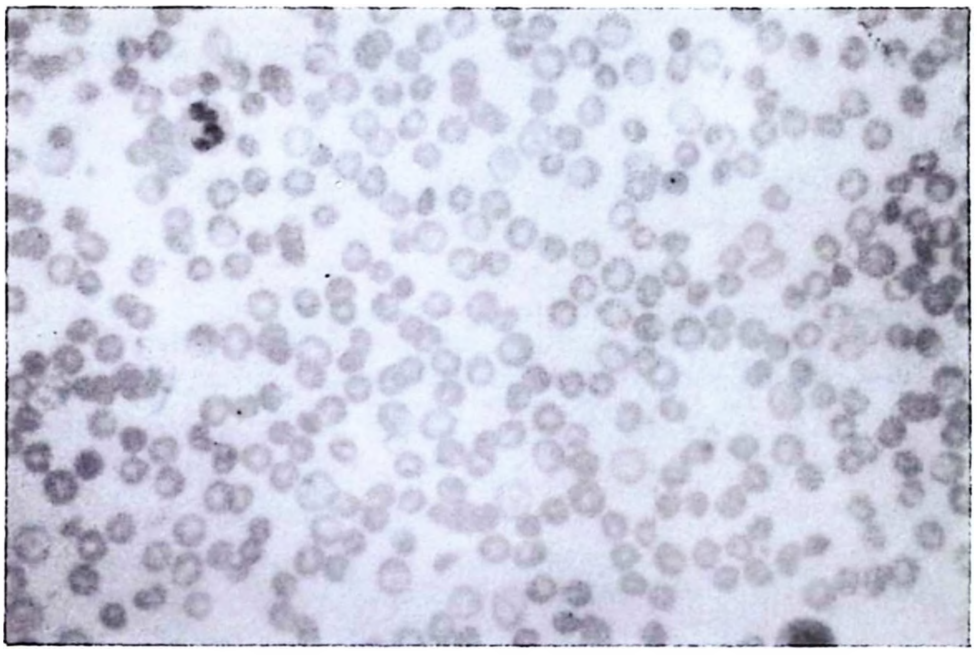

Figure 1

Peripheral smear of the propositus.

mg/dl, and direct bilirubin was 1.5 mg/dl on admission; the total bilirubin level taken one week later was 0.8 mg/dl. Hemoglobin electrophoresis on starch gel at pH 9.0 revealed that the propositus and one of his sisters were homozygous for a hemoglobin variant with Hb A_2 mobility while both parents were heterozygous for the variant[13] (Figure 2). The abnormal hemoglobin was segregated from the hemoglobin A in citrate agar gel electrophoresis at pH 6.4. The abnormal β^X chain was separated from β^A by acid urea triton X-100 polyacrylamid gel electrophoresis[14] (Figure 3). Abnormal hemoglobin was quantitated together with Hb A_2 by microcolumn chromatography[13] and the results are shown in Table I. Fetal hemoglobin was measured by Betke et al's alkali denaturation technique[15] and found to be within normal limits in all individuals. The serum iron was elevated in the propositus and the affected sister (40 and 47 µmol respectively). In the patient the red blood cell survival time by

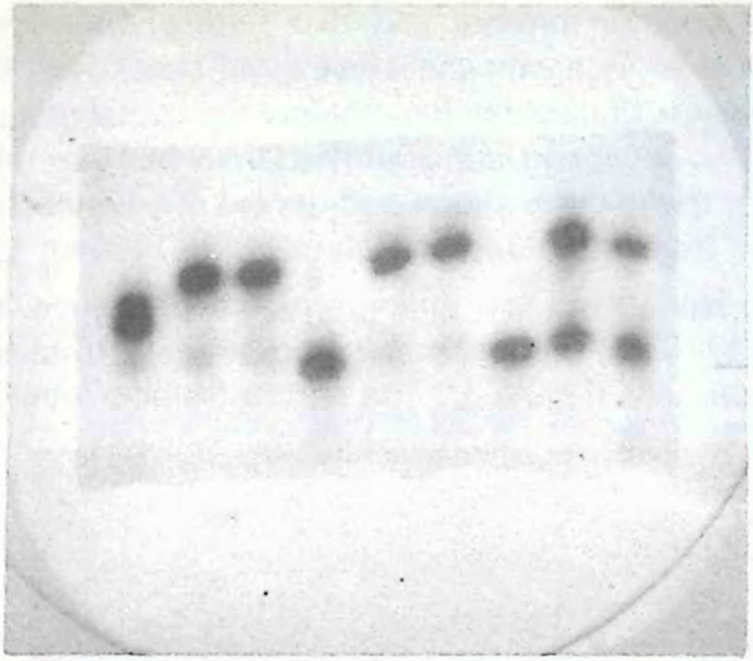

Figure 2

**Starch gel electrophoresis of the hemolysates at pH 9.0.
From left to right Hb A, Hb F, Hb A, Hb A_2 Hb O-Arab (propositus);
Hb A, Hb A_2, Hb O-Arab (affected sister);
Hb A, Hb O-Arab (mother); Hb A, Hb O-Arab (father).**

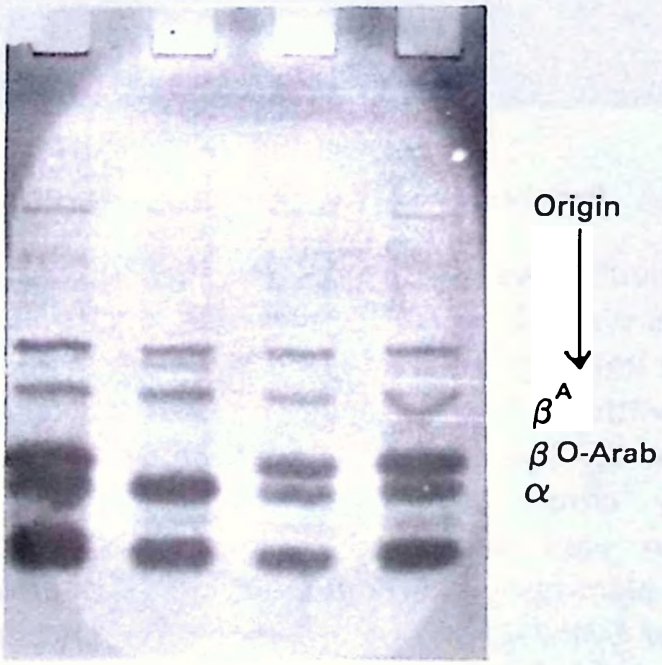

Figure 3

**Acid urea triton X-100 acrylamid gel electrophoresis.
From left to right $\alpha, \beta^A, \beta^{O\text{-}Arab}$, $\alpha, \beta^{O\text{-}Arab}$, $\alpha, \beta^A, \beta^{O\text{-}Arab}$,$\alpha, \beta^A, \beta^{O\text{-}Arab}$**

^{51}Cr was 21 days (normal 24-25 days) but without elevation of splenic sequestration. Red cell glucose 6-phosphate dehydrogenase (G6PD) activity was normal, the serum haptoglobin was zero in both affected siblings.

The scanning examination of the liver and spleen showed functionally inactive splenomegaly. A bone marrow smear from the propositus revealed an erythroid/ myeloid ratio of 2/1. One hundred ml of washed erythrocytes of the propositus were sent to the Protein Chemistry Laboratory, Augusta, Ga, USA, by air parcel post, for structural analysis and for quantitation of hemoglobins by HPLC (Table I). The structural analysis of the abnormal hemoglobin was performed by HPLC and showed that glutamic acid of the 121st residue of the β-chain was replaced by a lysine[16]. This indicates that the abnormal beta variant is Hb O-Arab.

The results, after 20, 60 and 120 minutes incubation, of *in vitro* hemoglobin synthesis analysis of the peripheral reticulocytes of the propositus and the affected sister, are shown in Table I. The α/non α ratio was 0.98 for one and 0.99 for the other homozygous individual, after two hours of incubation.

Discussion

The α /non α ratio is usually expected to be higher than 1.0 in individuals with a β-chain variant due to the instability of the abnormal hemoglobin or its decreased rate of synthesis. In these cases the α/non α ratios after 20 minute and 60 minute incubations were quite similar to those obtained after two hours of incubation; this indicates that the ratio of synthesis of $\beta^{O\text{-Arab}}$ is not less than that of the α-chain and there is no increased destruction of the newly synthesized $\beta^{O\text{-Arab}}$ The lower percentage of Hb O-Arab compared to Hb A in the peripheral blood and the β^A / β^O ratio of 1/6 in the *in vitro* Hb synthesis in heterozygotes may indicate that the affinity of the α-chain to $\beta^{O\text{-Arab}}$ may be lower than its affinity to the β^A chain[1-12].

The slight decrease in red blood cell survival, the absence of increased splenic sequestration in spite of splenomegaly in scanning studies and low reticulocyte counts, together with mild morphological abnormalities in the red cells indicate that hemolytic anemia is mild in Hb O-Arab disease. The episodes of jaundice observed in the propositus suggest that infections may increase the rate of hemolysis.

In the two similar cases previously reported the anemia was more severe than it was with the patients presented in this paper[3,11]. This indicates the need for more studies of new patients to get a clearer outline of the hematological picture of the disease. Splenomegaly is the common clinical finding in all four patients and this is probably the result of increased mild hemolysis[3,11]. The absence of reticulocytosis in spite of increased normoblastic activity in the bone marrow and the lack of increased splenic sequestration may be regarded as a sign of

ineffective erythropoiesis. That at least part of the hemolysis occurs intravascularly is suggested by the observation that haptoglobin was absent from the serum of both affected subjects.

Summary

A detailed clinical and hematological study – including *in vitro* hemoglobin synthesis analysis – of a family in which two siblings are homozygous for Hb O-Arab is presented and the relevant literature is discussed.

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