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ALPHA-THALASSEMIA IN A POOL OF INDIVIDUALS OF ETI-TURK ORIGIN WITH HEMOGLOBIN S (Hb S)*

*Hassan Jama MD***, Aytemiz Gürgey MD***, Çiğdem Altay MD*****

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Sickle cell disease is considered to be a heterogenous disease which includes a variety of sickling disorders with the gene for sickle hemoglobin (Hb S)[1-3] in common.

The characteristic clinical and hematologic diversities among homozygous sickle cell individuals have been the subject of various studies. Some patients have severe repetitive vaso-occlusive crises resulting in organ failure while others have no such clinical manifestations[1-3]. Alpha thalassemia has been given a particular position regarding the factors responsible for the clinical and hematologic variations in sickle cell anemia[3].

The incidence of Hb S has been reported to be high in the southern part of Turkey amongst an ethnic group known as the Eti-Turks[4-6], and α -thalassemia and hemoglobin H (Hb H) disease have been documented in the same area[7,8].

We reviewed our patients with homozygous sickle cell anemia for the interaction of α -thalassemia in sickle cell anemia.

Material and Methods

Twenty homozygous sickle cell anemia patients from 16 families and 31 heterozygous parents were included in this study. The ages of the patients ranged from nine months to 20 years (mean range 8.6 $\pm$ 6.0 years). There were 11 girls and 9 boys.

Standard hematologic techniques were used for routine hematologic studies and erythrocyte indices were taken with an electronic cell counter[9]. Hemoglobin electrophoresis was carried out on starch gel at pH 9.0 and agar gel

* From the Hematology Unit, Hacettepe University Institute of Child Health, Ankara.

** Formerly Resident in Pediatrics, Hacettepe University Institute of Child Health. Currently in Somalia.

*** Associate Professor of Pediatrics, Hacettepe University Institute of Child Health.

**** Professor of Pediatrics and Hematologist, Hacettepe University Institute of Child Health.

electrophoresis at pH 6.4. Hb S was determined by the cellulose ace method[10]. Hb A_2 was measured by microcolumn chromatography and Hb F Betke's alkali denaturation techniques[10,11]. The α : non α -ratio was determine by globin chain synthesis in the peripheral blood reticulocytes[12]. The serum iro and serum iron-binding capacities were calculated in all patients so that iro deficiency anemia could be excluded[9].

For the diagnosis of thalassemia interacting with SS, the criteria shown in Table were used[13].

TABLE I: Criteria for the Presence of α -thalassemia in Homozygotes (S and Heterozygotes (AS) Sickle Cell Anemia*

Suspected genotype	MCV (f1)	MCH (pg)	α:non-α	Hb S (%) in AS patients
1. $\alpha\alpha/\alpha\alpha$	≥ 81	27	≥ 0.85	≥ 36.8
2. $-\alpha/\alpha\alpha$	72-80	23.6-26.5	0.76-0.84	31-36.8
3. $-\alpha/-\alpha$ or $--/\alpha\ \alpha$	≤ 71	≤ 23.5	≤ 0.75	≤31

* Ref. 13

Results

Some of the hematologic findings are shown in Table II. Figures 1-4 indicate th distribution of the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), the α : non α -ratio and Hb in homozygous sickle cell anemia (SS) and heterozygous parents (AS).

The distribution of various types of α -thalassemia among SS patients and AS parents predicted by the MCV and the α : non α -ratio are shown in Tables III and IV.

Among the SS patients there was one patient (Table II, No. 14) with MCV of 71 fl. This patient also had a low MCH value and a α : non α -ratio of 0.75. These findings suggested the diagnosis of either homozygosity for α -thalassemia-2 or heterozygosity for an α-thalassemia-1-like trait Hematologic data of this patient's family is shown in Table V. Another patient (Table I, No. 13) presented with

TABLE II: Hematologic Data of Patients with Hb SS

Patient	Age (yrs.)	Sex	Hb (g/dl)	MCV (fl)	MCH (pg)	Hb A_2 (%)	Hb F %	α:nonα	Possible genotype	Clinical severity
1. H.K.	9	M	7.03	105	26	2.7	12	1.02	αα/αα	Mild
2. S.K.	6	M	6.03	92	26	2.9	9.1	1.03	αα/αα	Mild
3. D.K.	20	F	8.47	98	26.5	3	12.1	0.90	αα/αα	Mild
4. Ş.Ö.	10	F	8.64	97	37	3.5	0.5	0.97	αα/αα	Mild
5. D.S.	9/12	F	8.79	83	28.4	2.1	8.3	1.05	αα/αα	Mild
6. S.Ö.	6	M	6.23	92	24	2.6	10	0.99	αα/αα	Mild
7. A.Ç.	20	F	9.27	95	33.1	3	9	1.41	αα/αα	Mild
8. D.B.	3	M	7.51	84	32.7	3	4.1	0.89	αα/αα	Mild-moderate
9. L.T.	12	M	6.39	90	29	4	3.3	0.90	αα/αα	Mild-moderate
10. S.A.	1.5	F	9.11	80	26.8	5	3.5	1.07	αα/αα	Mild
11. E.A.	2	M	7.03	89	30.6	0.5	3.4	0.80		Mild
12. E.T.	8	M	7.19	95	37.8	5	3.5	0.80	-α/αα	Mild
13. S.D.	17	F	7.99	80	24.2	2.8	5	0.81	-α/αα	Severe
14. A.D.	15	M	10.23	71	24.4	3.7	15	0.75	-α/αα	Very severe
15. I.I.	18	M	9.59	95	33.1	4.2	5.8	0.70	-α/-α or --/αα	Very severe
16. Ö.S.	10	F	7.90	82	29.3	3.3	4.6	0.64	-α/-α or --/αα	Mild moderate
17. Y.D.	4	M	8.31	85	34.0	2	8.4	0.53	-α/-α or --/αα	Severe (finger necrosis)
18. F.Ç.	18	F	8.15	98	35.4	2	12	0.47	-α/-α or --/αα	Severe (multiple osteomyelitis) moderate
19. S.Ö.	2.5	M	7.19	88	27.7	2.6	10	0.50	-α/-α or --/αα	Mild-moderate-severe
20. L.K.	16	M	7.67	85	34	2	2.4	0.23	-α/-α or --/αα	Moderate-severe

TABLE III: Prediction of α-thalassemia in SS Patients According to the MCV and α:non α-ratio

Suspected Genotype	MCV (fl)	Number of patients	α:nonα-	Number of patients
αα/αα	≥ 81	18	≥ 0.85	10
- α/αα	80-72	2	0.76-0.84	3
- α/αα or --/αα	≤ 71	1	≥ .75	7

Note: The presence of severe or mild α-thalassemia in SS is not always predicted by changes in the MCV value.

TABLE IV: Prediction of α-thalassemia in AS Cases According to MCV, α:nonα-and Hb S (%)

Suspected genotype	MCV (fl)	α:nonα-	Hb S (< 36%)
αα/αα	23*	9	20
-α/αα	7	3	6
-α/-α or --/αα	1	10	4
Total Number	31	22	30

* Figures indicate number of subjects.

α -thalassemia-2 heterozygosity characterized by an MCV of 80 fl., an MCH value of 24 and an α : non α-ratio of 0.81. Two AS parents presented with an α -thalassemia-2 homozygosity or heterozygosity for an α -thalassemia-1-like trait with an MCV value of 66 and 71 fl respectively. The Hb S level was 24% in one parent and 32% in the other. Hematologic data of the latter is shown in Table V. Three AS parents had heterozygosity for an α -thalassemia-2-like trait which was characterized by an MCV value of 73-78 fl. (Figs. 1,5).

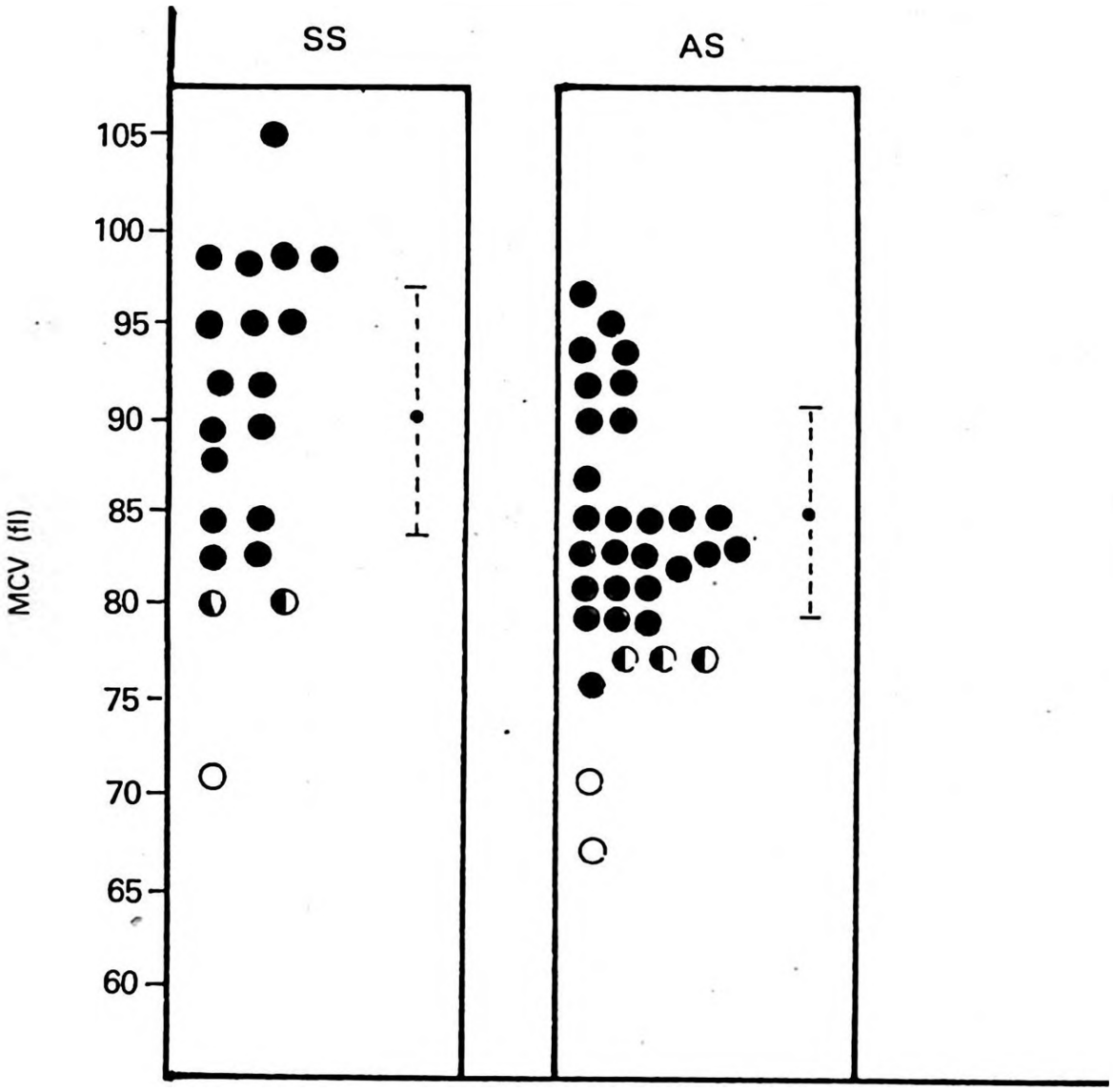

Fig. 1 : Distribution of MCV in AS and SS subjects:
- ● Four α-genes present
- ◑ α-thalassemia-2 heterozygosity
- ○ α-thalassemia-1 heterozygosity or α-thalasemia-2 homozygosity

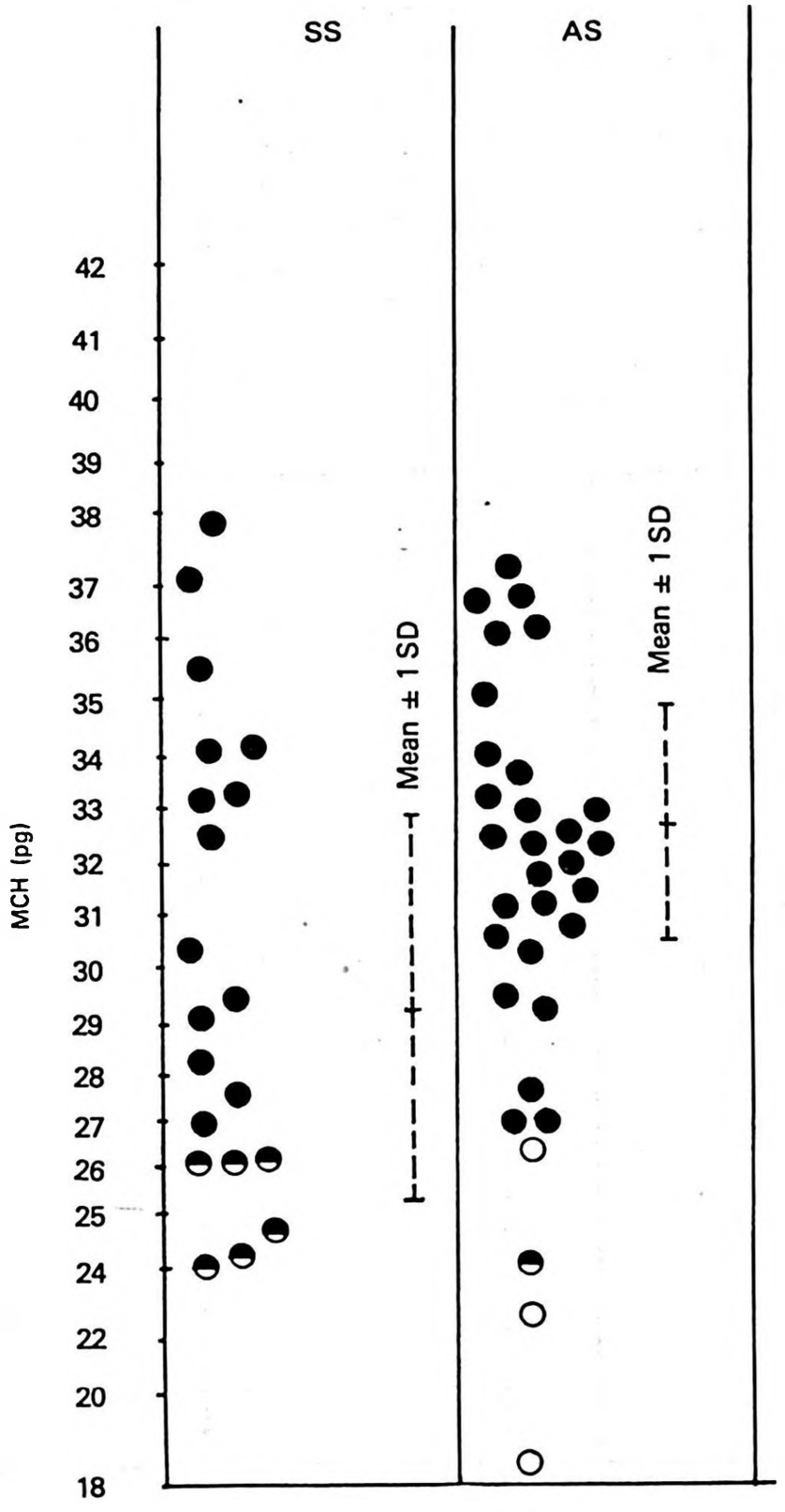

Fig. 2 : Distribution of MCH in SS and AS subjects

- ● Four α-genes present
- ◑ α-thalassemia-2 heterozygosity
- ○ α-thalassemia-1 heterozygosity or thalassemia-2 homozygosity

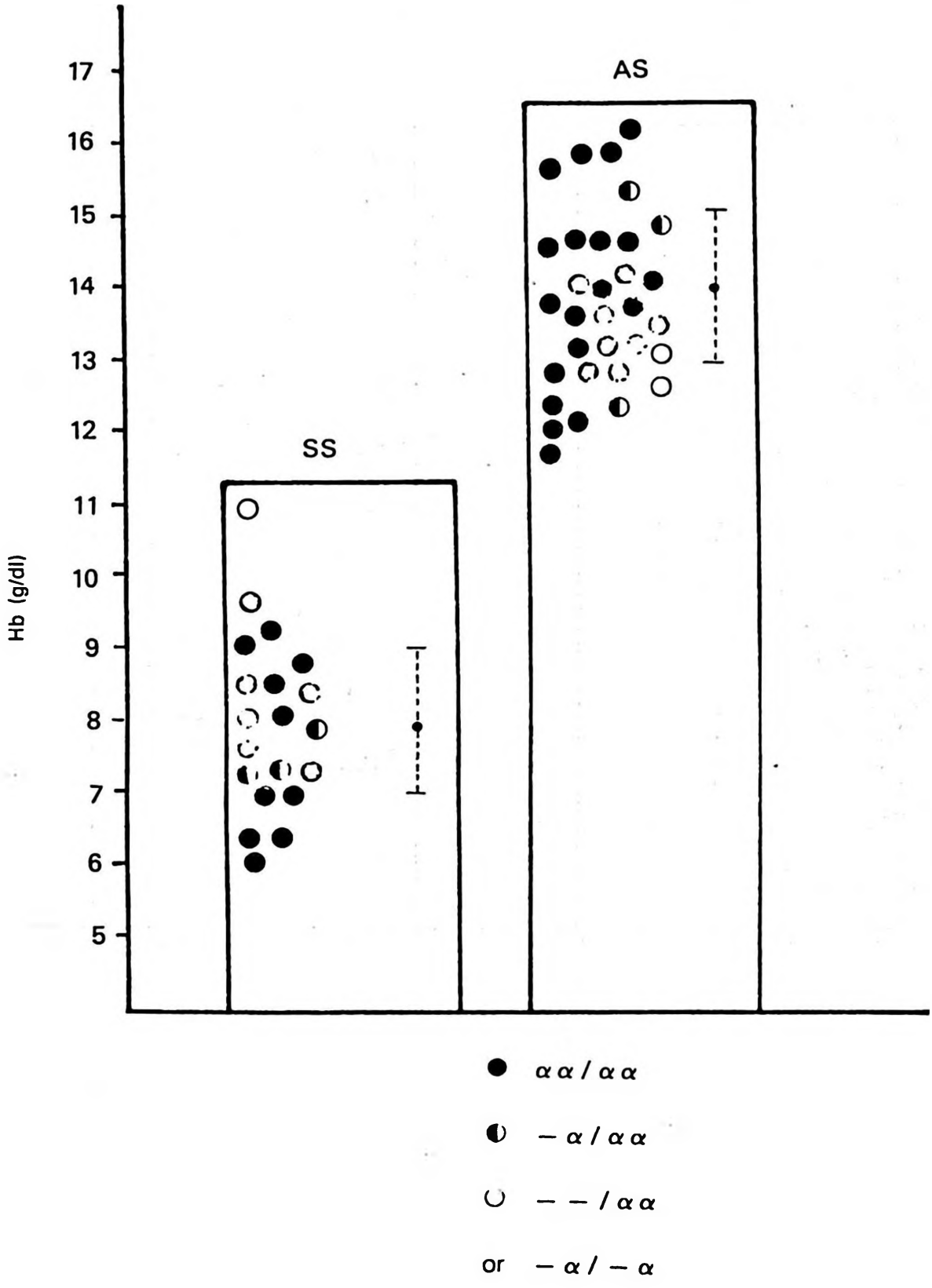

Fig. 3 : Distribution of hemoglobin among SS and AS subjects according to their genotypes predicted by the α: non α-ratio:

● Four α-genes present

◑ α-thalassemia-2 heterozygosity

○ α-thalassemia-1 heterozygosity or α-thalassemia-2 homozygosity

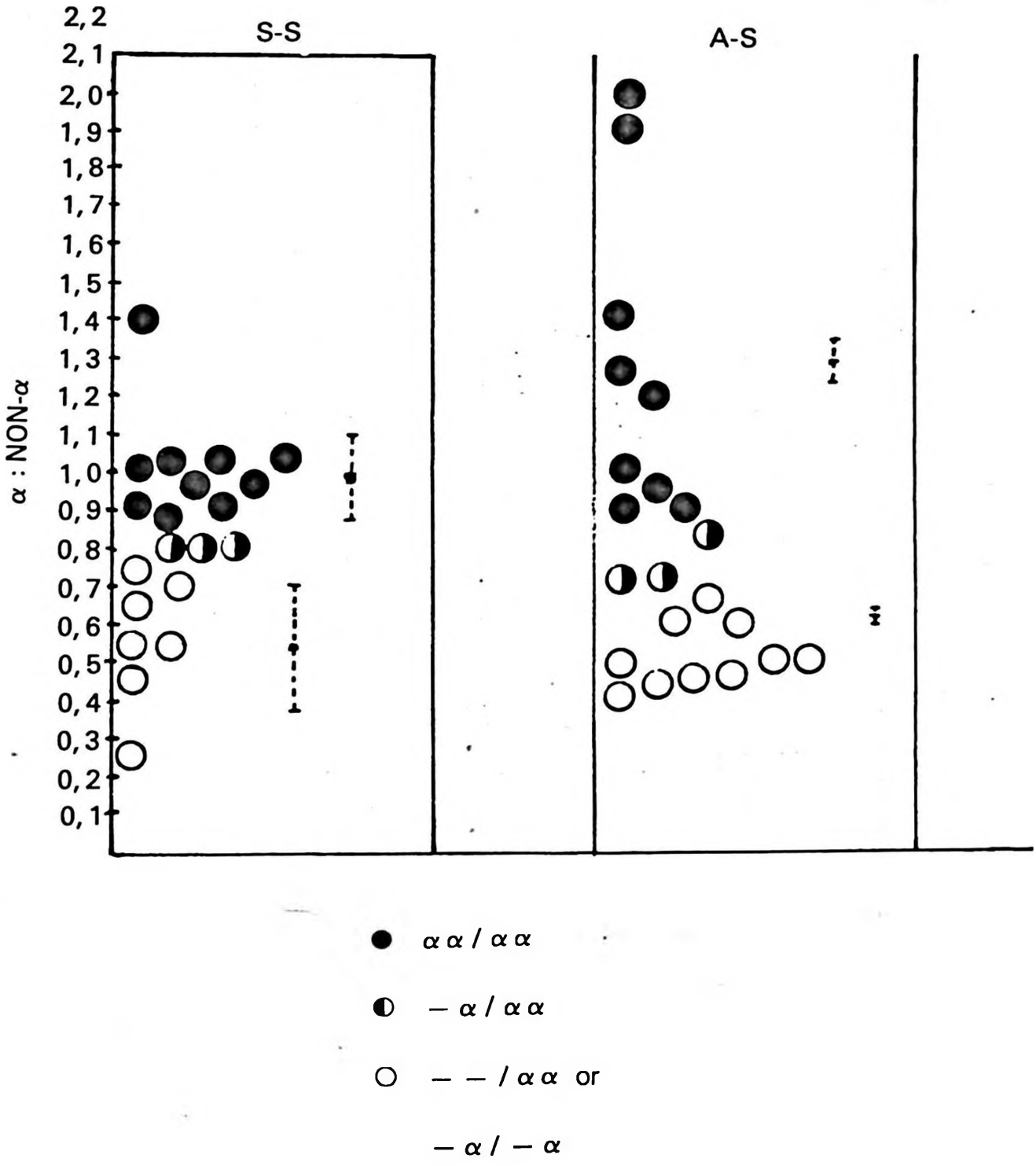

Fig. 4 : Distribution of α: non α-ratio of peripheral blood reticulocytes in SS and AS subjects:
- ● Four α-genes present
- ◑ α-thalassemia-2 heterozygosity
- ○ α-thalassemia-1 heterozygosity or α-thalassemia-2 homozygosity

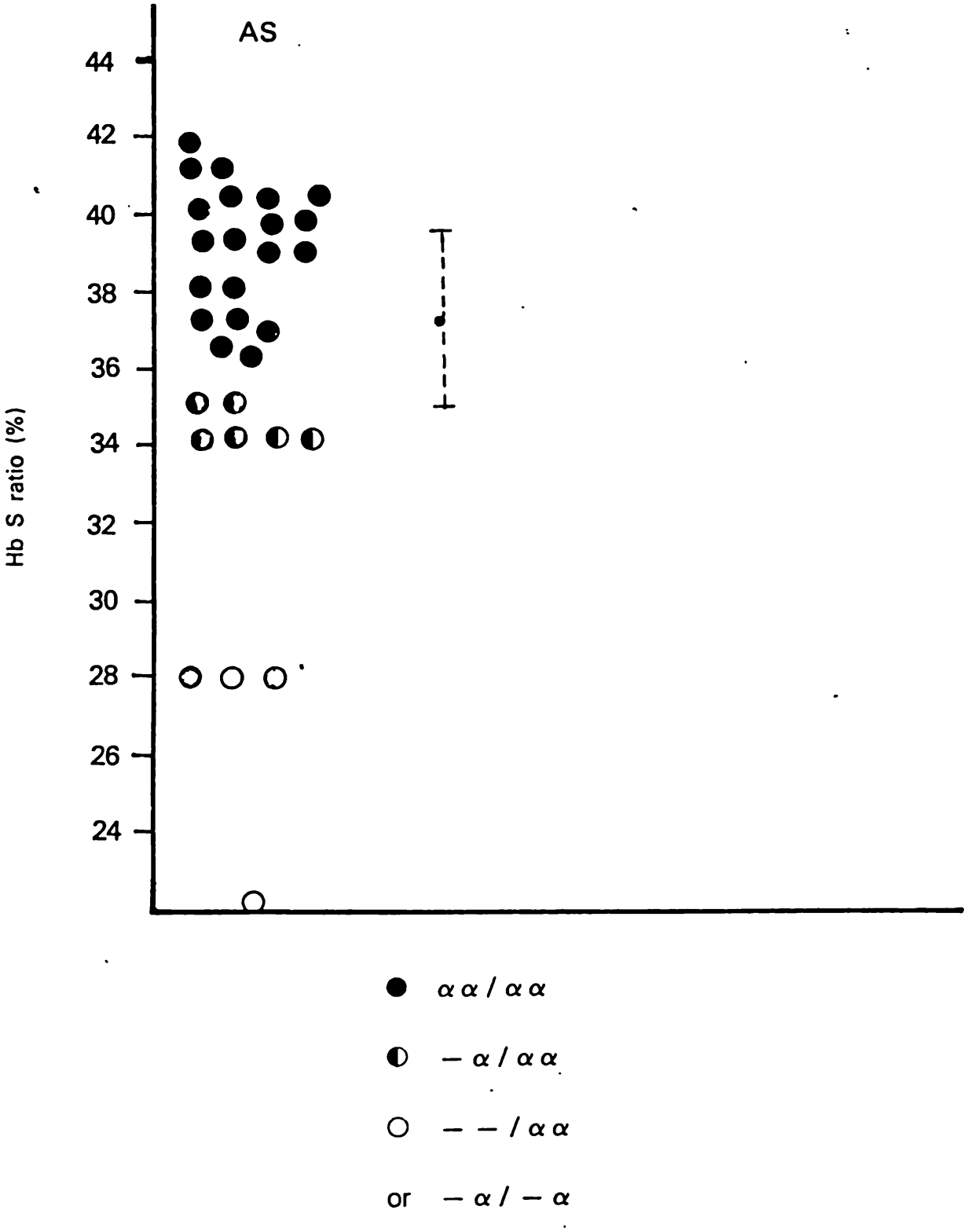

Fig. 5 : Distribution of Hb S % in AS subjects.
- ● Four α-genes present
- ◑ α-thalassemia-2 heterozygosity
- ○ α-thalassemia-1 heterozygosity or α-thalassemia-2 homozygosity

TABLE V: Hematologic Data of Family Members of Patients with Severe and Frequent Vasoocclusive Crises

Subject	Age (yrs.)	Sex	Hb (g/dl)	MCV (fl)	Hb A_2 (%)	Hb F (%)	Hb S (%)	α: non-α ratio	Possible	genotype
1. Mother	46		13.6	71	4.0	0.6	32.0	0.75	--/αα or -α/α-	AS
2. Father	44		15.5	77	3.5	1.5	34.0	–	-α/αα	AS
3. A.D. (propositus)	14	M	10.2	71	3.7	15.0	81.3	0.75	--/αα or -α/α-	S/S
siblings										
4. A.D.	26	F	12.5	75	2.1	2.0	28.0	–	--/αα or -α/α-	AS
5. S.D.	22	F	11.5	77	3.1	2.1	29.0	–	--/αα or -α/α-	AS
6. G.D.	20	F	13.7	74	3.3	1.5	35.0	–	-α/αα	AS
7. K.D.	16	M	14.9	73	4.2	1.5	36.0	–	-α/αα	A/S

The Hb levels were found to be low in all SS patients, except in one (Table V, No. 14, Fig. 3), where there was severe α-thalassemia (homozygote for α-thalassemia-2) or heterozygosity for the α-thalassemia-1-like trait (Fig. 3). Figure 4 shows the distribution of the α : non α-ratio in SS and AS parents. According to this ratio, there were seven patients with α-thalassemia-2 homozygosity (35%) and three with α-thalassemia-2 heterozygosity (15%) (Table III).

According to the α : non α-ratio in the AS parents there were ten cases with α-thalassemia homozygosity (32%) and three parents with thalassemia-2 heterozygosity (Table IV). The Hb S ratio in AS parents is shown in Fig. 5. The Hb F levels was found to be more than 9% in eight patients of which only three had coexistent α-thalassemia (Table II).

Discussion

This study indicates that α-thalassemia is not uncommon in the Hb S population in Turkey. This complies with previous studies which indicate that α-thalassemia, Hb H disease and Hb S are frequently observed in the Çukurova region[4-8]. Therefore the occurrence of α-thalassemia in association with Hb S can be expected. The diagnosis of α-thalassemia was made in 50% of the SS patients and in 41% of the AS parents by in vitro Hb synthesis. In some of these cases MCV, MCH and Hb S values were within normal limits. This indicates that MCV, MCH and quantitation of Hb S are not sufficient for the diagnosis of the coexistence of α-thalassemia in the SS and AS subjects. The cases of α-thalassemia were found to be even higher when the gene-mapping technique was used in the AS and SS subjects with normal MCV and MCH values[14]. More interestingly, α-tahalassemia was shown in cases with an α : non α-ratio of about 1.0. However in all of the cases with a low α : non α-ratio, α-thalassemia was found to be present by gene-mapping as well[14]. In sickle cell disease there are certain factors responsible for the clinical diversity. The coexistence of α-thalassemia was considered to be one of these factors[1-3,15]. Although there is no agreement on the role α-thalassemia plays in the amelioration of the clinical picture of Hb SS, it is accepted that α-thalassemia results in an increase in hemoglobin value[1,3].

In our study, seven patients were diagnosed as having a severe α-thalassemia trait. Four of these patients had experienced severe clinical manifestations which consisted of frequent painful crises, aseptic femoral necrosis, multiple osteomyelitis and amputation of a finger due to necrosis. With the other three patients, the course of the disease was relatively milder but still more severe than with the Hb SS patients without the α-thalassemia combination.

The highest Hb and Hb F levels were found in a patient with severe α-thalassemia. In the other six patients with severe α-thalassemia, the Hb F values were not found to be high. In eight patients, the Hb F level was more than 9%; and among these patients only three were diagnosed as having severe α-thalassemia.

Although in patients AD (Table II, No. 14, Fig. 2) and SD (No. 13) the high Hb F level was possibly the result of the coexistence of α-thalassemia, we do not believe that this could have been the case with FC (Table II, No. 18), since the Hb SS sibling of this patient (Table II, No. 7), who had no signs of thalassemia, did have a high Hb F level.

In this family and among the other five patients with an increased Hb F level without α-thalassemia, a haplotype associated with an increased Hb F might be present. In the literature there are suggestions regarding the positive influence of Hb F on the clinical severity of sickle cell anemia. Some authors, though, have disagreed regarding this point[16-20]. A high Hb F level, together with mild clinical symptoms is probably related to the haplotype in which Hb S mutation occurred. Recent studies have shown that in SS patients with haplotype 3 and 31, the Hb F levels are higher than among patients with other haplotypes[21-24]. In our study there were five patients with high Hb F levels. It appears that in our patients, the Hb F levels did not influence the clinical severity of the sickle cell anemia. Hb S and α-thalassemia are frequently observed in the Çukurova region. This study indicates that α-thalassemia often occurs in populations in Turkey where Hb S is present. In a recent study conducted among the Eti-Turks, it was indicated, with the gene-mapping technique, that the prevalence of α-thalassemia was 17%[23]. The figure for coexistent α-thalassemia in our patient pool was much higher than that in the above-mentioned study. The discrepancy in these figures is probably due to the fact that our patients were not selected at random. It is also well known that non-deletion types of alpha thalassemia (T)- determinants exist in Turkey[8]. Therefore to obtain more reliable data regarding the incidence of alpha thalassemia, mapping studies should include the restriction enzymes necessary to detect the mutations responsible for these α-thalassemia determinants.

The gene-mapping studies and haplotype analyses were necessary to obtain accurate data regarding the frequency of α-thalassemia and to better understand the factors responsible for the clinical diversity of our SS patients. The presence of α-thalassemia seems to cause an increase in hemoglobin levels but without any beneficial effect on the clinical severity. In fact, bone necrosis and painful crises seem to be more frequent in these subjects than in those without α-thalassemia.

Summary

We studied 2-0 homozygous sickle cell anemia patients from 16 families and 31 heterozygous parents. In vitro hemoglobin synthesis analysis indicated that

α-thalassemia was present in 50% of the Hb SS patients and in 41% of the Hb AS parents. The MCV and MCH values were not reduced in all the patients with α-thalassemia. Clinical and hematologic evaluations of the patients indicated that the coexistence of α-thalassemia in SS patients seems to result in an increase in hemoglobin levels without, however, having any beneficial effect on clinical severity.

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