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FATAL HEMOLYTIC ANEMIA PRESUMABLY DUE TO THE COMBINATION OF SICKLE CELL AND THALASSEMIA GENE

CASE REPORT

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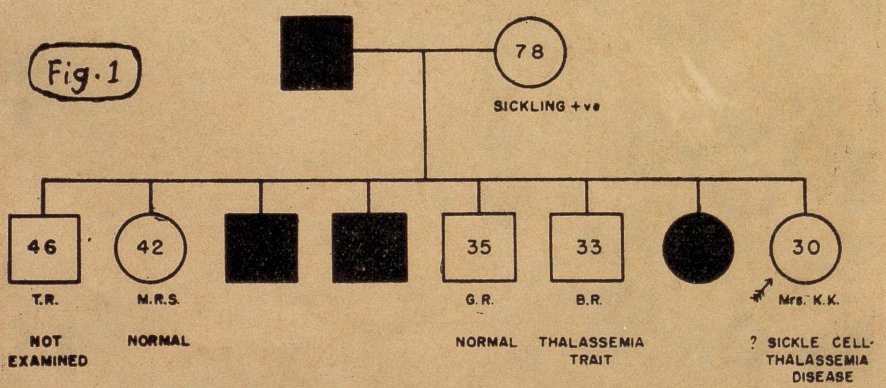
The subject of sickle cell anemia generally has been one of mere academic interest owing to the paucity or rather supposed absence of the disease in this country. Dunlop and Mozumdar[4], however, reported some cases of sickle cell anemia among a group of tea garden labourers in Upper Assam, and this according to them was the first time this anomaly was reported from India. Careful scrutiny of the data given shows that diagnosis of all the cases was based on the morphological study alone which can be often misleading. Sickle cell trait has been reported from the Nilgiri Hills in South India (Lehmann and Cutbush[5]) and from Uttar Pradesh by Bhatia *et al*[1]. Recently, Sukumaran *et al*[12] have reported sicklemia in tribal population in Western India. Thalassemia or Cooley's anemia, though once considered a rare entity in India, has been reported in fair numbers from various parts of this country since 1939. Reports available in the literature show that this condition is no longer uncommon in this sub-continent.

This paper presents a case report of a patient who died of hemolytic anemia attributable to the combination of sickle cell and thalassemia gene.

Case Report

Case history.—A Gujarati Hindu female (Mrs. K. K.) from Saurashtra, aged 30 years, was referred to Dr. Balabhai Nanavati Hospital, Bombay, on 3rd October, 1955 with a history of pain over the right hypochondrium and of passing dark coloured urine. The patient was married 11 years ago and was a primipara, 8 months pregnant at the time of admission. She had her splenectomy done 3 years before presumably for 'acholuric jaundice'. A history of breathlessness and edema of 15 days duration and of severe pain on right side of the chest and right hypochondrium one week ago, was given by the patient.

Family history (figure 1).—



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As shown in figure 1 the father of the patient was dead, while the mother aged 78 years was living when her blood was examined, but in very poor health. Subsequently she took a serious turn and was lately reported dead. Three brothers and one sister died in childhood from unknown causes. Three brothers and one sister are living and apparently healthy. The only paternal uncle was dead. Two uncles and some cousins are alive.

Clinical examination.—The patient was very anemic, with edema over the legs. The pulse was 130 per minute, and blood pressure 126/80 mm. of mercury. The neck veins were not seen with the patient in propped up position. The heart was enlarged, and there was systolic thrill and murmur in the mitral area. Over the lungs were observed hyper-resonance on the left side, and dullness over the right base. The liver was not palpable. Scar of splenectomy was seen. On fluoroscopy, the heart was found to be enlarged, and there was haziness in the right costophrenic angle.

A presumptive diagnosis of anemia of pregnancy with congestive cardiac failure was made. The patient was treated for the latter condition and was also given transfusion of 150 ml. of blood. She went into labour at 1-30 A.M. on the 5th of October, and expired 4 hours later. During the brief period the patient was alive since her admission to the hospital, a specimen of blood was all that could be examined. The result of the examination of this specimen of blood before transfusion is included in the table together with the result of a similar study on her two brothers and one sister.

Hematological examination showed no apparent abnormality in one brother G.R. and sister M.R.S. The other brother B.R. showed hypochromia and microcytosis with a mean corpuscular hemoglobin of 21 *rr*. The mean corpuscular volume was the lowest in this brother. Besides this, his blood smear showed a fair number of target cells (figure 2) and slight anisocytosis with a markedly decreased osmotic fragility. In spite of administration of iron his blood picture showed no improvement.

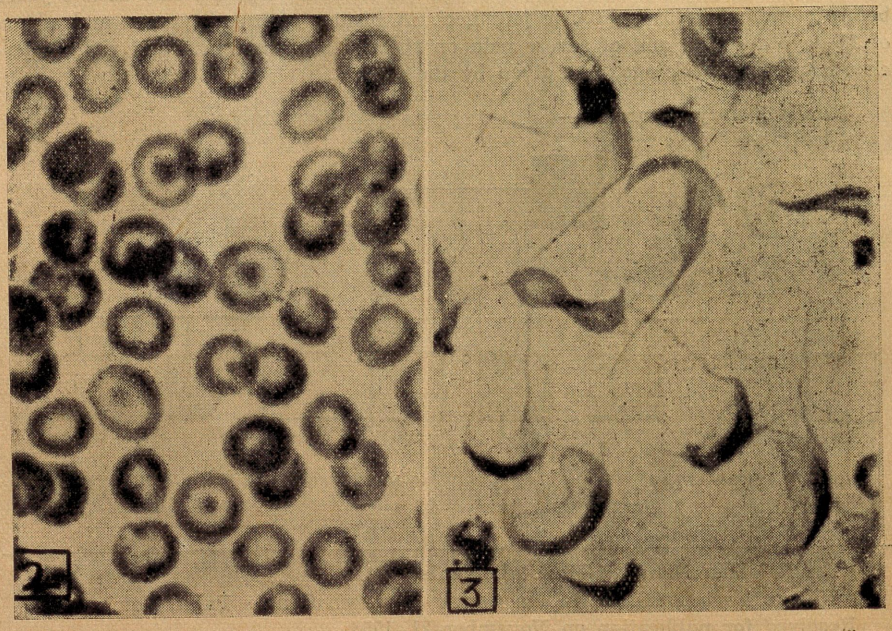

TABLE 1: *Hematological Data of the Propositus and Her Sibs*

	RBCx10^6 /cmm.	Hb.G. %	WBC/ cmm.	PCV %	MCH $\gamma\gamma$	MCV μ3	MCHC %	Blood picture	Osmotic fragility	Electro-phoresis
Mrs. K. K. (Propositus)	2.05	5.0	52,000*	22	24	107	22.7	Hypochromia ++ Anisocytosis ++ Polychromasia ++ Target cells ++	Not done	S†
B. R. (Brother)	5.70	12.0	5,500	41	21	72	29.3	Anisocytosis + Target Cells ++	Markedly Decreased	A
G. R. (Brother)	4.80	14.5	7,900	43	30	90	33.7	Nothing Abnormal	Normal	A
M. R. S. (Sister)	4.55	11.5	**	38	25	84	30.3	Nothing Abnormal	Not done	A

* This includes normoblasts 27,000/cmm.

** Total white blood cell count was not done; the cellularity of the peripheral smear, however, suggested a normal count.

† Refer text.

Serum and plasma of the propositus were markedly icteric.

Sickling test.—2% freshly prepared sodium metabisulphite was used for this test. The patient's blood was found to be positive, and nearly all the cells showed sickling. The sickling was of the filamentous type (figure 3). The blood of the patient's mother when tested by the same method gave sickling instantaneously, the sickling being holly-leaf type. A permanent preparation using formol-saline after overnight incubation at 37 C in the usual way under a layer of liquid paraffin was made with the patient's blood, but in the case of the mother, this could not be accomplished as a drop of blood was all that was available for this study. Repeated examination of the two brothers and one sister of the patient showed no sickling.

Abnormal hemoglobins.—Abnormal hemoglobin study was carried out by zone electrophoresis using Whatman 3MM filter paper strips. Horizontal type of apparatus with paper supported between siliconized glass plates according to the technique described by Smith and Conley[11] was used, with veronal buffer of pH 8.6 and ionic strength 0.05. Determination of alkali resistant hemoglobin was made by the method described by Singer et al[10].

Hemoglobin samples from the patient, her two brothers and one sister were examined for the type of hemoglobin present by the methods described above. A sample of hemoglobin A from a normal adult, and hemoglobin A and S from a case of sickle cell trait (Lehmann and Sukumaran[6]) were used as controls in this study. Hemolysate from the patient was found to move as almost a single band with a mobility similar to the hemoglobin S fraction in the control, while samples from two brothers and one sister showed pattern having only one type of hemoglobin, mobility being similar to adult type. Alkali denaturation technique showed the presence of 6 per cent of fetal hemoglobin in the patient and in others this fraction was found to be within normal range. Further investigation to determine the exact amount of non-sickle hemoglobin in the patient could not be made as the blood available for study was insufficient.

Discussion

The marked erythroblastemia, bilirubinemia and reticulocytosis (polychromasia++) taken in conjunction with the patient's history of passing dark coloured urine, incriminates a hemolytic factor and rules out hydremia and hematinic deficiencies from being the primary cause of anemia. The long filamentous type of sickling of erythrocytes though encountered in sickle cell anemia, and used as one of the diagnostic criteria, has also been found in severe cases of microdrepanocytic disease while holly-leaf type is seen in sickle cell trait (Powell *et al*[8]). The diagnosis of the case reported here would have been easily accomplished had the father been living or the paternal relatives available for investigation. In the absence of this, various possible conditions associated with sickling have to be considered and by elimination a most probable diagnosis has to be arrived at. It is now generally recognised that there are at least four other conditions simulating sickle cell disease and difficult to distinguish clinically or hematologically. There are described in literature five types of hemolytic anemia associated with sickling, and are in their order of frequency:

(1) Sickle cell anemia—a true sickle cell disease resulting from the inheritance of two sickle cell genes, one from each parent.

(2) Sickle cell-hemoglobin C disease—resulting from the inheritance of single sickle cell gene from one parent and Hb-C gene from the other.

(3) Microodrepanocytic anemia—results from the inheritance of a gene for sickling from one parent and thalassemia gene from the other.

(4) Sickle cell-hemoglobin D disease—caused by the inheritance of a single gene for sickling from one parent and hemoglobin D gene from the other.

(5) Sickle cell-hemoglobin G disease—wherein sickle cell gene is inherited from one of the parents and hemoglobin G from the other.

The introduction of electrophoretic technique by Pauling *et al*[7] and alkali denaturation method by Singer *et al*[10] to identify abnormal hemoglobins has seen the emergence of these four distinct entities until then indistinguishable from the classic sickle cell anemia. The presence of sickle cell hemoglobin in almost pure form or in relatively high concentration in association with any of the other abnormal hemoglobins is now accepted as the final arbiter in classifying these anemias, while its association in lower concentration with normal hemoglobin decides the sickle cell trait. The possibility of this case being sickle cell-hemoglobin C disease is ruled out due to the absence of hemoglobin C in the patient and other siblings. The finding of thalassemia trait in one of the brothers characterized by the hematological findings, and the reported incidence of Cooley's anemia in many parts of India in the last decade, makes it weigh more in favour of a combination of these two genes responsible for this malady. The absence of sickling in any of the siblings in spite of repeated examinations, supported by the electrophoretic findings makes it probable that it may not be a case of sickle cell anemia. To support this view is the presence of thalassemia gene in one of the siblings which in all probability could not have been inherited from the mother who was a sickle cell trait. Hemoglobin D indistinguishable from sickle cell hemoglobin by paper electrophoresis has been reported recently from this country (Bird and Lehmann[2]). Hemoglobin D was not found in any of the siblings examined. Sickle cell-hemoglobin G disease is also ruled out considering the reported absence of fetal hemoglobin in abnormal amounts and the rare incidence of this condition in literature (Chernoff[3]). It may be argued that in sickle cell-thalassemia disease the pattern of hemoglobin should be that of S and A together with hemoglobin F as demonstrated by alkali denaturation technique. Hemoglobin analysis on this case repeated several times showed a pattern which was rather difficult to distinguish from that found in classical sickle cell anemia. It has been reported that in some instances the percentage of S hemoglobin in double heterozygous condition may be within the range of those encountered in homozygous sickle cell anemia which may vary from 76 to 100 per cent (Singer *et al*[9]). Evidence is available that in individuals carrying a combinaton of thalassemia gene and a gene for sickling, the former enhances the expressivity of the gene for pathological (Hb-S) pigment. Sickle cell-thalassemia disease, with hemoglobin S about 80 per cent, and non-sickle hemoglobin as high as 20 per cent including about 8 per cent fetal pigment, showed pattern indistinguishable from sickle cell anemia by paper electrophoresis (Singer *et al*[9]). In the light of this it is possible that in the present case the pattern obtained by paper electrophoresis is hemoglobin S+A+F, though adult pigment may be comparatively insufficient to give a better differentiation by this method. A study of the children (young infants) of the brother with Cooley's trait and the maternal uncles and cousins of the patient is called for with a view to throw more light on the diagnosis, and attempts are being made to trace and investigate them. Till then, a presumptive diagnosis of sickle cell-thalassemia disease has been given.

Summary

A fatal case of hemolytic anemia in a Gujarati Hindu woman from Saurashtra is described. The severe hemolytic syndrome in the patient was associated with filamentous type of sickling of erythrocytes with an electrophoretic pattern indistinguishable from that of sickle cell anemia, and one of the brothers showed a picture characteristic of thalassemia trait. The mother—the only living parent—who carried the trait showed holly-leaf pattern of sickling. The various possibilities of a double heterozygous condition are discussed and reasons are given for a presumptive diagnosis of microdrepanocytic disease.

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