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HAEMOGLOBIN-M DISEASE IN A PUNJABI HINDU FAMILY

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Hb- M disease is described in a Punjabi Hindu boy and his mother. The abnormal hemoglobins in these patients were found to be similar to Hb-M Iwate having alpha chain abnormality. A point of additional interest in the boy was the presence of erythrocytosis.

Introduction

A methaemoglobin differing spectroscopically from "normal" methaemoglobin was first described by Horlein and Weber (1948) in a German family with hereditary methaemoglobinaemia. The designation of this "abnormal" methaemoglobin as Haemoglobin-M (Hb-M) was suggested by Singer (1955). The fundamental abnormality in this disorder lies in a structural alteration of one globin chain in the vicinity of the attachment of the haem-moiety, This renders the haemoglobin molecule unusually sensitive to oxidation which results in permanent methaemoglobin formation. The structural mutation leading to the occurence of Hb-M involves the substitution of an aminoacid in the alpha or the beta polypeptide chain of the haemogiobin molecule.

Hb-M disease is transmitted as autosomal dominant, whereas hereditary methaemoglobinaemia due to enzymatic defect is transmitted as autosomal recessive. This haemoglobinopathic disorder is extremely rare and only two families with this disorder have been reported from India (Chatterjea 1961-62; Bajaj *et al.* 1973).

This paper describes for the first time Hb-M disease in a Punjabi Hindu family. An additional point of interest was the association of erythrocytosis in the propositus.

Case Report :

K. L. 19 years old Hindu male born of Punjabi Hindu parents hailing from East Punjab was admitted to the local Nehru Hospital on 12.5 1973 for mild cough with expectoration, a vague sense of generalised weakness and dyspnoea on exertion. The boy worked in a steel factory in a non-technical job, which required hard phyiscal labour. The symptoms were of recent origin extending approximately over a period of one month. On physical examination, he was found to have a

peculiar slate-grey cyanosis apparent on the face, lips, tongue, nails and skin. Further enquiry revealed that this type of cyanosis was present since his birth. He had normal physical build, good nutritional status with no other physical or developmental anomaly. He had no fever radial pulse rate of 90/min., respiratory rate of 20/min. There was evidence of mild bronchial catarrh. There was no clubbing of fingers or toes. Detailed examinations of the cardiovascular system including X-rays of the chest and electrocardiogram revealed no significant abnormality. There was no evidence of congestive cardiac failure. Liver and spleen were not palpable.

Family History :

Parents of this boy belong to a low middle class peasant family of East Punjab. The propositus has two brothers and four sisters all healthy with no cyanosis. The parents were also healthy and of active habits. However, the mother of the boy on examination showed similar type of cyanosis as her affected son, but she had no other subjective or objective abnormalities.

The propositus was successfully treated for bronchitis after which he was relieved of his symptoms, but cyanosis persisted. Oral administration of ascorbic acid in doses of 500 mg. twice daily for seven days did not reduce cyanosis appreciably. Similarly slow intravenous injection of methylene blue (2mg/kg. body weight) was ineffective. These observations suggested that mathaemoglobinaemia in this patient was unlikely to be due to any enzymatic defect.

Laboratory studies :

Routine haematological investigations were carried out as recommended by Dacie and Lewis (1969). Red cell mass, plasma volume, were estimated by the use of Cr^{51} labelled sodium chromate, and I^{125} labelled human albumin (Dacie and Lewis, 1968). Plasma erythropoietin was estimated by hypertransfused polycythaemic mouse radio-bioassay technique as described by Das et al. (1974 a, b.). Electrophoretic studies of ferricyanide treated and untreated haemolysate were carried out in starchgel (phosphate buffer pH 7. 1). The haematological findings are summarized in Table-I.

TABLE I

HAEMATOLOGICAL FINDINGS IN THE PROPOSITUS AND MOTHER

	K.L.	Mother
Hb (gm%)	17.0	15.0
PCV%	51	45

WBC/mm^3	8000	6500
	P-70	P-65
	L-27	L-32
	M-2	M-0
	E-1	E-3
E.S.R. (mm/hr)	1 mm	2 mm.
Platelets $mm^3 \times 10^4$	22	20.5
Hb-electrophoresis	A+M	A+M
Hb A_2 (%)	1.9	2.1
Foetal Hb. (%)	1.6	1.4
Red cell mass (ml /kg.)	46.5	
Plasma volume (ml./kg.)	45 5	
Erythropoietin (I.U/ml. plasma	0.1 (Normal range .05 to .1)	

It is evident that the patient had haemoglobin and haematocrit at the higher range of normal. Estimation of the red cell mass and plasma volume revealed significant erythrocytosis without increase of other formed of blood. The plasma erythropoietin level of the propositus (K. L.) was found to be at the higher range of normal.

Electrophoresis of haemoglobin and ferrihaemoglobin showed Hb. A and M fractions (Fig. 2). The sample was referred to Prof. H. Lehman who identified the abnormal haemoglobin fraction as "Haemoglobin-M Iwate having alphg chain abnormality (Alpha$_2$ 87 Tyrosine beta$_2$)".

Oxygen carrying capacity of the arterial blood of the propositus was found to be mildly reduced which was carrected to normal after oxygen inhalation (Table-2 .

The mother of the propositus also had erythrocytosis and presence of Hb A and M in her blood. Other members of the family did not reveal any abnormality.

TABLE II

ARTERIAL OXYGEN SATURATION

Without 02 inhalation	With 02 inhalation	Vit. C. injection
Propositus 91% K. L.	97%	96.6%

Discussion

Methaemoglobinaemia may occur as an acquired or a hereditary disorder. In the intact red cells, methaemoglobin is continuously formed due to oxidation of ferrous porphyrin complex into the ferric form which is promptly reduced back into ferrous form by an efficient system of reducing enzymes. Normal blood contains a very small amount of methaemoglobin (less 1.7%) which is functionally innocuous (Lemberg and Legge, 1949).

Acquired methaemoglobinaemia may result from overexposure to various industrial chemicals or therapeutic agents which include aniline, phenacetin, acetanilid, sulphonamides, nitroglycerine, various nitrites, nitrates, chlorates, quinomes aminobenzenes nitrobenzenes, nitrotoluenes (Finch 1948, 1949). Withdrawal of the offending chemicals and administrations of ascorbic acid and methylene blue may reverse the process.

Hereditary methaemoglobinaemias may occur due to two fundamentally different types of genetic defects-one type being associated with enzymatic defect(s) of the red cells, the other due to a structural abnormality of the haemoglobin molecule (Hb-M) The enzymatic defects leading to methaemoglobinaemia include mainly deficiency of DPNH diaphorase (Gibson 1948), and rarely deficiency of TPNH-methamoglobin reductase activity (Muller et al. 1963), and inadequate synthesis of glutathione (Townes and Lovell, 1961). The enzymatic defects are transmitted by autosomal recessive genes excepting possibly when this is due to inadequate synthesis of glutathione, which has been stated to be inherited as a dominant trait. Methamoglobinaemias due to hereditary enzymatic defects appear to respond favourably to the administration of ascorbic acid and methylene blue.

In methaemoglobinaemias associated with the presence of Hb-M, the red cell enzyme systems for reducing methaemoglobin fuction normally, but are ineffective in reversing the condition presumably due to the formation of permanent methaemoglobin, an inherent property of the peculiar structural alterations of the haemoglobin molecule. Admnistration of ascorbic acid or methylene blue has little or no effect on methaemoglobinaemia due to Hb-M disease.

Numerous variants of Hb-M have been described which differ in spectroscopic properties, electrophoretic mobilities, the type of globin chain affected and the amino acid substitutions. These include Hb-M Boston (Gerald 1958 ; Gerald and George 1959), Hb-M Milwaukee (Pisciotta et al. 1959) Gerald and Gfron, 1961), Hb-M Saskatoon (Gerald and George, 1959), Hb-M Leipzig (Betke et al. 1960), Hb M Iwate (Shibata et al. 1960), Hb-M Chicago (Joseephson et al. 1962), Hb-M KanKakee (Heller et al. 1962) and others (Jaffe and Heller, 1964).

Hereditary methaemoglobinaemia described in this paper in two members of a Punjabi Hindu family was incidentally detected. The affected individuals were practically free from symptoms related to methaemoglobinaemia. The propositus (K.L.) had mild bronchitis which was successfully treated, but slate –grey cyanosis psrsisted. Administration of ascorbic acid and methylene blue produced no significant change. Starch-gel electrophoretic studies in phosphate buffer of pH 7.1 revealed an abnormal fraction which was further characterised by Prof. Lehman as "an abnormal haemoglobin similar to Hb-M Iwate, having alpha chain abnormality (alpha 2.87 Tyrosine $Beta_2$)". The study of the family members showed features consistent with autosomal dominant mode of inheritance.

An interesting finding in the propositus was the increased red cell mass without elevation of other formed elements of blood. Secondary polycythaemia (erythrocytosis) has been observed in association with several abnormal haemoglobins, which have increased oxygen affinity (Weatherall 1971). Oxygen affinity has been found to be variably affected in Haemoglobin M (Stamatoyannopoulos *et al.* 1971 ; Weatherall, 1971). Those with alpha chain substitution of the proximal or distal histidine (Hb-M Boston, Hb-M Iwate), have reduced oxygen affinity, whereas those with beta chain substitutions (Hb-M Saskatoon) have revealed nearly normal oxygen affinity. The oxygen carrying capacity of the propositus described here was found to be slightly below normal and was completely corrected to normal after oxygen inhalation. This suggested mild ventilatory deficit, which could be attributed to the bronchial catarrh. This was a transient process and the arterial oxygen saturation of the patient was normal after the successful treatment of bronchitis. Apparently normal arterial oxygen saturation of the propositus would appear to be surprising since only about 70 per cent of the haemoglobin (Hb-A) in the propositus would be expected fo be functional in oxygen transport. This could possibly be achieved by compensatory increase of the red cell mass, stimulated by reduced oxygen availability to the tissues, and this compensatory erythrocytosis developed, possibly due to increased production of erythropoietin in the kidneys. A state of equilibrium has been ultimately reached when the oxygen carrying capacity of the blood approached the normal level and the plasma erythropoietin level also came down to the upper range of normal.

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Addendum

In the report by Bajaj et al (1973), this disorder was found in a Muslim-Khoja family from Bombay which on subsequent study was shown to be similar to Hb-M Boston with a substitution of distal histidyl by a tyrosine at position 58 of the alpha chain (Sukumaran & Master, 1974).

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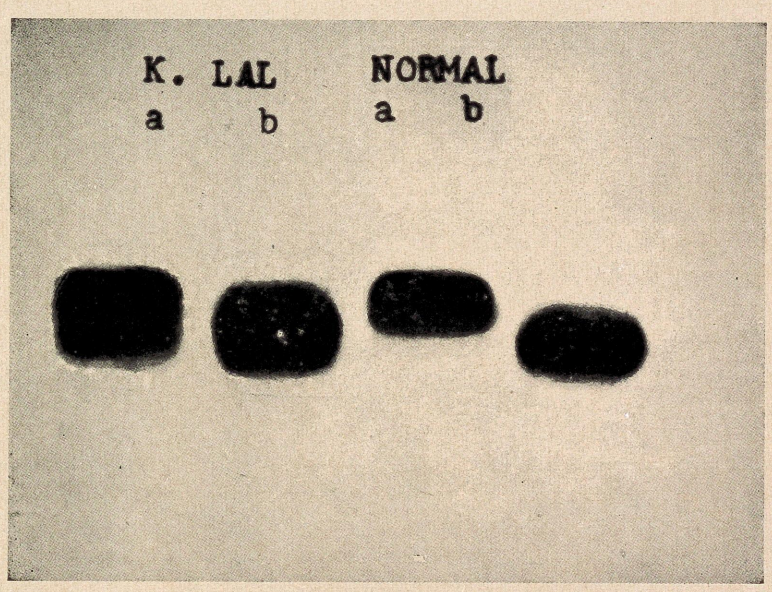

Figur Shows starch electophoresis of haemoglobin of the patient in phosphate buffer pH 7.1 ; a = untreated ; b = ferricyanide treated.