

Combination of Spherocytosis and a Variant of Beta Thalassaemia ('Isolated Raised Hb A₂') (‘Isolated Raised Hb A₂’)

T. A. CUNNINGHAM and F. VELLA

From the Departments of Pathology and Biochemistry, College of Medicine, University of Saskatchewan, Saskatoon, Sask., Canada

Hereditary spherocytosis is an intrinsic metabolic disorder of erythrocytes characterized by the presence of microcytes and spherocytes in the peripheral blood. It is believed to be due to a derangement in the energy-yielding reactions of glycolysis within the membrane of the erythrocyte. It has been calculated to occur once in every 20,000 births. Though it is not racially restricted, it has been reported most frequently in persons of northern European origin (Harris, 1963).

Beta thalassaemia is a hereditary disturbance in the mechanism of synthesis of the beta polypeptide chain characteristic of the major adult haemoglobin molecular species. It occurs more frequently than spherocytosis, particularly in countries around the Mediterranean littoral and in the Near and the Far East. There are various forms of beta thalassaemia and nearly all of them are characterized by an increase in the Hb A₂ fraction to approximately double the normal level.

One form of beta thalassaemia was described almost simultaneously in Italy by Silvestroni and Bianco (1957) and by Carcassi, Ceppellini, and Siniscalco (1957), in which the increased Hb A₂ concentration was not associated with the usual abnormal morphological characteristics of the erythrocytes, and which appeared to be transmitted as a Mendelian dominant character. Similar findings have been reported in Greek subjects (Gouttas 1962) and in a British family (Callender, Mallett, and Lehmann, 1961). This variant has been reported in three genetic forms: heterozygous, presumed homozygous, and in combination with classical beta thalassaemia (Silvestroni and Bianco, 1966; Quattrin, Dini, and Ventruto, 1964). It has been variously described as: ‘elevated A₂ haemo-

globin without morphologic thalassaemia’ (Bannerman, 1961), ‘beta chain thalassaemia in absence of a thalassaemia-like picture’ (Fessas, 1965), ‘isolated increase of haemoglobin A₂’ (Silvestroni and Bianco, 1966), and ‘elevated Hb A₂ without microcythaemia’ (Quattrin *et al.*, 1964). According to Quattrin *et al.*, this form accounts for 8% of all the thalassaemias found around Naples (Italy), though the frequency in Greece appears to be less than this (Gouttas, 1962). Weatherall (1965), however, considers it unlikely that this condition represents a separate genetic entity, and thinks of it as ‘rather one extreme of the continuous clinical variation from complete normality to a picture of severe thalassaemia, which seems to characterize heterozygous beta thalassaemia’. It is useful, for descriptive purposes, to consider an isolated raised Hb A₂ concentration as a distinct clinical form of beta thalassaemia.

The hereditary anomalies characteristic of spherocytosis and of beta thalassaemia affect different biochemical systems in the developing erythroblast. It is not surprising, that the genes concerned are not allelic and appear not to be closely linked. The occurrence of these genes in combination, though theoretically possible, must in practice be uncommon. There is only one published report in which the combination of spherocytosis, Hb S, and an unusual form of thalassaemia have been presumed to occur together in a Negro woman (Cohen, Zuelzer, Neel, and Robinson, 1959). In this patient the thalassaemia variant which was postulated produced the characteristic erythrocyte morphology but not the increase in Hb A₂. This gave rise to the concept of a ‘non-interacting’ form of thalassaemia. The suggestion was also made that the expression of this thalassaemia gene was masked by the presence of the gene for spherocytosis.

We would like to record the finding of spherocytosis and a raised Hb A₂ level, in the absence of the

morphological characteristics of thalassaemia, in a white subject. This is the first description of this combination of hereditary intra-erythrocytic defects.

Case Report

A man, 28 years old, was born in Jordan of Syrian parents and immigrated to Canada in 1958. His father and mother are alive and well, and an only brother is now 17 years old.

The patient was admitted into University Hospital, Saskatoon, for submucous resection of the nasal septum. He had complained of bouts of nasal bleeding and generalized headaches. There was no past history of jaundice, anaemia, allergy, or surgical operation. On physical examination his nasal septum was found to be deviated to the right. There were no physical or functional abnormalities, though the spleen was just palpable but not tender.

Laboratory Investigations. Hb 15.6 g./100 ml., WBC 6400/mm.³, platelets 113,000/mm.³ Reticulocytes 0.9%, ESR 7 mm. Bleeding time 1 min. 30 sec., clotting time (Lee and White three tube method) 19 min. 32 sec. Quick's prothrombin time 14 sec. (control 14 sec.). Clot retraction incomplete. Thrombin titre: 1/64 (control 1/64). Hicks Pitney partial thromboplastin time 38 sec. (control 37 sec.). Direct Coombs test negative. LE cells negative. Peripheral blood smears showed distinct microcytosis and spherocytosis, but slight anisocytosis and polychromasia. The osmotic fragility curve was shifted to the left both with and without incubation. Total bilirubin 0.7 mg./100 ml. X-ray films of the chest were normal. Urinalysis gave normal results.

A submucous resection of the septum was carried out. There was some bleeding and oozing after operation and a haematoma developed on the left side of the septum posteriorly. The pathological report on the resected material read 'degenerating osseous and cartilaginous tissue from the nasal septum'.

Special Investigations. Electrophoresis of the haemoglobin was performed on filter paper using a Tris-EDTA-borate/veronal discontinuous buffer system (pH 8.6) and on starch gel using Tris-EDTA borate buffer at the same pH. Both methods showed Hb A₁ and a marked Hb A₂ band. Elution of the Hb A₂ from a starch block after electrophoresis at alkaline pH showed it to account for 5.4% of the total haemoglobin. The 'one minute alkali denaturation test' for foetal haemoglobin gave normal results.

Family Study. The only relative available for study was the patient's brother. He was in good health. Laboratory investigations on him showed: Hb 16.2 g./100 ml., PCV 50%, RBC 5.0 million/mm.³ Prothrombin time 12 sec. (control 13 sec.), thrombin titre 1/64 (control 1/64), Hicks Pitney partial thromboplastin time 39 sec. (control 39 sec.). Peripheral blood smears

showed no morphological abnormality. Osmotic fragility curves with and without incubation were within normal limits. Hb electrophoresis showed the A₂ fraction to amount to 5%, while foetal Hb was normal.

Discussion

The diagnosis of hereditary spherocytosis in our patient was based on: (1) the presence of spherocytes in peripheral blood smears, (2) the increased osmotic fragility of the erythrocytes both with and without incubation, (3) the absence of any evidence of acquired haemolytic disease. The mild degree of reticulocytosis, the normal total bilirubin concentration, and the spleen, which was just palpable, suggested that any haemolysis taking place was minimal. The diagnosis of beta thalassaemia was based on the finding of an abnormal amount of Hb A₂.

There are several features of interest in our patient.

(1) The mildness—virtually complete absence—of symptoms attributable to either of the two inherited anomalies: the thalassaemia component would have been missed if electrophoretic studies on the Hb had not been carried out.

(2) The absence of any morphological characteristics of the erythrocytes attributable to thalassaemia in the patient's brother: this suggests that the morphological abnormalities (microcytosis, mild anisocytosis, and mild polychromasia) in the patient's erythrocytes were due to the spherocytosis component.

(3) The lack of any clinical or laboratory evidence of interaction between the two intra-erythrocytic defects or of accentuation of the pathogenicity of either.

(4) The prolonged clotting time.

There are reports of the combination of hereditary spherocytosis with a number of other hereditary anomalies of the erythrocyte. Thus, the combination of spherocytosis and sickling is well known (Cohen *et al.*, 1959; Smith and Conley, 1954; De Torregrosa, Ortiz, and Vargas, 1956; Jones and Klingberg, 1959; Martin, Kough, and Branche, 1959; Whitaker, Windmiller, Vietti, and Sartain, 1963). The simultaneous presence of three erythrocytic defects (spherocytosis, haemoglobin C, and sickling) has been found in one family (Thompson and Robertson, 1964), while the combination of spherocytosis, sickling, and an unusual variety of thalassaemia was presumed present in a Negro woman (Cohen *et al.*, 1959). It has been established for several years that haemoglobins S and C are beta polypeptide chain variants of normal adult

Hb and the reports of the simultaneous presence of these two haemoglobins together with spherocytosis demonstrate that spherocytosis is not allelic with the locus for the beta polypeptide chain of haemoglobin. The findings in our patient also suggest that the genes for spherocytosis and for the beta thalassaemia variant present in him are non-allelic.

There is no evidence that the simultaneous presence of the genes for spherocytosis and the beta thalassaemia variant in our patient in any way interfered with the expression or clinical manifestation of either. A similar lack of interaction can be inferred from study of the published reports on patients with the combination of hereditary elliptocytosis and classical beta thalassaemia (Brumt, Delabarre, and De Traverse, 1960; De Vries, De Jong, and Frenkel, 1959; Aksoy, 1963; Perillie and Chernoff, 1965).

Absence of the haematological stigmata of a postulated thalassaemia gene in the presence of spherocytosis was noted by Cohen *et al.* (1959) in their patient. This they attributed to a suppression or masking of the thalassaemia effect, by the spherocytosis gene. However, the thalassaemia gene they postulated did not produce an increase in haemoglobin A₂ level. In our patient this explanation is not tenable since his brother also had a raised haemoglobin A₂ level in the absence of any noticeable morphological abnormalities. The thalassaemia gene in our family, therefore, is of the kind that is expressed as an isolated raised haemoglobin A₂ level.

No explanation can be offered for the finding of a prolonged clotting time and incomplete clot retraction in our patient. There was no history of a haemorrhagic diathesis in the patient or his family. The situation is different, therefore, from that reported by Chatterjea (1956) in a Hindu family in which both spherocytosis and 'pseudo haemophilia' were present and occurred together in one member.

Summary

A case report is presented of a male Jordanian of Syrian origin in whom the combination of spherocytosis and a beta thalassaemia variant (isolated raised haemoglobin A₂ level) occurred. This is the first description of this combination of hereditary intrinsic abnormalities of the erythrocyte. There was no evidence of interaction between the two

genes. The genetic combination was asymptomatic and was discovered during routine pre-operative investigation.

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