HEREDITARY DIAPHORASE DEFICIENCY METHAEMOGLOBINAEMIA IN MALTESE FAMILIES

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Summary

Hereditary Diaphorase Deficiency Methaemoglobinaemia was discovered in seven persons belonging to four Maltese families. These are the first families of diaphorase deficiency methaemoglobinaemia to be recorded from the Maltese Islands. It is suggested that the gene frequency for this type of congenital methaemoglobinaemia is high in the Maltese Islands and that wider systematic surveys would reveal many more cases.

Methaemoglobin (ferrihaemoglobin) normally constitutes only 1% of the total haemoglobin of the red cells. Its presence in a greater concentration is pathological and may be due to a number of different causes, congenital or acquired. The congenital forms are rare and are of two types which are completely different from one another. One type, inherited recessively, is due to an enzyme deficiency in the red cell, (Gibson, 1948), while the other type, showing dominant inheritance, is due to a defect in the globin moiety of the haemoglobin molecule (Horlein and Weber, 1948).

Seven affected individuals in four Maltese families with methaemoglobinaemia secondary to deficiency of the enzyme diaphorase have been discovered in the Maltese Islands and will be described in this paper.

Methods

Methaemoglobin estimation was carried out using the method of Evelyn and Malloy (1938) as described by Varley (1967).

Diaphorase Activity in the red cell was estimated by the method described by Scott (1960). Levels of diaphorase (measured as units of change in optical density per minute $\times 10^4$) below 100 are usually taken as characteristic of the methaemoglobinaemic homozygote, while levels between 100 and 300 represent the heterozygote. Levels in excess of 300 represent the normal state.

Haemoglobin F Estimation: The degree of denaturation of cyanmethaemoglobin was determined using the modification of Bethke, Marti and Schlicht.

G-6 PD Estimation: The Brilliant Cresyl Blue dye test (Motulsky and Kampbell-Kraut, 1964) was used.

Haemoglobin estimation, blood smears and reticulocyte counts were performed by standard methods. PCV was determined using a microhematocrit technique.

Case Reports

Family A. None of our patients had been taking any analgesics or any other drugs. The father and the mother are apparently healthy, look normal and are not related to one another. They hail from an inland village in Gozo, the sister island of Malta. There are six offsprings from the marriage of whom two have been affected.

Case 1. The eldest sibling, a 16 year old male (J.V.) was noted to have had a cyanotic tinge since early childhood, and had been seen by various specialists who had not found any cardiac or pulmonary abnormalities. Intelligence is average and his only complaints have been occasional dizzy spells. When seen by one of us (L.V.), the patient had just undergone an emergency appendicectomy, and had caused some concern to the anaesthetist as the blood oozing at operation was unduly dark. Physical examination revealed no other abnormality. His Hb level was 13.6 g., PCV 40%, MCHC 34, reticulocyte count 0.5%, G6PD activity 50, and Hb F 0.32%. His methaemoglobin level was 10.5% (1.43Gm). His diaphorase level was 109. (Control 350).

Case 2. T.V., the youngest child of the marriage (d.o.b. 4.5.65) was noted to be cyanosed on the day of birth. Cyanosis persisted. There were otherwise no other abnormalities. At the age of nine months, he was seen by a specialist because of the worrying cyanosis, but no physical abnormalities were found. His lungs and heart, in particular, were thought to be normal. Apetite, developmental milestones, both mentally and physically appeared normal. At the age of 3, he developed peritonitis from a perforated appendix. He died a few days after an emergency laparatomy. During his terminal illness, intravenous ascorbic acid, 500 mg daily was given. Investigation results (taken when he was still healthy) were as follows: Hb level 13.2 g; PCV 44%; MCHC 30; reticulocyte count 1%; G6PD activity 50; Hb F 0.9%. His methaemoglobin level was 8.3% (1.07Gm). His diaphorase level was only 47. (Control 350).

Family B. The mother of this family is dead. The marriage is not consanguineous. There were eight sublings from this marriage, and two are affected.

Case 3. The eldest sibling (J.V.) a thirty year old male, was the most cyanosed member of the family. His only complaints have been dizzy spells especially after exertion. Cardiac and pulmonary disease have been excluded on medical examination. He works as a heavy manual labourer. He has emigrated to Australia, and investigations to prove the presence of methaemoglobinaemia secondary to diaphorase deficiency have unfortunately not been carried out. He has presumed diaphorase deficiency because the deficiency in his sister has been proved.

Case 4. (S.S., a 27 year old female, now married, is the third child of the marriage. She has always been noted to be moderately cyanotic, but less so than her elder brother (Case 3). Her health has always been good but she has been examined by specialists in the past, as her

parents were afraid there was serious underlying disease. The cyanosis had become especially marked when she was pregnant. The pregnancy was otherwise uneventful. The cyanosis diminished after pregnancy only to become more marked when she was again pregnant. She has now two children who appear normal. Her Hb level was 14.2g; PCV 43%; MCHC 33%; reticulolocyte count 0.1%; G6PD activity 50; Hb F 1.4%. Her methaemoglobin level is 16.6% (2.35g). Her diaphorase level is 0. (Control over 400)

Family C. There are ten siblings from this union, which is not consanguineous. Only one child is affected.

Case 5. (R.V.), a fourteen year old girl is the fifth child of this family. She is of normal height and development. Her intelligence is average. She is symptomless. There are no physical abnormalities apart from the presence of a mild cyanotic or lavender hue. Her Hb level is 1218/g; PCV 41%; MCHC 31%; reticulocyte count 0.6%; G6PD activity 50; Hb F 0.7%. Her methaemoglobin level is 22.5% (2.82g). Her diaphorase level was 5. (Control 290).

Family D. The father and mother are unrelated though both hail from the same town in Malta. There are ten siblings in the family of whom two are affected.

Case 6. Y.B., is a 26 year old female. She is the eldest offspring. She had been noted by her parents to have had mild intermittent cyanosis when she was a child and again when she was pregnant. She is otherwise perfectly normal, and has a three year old son. Her methaemoglobin level is 6.2% (0.81g.). Her diaphorase level was 0. (Control 290)

Case 7. The condition had been recognised in N.B. the fifth child now 19 years old, because of the colour of her blood at appendicectomy. Diaphorase levels were not estimated then. She had never complained of symptoms and was of normal intelligence and development. Her Hb level is 13.2g; PCV is 40%; MCHC 33%; G6PD activity 40; Hb F 0.6%. Her diaphorase level is 0 (Control over 400). She has since married and is now pregnant. The diaphorase of six healthy siblings of the above two cases (6 and 7) varied from 95 to 304.

The father's diaphorase level was 115 while the mother's was 90. (Control over 400).

Discussion

These are the first families of diaphorase deficiency methaemoglobinaemia to be recorded from the Maltese Islands. Though most of the cases so far described in the world literature have been in Europeans or in persons of European stock, interesting geographical concentrations of the disease have been described. Thus Scott and Hoskins (1958) found fifteen affected persons arising from 9 families of Alaskan Eskimos and Indians in four areas of Alaska. Four of these nine families were interrelated. A relatively high gene frequency has also been found among the Navajo Indians (Balsano *et al.*, 1964). The condition is of world wide distribution as cases have been recorded among North Africans (Rousell *et al.*, 1963), Chinese (Chang and Wu, 1954), Hindus (Raj *et al.*, 1959) and Puerto Ricans (Jaffe *et al.*, 1966). The incidence of this recessively inherited disease therefore is relatively high in the Maltese Islands whose total population is only one third of a million inhabitants.

The degree of methaemoglobinaemia has been noted to fluctuate from season to season and with age. Thus Scott and Hoskins (1958) found that methaemoglobin levels in their Alaskan patients were less in September than in December fifteen months later. They postulated an environmental factor such as ascorbic acid. The same workers also noted a tendency for the methaemoglobin levels to fail with age. They recorded a patient who had a level of 22 per cent methaemoglobin when eight years old, and 4.8 per cent when re-examined eight years later. It is interesting to note in this connection that Case 6 was described by her parents to have had obvious cyanosis in early childhood and that this had slowly disappeared with the years only to reappear when she became pregnant. The methaemoglobinaemia also

became more aggravated with pregnancy in Case 4. The cyanosis of methaemoglobinaemia in pregnancy has been mistaken as being due to cardiac disease as has been pointed out by Vassallo and Cauchi, (1970). This aggravation by pregnancy has also been noted by other workers, and pregancy may be the factor that leads to the diagnosis of methaemoglobinaemia. (Pepper, Weinstein and Heller, 1961).

None of the patients had been taking any drugs. The recessively inherited form of congenital methaemoglobinaemia appears to have been first described by Hitzenberger (1932) who described a mentally defective dwarf with a strong family history. Sievers and Ryan (1945) suggested that the defect lies in the reduction system of methaemoglobin. Gibson (1948) showed that the enzyme diaphorase was missing in methaemoglobinaemic cells as methylene blue was extremely effective in reducing methaemoglobin. Diaphorase (diphosphopyridine reductase DPNH, NADPH, coenzyme factor 1) is an intermediate carrier which catalyses the reduction of methaemoglobin by reduced diphosphoryridine nucleotide (DPNH). Another far less important pathway for the reduction of methaemoglobin is mediated by Diaphorase 11 (reduced triphospopyridine nucleotide, TPNH, NATPH). (Huennekers *et al.*, 1957). Other unimportant systems known for the reduction of methaemoglobin in normal cells are ascorbic acid (Lian, Frumusan, and Sassier, 1939) and by means of reduced gluathione (Scott, Duncan, Ekstrand, 1965). Lately another enzyme variant in a case of congenital methaemoglobinaemia has been demonstrated (West *et al.*, 1967).

Treatment of hereditary methaemoglobinaemia secondary to diaphorase deficiency is not necessary except in unusual circumstances. Both ascorbic acid (Lian, Frumusan and Sassier, 1939) and methylene blue (JJaffe and Neuman, 1964) are both very efficacious and act by direct chemical reduction of methaemoglobin. Ascorbic acid was used intravenously in our Case 2 during his terminal illness as it was necessary for the oxygen transport system of haemoglobin to be utilised to the fullest extent and for the dead load of methaemoglobin to be minimised.

The discovery of these seven cases clinically would suggest that the relative gene frequency for this recessive type of congenital methaemoglobinaemia is high in the Maltese Islands and that a future systematic survey for diaphorase deficiency among a suitable section of the population such as school children may reveal many more cases.

Acknowledgements

We wish to acknowledge the kindness of the Editor of "The Journal of Obstetrics and Gynaecology of the British Commonwealth" for permission to quote Case 4 (Family B) and Case 6 (Family D).

We wish to thank the personal doctors of the families concerned, for their cooperation.

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