

Haemolysis during diabetic ketoacidosis treatment in two girls with incomplete glucose-6-phosphate dehydrogenase deficiency

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Abstract. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a typical X-linked enzymopathy causing severe haemolytic anaemia in males, and mild to moderate anaemia in homozygous females. Haemolysis due to G6PD deficiency in patients with type 1 diabetes mellitus (T1DM) has been principally reported in males, but is uncommon. During the last 10 years 2 girls with an unknown incomplete G-6-PD deficiency showed haemolysis during the treatment of DKA at the onset of T1DM. We speculate that the patients here described showed haemolytic anaemia as a phenotypic expression of the lyonization process and/or an uncommon penetrance of the defective gene. Haemolysis occurred when blood glucose levels were returning to normal values. In normal red blood cells, G6PD provides a source of reducing power for maintaining sulphhydryl groups (SH) and facilitating the detoxification of free radicals and peroxides. During insulin i.v. infusion the copious glucose available due to the hyperglycaemia progressively decreased and affected the old red blood cells to generate nicotinamide adenine dinucleotide (NADPH), a crucial source for energy-dependent functions. This NADPH loss could have enhanced the rate of all factors such as methaemoglobin generation, Heinz body formation, and lipid peroxidation, which occur in G6PD deficient cells in response to both endogenous and exogenous oxidants. The direct consequence of this phenomenon is an increased erythrocyte oxidant sensitivity and a loss of sulphhydryl group availability causing premature red blood cell destruction. (www.actabiomedica.it)

Key words: G6PD, deficit of G6PD, type 1 Diabetes, haemolysis

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a typical X-linked enzymopathy causing severe haemolytic anaemia in males, and mild to moderate anaemia in homozygous females. The haemolysis is usually triggered by the ingestion of fava beans, peas, *Verbena Hybrida*, many vegetable drugs or some pharmaceutical drugs (i.e. sulphamidic derivatives, salicylic acid, chinidine) which act as “trigger factors” (1). The highest prevalence rate of G6PD deficiency has been

reported in Africa, Middle East, southeast Asia and in some Mediterranean areas, particularly in Sardinia where gene frequency ranges from 5 to 25% (2-7).

Haemolysis due to G6PD deficiency in patients with type 1 diabetes mellitus (T1DM) has been reported but is uncommon (8-12). Haemolysis has been related to diabetic ketoacidosis (DKA) (8, 9, 11, 13) bacterial infections, hemolytic drugs (14), hypoglycemia (15), and glucotoxicity (2, 10). Only a few diabetic boys experienced a haemolytic event (2, 10). No young girls with diabetes has been reported so far.

During the last 10 years two young girls with an unknown G-6-PD deficiency were admitted to our department because of diabetes at onset, and unexpectedly developed haemolytic anaemia during DKA treatment.

Case report n. 1

In march 1998 E.E., a 9 year-old girl, was admitted to the Emergency Room of the Children's Hospital of Parma, for dehydration (8%), weight loss (3 kg within 2 months) and nicturia, polyuria and polydipsia over a period of 2 weeks. Diagnosis of T1DM was confirmed by laboratory findings that showed a moderate DKA (16) (Table 1). E.E. presented no bacterial infections and was not on any medication. On admission, haemoglobin, bilirubin plasma concentration and reticulocytes were within normal ranges. DKA was treated according to a protocol previously described (17). Normoglycaemia recovery and the disappearance of urine ketone bodies were achieved within 18 hours, and a basal-bolus insulin regimen was introduced. Fasting and post-prandial blood glucose levels ranged from 134 to 180 mg/dL and from 166 to 214 mg/dL respectively. On the 4th day, E.E. developed pallor, jaundice and asthenia. Blood tests showed haemolytic anaemia; on the 12th day haemoglobin levels reached the lowest value of 8.1 gr/dL (Table 2). Between the 13th and the 21th day haemoglobin levels gradually rose up spontaneously to 10.3 g/dL. Since the girl's father and mother referred to be originated from Sardinia a quantitative assay of G6PD was carried out. G6PD was undetectable in the father and in the normal range in the mother (121 u/g, n.v. 118-144 u/g). E.E. had an incomplete G6PD deficiency (23 u/g). DNA analysis of G6PD gene showed the presence of the typical G6PD Mediterranean mutation (exon 6, 563 C → T).

Table 1. Laboratory investigations at admission

	Blood glucose mg/dL	pH	HCO ₃ ⁻	Beta OH mmol/L	Hb gr/dL	Hct
Patient 1	310	7.24	14.8	6.04	12.7	36.9
Patient 2	385	7.06	3.5	5.2	15.8	47.9

Case report n. 2

In November 2008 G.F., 12 years old, was admitted to the same Emergency Room, for dehydration (9,5%), weight loss (4,5 kg within 2 months) and for nicturia, polyuria e polydipsia over a period of 11 days. Laboratory tests confirmed the diagnosis of T1DM that was complicated by a severe DKA (16) (Table 1). E.E. had no clinical history of infection or drug administration before admission. DKA was managed according to the same protocol previously described (17). Normoglycaemia recovery and the disappearance of urine ketone bodies were achieved within twenty-five hours without hypoglycaemic episodes. On the twenty-fourth hour of DKA treatment G.F. developed jaundice and blood tests showed haemolytic anaemia. Haemoglobin decreased up to 8.8 gr/dL on the fourth day (Table 2). Seven days after, haemolysis was spontaneously resolved and haemoglobin raised to 10.8 g/dL. A basal-bolus insulin regimen was introduced. The anamnesis advised that the girl's father suffered from G6PD deficiency, confirmed by the laboratory value (G6PD undetectable). Quantitative G6PD assay in the mother was in the normal range (124 u/g). The same dosage was performed in G.F. who resulted to have an incomplete G6PD deficiency (6,8 u/g, n.v. 118-144 u/g). DNA analysis showed the presence of the typical G6PD Mediterranean mutation.

Discussion

In a previous work we reported three boys with T1DM at onset who developed haemolytic anaemia due to an unknown G6PD deficiency during the DKA treatment (10). We speculated that blood glucose normalization during DKA treatment produced a stressing glucose deprivation for the energy-depen-

Table 2. Laboratory investigations during DKA treatment

	RBC/mm ³	Hb (g/dL)	Hct %	Reticulocytes %	Total bilirubin (mg/dL)
Patient 1	2940	8.1	27.4	8.51	0.9
Patient 2	3120	8.8	26.1	6.54	1.1

dent function of the red blood cells causing premature red blood cell destruction. The same mechanism was used for explaining the appearance of haemolytic anaemia during repeated episodes of hypoglycaemia (15). The mothers of these three patients originated from Sardinia and suffered from incomplete G6PD deficiency, and transmitted the X-linked recessive gene enzymopathy to their male children (10).

In this report, we described the first two girls with T1DM at onset who developed haemolytic anaemia due to an unknown G6PD deficiency during DKA treatment. In both females, all parents originated from Sardinia. The fathers resulted to have complete G6PD deficiency, while the mothers were healthy. In the two young girls the quantitative G6PD assay showed an incomplete G6PD deficiency.

Females have two copies of the G6PD gene, one for each X chromosome, and can express the normal or the defective gene. In the Sardinian population, where the G6PD mutation prevalence is very high, homozygous females are numerous. In the same population heterozygous females have a functional genetic mosaicism resulting from the inactivation of one of the two X chromosomes. When heterozygous female cells undergo inactivation of the X-chromosomes it is possible that the X-chromosome carrying the normal gene may be preferentially inactivated due to chance and a small amount of working enzyme is therefore produced because the cells mainly possess the defective gene. Thus the lyonization process seems to be a likely cause of G6PD deficiency in female carriers.

In addition some heterozygous individuals may show a recessive phenotype because of unusually severe penetrance of G6PD gene (18).

We speculate that the patients here described showed haemolytic anaemia such as a phenotypic expression of the lyonization process and/or an uncommon penetrance of the defective gene.

The clinical history of the two girls did not point out any infection or drugs administration that could be responsible for haemolysis. Therefore the pathogenesis must be found in the DKA treatment. Insulin was the only drug used during the treatment but in the literature no evidence of its role as a cause of haemolysis in individuals with G6PD deficiency is present.

As previously reported we can assume that also in the present young girls, haemolysis occurred when blood glucose levels were returning to normal values (10). In normal red blood cells, G6PD provides a source of reducing power for maintaining sulphhydryl groups (SH) and facilitating the detoxification of free radicals and peroxides. This process needs energy which is exclusively supplied by glucose that was present in large quantity due to the hyperglycaemia in these young girls. During insulin i.v. infusion this copious glucose availability progressively decreased and may have increased the inability of the old red blood cells to generate nicotinamide adenine dinucleotide (NADPH), and to preserve all energy-dependent functions. This loss of NADPH could have enhanced the rate of all factors such as methaemoglobin generation, Heinz body formation, and lipid peroxidation, which occur in G6PD deficient cells in response to both endogenous and exogenous oxidants (10). The direct consequence of this phenomenon is an increased erythrocyte oxidant sensitivity and a loss of sulphhydryl group availability causing premature red blood cell destruction.

Diabetic patients who develop haemolysis during DKA treatment rarely need blood transfusions (2). Haemolytic anaemia progressively and spontaneously disappears because new red blood cells, grown in a normal glycemic medium, find a physiologic source of energy for generating a sufficient quantity of NADPH to prevent oxidative damage and to preserve other energy-dependent functions.

Acknowledgements

Thanks go to doctor Rossana Di Marzio for the manuscript revision

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Accepted: April 9th 2009

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