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Case Report

Contribution of laboratory clinical consultation for excessively low Hba1c results to the diagnosis

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Abstract

When interpreting the results of a glycosylated haemoglobin (HbA1c) level, factors affecting the life span of the red blood cells should be kept in mind as it may affect the measurement of the HbA1c. Severe hemolysis may result in a falsely low HbA1c level. In this case study, in the diagnosis of hemolysis, a case who has switched the laboratory to an alarm condition is presented. In a 21-year-old male patient with hemolytic anemia, the HbA1c level was incompatible with blood glucose. Peripheral blood smear was performed by clinical laboratory consultation revealed Heinz bodies in erythrocytes, which were deficient in glucose 6 phosphate dehydrogenase enzyme. In the clinic, the diagnosis of favism due to glucose 6 phosphate dehydrogenase enzyme deficiency was considered. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymatic disorder of red blood cells in humans. In the diagnosis of hemolysis case with clinical laboratory consultation, attention was drawn to the low HbA1c results that did not match the blood glucose measurement result. This study emphasizes that an extremely low HbA1c level can serve as a marker of hemolysis.

Keywords: HbA1c, hemolysis, laboratory consultation

Glucose-6-phosphate dehydrogenase (G6PDH) enzyme deficiency is the most commonly seen erythrocyte enzyme deficiency and has an X-linked recessive inheritance pattern. As in our case, one of the clinical pictures that the disease manifests itself is favism. In favism, clinical signs appear 5-24 hours after ingestion of fava beans and it is often seen in boys between one to five years of age. Anemia, which develops suddenly as a result of hemodialysis, is severe and may cause renal failure [1]. When interpreting glycosylated hemoglobin (HbA1c) results, factors affecting erythrocyte life span should be kept in mind because they can have an impact on analysis. Our case was an adult patient, and, what is more, did not speak our language, Turkish. As a result of noticing the abnormally low glycosylated hemoglobin (HbA1c) levels among the laboratory tests, the data supporting early and correct diagnosis were obtained. Attention was drawn low HbA1c results in multiple cases of hemolysis and low HbA1c results at similar levels were shown to be markers for severe hemolysis. It is emphasized that laboratory results should be in alignment with the clinic in such cases [2].

Case Report

Twenty-one-year-old male patient was admitted to our Internal Medicine department because of anemia and icterus of sudden onset. Blood work up of the patient on the same day and on previous days revealed hemolytic anemia due to hemodialysis. An abnormally low HbA1c level among the blood workup performed before admission at presentation to our outpatient clinic because of hematuria drew our attention during the consent for the test. HbA1c level was measured using high pressure liquid chromatography (HPLC) analyzers ADAMS™ A1c V-8180 (Arkray, Kyoto, Japan). HbA1c test was performed with the HPLC method and a value of 2.9% was found which was below the lower limit of reference which is 4%. The glucose level in the blood simultaneously obtained from the patient was 124 mg/

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dl and was incompatible with the HbA1c value. The internist of the patient at the outpatient clinic was advised about the low HbA1c level, and a request for HbF was recommended regarding differential diagnosis algorithm for anemia because of the elevated MCV value of >95 fl in the whole blood count [3]. Hb electrophoresis was carried out with the HPLC method from the present HbA1c blood tube and HbF value was found to be normal. We used to Hb electrophoresis analyzers ADAMS™ A1c T-8180 (Arkray, Kyoto, Japan). Abnormal values noted in the laboratory results before and during hospital admission (Table 1) were similar to those seen in hemolysis [4].

Among the results of blood tests of the patient, levels of urea, BUN, total and indirect bilirubin, LDH, ferritin and reticulocytes were elevated and those of hemoglobin, hematocrit, RBC were low, drawing attention for a case of hemolysis. The patient was admitted after laboratory clinical consultation and contact was made with the physician responsible for the service, drawing attention to the picture of hemolytic anemia and the tendency for low Hb values in the patient and comment was made that the patient's laboratory results suggested hemolysis. Haematological samples were measured using Sysmex XN-9000 (optical (O), impedance (I), fluorescence (F)), biochemical tests were determined using the Roche Cobas 6000 (Roche Diagnostics, Germany); Serum ferritin was measured in Cobas e 602 device with electrochemiluminescence (ECLIA). Serum glucose, urea, BUN, total and indirect bilirubin, LDH, were measured in Cobas c 501 device with enzymatic-spectrophotometric method. The picture of hemolysis in our case in whom glucose-6-phosphate dehydrogenase enzyme deficiency was detected was based on favism which became apparent with the ingestion of fava beans in the history of the patient. Following clinical consultation, Heinz bodies were seen within the erythocytes in the peripheral smear in the laboratory (Fig. 1) and this finding supported the early diagnosis. Heinz bodies are characteristic for favism [5]. In fact, the diagnosis of glucose-6-phosphate dehydrogenase enzyme deficiency was made a few days later when this enzyme was measured in the patient's blood and was found to be 3.5 u/gHb which is below the reference range (7-20.5 u/gHb). Glucose-6-phosphate dehydrogenase was measured in Cobas c 501 device with enzymatic-spectrophotometric methods. In the meantime, the patient was treated at his clinic and his blood levels and clinical condition were reported to have improved. The patient's consent was obtained for this case report.

Discussion

Hemolytic anemia is defined as decrease in the number of erythrocytes due to accelerated breakdown of circulating erythrocytes. In hemolytic syndromes, breakdown of erythrocytes by the reticuloendothelial system may be increased (extravascular hemolysis) or else cells may be broken down inside the circulation (intravascular hemolysis). On the other hand, in some conditions like glucose six phosphate dehydrogenase deficiency, both intrinsic and extrincic factors have a combined role in the development of hemolysis. In conclusion, while the normal survival of erythrocytes is 120 days, it is reduced to less than 100 days4. HbA1c measures glycosylated hemoglobin. Glucose is irreversibly bound to hemoglobin using the amino group of N-terminal amino acid (valine) of the β-globin chain. Glycation continues throughout the life span of the red blood cell (RBC) and HbA1c increases. In disorders causing a decrease in RBC survival (e.g., recovery period after acute blood loss, hemolytic anemia), the time of encounter with glucose is reduced, therefore, hemoglobin exposed to glycation has a lower percentage and causes a false decrease in test results regardless of the method [6, 7]. Considering that a shortened RBC life span

Table 1. Noteworthy blood test results of the case

Test /unit	Day 1	Day 2	Day 3	Day 4	Reference range
Glucose mg/dl	97	124 ↑	90	-	74-106
Urea mg/dl	61.0 ↑	-	57 ↑	-	17-43
BUN mg/dl	28.5 ↑	-	26.64 ↑	-	8-24
LDH U/L	441	780 ↑	532 ↑	419	<480
T. Bilirubin mg/dl	18.37 ↑	16.69 ↑	6.19 ↑	5.23	<1.2
D.Bilirubin mg/dl	0.69 ↑	1.04 ↑	0.54 ↑	0.54	<0.3
I Bilirubin mg/dl	17.68 ↑	15.65 ↑	5.65 ↑	4.69	0.1-1.0
Ferritin ng/ml	-	5191 ↑	-	-	30-400
RBC 10 (6)/µL	3.54 ↓	2.49 ↓	2.15	2.02	4.44-5.61
Hgb g/dl	12.1	8.6 ↓	7.3 ↓	6.8 ↓	11.9-15.4
Hct %	34.7 ↓	25.5 ↓	21.4 ↓	20.04 ↓	40.0-49.4
MCV fL	98 ↑	102.4 ↑	99.5 ↑	101 ↑	80.0-93.6
MCH pg	34.2 ↑	34.5 ↑	34 ↑	33.7 ↑	26.5-31.4
Reticulocyte 10 (6)uL	-	5.05 ↑	-	-	0.039-0.057
HbA1c %		2.9 ↓			4-6

BUN: Blood Urea Nitrogen, D. Bilirubin: Direct Bilirubin, Hct: Hematocrit, Hgb: Hemoglobin, I. Bilirubin: Indirect Bilirubin, LDH: Lactate Dedhydrogenase, MCV: Mean corpuscular value, RBC: Red blood cell counts, T. Bilirubin: Total Bilirubin

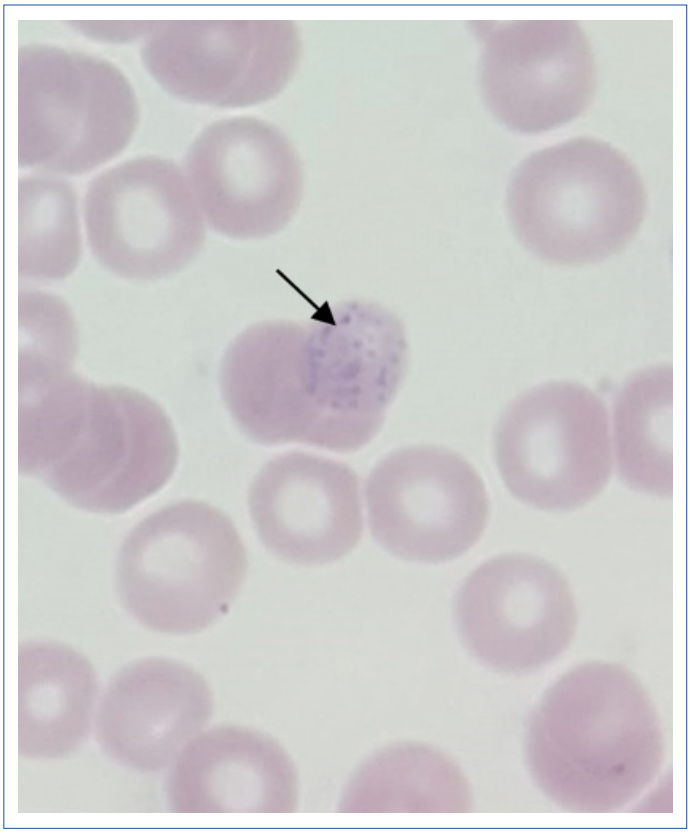

Figure 1. Heinz body noted between erythrocytes in peripheral smear- in stained microscopic image with 100x magnification.

may lower HbA1c values, it is shown that hemolytic disorders should be considered when evaluating an incompatible HbA1c result. In the case report by Danzing et al., the unexpected diagnosis of glucose-6-phosphate dehydrogenase deficiency was explained starting from blood glucose values incompatible with HbA1c values [8]. When interpreting HbA1c results (especially when unexpected results are encountered), it should be checked whether the patient carries the factors that can affect measurements (such as hemoglobinopathy, hemolysis, uremia) [9]. Wiener has reported that while the blood sugar of a diabetic patient was elevated (200 mg/dl) a very low glycosylated Hb result (1.4%) has obtained and hematologic work up performed later revealed that this has arisen from autoimmune hemolysis (Hb 7.7 g/dl) and has drawn attention to that a decrease in glycosylated Hb levels may not always mean an improvement in diabetes control [10]. It is stated in publications that "All samples with HbA1c levels below the lower limit of the reference range should be repeated by the laboratory and if confirmed, the clinicians should be warned by laboratory personnel that it could be breakdown of erythrocytes, and abnormal hemoglobin" [11, 12].

Conclusion

In addition to the adult age of the case which is rare for the diagnosis of the relevant enzyme deficiency, the aid of the laboratory in the diagnosis by refusing the HbA1c result as an incorrect sample has made a contribution to making this case extraordinary. We think that this study is significant regarding the motivation of consultation among medical laboratories and clinics.

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