Hemoglobin calabria leading to undetectable hemoglobin A1C

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Abstract
More than 12,000 hemoglobin (Hb) variants have been identified and listed on the HbVar database. Reliable detection of these Hb variants is important for planning hemoglobinopathy screening and genetic counseling. Presumptive diagnosis is usually dependent on dedicated Hb analyzers, e.g. high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) systems specifically developed for Hb fraction separation and quantification. In most cases, simple Hb variants can be easily identified. Rarely, a novel Hb molecule formed by the assembly of different defective globin chains could complicate clinical and laboratory diagnostics and requires DNA testing.

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Introduction
Glycated hemoglobin (HbA1c) is the gold standard for evaluating long-term glycemic control in patients with diabetes. HbA1c is proposed as an early diagnostic marker and also is used in long term follow up of Diabetes in guidelines published in 2019 by the American Diabetes Association (ADA) [1-4]. Numerous assays were subsequently developed to measure glycated hemoglobins. The principle of all methods is to separate the glycated and nonglycated forms of hemoglobin. This can be accomplished based on differences in charge (usually by HPLC) or structure (usually immunoassays or boronate affinity chromatography) [5]. The Diabetes Control and Complications Trial's reference method for measurement of HbA1c is cation-exchange HPLC(CE-HPLC). Although CE-HPLC is widely used for the measurement of HbA1c, presence of hemoglobin variants may interfere this test, producing falsely high or low values when the variant hemoglobin or its glycated form cannot be separated from either hemoglobin A or HbA1c [6].

Here we report a case of a variant Hb detected during HbA1c measurement with CE-HPLC in a diabetic patient. This variant Hb was Hemoglobin J-Calabria (c.194G>A (p.Gly65Asp)). We would like to discuss the consequences of Hb variants in interpretation of HbA1c tests.

Case
A 56-year-old woman was referred to internal medicine outpatient clinic for diabetes screening. She was a native of Ankara, the capital of Turkey. She reported a normal physical development and no history of anemia, jaundice, abdominal distension or blood transfusions. Her family history was not significant. General physical and systematic examinations were unremarkable. Complete blood count (CBC), fasting plasma glucose and HbA1c tests ordered. HbA1c was measured by CE-HPLC method in ARKRAY ADAMS A1c HA-8180V. CBC (complete blood count) results were RBC: 4.8x10^12/L, Hb: 9.8 g/dL, Hct:28.2%, MCV: 58.6 f/L. Fasting glucose was 126 mg/dL. An unusual peak was observed in the labile HbA1c and stabile HbA1c was 0.0%. Presence of such a high level of labile HbA1c alerted us for a possible presence of hemoglobinopathy. Then the patient was advised to have a hemoglobin variant analysis with HPLC (Thermo Scientific Dionex Ultimate 3000). HPLC revealed HbA: 11.3%, HbA2: 4.7%, and Hbf: 4.3%. A peak of 76.8% unknown hemoglobin. HbA1c level was measured by immunoturbidimetric assay in Dimension RxL Max instrument. HbA1c level was reported as 6.5%. DNA mutation analysis was performed to detect the unusual hemoglobin variant in the department of genetics, University of Health Sciences Dr Sadi Konuk Training and Research Hospital. DNA isolation was achieved by the salt precipitation method and presence of heterozygote Hb J-Calabria variant (c.194G>A (p.Gly65Asp)) was detected in our patient.

Discussion
Hb J-Calabria (beta64 (E8) Gly->Asp, also known as J-Bari; J-Cosenza,) is usually detected incidentally. Hematologic parameters are normal in the heterozygote patients. In peripheral smear with cresyl blue, Heinz bodies may be seen. CE-HPLC separates Hb species based on charge differences between HbA1c and other hemoglobins. HPLC shows Hb J-Calibri and HbA separation at alkaline pH; Hb J-Calibri...
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As far as we know, HB Calabria has been reported only in members of a French family and in a Chinese male. In one of these cases it is found in combination with beta-thalassemia [10,11]. We could not find any cases in the literature about the effect of Hb J-Calabria on the false measurement of HbA1c. As far as we know, this is the first case reported of Hb J-Calabria variant in Turkey. Hb J-Calabria does not lead to clinical abnormalities, it also does not affect hematologic parameters. However, in our case microcytic anemia was present. By further investigations it was found that this microcytic anemia was due to iron deficiency so oral iron replacement therapy was started. We think that Hb J-Calabria and other Hb variant cases are mostly overlooked. Hb J-Calabria variant should be taken into account for cases that HbA1c levels cannot be evaluated by CE-HPLC method. If the CE-HPLC method is used to measure HbA1c, then careful inspection of chromatograms may identify the presence of aberrant peaks produced by variants. When the HPLC method shows the presence of an uncommon hemoglobin variant, an alternative method to measure HbA1c should be used [12].

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