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High peak of carbamylated hemoglobin discordant with urea level: a case report

Pico alto da hemoglobina carbamilada discordante com o nível de ureia: relato de caso

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ABSTRACT

Hemoglobin A1c (HbA1c) measurement is commonly performed in diabetes mellitus patients to monitor glycemic control over the last three to four months. Carbamylated hemoglobin, which is the hemoglobin that binds to isocyanic acid derived from urea, is one of the possible analytical interference in the uremic patient. When measured by ion-exchange high-performance liquid chromatography (HPLC), carbamylated hemoglobin forms a peak that overlaps the peak of HbA1c, causing a falsely elevated HbA1c result. We report a case of a 60-years-old man who had a spurious increase in HbA1c, with a high carbamylated hemoglobin peak disproportionate to the urea value. Subsequent hemoglobin analysis using hemoglobin electrophoresis and HPLC hemoglobin testing system suggested hemoglobin J (Hb J) variant. Our case highlighted the possibility of misleading HbA1c interpretation in the presence of a high carbamylated hemoglobin peak, but not proportionate to urea value. In this study, Hb J was detected. A method free from hemoglobin variant interference should be used ideally, and monitoring glycemic control should be performed using alternative methods, such as serum fructosamine or continuous glucose monitoring.

Key words: glycated hemoglobin A; chromatography high-pressure liquid; carbamylated hemoglobin; urea.

RESUMO

A dosagem de hemoglobina A1c (HbA1c) é comumente realizada em pacientes com diabetes mellitus *para monitorar o controle glicêmico nos últimos três a quatro meses. A hemoglobina carbamilada – hemoglobina que se liga ao ácido isociânico derivado da ureia – é uma das possíveis interferências analíticas no paciente urêmico. Quando medida por cromatografia líquida de alta eficiência (HPLC) por troca iônica, a hemoglobina carbamilada forma um pico que se sobrepõe ao pico da HbA1c, causando um resultado falsamente elevado da HbA1c. Relatamos o caso de um homem de 60 anos de idade que apresentava um aumento espúrio de HbA1c, com alto pico de hemoglobina carbamilada desproporcional ao valor de ureia. A análise subsequente da hemoglobina usando eletroforese de hemoglobina e sistema de teste de hemoglobina por HPLC sugeriu a variante de hemoglobina J (Hb J). Nosso caso destacou a possibilidade de interpretação enganosa da HbA1c na presença de um pico alto de hemoglobina carbamilada, mas não proporcional ao valor da ureia. Neste estudo, foi detectada a Hb J. Um método isento de interferência de variantes da hemoglobina deve ser idealmente usado, e o monitoramento do controle glicêmico deve ser feito usando métodos alternativos, como frutosamina sérica ou monitoramento contínuo da glicose.*

Unitermos: *hemoglobina A glicada; cromatografia líquida de alta pressão; hemoglobina carbamilada; ureia.*

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RESUMEN

La prueba de la hemoglobina glicosilada A1c (HbA1c) se realiza comúnmente en pacientes con diabetes mellitus *para monitorear el control glucémico durante los últimos tres o cuatro meses. La hemoglobina carbamilada, que es la hemoglobina que se une al ácido isociánico derivado de la urea, es una de las posibles interferencias analíticas en el paciente urémico. Cuando se mide mediante cromatografía líquida de alta eficacia (HPLC) de intercambio iónico, la hemoglobina carbamilada forma un pico que se superpone al pico de HbA1c, lo que provoca un resultado de HbA1c falsamente elevado. Presentamos el caso de un hombre de 60 años que tuvo un aumento espurio de HbA1c, con un pico de hemoglobina carbamilada alto desproporcionado al valor de urea. El análisis de hemoglobina posterior mediante electroforesis de hemoglobina y el sistema de prueba de hemoglobina HPLC sugirió una variante de hemoglobina J (Hb J). Nuestro caso destacó la posibilidad de una interpretación engañosa de la HbA1c en presencia de un pico de hemoglobina carbamilada alto, pero no proporcional al valor de urea. En este estudio, se detectó Hb J. Lo ideal sería utilizar un método libre de interferencia de variantes de hemoglobina, y la monitorización del control glucémico debería realizarse mediante métodos alternativos, como la fructosamina sérica o la monitorización continua de la glucosa.*

Palabras clave: *hemoglobina A glicada; cromatografía líquida de alta eficacia; hemoglobina carbamilada; urea.*

INTRODUCTION

HbA1c is a hemoglobin molecule that is irreversibly glycated at one or both N-terminal valine of beta chains[1]. It provides an estimate for the glucose control of a diabetic patient over the past three to four months, reflecting the erythrocytes' lifespan of 120 days[1]. Using cation-exchange high-performance liquid chromatography (HPLC), HbA1c is separated from another hemoglobin fractions in a column due to the charge difference[2]. This method, however, is subjected to analytical interferences from many factors, such as the presence of chemically modified hemoglobin as carbamylated hemoglobin[1]. The carbamylation process occurs when isocyanate derived from urea prevents glycation of hemoglobin molecule at its N-terminal valine[3]. It has been described as causing interference in HPLC when urea level is 180 mmol/l or higher[4].

We present a case of persistently high HbA1c value, secondary to a high peak of carbamylated hemoglobin with urea level ranging from 3.4 mmol/l to a maximum of 91 mmol/l over a 4-year period. Subsequent hemoglobin analysis revealed the presence of a hemoglobin J (Hb J) variant.

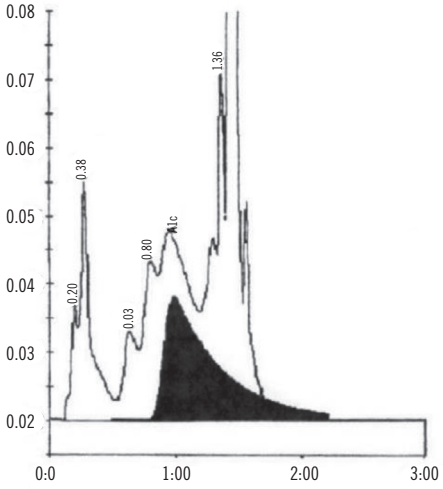
CASE REPORT

A 60-years-old man with underlying ischemic heart disease, hypertension, and type 2 diabetes mellitus presented at the emergency room with a week-long history of intermittent palpitation associated with loss of appetite. Upon examination, he appeared lethargic, dehydrated, and hypotensive (blood pressure of 83 × 50 mmHg) with an initial heart rate of 53 beats per minute. He then complained of a sudden onset palpitation during which his heart rate changed from 140 to 160 beats/minute. The electrocardiogram (ECG) showed atrial fibrillation, requiring intravenous amiodarone infusion and a subsequent ECG repetition, which revealed changes compatible with myocardial infarction, non-ST-elevation. The patient was then admitted to the medical ward and treated for acute myocardial infarction with atypical presentation. He was also treated for sepsis (leukocytosis, positive C-reactive protein, and a positive blood culture) associated with acute kidney injury with 91 mmol/l urea level and 2.52 µmol/l creatinine level. Because of his diabetic background, bedside capillary blood glucose during the acute illness was monitored and ranged from 241 mmol/l to 378 mmol/l, requiring regular subcutaneous Actrapid. The plasma glucose level was 31.4 mmol/l and HbA1c measured using the HPLC Bio-Rad D-10 HbA1c program was considered very high (21.9%), outside the reportable limit. The HPLC chromatogram showed a very high carbamylated hemoglobin peak of 12.6% (**Figure 1**) alerting to the possibility of positive interference in the HbA1c measurement. After the disease subsided and the insulin was discontinued, capillary blood glucose normalized and he was discharged with plasma blood glucose of 6.5 mmol/l. Renal function also returned to normal with urea level of 2.3 mmol/l and creatinine level of 97 µmol/l.

Apparently, this patient was previously diagnosed with type 2 diabetes mellitus in 2015, during an admission to the sepsis ward based on fasting blood glucose of 14.4 mmol/l and a very high HbA1c, outside the reportable range. Since then he started a treatment with an oral hypoglycemic agent (T. Gliclazide MR 30 mg per day), and HbA1c was regularly measured to monitor

FIGURE 1 – HPLC (Biorad D10) chromatogram for HbA1c analysis with high carbamylated Hb peak co-eluted with HbA1c (HbA1c peak highlighted in black)

Peak name	Retention time	Height	Area	Area (%)
A1a	0.2	16526	58905	1.9
A1b	0.28	34839	202876	6.6
LA1c/CHb-1	0.63	13038	95742	3.1
LA1c/CHb-2	0.8	22825	375871	12.1
A1c	0.99	18096	555061	21.9
P3	1.36	51313	351875	11.4
Ao	1.43	420664	1453518	47

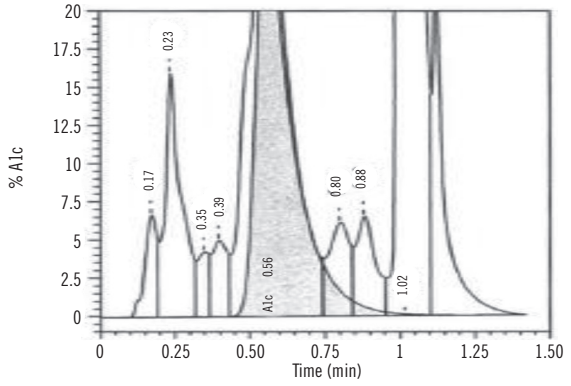


HPLC: high performance liquid chromatography; HbA1c: glycated hemoglobin A1c.

his glycemic control. Interestingly, from previous records, HbA1c has always been very high and unreportable, the chromatogram showed a persistently high carbamylated hemoglobin peak even with not very high blood urea level, ranging only from 2.1 mmol/l to a maximum of 5.7 mmol/l. Blood glucose over the past four years from, 2015 to 2018, was also well-controlled, ranging from 6.9 mmol/l to a maximum of 10 mmol/l, and he used an oral hypoglycemic agent, which was discontinued after the recent admission. For that reason, the same sample was sent for analysis using another HPLC (Bio-Rad V-II Turbo), and the HbA1c was still detected as very high (27.1%), outside the reportable range, but this time, no carbamylated hemoglobin peak was observed, which suggested the possibility of a hemoglobin variant (**Figure 2**). The sample was then sent for hemoglobin analysis using HPLC (Bio-Rad Variant II, Beta Thalassaemia Short Programme, United State) and hemoglobin electrophoresis on an agarose gel (Interlab G26, Italy), which revealed the presence of an abnormal hemoglobin variant, probably Hb J (**Figure 3**). However, the molecular study for confirmation was not sent due to a logistic problem.

FIGURE 2 – HPLC (variant 2 turbo) chromatogram for Hb A1c analysis shows very high HbA1c level

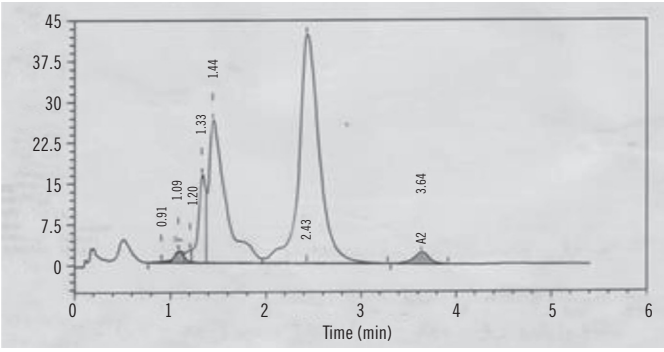
Peak name	Calibrated area (%)	Area	Retention time (min)	Peak area
A1a	---	2.1	0.169	59436
A1b	---	6.8	0.233	189205
Unknown	---	1.3	0.346	35308
Unknown	---	2	0.394	55695
A1c	27.1	---	0.561	700548
P3	---	3.3	0.800	92468
P4	---	3.3	0.880	91501
Ao	---	55.8	1.016	1543847



HPLC: high performance liquid chromatography; HbA1c: glycated hemoglobin A1c.

FIGURE 3 – HPLC (variant II, Beta-Thalassemia Short Program) revealed an increased P3 peak (29.2%) with low HbA (58.1%), and normal HbA2 and HbF (2.1% and 1% respectively). A further analysis with alkaline agarose gel electrophoresis (Interlab G26), revealed an abnormal band anodal to A band. These findings were suggestive of hemoglobin variant, probably Hb J

Peak name	Calibrated area (%)	Area (%)	Retention time (min)	Peak area
P1	---	0.1	0.91	2711
F	1.3	---	1.09	34790
Unknown	---	0.8	1.2	21745
P2	---	8.3	1.33	213733
P3	---	29.2	1.44	754041
Ao	---	58.1	2.43	1501669
A2	2.1	---	3.64	57129



HPLC: high performance liquid chromatography; HbA: hemoglobin A; HbA2: hemoglobin A2; HbF: hemoglobin F; Hb J: hemoglobin J variant.

DISCUSSION

Carbamylated hemoglobin is the hemoglobin condensed with isocyanic acid at the N-terminal valine of its molecule, the reactive form of cyanate derived from the spontaneous decomposition of urea[3]. The change at the N-terminal valine for both HbA1c and carbamylated hemoglobin molecules causes them to become less positive, therefore less attracted to the negatively charged resins in the separation column, hence they migrate faster than hemoglobin A (HbA)[5]. Carbamylated hemoglobin may represent a possible interference in HPLC during the HbA1c measurement because the chemical modification at the N-terminal valine results in both molecules co-elute almost at the same time, subsequently producing an overlapping chromatogram peak[6].

Carbamylated hemoglobin has been stated to cause significant interference in the HbA1c HPLC measurement when urea is equal to or greater than 30 mmol/l[4]. However, this patient's urea level was never very high, but carbamylated hemoglobin persisted causing a consistently very high HbA1c value, outside the reportable range. Besides, the HbA1c value has always been greater than 15%, not corresponding to his blood glucose level. This raises the suspicion of a hemoglobin variant that is not separable from the HbA1c fraction[7]. Unlike labile A1c which can also cause similar interference, neither carbamylated hemoglobin nor the Hb variant can be resolved using incubation due to the permanent modification[6].

Furthermore, repeated analysis using a different HPLC did not even show any presence of carbamylated hemoglobin peak, despite the high HbA1c value of more than 15%. The patient also had no other risk factors that could lead to a falsely high HbA1c, such as in cases where erythrocyte lifespan is prolonged, reducing red cell turnover (eg. iron deficiency anemia), or recent blood transfusion[1]. For this reason, hemoglobin analysis was performed and reported to be consistent with the Hb J variant. The interference mechanism of Hb variants can vary, therefore a different type of variant can cause either a falsely high or low HbA1c (Figure 1C)[1, 5]. However, the Hb J variant has been reported to causing an abnormally high HbA1c value and disproportionate to plasma glucose[8]. In such cases, an alternative methodology should be chosen, such as immunoassay that uses specific HbA1c antibodies or capillary electrophoresis that can differentiate HbA1c from a hemoglobin variant[2, 9]. However, at our center, these methods are not available. Another option is serum fructosamine, a glycoprotein that provides information on glycemic control over 2-4 weeks and is unaffected by the erythrocytes and hemoglobin characteristics.

Nevertheless, an abnormally high HbA1c reading such as this should not be overlooked, as a misleading interpretation can lead to an overestimation of glycemic control and misdiagnosis. In the presence of high carbamylated peak or hemoglobin variants, the alternative to HbA1c measurements should be considered, such as serum fructosamine that measures glycated albumin, or continuous glucose monitoring[1, 10]. However, unlike HbA1c, no other alternative measures were found to be superior to HbA1c in predicting complications related to diabetes mellitus[1, 10]. Therefore, their use should be limited only to cases where the HbA1c results can be misinterpreted[1].

CONCLUSION

Interpreting HbA1c must consider both its clinical and analytical aspects, especially when the discordant HbA1c value and its plasma glucose are observed. When reporting HbA1c, a suspicious peak and very high or low HbA1c value that fall outside the reportable range should alert the possibility of a hemoglobin variant. Therefore, ideally, further investigations may include measurement of HbA1c with a different assay method and hemoglobin analysis to determine the type of variant. Meanwhile, monitoring glycemic control should be performed using other modalities, such as serum fructosamine or continuous glucose monitoring.

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