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Case Report

Hyperleukocytosis: a unique cause of an unidentifiable hemoglobin A1c peak

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ABSTRACT

Background: Glycosylated hemoglobin (HbA1c) serves as a crucial biomarker for the diagnosis and monitoring of diabetes. It can be measured via different methods. Interference during analysis can potentially arise from various factors, including rare occurrences such as hyperleukocytosis. **Case presentation**: Here, we present the case of a 54-year-old male patient with a 20-year history of type 2 diabetes mellitus who complained of prolonged lethargy, epigastric discomfort, and constitutional symptoms of malignancy. Further investigation revealed a diagnosis of T-cell prolymphocytic leukemia accompanied by hyperleukocytosis, indicated by a white cell count of 574.60 × 10^9/L with predominant lymphocytes. Chemotherapy and tumor lysis syndrome prophylaxis were initiated. During diabetic monitoring, analysis of HbA1c using capillary electrophoresis revealed an absent HbA1c peak; this has not previously been observed. To address this finding, the sample underwent repeated saline washing and centrifugation. Subsequent analysis demonstrated an improvement, with a well-fractionated HbA1c peak present at 8.7% (71 mmol/mol). Various factors can interfere with HbA1c analysis. Drug and hemoglobin variant interference was ruled out following the recovery of the peak post saline washing. The accelerated migration speed of the sample caused by interfering substances in the plasma was postulated to result in a profile shift, leading to the non-recognition of HbA1c fractions. **Conclusion**: By implementing the important step of washing out interfering molecules, the shift was eliminated, allowing for a true HbA1c level measurement. The appearance of an HbA1c peak post saline wash suggests the presence of endogenous substances that interfere with the assay's analytical method.

Key words: HbA1c, hyperleukocytosis, T-PLL, assay interference

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INTRODUCTION

HbA1c is a glycosylated hemoglobin (Hb) adduct that is well-accepted as a versatile marker of hyperglycemia in diabetes mellitus (DM). It can be used to screen, diagnose, and monitor DM patients so that optimum treatment can be given to slow down disease progression. However, it may not represent the actual glycemic control status in certain patients. The presence of Hb variants, diseases that impair red blood cell (RBC) survival (such as hemolytic anemia), as well as the administration of certain drugs that may affect RBC integrity (*e.g.*, dapsone, hydroxyurea) or vitamins (*e.g.*, vitamins C and E) are known factors that may adversely affect HbA1c levels. Here, we report a rare case of undetectable HbA1c associated with hyperleukocytosis secondary to T-cell prolymphocytic leukemia (TPLL).

CASE PRESENTATION

A 54-year-old Malay gentleman with underlying hyperlipidemia and type 2 diabetes mellitus (T2DM) on insulin therapy since 2004 initially presented with a few weeks' history of lethargy and epigastric discomfort associated with abdominal distention and pedal edema. He was admitted for further assessment. The initial workup, including a full blood count (FBC), showed that the total white cell (TWC) count was markedly raised (574.60 × 10^9/L), with lymphocytes predominating (558.42 × 10^9/L [97.2%]), a red blood cell (RBC) count of 3.81 × 10^9/L, Hb = 11.3 g/dL, and platelets = 81 × 10^9/L. A full blood picture (FBP) revealed significant hyperleukocytosis, with 98% abnormal lymphoid cells (**Figure 1**). Based on these features, lymphoproliferative disorder needed to be considered. To explain the patients' white cell count of over 50,000 × 10^9/L, a bone marrow aspiration and trephine (BMAT) biopsy and flow cytometry immunophenotyping were performed, revealing TPLL. The patient was started on induction chemotherapy with 3-cycle fludarabine, mitoxantrone, and cyclophosphamide, as well as prophylactic allopurinol, hydroxyurea, and hyperhydration as a preventive

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strategy against tumor lysis syndrome (TLS). In view of the patient's comorbidity of T2DM, fasting blood glucose (FBS) and HbA1c were measured to assess glycemic control. The FBS value was 11.2 mmol/L. However, surprisingly, no HbA1c peak was detected in the initial run using the capillary electrophoresis (CE) method (**Figure 2**).

Thus, the sample was treated by removing the plasma, and the remaining RBCs were washed 3 times with saline (the sample was centrifuged after each wash step). The analysis was repeated, and the result is shown in **Figure 3**.

DISCUSSION

DM is a common occurrence in cancer patients due to the effects of cancer treatment regimes. Many patients with pre-existing DM undergoing cancer treatment develop iatrogenic hyperglycemia most commonly associated with steroid administration [1]. This may pose a significant challenge in monitoring the patient's actual glycemic control status. Clinical management should be tailored to the individual patient to maintain euglycemia and prevent further complications [2]. In the present case, the patient was diagnosed with TPLL on top of having T2DM and being on insulin therapy for the past 20 years; this presentation posed a challenge to the managing team.

TPLL is characterized by the malignant proliferation of mature T lymphocytes. The morphology is described as hyperleukocytosis with the marked proliferation of small- to medium-sized prolymphocytes, showing distinct cytoplasmic blebbing, similar to our case. The cells in B- and T-CLLs are small and mostly well-differentiated. Immunophenotypically, they are positive for CD7, CD2, CD5, and CD3, with CD52 usually also exhibiting bright expression. Patients will usually be given high-dose chemotherapy, and in certain cases, hematopoietic stem cell transplantation may offer a curative outcome. Nevertheless, it is generally resistant to conventional chemotherapy and has a poor prognosis [3].

The presence of hyperviscosity and leukostasis syndromes in TPLL is uncommon. To increase whole-blood viscosity, a cell count of at least 1,000,000 cells/μL is needed to reach a leucocrit of 20% [4]. Hyperleukocytosis occurs when the white blood cell (WBC) count exceeds 100,000 cells/μL. When hyperleukocytosis becomes clinically evident, it is known as leukostasis. It can result in complications such as TLS and disseminated intravascular coagulopathy (DIC) [5]. Various methods can be used to improve patients' outcomes in cases of symptomatic hyperleukocytosis. These include induction chemotherapy, supportive treatments, and cytoreductive therapy using corticosteroids, leukapheresis, and chemotherapeutic agents such as hydroxyurea [6].

At presentation, the current patient had hyperleukocytosis without leukostasis. He was given fluid therapy, allopurinol, and hydroxyurea as preventive measures against TLS at the commencement of induction chemotherapy. In addition to the assessment of liver and renal function and electrolyte abnormalities, HbA1c was measured as part of the monitoring process in view of the patient's comorbidity. However, the initial HbA1c result was unreportable as no HbA1c peak was detected by the assay. HbA1c was measured using CE, which is reported to have better resolution and less interference from interfering substances. In the present case, hydroxyurea was given to the patient; this drug is known to interfere with HbA1c measurement. The drug causes a shift from HbA to HbF, leading to a lower HbA1c level [7,8]. The drug should thus be withheld to stop the alteration process to measure the HbA1c level [9]. However, the false HbA1c result of the initial run in this report is not likely to be due to interference with hydroxyurea as the HbA1c peak was detected after washing the sample with saline. If hydroxyurea had affected the HbA1c peak, the result should have remained the same even after the washing step.

Other causes of an absent HbA1c peak include the presence of Hb variants that can interfere with HbA1c measurement methods. For example, HbA synthesis is reduced in β-thalassemia and hereditary persistence of fetal Hb (HPFH), causing falsely low HbA1c regardless of the analytical methods used [10]. Drugs that can cause hemolysis, like dapsone and certain antiretroviral agents, can also result in spuriously low HbA1c levels [11].

The absence of an HbA1c fraction in this patient is postulated to be due to accelerated migration caused by the presence of some unknown molecules secreted by the malignant cells. It is unlikely to be due to factors endogenous to the erythrocytes since the HbA1c peak was recognizable after the saline wash [12].

CONCLUSION

Hyperleukocytosis is a potential cause of interference in HbA1c measurement, possibly due to proteins or other substances secreted by the malignant cells. As presented, in our patient with hyperleukocytosis, no HbA1c peak was detected in the pre-treatment sample. This hypothesis arises based on the observation that washing the plasma out of the sample could eliminate the interference. Unfortunately, the exact mechanism that underlies the condition is still unclear, and further studies are needed to identify its true cause.

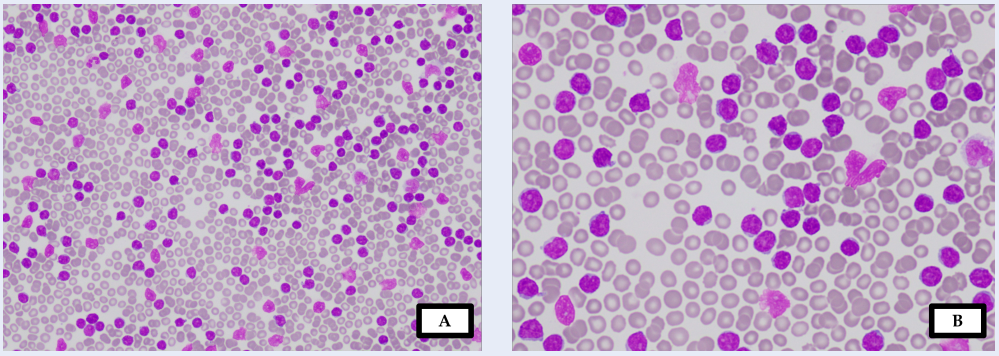

Figure 1: **FBP with microscopic and Wright's staining features. (A: 10x HPF) and (B: 40x HPF)**. Hyperleucocytosis with abundant abnormal lymphoid cells described as varying sizes ranging from small to medium, scanty with some cytoplasmic blebbing, and irregular nuclear outline. Occasional smudges are seen. Otherwise, normochromic normocytic RBC with thrombocytopenia.

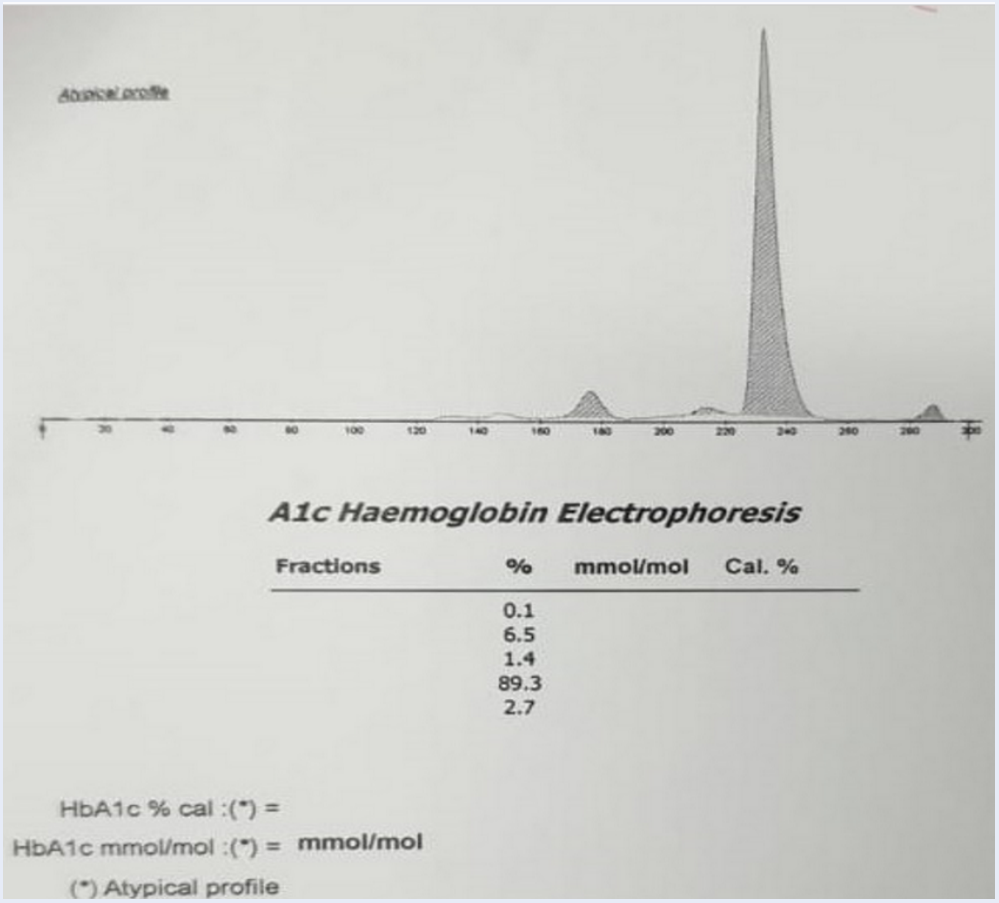

Figure 2: **Pre-treatment sample**. Electropherogram from CE showed unidentifiable peak with absent of HbA1c fraction at the initial run.

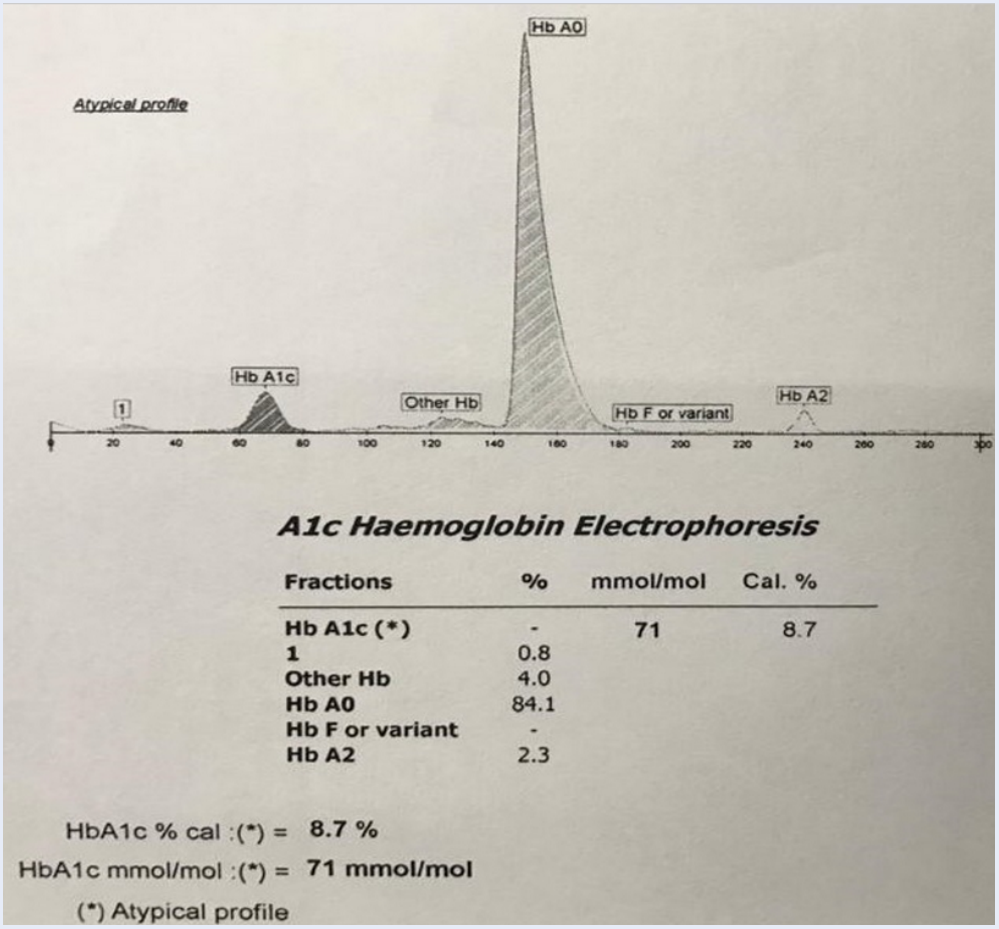

Figure 3: **Post-treatment sample**. Electropherogram from CE showed amelioration of Hb peak with detectable HbA1c of 8.7% (71 mmol/mol) in post washing and centrifugation sample.

ABBREVIATIONS

BMAT: bone marrow aspiration and trephine biopsy
CE: Capillary Electrophoresis
DIC: Disseminated Intravascular Coagulopathy
DM: Diabetes Mellitus
FBP: Full Blood Picture
FBS: Fasting Blood Glucose
HbA1c: Glycosylated Haemoglobin
Hb: Haemoglobin
HPF: High Power Field
HPFH: Hereditary Persistence of fetal Hb
RBC: Red Blood Cell
T2DM: Type 2 Diabetes Mellitus
T-PLL: T-cell prolymphocytic Leukemia
TWC: Total White Cell Count
TLS: Tumor Lysis Syndrome

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AUTHOR'S CONTRIBUTIONS

Abdul Razak AA, Tuan Ismail TS, Zulkeflee RH, Ab Rahim SN, Zulkeflee HA, and Wan Nik WNFH, contributed equally to this work; Wan Nik WNFH supervised and directed the focus of this review; Abdul Razak AA, Tuan Ismail TS, Zulkeflee RH, Ab Rahim SN, Zulkeflee HA, Wan Nik WNFH performed the literature review. All authors have reviewed and agreed upon the final version of this case report.

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AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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