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Case Report Open Access

Falsely Low HbA1c Unmasking a Latent HbE Defect

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Abstract

The current case report illustrates role of vigilant laboratory services that helped in unmasking asymptomatic HbE variant in a middle aged known diabetic woman who came as an outdoor patient and on subsequent HbA1c testing was found to have lower value -2.9 %. It triggered extensive workup after looking for common causes and an electrophoresis of Hemoglobin revealed HbE variant.

The case also summarizes common causes of spurious low and high HbA1c values which are encountered on regular basis by laboratories. Such abnormal values that do not correlate with blood glucose values mandates workup and shouldn't be left without work up.

Keywords: Spurious glycated hemoglobin; Latent hemoglobinopathy

Abbreviations: Glycated hemoglobin - HbA1c; Hemoglobin E - HbE Hemoglobinopathies are known to arti factually alter hemoglobin A1c (HbA1c) concentration. [1] The American diabetes association recommends testing for glycated hemoglobin (Hb A1C) for diagnosis and monitoring of diabetes. [2] Here we report a case of diabetes with normal hemoglobin level but reduced HbA1c. The case holds clinical relevance as A1c is frequently ordered test and therefore close attention at the part of pathologists & laboratory personnel could enable us to unfold asymptomatic Hemoglobinopathies.

Case Report

A middle aged 55yearold known diabetic woman came to OPD for routine investigations. The investigations showed Hb- 12.0 g/dl WBC Count- 10.5 x 10^3/ uL, MCV-75.2 fl, MCH- 24.6 pg, MCHC-32.8 g/dl, Platelet count – 353 x 10^3 /uL and WBC differential showing – Neutrophils- 63%, Lymphocytes- 32%, Monocytes- 02% & Eosinophils- 03%. The biochemical investigations showed serum creatinine- 0.6 mg/dL, SGPT- 57 IU, Total cholesterol- 195 mg/dl, HDL- 41 mg/dl, LDL- 117 mg/dl, VLDL- 37 mg/dl & Triglyceride- 185 mg/dl. (Table -1) The patient serum blood glucose was 236 mg/dl but surprisingly the HbA1c was far lower than expected value at 2.9 % (Fully automated HPLC Variant II Turbo, Bio rad Laboratories, Munich, Germany) (Figure1) The abnormal A1c value prompted laboratory to repeat test to eliminate any potential source of preanalytical error. The repeat testing showed A1c -2.9% and fasting blood glucose – 230 mg/dl. The exceptionally low level of A1c prompted further work up to evaluate the cause and a hemoglobin electrophoresis revealed patient presence of Hemoglobin E. (Figure 2).

Parameter	Value	Unit
Hb	12	g/dL
WBC count	10.5	/cumm
MCV	75.2	fl
MCH	24.6	pg
MCHC	32.8	g/dl
Platelet count	353000	Ul
Serum Creatinine	0.6	mg/dl
SGPT	57	mg/dl
Total Cholesterol	195	mg/dl
HDL	41	mg/dl
LDL	117	mg/dl
VLDL	37	mg/dl
Triglyceride	185	mg/dl
Blood glucose	236	mg/dl
HbA1C	2.9	%

Table 1: Summary of Investigations

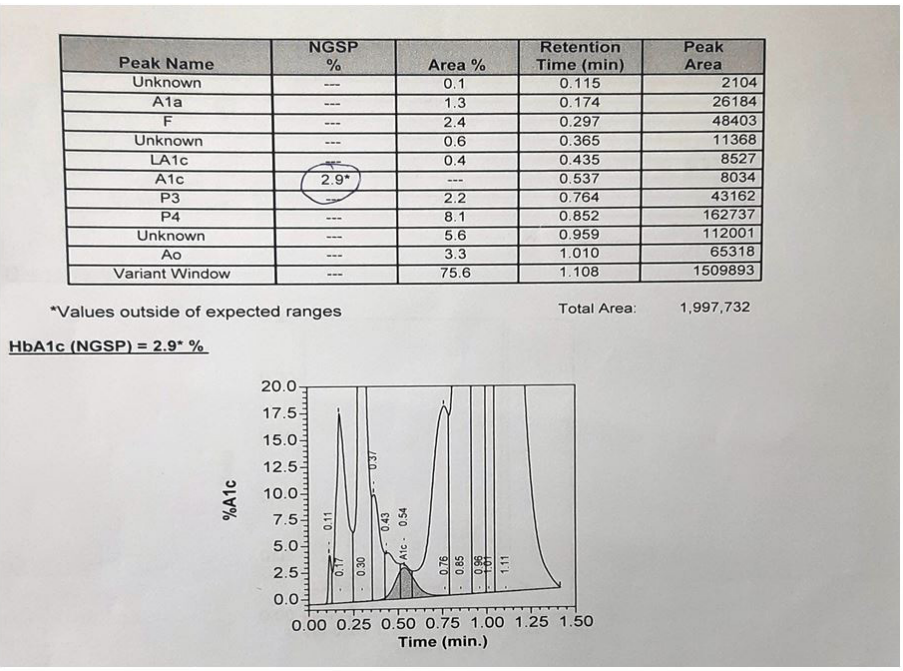

Figure 1: HbA1c Bio rad variant II

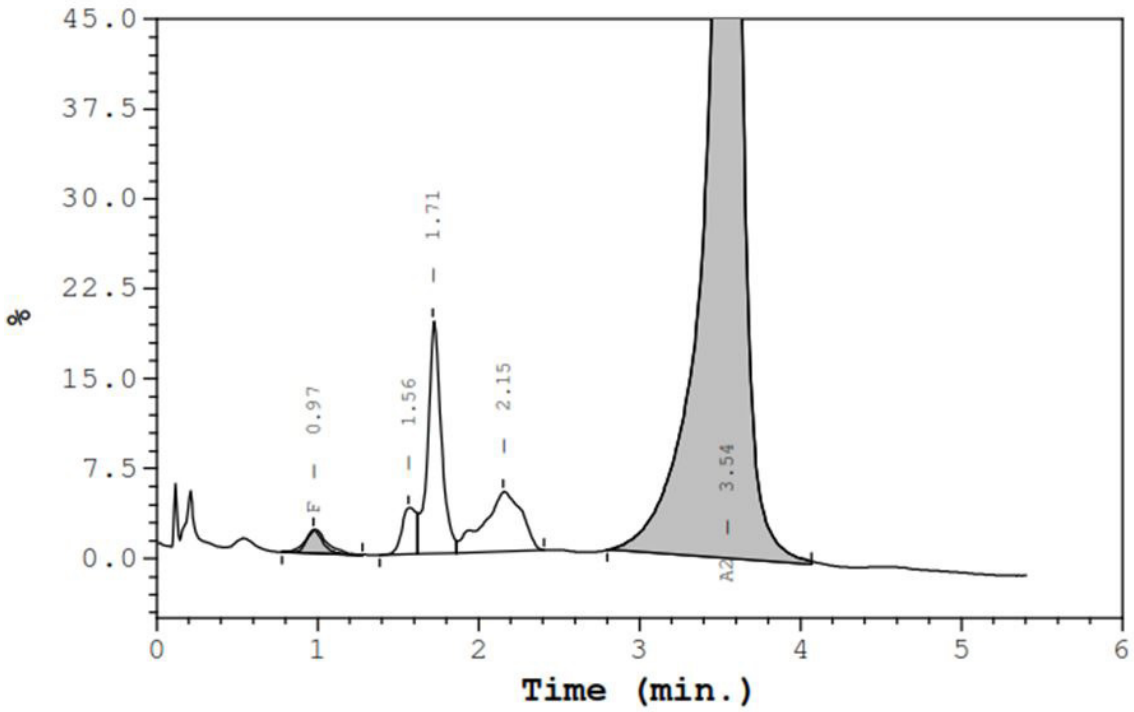

Figure 2: Hemoglobin electrophoresis showing abnormal high HbA2 due to presence of Hb E (Bio rad Laboratories, Munich, Germany) which was confirmed by second electrophoresis

Discussion

HbA1c was available for testing in 1978 and was put in to clinical use in 1980's. [3] Glycated hemoglobin (HbA1c) is formed by non enzymatic glycation of the beta chain of hemoglobin A by plasma glucose. [4] The initial reaction is reversible which results in the formation of an aldehyde Shiff's base and later an irreversible reaction takes place that forms a stable ketomine by Amadori rearrangement. The level of A1c is directly proportional to the blood glucose level and life span of RBC. [5,6] The Schiff's base formed is unstable known as Labile hemoglobin A1c or Pre Hb A1c or LA1c or pre glycohemoglobin which cannot be separated from stable ketoamine fraction by the currently available methods.[7] The latter stable ketomine undergoes irreversible glycation and occurs continuously throughout the life span of red blood cells i.e 120 days [8,9] This property serves as principle for using A1c as long term glycemic control as diabetes is rampant worldwide.

The American Diabetic association (ADA) in 2010 recommended the use of HbA1c as diagnostic test for screening for Diabetes mellitus type II. [10,11] ADA standards of medical care in diabetes, consensus statement by American association of clinical endocrinologists (AACE) and guidance statement update from American college of physicians (ACP) , published guidelines for HbA1c target for glycemic control for non pregnant adults with type II diabetes mellitus in 2018. [12, 13] As per ADA guideline the diagnosis of diabetes mellitus is made when A1c value is more than or equal to 6.5%. (154.1 mg/dl). Prediabetes is defined as A1c value between 5.7% to 6.4%. The diagnosis of type I DM in individuals with overt symptoms of hyperglycemia in pediatric age group to be made using plasma glucose concentration rather than HbA1c levels [14] whereas for the diagnosis of type 2 DM in younger as well as older patients – A1c, fasting plasma glucose and 2 hour Post prandial values after oral glucose tolerance test may be used. [15] The guideline also recommends the testing for HbA1c levels twice a year for patients who have achieved stable glycemic control and for those who have not achieved glycemic control, a quarterly testing of A1c is recommended. [16]

A1c testing is advantageous than other glucose estimation methods as it does not involve any special preparation like overnight fasting requirement and therefore can be done at any point of time during the day. There is also less variability in levels of A1c as compared to fasting blood sugar [17,18]

The commonly methods used for measuring A1c [19,20]

1. Liquid chromatography
2. Immunoassay
3. Boronate affinity
4. Ion exchange high performance liquid chromatography
5. Capillary electrophoresis

A1c measurement is advantageous as it eliminates preanalytical issues of testing because it does not require fasting state of patient and is less affected by physiological changes. It results from post translational addition of glucose to N-terminal valine of beta chain of hemoglobin.

However, A1c is not full proof and it is affected by many factors like race & age, African- American having higher A1c values in comparing with non Hispanic whites. [14,21] One of the most important condition that results in erroneous A1c estimation is hemoglobinopathy. Hemoglobinopathies affect the reliability of A1c testing by threefold mechanism- altering normal glycation of HbA to HbA1c, abnormal chromatography peak and shorter red cell survival. Table -1 depicts various situations that may result in spurious HbA1c values.

Falsely decreased HbA1c	**Falsely increased HbA1c**
Physiological	
Pregnancy in II trimester	Pregnancy III trimester, Hypertriglyceridemia, Hyperbilirubinemia
Hemoglobinopathy	
Hemoglobin variants (Thal trait, HbE etc)	
Other Pathology	
Splenomegaly	Asplenia, Chronic liver disease, Renal insufficiency, uremia
RBC disorder	
RBC transfusion	Anemia – Defective erythropoiesis (Vit B12, Folate, Iron deficiency)
Drug intake	
Vitamin C ingestion	Chronic alcohol consumption, Chronic salicylate ingestion, Chronic opiod ingestion, Lead poisoining, Dapsone, Ribavarin

Table 1: HbA1c values affected by various factors [22]

The situations where A1c does not yield accurate results one has to adopt alternative methods to assess long term glycemic control like fructosamine level, glycated albumin or 1,5 anhydroglucitol [23]. Fructosamine test is useful in patients having renal disease however they have limitation in states associated with hypoproteinemia. [24,25] Similarly, glycated albumin may be used for assessment of glycemic control over 2to3 week period. [24,25] 1,5 anhydroglucitol may also be used as higher glucose concentration competitively inhibit its renal absorption resulting in inverse relationship with blood glucose concentration. Mendlovic et al [26] in their review found fructosamine estimation as a good alternative method for cases where long term glycemic control using A1c cannot be performed in presence of hemoglobinopathy.

HbA1c has been used for long term glycemic control and therefore low HbA1c values below reference range are not given clinical relevance rather neglected and should be worked up as the literature studies which have shown that low HbA1c values of (<5%) are associated with increased risk of mortality showing a J shaped or Ushaped association between HbA1c & all cause mortality. [27-30] The low HbA1c values are general marker of ill health, liver disease and early stage of cancer. [27, 30, 31] Our case highlights the importance of evaluating very low HbA1c levels that unfold in asymptomatic hemoglobinopathy HbE and therefore mandates work up for all extremely low HbA1c levels for better health surveillance.

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References

Little RR, Roberts WL (2019) A review of variant hemoglobins interfering with hemoglobin A1c measurement. *J Diabetes Sci Technol.* 3: 446-51.

2. American Diabetes A (2019) Standards of medical care in diabetes-2019 abridged for primary care providers. Clin Diabetes. 37: 11-34 doi: 10.2337/cd18-0105.

3. Sacks DB (2012) Measurement of hemoglobin A(1c): a new twist on the path to harmony Diabetes Care 35: 2674-80.

4. Lahousen T, Roller RE, Lipp RW, Schnedl WJ (2002) Determination of glycated hemoglobins (Hb A1c). When Klin Wochenschr. 114: 301-5.

5. Bunn HF, Haney DN, Kamin S, Gabbay KH, Gallop PM (1976) The biosynthesis of human hemoglobin A1c: Slow glycosylation of hemoglobin in vivo. J Clin Invest. 57: 16529

6. Ping Wang, PhD, DABCC, FACB (2017) What Clinical Laboratorians Should Do in Response to Extremely Low Hemoglobin A1c Results Laboratory Medicine 48: 89-92.

7. John WG (1984) Effect of Schiff base (labile fraction) on the measurement of glycated hemoglobin by affinity chromatography Clin Chem 30: 1111-2.

8. Nathan DM, Turgeon H, Regan S (2007) Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia 50: 223944.

9. Ryden L, Standl E, Bartnik M, Van den Berghe G, Betteridge, et al (2007). Guidelines on diabetes, prediabetes, and cardiovascular disease. Eur Heart J 28: 88-136.

10. World Health Organization. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus [Internet]. Available from http://www.who. int/diabetes/publications/reporthba1c_2011.pdf.

11. American Diabetes Association (2011) Diagnosis and classification of diabetes mellitus Diabetes Care 34: S62-9.

12. American Diabetes Association (2018) Chapter 6. Glycemic targets: standards of medical care in diabetes Diabetes Care 41: S55-64.

13. Little RR, Rohlng C (2013) The long and winding road to optimal HbA1c measurement Clin Chim Acta. 418: 63-71.

14. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes Ch 2. Diabetes Care. 41: S13-27.

15. American Diabetes Association Standards of Medical Care in Diabetes. Diabetes Care 36: S11-66.

16. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Report of a World Health Organization Consultation Diabetes Res Clin Pract. 93: 299-309.

17. Nayal B, Raghuveer CV, Suvarna N, Goud MBK, Devi SO, Devaki RN (2011). Glycated haemoglobin - the clinical and Biochemical divide: A review Int J Pharm Sci Rev Res. 6: 122-4.

18. Rhea JM, Molinaro R (2014) Pathology consultation on HbA1c methods and interferences Am J Clin Pathol. 141: 5-16.

19. Gupta S, Jain U, Chauhan N (2017) Laboratory diagnosis of HbA1c: a review J Nanomed Res 5: 00120.

20. Menke A, Rust KF, Savage PJ, et al (2015) Hemoglobin A1c, fasting plasma glucose, and 2-hour plasma glucose distribution in US population subgroups: NHANES. Ann Epidemiol. 24: 83-9.

21. Radin MS (2013) Pitfalls in hemoglobin A1c measurement when results may be misleading J Gen Intern Med. 29: 388-94.

22. Bazerbachi F, Nazarian S, Alraiyes AH, et al. (2014) One-minute consult: is hemoglobin A1c an accurate measure of glycemic control in all diabetic patients? Cleveland Clin J Med. 81: 146-9.

23. Hare MJL, Shaw Je, Zimmet PZ (2012) Current controversies in the use of hemoglobinA1c. J Intern Med. 271: 227-36.

24. Sofronescu AG, Williams LM, Andrews DM, et al (2012) *Clin Chem*. 57: 1537.

25. Mendlovic DB, Whitehouse FW, Foreback CC (1992) The why and wherefore of fructosamine Henry Ford Hosp Med J 40: 149-51.

26. Selvin E, Steffes MW, Zhu H, et al (2010) Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults N Engl J Med 362: 800-11.

27. Brewer N, Wright CS, Travier N, et al (2008) A New Zealand linkage study examining the associations between A1C concentration and mortality. Diabetes Care 31: 1144-9.

28. Levitan EB, Liu S, Stampfer MJ, et al (2008) HbA1c measured in stored erythrocytes and mortality rate among middle-aged and older women. Diabetologia 51: 267-75.

29. Carson AP, Fox CS, McGuire DK, et al. (2010) Low hemoglobin A1c and risk of all-cause mortality among US adults without diabetes Circ Cardiovasc Qual Outcomes 3: 661-7.

30. Saydah S, Tao M, Imperatore G (2009) Gregg E GHb level and subsequent mortality among adults in the U.S. Diabetes Care 32: 1440-6.

31. Jacobs D, Blackburn H, Higgins M, et al. (1992) Report of the Conference on Low Blood Cholesterol: Mortality Associations. Circulation 86: 1046-60.