Case Report

Limitation of Estimating Glycosylated Hemoglobin to diagnose Diabetes Mellitus in a case of Spherocytosis

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ABSTRACT

Here we report identification of a case of spherocytosis, which is peculiar in the sense that detection was done due to mismatch of blood glucose and HbA1c level. The stepwise identification is discussed in detail. It is also recommended that monitoring of patients with Diabetes mellitus should not be done by estimation of Hb A_{1c} level, if the patient is suffering from spherocytosis at the same time. In such case, estimation of glycosylated pre-albumin and fructosamine may be helpful.

Keywords: Glycosylated Hemoglobin**,** Spherocytosis, Monitoring, Diabetes, Mellitus

1. INTRODUCTION

Glycosylated hemoglobin or hemoglobin A1c (HbA1c) has been recommended to diagnose Type 2 Diabetes Mellitus (T2DM by The International Expert Committee and American Diabetes Association (ADA). The reasons behind are that, overnight fasting is not required for the test. So blood collection can be done at any time, and not related to food. It is stable for 90 to 120 days and worldwide standardization has been achieved for the test. Moreover, HbA1c has been found to have good reproducibility [1].

The cut off value of HbA1c for diagnosis of T2DM has been proposed to be $\geq$ 6.5% by World Health Organization (WHO) & ADA [2]. But HbA1c has some limitations to be used as diagnostic parameter. In blood, glucose is bound to hemoglobin to form HbA1c. Hence, changes in RBC lifespan due to hemoglobinopathies, blood loss, blood transfusion, deficiency of micronutrients like vitamin B12, folic acid, iron, hemolytic anaemia, deficiency of G6PD enzyme can alter the value of HbA1c. any condition, when RBC lifespan is decreased can cause lowering of HbA1c level whereas, prolongation of RBC lifespan can result in increased HbA1c value. A tabular form of the conditions altering the level of HbA1c is given in Table 1 [3].

Some drugs can also alter the level of HbA1c, which is depicted in Table 2 [4]. Sometimes labile fractions of Hb A1 (i.e. HbA1q and HbA1b) are eluted with Hb F in High Performance liquid chromatography (HPLC) and can cause confusion.

Table 1: Conditions giving rise to inappropriate value of HbA1c

Inappropriately Low HbA1c	**Inappropriately High HbA1c**	**Variable Effect on HbA1c**
Hemolysis	Iron deficiency	Fetal hemoglobin
Certain hemoglobinopathies	Vitamin B12 deficiency	Methaemoglobin
Recent blood transfusion	Alcoholism	
Acute blood loss	Uremia	
Hypertriglyceridemia	Hyperbilirubinemia	
Drugs	Drugs	

Table 2: Drugs Causing Inappropriately Low or High HbA1c

Postulated Mechanism	Falsely Low HbA1c	Falsely High HbA1c
Increased erythrocyte destruction	• Antiretrovirals • Trimethoprim • Sulfamethoxazole • Hydroxyurea	
Altered hemoglobin / Altered glycation	Vitamin C Vitamin E Aspirin (small doses)	Aspirin (large doses) Chronic opiate use

2. Case History

A 30-year female patient was suspected to suffer from Type 2 Diabetes Mellitus and was referred to Micro Clinical Laboratory & Diagnostic Centre, Haldia, Purba Medinipur (NABL and ISO accredited) for examination of fasting and post prandial blood glucose level and estimation of Hb A1c. The report was as follows:

- Fasting Blood Glucose: 196 mg%
- PP Blood glucose: 384mg%
- Hb A1c by HPLC (Bio-Rad): 3.5%

The parameters were re estimated in a fresh sample but same results were found. On enquiry, patient gave history of blood transfusion 8 years ago, Recurrent episodes of Jaundice, 2 – 3 times a year for last 10 years, Abnormal fullness and heaviness of abdomen along with Weakness, Lethargy, Malaise. Unfortunately, no Complete blood count (CBC) or Liver Function test (LFT) was done earlier.

To eliminate drug induced causes of abnormally low glycosylated hemoglobin (Table 2), history of intake of Vitamin C, Vitamin E, Hydroxyurea, Aspirin, Trimethoprim, Sulfamethoxazole, Dapsone were taken but a negative response was obtained.

Though not asked for, we performed a CBC by automated blood cell analyser (Sysmex 121), as the reports of blood glucose and glycosylated hemoglobin could not be matched.

On examination of blood slide more than 70% micro spherocytes were found to be present in peripheral smear. The report of other parameters was as follows:

- RBC: 5.75 (10^{12}/l)
- MCV: 67.8 (fL) 80 – 100 femtoliters (fL)
- MCH: 21.4 (pg) (27 -33)
- MCHC: 31.5 (g/dL) (**32 – 36 grams per deciliter (g/dL)**
- RDW-CV: 18.2% 11.5% – 14.5%
- Hb%: 12.3 g%

Osmotic Fragility Test: RBCs were placed in serial solutions of saline at concentrations ranging from 0.1% to 0.9% NaCl. Hemolysis is evaluated by spectrophotometric measurement of the hemoglobin concentration in the solution

It was found that initial hemolysis of sample started at 0.75% of NaCl solution (Control: 0.45 % NaCl solution) whereas complete hemolysis of sample was observed at 0.45% solution in contrast to 0.1% NaCl solution for the control. (Fig. 1 and Table 3)

A written Informed consent was obtained from the patient. Patient anonymity was carefully maintained.

Table 3: Median corpuscular fragility with Initial and Final hemolysis in Concentration of saline (gm/L of NaCl)

Osmotic Fragility test	Initial hemolysis	Final hemolysis	Median corpuscular fragility
Control (gm/L of NaCl)	7.5	4.5	6.2
Test (gm/L of NaCl)	4.5	1.0	3.8

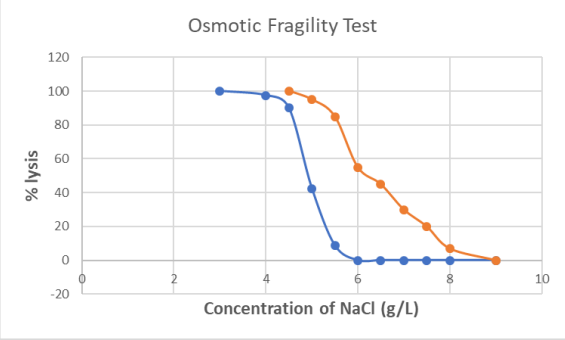

Fig 1: Legend: Red colored line for Sample, Blue colored line for Control

3. DISCUSSION

It is now concluded that patient is suffering from hereditary micro spherocytosis, which explains episodes of jaundice. This also explains low level of Hb A1c due to shortened lifespan of RBC, i.e. spherocytes.

Hereditary spherocytosis is one of the causes of hemolytic anemia. Pathogenesis involves mutation in one of genes coding for proteins (Spectrin, Ankyrin, band 3 and band 4.2). This results defect in the cytoskeleton of the phospholipid bilayer of erythrocyte membrane

which makes RBC rigid and give it a spherical shape. Due to this morphological change, RBCs are sequestered early in the spleen resulting into short half-life of 15 to 30 days and hemolytic anemia [5]. The first step to identify hereditary spherocytosis is the complete blood count (CBC). It has been shown that cytoplasmic viscosity affecting RBC deformability is reflected in Mean cell hemoglobin concentration. MCHC is considered as a marker for hereditary spherocytosis it has a sensitivity of 82% and specificity of 94% to 98% [6]. The CBC report in this case does not show increased MCHC. However, our result corroborates to findings of Renuka *et al.,* [7].

Studies have also reported that an elevated MCHC alone is not diagnostic for hereditary spherocytosis but may be suspicious as a marker of disease [8]. Other indices may be used to help diagnose hereditary spherocytosis. In hereditary spherocytosis the red blood cell distribution width (RDW) is elevated. They have a smaller red blood cell volume (MCV) than normal erythrocytes [9]. This result is in accordance to these findings.

So far osmotic Fragility test is concerned, its increased value is the confirmatory test for Hereditary Spherocytosis (HS) but at least 2% RBCs must be spherocytes for the test to be accurate [10]. Spherocytes are unable to increase in volume due to their surface-to-volume area. So they cannot absorb hypotonic solutions. As a result, they hemolyze in higher concentrations of NaCl, thus presenting lower osmotic resistance than normal biconcave RBCs. The same has been found in this case.

Previous study has also reported that anemia is one of the most prevalent diseases to influence the level of HbA1c; however, based on variable pathophysiology of anaemia the effect varies on HbA1c [11].

The International Expert Committee has advised clinicians to be mindful of the conditions affecting the red cell turnover since they affect the glycated hemoglobin levels. These changes may be reflected by alteration in any one or all RBC parameters such as Hct, MCV, MCH, MCHC and RDW [12]. For monitoring of this patient, other methods like glycosylated prealbumin and fructosamine can be tried.

One major limitation of this study is that the family History of this patient for spherocytosis is not available. We could not perform Osmotic Fragility test for parents of the patients. Mutation study in patients and the parents could have been confirmed the condition.

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Conflict of Interest

"We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome."

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Ethical Information

A written Informed consent was obtained from the patient. Patient anonymity was carefully maintained. Ethical Clearance was also obtained from the IEC.

REFERENCES:

1. American Diabetes Association. 2. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care.* 2018;41(Suppl. 1):S13-S27.
2. English E, Idris I, Smith G, Dhatariya K, Kilpatrick ES, John WG. The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. *Diabetologia.* 2015 Jul;58(7):1409-21.
3. Kim J, Lee H, Shin S. Advances in the measurement of red blood cell deformability: A brief review. *Journal of Cellular Biotechnology.* 2015;1(1):63-79.
4. Suryavanshi Chinmay, Manjula SD, Ragini Bekur, Raghavendra Rao K. Association of increased levels of Glycated hemoglobin with variations in Red blood cell parameters

in Diabetes mellitus. *International Journal of Advanced Research.* 2015; 3 (6): 31-37.

5. Perrotta S, Gallagher PG, Mohandas N. Hereditary spherocytosis. *Lancet.* 2008 Oct 18;372(9647):1411-26.
6. Liao L, Xu Y, Wei H, Qiu Y, Chen W, Huang J, Tao Y, Deng X, Deng Z, Tao H, Lin F. Blood cell parameters for screening and diagnosis of hereditary spherocytosis. *J Clin Lab Anal.* 2019;33(4):e22844
7. Renuka P, Bag S, Vinodhini V. M. Hemorheological Indices and Glycated Hemoglobin in Type 2 Diabetes Mellitus. *Biomed Pharmacol J.* 2020;13(4). Available from: https://bit.ly/34YTuCw
8. Manivannan P, Tyagi S, Chandra D, *et al.*, Flow cytometric analysis of patients with hereditary spherocytosis - an Indian scenario. *Hematology.* 2018;23:175-80.
9. Malandrino N, Wu WC, Taveira TH, Whitlatch HB, Smith RJ. Association between red blood cell distribution width and macrovascular and microvascular complications in diabetes. Diabetologia. 2012 Jan;55(1):226-35.Zgodzinska A, Ciepiela O. Osmotic fragility of red blood cells – a review of diagnostic methods. *Diagn Lab.* 2015;51:229-34.
10. Son HJ, Rhee SY, Woo J, Hwang JK, Chin SO, Chon S, Seungjoon Oh, Kim SW, Kim YS. Hemoglobin A1c May Be an Inadequate Diagnostic Tool for Diabetes Mellitus in Anemic Subjects. *Diabetes Metab J.* 2013 Oct 17;37(5):343-348.
11. International Expert Committee. International expert committee report on the role of A_1C assay in the diagnosis of diabetes. *Diabetes Care*. 2009; 32:1327