

Hemorheological Indices and Glycated Hemoglobin in Type 2 Diabetes Mellitus

Renuka P*, Somnath Bag and VM Vinodhini

Department of Biochemistry, SRM Medical College Hospital & Research Centre, Faculty of Medical & Health Sciences, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur 603203, Kanchipuram, Chennai, Tamil Nadu, India.

*Corresponding author E-mail: renukap@srmist.edu.in

<https://dx.doi.org/10.13005/bpj/2066>

(Received: 12 March 2020; accepted: 30 October 2020)

The use of HbA1c in diagnosis of diabetes mellitus by WHO and ADA has come under scrutiny. Aim: To study the erythrocyte index alterations and their relationship with HbA1c for proper interpretation of HbA1c values in diabetic subjects. Data from 301 diabetic patients was collected. The parameters included were fasting plasma glucose, red blood cell count, hemoglobin level, hematocrit, glycated Hemoglobin (HbA1c), MCV, MCH, MCHC and RDW. A negative correlation was found between HbA1c and MCV, MCH and MCHC and positive correlation with RDW. Hematological parameters like MCH, MCV and MCHC should be taken into account in interpreting HbA1c levels in diagnosis and management of pre diabetes and diabetes.

Keywords: Diabetes mellitus, Glycated hemoglobin, MCH, MCV, MCHC, RDW.

The World Health Organization (WHO) & American Diabetes Association (ADA) have both advocated the use of HbA1c for diagnosis of diabetes at a value of $\leq 6.5\%$ ¹. Changes in RBC lifespan due to blood loss, blood transfusion, anemias, chronic renal or liver disease, erythropoietin treatment and hemoglobinopathies impede the use of HbA1c as a diagnostic tool. The erythrocyte lifespan is decreased resulting in lowering of HbA1c due to deficiency of micronutrients like vitamin B₁₂, folic acid, iron and G6PD enzyme. Reports have suggested that hemolytic anemia can lead to decreased HbA1c values due to reduced RBC lifespan and IDA (Iron Deficiency Anemia) results in increased HbA1c values due to prolongation of erythrocyte lifespan². A key determinant of red

cell deformability is RBC cytoplasmic viscosity. It has been shown that cytoplasmic viscosity affecting RBC deformability is reflected in Mean cell hemoglobin concentration. Clark *et al* observed that a decrease in RBC deformability is seen as increase in MCHC in hereditary xerocytosis³.

Red cell indices like hematocrit (Hct), Hemoglobin (Hb%), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and red cell distribution width (RDW) are measures of red cell deformability^{4,5}. Several factors like cellular aging, genetic disorders affecting the red cells, nutritional deficiencies, blood loss and pregnancy in women and other illnesses cause alteration in these red cell indices.

The International Expert Committee has advised clinicians to be mindful of the conditions affecting the red cell turnover since they affect the glycated hemoglobin levels⁶. These changes may be reflected by alteration in any one or all RBC parameters such as Hct, MCV, MCH, MCHC and RDW². Hence this study was undertaken to study the erythrocyte index alterations and their relationship with HbA1c for proper interpretation of HbA1c values in diabetic subjects.

MATERIALS AND METHODS

After getting the institutional Ethics committee clearance certificate (ECN: 1184/ICE/2017), the study was performed on patients diagnosed with type 2 diabetes mellitus, from the OPD of SRM Medical College Hospital & Research Centre, Kattankulathur. The patients diagnosed with type 2 diabetes mellitus were according to ADA & WHO: HbA1c \geq 6.5% or FBG \geq 126 mg/dL or 2 hour plasma glucose \geq 200 mg/dL. 301 patients for whom the reports are available for HbA1c, FPG, RFT and CBC in the outpatient department at SRM MCH & RC were included in the study. The patients with anemia, liver disease, renal failure, hemolytic disorders including infections like malaria were excluded from the study.

The following data was collected from the records of patients: Fasting Plasma Glucose, Red blood cell count, Hemoglobin level, Hematocrit, Glycated Hemoglobin level (HbA1c), MCV, MCH, MCHC and RDW. The complete blood count was analyzed by using Automated Hematology Analyzer– Sysmax XT- 1800 using impedance method. FPG was analyzed by Hexokinase method on Olympus AU 400 analyzer. HbA1c was analyzed

by automated hemoglobin analyzer- Biorad Variant II using cation exchange HPLC method.

Analysis of data was performed using SPSS 16 (Statistical Package for Scientific Studies) for Windows. Data were statistically described in terms of mean and standard deviation (SD). Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables. p value less than 0.05 was considered statistically significant.

RESULTS

Data from 301 diabetic patients was included in the study. The mean age of the study population was 47.5 years. Mean FPG was 146.93 mg/dL. The highest value was 351 mg/dL and the lowest was 88 mg/dL. Mean HbA1c was 7.25%. The highest value was 14.3% and the lowest was 5.5%. Mean MCV was 83.59 fL. Mean MCH was

Table 1. Characteristics of the Study Participants

Number	301
Age (Years)	47.50 \pm 14.4
Measures of glycemia	
Fasting blood glucose (mg/dL)	147 \pm 25.3
HbA1C (%)	7.25 \pm 1.66
Hematological profile	
Hemoglobin (gm/dL)	12.9 \pm 1.8
RBC ($10^{12}/l$)	4.6 \pm 0.5
PCV (%)	38.5 \pm 4.8
MCV (fL)	83.5 \pm 7.2
MCH (pg)	28.0 \pm 3.0
MCHC (g/dL)	33.5 \pm 1.8
RDW (%)	14.3 \pm 1.9
Renal profile	
Urea (mg/dL)	21.21 \pm 8.48
Creatinine (mg/dL)	0.79 \pm 0.44

Table 2. Shows the Pearson's Correlation analysis between HbA1c (7.25 \pm 1.66) and some hematological parameters in type 2 diabetes mellitus subjects

Parameters	Mean + SD	r- Value	p- Value
MCV (fL)	83.59 \pm 7.22	-0.23	< 0.05 *
MCH(pg)	28.02 \pm 3.09	- 0.24	< 0.05 *
MCHC(g/dL)	33.48 \pm 1.82	-0.11	< 0.05 *
RDW(%)	14.36 \pm 1.99	0.20	< 0.05 *

* P value < 0.05 is considered significant

28.02 pg. Mean MCHC was 33.48 mg/dL. Mean RDW was 14.36 % (Table 1).

A Pearson product-moment correlation coefficient was computed to assess the relationship between HbA1c, RBC count, Hb%, PCV, MCV, MCH, MCHC and RDW. Among them MCV, MCH, MCHC and RDW were significantly correlated with HbA1c (Table No 2). An inverse correlation was seen between HbA1c and MCV, MCH and MCHC. A positive correlation was seen between HbA1c and RDW.

DISCUSSION

In our current study, we found a significant negative correlation between HbA1c and MCV, MCH and MCHC.

Our findings are in concurrence with a study from Spain. The authors demonstrated that there existed a substantial negative correlation in HbA1c from 6.09- 6.79 (expressed as %) across deciles of MCH & MCV. They showed an increased risk of erroneous HbA1c based identification of glycemic status in at least 25% of patients, concluding that MCH and MCV should be taken into account while interpreting HbA1c levels in clinical practice⁷.

Even in apparently healthy individuals, inverse correlation existed between MCV, MCH, MCHC and HbA1c. These were the findings of Hardikar *et al.*⁸ in 21 year old healthy male subjects misclassifying 5.9% subjects as prediabetic and diabetic based on HbA1c.

A systemic review by Emma English (2015) of 12 articles suggested that consideration must be given to abnormalities in RBC indices when there is discordance between glucose and HbA1c for diagnosis or monitoring of diabetic patients. Correction of the abnormality was recommended before utilizing HbA1c for diagnosis or monitoring. RDW may be used as an additional indicator of normalization of the RBC population and lifespan. RDW is the measure of the heterogeneity of the volume of RBCs².

Koga *et al.*⁹ studied the relationship between erythrocyte indices and HbA1c in premenopausal women. They found that MCV and MCHC were negatively associated with HbA1c as found in our study. Every 1 pg decrease in MCH seemed to correlate with 0.03% in increase in

HbA1c and the authors suggested that erythrocyte indices influence HbA1c values in pre menopausal women in the absence of overt anemia.

Using cell separation techniques (based on the cell density and size), Bosch *et al.* found a strong inverse correlation ($r = -0.97$) between HbA1c and mean cell volume. This implies that MCV and HbA1c are both highly dependent on a common variable, presumably red cell age¹⁰.

We found a positive correlation between HbA1c and RDW. Nada (2015) suggested that high RDW value in diabetic subjects is due to the presence of anisocytosis caused by inefficient erythropoiesis or destruction. Hyperglycemia leads to decreased RBC deformability, alteration in mechanical properties and result in various hemodynamic characteristics¹¹.

Malmö Diet and cancer study in Sweden showed similar correlation in non diabetic individuals indicating a relationship between variability in erythrocyte volumes and high population of old dense cells exposed to glucose for a long time¹².

RBC lifespan is a physiological variable influencing HbA1c and is independent of blood glucose. There is evidence to show a linear increase in HbA1c as RBCs age in vivo using labeled autologous RBCs in both non diabetic and diabetic subjects^{13, 14}.

Both MCH and MCV decrease linearly during the lifespan of RBC. MCH is considered as an indirect measure of RBC survival⁷.

A cross sectional study from South India showed that glycated hemoglobin levels below 7% were influenced significantly by red cell turnover indices and recommended the usage of oral glucose tolerance test to determine the diabetic status of the patients¹⁵.

In view of the alterations in the hematological indices, the proper interpretation of HbA1c values for diagnostic or management purpose would seem to require the RBC indices especially MCH and MCV which reflect the RBC lifespan. Further studies are needed to establish the use of MCH dependent HbA1c threshold for proper management of diabetic subjects.

Limitations of our study: We did not study the nutritional influence (iron, vitamin B12 and folic acid) on HbA1c.

CONCLUSION

We demonstrated a negative correlation between HbA1c levels and MCV, MCH and MCHC and a positive correlation with RDW. The association between HbA1c and hematological parameters mandates that caution be exercised while categorizing patients in pre-diabetes and diabetes and also monitoring of treatment, based on the HbA1c values. It is important to consider the erythrocyte indices especially MCH and MCV for proper diagnostic or management purposes in diabetic subjects.

Conflict of interest

None.

Author's contributions

Renuka Pangaluri - Conception and design, data analysis and interpretation and drafting the article. Somnath Bag - Data collection, data analysis. Vinodhini V M - Data interpretation and final approval of version to be published.

REFERENCES

- American Diabetes Association. 2. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care*; **41**(Suppl. 1):S13–S27 (2018).
- Emma English, Iskandar Idris, Georgina Smith, Ketan Dhatariya, Eric S. Kilpatrick & W. Garry John. The effect of anaemia and abnormalities of erythrocyte indices on HbA1c analysis: a systematic review. *Diabetologia.*, **58**(7): 1409–1421 (2015).
- Jeongho Kim, HoYoon Lee and Sehyun Shin. Advances in the measurement of red blood cell deformability: A brief review. *Journal of Cellular Biotechnology*; **1**: 63–79 (2015). DOI 10.3233/JCB-15007.
- 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*: **3**(6): 31-37 (2015).
- N. Malandrino & W. C. Wu & T. H. Taveira & H. B. Whitlatch & R. J. Smith. Association between red blood cell distribution width and macrovascular and microvascular complications in diabetes. *Diabetologia*, **55**: 226–235 (2012).
- International Expert Committee. International expert committee report on the role of A1C assay in the diagnosis of diabetes. *Diabetes Care.*; **32**:1327–34 (2009). [PMCID: PMC2699715] [PubMed: 19502545]
- Santiago Rodriguez-Segade, Javier Rodriguez Garcia, Jose M. Garcia-Lopez, Francisco Gude, Felipe F. Casanueva, Santiago RS-Alonso and Felix Camina. Impact of Mean Cell Hemoglobin on HbA1c– Defined Glycemia Status. *Clinical Chemistry* **62**:12 (2016).
- Pallavi S. Hardikar, Suyog M. Joshi, Dattatray S. Bhat, Deepa A. Raut, Prachi A. Katre, Himangi G. Lubree, Abhay Jere, Anand N. Pandit, Caroline H.D., Chittaranjan S. Yajnik. Spuriously High Prevalence of Prediabetes Diagnosed by HbA1c in Young Indians Partly Explained by Hematological Factors and Iron Deficiency Anemia. *Diabetes Care*, **35**: (2012).
- Koga M, Saito H, Mukai M, Matsumoto S, Kasayama S. Influence of iron metabolism indices on glycated haemoglobin but not glycated albumin levels in premenopausal women. *Acta Diabetol* **47**(Suppl 1):S65–S69 (2010).
- Bosch FH, Werre JM, Roerdinkholder Stoelwinder B, Huls TH, Willekens FL, Halie MR. Characteristics of red blood cell populations fractionated with a combination of counterflow centrifugation and Percoll separation. *Blood*; **79**:254 – 60 (1992).
- Aml Mohamed Nada. Red cell distribution width in type 2 diabetic patients. *Diabetes Metab Syndr Obes.*; **8**:525–33 (2015). [PMCID: PMC4634828] [PubMed: 26586957].
- G. Engstrom, J.G Smith, M. Persson, P.M. Nilsson, O. Melander & B. Hedblad. Red cell distribution width, haemoglobin A1c and incidence of diabetes mellitus. *Journal of Internal Medicine*, **276**: 174-183 (2014).
- Cohen RM, Herman WH. Are glycated serum proteins ready for prime time? *Lancet Diabetes Endocrinology*; **2**: 264 –265 (2014).
- Gallagher EJ, Bloomgarder ZT, Le Roith D. Review of hemoglobin A1c in the management of diabetes. *Journal of Diabetes* **1**: 9–17 (2009).
- Kannan S, Jaipalreddy C, Annapandian VM, Murali Mohan BV, Damodar S, Khadilkar KS, Shivaprasad KS. Impact of anemia and red cell indices on the diagnosis of pre-diabetes and diabetes in Indian adult population: Is there a cut-off guide for clinicians?. *Indian J Endocr Metab.*; **23**:91-6 (2019).