Original Article

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?

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Abstract

Background: It is well known that anemia and red cell turn over affects the HbA1c value. Iron deficiency anemia increases the HbA1c and haemolytic anemia lowers it. However, the cut-off of haemoglobin (Hb) or red-cell indices when the HbA1c value becomes unreliable is not known. **Aim:** We sought to find out values of HbA1c and red-cell indices where there is considerable discordance between HbA1c and plasma glucose (PG) values in the diagnosis of diabetes (DM) and pre-diabetes (Pre-DM) making HbA1c values unreliable. **Methods:** A cross-sectional study of 237 non-diabetic subjects who attended our out-patient division of preventive health check-up clinics, between November 2016 and December 2017. Data was collected only from relatively healthy subjects who had voluntarily opted for undergoing a preventative health check-up (including a diabetes screening). Patients were classified as concordant (fasting and 2-hr post meal glucose values are in agreement with HbA1c) and discordant (values are not in agreement with HbA1c). **Results:** A total of 237 patients (73% males) with mean age was 47.2±9.7 years (range 25-75) were included in the study. The HbA1c definition group had more diagnosis of DM (153 vs 96) and but lesser numbers of pre-DM (66 vs 102) compared to the PG group. Out of 237 patients, 133 (56%) showed concordance and 104 (44%) were discordant. The FPG, 2h-PPBG and HbA1c are significantly higher in the concordant group. The Hb value and MCV were significantly higher ($p<0.05$) in concordant group whereas, RDW and platelets are significantly higher ($p<0.05$) in discordant group. The highest rate of discordance was noted in the HbA1c strata of 6.5-7% (72%) followed by HbA1c of 5-6.4% (42%) and least in the HbA1c strata >7% (20%). While no single Hb or MCV value could predict discordance, a RDW value >17 was consistently associated with discordance across all the HbA1c strata. **Conclusion:** A HbA1c below 7% is significantly influenced by red-cell turn over indices and clinicians need to perform additional testing using plasma glucose levels to confirm the presence of diabetes or pre-diabetes. In patients whose RDW >17, HbA1c should be replaced by 75gm OGTT as a test of choice for diagnosis of diabetes or pre-diabetes.

Keywords: Anemia, concordance, diabetes, discordance, HbA1c, pre-diabetes

Introduction

Diabetes mellitus (DM), especially type 2 diabetes, is a massive health problem and a significant contributor to morbidity and mortality worldwide. A significant proportion of DM cases still remain undiagnosed in India. While fasting and post-glucose load plasma glucose (PG) tests have a well-established role in the diagnosis of DM and pre-diabetes (pre-DM), the American Diabetes Association (ADA) recommended glycosylated hemoglobin (HbA1c) values of ≥6.5% for diagnosing DM and 5.7–6.4% (39–46 mmol/mol) for pre-DM in 2010.[1] The World Health Organization (WHO) also endorsed HbA1c ≥6.5% as a diagnostic test for diabetes, with a caveat that values <6.5% cannot exclude diabetes diagnosed by PG tests.[2] Conditions that affect erythrocyte turnover influence HbA1c concentrations

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and the International Expert Committee has warned clinicians to be aware of any conditions that could affect the turnover of red blood cells (RBC).[3] Uremia, hyperbilirubinemia, hypertriglyceridemia, chronic alcoholism, chronic ingestion of salicylates, vitamin C ingestion, and opiate addiction have all been reported to interfere with some assay methods, falsely increasing results.[4] Racial and ethnic differences in HbA1c have also been described.[5] The association between anemia and concentrations of HbA1c has been reviewed in the literature and noted that anemia with more rapid erythrocyte turnover lowers HbA1c, and those with slower erythrocyte turnover or changes in the three-dimensional configuration of hemoglobin (Hb) (malondialdehyde-increased glycation of N-terminal valine), leads to higher HbA1c.[6] Vitamin B12, folate, and iron deficiency anemias (IDA) and its treatment have all been shown to affect HbA1c levels. In 2004, Coban *et al*. found that the mean HbA1c (7.4%) level in patients with IDA was higher than in a healthy group (5.9%) and in patients with IDA and HbA1c decreased significantly to 6.2% after iron supplementation of 100 mg/day for 3 months.[7] Studies measuring HbA1c pre- and post-treatment for anemia showed changes as large as −1.2% HbA1c post-treatment.[8] Kim *et al*. found that iron deficiency shifted the HbA1c slightly upward independent of glucose levels, but this shift occurred primarily at the lower end of the HbA1c spectrum, i.e., between <5.5 and 5.5–6.0% in the National Health and Nutrition Examination Survey (NHANES 1999–2006).[9] Hardikar *et al*. found that using HbA1c to diagnose pre-DM and DM in iron-deficient young populations leads to spuriously exaggerated prevalence (26%) compared to the oral glucose tolerance test (OGTT) (10%) and also observed that low mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), high red cell distribution width (RDW), and low ferritin concentrations predicted higher HbA1c.[10] IDA was sufficient to re-categorize patients from pre-DM to DM in the anemic group.[10] Koga *et al*. demonstrated that Hb, MCH, and MCV are negatively associated with HbA1c and a decrease of 1 pg in MCH corresponding to an increase of 0.03% in HbA1c.[11] Simmons and Hlaing concluded that the mean HbA1c is higher with a lower MCHC and those with MCHC at either end of the range and an HbA1c within 0.5% of criteria for diabetes and pre-diabetes.[12]

However, the cut-off values for Hb and red cell indices when the HbA1c becomes unreliable has not been defined in the literature. Clinicians who review HbA1c and PG levels need a guide from the lab on when HbA1c becomes unreliable so that decisions on diagnosis and management need not be based solely on HbA1c, but also include plasma and capillary glucose levels.

Aim of the Study

We sought to find out values of Hb and red cell indices where there is considerable discordance between HbA1c and PG values in the diagnosis of diabetes and pre-diabetes.

Study Type

Cross-sectional prospective study.

Materials and Methods

After obtaining the Ethics Committee and Institutional Review Board approval, we have collected data of patients who attended our preventive health checkup clinics between November 2016 and December 2017 at a tertiary care teaching hospital, Bangalore, India. Data included complete blood count, red cell indices (including MCV, MCHC, RDW, PG [fasting (FPG) and postprandial blood glucose (PPBG)], HbA1c, renal, liver functions, and lipid profile. Fasting blood glucose (FBG) and PPBG levels were measured by hexokinase-glucose-6-phosphate dehydrogenase method, using the GLUC method for the Dimension® (Siemens Healthcare Diagnostic Inc., Newark, DE, USA) clinical chemistry system. The HbA1c was estimated by ion exchange high-performance liquid chromatography (HPLC; VariantTM II Turbo, Bio-Rad laboratories Inc., Hercules, CA). Patients were classified into three categories based on their HbA1c, FPG, and PPBG values: normoglycemic (HbA1c < 5.7; FPG < 100 mg/dl; PPBG < 140 mg/dl), pre-DM (HbA1c 5.7–6.4%; FPG 100–125; PPBG 140–199 mg/dl), and DM (HbA1c > 6.5%; FPG > 126 mg/dl; PPBG > 200 mg/dl). Any patients with previously known history of diabetes or on anti-diabetic medicines, blood transfusions in the last 6 months, on treatment for anemia including iron, B-vitamins or erythropoietin, and history of chronic liver or chronic kidney disease [glomerular filtration rate (GFR) <60 ml/min] were excluded. All the study patients were classified into concordance group (FBG and 2-h PPBG values are in agreement with HbA1c) and discordance group (FPG or 2-h PPBG values are not in agreement with HbA1c).

Sample size calculation

Assuming the expected percentage of diabetes (pre-diabetes and diabetes) about 20% and absolute precision is 5% with 95% confidence interval, the sample size was arrived by the following calculation.

$n = Z^2\ 1-\alpha/2\ P\ (1-p)/d^2$

P: expected percentage of diabetes = 20% or 0.20

d: absolute precision = 5% or 0.05

$1-\alpha/2$ = confidence interval 95%

Normal table value 1.96 (two sided)

$n = (1.96)^2\ (0.20)\ (0.80)/0.05 \times 0.05$

$= (3.84) \times (0.16)/0.0025$

$= 245$

Statistical methods

The statistical analyses were performed by the SPSS software (Version 22.0; IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). Continuous variables were

Table 1: Baseline characteristics of study subjects

Variables	Mean±SD	Median	Range
Age (years)	47.18±9.68	47	(25-75)
Weight (kg)	75.82±11.68	75.00	(54-102)
Height (cm)	164.50±8.13	164	(144-190)
BMI	28.01±3.3.27	27.01	(21.95-39.76)
FPG (mg%)	126.99±40.78	114	(72-282)
2-h PPBG (mg%)	173.63±59.76	154	(86-380)
HbA1c	7.12±1.56	6.8	(4.5-16.2)

BMI: Body mass index, FBG: Fasting blood glucose, 2-h PPBG: 2-h postprandial blood glucose, HbA1c: Glycosylated hemoglobin

Table 2: Details of complete blood counts (red cell indices)

Variables	Mean±SD	Median	Range
Hemoglobin (g/dl)	13.35±1.69	13.305	(8.1-17)
Hematocrit	34.73±6.78	34.00	(23-51)
Mean corpuscular hemoglobin	29.58±4.52	29	(19-45)
Mean corpuscular volume	83.52±7.18	83.70	(61.8-109)
Mean corpuscular hemoglobin concentration	32.39±2.98	32.50	(28-45)
Red blood cell distribution width	14.34±1.43	14	(11.9-21.4)
Platelets ($\times 10^3$ cumm)	243.36±75.01	235	(100-490)
Total white blood cells ($\times 10^3$ cumm)	7.78±6.53	7.00	(4-10.3)

expressed in terms of mean with standard deviation (SD), median, and range. Categorical variables were expressed in terms of frequencies or percentages. Independent sample *t*-test was used to find the influence of anemia and RBC indices on concordant and discordant groups. *P* value <0.05 is considered as statistically significant.

Results

A total of 237 patients (73% males) with mean age was 47.2 ± 9.7 years (range 25–75) were included in the study. The baseline characteristics, their glycemic profiles, red cell indices, liver, and renal functions are summarized in Tables 1-3. The population cohort represented a geographical preponderance of southern and north-eastern part of the country: Tamilnadu – 23.8%; Karnataka – 23.4%; West-Bengal – 21%, Assam – 11.7%; Andhra-Pradesh – 5.2%; other states – 4.8%, and Bangladesh – 10.1%. We observed elevated ALT (33%), AST (22%), and GGT (6%) in our study population. About 75% had high-density lipoprotein (HDL) values <40 mg/dl and 25% patients had triglycerides (TGL) level >200 mg/dl.

Out of 237 patients, 133 (56%) showed concordance and the remaining 104 (44%) were discordant [Table 4]. HbA1c definition had more diagnosis of DM (153 vs 96) and but lesser numbers of pre-DM (66 vs 102) compared to the PG group. The FPG, 2-h PPBG, and HbA1c are significantly different between

Table 3: Liver enzymes, lipid profile, renal profile, and thyroid status of the study subjects

Variable	Mean±SD	Median	Range
ALT	41.12±27.96	33	(8-214)
AST	32.41±16.99	29	(12-135)
GGT	53.08±40.04	44	(14-379)
LDL	112.70±30.87	109	(40-239)
HDL	38.33±3.48	37	(15-74)
TGL	170.10±92.67	146	(45-842)
Uric acid	4.83±2.30	4.60	(2.2-33.0)
Serum creatinine	0.926±0.21	0.90	(0.40-1.60)
GFR	93.66±16.32	94.0	(60.50-153)
TSH	3.07±2.16	2.63	(0.10-14.69)

Table 4: Comparison between FPG/2-h PPG and HBA1c levels in the study subjects

Parameter for diagnosing dysglycemia	HbA1c		
	Normal (*n*=18) (%)	Pre-diabetic (*n*=66) (%)	Diabetic (*n*=153) (%)
FPG/2-h PPG			
Normal (*n*=39)	9 (23.1)	19 (48.7)	11 (28.2)
Pre-diabetic (*n*=102)	7 (6.9)	37 (36.3)	60 (56.9)
Diabetic (*n*=96)	3 (2.1)	10 (10.4)	84 (87.5)

concordant and discordant group [Table 5], with a higher level of glycemia in the concordant group. The Hb value and MCV were significantly higher ($P < 0.05$) in concordant group, whereas RDW and platelets are significantly higher ($P < 0.05$) in discordant group [Table 6].

We divided the concordant and discordant group into three strata of HbA1c [5–6.4% (n = 79), 6.5–7% (n = 77), and >7% (n = 81)]. The highest rate of discordance was noted in the HbA1c strata of 6.5–7% (72%) followed by HbA1c of 5–6.4% (42%), and least in the HbA1c strata >7% (20%) [Figure 1a-d]. While no single Hb (g/dl) or MCV (fl) value could predict discordance, an RDW value >17 was consistently associated with discordance across all the HbA1c strata [Figures 2a-d and 3a-d].

Discussion

The results of the current study is consistent with findings in the literature that lowers Hb, lower MCV, higher RDW, and higher platelet counts (all parameters related to IDA) were noted to be more prevalent in the discordant group. Also, as reported by Hardikar *et al*. and Simmons and Hlaing the discordance tends to influence the lower levels of HbA1c cut-off used in the diagnosis of pre-DM and in some cases early DM (HbA1c ranges of 5–6.4% and 6.5–7%).[10,12] This is likely because a higher glucose value tends to elevate the HbA1c sufficient enough to be categorized as diabetes. Whether a linear relationship exists between degree of hyperglycemia and HbA1c (>7%) in cases of altered red cell indices is not

Table 5: Overall comparison of FBB, 2-h PPBG, and HbA1c in concordant and discordant groups

Variable	Concordant (n=133)			Discordant (n=1040)			95% Confidence interval of the difference	*P*
	Mean±SD	Median	(Q1, Q3)	Mean±SD	Median	(Q1, Q3)		
FBG	143.44±42	131.0	(106.5-176.5)	105.96±17	104	(95.5-116.75)	(28.1-46.8)	0.000
2-h PPBG	195.94±67	185.0	(139.0-247.5)	145.11±31	143.5	(123.5-164.75)	(36.8-64.8)	0.000
HbA1c	7.49±1.68	7.0	(6.1-8.1)	6.65±0.73	6.6	(6.0-7.0)	(0.45-1.23)	0.000

Q1=25th percentile, Q3=75th percentile

Table 6: Overall comparison of red cell indices in concordant and discordant groups

Variable	Concordant (n=138)			Discordant (n=110)			*P*
	Mean±SD	Median	(Q1, Q3)	Mean±SD	Median	(Q1, Q3)	
Hb	13.61±1.68	13.8	(12.1-15.0)	13.01±1.64	13.0	(11.9-14.0)	0.007
MCH	29.54±4.03	29.0	(27.0-31.0)	29.60±5.09	29.0	(26.9-32.0)	0.928
MCV	84.58±7.12	85.0	(79.8-89.0)	82.14±7.07	82.0	(78.0-87.0)	0.010
MCHC	33.34±2.71	32.8	(32-33.5)	33.44±3.30	32.2	(32.0-34.0)	0.791
RDW	14.09±1.09	14.0	(13.2-15.0)	14.63±1.72	14.0	(13.5-15.5)	0.004
Platelets	232.39±69.93	225.5	(178.0-279.5)	257.70±79.26	254.5	(189.3-323.5)	0.012

Q1=25th percentile, Q3=75th percentile

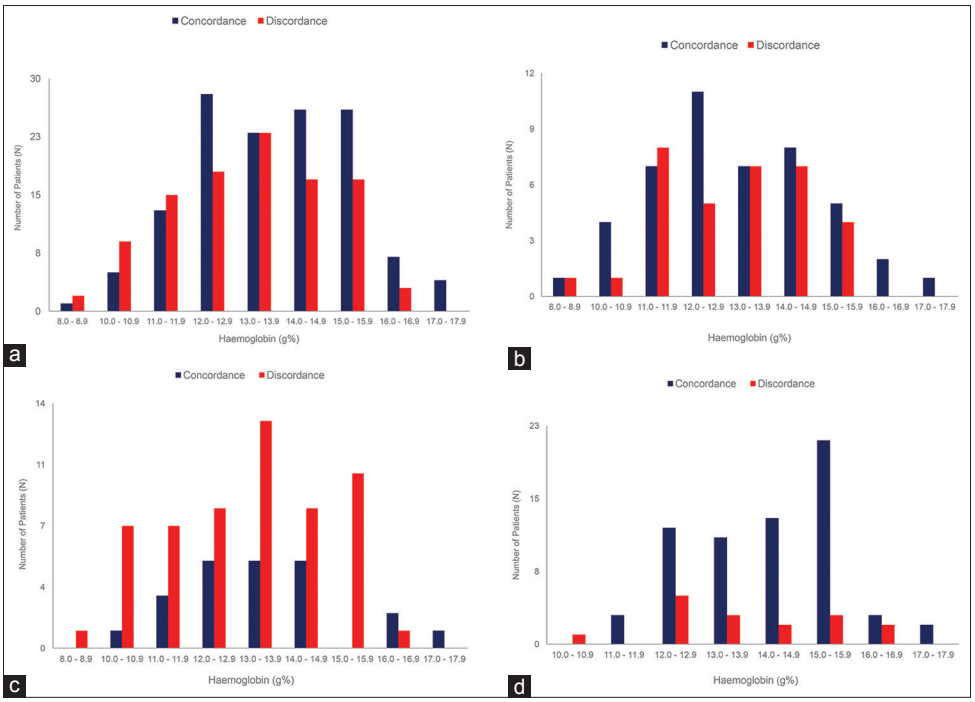

Figure 1: Hemoglobin distribution (a) across entire cohort (n = 237), (b) HbA1c 5.0–6.4% (n = 79), (c) HbA1c 6.5–7% (n = 77), (d) HbA1c > 7% (n = 81)

clear and scope of future studies. In addition, our study notes that no single Hb or MCV value could predict discordance, but RDW value >17 resulted in discordant results in almost all cases across the spectrum of HbA1c.

The RDW is a measure of the variation in RBC sizes, based on the width of the MCV histogram (bell-shaped curve around the mean) corresponding to anisocytosis on the peripheral smear. The normal range for the RDW is 11.5 to 14.5%. An RDW in the normal range indicates that the size distribution of RBCs has the normal degree of variation (i.e., a relatively homogenous population of cells). An increased RDW suggests greater-than-normal variation in RBC size frequently seen in anemias caused by vitamin or mineral deficiencies (e.g., folate, vitamin B12, and iron). As noted above, an increased RDW may also be seen with two homogenous populations of cells (e.g., a population of microcytic cells from hemolysis plus a population of reticulocytes, which are larger than normal RBCs) or following RBC transfusion. While there

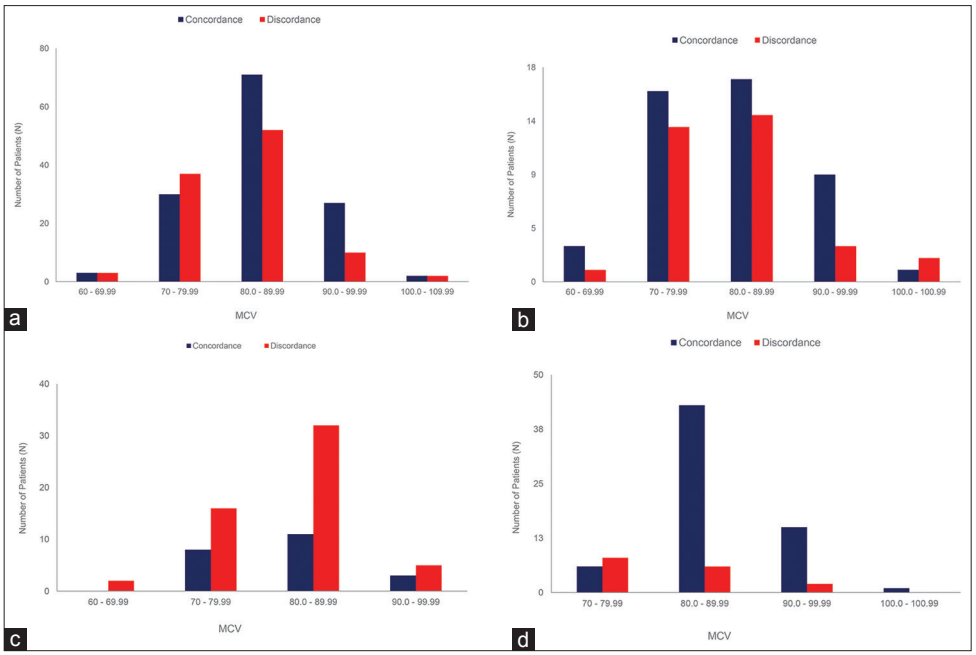

Figure 2: Mean corpuscular volume (MCV) distribution (a) across entire cohort ($n = 237$), (b) HbA1c 5.0–6.4% ($n = 79$), (c) HbA1c 6.5–7% ($n = 77$), (d) HbA1c > 7% ($n = 81$)

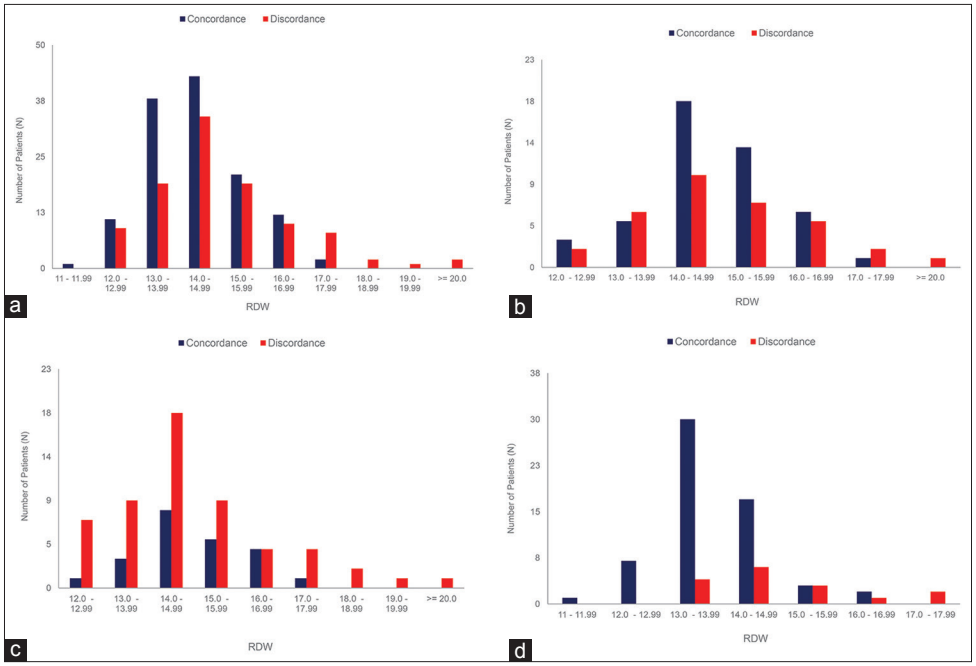

Figure 3: Red cell width (RDW) distribution (a) across entire cohort ($n = 237$), (b) HbA1c 5.0–6.4% ($n = 79$), (c) HbA1c 6.5–7% ($n = 77$), (d) HbA1c > 7% ($n = 76$)

have been studies on association of RDW and prediction of metabolic syndrome as well as complication of diabetes,[13,14] we have noted that RDW (>17) in comparison to MCV, MCHC, or Hb proved to be a reliable indicator of discordance between HbA1c and PG values across the HbA1c strata from pre-DM to DM.

Since the present study data obtained from the preventive health checkup, the study did not have the OGTT for diagnosis of DM or pre-DM. We used the glucose values from 2-h PPBG as a surrogate for post-75 g glucose level. Also, this was a cross-sectional study rather than a longitudinal study, the FPG and PPBG is likely to be influenced by the carbohydrate intake overnight and on the day of the test. These unmeasured factors could affect the results of PG and hence the categorization of cohort. Finally, the results of the investigation of abnormal Hb (iron profile,

B12, folate levels, and electrophoresis) were not collected as part of the study.

However, the strengths of the study included the spectrum of Hb, MCV, RDW range of the population, and the high prevalence of DM and pre-DM (as expected from tertiary care hospital) has helped us study the concordance and discordance in a geographically variable population attending a single center.

Conclusion

HbA1c <7% is significantly influenced by red cell turnover indices and clinicians need to perform additional testing using PG levels to confirm whether they have diabetes or pre-diabetes. In those with RDW >17, HbA1c should be replaced by 75 g OGTT as a test of choice for diagnosis of diabetes or pre-diabetes.

Ethical statement

This study was approved by the Ethics Committee and Institutional Review Board of Narayana Hrudayalaya Multispeciality Hospital, Bangalore.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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