Correlation of Red Blood Cell Distribution Width (RDW) and Haemoglobin A1C (HbA1C) Levels in Diabetic Individuals

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ABSTRACT: Diabetes Mellitus (DM) is a metabolic disease characterized with persistent hyperglycemia due to, inappropriate glucose use or insufficient insulin production of pancreas. There is a defect in insulin effect, secretion or both. Due to sedentary life style and obesity, incidence of type 2 diabetes mellitus is increasing. Oral hypoglycaemic drugs or insulin therapy along with diet control and exercise helps the diabetic individual to keep proper glycemic control. Glycemic control is must in DM. Glycemic control is the main target of treatment in protection against organ damage and other complications of DM. Glycatedhemoglobin (HbA1C) follow-up is made in order to measure the efficiency of the treatment in DM and observe the glycemic control. HbA1C is formed as the result of non-enzymatic reaction of glucose to hemoglobin. It provides information on the average blood glucose levels of past 8-12 weeks in DM treatment. HbA1C is the golden standard in glycemic control of patients. Hyperglycemia tend to have effect on RBC survival and glycation of haemoglobin. Red blood cell distribution width (RDW) is the symbol of red blood cell volume distribution width coefficient of variation. It may be considered as heterogeneity index and is the equivalent of anisocytosis observed in peripheral blood smear. The present study was undertaken to explore the correlation between RDW and HbA1c in diabetic individuals presented at Laboratoty, Ruby Hall Clinic, Pune. The results of study will help to determine the risk of future cardiovascular disease and organ damage due to hyperglycemia.

KEYWORDS: Red Cell Distribution Width, Glycosylated Hemoglobin, Glycated Hemoglobin, Coronary Artery Disease, Cardiovascular Disease, Diabetes Mellitus, American Diabetes Association

I. INTRODUCTION

The term “diabetes mellitus” refers to a group of disorders of abnormal carbohydrate metabolism sharing in clinical finding of hyperglycaemia. Incidence of diabetes mellitus continues to rise with increase in elderly population, sedentary lifestyle and obesity. Diabetes mellitus is a chronic illness characterized by increased risk of macrovascular and microvascular complications. These complications of diabetes mellitus are responsible for the majority of morbidity and mortality associated with the disease. The risk of these complications is related with the degree and duration of hyperglycaemia. When plasma glucose is episodically elevated over time, small amount of hemoglobin A are nonenzymatically glycated to form glycosylated hemoglobin – HbA1c. HbA1c has a glucose attached to the terminal valine in each β chain. The concentration of HbA1c depends on concentration of glucose in the plasma and the duration of hyperglycaemia. HbA1c concentration is measured clinically as an index of diabetic (glycemic) control for a period over past 8 to 12 weeks. High levels of HbA1c have been shown to impair endothelium mediated vasoactive responses, which can lead to hypertension and vascular diseases in diabetic patients.
effects on the red blood cell (RBC), including glycation of haemoglobin, reduced deformability and reduced lifespan of red cells. Slow glycosylation and consistent elevation of HbA1c can be associated with functional and structural change in Hb molecule and cytoplasmic viscosity within each red blood cell, along with osmotic disturbances within the cell. These changes can be reflected by red cells analytic parameters like RDW. Red cell distribution width (RDW) is a coefficient of variation of the distribution of individual RBC volume. It is routinely reported as a part of complete blood count. It may be considered as heterogeneity index and is the equivalent of anisocytosis observed in peripheral blood smear. The latest studies have reported that RDW is an independent prognostic marker strong in many pathophysiological cases such as cardiovascular disease, pulmonary disease, rheumatoid arthritis and progressive inflammatory diseases and even cancer. Red cell distribution width (RDW) and hemoglobin A1c (HbA1c) are both known to be predictive of future cardiovascular disease (CVD). Hence, the present study was undertaken to study the correlation of RDW and Hba1c in diabetic individuals presented to Laboratory, Ruby Hall Clinic, Pune. This will include measurement of Hba1c by HPLC (High Performance Liquid Chromatography) and RDW to be calculated from RBC histogram. Results will be helpful in determining the risk of future cardiovascular disease and to educate patients to take proper measures to reduce the risk of same.

II. AIM AND OBJECTIVES

I. To estimate Hb1AC and RDW from collected blood samples of diabetic individuals presented to the laboratory.

II. To study the correlation between Hb1AC and RDW in the diabetic individuals.

III. MATERIALS AND METHODS

Selection of subjects

Patient Group

In this study 50 patients with type two diabetes mellitus were selected. Subjects for this study were randomly selected from patients coming to laboratory for routine investigations as prescribed by clinicians at Ruby Hall Clinic, Pune. Measurements of glycosylated haemoglobin (Hba1c) and Hemogram (For Red Cell Distribution Width) were easily accepted by patients with diabetes.

Clinical information and data of these subjects were obtained like age, gender, registration number, duration of disease and associated clinical conditions, etc.

Blood sample collection

A minimum of 3 ml of blood was collected by venepuncture into the tubes containing EDTA anticoagulant (Lavender Top), which was inverted up and down to avoid clotting with all aseptic precautions.

Sample Processing and estimation of Hba1c and RDW

1) Hba1c (Glycosylated Hb) [4]:

HPLC method

Bio-Rad D-10 Hemoglobin A1c program which is intended for the percent determination of Hba1c in human whole blood was used for estimation of Hba1c.
The D-10 Hemoglobin A1c Program is based on chromatographic separation of HbA1c on a cation exchange cartridge. Separation is optimized to minimize interferences from hemoglobin variants, labile A1c and carbamylatedhemoglobin. It also offers automatic sampling from a primary whole blood tube, followed by sample dilution and an analysis time of three minutes per sample.

The samples are automatically diluted on the D-10 and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge where the hemoglobin is separated based on their ionic interactions with the cartridge material. The separated hemoglobin then passes through the flow cell of the filter photometer where changes in the absorbance are measured at 415 nanometer wavelength. The D-10 software performs reduction of raw data collected from each analysis. Two-level calibration is used for quantification of the HbA1c values. A sample report and a chromatogram are generated for each sample.

The A1c area is calculated using an exponentially modified Gaussian (EMG) algorithm that excludes the labile HbA1c and carbamylated peak area from the A1c peak area.

Materials:

- **Elution Buffer 1.** Two bottles containing 2000 mL of a Bis-Tris/Phosphate buffer, pH 6.0. Contains <0.05% sodium azide as a preservative.
- **Elution Buffer 2.** One bottle containing 1000 mL of a Bis-Tris/Phosphate buffer, pH 6.7. Contains <0.05% sodium azide as a preservative.
- **Wash/Diluent Solution.** One bottle containing 1600 mL of deionized water with <0.05% sodium azide as a preservative.
- **Analytical Cartridge.** One cation exchange cartridge, 4.0 mm ID x 30 mm.
- **Floppy Diskette** with D-10 Hemoglobin A1c Program parameters.
- **Calibrator/Diluent Set.** One set consisting of three vials of Calibrator Level 1, three
vials of Calibrator Level 2, and one bottle of Calibrator Diluent. The calibrator vials contain lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 7 mL per vial. Calibrator Diluent contains 100 mL of deionized water with <0.05% sodium azide as a preservative.

- **Whole Blood Primer.** Four vials of lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 1.0 mL per vial.
- **Sample Vials.** 100 polypropylene vials with pierceable caps, 1.5 mL.
- **Thermal Paper.** One roll.
- **Specimen:** Whole blood specimen in EDTA

Sample can be stored up to 7 days at 2–8 °C or 3 days at room temperature (15–30 °C).

**Sample Preparation**

1. Allow sample tubes to reach room temperature (15–30 °C) before performing the assay. No sample preparation is required. Mixing has no impact on HbA1c value as long as the total area is in range.

   - The sample tubes are loaded into the D-10 sample rack and placed in the D-10. Ensure that the sample barcodes are facing towards the back of the instrument.
   - Use special rack inserts for 12, 13, and 14 mm diameter tubes. Remove all inserts for 16 mm diameter tubes. Tubes with a height of 75 mm to 100 mm are acceptable for use.

2. If the sample is in an abnormal size/type tube, or if there is less than 2.0 mL of sample in the tube, then the sample must be prediluted. Before pipetting, thoroughly mix the sample by gently inverting the tube.

   - To predilute, pipet 1.5 mL of Wash/Diluent Solution into a labeled 1.5 mL vial, followed by 5 μL of the whole blood sample. Cap the sample vial and mix thoroughly. Use a sample vial adapter for 1.5 mL vials.

**Procedure:**

- Pipet 1 mL of reconstituted Whole Blood Primer into a sample vial. Put the sample vial into a sample vial adapter labeled with a Primer barcode, then place the adapter into sample rack position 1. Start a run. When finished, the cartridge is ready for calibration.

**Calibration:**

- Calibration must be performed once, following the installation and priming of every new analytical cartridge. Additional calibration may be performed at the discretion of the laboratory.

Prepare samples as described in Sample Preparation section.

- Place calibrators and controls on the D-10. Use sample vial adapters for 1.5 mL vials with calibrators and controls. The adapters should have barcodes identifying sample type.

2) **RDW (Red Cell Distribution Width)**

The COULTER LH 750 Hematology Analyzer was used for estimation of RDW.

It utilizes the Coulter Principle for enumerating and sizing blood cells, automatic diluting and mixing for sample processing and a single beam photometer for hemoglobinometry. It uses COULTER VCS (volume, conductivity, light scatter) technology for WBC Differential analysis and classification and reticulocyte analysis.

The analyzer uses a reagent system consisting of an isotonic diluent, lytic reagents to lyse the red cells without significantly affecting the white cells and an instrument cleaner.
RBC count and RBC indices including RDW like MCH, MCV and MCHC are important parameters of CBC in evaluating various RBC disorders including anemia.

Principle for RBC count is impedance. Other parameters which help in derivation of RDW are:

- **MCV (Mean Corpuscular Volume)**: Calculated from RBC histogram
- **MCH (Mean Corpuscular Hemoglobin)**: Hemoglobin / RBC count × 10
- **MCHC (Mean Corpuscular Hemoglobin Concentration)**: Hemoglobin / Hematocrit × 100
- **RDW**: CV(%) of RBC histogram: (SD/MCV) × 100. [SD is determined according to statistical studies on cell distribution]

Sample Type: EDTA anticoagulated blood fresh (Within 6 hours at room temperature or stored at 2-8°C (upto 24 hours).

**Beckman Coulter LH 750 Hematology Analyser**

**Sample processing**

Anticoagulated sample is run through a sample probe if it is single sample. In case of multiple samples, sample are kept as per position in a sample rack and kept at sampling area. Sample is aspirated and results are displayed in the computer and they are noted as required.

After collection of data i.e Hba1c and RDW, it was recorded in the sheet along with age, gender, registration number and necessary statistical analysis was done.
IV. REVIEW OF LITERATURE

Historical Aspects

Glycosylated Hemoglobin (HbA1c) (6)

1958: Hemoglobin A1c was first separated from other forms of hemoglobin by Huisman and Meyering in 1958 using a chromatographic column.

1966: Holmquist and Schroeder identify five subtypes of hemoglobin A, including HbA1c. 1968: Rahbar recognizes that HbA1c is elevated in people with diabetes.

1975: Koenig and Cerami suggest that HbA1c is related to metabolic control.

1993: DCCT (Diabetes Control and Complications trial) establishes HbA1c as a valuable clinical marker in people with type 1 diabetes.

1998: UKPDS (The UK Prospective Diabetes Study) establishes HbA1c as a valuable clinical marker in people with type 2 diabetes.

2010: ADA (American Diabetes Association) recommends using the HbA1c test to diagnose diabetes and prediabetes.

One of the first systematic demonstration of HbA 1c increase in diabetic patients was made by Trivelli et al. in 1971 (7). During this period, a number of methods based on various principles (i.e., ion-exchange chromatography, affinity chromatography, electrophoresis, colorimetric assay, immunoassay) and methodologies [i.e., micro- and minicolumns of chromatography, low pressure (LPLC), high pressure liquid chromatography liquid chromatography (HPLC), manual assays, automated assays] were proposed for assaying Hba1c , evaluated and compared (8,9).

Red Cell Distribution Width (RDW)

In 1929, Maxwell Wintrobe described his method for obtaining the hematocrit or packed red cell volume (PCV) by centrifugation of blood in a glass tube. He then defined red cell indices, the mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and mean corpuscular Hb concentration (MCHC), which proved of enormous value in classifying the various forms of anemia. (10)

The latest advance in blood cell quantitation began in 1956 with the invention by Wallace H. Coulter of a sophisticated, computer driven electronic instrument that very accurately measured Hb, the numbers of all of the blood cells, as well as the red cell indices and the RDW (red cell distribution width) (11).

Haemoglobin A1C (Hba1c) (12)

Glycated Hb is derived from the nonenzymatic addition of glucose to amino groups of haemoglobin. HbA1c is a specific glycated haemoglobin that results from the attachment of glucose to the N-terminal valine of the haemoglobin beta chain.

Total glycated Hb includes all glycated fractions including HbA1c as well as haemoglobin glycated at sites other than the N-terminus of the beta chain.

Glucose combines with Hb continuously and nearly irreversibly during the life span of RBC (120 days). Therefore, glycosylated Hb will be proportional to mean plasma glucose level during previous 6-12 weeks. Hence, it is therefore, free of the large fluctuations that occur daily in blood glucose concentrations.
Measurement of Hba1c

Numerous assays have been developed to measure glycated haemoglobin.

The principle of all methods is to separate the glycated and nonglycated forms of haemoglobin. This can be accomplished based on differences in charge (usually by HPLC) or structure (usually immunoassays or boronated affinity chromatography).

The interpretation of Hba1c levels is not intuitively obvious to many patients with DM, who are accustomed to thinking in terms of blood glucose levels.

The American Diabetes Association has called for laboratories to express Hba1c results as estimated average blood glucose (eAG). ADA believes that eAG will be easier for patients to understand and will lead to improved management of DM.

Formula recommended to calculated eAG is

\[
eAG (\text{mg/dl}) = 28.7 \times \text{Hba1c} – 46.7
\]

Normal range (ADA 2017) guidelines

Hba1c levels for diagnosis of Diabetes Mellitus

- \(< 5.7\%\) - Normal
- \(5.7-6.4\%\) - High risk / Prediabetes
- \(\geq 6.5\%\): Diabetes (to be confirmed by repeating test on different day).

For monitoring of Diabetes mellitus, HBA1C target goal should be \(< 7\%\).

Uses

- Monitoring compliance and long term glucose level control in patients with diabetes
- Index of diabetic control (Direct relationship between poor control and development of complications)
- Predicting development and progression of diabetic microvascular complications
- Possibly for diagnosis of DM.

Increased in

- Chronic renal failure
- Iron deficiency anemia
- Splenectomy
- Increased serum triglycerides
• Alcohol ingestion
• Fetal Hb above normal or 0.5%

**Decreased in**

• Shortened RBC life span (e.g., haemolytic anemias, blood loss)
• Following blood transfusions
• Pregnancy
• Hemoglobinopathies (e.g., spherocytes)

**Importance and prerequisite for testing**

• Fasting or dietary preparation is not required
• One percent increase in HbA1c is related approximately 30 mg/dl increase in glucose levels
• Test should be performed at least twice in a year to keep check on glycemic control.

**Red Cell Distribution Width (RDW)**

**Definition**

RDW is a coefficient of variation of the distribution of individual RBC volume.

Normal range: 12.1 – 14 fL.

**Use**

An elevation in RDW is useful in drawing attention to anisocytosis, a marker for various anemias.

RDW is defined as the quotient of standard deviation of red blood cell volume and its mean volume and is expressed as a percentage according to the following formula:

RDW = (standard deviation of red blood cell volume / mean cell volume) x 100.

**Higher RDW values reflect greater variations in red blood cell volume**

**Interpretation**

The RDW is particularly helpful in separating iron deficiency anemia (high RDW, normal to low MCV), from β thalassemia trait (normal RDW, low MCV).

Increased RDW is also useful in identifying red cell fragmentation, agglutination, or dimorphic cell population.
Limitation

Very high WBC, numerous large platelets, and auto agglutination result in falsely elevated RDW.

Recent studies:
Glycemic Disturbances and RDW.

In 2014, Lippi et al. observed the existence of a relationship between HbA1C and RDW in an unselected population of elderly patients.\(^{(13)}\)

Additional studies have noted an increased prevalence of diabetes complications such as nephropathy and cardiovascular disease in patients with diabetes and elevated RDW levels.

In 2014, Garg et al. demonstrated that HbA1C levels had an impact on CAD severity in patients without diabetes; therefore, these levels may be considered a mechanism linking RDW values to impaired glucose metabolism.\(^{(14)}\)

Veeranna et al described in 2012 that RDW significantly predicts HbA1c independent of FBG in healthy nondiabetic adults, suggesting the possibility of chronic hyperglycemia mediating the association between RDW and CVD.\(^{(15)}\)

As per the study done by Suryavanshi et al in 2015, RDW significantly correlated inversely with HbA1c.\(^{(1)}\)

As per the study done by Nada AM in 2015, RDW which is recently considered as an inflammatory marker with a significant predictive value of mortality in diseased and healthy populations, is significantly higher in diabetic patients than healthy subjects and is particularly higher in uncontrolled glycemia.\(^{(16)}\)

As per the study done by Engstrom et al in 2014, RDW was significantly and positively associated with HbA1c, corresponding an increase in HbA1c of 0.10% per 1 SD increase in RDW.\(^{(17)}\)

V. EXPERIMENTAL RESULTS

1. Gender wise separation of sample size

Total 50 patients with diabetes were included in the study, out of which 18 were male and 32 were female.

2. Age wise distribution

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Age group (Years)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21-30</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>31-40</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>41-50</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>51-60</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>61-70</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>71-80</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>81-90</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>18</td>
<td>32</td>
</tr>
</tbody>
</table>
3. RDW levels (%) according to gender among all studied patients

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDW (Mean)</td>
<td>15.2 ± 0.35</td>
<td>16.7 ± 0.63</td>
</tr>
</tbody>
</table>

Mean value for RDW in male was 15.2 and female it was 16.7

4. Hba1c levels according to gender among all studied patients

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hba1c (Mean)</td>
<td>8.6 ± 1.70</td>
<td>8.5 ± 4.38</td>
</tr>
</tbody>
</table>

Mean Hba1c in males was 8.6% and in females it was 8.5%

5. Correlation between RDW and Hba1c in males and females

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient (r) between Hba1c and RDW</td>
<td>0.400 (Moderate Positive Correlation)</td>
<td>0.04 (Weak Positive Correlation)</td>
</tr>
</tbody>
</table>

Moderate positive correlation was noted between RDW and Hba1c in males whereas weak positive correlation was noted in females.
V. DISCUSSION

HbA1c measurement is a standard part of diabetes care including diagnosis and management.

HbA1c is shown to be a predictor of complications of diabetes as well as mortality and morbidity.

Red blood cell distribution width (RDW) is a measure of variation of the volume of red blood cells. The “width” referring to the width of the distribution curve and not the width of the cells.

Increased RDW results from heterogeneity of erythrocyte size. Increased erythrocyte size heterogeneity may result from iron or folate deficiency, decreased erythrocyte life-span, or impaired erythropoiesis.

Both HbA1c and RDW are shown to be as prognostic markers in diabetic as well as in nondiabetic patients.
This study shows a significant correlation between RDW and HbA1c in males (r = 0.400), whereas in females it was weak correlation (r = 0.04). This agrees with Lippi et al in 2014(13) who also suggested that in the entire study population, HbA1c was significantly associated with RDW values (r = 0.11; p < 0.001), even after adjustment for age and gender.

Sharif et al.(2013)(18) who also observed correlation between RDW and HbA1c, but it was statistically insignificant.

In a study done by Veerannaet al.(2012)(15) reported that RDW was highly significantly correlated with HbA1c, however the correlation itself was relatively mild (r=0.27), but it was done in healthy non diabetic individuals.

In a study done by Engstrom et al (2013)(17), RDW was significantly and positively associated with HbA1c, corresponding an increase in HbA1c of 0.10% per 1 SD increase in RDW.

VI. CONCLUSION

The present observational study shows a statistically significant but moderate positive correlation between HbA1c and RDW in case of males. No significant correlation was noted between HbA1c and RDW was observed in case of females. However there are discrepancies between the correlation between HbA1c and RDW in various studies. HbA1c is formed by a slow irreversible reaction between haemoglobin and glucose. The percentage of HbA1c is mainly determined by plasma glucose levels and the time the RBCs have been exposed to glucose. The average lifespan of the RBC is considered to be approximately 120 days; during this time, the density of the cells increases and the surface area gradually decreases. However, available data show that there are substantial differences in RBC lifespan. Variations in RBC lifespan of this magnitude are sufficient to cause considerable alterations in HbA1c. Hence, the positive correlation between RDW and HbA1c could reflect a relationship between high variability of the RBC volumes and a high proportion of old dense cells which have been exposed to glucose for a long time. Furthermore, it could be speculated that the previously reported relationships between high RDW, heart failure and mortality could be related to the properties and functions of senescent RBCs. The results seem to be of clinical importance for screening and diagnosis of DM, as HbA1c overestimates the degree of hyperglycaemia if RDW is high and underestimates the DM risk in subjects with low RDW. Hence, RDW along with HbA1c may be considered as a marker of glycaemic control and prognostic marker for future cardiovascular disease in diabetic individuals.

REFERENCES