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Original Article

Pathology Section

Red Blood Cell Morphology in Diabetic Patients: A Case-control Study

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ABSTRACT

Introduction: According to earlier studies, patients suffering from diabetes frequently experience haematological changes. India is the diabetes capital, with 69.1 million residents suffering from the disease.

Aim: To study Red Blood Cell (RBC) morphological changes and grading on percentage of cells that differ in size and shape from normal erythrocytes in diabetic patients.

Materials and Methods: The case-control study was conducted at Division of Central Clinical Laboratory in Department of Pathology in coordination with Department of General Medicine at Jawaharlal Nehru Medical College, Wardha, Maharashtra, India, from October 2019 to October 2021. Total 90 known cases of diabetes mellitus, aged between 18-80 years and 90 subjects (control group) with normal blood glucose levels were included for the study. Demographic details, anthropometric, parameters i.e, Fasting Plasma Glucose (FPG), Random Blood Sugar (RBS), Glycated haemoglobin (HbA1c) and morphology of blood smear were assessed.

Results: The mean age of study group was 54.98±2.31 years and control group was 50.45±17.94 years (p-value= 0.0004). The mean random blood sugar of study group was 163.92±91.31 mg/dL and of control group was 96.92±22.31 mg/dL (p-value=0.0001). Mean HbA1c for the study group was 7.73±1.71%, while in control group was 5.24±0.62% (p-value=0.0001). In the study group, 9 (10%) cases showed a slight anisocytosis (1+) and 3 (3.3%) cases showed moderate anisocytosis (2+), 2 (2.2%) cases showed marked anisocytosis (3+) (variation in size of RBCs with respect to normal) in respect to Mean Corpuscle Volume (MCV), and 11 (12.2%) cases showed MCV <80 fL, 2 (2.2%) cases showed MCV >80 fL, while majority of the cases; 77 (85.5%) had normal sized RBCs, had MCV between 80-99 fL.

Conclusion: The findings of the study suggest that there is need for some simple and effective techniques like ectocytometry, micropore filtration for routine haematological tests in type 2 diabetes mellitus.

Keywords: Anisocytosis, Poikilocytosis, Type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus is a collection of metabolic illnesses that have hyperglycaemia as one of their shared underlying characteristics. The vast majority of diabetes cases can be broadly divided into type 1 and type 2. Between 90% and 95% of diabetic individuals have type 2 diabetes and the majority of them are overweight [1].

With 69.1 million people living there who have the disease, India is the diabetes capital and its prevalence in India ranges from 5-17% [2]. Risk factors include, age group (45-69) years, sedentary life style, marital status, hypertension, obesity, reduced physical activity and unhealthy behaviour and family history [3]. Diabetes is a leading cause of morbidity and mortality globally [3].

Glycaemic control can be assessed by self monitoring of blood glucose or interstitial glucose, HbA1c levels determination [4]. For the diagnosis of diabetes, value of HbA1c should be >6.5% as recommended by American Diabetes Association [4].

Red Blood Cells (RBCs) have smooth, rounded shapes and their diameters appear to be the approximately 7 µm [5]. Sometimes there exists pathological states like pathological sideroblasts, Reticulofilamentous substances, Howell-Jolly bodies, Cabot rings, basophillic stippling, heinz bodies, haemoglobin H inclusions [6].

Abnormal erythropoiesis includes, anisocytosis (variation in size) and poikilocytosis or both and the term poikilocytosis implies more variation in shape than is normally present, example-spherocyte, elliptocyte, ovalocyte, fragmented RBCs [5]. Function of erythrocytes is affected in diabetes mellitus through interaction with its membrane and intracellular constituents. Diabetic RBCs have increased Malonyldialdehyde (MDA), decreased glutathione and membrane Sulfhydryl (SH) group as compared to normal RBCs [7].

Effects of hyperglycaemia on RBCs include RBC count, glycation of haemoglobin, reduced deformability and lifespan of RBCs [8]. Major determinents of erythrocytes deformability include: average membrane Surface Area (SA), Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Complete Blood Count (CBC), Haematocrit, Red cell Distribution Width (RDW), RBC count [7]. An increase in RDW is related to impairment of erythropoiesis, reflected by chronic inflammation and oxidative stress, both of which are cornerstone in pathogenesis of Type 2 Diabetes Mellitus (T2DM) [9].

In order to better understand how diabetes mellitus affects blood cells' structure, shape and function, this study was carried out as a research priority. With the help of biomarkers and parameters like Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and RDW risk assessment for individuals at risk of developing diabetes mellitus can be improved. Thus, changes in RBC morphology is an inextricable part to be studied in diabetic patients.

Higher RDW readings are strongly linked to an increased risk of developing cardiovascular disease and nephropathy. So, estimation of their values may be helpful in estimating the disease progression and thereby the need for modification in treatment part and thereby a better outcome. The present study was carried out to study the changes in RBC morphology in diabetes patients. Also to study erythrocyte deformability and its variation in diabetes mellitus, grading of RBCs based on percentage of cells that differ in size and shape from normal erythrocytes and to compare the results with same measurements in 90 subjects without diabetes mellitus.

MATERIALS AND METHODS

The case-control study was conducted at Division of Central Clinical Laboratory in Department of Pathology in co-ordination with Department of General Medicine at Jawaharlal Nehru Medical

College, Wardha, Maharashtra, India, from October 2019 to October 2021. No ethical issue involved in to it as the study is diagnostic procedure based and data will not be published by disclosure of identity of patient. Informal consent was be obtain of all the patients as per the Acharya Vinoba Bhave Rural Hospital (AVBRH) policy.

Inclusion criteria:

Case group: Patients (n=90) known cases of diabetes mellitus; minimum since five years, on oral hypoglycaemic drugs since five years, aged between 18-80 years.

Control group: Patients (n=90) with normal blood glucose levels were included for the study.

Exclusion criteria: All patients with DM >5 years duration were excluded.

Diagnostic criteria: Using reference guide for grading RBC morphology [Table/Fig-1] [10]. Age parameter was used as a guide to subtype diabetes according to World Health Organisation (WHO) guideline [11]. Patient demographics, personal history along with history of smoking, hypertension was noted.

Red blood cells (cell type)	Normal (non specific)	1+ (Mild)	2+ (Moderate)	3+ (Marked)
Hypochromasia (MCH-pg)	27-34 pg	22-26	18-21	<18
Polychromasia		3-5	6-20	>20
Macrocytes (MCV- fL)	80-99	70-79	60-69	<60
	80-99	100-115	115-125	>125
Schistocytes		1-5	6-15	>15
Elliptocytes		6-20	21-50	>50
Rouleaux		-	11-50	>50
Spherocytes		1-5	6-20	>20
Target Cells		5-10	11-25	>25
Acanthocytes		1-10	11-30	>30

[Table/Fig-1]: Reference guide [10].

Anthropometry, biochemical parameters like fasting plasma glucose, random blood glucose, HbA1c was measured and morphology of blood smear was assessed. Patients in which fasting blood glucose was inaccessible, random blood sugar was recorded.

- Referance range of RBS was used and divided into five categories as follows: <120 mg/dL , 120-160 mg/dL, 161-200 mg/dL, 201-240 mg/dL, >241 mg/dL [7].
- Reference range for HbA1c was followed as: between 4.5 and 5.7% (1+), between 5.8 and 6.4% (2+) and above 6.5% (3+) [4].
- RDW values were divided into categories using the following cut-off values: 11.1 to 12.6% (1+), >12.6 to <13.2 (2+), 13.2 to <13.9 (3+),13.9 to 24.1 (4+) and >24.1 (5+) [12].

Study Procedure

To access the staining and cellular distribution, a 10X magnification objective microscopic inspection was first used, achieving an estimate number of leucocytes and presence of abnormal cellular elements like blasts, platelet aggregates, any abnormal red cell type. Subsequently smear were evaluated with objective of 100X magnification. Each cell was evaluated for quantitative and qualitative disturbances. Images were taken after the examination of preparation obtained with 100X objective.

STATISTICAL ANALYSIS

Chi-square test and z-test for difference between two means were employed in the statistical analysis, which was conducted using descriptive and inferential statistics. Statistical Package for Social Sciences (SPSS) version 27.0 and GraphPad Prism 7.0 versions of software were used in the study. A p-value<0.05 was considered as statistically significant.

RESULTS

In study group; 90 cases (23 women and 67 men) were aged between 25-87 years, mean age 54.98±12.31 years. The subjects of control group; 90 (42 women and 48 men) were aged between 18-92 years, median age 48.41±18.04 years. Average blood pressure in case group was recorded as 120/78 with standard deviation of 27.32 and in control group as 116/72 standard deviation of 27.09 mmHg. The age distribution of the study group's patients and the control group's participants was similar for both groups [Table/Fig-2,3].

Among study group, 32 (35.55%) out of the 90 subjects had a history of smoking and 27 (84.3%) of them were males, while all the non smokers were included in the control group. Among study group, 38 (42.22%) out of the 90 subjects had a history of hypertension and 20 (52.6%) of them were males, while all the subjects under the control group had blood pressure under normal limits.

Age group (years)	Control group	Study group	p-value
≤20	4 (4.44%)	-	χ^2=22.67; p-value=0.0004
21-30	17 (18.89%)	3 (3.33%)	
31-40	13 (14.44%)	9 (10%)	
41-50	19 (21.11%)	17 (18.89%)	
51-60	13 (14.44%)	31 (34.44%)	
>60	24 (26.67%)	30 (33.33%)	
Total	90 (100%)	90 (100%)	
Mean±SD	48.41±18.04	54.98±12.38	
Range	18-89	25-87	

[Table/Fig-2]: Distribution according to age in years.
p-value<0.05 was considered as statistically significant.

Gender	Control group	Study group	χ^2-value
Male	48 (53.33%)	67 (74.44%)	8.69 p=0.0032,S
Female	42 (46.67%)	23 (25.56%)	
Total	90 (100%)	90 (100%)	

[Table/Fig-3]: Distribution of patients according to gender.
p-value<0.05 was considered as statistically significant

The prevalence of diabetes was found more in the age group ≥51 years of age, 61 (67.7%) out of 90 cases. Random blood sugar in the study group were recorded averages of 163.92 mg/dL with a standard deviation of 91.31 compared with controls that had a blood glucose value of 96.92 mg/dL with a standard deviation of 22.31 as shown [Table/Fig-4]. Majority of cases, 36 (40%) out of 90 were found to be having RBS <120 mg/dL.

Random blood sugar (mg/dL)	Control group	Study group	χ^2-value
<120	77 (85.56%)	36 (40%)	48.69 p=0.0001
120-160	11 (12.22%)	16 (17.78%)	
161-200	2 (2.22%)	16 (17.78%)	
201-240	-	8 (8.89%)	
>240	-	14 (15.56%)	
Total	90 (100%)	90 (100%)	
Mean±SD	96.92±22.41	163.92±91.31	
Range	55-179	46-625	

[Table/Fig-4]: Distribution of patients according to random blood sugar.
p-value<0.05 was considered as statistically significant

In the study group, there were 2 (2.2%) out of 90 cases with values of HbA1c between 4.5-5.7%, 24 (26.6%) cases between 5.8-6.4%, and 64 cases (71.1%) recorded values above 6.5%. Mean HbA1c for the study group was 7.73±1.71%, while in control group, the mean value of HbA1c was 5.24±0.62% [Table/Fig-5].

HbA1c (%)	Control group n (%)	Study group n (%)	χ^2-value
4.5-5.7	76 (84.44%)	2 (2.22%)	136.8 p-value=0.0001
5.8-6.4	14 (15.56%)	24 (26.67%)	
≥6.5	-	64 (71.11%)	
Total	90 (100%)	90 (100%)	
Mean±SD	5.24±0.61	7.73±1.72	
Range	4.21-6.37	5.50-13.20	

[Table/Fig-5]: Distribution according to HbA1c.
p-value<0.05 was considered as statistically significant

Blood cytology analysis: In the study group, 2 (2.2%) cases showed a slight anisocytosis (1+) and 3 (3.3%) cases showed moderate anisocytosis (2+), 2 (2.2%) cases showed marked anisocytosis (3+) (variation in size of RBCs with respect to normal) in respect to mean corpuscle volume, and 11 (12.2%) cases showed MCV <80 fL, 2 (2.2%) cases showed MCV >80 fL, while majority of the cases; 77 (85.5%) had normal sized RBCs, had MCV between 80-99 fL.

In control group, 5 (5.5%) cases showed a slight anisocytosis (1+), 1 (1.1%) cases showed moderate anisocytosis (2+), and 4 (4.4%) cases showed MCV <80 fL, 2 (2.2%) cases showed MCV >80 fL, while majority of the cases; 84 (93.3%) had normal sized RBCs. The distribution of patients according to RBC cell type has been shown in [Table/Fig-6].

RBC cell type	Control group	Study group	χ^2-value
Normocytic mildly hypochromic RBCs	23 (25.56%)	3 (3.33%)	χ^2=34.89; p-value= 0.0001
Normocytic moderately hypochromic RBCs	1 (1.11%)	7 (7.78%)	
Normocytic normochromic RBCs	66 (73.33%)	65 (72.22%)	
Predominantly normocytic mildly hypochromic RBCs	-	14 (15.56%)	
Predominantly normocytic mildly to moderately hypochromic RBCs	-	1 (1.11%)	
Total	90 (100%)	90 (100%)	

[Table/Fig-6]: Distribution according to RBC cell type.
p-value<0.05 was considered as statistically significant

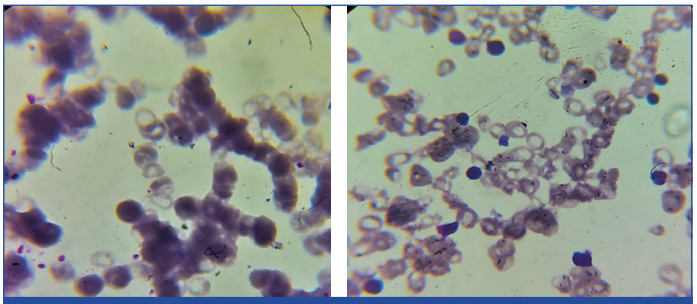
In study group, 11 (12.2%) cases out of 90 showed poikilocytosis and among 11, seven cases showed slight poikilocytosis (1+), three showed moderate poikilocytosis (2+), and one case showed marked poikilocytosis (3+), and all these cases were associated with increased red cell distribution width (>15.9), as shown in [Table/Fig-7].

Red cell distribution width (fL)	Control group	Study group	χ^2-value
11.1-12.6	26 (28.89%)	2 (2.22%)	χ^2=31.53; p-value=0.0001
>12.6-<13.2	8 (8.89%)	12 (13.33%)	
13.2-<13.9	15 (16.67%)	9 (10%)	
13.9-24.1	41 (45.56%)	63 (70%)	
>24.1	-	4 (4.44%)	
Total	90 (100%)	90 (100%)	
Mean±SD	13.74±1.94	15.71±3.25	
Range	9.70-21.60	12.50-30.90	

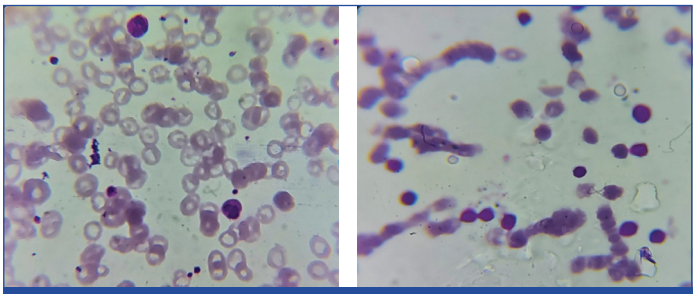
[Table/Fig-7]: Distribution according to RDW.
p-value<0.05 was considered as statistically significant

In control group, 5 (5.5%) out of 90 cases showed poikilocytosis and among them, four cases showed slight poikilocytosis (1+), one case showed moderate poikilocytosis (2+). Combined ansiopoikilocytosis was found in 9 (10%) out of 90 cases in the study group, while it was found in 4 (4.4%) out of 90 cases in control group. Variation in blood smear are shown in [Table/Fig-8-11] and the changes recorded as tear drop cells, target cells, pencil cells, schistiocytes in terms of red cell morphology.

Among the both groups; study group as well as control group, prevalence of hypochromia was found out to be 24 (26.6%). The [Table/Fig-12] depicts distribution of cases according to grading in case as well as control group. The [Table/Fig-13] depicts the study's participant demographics for the T2DM group and the control group, and statistically significant differences are found for the recorded parameters between the study group and the control group.



[Table/Fig-8]: Hypochromasia 1+; Anisocytosis 1+;Poikilocytosis 2+, Low power magnification, Leishman stain. **[Table/Fig-9]:** Hypochromasia 2+; Anisocytosis 1+;Poikilocytosis 2+, Low power magnification, Leishman stain.
(Images from left to right).



[Table/Fig-10]: Hypochromasia 3+; Anisocytosis 1+; Poikilocytosis 2+, Low power magnification, Leishman stain.
[Table/Fig-11]: Poikilocytosis 1+ showing fragmented RBC, Low power magnification, Leishman stain. (Images from left to right).

Grading	Control group n (%)	Study group n (%)	χ^2-value
1+ (slight poikilocytosis)	6 (6.67%)	9 (10%)	3.90 p-value =0.27
2+ (moderate poikilocytosis)	1 (1.11%)	3 (3.33%)	
3+ (marked poikilocytosis)	-	2 (2.22%)	
Normal	83 (92.22%)	76 (84.44%)	
Total	90 (100%)	90 (100%)	

[Table/Fig-12]: Distribution according to grading.
p-value<0.05 was considered as statistically significant

Parameters	Study group (n=90)	Control group (n=90)	p-value
Age (in years)	54.98±2.31	50.45±17.94	0.0004
Random blood sugar (mg/dL)	163.92±91.31	96.92±22.31	0.0001
HbA1c (%)	7.73±1.71	5.24±0.62	0.0001
Red cell distribution width (fL)	15.71±3.23	13.86±2.27	0.0001

[Table/Fig-13]: Characteristics of patients with T2DM and control group recruited in study.
p-value<0.05 was considered as statistically significant

DISCUSSION

Between 5-17% of Indians have diabetes. RBC morphology is significantly affected in diabetes. Diabetes should be a top priority for study since it causes changes to the structure, function and makeup of blood cells [1]. Parameters used in the present study include random blood sugar, HbA1c, RBC type, polychromasia, hypochromasia, RDW, any other abnormal cell found. The prevalence of hypochromia in both groups; study group as well as control group, prevalence of hypochromia was found out to be 24 (26.6%).

In study group, 11 (12.2%) cases out of 90 showed poikilocytosis and among 11, six cases showed slight poikilocytosis (1+), three cases showed moderate poikilocytosis (2+), and one case showed marked poikilocytosis (3+), according to reference guide for grading RBC morphology, and all these cases were associated with increased RDW (>15.9).

In control group, five (5.5%) out of 90 cases showed poikilocytosis and among them, four cases showed slight poikilocytosis (1+), one case showed moderate poikilocytosis (2+). Combined anisopoikilocytosis was found in 9 (10%) out of 90 cases in the study group, while it was found in 4 (4.4%) out of 90 cases in control group. In this study, there are variations between the study group's patients and the control individuals for the collected parameters that are statistically significant.

Atalay H, found in a study that RDW/MCV ratio was found significantly high in patients with diabetic ketoacidosis than in the non diabetics and Hyperosmolar Non Ketotic acidosis (HONK) group. In this study, the sensitivity and specificity for a cut-off of 0.15 for RDW/MCV was 90% and 50%, for MCV they were 76.67% and 70% and for RDW 76.67% and 63.33% [9]. Higher RDW readings were linked to a higher risk of developing cardiovascular disease and nephropathy, according to research by Malandrino N et al., [12].

Rajab A et al., found in a study that persistent hyperglycaemia changes shape, size and haemoglobin contents of RBCs [13]. Liu DS et al., reported that RDW/MCV ratio values higher then 45.40% had 75.0% sensitivity and 99.9% specificity for predicting diabetic ketoacidosis. Also, the sensitivity and specificity values for MCV were 23.1% and 91.7% and for RDW they were 53.8% and 87.5% respectively [14].

Engstrom G et al., found that a low RDW was independently associated with increased incidence of diabetes mellitus [8]. NeamȚu MC et al., found that the prevalence of anisocytosis and poikilocytosis in control group was 46.66%, 30.30% respectively as compared to control group 3.33% [4]. Findings of all these studies were concordant with the present study as in all these studies, high pravalence of anisocytosis and poikilocytosis was found in study group and statistically significant differences were found in study and control groups. Although poikilocytosis lacks specificity from a diagnostic standpoint, the identification of particular poikilocyte types indicates the presence of particular diseases [4].

Limitation(s)

This was a short-term interval study. The other confounding factors for RBC morphology changes (anaemia) were not considered for the study. Due to case-control design, the causal relationships between RDW and diabetic complications could not be established.

CONCLUSION(S)

The study concluded that the patients with diabetes mellitus had more morphological abnormalities with respect to anisopoikilocytosis, RDW, RBC, grading than the patients in the control group, even the two groups were age matched. These changes may have a direct impact on erythrocyte function and may contribute to the patient complex pathology. Authors conclude by suggesting that the morphological assessment of RBCs might give valuable information about the disease status of the patients with diabetes, ststus of diabetes control as well as different associated risks; such as macrovacular and microvascular complications. This study raises the possibility that morphological analyses of RBCs could provide insightful data regarding the illness status of diabetic patients.

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