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Hyperglycemia effect on red blood cells indices

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Abstract. – OBJECTIVE: Hyperglycemia has an effect on all body tissues; one of them is the bone marrow. This effect is related to protein glycation and other chemical and physiological changes of red blood cells (RBCs). The aim of this study was to assess the effect of hyperglycemia on different RBCs indices along with evaluating these changes in the normal physiology and chronic diabetes complication pathology.

PATIENTS AND METHODS: This is a cross-sectional hospital-based study of 1000 type 2 Saudi diabetic patients without any hematological diseases. Patients were fully evaluated clinically and biochemically with full blood hematological parameters assessment. The studied cohort matched the general characteristics of Saudi type 2 diabetic patients.

RESULTS: This study shows that hyperglycemia increases the red blood cells count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). Red blood cell distribution width (RDW) was negatively correlated with poor glycemic control. Concurrently, the presence of micro and macroangiopathies with hyperglycemia shortens the lifespan of RBCs.

CONCLUSIONS: We conclude that hyperglycemia has an imposing effect on RBCs count and its physiological function, which can be normalized effectively with good glycemic control.

Key Words:
Red blood cells indices, Red blood cell distribution width (RDW), Hematology, Diabetes mellitus.

Introduction

Overwhelming data demonstrated the effect of hyperglycemia on different body tissues resulting from the glycation of different proteins. Bone marrow is one of the body tissues with a high proliferation rate that produces all the different types of blood cells on a daily basis, one of which is red blood cells through the erythropoiesis system[1]. The persistent elevation of glycosylated hemoglobin as a result of diabetes-related hyperglycemia is associated with the structural and functional changes in hemoglobin (Hb) molecule, the osmotic disturbance and the cytoplasmic viscosity within each cell. All these changes could have an imposing effect on any of the red blood cell indices, which include the red blood cells (RBCs) count, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and the cell shape and deformability represented by red blood cell distribution (RDW)[2]. Recently, researchers[2,3] demonstrated the effect of different levels of glycemia on hematological parameters, wherein it has been reported that hyperglycemia with insulin resistance has an imposing effect on red blood cell and hematocrit (Hct). On the other hand, a high value of glycosylated hemoglobin was reported to correlate with decreased deformability of erythrocytes[4]. Additionally, RBCs count was positively associated with hyperglycemia and was reflected by higher HbA1c, as shown in both diabetes and prediabetes state[5]. The effect of hyperglycemia on the hematological parameters does not manifest any pathological phenomena, but it could well be the reason behind different abnormal observations among diabetic patients including delayed wound healing and a defect in the normal physiology of hematopoietic system[6].

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These changes may also contribute to chronic diabetes complications, wherein, the RDW, Hct and RBCs count have been found to be associated with microvascular and macrovascular complications[7,8]. Since the data is scarce related to the association of different erythropoietic parameters with hyperglycemia and diabetes complications, the main aim of the current work was to evaluate the correlation between glycemic markers and different erythropoietic parameters among type 2 diabetic patients along with their association in the presence of diabetes chronic complications.

Patients and Methods

Patient Selection and Data Collection

This is a cross-sectional study and the cohort was selected from the University Diabetes Center (UDC), King Saud University from January to August 2016. A total of 1000 patients with type 2 diabetes who were recently referred to UDC and aged ≥18 years with complete erythropoietic parameters, namely RBCs count, Hct, MCV and MCHC, RDW and erythrocyte sedimentation rate (ESR) were recruited in a convenience series manner. These parameters were then correlated with simultaneous glycemic control parameters, namely HbA1c, fasting blood glucose (FBG), 2h postprandial and fasting lipids, which include total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides. Patients with hematological diseases like hemoglobinopathies and myeloproliferative disorders or chronic diseases like cancer, liver cirrhosis and renal diseases were excluded. This exclusion also included patients with infections, inflammatory bowel disease, hypothyroidism and all those patients on drugs which may suppress bone marrow activity. The clinical data collected from patients' charts included age, gender, diabetes duration, and history of smoking. Weight, height, both systolic (SBP) and diastolic blood pressures (DBP) were collected during the same visit when the biochemical and hematological evaluations were performed.

Laboratory Analysis

A venous blood sample was collected from the median cubital vein using a Becton Dickinson vacutainer heparinized tube and was then transferred to the central laboratory for analysis. All the erythropoietic parameters were measured and analyzed by a COULTER LH 500 hematology analyzer (Beckman Coulter, Fullerton, CA, USA) machine. Simultaneously, another sample was collected in a plain tube for metabolic markers including glycemic and lipid markers in serum. Blood glucose assessment was performed using the glucose oxidase-peroxidase methodology. The serum cholesterol assessment was performed using the cholesterol oxidase-peroxidase methodology and the HDL, LDL and triglyceride assessments were performed using direct and glycerokinase oxidase-peroxidase methodology. A third sample was also collected in potassium EDTA tube for HbA1c measurement based on the Randox Daytona (United Kingdom) latex agglutination inhibition assay.

Statistical Analysis

Data were analyzed using Statistical Package for social science (SPSS software version 21, IBM, Armonk, NY, USA). The *t*-test was used to measure mean±SD and both descriptive and frequency measurement. Pearson's correlation, ANOVA and regression analysis were used to assess the correlation between hematological parameters and glycemic control. Least Significant Difference (LSD) test was used as a post-hoc test to validate ANOVA. Odds ratio (OR) and its 95% confidence intervals (CI) were used to express different risks. p-value of < 0.05 was considered statistically significant.

Results

The mean age of the total sample was 51.1 ± 12.7 years, which was identical to the median with a range between 18-95 years. The mean duration of diabetes exceeded 8 years and the mean HbA1c was 8.9 ± 2.0% with a median of 8.5% ranging between 5.2 and 14.6%. The values for erythropoietic parameters including Hb, Hct, RBC, MCV, MCH, MCHC, RDW, and ESR demonstrated normal distribution based on their mean, median and range values, as shown in Appendix 1. Table I demonstrates the metabolic and erythropoietic parameters in relation to clinical categories, wherein older patients show a significant increase of RBC count, Hb, Hct, and ESR. Men had significantly higher RBCs count, Hb, and Hct, while lower ESR when compared to women. Diabetic patients showed a lower mean value of RBCs count and Hct with diabetes duration > 10 years. The results show a marked increase in RBCs count among smokers, Hb and

Appendix I. Measures for central tendency for clinical and metabolic and erythropoietic markers.

	Mean (± SD)	Median	Range
Age (years)	51.1 ± 12.7	51.0	18.0-95.0
Diabetes duration (years)	8.4 ± 7.8	6.0	1.0-47.0
SBP (mmHg)	132.4 ± 17.8	132.0	82.0-181.0
DBP (mmHg)	76.5 ± 9.8	77.0	51.0-105.0
BMI (kg/m^2)	31.2 ± 6.2	30.5	15.5-63.6
HbA1c (%)	8.9 ± 2.0	8.5	5.2-14.6
FBG (mg/dL)	9.4 ± 3.4	8.8	3.4-18.6
2 hr PC	13.9 ± 5.4	13.3	4.3- 29.0
Hemoglobin g/dL	13.8 ± 1.7	13.8	9.2-17.5
Hct %	40.7 ± 4.7	41.0	27.8-53.2
RBC (10^{12}/L)	4.7 ± 0.5	4.7	3.3-6.2
MCV(fL)	86.5 ± 4.6	86.8	74.3-98.3
MCH (pg)	29.3 ± 1.9	29.4	23.8-33.9
MCHC (g/dL)	33.8 ± 0.1	33.8	31.0-35.8
RDW (%)	13.6 ± 0.1	13.5	11.5-16.4
ESR (mm/h)	20.6 ± 15.8	18.0	0.0-65.0
Cholesterol (mg/dL)	4.8 ± 1.1	4.7	2.4-7.9
LDL (mg/dL)	2.8 ± 0.9	2.7	0.7-5.9
HDL (mg/dL)	1.2 ± 0.4	1.2	0.6-2.5
Triglyceride (mg/dL)	1.8 ± 0.1	1.5	0.5-7.5

Hct, but lower ESR. SBP ≥ 140 mmHg did not have any effect on those parameters, while DBP ≥ 80 mmHg showed a remarkable increase in the mean value of RBC's count, Hb, Hct, but decreased ESR. Patients with BMI ≥ 30 kg/m^2 had significantly lower mean values of Hb and Hct but increased mean ESR value. The mean values for MCV, MCH, and MCHC were markedly lower in women and patients with high mean BMI values, except for MCHC. Both MCV and MCH were significantly higher among older patients and smokers. RDW was remarkably increased among women and subjects with higher mean values of BMI, but markedly lower with high DBP. Patients with poor glycemic control represented by HbA1c > 7% or FBG > 130 mg/dL or 2-h postprandial glucose of > 180 mg/dL showed a significant increase in their mean values of RBCs count, Hb and Hct. Patients with total cholesterol > 4.0 mg/dL, LDL > 2 mg/dL and triglycerides > 1.7 mg/dL showed markedly higher mean values of RBCs count, Hb, and Hct, while the reverse was observed with a high value of HDL. The mean values of ESR were remarkably lower in patients with higher FBG and 2-h postprandial glucose. None of the metabolic parameters with the exception of higher FBG and lower HDL had any significant effect on MCV, MCH and MCHC. RDW was remarkably higher among patients with good glycemic control, but not with any lipid parameters values, as shown in Table II. Table III shows the significant decrease in the mean values of RBCs count, Hb and Hct among patients with diabetic neuropathy, retinopathy, nephropathy and vasculopathy. This study has also shown an increase in RDW, but a decrease in the mean values of MCV and MCH among patients with vasculopathy. When looking for different quartile levels of the hematopoietic parameters in relation to chronic complications, the only significant ones were higher quartile for ESR among patients with nephropathy and MCV with retinopathy, while lower quartile for Hct and RBCs count for neuropathy and retinopathy, as shown in Appendix 2. Pearson correlation for erythropoietic parameters demonstrated a positive correlation with RBCs count, Hb and Hct, while it demonstrated a negative correlation with RDW in relation to poor glycemic control presented by higher HbA1c, FBG and 2-hour postprandial blood glucose. On the other hand, both MCV and MCH had a negative correlation with higher HbA1c values. High FBG had a negative correlation with ESR, but a positive correlation with MCHC, as shown in Table IV and Figure 1A and 1B.

Discussion

The collected study sample represents the normal distribution for the Saudi diabetic population based on data from the Saudi National Diabetes

Table I. The mean values of erythropoietic and metabolic parameters in relation to baseline clinical categories.

Parameter (number)	HbA1c (978)	FBG (970)	2hrspp (830)	RBC (967)	HGB (966)	HCT (966)	ESR (894)	MCV (927)	MCH (933)	MCHC (957)	RDW (913)
Age (years)											
≤ 45	8.5 ± 2.0	8.9 ± 3.5	12.6 ± 5.4	4.7 ± 0.5	13.5 ± 1.8	40.0 ± 5.1	22.1 ± 16.5	85.1 ± 4.8	28.8 ± 1.9	33.7 ± 1.0	13.6 ± 1.0
46-64	9.0 ± 2.0	9.8 ± 3.3	14.3 ± 5.3	4.8 ± 0.5	14.0 ± 1.6	41.3 ± 4.6	18.5 ± 14.7	86.8 ± 4.3	29.4 ± 1.8	33.8 ± 1.0	13.5 ± 0.9
≥ 65	9.2 ± 2.0	9.5 ± 3.3	15.4 ± 5.1	4.6 ± 0.5	13.6 ± 1.4	40.4 ± 4.1	23.9 ± 16.9	88.2 ± 4.5	29.9 ± 1.7	33.8 ± 0.9	13.5 ± 0.9
p-value	< 0.001	0.002	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001	0.213	0.179
Gender											
Men	9.0 ± 2.0	9.7 ± 3.3	14.7 ± 5.5	5.0 ± 0.5	14.7 ± 1.4	43.5 ± 4.0	14.2 ± 13.0	87.5 ± 4.4	29.7 ± 1.8	33.9 ± 0.9	13.4 ± 0.9
Women	8.8 ± 2.0	9.2 ± 3.5	13.1 ± 5.2	4.5 ± 0.4	12.7 ± 1.3	37.8 ± 3.6	27.7 ± 15.5	85.3 ± 4.5	28.8 ± 1.8	33.6 ± 1.0	13.7 ± 1.0
p-value	0.111	0.015	< 0.001	< 0.001	<0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
DM duration											
≤ 5	8.3 ± 2.0	8.6 ± 3.1	12.5 ± 5.1	4.8 ± 5.2	13.8 ± 1.8	40.8 ± 4.9	19.5 ± 15.5	86.2 ± 4.7	29.3 ± 1.9	33.8 ± 1.0	13.6 ± 1.0
6-10	9.2 ± 2.0	10.0 ± 3.5	14.8 ± 5.4	4.7 ± 5.0	13.9 ± 1.6	41.3 ± 4.5	21.2 ± 16.3	86.4 ± 4.4	29.2 ± 1.8	33.7 ± 0.9	13.6 ± 0.9
> 10	9.6 ± 1.8	10.4 ± 3.4	15.6 ± 5.2	4.6 ± 5.0	13.6 ± 1.5	40.2 ± 4.4	21.9 ± 15.7	86.9 ± 4.4	29.4 ± 1.8	33.7 ± 0.9	13.5 ± 0.9
p-value	< 0.001	< 0.001	< 0.001	< 0.001	0.055	0.036	0.123	0.101	0.309	0.461	0.631
Smoking											
Yes	9.0 ± 2.0	9.8 ± 3.5	14.8 ± 5.4	5.0 ± 0.5	14.6 ± 1.6	43.5 ± 4.5	15.4 ± 13.2	87.5 ± 4.5	29.7 ± 1.8	33.8 ± 0.9	13.5 ± 1.0
No	8.8 ± 2.0	9.4 ± 3.4	13.7 ± 5.4	4.7 ± 0.5	13.5 ± 1.6	40.0 ± 4.5	21.7 ± 16.1	86.2 ± 4.6	29.2 ± 1.8	33.8 ± 1.0	13.6 ± 0.9
p-value	0.125	0.108	0.030	<0.001	<0.001	< 0.001	< 0.001	< 0.001	0.006	0.601	0.580
SBP											
< 140	8.7 ± 2.0	9.2 ± 3.4	13.4 ± 5.5	4.7 ± 0.5	13.7 ± 1.7	40.6 ± 4.7	20.7 ± 15.9	86.4 ± 4.6	29.2 ± 1.9	33.8 ± 1.0	13.6 ± 1.0
≥ 140	9.1 ± 1.9	9.9 ± 3.2	14.8 ± 5.1	4.7 ± 0.5	13.8 ± 1.7	40.9 ± 4.7	20.0 ± 15.6	86.6 ± 4.5	29.3 ± 1.8	33.8 ± 0.9	13.5 ± 0.9
p-value	0.004	0.001	< 0.001	0.867	0.291	0.451	0.569	0.548	0.287	0.466	0.147
DBP											
< 80	8.8 ± 2.0	9.3 ± 3.4	13.7 ± 5.4	4.7 ± 0.5	13.6 ± 1.6	40.3 ± 4.7	21.6 ± 15.9	86.4 ± 4.6	29.3 ± 1.9	33.8 ± 0.9	13.6 ± 1.0
≥ 80	9.0 ± 2.0	9.7 ± 3.3	14.1 ± 5.3	4.8 ± 0.5	14.0 ± 1.7	41.3 ± 4.8	18.7 ± 15.5	86.4 ± 4.5	29.3 ± 1.8	33.8 ± 1.0	13.5 ± 0.9
p-value	0.127	0.033	0.284	<0.001	0.001	0.002	0.008	0.992	0.615	0.627	0.031
BMI											
< 25	9.4 ± 2.3	9.8 ± 3.6	15.7 ± 6.0	4.7 ± 0.5	14.1 ± 1.6	41.7 ± 4.6	17.5 ± 14.9	88.1 ± 4.3	30.0 ± 1.8	33.9 ± 1.1	13.3 ± 0.8
25-30	9.0 ± 2.0	9.7 ± 3.5	14.2 ± 5.6	4.8 ± 0.5	14.1 ± 1.6	41.7 ± 4.6	16.4 ± 14.5	86.9 ± 4.5	29.4 ± 1.8	33.8 ± 0.9	13.4 ± 0.9
≥ 30	8.6 ± 1.9	9.2 ± 3.2	13.1 ± 5.0	4.7 ± 0.5	13.5 ± 1.7	40.0 ± 4.6	23.5 ± 16.0	85.8 ± 4.6	29.0 ± 1.9	33.7 ± 0.9	13.7 ± 1.0
p-value	< 0.001	0.081	< 0.001	0.063	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.415	< 0.001

Table II. The effect of metabolic control on the studied erythropoietic parameters.

		No. (%)	RBC	HGB	Hct	RDW	ESR	MCV	MCH	MCHC
HbA1c (%)	< 7.0%	194 (19.8%)	4.6 ± 0.5	13.4 ± 1.7	39.6 ± 4.8	13.7 ± 1.0	21.4 ± 17.0	86.7 ± 5.0	29.4 ± 2.0	33.8 ± 1.0
	> 7.0%	784 (80.2%)	4.8 ± 0.5	13.9 ± 1.6	41.0 ± 4.6	13.5 ± 0.9	20.2 ± 15.4	86.4 ± 4.5	29.3 ± 1.8	33.8 ± 1.0
	p-value		< 0.001	0.001	< 0.001	0.044	0.395	0.387	0.386	0.760
FBG (mg/dL)	< 130	299 (30.8%)	4.6 ± 0.5	13.3 ± 1.6	39.4 ± 4.6	13.8 ± 1.0	22.8 ± 16.8	86.2 ± 5.0	29.1 ± 2.0	33.7 ± 1.0
	> 130	671 (69.2%)	4.8 ± 0.5	14.0 ± 1.6	41.4 ± 4.6	13.5 ± 0.9	19.4 ± 15.0	86.6 ± 4.4	29.4 ± 1.8	33.8 ± 0.9
	p-value		< 0.001	< 0.001	< 0.001	< 0.001	0.004	0.208	0.029	0.141
2hrs PC (mg/dL)	<180	228 (27.5%)	4.6 ± 0.5	13.3 ± 1.7	39.2 ± 4.7	13.8 ± 1.0	23.0 ± 17.0	86.1 ± 4.8	29.1 ± 1.9	33.7 ± 1.0
	> 180	602 (72.5%)	4.8 ± 0.5	13.9 ± 1.7	41.1 ± 4.7	13.5 ± 0.9	20.1 ± 15.4	86.4 ± 4.5	29.3 ± 1.9	33.8±1.0
	p-value		< 0.001	<0.001	< 0.001	< 0.001	0.036	0.409	0.237	0.350
Cholesterol (mmol/l)	< 4.0	239 (24.2%)	4.6 ± 0.5	13.5 ± 1.7	40.0 ± 4.8	13.7 ± 0.9	20.9 ± 15.1	86.9 ± 4.8	29.5 ± 1.9	33.7 ± 0.9
	> 4.0	747 75.8%)	4.8 ± 0.5	13.8 ± 1.6	41.0 ± 4.6	13.5 ± 0.9	20.5 ± 16.0	86.4 ± 4.5	29.5 ± 1.8	33.8 ± 1.0
	p-value	–	< 0.001	< 0.004	< 0.006	0.066	0.787	0.092	0.128	0.523
LDL (mmol/l)	< 2.0	228 (23.5%)	4.6 ± 0.5	13.4 ± 1.6	39.6 ± 4.6	13.6 ± 0.9	22.0 ± 15.7	86.4 ± 4.8	29.3 ± 1.9	338.0 ± 1.0
	>2.0	741 (76.5%)	4.8 ± 0.5	13.9 ± 1.7	41.1 ± 4.7	13.5 ± 0.9	19.9 ± 15.6	86.5 ± 4.5	29.3 ± 1.8	33.8 ± 1.0
	p-value		<0.001	< 0.001	< 0.001	0.383	0.094	0.599	0.905	0.873
HDL (mmol/l)	< 1.0 men /1.2 women	227 (23.4)	4.8 ± 0.5	14.2 ± 1.7	41.8 ± 4.8	13.5 ± 0.9	17.1 ± 13.6	87.0 ± 4.4	29.5 ± 1.7	33.8 ± 1.0
	> 1.0 men /1.2 women	742 (76.6%)	4.7 ± 0.5	13.7 ± 1.6	40.5 ± 4.6	13.6 ± 0.9	21.2 ± 16.0	86.3 ± 4.6	29.2 ± 1.9	33.8 ± 1.0
	p-value		0.001	<0.001	<0.001	0.770	< 0.001	0.036	0.052	0.886
Triglyceride (mmol/l)	< 1.7	557 (58.6%)	4.7 ± 0.5	13.6 ± 1.7	40.4 ± 4.8	13.6 ± 0.9	21.2 ± 15.9	86.2 ± 4.7	29.3 ± 1.9	33.8 ± 0.9
	> 1.7	408 (41.4%)	4.8 ± 0.5	13.9 ± 1.6	41.2 ± 4.6	13.5 ± 0.9	19.7 ± 15.5	86.3 ± 4.4	29.3 ± 1.7	33.8 ± 1.0
	p-value		< 0.001	0.002	0.006	0.105	0.165	0.292	0.983	0.310

Table III. The mean ± SD of the different erythropoietic parameters and presence of chronic complications.

Chronic complications	Number (%)	RBC	HGB	Hct	RDW	ESR	MCV	MCH	MCHC
Neuropathy	Absent 672 (67.2%)	4.8 ± 0.5	13.9 ± 1.6	41.0 ± 4.6	13.5 ± 0.9	20.2 ± 15.6	86.5 ± 4.6	29.3 ± 1.9	33.8 ± 0.9
	Present 328 (32.8%)	4.7 ± 0.6	13.5 ± 1.7	40.1 ± 4.9	13.7 ± 0.1	21.4 ± 16.1	86.5 ± 4.6	29.3 ± 1.8	33.8 ± 0.1
	p-value	0.001	0.002	0.004	0.076	0.319	0.848	0.804	0.663
Retinopathy	Absent 794 (79.4%)	4.8 ± 0.5	13.8 ± 1.7	40.9 ± 4.7	13.6 ± 0.1	20.1 ± 15.8	86.4 ± 4.6	29.3 ± 1.9	33.8 ± 0. 1
	Present 206 (20.6%)	4.6 ± 0.5	13.5 ± 1.6	39.99 ± 4.7	13.5 ± 0.1	22.4 ± 15.9	86.8 ± 4.4	29.4 ± 1.8	33.9 ± 0.9
	p-value	< 0.001	0.020	0.012	0.439	0.092	0.277	0.257	0.209
Nephropathy	Absent 915 (91.5%)	4.8 ± 0.5	13.8 ± 1.6	40.9 ± 4.6	13.6 ± 0.1	20.3 ± 15.7	86.5 ± 4.6	29.3 ± 1.9	33.8 ± 0.1
	Present 85 (8.5%)	4.6 ± 0.6	13.3 ± 1.9	39.4 ± 5.7	13.7 ± 0.9	24.3 ± 16.7	86.0 ± 4.7	29.0 ± 1.8	33.8 ± 0.1
	p-value	0.015	0.010	0.022	0.367	0.050	0.414	0.248	0.905
Vasculopathy	Absent 879 (87.9%)	4.8 ± 0.5	13.8 ± 1.7	40.9 ± 4.7	13.6 ± 0.1	20.3 ± 15.7	86.6 ± 4.6	29.3 ± 1.9	33.8 ± 0.1
	Present 121 (12.1%)	4.7 ± 0.6	13.4 ± 1.7	39.8 ± 4.8	13.8 ± 0.1	23.0 ± 16.1	85.6 ± 4.6	29.0 ± 1.9	33.6 ± 0.1
	p-value	0.083	0.030	0.027	0.012	0.103	0.028	0.049	0.057

Registry (SNDR) in terms of important characteristics, which include mean age, duration of diabetes and BMI. In this work, we focused on the effect of hyperglycemia, hyperlipidemia and chronic diabetes complications on erythropoiesis. During erythropoiesis, RBCs structure or chemistry will be affected by hyperglycemic state among diabetic patients when compared with the normal subjects[9].

Red Blood Cells (RBCs) Count

Among diabetic patients, chronic hyperglycemia will lead to non-enzymatic glycation of RBC membrane proteins that would accelerate RBCs aging as a result of reduced negative surface electrical charge[10]. Our study has demonstrated an increase in RBCs count among older patients, especially men, although there was a significant reduction in RBCs count for those with diabetes duration of more than 10 years. This increase in RBCs count observed during hyperglycemia is similar to the observation of other studies and could be explained by the effect of insulin resistance, especially when it has been demonstrated that insulin regulates erythropoiesis *in vitro*[11]. The hyperinsulinemia effect on erythropoiesis has been well explained by different mechanisms, since the presence of insulin receptors in human erythropoietin cells during different developmental stages, suggests the role of insulin as a co-factor in erythropoiesis[12]. Polycythaemia observed in newborn babies of diabetic mothers give another clue for hyperinsulinemia's relationship with erythropoiesis[13]. The insulin resistance effect on erythropoiesis could explain the increase in RBCs count in older patients and men[14]. Smoking among the studied diabetic patients was associated with increased RBCs count as expected due to the effect of carboxyhemoglobin (HbCO), which would stimulate the production of RBCs as its hypoxic effect would eventually lead to the development of secondary polycythemia[15]. The association between increased RBCs counts with higher DBP and lipid parameters namely cholesterol, triglycerides and LDL, could also be explained by the insulin resistance effect, since such conditions are associated with metabolic syndrome[16], as reported by Hosseini et al[17]. The RBCs count in this study has shown to be reduced with microvascular complications, especially with longer diabetes duration and was also reported by other researchers[8]. The decreased RBCs count could be a consequence of erythropoietin deficiency observed among patients with diabetic nephropathy or as a result of the increased RBCs destruction, secondary to macroangiopathic or microangiopathic changes[18]. The decreased negative charge of RBCs among patients with hyperglycemia would also result in reduced average lifespan of RBCs or increased microviscosity and aggregation or adhesion of RBCs and consequently would reduce RBCs count[19].

Hemoglobin (Hb)

The heme part of hemoglobin is the protein that is subjected to glycation and is affected by the duration and level of hyperglycemia[20]. In this work, patients with anemia were excluded to eliminate the effect of other hematological factors that may affect the concentration of Hb in

Appendix II. Association between erythropiotic parameters and macrovascular and microvascular complications.

Parameters	Nephropathy 95% CI				Retinopathy 95% CI				Neuropathy 95% CI				Vasculopathy 95% CI			
	OR	Lower	Upper	*p*-value	OR	Lower	Upper	*p*-value	OR	Lower	Upper	*p*-value	OR	Lower	Upper	*p*-value
Hemoglobin g/dL																
1st quartile	1.27	0.57	2.83	0.563	1.53	0.92	2.56	0.103	1.46	0.96	2.24	0.078	0.97	0.47	2.03	0.945
2nd quartile	1.93	0.89	4.17	0.309	1.75	1.04	2.94	0.036	1.36	0.88	2.12	0.168	2.06	1.06	4.02	0.033
3rd quartile	1.26	0.56	2.85	0.580	1.34	0.79	2.28	0.275	1.12	0.72	1.74	0.605	1.87	0.96	3.64	0.064
4th quartile	1.00				1.00				1.00				1.00			
Hct %																
1st quartile	1.09	0.51	2.36	0.823	1.95	1.14	3.32	0.015	1.69	1.11	2.58	0.015	0.94	0.44	1.97	0.861
2nd quartile	1.12	0.52	2.43	0.770	1,90	1.11	3.25	0.020	0.86	0.55	1.34	0.499	1.77	0.91	3.47	0.093
3rd quartile	1.10	0.51	2.39	0.802	1.60	0.92	2.76	0.093	1.03	0.66	1.60	0.901	1.82	0.94	3.54	0.078
4th quartile	1.00				1.00				1.00				1.00			
RBC (10^{12}/L)																
1st quartile1.16	0.56	2.43	0.692	1.65	1.00	2.73	0.050	1.74	1.14	2.66	0.010	0.63	0.34	1.18	0.146	
2nd quartile	0.97	0.45	2.09	0.931	1.40	0.84	2.34	0.201	0.86	0.55	1.35	0.517	0.72	0.39	1.33	0.299
3rd quartile	0.86	0.39	1.88	0.704	0.99	0.58	1.70	0.985	0.96	0.62	1.49	0.850	0.55	0.29	1.05	0.069
4th quartile	1.00				1.00				1.00				1.00			
MCV (fL)																
1st quartile	1.55	0.73	3.32	0.257	0.69	0.43	1.13	0.140	0.95	0.62	1.46	0.827	1.65	0.88	3.10	0.119
2nd quartile	1.15	0.52	2.56	0.725	0.75	0.46	1.20	0.229	0.97	0.63	1.48	0.888	1.10	0.56	2.15	0.784
3rd quartile	1.29	0.59	2.84	0.522	0.57	0.34	0.94	0.028	0.80	0.52	1.24	0.312	0.89	0.44	1.81	0.756
4th quartile	1.00				1.00				1.00				1.00			
MCH (pg)																
1st quartile	1.30	0.63	2.67	0.480	0.86	0.56	1.34	0.516	0.86	0.56	1.34	0.516	1.34	0.71	2.55	0.370
2nd quartile	1.02	0.48	2.18	0.951	1.11	0.72	1.70	0.648	1.11	0.72	1.70	0.648	1.07	0.55	2.09	0.850
3rd quartile	0.75	0.33	1.70	0.492	1.06	0.69	1.64	0.787	1.06	0.69	1.64	0.787	0.97	0.49	1.93	0.933
4th quartile	1.00				1.00				1.00				1.00			
MCHC (g/dL)																
1st quartile	0.88	0.42	1.84	0.736	0.71	0.42	1.19	0.197	0.95	0.62	1.48	0.831	1.59	0.81	3.14	0.180
2nd quartile	1.03	0.51	2.06	0.940	0.81	0.49	1.33	0.399	1.00	0.65	1.53	0.991	1.36	0.69	2.69	0.375
3rd quartile	0.59	0.26	1.38	0.226	1.23	0.75	2.02	0.407	1.33	0.85	2.06	0.209	1.52	0.75	3.07	0.246
4th quartile	1.00				1.00				1.00				1.00			
RDW (%)																
1st quartile	1.00				1.00				1.00				1.00			
2nd quartile	1.05	0.46	2.40	0.907	1.06	0.65	1.72	0.812	1.07	0.69	1.65	0.770	0.99	0.49	1.98	0.973
3rd quartile	1.20	0.55	2.63	0.650	0.96	0.60	1.55	0.877	1.14	0.75	1.74	0.539	1.30	0.68	2.47	0.428
4th quartile	1.72	0.80	3.71	0.168	0.67	0.39	1.15	0.145	1.34	0.87	2.08	0.187	1.64	0.86	3.14	0.135
ESR (mm/h)																
1st quartile	1.00				1.00				1.00				1.00			
2nd quartile	0.34	0.10	1.07	0.065	1.07	0.62	1.85	0.809	0.81	0.52	1.26	0.350	0.71	0.34	1.52	0.381
3rd quartile	2.40	1.14	5.05	0.021	1.56	0.92	2.64	0.097	1.08	0.70	1.66	0.743	1.64	0.86	3.15	0.132
4th quartile	1.45	0.65	3.24	0.371	1.50	0.88	2.54	0.138	1.02	0.66	1.59	0.923	1.31	0.67	2.58	0.430

Table IV. Pearson correlation coefficient of each erythropbiotic parameters and glycemic control.

Characteristic	HbA1c		FBG		2 hours postprandial	
	r	p	r	p	r	p
RBC	0.21	< 0.001	0.25	< 0.001	0.22	< 0.001
HGB	0.14	< 0.001	0.24	< 0.001	0.22	< 0.001
HCT	0.15	< 0.001	0.23	< 0.001	0.23	< 0.001
ESR	0.01	0.791	-0.09	0.009	-0.03	0.370
RDW	-0.09	0.006	-0.21	< 0.001	-0.18	< 0.001
MCV	-0.06	0.054	0.00	0.970	0.07	0.053
MCH	-0.07	0.049	0.04	0.218	0.08	0.021
MCHC	-0.02	0.485	0.08	0.011	0.05	0.175

the presence of metabolic abnormalities, namely hyperglycemia and hyperlipidemia. Since Hb levels are directly correlated with RBCs count, all the factors as discussed above that would decrease or increase RBCs count, would reflect on Hb concentration. Patients with a higher BMI have shown lower Hb concentrations which could be related to the fact that obesity is characterized by a state of chronic inflammation with high circulating pro-inflammatory cytokines affecting the hematopoietic system, and may lead to the depletion of these cells[1]. Thus, we could suggest that obesity and the associated metabolic changes or inflammation may affect bone marrow niches through pathways that have not yet been investigated before. Hyperlipidemia, in this study, is associated with the increased hemoglobin mean concentration and is similar to what has been earlier reported by other researchers[17] thereby warranting further analysis. The presence of chronic complications in this work was associated with low Hb concentrations in non-anemic type 2 diabetic patients. In addition to the prevalence of chronic kidney disease among diabetic patients and its association with low erythropoietin production, this hormone could be affected by autonomic neuropathy that decreases sympathetic stimulation of erythropoietin production related to renal denervation[21]. Reduced androgens observed in diabetic patients is associated with decreased erythropoietin synthesis in the kidney. These factors could explain the reduced mean hemoglobin concentration in diabetic patients with chronic complications. Another explanation could well be related its resistance to erythropoietin as a result of the inflammatory process and due to the rise of cytokines that would suppress cell proliferation[21].

Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC)

It was observed that there was an insignificant increase of MCV with poor glycemic control, which could be further explained by the fact that membrane proteins can be non-enzymatically glycosylated displacing sodium and chloride ions in the immediate environment of the cells. Such displacement could explain the changes in cell size (increased MCV)[22]. The MCV, MCH and MCHC were reduced in obese subjects, which could be the result of hyposideremia that lowers the erythrocytes indices as a result of low-calorie diet, as practiced by these obese subjects[23]. The current work has also shown that vasculopathy had significantly decreased MCV and MCH and is similar to what has been observed among Taiwanese subjects[24].

Red Blood Cell Distribution Width (RDW)

Our observation correlates with the findings of other studies[3,19,25] that there is a significant negative correlation of RDW with poor glycemic control. This could result from the decreased lifespan of erythrocytes that reduces HbA1c concentration, as the average time of RBCs exposure to hyperglycemia is reduced. Chronic inflammatory process related to diabetes may influence erythropoiesis and would reduce RBCs half-life and deformability, thereby increasing RDW. This inflammatory theory could also explain the positive correlation between RDW and obesity[2,23,26]. Our work also shows a positive correlation between RDW and DBP, but not between RDW and SBP, which is similar in part to the finding of Tanindi et al[27], that can be ex-

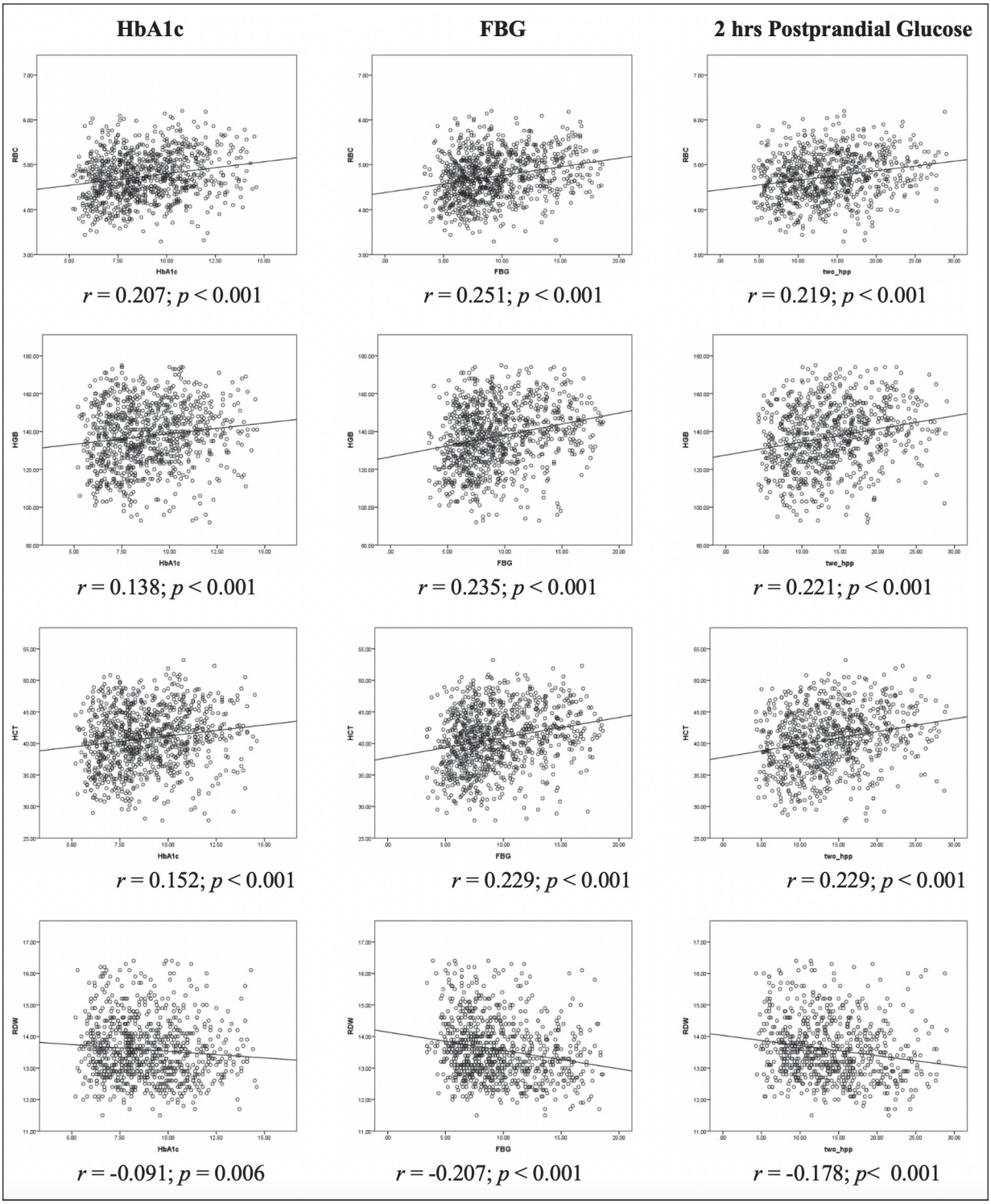

Figure 1. ***A*,** Pearson correlation of each erythropbiotic parameters and glycemic control.

Figure continued

plained on the basis of the inflammatory effect that might results in end-organ damage among patients with hypertension. The activation of the renin-angiotensin-aldosterone system could increase erythropoietin production *in vivo* and directly stimulate the proliferation of normal early erythroid progenitors. Additionally, increased sympathetic nervous system activation

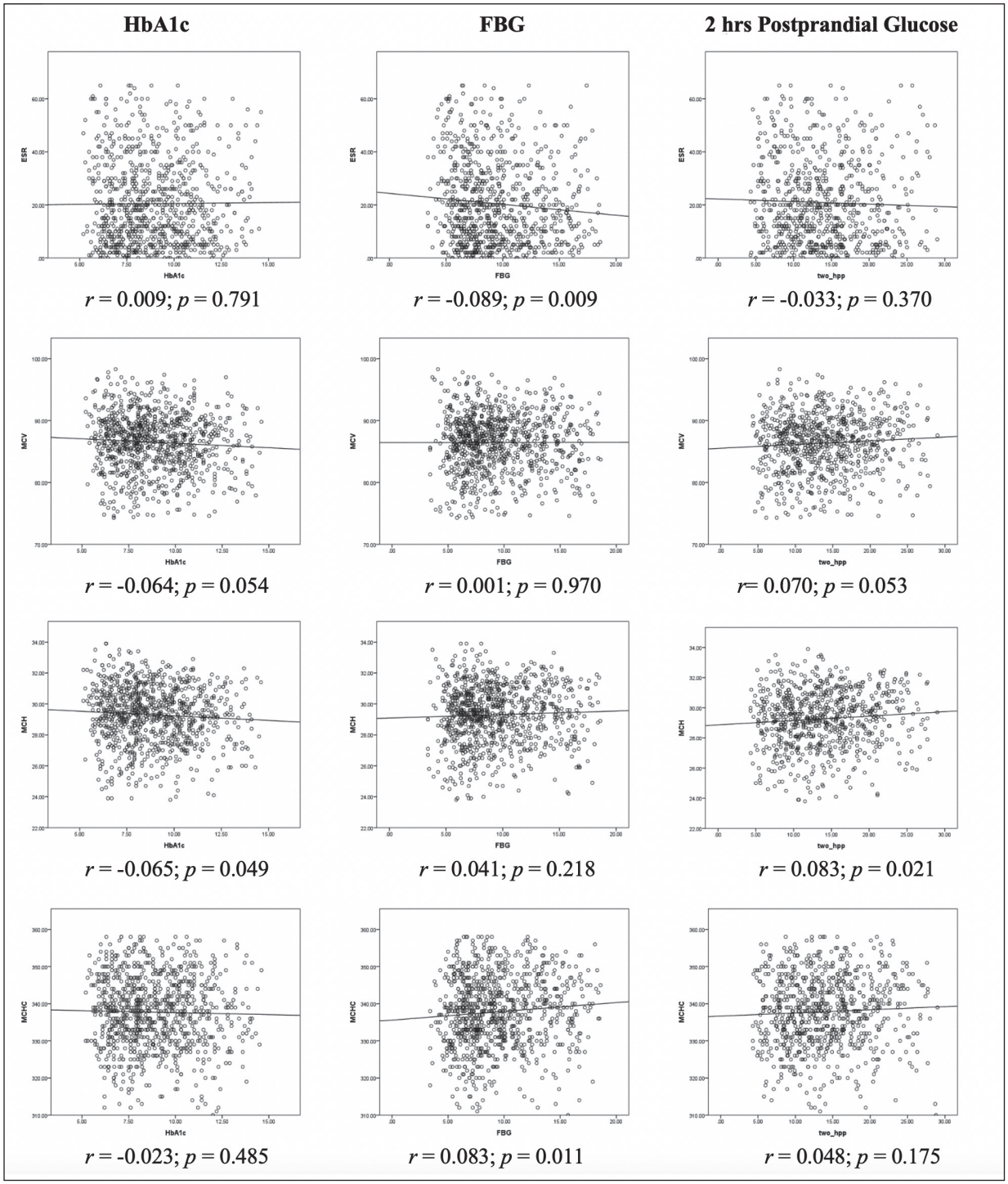

Figure 1 ***(Continued). B,*** Pearson correlation of each erythropbiotic parameters and glycemic control.

stimulates erythropoiesis in humans through increased erythropoietin production[27]. The correlation of RDW with vasculopathy, which is one of our observations, is further supported by the fact that vasculopathy is associated with a pro-inflammatory state and oxidative stress that impairs membrane fluidity of the erythrocytes and reduces RBCs lifespan. In addition to this, it blocks iron metabolism and erythropoietin response as a result of inflammation. Our work

is limited by being a cross-sectional study and therefore causality could not be established. However, the main aim of this paper was to determine an association rather than causality. Additionally, the results represent a single center study which might not allow us to generalize the results, but the main demographic characteristics were matching with a large countrywide cohort of the Saudi National diabetes registry. The primary strength of our work is the large sample size which increases the power of this study. The secondary strength of this paper is the exclusion of patients with anemia, to avoid any confounding effects on the association between the studied indices and metabolic parameters or chronic complications.

Conclusions

We showed that hyperglycemia affected RBCs production, its function and other physical properties. This may eventually affect the normal functional physiology of erythrocytes or have a direct effect on its vascular structure, leading to micro or microangiopathies. At the same time, the presence of diabetes chronic complications would affect RBCs life span, its cytoplasmic viscosity, and deformability. However, these changes are not fully understood and more studies are needed to evaluate the importance of such changes on the progression of diabetes complications, especially cardiovascular. The effect of hyperglycemia on RBCs shape and its function should also be considered among diabetic patients, especially when evaluating for their hematological diseases. The findings of the current study highlight the importance of strong glycemic control in improving the structure and function of RBCs and consequently, their indices. Additionally, diabetic patients with poor control should not be encouraged to donate blood.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Author Declaration

The authors certify that the manuscript represents valid work and neither this manuscript nor one with substantially similar content under named authorship has been published or is being considered for publication elsewhere.

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Ethical Approval

This study was reviewed and approved by the Institutional Review Board (IRB) at the College of Medicine, King Saud University. The data used in this publication were not consented since it does not compromise anonymity, confidentiality or breach of the local data protection laws. Additionally, all the collected investigations were part of the routine full assessment investigations for newly registered patients in the center, which did not require the patients' consents to be obtained..

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Authors' Contribution

BNA: Study design, data collection, manuscript preparation, AB: Study design, data collection, manuscript preparation. AAA: Study design, data collection, manuscript preparation. MA: Study design, data collection, manuscript preparation. MMA: Study design, data collection, manuscript preparation. DA: Study design, data collection. EA: Literature Search. NSM: Literature Search. OAA: Literature Search. KAR: Study design, data collection, manuscript preparation, Literature Search, Statistical Analysis, Data Interpretation.

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