Association of erythrocytes indices and interleukin-1 beta with metabolic syndrome components

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Abstract

Background: The relationship between hematological studies and cytokines are of great importance in metabolic syndrome (MetS) pathophysiology. The study aimed to illuminate the association between red blood cell (RBC) indices and interleukin-1-beta (IL-1β) with MetS components among adult Egyptian patients.

Methods: A total of 100 healthy subjects and 200 patients diagnosed with MetS components were enrolled in the study. MetS patients have at least three of the following MetS components: abdominal obesity, hypertriglyceridemia, low high-density lipoprotein levels, hypertension, and hyperglycemia. Eligible patients were classified into 4 groups of 50 patients each. Group 1 included patients with 2 MetS criteria, Group 2 patients met 3 MetS criteria, Group 3 patients had 4 MetS criteria, and Group 4 patients fulfilled all 5 MetS criteria. Patients in Groups 2 to 4 are considered MetS patients, while those in Group 1 are classified as being at risk of MetS.

Results: Among MetS patients, the data disclosed significantly ($p<0.001$) elevated RBC count and hematocrit percentage (HTC%) in Groups 2 and 3. However, higher hemoglobin (HB) content and elevated mean corpuscular volume values were recorded in Group 3 compared to healthy controls. Group 4 had noticeably lower RBC count and HB level compared to healthy controls. Moreover, values of red blood cell distribution width and IL-1β were significantly higher ($p<0.001$) in all MetS patient groups compared to the healthy group. The continuous MetS score showed a graded significant ($p<0.001$) elevation with the increase in MetS components. Concerning Group 3, RBC count recorded positive correlations with systolic blood pressure and IL-1β values, while red cell distribution width percentage (RDW%) was correlated with the homeostatic model assessment of insulin resistance (HOMA-IR) in Groups 3 and 4.

Conclusions: Erythrocyte profile, IL-1β, and continuous MetS score values were associated with the increase in MetS components. The study provides additional evidence to use hematological and cytokine biomarkers as well as continuous MetS score values in early identification of individuals at risk of MetS.

Background

In recent years, metabolic syndrome (MetS) has emerged as a global public health problem because of its increased prevalence around the world, affecting nearly 20%–30% of adults in many countries.[1] MetS patients have at least three of the following MetS components: abdominal obesity, hyper-triglyceridemia, low HDL-cholesterol (HDL-C) levels, hypertension, and hyperglycemia.[2] Unfortunately, MetS is associated with an increased risk of type 2 diabetes (T2DM) and cardiovascular disease (CVD).[3] Currently, the pathophysiology of MetS is not understood clearly. Generally, MetS is correlated with insulin resistance and/or chronic low-grade inflammation.[4] Erythrocyte or red blood cell (RBC) indices are individual components of a routine blood test used to measure the quantity and physical characteristics of different types of red blood cells in blood. Moreover, several studies have associated erythrocyte indices and oxidative stress with inflammatory status.[5] Nebeck et al. concluded that hemoglobin (HB), hematocrit (HTC), and RBCs were correlated with MetS components in both men and women.[6] Furthermore, Huang et al. hypothesized that the association of erythrocyte parameters with MetS components may be indicative of the development of insulin resistance. The authors also added that the pathogenesis of insulin resistance may, in part, be causative of the association between RBC values and MetS due to the role of insulin in the proliferation and differentiation of erythropoietic cells.[7]

Erythrocyte morphology is of great importance in the field of hemorheology as the deformability of the circulating cells has a fundamental influence on the rheological properties of the blood.[8] Adipocytokines generated in MetS alter erythrocyte morphology, decrease erythrocyte deformability, and increase whole blood viscosity (WBV). Increased WBV has been attributed to erythrocyte aggregation which, in turn, is greatly influenced by other rheological parameters, including its membrane surface charge.[9] Also, WBV is dependent on the number (and volume) of erythrocytes in the blood and is thus linearly related to HTC%. Furthermore, elevated HTC levels promote increased WBV, thus dynamically altering the blood's rheological parameters.[10] Unfortunately, alterations of erythrocyte morphology could have a negative impact on the circulation and gaseous exchange in the small blood vessels that

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lead to several complications.[11] Additionally, morphologically-altered erythrocyte and erythrocyte aggregates may interact with the existing endothelium inflammation. Increased erythrocyte aggregation has been associated with MetS cardiovascular complications.[12] Furthermore, insulin resistance and obesity have been suggested as the main factors behind altered rheology in MetS.[13,14] Hence, the increase in erythrocyte aggregation in MetS may be due to the alterations in the erythrocytes' intrinsic properties. Red blood cell distribution width (RDW) represents a measure of heterogeneity in the size of circulating erythrocytes and maybe an exceptional inflammatory biomarker.[15] Several studies have investigated the hematological profile of MetS patients in different parts of the world: a) RBC, HB, HTC, and platelet counts among working adults in Ethiopia,[6] b) HB, HTC, platelet counts among professional- and office-working women in Bangkok, Thailand,[16] and c) RBCs, HTC, HB and RDW, stratified by sex, in the Pearl River Delta region of China.[7]

MetS is an inflammatory state described clinically by the presence of several metabolic disturbances. Chronic inflammation is known to be associated with obesity and insulin resistance which is characterized by production a higher level of serum tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL)-1β and IL-6, all of which are produced by macrophages derived from adipose tissue.[17] IL-1β is a key regulator of the body's inflammatory response and plays a role in various diseases, including autoimmune diseases and MetS-associated diseases, such as atherosclerosis, chronic heart failure, and T2DM.[18] In this regard, IL-1β is capable of impairing insulin signaling and action as well as participating in the development of insulin resistance. *In vitro* studies have also revealed the pathogenic role of IL-1β in the development of insulin resistance and indicate that IL-1β signaling inhibition enhances insulin sensitivity.[19] Indeed, IL-1β treatment of adipocytes disturbs insulin signaling via down-regulation of insulin receptor substrate-1 expression, leading to a marked reduction of insulin-mediated GLUT-4 translocation. To date, no attention has been paid to the association of IL-1β level with the increase in MetS components.[20]

The aforementioned studies indicate the need for further investigations on hematological profiles in different world populations, assessing the relationship of proinflammatory cytokines and MetS components. To the best of our knowledge, this study is the first investigation on the population of the Middle East and North Africa (MENA) region, particularly in Egypt, on the association of increasing MetS components with changes in erythrocyte indices and IL-1β. Therefore, the study aims to explore the association between RBCs profile and IL-1β with the increase in MetS components among adult Egyptian patients.

Material and Methods

Study population

Our survey included 200 patients diagnosed with MetS components who visited the outpatient laboratory of the Department of Endocrinology and Metabolism, National Nutrition Institute (NNI) in Cairo, Egypt, from July 2016 to March 2018.[21] The study population included both urban-dwelling (40%) and rural-dwelling patients (60%), and individuals who visited the outpatient laboratory were randomized. The data was collected using a paper questionnaire and clinical lab reports. Participants were interviewed by doctors to answer the questionnaire data. The paper questionnaire contained questions on the patient's history and demographic data, while the clinical laboratory report contained the results of blood and serum analysis. The study protocol was performed in compliance with the Declaration of Helsinki and good clinical practice guidelines. Written informed consent was obtained from each patient after the study protocol was approved by the ethical committee of the National Nutrition Institute (Ref: NNI/FS/16/4).

The patients who had MetS were diagnosed according to WHO (1999) and NCEP ATP III guidelines.[22] The individual was classified as having MetS if he/she fulfilled 3 of the following 5 criteria: a) waist circumference >102 cm in males and >88 cm in females and/or obesity (body mass index >30 kg/m^2), b) serum triglycerides >150 mg/dL (1.7mmol/L) or patients receiving treatment for hypertriglyceridemia, c) serum high-density lipoprotein cholesterol (HDL-C) <40 mg/dL (1.03 mmol/L) in men or <50 mg/dL (1.29 mmol/L) in women or a previously treated dyslipidemia, d) arterial blood pressure >130/85 mmHg in two different determinations or if the patients were receiving treatment with drugs, and e) fasting glucose ≥110 mg/dL. However, key exclusion criteria include severe illnesses such as hematological disorders, infectious diseases, autoimmune disorders, thyroid dysfunction, and allergies, as well as chronic cardiovascular, respiratory, kidney, and liver diseases.

Study design

Eligible MetS 200 patients aged 20-70 years were classified into 4 groups of 50 patients according to the number of MetS criteria as mentioned in our previous study.[21] Group 1 consisted of patients with hypertriglyceridemia and low HDL (dyslipidemia), Group 2 of patients with dyslipidemia plus obesity, Group 3 of patients with dyslipidemia, obesity, and hypertension. Group 4 was comprised of patients with dyslipidemia, obesity, hypertension, and hyperglycemia.

In addition to the four patient groups, a healthy control group of 100 subjects was added to the study. The patients in Groups 2 to 4 (fulfilling 3 or more MetS criteria) are typical MetS patients.[22]

Laboratory assays

Serum cholesterol, HDL-C, triglycerides, and fasting blood glucose concentrations were estimated using reagent kits purchased from Reactivos Spinrect, Spain. Glycated hemoglobin (HbA1c) levels were determined using reagent kits purchased from Spectrum. Insulin concentration was determined using reagent kits purchased from Biovendor. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the formula: [fasting insulin (U/L) x fasting glucose (mg/dL)]/405. The methodology of the continuous MetS score (cMetS score) measurement was previously reported in detail.[23] Serum IL-1β concentration was estimated using the Human IL-1β PicoKine™ ELISA Kit purchased from MyBio-source according to the instructions provided. However, red blood cell indices, RBC count, HB, HTC, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC), and RDW values were determined using the MICROS ABX auto-analyzer according to the manufacturer's protocol.

Statistical analysis

The collected data was entered into a protected Excel sheet and exported to our statistical software. The experimental results were expressed as mean (M) ± standard deviation (SD) and subjected to One-Way Analysis of Variance (ANOVA), using a computer software package (Statistical Package for Social Sciences (SPSS) version 20, IBM Corp., 2011), followed by Duncan's Multiple Range Test (DMRT) to determine the significant differences between groups. A simple linear correlation analysis was processed by Pearson's method to measure the degree of dependency between variables. The results were adjusted for confounding factors using multivariate analysis. Multivariable analysis was employed to calculate odd ratios (OR) of potential risk factors associated with MetS. Values of $p<0.05$ were considered statistically significant.

Results

The group of all patients with metabolic syndrome components (2 to 5 criteria) showed significantly ($p<0.001$) higher body mass index (BMI) and systolic and diastolic blood pressure compared with healthy controls. Also, serum triglyceride concentrations and the atherogenic index of patients with MetS components were significantly ($p<0.001$) elevated, while serum HDL-C was significantly ($p<0.001$) lowered compared to healthy controls. In addition, both HbA1c and HOMA-IR values were noticeably elevated in overall patients with MetS compared to healthy controls as shown in Table 1.

Table 1. Demographic and metabolic syndrome-related characteristics of participants.

Group Characteristic	Healthy group N = 100	Patients group N = 200	p-values
Age (years)	32.68 ± 7.76	46.94 ± 8.78***	<0.001
Female/Male	36/64	107/93	0.027
BMI (Kg/m²)	25.35 ± 3.39	33.25 ± 5.97***	<0.001
SBP (mmHg)	116.80 ± 6.61	132.88 ± 15.94***	<0.001
DBP (mmHg)	75.70 ± 5.63	83.25 ± 10.95***	<0.001
TG (mg/dl)	107.38 ± 24.31	203.47 ± 83.99***	<0.001
HDL-C (mg/dl)	47.98 ± 6.81	38.28 ± 7.88***	<0.001
Atherogenic Index	2.09 ± 0.50	4.90 ± 2.40***	<0.001
HbA1c %	5.42 ± 0.47	6.21 ± 1.33***	<0.001
HOMA-IR	2.47 ± 0.37	3.37 ± 1.21***	<0.001

*Significance compared healthy group, ***p < 0.001. Values significantly different from control at (P<0.05). Data are expressed as mean ± SD.
BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, TG = triglycerides, HDL-C = high-density lipoprotein-cholesterol, HbA1c = glycosylated hemoglobin, HOMA-IR = homeostatic model assessment for insulin resistance.

Current data observed that RBC count and HTC% were elevated significantly ($p<0.001$) in both Groups 2 and 3. However, in Group 4, RBC count and HTC% were significantly lowered compared to healthy controls and other MetS groups. The recorded values of HB and MCV showed a non-significant change in Groups 1 and 2, but there was a significant ($p<0.001$) elevation in Group 3 only. Furthermore, HB and MCV values were markedly lower in Group 4 compared to the healthy group. Among MetS components groups, the results indicated that MCH values were lower in all MetS groups compared to healthy controls. RDW% was significantly

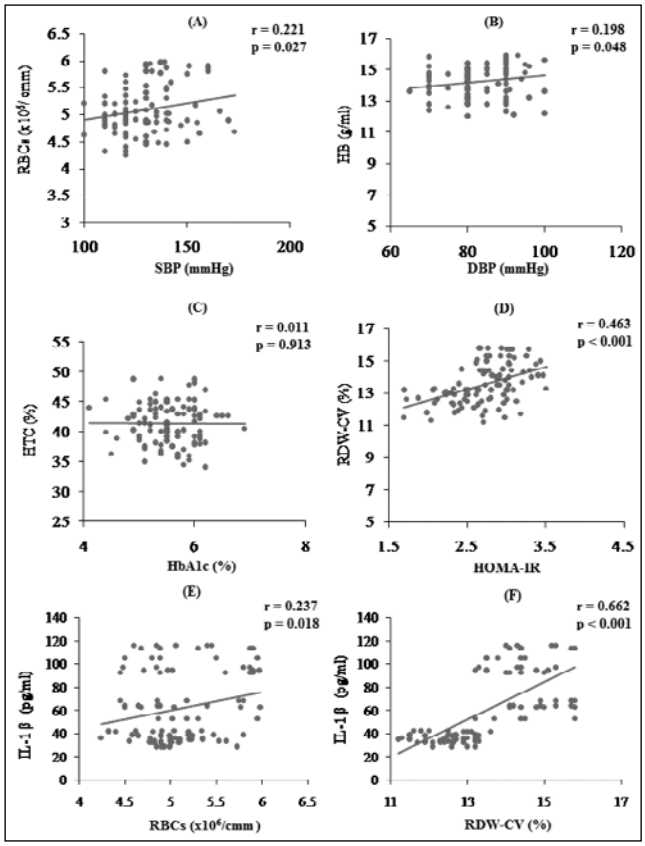

Figure 1. The correlations between RBC count with SBP (A), HB content with DBP (B), HTC% with HbA1c% (C), RDW% with HOMA-IR (D), and IL-1β level with RBC count (E) and RDW% (F) in Group 3.
RBC = red blood cell, SBP = systolic blood pressure, DBP = diastolic blood pressure, HB = hemoglobin, HTC = hematocrit, HbA1c = glycosylated hemoglobin, RDW = red cell distribution width, HOMA-IR = homeostatic model assessment for insulin resistance, IL-1β = interleukin-1-beta.

higher ($p<0.001$) in patients with different MetS components compared to the healthy group. Moreover, our data showed that IL-1β concentration was significantly ($p<0.001$) higher in all groups of MetS components (2 to 5 criteria) compared to healthy controls. Additionally, cMetS score exhibited a significant ($p<0.001$) elevation with the increase in MetS components (Table 2).

The multivariate analysis revealed statistically significant associations between age, BMI, and triglyceride values with MetS components [OR (95% CI): 1.268 (1.059–1.518), 1.713 (1.147–2.558), and 1.051 (1.009–1.095), respectively; p=0.05, 0.01, and 0.05, respectively]. Systolic and diastolic blood pressure as well as HDL values showed a non-significant association (Table 3). In addition, RBC count, HB, HTC, MCH, and RDW values were associated with MetS components [OR (95% CI): 0.634 (0.522–0.773), 1.027 (1.001–1.054), 1.105 (1.002–1.017), 0.980 (0.965–0.996), 1.075 (1.041–1.108), respectively; $p<0.001$ for RBC and RDW, $p<0.01$ for HTC, and $p<0.05$ for HB for MCH]. Furthermore, IL-1β and cMetS scores revealed a marked association with MetS components [OR (95% CI): 1.009 (1.008–1.011), 1.234 (1.188–1.280), respectively; $p<0.001$ and $p<0.05$, respectively] as shown in Table 3.

Table 2. Red blood cell indices, continuous metabolic syndrome score and interleukin-1β of healthy controls and metabolic syndrome criteria groups.

Groups	Healthy controls No criteria N = 100	Group 1 2 criteria N = 50	Group 2 3 criteria N = 50	Group 3 4 criteria N = 50	Group 4 5 criteria N = 50	p-values
RBCs (x10^6/mm)	4.97 ± 0.34^b	5.13 ± 0.51^{bc}	5.18 ± 0.47^c	5.21 ± 0.52^c	4.41 ± 0.47 ^{a}	<0.001
HB (g/mL)	13.98 ± 0.72^b	14.30 ± 0.80^{bc}	14.41 ± 1.25^c	14.46 ± 1.28^c	13.04 ± 1.02^a	<0.001
HTC (%)	40.37 ± 3.17^{ab}	41.87 ± 5.00^{bc}	42.35 ± 4.58^c	42.42 ± 3.45^c	39.05 ± 3.67^a	<0.001
MCV (Fl)	84.36 ± 3.53^{ab}	85.09 ± 3.94^{bc}	85.48 ± 4.18^{bc}	86.55 ± 5.79^c	83.27 ± 5.24^a	0.008
MCH (pg)	28.60 ± 0.83^b	27.39 ± 3.11^a	27.50 ± 2.34^a	27.53 ± 3.07^a	27.45 ± 2.20^a	0.068
MCHC (g/dL)	33.20 ± 0.90^a	32.88 ± 2.01^a	32.78 ± 2.07^a	33.38 ± 1.95^a	33.24 ± 1.30^a	0.352
RDW (%)	12.56 ± 0.60^a	13.59 ± 1.02^b	14.47 ± 0.90^c	14.53 ± 0.77^c	14.38 ± 1.00^c	<0.001
cMetS score	-0.245 ± 0.30^a	0.680 ± 0.52^b	0.968 ± 0.35^c	1.115 ± 0.424^c	2.592 ± 1.105^d	<0.001
IL-1β (pg/mL)	36.16 ± 4.07^a	71.70 ± 5.46^b	93.48 ± 14.54^d	86.88 ± 21.94^c	111.93 ± 8.53^e	<0.001

Values that share the same superscript symbol are not significantly different. Values significantly different from control at p<0.05. Data are expressed as mean ±SD.
RBC = red blood cell, HB = hemoglobin, HTC = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin contents, RDW = red cell distribution width, cMetS score = continuous metabolic syndrome score, IL-1β = interleukin-1-beta.

Table 3. Odds ratio (OR) and 95% Confidence interval (CI) for demographic, biochemical, hematological and IL-1β with metabolic syndrome.

Group	Answered correctly	Answered incorrectly
Age (years)	1.268 (1.059 – 1.518)	<0.050
Gender (M/F)	0.199 (0.015 – 2.575)	0.217
BMI (kg/m^2)	1.713 (1.147 – 2.558)	<0.010
SBP (mmHg)	1.159 (0.978 – 1.373)	0.088
DBP (mmHg)	1.072 (0.854 – 1.347)	0.549
HDL (mg/mL)	1.125 (0.929 – 1.364)	0.228
TG (mg/mL)	1.051 (1.009 – 1.095)	<0.050
RBCs (x10^6/mm)	0.634 (0.522 - 0.773)	< 0.001
HB (g/mL)	1.027 (1.001 – 1.054)	<0.050
HTC (%)	1.105 (1.002 – 1.017)	<0.010
MCV (Fl)	1.005 (0.998 – 1.011)	0.178
MCH (pg)	0.980 (0.965 – 0.996)	<0.050
MCHC (g/dL)	1.006 (0.985 – 1.028)	0.580
RDW (%)	1.075 (1.041 – 1.108)	< 0.001
IL-1β (pg/ml)	1.009 (1.008 – 1.011)	< 0.001
cMetS score	1.234 (1.188 – 1.280)	<0.050

95% CI = 95% confidence interval, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, TG = triglycerides, HDL-C = high-density lipoprotein-cholesterol, RBC = red blood cell, HB = hemoglobin, HTC = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin contents, RDW = red cell distribution width, IL-1β = interleukin-1-beta, cMetS score = continuous metabolic syndrome score.

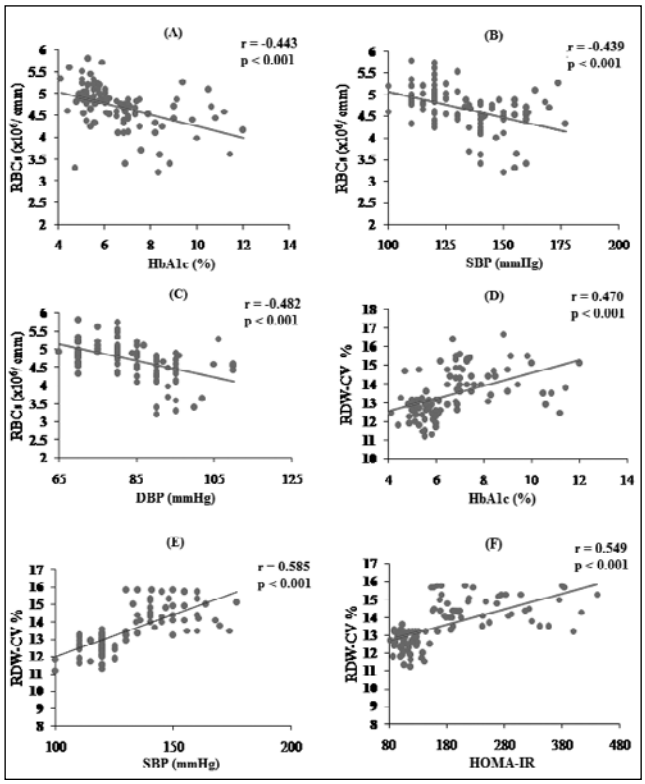

Figure 2. The correlations between RBC count with HbA1c% (A), SBP (B), DBP (C), and RDW% with HbA1c% (D), SBP (E), and HOMA-IR (F) in Group 4. RBC = red blood cell, HbA1c = glycosylated hemoglobin, SBP = systolic blood pressure, DBP = diastolic blood pressure, RDW = red cell distribution width, HOMA-IR = homeostatic model assessment for insulin resistance.

Regarding MetS patients, we examined the results from the 2 groups fulfilling the highest levels of MetS criteria. We included Groups 3 (4 criteria) and 4 (5 criteria) in the current study and results of the other groups were provided as supplementary data. In Group 3 (4 criteria), the present results revealed a positive correlation between RBC count and systolic blood pressure (r=0.221; p<0.05), while HB concentration revealed a positive correlation with diastolic blood pressure (r=0.198; p< 0.05). Furthermore, RDW% was positively correlated with HOMA-IR (r= 0.463; p<0.001), while HTC% showed a non-significant correlation with HbA1c (r=-0.011; p= 0.913). Additionally, our study showed a positive correlation between IL-1β concentration and RBC count (r=0.237; p<0.05) and RDW% (r=0.662; p<0.001) in Group 3 as shown in Figure 1. Among MetS patients in Group 4, RBC count showed a negative correlation with HbA1c (r=-0.443; p<0.001), systolic blood pressure (r=-0.439; p<0.001), and diastolic blood pressure (r=-0.482; p<0.001). The result also indicated a positive correlation between RDW% and HbA1c% (r=0.470; p<0.001), systolic blood pressure (r= 0.585; p<0.001), and HOMA-IR (r= 0.549; p<0.001) (Figure 2).

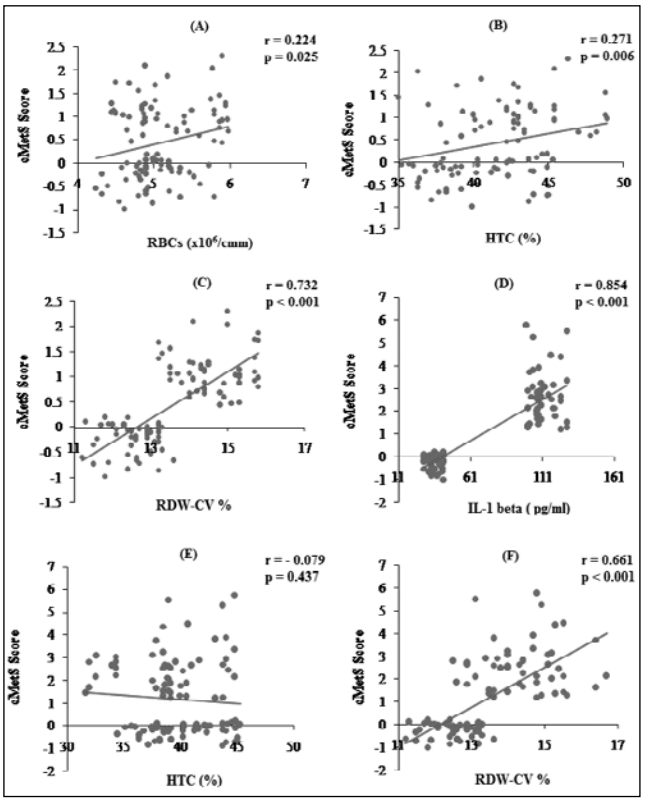

Figure 3. Correlations between cMetS score with RBC count (A), HCT% (B) and RDW% (C) in Group 3, and with IL-1β level (D), HCT% (E), and RDW% (F) in Group 4.
cMetS score = continuous metabolic syndrome score, RBC = red blood cell, HTC = hematocrit, RDW = red cell distribution width, IL-1β = interleukin-1-beta.

In Group 3, the cMetS score revealed significant positive correlations with RBC count (r= 0.224; p<0.05), HCT% (r=0.271; p<0.01), and RDW% (r= 0.732; p<0.001) (Figure 3). In Group 4, the cMetS score exhibited positive correlations with IL-1β (r=0.854; p<0.001) and RDW% (r=0.661; p<0.001), and a negative correlation with HCT % (r= 0.079; p<0.437) as illustrated in Figure 3.

Discussion

Our data revealed non-significant changes in RBC count, HTC%, and HB concentration among patients in Group 1 and a significant elevation (p< 0.001) in Groups 2 and 3. RBC count and HB concentration were lowered significantly in Group 4 compared to healthy controls. Huang et al. concluded that RBC count, HTC%, and HB levels were linearly associated with the number of MetS components from 0 to 5, identified in both men and women.[7] In fact, previous studies demonstrated that erythrocyte indices, RBC count, HB, HTC, and RDW values increased with the number of MetS components, which reflects our outcomes (1 to 4 criteria).[6,7,16] It is worthwhile to note that MetS is a condition of oxidative stress and chronic low-grade inflammation, which are contributing to the development of MetS and altered hemorheology in MetS patients.[24,25] Free radicals are increased in MetS that decrease deformability and change the mechanical characteristics of erythrocytes, which could be affected in other hemorheological parameters.[11] Moreover, a high RBC count and HTC have been hypothesized to contribute to insulin resistance through rheological alterations and impaired tissue blood flow, and RBC count, HTC, and HB values have been reported to be positively correlated with insulin resistance.[26] Additionally, increased levels of RBCs and HTC% can lead to reduced blood flow, via increased WBV, and subsequently diminish the flow of oxygen, insulin, and glucose to fundamental tissues.[27] The fundamental pathophysiology is apparently inseparable from blood as there is always the propensity for erythrocytes to be involved in cellular oxidative stress function, subsequently making them a common cellular factor in all components of MetS.[9]

Regarding RDW%, recent studies reported that it is a potential metabolic marker to recognize metabolic diseases.[28] Our data showed that RDW% values in patients with MetS components were higher (p<0.001) than healthy controls. Furthermore, our results indicated a positive correlation between RDW% and HOMA-IR, systolic blood pressure, and HbA1c% in Group 4. In addition, RDW% was markedly associated with MetS components [OR (95% CI): 1.075 (1.041–1.108), p<0.001]. To date, the mechanism for the relationship between RDW% and MetS remains obscure; however, chronic inflammation linked to RDW% may play a vital role as MetS has been associated with chronic inflammation and RDW% reflects an underlying inflammatory state.[4,15] Moreover, systemic inflammation can cause bone marrow dysfunction leading to the release of immature erythrocytes and subsequent anisocytosis which influence the level of RDW%.[29]

In the concept of MetS components, dyslipidemia refers to high triglycerides and/or low HDL-C. Hypertriglyceridemia is cytotoxic to erythrocytes via inducing morphological changes and consequential decreased deformability, membrane fluidity, and increased osmotic fragility. In addition, morphologic changes in erythrocytes may occur due to the toxic effect of free fatty acids and oxidative stress in obese subjects.[30] Guiraudou et al. concluded that overall adiposity evaluated with the body mass index (BMI) was associated with increased plasma viscosity and red cell rigidity, while abdominal fat increases blood viscosity due to a rise in hematocrit level.[31] Also, RDW% is elevated in overweight patients and reflects an inflammatory state.[32] Moreover, hypertension and dyslipidemia are coexisting synergizing risk factors for cardiovascular diseases. Some hematological parameters, such as RBC count are also elevated as the severity of hypertension is increased.[33] In parallel with our results, Shimizu et al. revealed that three erythrocyte parameters (RBC, HB, and HTC) were observed to be related to hypertension.[34] Our findings provide evidence in support of using some hematological biomarkers clinically for the early detection of individuals at risk for cardiovascular disease.

MetS increases the risks of diabetes with the association of insulin resistance.[35] Considering that reality, the levels of RBC, HTC, and HB are significantly associated with insulin resistance, which is compatible with our study.[36] The pathogenesis of insulin resistance may, in part, cause the association between RBC count and MetS. He et al. suggest that anemia in Chinese patients with T2DM was related to both micro- and macrovascular complications but was only an independent risk factor of microvascular complications.[37] The present data revealed that RBC count, HB, and HTC levels were significantly lower in Group 4 (which included hyperglycemic patients) compared to healthy controls. To clarify this finding, the

mechanisms of anemia in diabetic patients are multifactorial and often not very well understood. In diabetic patients, the lifespan of RBCs may be affected by various disturbances in the hematopoietic microenvironment such as chronic hyperglycemia (glucose toxicity), hyperosmolarity, and advanced glycation end-products (AGEs).[38] The manifestation of anemia in diabetics has been attributed to the increase of non-enzymatic glycosylation of RBC membrane proteins, which was correlated with hyperglycemia.[39] Some recent epidemiological studies have also reported that diabetes is characterized by increased erythrocyte osmotic fragility.[40] Furthermore, oral antidiabetic drugs (mainly metformin) and renal nephropathy are the major causes of HB level decrease in diabetic patients.[5] Metformin disturbs the absorption of vitamin B12 and serum vitamin B12 levels are conversely associated with the dose and term of metformin treatment.[41] The current study recommended the need for follow up of individuals receiving daily high dose metformin using regular hematological biomarkers.

Additionally, our study showed that the level of IL-1β, a pro-inflammatory cytokine, was significantly elevated in the different groups of MetS (2 to 5 criteria) compared with healthy controls. Moreover, our study showed a positive correlation between IL-1β and RBC count and RDW% in Group 3. Also, IL-1β levels revealed a marked association with MetS components [OR (95%CI): 1.009 (1.008 – 1.011), p<0.001]. These data are supported by recent studies that have shown a relationship between IL-1β and MetS and increased production of IL-1β has been linked to various

Table S1. Correlations between HOMA-IR, systolic and diastolic blood pressure, HbA1c and Interleukin-1β with RBCs indices in all metabolic syndrome patients.

Variables	HOMA-IR	Systolic	Diastolic	HbAc1	IL-1β
			r (p value)		
RBC	-0.342***(<0.001)	-0.227***(<0.001)	-0.200**(0.001)	-0.362***(<0.001)	-0.167**(0.008)
HB	-0.236***(<0.001)	-0.148* (0.019)	-0.169**(0.007)	-0.299***(<0.001)	-0.114 (0.073)
HTC	-0.116 (0.068)	-0.078 (0.216)	-0.038 (0.552)	-0.235***(<0.001)	0.005 (0.938)
MCV	-0.198**(0.002)	-0.009 (0.884)	-0.006 (0.926)	-0.218** (0.001)	0.005 (0.935)
MCH	-0.107 (0.093)	-0.102 (0.107)	-0.076 (0.229)	-0.169** (0.008)	-0.062 (0.327)
MCHC	0.006 (0.929)	0.072 (0.255)	0.116 (0.066)	0.039 (0.537)	0.046 (0.470)
RDW-CV	0.208**(0.001)	0.307***(<0.001)	0.250***(<0.001)	0.157*(0.013)	0.537***(<0.001)

HbA1c = glycosylated hemoglobin, HOMA-IR = homeostatic model assessment for insulin resistance, RBC = red blood cell, HB = hemoglobin, HTC = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin contents, RDW = red cell distribution width, IL-1β = interleukin-1beta. ***Correlation is significant at the 0.001 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed).

Table S2. Correlations between HOMA-IR, systolic and diastolic blood pressure, HbA1c and Interleukin-1β with RBCs indices in Group 1.

Variables	HOMA-IR	Systolic	Diastolic	HbAc1	IL-1β
			r (p value)		
RBC	-0.023 (0.824)	0.168 (0.096)	0.197* (0.049)	0.084 (0.409)	0.139 (0.167)
HB	0.027 (0.790)	0.087 (0.390)	0.012 (0.906)	0.128 (0.204)	0.172 (0.087)
HTC	0.015 (0.881)	0.151 (0.134)	0.192 (0.056)	-0.076 (0.452)	0.164 (0.102)
MCV	0.004 (0.970)	-0.046 (0.650)	0.103 (0.309)	-0.088 (0.383)	0.108 (0.285)
MCH	-0.170 (0.091)	-0.191 (0.057)	-0.007 (0.947)	-0.190 (0.058)	-0.237* (0.018)
MCHC	-0.036 (0.725)	-0.164 (0.104)	-0.033 (0.746)	-0.154 (0.126)	-0.060 (0.554)
RDW-CV	0.129 (0.202)	0.230* (0.021)	0.009 (0.932)	0.017 (0.866)	0.508***(<0.001)

***Correlation is significant at the 0.001 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Table S3. Correlations between HOMA-IR, systolic and diastolic blood pressure, HbA1c and Interleukin-1β with RBCs indices in Group 2.

Variables	HOMA-IR	Systolic	Diastolic	HbAc1	IL-1β
			r (p value)		
RBC	0.153 (0.130)	-0.068 (0.499)	0.061 (0.548)	0.028 (0.783)	0.277**(0.005)
HB	0.070 (0.492)	0.153 (0.128)	-0.077 (0.445)	0.075 (0.460)	0.201* (0.045)
HTC	0.152 (0.132)	-0.153 (0.130)	0.058 (0.564)	0.077 (0.445)	0.257**(0.010)
MCV	0.108 (0.284)	-0.055 (0.587)	0.016 (0.874)	0.039 (0.701)	0.212* (0.034)
MCH	-0.164 (0.104)	-0.060 (0.554)	0.019 (0.854)	-0.139 (0.169)	-0.179 (0.074)
MCHC	-0.188 (0.061)	0.145 (0.146)	0.063 (0.534)	-0.128 (0.206)	-0.083 (0.412)
RDW-CV	0.443***(<0.001)	0.147 (0.145)	- 0.051 (0.612)	0.197* (0.049)	0.764***(<0.001)

***Correlation is significant at the 0.001 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

autoimmune and auto-inflammatory diseases in addition to metabolic abnormalities.[19,42] IL-1β levels increase in the presence of obesity and its production by adipose tissue could alter insulin signaling, leading to increased inflammation and insulin resistance.[43] Expression of both IL-1β and its receptor increases in visceral adipose tissue of obese subjects.[40] Pro-oxidants and adipocytokines generated in MetS impaired erythrocyte morphology and erythrocyte deformability. In addition, the elevation of pro-inflammatory cytokines induces diabetic nephropathy and anemia, which triggers suppression of renal erythropoietin production and erythropoiesis. This in turn decreases the number of erythrocytes and consequently reduces the circulating HB level.[44]

cMetS is a more robust measure of MetS and predicted MetS with moderate to high accuracy. As a continuous variable, cMetS has greater statistical power, is more sensitive, and is less error-prone compared with categorical measures of MetS.[45] Our findings documented a graded relationship between cMetS and the number of MetS components. The cMetS score also revealed positive correlations with RBC count, HCT %, RDW%, and IL-1β levels. Additionally, cMetS scores revealed a marked association with MetS components [OR (95%CI): 1.234 (1.188 – 1.280), $p<0.05$]. The cMetS scores exhibited high sensitivity and specificity in predicting MetS with these hematological and inflammatory biomarkers.

Study strengths and limitations

The study's strength is related to a pioneer investigation on the Middle Eastern population about the relation between MetS components, cMetS score, inflammation status, and erythrocyte profiles. Our study has several limitations. First, the sample size of the 4 groups could be increased in future studies, while continuing to highlight important confounders such as age, sex, occupation, medications, physical activity, GFR data, and smoking status. Second, it was a cross-sectional study, so a causal relationship cannot be pinpointed. Finally, further prospective studies in a large cohort are necessary to better explore the causality question, including detailed food recall, vitamin B12 and folate levels, and another pro-inflammatory level of cytokines that could influence hematological parameters or metabolism in MetS patients.

Conclusions

The current study indicated an association between erythrocyte indices, IL-1β, and cMetS scores, and an increasing number of MetS components. Furthermore, the results provided additional evidence that RBC indices are particularly sensitive to changes in cytokine levels and inflammation status. The study also confirmed the previous clinical data for using hematological markers and the cMetS score in the early detection of individuals at risk for MetS over time.

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