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Original Article

Correlation between oxidative stress and hematological profile abnormalities in diabetic nephropathy

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ABSTRACT

Aims: Diabetes patients with renal impairment commonly have a degree of hematological abnormalities than those non-diabetics with chronic kidney disease. The present study aimed to clarify the association between oxidative stress and hematological abnormalities with the progression of diabetic nephropathy.
Methods: A total of 20 healthy subjects and 100 patients were enrolled in the study. Eligible renal dysfunction patients were classified according to biochemical markers into five groups (20 patients); diabetic patients, pre-renal failure patients, diabetic pre-renal failure patients, renal failure patients, and diabetic renal failure patients.
Results: Erythrocytes and platelets count, hemoglobin and hematocrit levels revealed a significant decrease in all renal dysfunction groups, while leukocytes count, red cell distribution width, platelet distribution width, and mean platelet volume showed significant increases in diabetic and renal dysfunction groups as compared to the healthy control. Nitric oxide level increased significantly, while reduced glutathione showed a marked decrease in diabetic and all renal dysfunction groups compared to the healthy control.
Conclusion: Nitric oxide and reduced glutathione were associated with the inflammatory status in diabetic renal dysfunction patients which reflected by elevation in leukocytes and neutrophils count, red cell distribution width as well as the reduction in values of erythrocytes, platelets count, hemoglobin and hematocrit. Therefore, hematological indices can play a role in predict the progression of diabetic nephropathy.

1. Introduction

Diabetes is the most cause of chronic kidney disease (CKD) and 20–30% of diabetic patients have diabetic nephropathy which is considered the main cause of end-stage renal diseases (ESRD) [1,2]. Several hematological alterations affecting the red blood cells (RBCs), hemoglobin concentration (HB), white blood cells (WBCs), and the coagulation factors are related to CRD [3] and diabetic nephropathy [4]. Anemia is reported as the most common hematologic change in CKD, and anemia develops earlier in diabetic patients than in those with renal impairment from other causes. Diabetic nephropathy patients commonly have a greater degree of anemia correlated with their degree of renal impairment than those presenting with other causes of renal failure [5]. The red blood cell distribution width (RDW), a parameter reflects the degree of heterogeneity of erythrocyte volume and used for diagnoses of anemia [6], was significantly increased from stages 1–5 of CKD [7].

Several investigations have emphasized the importance of platelet (PLT) dysfunction in microvascular (nephropathy, neuropathy, and retinopathy) complications, lead to increased morbidity and mortality in type 2 diabetes [8]. PLT dysfunction is the main factor responsible for hemorrhagic tendencies in CRD [9]. White blood cells (WBCs), one of the main inflammatory markers, are activated and secrete cytokines in the diabetic state and leptin stimulates leukocyte proliferation and differentiation that contribute to the development and progression of nephropathy [10].

There are significant evidences supporting the increased level of

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nitric oxide and depletion of reduced glutathione content with the reduction in the activities of glutathione peroxidase and glutathione-S-transferase in diabetic rats [11]. Prabhakar [12] found that early diabetic nephropathy is associated with increased intrarenal nitric oxide (NO) production which contributes to hyperfiltration and microalbuminuria that characterizes early nephropathy. Otherwise, Singh and Singh [13] concluded that depletion of GSH in diabetic patients reduced the activity of antioxidant enzymes that induced oxidative damage which subsequently initiated diabetic nephropathy.

Thus, the exploration of mechanisms underlying metabolic and hematological changes that may associate the progression of diabetic nephropathy were, therefore, becomes increasingly important. The present study aimed to clarify the association between oxidative stress and hematological alterations in the progression of diabetic nephropathy in diabetic Egyptian patients.

2. Subjects, material and methods

2.1. Study population

One hundred volunteer patients were enrolled in the study from patients at Kidney Hospital, Beni-Suef, Egypt. Eligible patients were classified according to biochemical markers into five groups (20 patients); diabetic patients, prerenal failure patients, prerenal failure patients with diabetes, renal failure patients, and renal failure patients with diabetes. Furthermore, twenty healthy subjects were included in the study as healthy control. The study protocol was performed as per the declaration of Helsinki and good clinical practice guidelines. After the study protocol was approved by the Hospital ethical committee, the blood samples were collected during the period from December 2017 to May 2018.

2.2. Inclusion and exclusion criteria and experimental design

Adult males and females' normal subjects, diabetes patients (HbA1c > 6.5%, serum creatinine <1.5 mg/dl), establish nephropathy or pre-renal failure patients (creatinine; > 1.5–6.5 mg/dl), diabetic patients with pre-renal failure patients, renal failure (hemodialysis patients or end-stage renal disease) and diabetic patients with renal failure were enrolled in the study. Patients with infectious diseases, autoimmune disorders, asthma, eczema, allergies, thyroid diseases, liver and heart dysfunction, and alcohol abuse were excluded from the study. The enrolled patients and healthy subjects were classified into six groups, each comprising twenty patients as follows;

Group 1: Healthy control (healthy subjects).
Group 2: Diabetic patients.
Group 3: Pre-renal failure (moderate renal dysfunction) patients.
Group 4: Diabetic patients with pre-renal failure (moderate renal dysfunction).
Group 5: Patients with renal failure.
Group 6: Diabetic patients with renal failure.

2.3. Biochemical assays

Blood samples will be taken from participants after overnight fasting in EDTA and plain tubes (4 ml each). EDTA blood samples will be used for CBC count and HbA1C measurement. Samples will be stored at −40 °C until used. Blood glucose, urea, uric acid, and creatinine concentrations were estimated using reagent kit purchased from Spinreact Co. (Spain). Blood glycohemoglobin percentage (HbA1c%) was estimated using reagent kits purchased from Stanbio Company (Texas). Moreover, reduced glutathione (GSH) and nitric oxide (NO) were assayed using reagent kit purchased BioVision, Milpitas, USA. The procedures were performed according to the kit instructions provided. Hematology profile was determined using a MICROS ABX autoanalyzer according to the manufacturer's protocol.

2.4. Statistical analysis

The Statistical Package for the Social Sciences (IBM SPSS for WINDOWS, version 20; SPSS Inc, Chicago) was used for the statistical analysis of the results. Comparative analysis (one-way ANOVA) was conducted by using the general linear models' procedure (IBM SPSS) and the data were presented as mean ± SE. A simple linear correlation analysis was processed by Pearson's method to measure the degree of dependency between variables (IBM SPSS)·$P < 0.05$ was considered statistically significant.

3. Results

In the present study, the patients were in the age range of 46.9–56.5 years and the majority of patients (56%) are males. The current results revealed that blood urea and creatinine levels revealed a significant increase in the groups of; prerenal failure,

Table 1
Demographic, biochemical and hematological profile of control, diabetic and renal dysfunction groups.

Group Parameter	Normal healthy	Diabetic	Pre-renal failure	Diabetic pre-renal failure	Renal failure	Diabetic renal failure
Age (Year)	46.90 ± 2.83^{a}	49.50 ± 2.85^{ab}	51.85 ± 2.53^{ab}	55.70 ± 1.96^{ab}	47.80 ± 3.42^{ab}	56.50 ± 2.30^{b}
Gender, n (%) Male Female	9 (45) 11 (55)	9 (45) 11 (55)	12 (60) 8 (40)	10 (50) 10 (50)	13 (65) 7 (35)	14 (70) 6 (30)
Urea (mg/dl)	27.35 ± 1.15^{a}	33.06 ± 1.71^{a}	71.25 ± 2.45^{c}	58.84 ± 1.28^{b}	128.65 ± 7.53^{e}	113.70 ± 4.33^{d}
NO (μmol)	11.30 ± 0.33^{a}	22.45 ± 0.82^{b}	53.49 ± 2.63^{c}	66.82 ± 1.64^{d}	48.47 ± 3.53^{c}	64.28 ± 1.91^{d}
GSH (mg/dl)	66.18 ± 1.86^{d}	39.93 ± 1.15^{bc}	40.21 ± 0.75^{c}	35.90 ± 1.17^{b}	36.85 ± 1.66^{bc}	30.34 ± 1.55^{a}
RBCs ($x10^{6}/cm^{3}$)	4.74 ± 0.10^{de}	4.43 ± 0.13^{cd}	4.39 ± 0.15^{cd}	4.14 ± 0.09^{bc}	3.98 ± 0.16^{ab}	3.69 ± 0.12^{a}
MCV(fl)	76.17 ± 1.59^{bc}	76.68 ± 1.53^{bc}	65.83 ± 0.47^{a}	73.99 ± 1.85^{b}	76.74 ± 1.02^{bc}	79.63 ± 1.42^{c}
MCH (fl)	27.13 ± 0.52^{b}	26.26 ± 0.73^{b}	24.12 ± 0.38^{a}	25.65 ± 0.49^{ab}	27.02 ± 0.48^{b}	26.79 ± 0.79^{b}
RDW (%)	12.86 ± 0.13^{a}	13.59 ± 0.16^{bc}	13.17 ± 0.18^{ab}	13.80 ± 0.16^{c}	14.28 ± 0.23^{d}	13.98 ± 0.16^{cd}
MPV (fl)	9.59 ± 0.41^{a}	10.30 ± 0.10^{b}	11.21 ± 0.14^{c}	11.47 ± 0.13^{c}	11.76 ± 0.13^{c}	11.82 ± 0.17^{c}
Neutrophils (x103/cm3)	3165 ± 234^{a}	4083 ± 425^{ab}	6985 ± 190^{d}	$4827 \pm 351b^{c}$	5099 ± 333^{bc}	5340 ± 507^{c}
Lymphocytes (x103/cm3)	2243 ± 137^{bc}	2578 ± 184^{c}	1788 ± 115^{ab}	1800 ± 101^{ab}	1652 ± 115^{a}	1788 ± 265^{ab}
Monocytes (x103/cm3)	432 ± 30^{a}	527 ± 49^{ab}	642 ± 18^{b}	532 ± 36^{ab}	543 ± 45^{ab}	577 ± 57^{b}
P/L ratio	0.11 ± 0.01^{ab}	0.12 ± 0.01^{ab}	0.12 ± 0.01^{ab}	0.14 ± 0.02^{b}	0.13 ± 0.01^{ab}	0.14 ± 0.02^{b}

Data are expressed as mean ± SE. Number of patients = 20/group. Values which share the same superscript symbol are not significantly different. NO: nitric oxide, GSH: reduced glutathione, RBCs: Red blood cells, MCV: Mean corpuscular volume, MCH: mean corpuscular hemoglobin, RDW: red cell distribution width, MPV: Mean platelet volume, P/L, platelet/lymphocyte ratio.

diabetic prerenal failure, renal failure, and diabetic renal failure patients when compared to the healthy control. In addition, HbA1c % was increased significantly in diabetic groups as compared to healthy control. RBCs count revealed a significant decrease in diabetic prerenal renal failure, renal failure, and diabetic renal failure groups compared to healthy control. Additionally, both HB and HCT values were markedly decreased in different renal dysfunction groups, while, MCV and MCH values had decreased significantly in the renal failure group only. However, RDW % showed significant increases in diabetic and renal dysfunction groups compared to healthy control (Table 1, Fig. 1).

Concerning PLT indices, PLT count was significantly decreased in prerenal renal failure, renal failure, and diabetic renal failure groups, while MPV and PDW values revealed noticeable increases in diabetic and all renal dysfunction groups compared to healthy control. Otherwise, WBCs count increased significantly in diabetic and all renal dysfunction groups except diabetic prerenal renal failure group, while neutrophils count increased significantly in different renal dysfunction groups compared to healthy ones. In addition, lymphocytes count decreased markedly in renal failure group only, while monocytes count showed a significant increase in diabetic renal failure group compared to the healthy control. Regarding P/L ratio, the results observed a non-significant increase in all investigated groups, while N/L ratio indicated a marked significant increase in all renal dysfunction groups. Concerning NO level, NO was increased significantly in diabetic and renal dysfunction groups, while GSH content showed a noticeable decrease in all tested groups compared to the healthy control

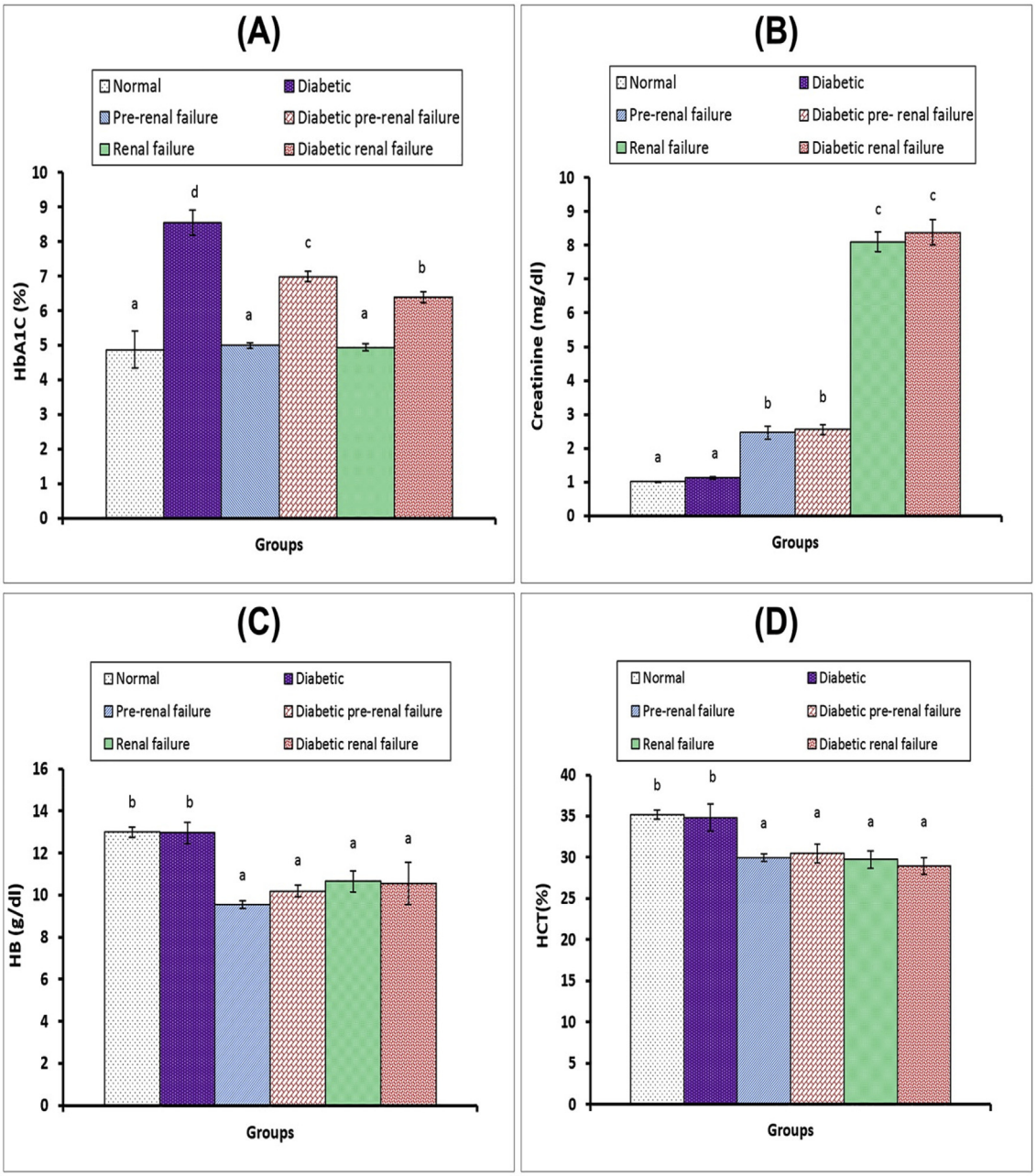

Fig. 1. The levels of (A) HbA1c, (B) creatinine, (C) HB, (D) HCT% in healthy control, diabetic and renal dysfunction groups. HbA1c: glycosylated hemoglobin, HB: hemoglobin, HCT: hematocrit. Data are expressed as mean ± SE. Values which share the same superscript symbol are not significantly different.

(Table 1, Fig. 2).

Among diabetic pre-renal failure group, our finding revealed a negative correlation between NO with RBCs count (r = - 0.834, P < 0.001), HCT % (r = - 0.706, P < 0.001), and PDW% (r = −0.656, P < 0.01), while NO showed a positive significant correlation with N/L ratio (r = 0.624, P < 0.01) (Fig. 3). Additionally, GSH had a positive correlation with RBCs (r = 0.568, P < 0.01), HCT (r = 0.715, P < 0.001), PLT (r = 0.616, P < 0.01), and PDW (r = 0.574, P < 0.01) (Fig. 4).

Concerning diabetic renal failure group, NO exerts a negative correlation with PDW % (r = - 0.601, P < 0.01), M/L (r = −0.654, P < 0.01), and N/L (r = −0.055, P < 0.818), while GSH exhibited a negative correlation with N/L ratio (r = - 0.567, P < 0.01), and PLT (r = −0.075, P < 0.752) (Fig. 5, Table 2). On the other hand, NO revealed a positive correlation with WBCs (r = 0.096, P < 0.686) and RDW (r = 0.201, P < 0.396), however, GSH exhibited a positive correlation with WBCs (r = 0.217, P < 0.358), and PDW (r = 0.071, P < 0.766) (Table 2).

4. Discussion

It has been observed in the current study that RBCs count, HB concentration, and HCT% were decreased significantly in renal impairment groups when compared to healthy control. Our findings were comparable with the results reported by McClellan et al. [14] and Nasri, [15]. In diabetes, hyperglycemia may cause changed in erythrocyte properties including, altered red cell deformability [16], and reduced RBC lifespan [17]. Further, RBCs are affected by various disturbances in the hematopoietic milieu, and these disturbances lead to a decrease in RBC numbers. On the other hand, in

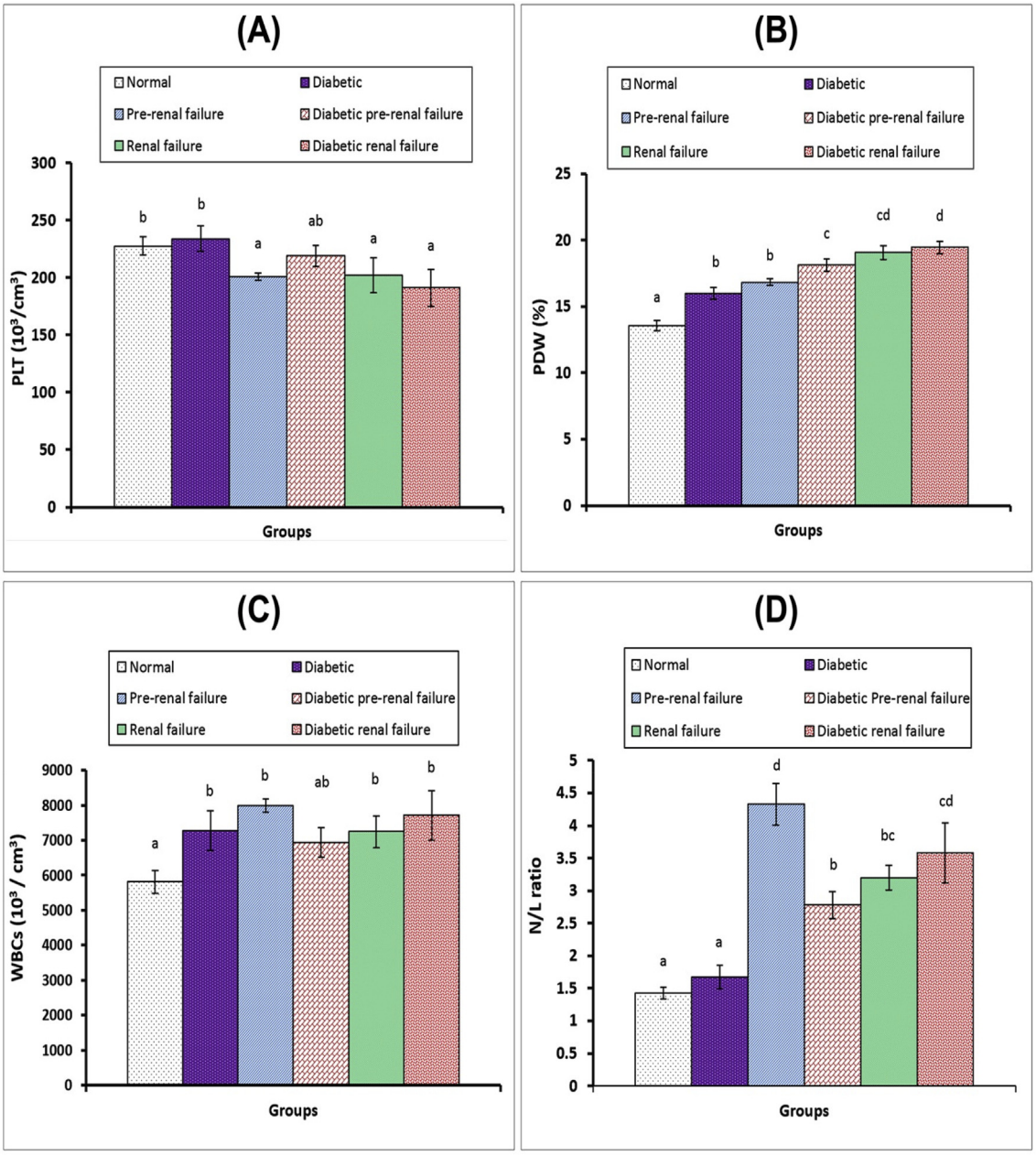

Fig. 2. (A) PLT count, (B) PDW %, (C) WBCs count, (D) N/L ratio in healthy control, diabetic and renal dysfunction groups. PLT: platelets, PDW: platelet distribution width, WBCs: white blood cells, N/L, neutrophil/lymphocyte ratio. Data are expressed as mean ± SE. Values which share the same superscript symbol are not significantly different.

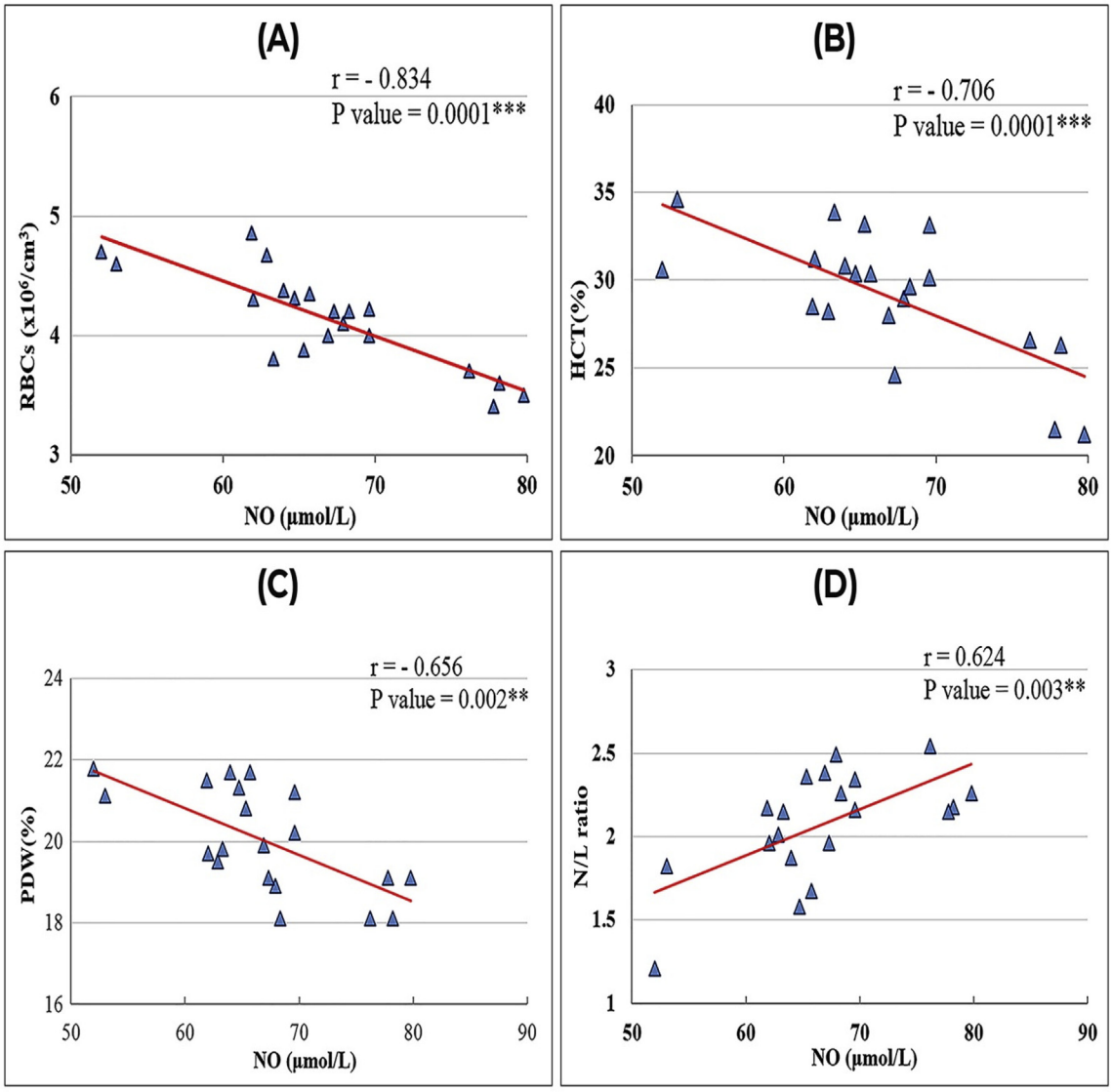

Fig. 3. Correlations between NO with RBCs (A) HCT (B), PDW (C), and N/L ratio (D) among diabetic pre-renal failure group. Correlation is significant at the 0.05 level, ** at the 0.01 level, *** at the 0.001 level. NO: nitric oxide, RBCs: red blood cells, PDW: platelet distribution width.

uremic patients, erythrocytes are decreased in their number, impairments of their structural and functional, alterations in the nitric oxide production, reduced antioxidant activity, and pro-coagulant activity as well as modification in the structure of plasma membrane [18]. Moreover, uremic RBCs exhibited reduced surface charge and deformability which triggers hemolysis in the capillaries and reduced the RBCs count [19]. Additionally, Nasri attributed the reduction in RBC count, HB concentration and HCT% in chronic renal disease patients to the depletion in the production of erythropoietin and factors suppress marrow erythropoiesis and shortened red cell survival [15]. Anemia is a common sign of renal dysfunction and develops earlier in patients with diabetes than in patients with renal impairment from other causes [20]. Although anemia is found at different stages of chronic renal disease, a strong correlation exists between the incidence of anemia and the severity of renal disease [14]. However, it was clarified that the failure to increase erythropoietin concentrations in response to falling HB levels is the main factor in the genesis of anemia associated with diabetic nephropathy [21].

Anemia is also closely correlated with oxidative stress, as erythrocytes represent a major antioxidant component of the blood [22]. In addition, uremic anemia is correlated with increased production of free radicals [23]. Among diabetic pre-renal failure patients, the present results exhibited a negative correlation between nitric oxide (NO) with RBCs count and HCT%, while GSH had a positive correlation with RBCs and HCT%. Several evidences exhibited that excessive oxidative stress may be contributing to the initiation and development of diabetic nephropathy [24]. Glutathione depletion may have adverse consequences in diabetic patients, independent of glycemic control, and it may weaken the defense against ROS, which causes damage to protein, DNA, or membrane lipids and thus potentially lead to cell dysfunction in various tissues [25]. Ozdemir et al. [26] showed lower values of GSH in diabetic subjects with microalbuminuria than normoalbuminuric subjects. In diabetic nephropathy, hyperglycemia is preferentially used in polyol pathways that consumes NADPH necessary for GSH regeneration by the GSH-Red enzyme and indirectly decrease the GSH level. Further, patients with type 2 diabetes have a lower concentration of intracellular GSH, which increases the susceptibility of RBCs to be affected by ROS, subsequently, alter RBCs

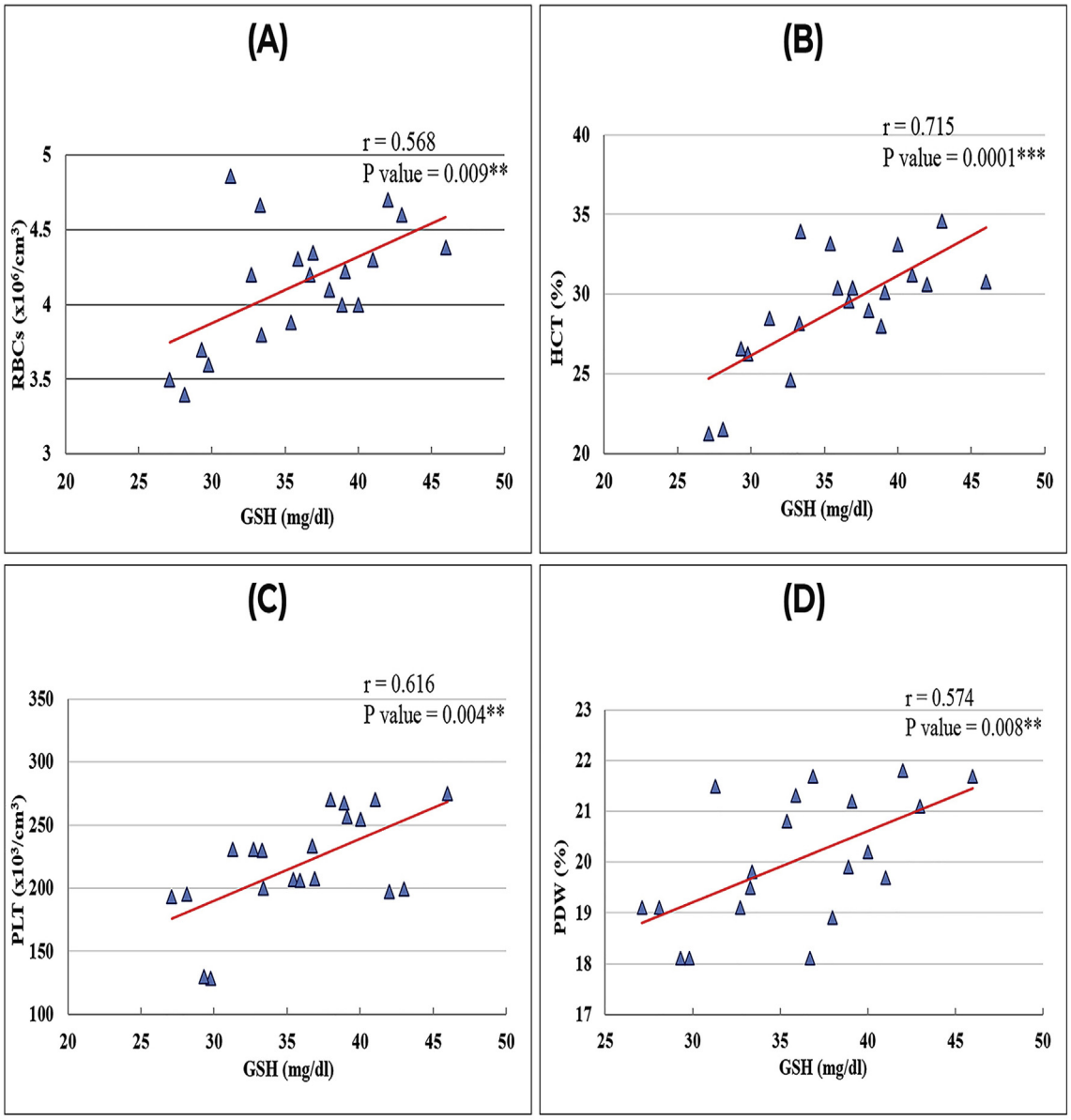

Fig. 4. Correlations between GSH with RBCs (A) HCT (B), PLT(C), and PDW (D) among diabetic pre-renal failure group. GSH: reduced glutathione.

properties as well as reduced the cell number. Additionally, Waggiallah and Alzohairy [27] reported that there is a strong significant effect of oxidative stress (reduced glutathione) on reducing HB concentration, which indicated that oxidative stress of diabetes is the possible cause of anemia in diabetics with or without nephropathy. Otherwise, early nephropathy in diabetes is associated with increased intrarenal NO production mediated primarily by constitutively released NO (endothelial nitric oxide synthase [eNOS] and neuronal nitric oxide synthase [nNOS]). Furthermore, the enhanced NO production may be associated with hyperfiltration and microalbuminuria that characterizes early diabetic nephropathy [28].

Recently, RDW has received attention in various populations and inflammatory diseases. In the present study, RDW showed significant increases in diabetic and renal dysfunction patients compared to the healthy subjects. Numerous investigations reported that RDW has been associated with mortality and other adverse outcomes in various clinical conditions, including kidney injury [29]. In addition, increased RDW values have been associated with diabetic complications, diabetic nephropathy, and peripheral arterial disease [30]. Recently, Solak et al. measured RDW in 367 patients with chronic kidney disease stages from 1 to 5, and mention that RDW significantly increased from stages 1–5, and also exhibiting a significant inverse correlation with estimating glomerular filtration rate values [31].

Concerning PLT indices in the current study, PLT count was significantly decreased in renal dysfunction groups, however, MPV values and PDW% revealed noticeable increases in diabetic and renal dysfunction groups when compared to healthy control. Regarding diabetic pre-renal failure patients, the present results exert a negative correlation between NO level with PDW, while it showed a positive correlation with N/L ratio. Additionally, GSH showed a positive correlation with PLT count and PDW%. The results of Vinik et al. [32] indicated that PLT in diabetic individuals adheres to vascular endothelium and aggregate more readily than those in healthy people. Moreover, patients with chronic kidney

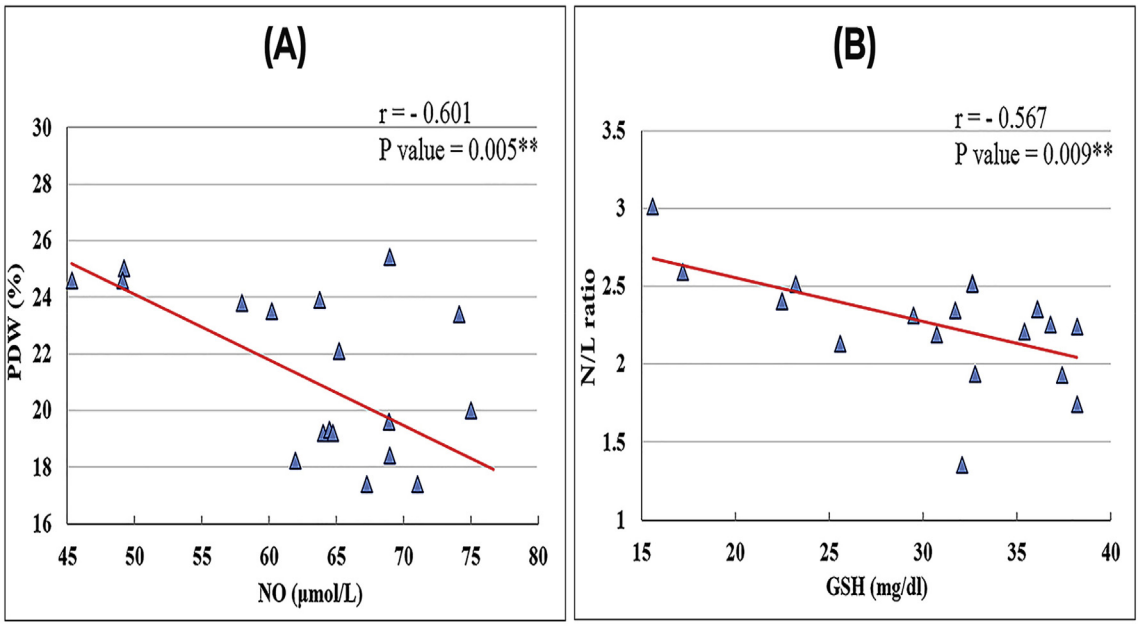

Fig. 5. Correlations between NO with PDW (A) and GSH with N/L ratio (B) among diabetic renal failure group.

Table 2
Correlations between NO and GSH with some hematological parameters among diabetic pre-renal failure group and diabetic renal failure group.

Group Parameters	Diabetic pre-renal failure group				Diabetic renal failure group			
	NO		GSH		NO		GSH	
	r	p value	r	p value	r	p value	r	p value
WBCs	−0.399	0.081	0.072	0.761	0.096	0.686	0.217	0.358
PLT	−0.337	0.147	0.616	0.004	0.107	0.653	−0.075	0.752
RBCs	−0.834	0.0001	0.568	0.009	−0.095	0.692	0.050	0.834
HB	0.142	0.549	−0.250	0.289	−0.198	0.403	−0.300	0.198
HCT	−0.706	0.001	0.715	0.0001	−0.155	0.513	0.338	0.145
RDW	−0.059	0.805	0.316	0.174	0.201	0.396	−0.273	0.244
PDW	−0.656	0.002	0.574	0.008	−0.601	0.005	0.071	0.766
N/L	0.624	0.003	−0.404	0.077	−0.055	0.818	−0.567	0.009
M/L	0.176	0.457	−0.343	0.139	−0.654	0.002	0.024	0.920

Correlation is significant at the 0.05 level, NO: nitric oxide, GSH: reduced glutathione, WBCs: white blood cells, PLT: platelet, RBCs: Red blood cells, HB: hemoglobin, HCT: hematocrit, RDW: red cell distribution width, PDW: platelet distribution width, N/L: Neutrophil/lymphocyte ratio, M/L: Monocyte/lymphocyte ratio.

disease had significantly lower PLT count, impaired erythropoietin secretion leads to a decrease in PLT count [33]. The detection of receptors for erythropoietin in megakaryocytes is understandable because erythropoietin levels can affect PLT numbers and extensive homology between erythropoietin and thrombopoietin [34]. Otherwise, NO may causes PLT dysfunction by inhibiting platelet–platelet interaction and also by affecting platelet–vessel wall interaction [35], and also, in end-stage renal disease patients, excessive production of NO and cGMP were reported [36].

Larger PLT, demonstrated by elevated MPV, is known as more active PLT. Ünübol et al. [37] determined a positive relationship between microalbuminuria and MPV value. In a study compare MPV in diabetics in different stages of CKD, the highest MPV values were recorded in diabetics and stage 2–4 of renal disease [38]. Also, in agreement with our outcome, Ju et al. [39] have been reported that as chronic kidney disease stage preceded, the MPV values also significantly increased, the authors also claimed that MPV can predict chronic kidney disease severity. Moreover, systematic inflammation, oxidative stress, impaired calcium metabolism, increased phosphorylation and glycosylation of cellular proteins are responsible for increased PLT activation and increased release of prothrombotic and proinflammatory agents in diabetic patients [40]. In patients with renal failure, anemia directly influences the bleeding time, and hemodialysis is also associated with enhanced PLT aggregation which may contribute to thrombotic events [41]. Furthermore, the thrombophilic tendency of uremic PLT was attributed partly to the translocation of phosphatidyl-serine to the outer membrane due to chronic PLT activation in chronic hemodialysis patients [42].

Chronic inflammation, as indicated by a higher WBCs count, may play a linkage role in the development of macro- and microvascular complications in diabetes [43]. The current study showed that both WBCs and neutrophils count and N/L ratio were increased significantly in diabetic and renal dysfunction patients compared to healthy control. However, there is growing support that inflammation plays a key role in the pathogenesis of diabetic nephropathy. The patients with chronic kidney disease had leukocytosis and the higher WBCs count may indicate a clinical or subclinical inflammatory status. The increase in pro-inflammatory state enhances the activation of WBCs and endothelial cells, thereby promoting PLT aggregation and thrombus formation [44,45]. Additionally, in parallel with our results, elevated levels of N/L ratio

were reported in diabetes and diabetic nephropathy [46], which reflects the inflammatory condition of diabetic nephropathy. Furthermore, leukocytes, monocytes, and macrophages have all been implicated in the process of diabetic nephropathy [47], and circulating inflammatory markers and proinflammatory cytokines are strongly associated with the risk of developing of diabetic complications, like nephropathy [48]. In addition, neutrophils in diabetic patients showed an increase in the release of oxygen free radicals that might damage endothelial cells and accelerate the progression of diabetic nephropathy [49].

Limitations of the study included the small number of patients (100), and the stages of renal dysfunction groups. Additionally, the limitations of investigated parameters like eGFR, serum iron, Ferritin, Fe, TIBC, vitamin B12 and folic acid levels.

5. Conclusion

The study indicated that nitric oxide and reduced glutathione were associated with the inflammatory status which characteristic diabetic nephropathy and reflected by elevation in leukocytes and neutrophils count, red cell distribution width and platelet distribution width as well as neutrophils/lymphocytes ratio. Additionally, the effects were extended to the reduction in the values of erythrocytes, platelets, hemoglobin, and hematocrit. Therefore, hematological indices can play a role in predict the development and severity of diabetic nephropathy.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.06.014.

Conflicts of interest

The authors state that they have no conflicts of interest regarding the publication of this article.

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