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Original Article

Association of glycemic status and interferon-γ production with leukocytes and platelet indices alterations in type2 diabetes

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ABSTRACT

Aims: The present study aimed to evaluate the correlation between glycemic status and the inflammation biomarkers; leukocytes, platelets indices and interferon gamma (IFN-γ) production in type 2 diabetes mellitus (T2DM) patients regarding diabetic complications.
Methods: Study was conducted on 158 patients allocated as normal healthy subjects (50) and 108 patients diagnosed as T2DM. The diabetic patients were subdivided into six groups according to metformin administration as mono-or dual therapies.
Results: The current results exhibited a significant elevation in systolic blood pressure, total and LDL-cholesterol levels and IFN-γ as well as a noticeable decrease in HDL-cholesterol and anti-atherogenic factor values compared to the healthy patients. Leukocytes and neutrophils count, main platelets volume (MPV) and platelet distribution width (PDW) values revealed noticeable elevations in most treated T2DM groups, while a marked depletion was recorded in platelets count compared to healthy subjects. Glycemic control, most treated diabetic patients with metformin mono- and dual therapies showed an ameliorative effect in HbA1c, IFN-γ, MPV, and PDW values compared to recent diabetic ones.
Conclusion: Diabetes was correlated significantly with dyslipidemia and atherogenic risk in parallel with an increase in IFN-γ production and hematological inflammatory biomarkers; leukocytes, neutrophil/lymphocyte and platelet/lymphocyte ratios, MPV and PDW values. The amelioration in inflammatory biomarkers was associated with improvement in glycemic control.

1. Introduction

Diabetes mellitus (DM) is a chronic global epidemic disease includes a heterogeneous group of disorders characterized by hyperglycemia associated with metabolic, cellular and blood disturbances leading to vascular complications [1,2]. Altered hematological indices are directly correlated with glycosylated hemoglobin (HbA1c) levels in subjects with or without diabetes. These associations may be explained by linked hematological indices, inflammation, and the tendency to coagulation and thrombosis in diabetic patients [3]. Platelets play a vital role in the development of atherothrombosis, the most leading cause of morbidity and mortality in patients with diabetes [4]. Also, platelets aggregation and adhesion play a major role in intravascular thrombosis, resulting in cardiovascular and cerebrovascular events [5]. Several hematological abnormalities affecting the white blood cells (WBCs) and the coagulation factors are shown to be associated with DM [6]. Chronic inflammation indicated by elevated leukocytes count may play a central role in the development of diabetic macro-and microvascular complications [7]. Different studies have discussed the role of hyperglycemia and insulin resistance (IR) in enhanced ROS production by peripheral blood leukocytes, the increase of proinflammatory cytokines, and an increase in the number of leukocytes-endothelium interactions [8].

Interferon-γ (IFN-γ), a Th1 cytokine, plays the main role in defense against viruses and intracellular pathogens as well as the induction of immune-mediated inflammatory responses. Furthermore, INF-γ may participate in the development of type 2 diabetes

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mellitus [9]. Metformin has long been used to accelerate insulin-glucose uptake in adipocytes and skeletal muscle, which attenuate hyperglycemia and insulin resistance (IR). Furthermore, metformin exhibited antioxidant and anti-inflammatory effects in T2DM patients [10]. Numerous reports have connected metformin therapy with reduced mortality and diabetes-associated cardiovascular and cerebrovascular events in diabetic patients [11]. In diabetic patients, the correlation between inflammatory biomarkers and glycemic status is critical to be investigated [12]. Moreover, the relationship between hematological inflammatory parameters and IFN-γ production in T2DM patients remains controversial and has not been discussed previously. Thus, the present study aimed to evaluate the correlation between glycemic status and inflammation biomarkers; leukocytes, platelets indices and IFN-γ production, in T2DM patients.

2. Subjects, material and methods

2.1. Study groups

The current study was conducted on 158 patients of both sexes; 83 male and 75 females (aged between 30 and 75 years) who were followed up at the diabetic section of General Institution of Healthy Insurance, Beni Suef, Egypt, from December 2016 to March 2018. Enrolled patients were allocated to normal healthy subjects (control group) who had no previous history of chronic diseases, and 108 patients diagnosed as T2DM patients according to WHO 1999 criteria. Written agreements were obtained from all patients before participation in the experiment. The study protocol was performed in accordance with the declaration of Helsinki and good clinical practice guidelines and approved from the committee of General Institutions of Health Insurance. Trial registration no; PACTR201801002776119.

Pregnant and lactating women, patients receiving immunomodulatory drugs and patients with medical conditions such as infections, cerebrovascular diseases, ischemic heart disease, malignancies, autoimmune disorders, eczema, respiratory disorder, thyroid dysfunction, kidney failure, liver dysfunction, and alcohol abuse were excluded from the study. In addition, diabetic patients who underwent medication changes during the 2 months preceding participation will be also excluded.

The diabetic patients were subdivided into six groups according to the treatment:

1) Group 1: normal healthy subjects (control).	(50 subjects)
2) Group 2: diabetics (recent diagnoses) without treatment	(20 patients).
3) Group 3: diabetics treated with metformin only	(15 patients).
4) Group 4: diabetics treated with glimepiride only	(19 patients).
5) Group 5: diabetics treated with metformin + glimepiride	(15 patients).
6) Group 6: diabetics treated with insulin only	(20 patients).
7) Group 7: diabetics treated with metformin + insulin	(19 patients).

The demographic data regarding anthropometric variables such as height, weight, gender, duration of disease and blood pressure (BP) were collected. Serum samples were rapidly separated, aliquoted and stored at −40 °C until the biochemical and IFN-γ measurements.

2.2. Laboratory assays

The levels of glucose, total cholesterol, triglyceride, and HDL-c were determined using commercially available assay kits obtained from Reactivos Spinrect, Spain. However, LDL cholesterol level calculated according to Friedewald formula [13]. The cardiovascular risk (CVR) and anti-atherogenic factor indices were calculated according to Ross formula [14]. Moreover, HbA1c% was measured by the turbidimetric inhibition immunoassay for hemolyzed whole blood (Cobas Intgra 800, Roche, Basel, Switzerland). IFN-γ level was estimated using high sensitivity sandwich ELISA kit (Cusabio Wuhan, China) according to manufacturer instructions. Hematology parameters; platelet count (PLT), Mean platelet volume (MPV), and platelet distribution width (PDW), white blood cell (WBCs) count, differential leukocytes count (neutrophil, lymphocyte, and monocyte) was determined using a MICROS ABX autoanalyzer according to the manufacturer's protocol.

2.3. Statistical analysis

Data are presented as means ± SD. The data were analyzed by one-way analysis of variance (ANOVA). *P* value less than 0.05 was considered statistically significant. To compare the difference among the groups, post hoc testing was performed by the Duncan test with LSD. Pearson-year's analysis method was used to determine the correlation coefficients between different studied parameters. Statistical analysis was performed using Statistical Package for the Social Science (SPSS) for Windows (version 22.0, Chicago, IL, USA).

3. Results

All T2DM patients' groups showed a significant increase in systolic blood pressure (Bp1) and HbA1c% when compared to the healthy group, however, HbA1c% showed a noticeable decrease in diabetic treated groups as compared to recent diabetic group (Table 1 & Fig. 1). Also, serum total and LDL-cholesterol concentrations and risk factor 1 of all T2DM patients were significantly increased, while serum HDL-cholesterol and anti-atherogenic factor were generally decreased in most groups compared to the healthy group. Additionally, cytokine IFN-γ production revealed a marked elevation in all T2DM patients compared to the healthy group, while, an obvious amelioration has been recorded in all treated T2DM groups compared to the recent diabetic ones. On the other hand, leukocytes (WBCs), neutrophils count revealed a significant increase in different T2DM groups, while lymphocytes count observed a significant decrease in the recent diabetic group only. Otherwise, platelets count showed a marked depletion in most diabetic groups, while MPV values revealed a noticeable increase in most T2DM groups compared to normal healthy subjects. Furthermore, PDW values and N/L ratio observed a significant elevation in all T2DM groups (Fig. 1& Table 1).

Regarding T2DM patients, the present results revealed a positive correlation between HbA1c% and IFN-γ ($r = 0.309$, $P < 0.001$; 95% confidence interval (CI) = 0.193–0.413) (Table 2). Also, HbA1c% and IFN-γ observed a positive correlation with both systolic and diastolic blood pressure (Bp1; $r = 0.125$, $P < 0.05$; $r = 0.222$, $P < 0.001$; Bp2; $r = 0.143$, $P < 0.05$; $r = 0.138$, $P < 0.05$, respectively) (Table 2). Additionally, the current results also showed a positive correlation between both HbA1c% and IFN-γ with total cholesterol ($r = 0.381$, $P < 0.001$; $r = 0.195$, $P < 0.01$, respectively). On the other hand, a negative correlation was recorded between HbA1c% and IFN-γ with platelets count ($r = -0.177$, $P < 0.01$; $r = -0.225$, $P < 0.001$, respectively). Otherwise, a noticeable positive correlation was recorded between HbA1c% and IFN-γ with P/L ratio ($r = 0.275$, $P < 0.001$; $r = 0.131$, $P < 0.05$, respectively). Also, MPV and PDW values showed a positive correlation with IFN-γ only ($r = 0.249$; $P < 0.001$, 95% CI = 0.166–0.350 & $r = 0.129$; $P < 0.05$, 95% CI = 018–0.238, respectively). Similarly, WBCs count and N/L ratio showed the

Table 1
Demographic, IFN-γ production, leukocytes and platelet indices in control, recent diabetic, and different treated groups.

Parameters	Control	Diabetic	Metf.	Sulf.	Ins.	Metf.+Sulf.	Metf.+Ins
n =	50	20	15	19	20	15	19
Gender:							
M, n (%)	25 (50)^{c}	10 (50)^{a}	8 (53.3)^{a}	9 (47.4)^{a}	13 (65) ^{ab}	8 (53.3)^{a}	10 (52.6)^{a}
F, n (%)	25 (50)^{c}	10 (50)^{b}	7 (46.7)^{a}	10 (52.7)^{b}	7 (35)^{a}	7 (46.7)^{a}	9 (47.4)^{b}
Age (y)	42 ± 16^{a}	49 ± 15^{a}	57 ± 08^{bc}	63 ± 11^{c}	62 ± 07^{c}	55 ± 09^{bc}	57 ± 08^{bc}
Bp1 (mmHg)	123 ± 05^{a}	143 ± 26^{b}	154 ± 25^{c}	153 ± 19^{cd}	158 ± 20^{d}	148 ± 12^{b}	152 ± 28^{cd}
Bp2 (mmHg)	84 ± 06	92 ± 19	86 ± 14	90 ± 09	89 ± 11	86 ± 09	87 ± 13
T.C. (mg/dl)	167 ± 28^{a}	218 ± 49^{bcd}	191 ± 51^{ab}	197 ± 55^{bc}	220 ± 42^{cd}	227 ± 41^{d}	194 ± 24^{abc}
T.G. (mg/dl)	91 ± 41^{a}	112 ± 50^{ab}	107 ± 48^{ab}	112 ± 43^{ab}	125 ± 44^{ab}	91 ± 45^{a}	99 ± 41^{a}
HDL (mg/dl)	48 ± 10^{b}	45 ± 06^{ab}	44 ± 08^{ab}	39 ± 10^{a}	40 ± 08^{a}	38 ± 06^{a}	37 ± 12^{a}
LDL (mg/dl)	99 ± 38^{a}	151 ± 45^{bc}	124 ± 49^{ab}	136 ± 46^{bc}	154 ± 39^{cd}	165 ± 39^{d}	128 ± 23^{bc}
Risk F.1	3.71 ± 1.1^{a}	4.97 ± 1.3^{b}	4.4 ± 1.2^{ab}	5.24 ± 1.7^{b}	5.23 ± 1.4^{b}	5.19 ± 1.5^{b}	4.47 ± 1.1^{ab}
Anti-ath. F.	44 ± 13^{b}	28 ± 12^{a}	33 ± 13^{ab}	27 ± 09^{a}	26 ± 09^{a}	27 ± 09^{a}	32 ± 09^{ab}
Lym. (x10^{3}/cmm)	2.37 ± 0.5^{b}	1.98 ± 0. 7^{a}	2.03 ± 0.6^{a}	2.30 ± 0.7^{ab}	1.99 ± 0.7^{a}	2.49 ± 0.3^{ab}	2.65 ± 0.6^{ab}
Mon. (x10^{3}/cmm)	0.33 ± 0.14^{a}	0.33 ± 0.15^{a}	0.37 ± 0.10^{ab}	0.45 ± 0.18^{bc}	0.48 ± 0.15^{c}	0.37 ± 0.12^{ab}	0.36 ± 0.07^{ab}
Neut. (x10^{3}/cmm)	2.50 ± 0.3^{a}	5.70 ± 1.5^{d}	4.05 ± 0.6^{bc}	3.56 ± 1.4^{b}	4.54 ± 1.3^{cd}	5.06 ± 1.4^{d}	4.74 ± 1.8^{cd}
PLT (x10^{3}/cmm)	277 ± 54^{c}	225 ± 77^{b}	199 ± 25^{a}	199 ± 42^{a}	215 ± 54^{b}	266 ± 24^{c}	272 ± 55^{c}
PDW (fl)	10.93 ± 1.89^{a}	14.40 ± 1.35^{c}	12.65 ± 1.18^{b}	13.78 ± 2.15^{c}	12.56 ± 0.53^{b}	12.22 ± 0.28^{b}	12.62 ± 0.91^{b}
N/L	1.05 ± 0.06^{a}	2.88 ± 0.05^{d}	2.00 ± 0.09^{c}	1.55 ± 0.07^{b}	2.28 ± 0.09^{c}	2.03 ± 0.05^{c}	1.79 ± 0.17^{b}
P/L	111 ± 11^{b}	139 ± 16^{bc}	98 ± 04^{a}	86 ± 08^{a}	108 ± 08^{b}	107 ± 07^{b}	103 ± 05^{b}

Data are expressed as mean ± SD. Values which share the same superscript symbol are not significantly different. Metf.: Metformin, Sulf.: Sulfonylurea, Ins.: Insulin, M: male, F: female, Bp1: systolic blood pressure, BP2; diastolic blood pressure, T.C.: total cholesterol, T.G.: triglyceride, HDL: high-density lipoprotein, LDL: low-density lipoprotein, *Anti*-ath., F.: Anti-atherogenic factor. Lym.: lymphocyte, Mon.: monocyte, Neut.: neutrophil, PLT: platelet, PDW: platelet distribution width, P/L: platelet-to-lymphocyte ratio, and N/R: neutrophil-to-lymphocyte ratio.

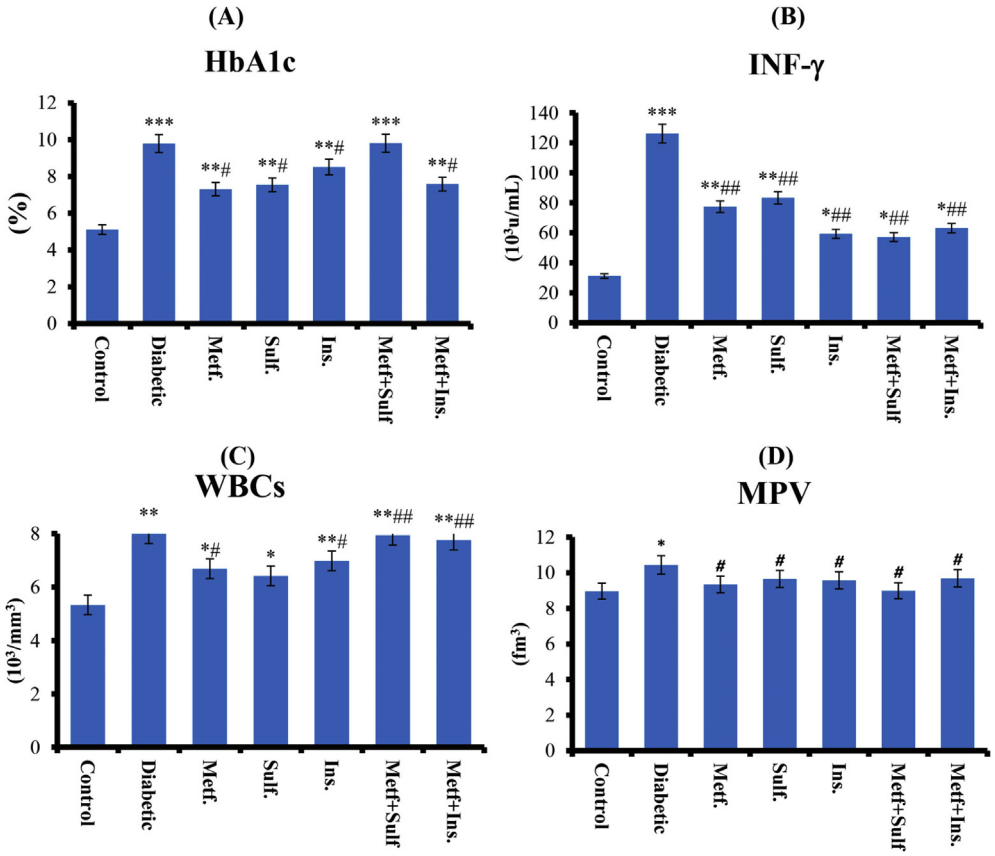

Fig. 1. HbA1c% (A), IFN-γ level (B), WBCs count (C), MPV values (D), among control, recent diabetic and different treated groups. *significant between diabetics compared to control at the 0.05 level, **: significant at the 0.01 level, ***: is significant at the 0.001 level; #significant between treated diabetics compared to recent diabetics at the 0.05; # #: significant at the 0.01 level, # # #: is significant at the 0.001 level, HbA1c Glycosylated hemoglobin, IFN-γ: interferon-gamma, WBCs: white blood cells, MPV: mean platelet volume.

identical correlation with HbA1c% (r = 0.183, P < 0.01; r = 0.342, P < 0.001, respectively) as represented in Table 2 and Figs. 2 and 3. Overall, the obtained data exhibited that Bp1, PLT, MPV and PDW correlated significantly more with IFN-γ production more than HbA1c%, however, total cholesterol, WBCs, N/L and P/L ratios correlated markedly with HbA1c% than IFN-γ (Table 2).

Table 2
Pearson correlation between HbA1c% and IFN-γ with inflammatory biomarkers and atherogenic factors in control, recent diabetic and different treated groups.

Parameters	HbA1c%			IFN-γ		
	r	95% C.I.	P value	r	95% C.I.	P value
HbA1c%	–	–	–	0.309***	(0.193–0.413	<0.001
Bp1	0.125*	(0.009–0.234)	0.040	0.222***	(0.102–0.343)	<0.001
Bp2	0.143*	(0.043–0.244)	0.018	0.138*	(-0.002-0.268)	0.023
T. Cholesterol	0.381***	(0.261–0.507)	<0.001	0.195**	(0.080–0.311)	0.001
WBCs	0.183**	(0.090–0.278)	0.002	0.027	(0.157–0.110)	0.652
PLT	−0.177**	(-0.281–0.069)	0.003	−0.225***	(-0.351–0.087)	<0.001
MPV	0.040	(-0.110-0.230)	0.509	0.249***	(0.166–0.350)	<0.001
PDW	0.099	(0.029–0.223)	0.102	0.129*	(0.238–0.018)	0.033
N/L Ratio	0.342***	(0.218–0.456)	<0.001	0.042	(-0.080-0.160)	0.491
P/L Ratio	0.275***	(0.148–0.404)	<0.001	0.131*	(-0.024-0.290)	0.030

*Correlation is significant at the 0.05 level, ** at the 0.01 level, *** at the 0.001 level. HbA1c: glycosylated hemoglobin, IFN-γ: interferon-gamma, Bp: Blood pressure, WBCs: white blood cells, MPV: Mean platelet volume, PLT: platelet, PDW: platelet distribution width, P/L: platelet-to-lymphocyte ratio, and N/R: neutrophil-to-lymphocyte ratio, T.: total.

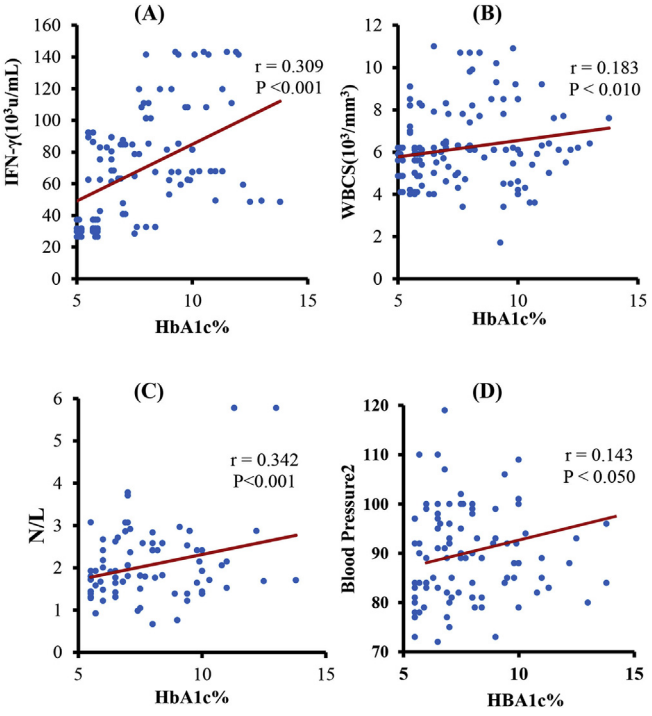

Fig. 2. HbA1c% correlation with IFN-γ level (A), WBCs count (B), N/L ratio (C) and Blood Pressure 2 (D) in control, recent diabetic and different treated groups. N/L: neutrophil-to-lymphocyte ratio.

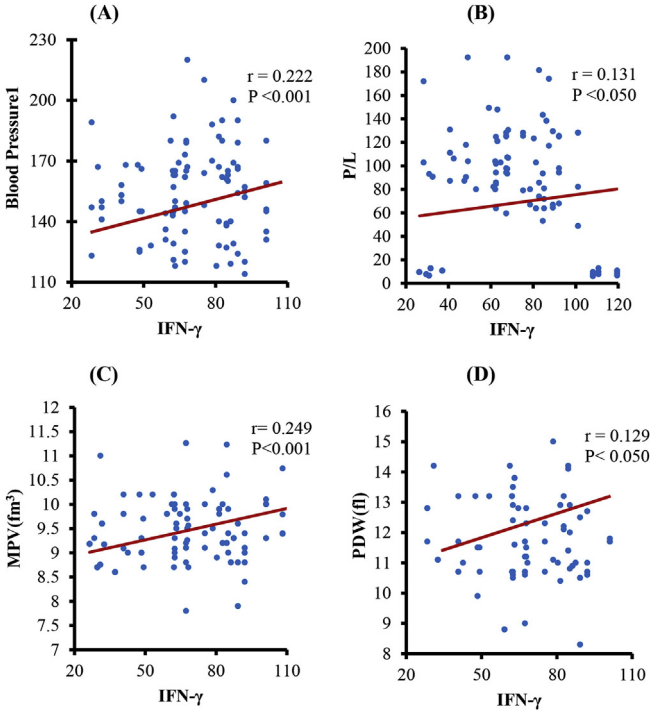

Fig. 3. IFN-γ level correlation with Blood Pressure 1 (A), P/L ratio (B), MPV (C) and PDW (D) in control, recent diabetic and different treated groups. P/L: platelet-to-lymphocyte ratio and PDW: platelet distribution width.

4. Discussion

The current results exhibited a significant elevation in a cardiovascular indicator, systolic blood pressure, in all diabetic patients as well as noticeable atherogenic activities indicated by marked increases in serum total and LDL-cholesterol concentrations and in risk factor 1, while a noticeable decrease in HDL-cholesterol level and anti-atherogenic factor was observed compared to the healthy group. Atherogenic dyslipidemia is particularly common in T2DM and has been correlated with both microangiopathy and residual cardiovascular risk in T2DM patients [15]. Generally, hyperlipidemia-induced inflammation contributes to pathological processes which can be lead finally to adverse diabetic complications [16]. In diabetic patients, hyperlipidemia may initiate vascular occlusion and atherothrombotic diseases through activation of platelet aggregation exceeding the normal biological responses [17]. Platelets function is critical to understanding the pathophysiology of vascular disease in diabetes. The metabolic abnormalities in diabetics, hyperglycemia and hyperlipidemia, may change the functions of blood cells as well as the coagulation system leading to hypercoagulability [18]. On the other hand, numerous evidences reported that platelet hyperactivity participates in the pathogenesis of abnormal platelet function which plays a role in the pathogenesis of several diabetic complications [19].

On the other hand, T2DM patients showed high levels of cytokines of both patterns Th1 proinflammatory cytokines and Th2 anti-inflammatory cytokines [20]. Our data revealed a marked elevation the cytokine IFN-γ production in all T2DM patients as compared to healthy subjects, however, a significant attenuation was recorded in different treated diabetic groups compared to recent diabetic ones. However, IFN-γ showed a positive correlation

with blood pressure, HbA1c%, and total cholesterol level. In parallel with our result, Fachinan et al. [21] reported that diabetic rats showed a significant increase in plasmatic levels of IL-2 and IFN-?? (Th1 cytokines). Also, Johnsen-Soriano et al. mention that immune-regulatory cytokines, Th-1 group (IL-2 and IFN-γ), were increased in the retinal homogenate of experimental diabetic rats [22]. Furthermore, due to the elevation of IFN-γ level in nephropathic patients, Nosratabadi et al. concluded that IFN-γ was highly contributable to nephropathy complication of T2DM [23]. The expression of inflammatory cytokines including IFN-γ participates in the increased production of fibrinogen to induce a prothrombotic milieu [24]. Additionally, IFN-γ increases the secretion of *C*-reactive protein (CRP) which acts as an inflammatory mediator in the development of atherothrombosis [25]. Our study showed a noticeable positive correlation between IFN-γ with MPV, PDW, and P/L ratio. INF-γ is a master regulator of immune function, atherogenesis and its expression is upregulated in atherosclerotic lesions, it accelerates the formation of foam cell via the pathological disruption of cholesterol homeostasis [26], promotes the activity of the tissue factor which enhances thrombosis upon blood contact [27] and progression of atherosclerotic diseases even at advanced stages. This, in turn, contributes to the interaction between platelets, leucocytes and vascular endothelium, inducing microvascular complications [28].

Recently, MPV, PDW, P/L ratio, and N/L ratio (NLR) have been considered as predictors of microvascular complications of diabetes and were introduced as novel markers of inflammation in cardiac and non-cardiac disorders [3,29]. Soma et al. suggested that platelets showed increased reactivity and baseline activation in T2DM patients compared to healthy controls [30]. So, MPV and PDW are considered biomarkers of platelet activation [31]. In the present investigation, platelets count showed a marked depletion, while MPV and PDW values revealed noticeable elevations in most treated T2DM groups compared to healthy subjects. In addition, HbA1c revealed a positive correlation with P/L ratio. The present study also revealed a noticeable positive correlation between IFN-γ with MPV, PDW, and P/L ratio. In accordance to the present results, Ulutas et al. [32] reported that MPV and PDW showed statistically significant increment in T2DM patients like several studies which showed an increasing number of circulating large platelets (MPV) compared with controls. Gasparyan et al. suggested that larger platelets are more active because of elevated prothrombic contents, such as thromboxane A2, thromboxane B2, platelet factor 4, serotonin, and platelet-derived growth factor. These mediators can contribute to inflammation and atherogenesis and may explain an association between MPV and severity of atherosclerosis [33]. Moreover, osmotic swelling of platelets, as a result of hyperglycemia and the platelet granule secretions may contribute to platelet size variation and MPV elevation in T2DM patients [34]. On the other hand, PDW is a specific marker of platelet activation and increases in the heterogeneity of platelet, increased PDW is also reported to be associated with diabetes and vascular complications [35]. Vagdatli et al. [36] reported that MPV and PDW were elevated together in platelet activation states, but PDW is the more specific marker.

Association of MPV and impaired glucose regulation in diabetic patients was reported previously [3]. With regarding glycemic control in the current study, IFN-γ, MPV, and PDW revealed a marked increase in most T2DM patients compared to the healthy group, however, an ameliorative effect has been observed in most treated diabetic patients with metformin (mono- and dual therapy) compared to recent diabetic ones. In parallel with the current data, Demirtunc et al. [37] showed a close relationship between poor glycemic control and increased platelet activity in T2DM patients. Also, an increase in HbA1c% was associated by increased MPV values, however, improved glycemic control decreases MPV values and recovered platelet functions and activity, and may prevent the crucial role of platelets in cardiovascular events in T2DM. Metformin, the first-line antidiabetic therapy for T2DM, has been shown to exhibit antiatherogenic action through ameliorative effects on cholesterol levels, inflammatory markers, vascular adhesion molecules, decreased MPV values, and platelet activation in diabetic patients [38]. In addition, Diaz-Morales et al. [39] findings supported the beneficial effects of metformin on oxidative stress, endothelial function and leukocyte-endothelium interactions, which suggest the positive outcome on development of an atherogenic process in T2DM. Franco et al. study showed a marked elevation of IFN-γ level in patients with poorly controlled T2DM, demonstrating that glycemic control may be correlated with immune inflammatory response [20]. Otherwise, the increased levels of INF-γ released from peripheral blood mononuclear cells (PBMC) of T2DM patients with adequate metabolic control indicate that glycemic control modulates the immune response and reduced the susceptibility to any infections [40].

The hematological changes in chronic diseases open the possibility that circulating leukocytes including lymphocytes and neutrophils could serve as indices of diagnostic significance in the routine clinical evaluation of diabetes [41]. Also, advanced glycation end products (AGEs) and cytokines can activate polymorphonuclear and mononuclear leukocytes in hyperglycemic individuals [42]. So, the present data found that leukocytes count, and N/L ratio showed a positive correlation with HbA1c%. Further, leukocytes (WBCs), neutrophils count revealed a significant increase in different T2DM groups, while lymphocytes count observed a significant decrease in the recent diabetic group only. The available previous investigations have referred to markedly elevated WBC in diabetic patients with significant correlation with HbA1c%. Additionally, leukocyte is a classical inflammatory marker and is associated with several cardiovascular disease risk factors [43]. The process of atherosclerosis is known to involve inflammatory mechanisms [44], reported that leukocytosis is directly associated with the pathogenesis of both atherosclerosis and metabolic syndrome [45]. On the other hand, P/L ratio has been designated as predictors of microvascular complications of diabetes and was introduced as novel markers of inflammation in both cardiac and non-cardiac diseases [36]. In addition, N/L ratio exhibits a balance of two interdependent components of the immune system; neutrophils that are the active nonspecific inflammatory mediator form the first line of defense whereas lymphocytes are the regulatory or protective component of inflammation [46]. Hussain et al. [47] suggested that increased N/L ratio may be independently associated with poor glycemic control in T2DM patients. Correspondingly, Shung et al. [42] have shown that N/L ratio is associated to increase the severity of hyperglycemia and insulin resistance and can be used as a prognostic marker for macro- and microvascular complications in patients with glucose intolerance. Furthermore, chronic diseases such as hypertension and diabetes have a significant association with systemic inflammation reflected by an increase in N/L ratio [48]. The microvascular dysfunction, an important manifestation of a pathophysiological response to inflammation, includes the activation of vascular endothelial cells, circulating leucocytes and platelets [28]. IFN-γ and nitric oxide (NO) produced at the inflammation site, cause local dilation of the blood vessels, decreasing the local blood flow rate and causing the gathering of blood in leaky vessels. In addition, specific leukocyte subsets are instructed by the cytokine/chemokine milieu to extravasate into the tissue via interactions between adhesion molecules presented on leukocyte and endothelial surfaces [49]. Further, IFN-γ is produced predominantly by natural killer (NK) and natural killer T (NKT) cells, T cells promote chronic

inflammation in diabetes patients through IFN-γ and IL-17 production [50].

5. Conclusion

Diabetes is correlated significantly with dyslipidemia and atherogenic risks in parallel with the increase in inflammatory cytokine, IFN-γ, and hematological inflammatory biomarkers, leukocytes, N/L and P/L Ratios, MPV and PDW values. The recorded amelioration in IFN-γ, hematological inflammatory biomarkers, and atherogenic risks followed metformin (mono and dual) therapies suggested their anti-inflammatory effect beside hypoglycemic action. The results also supported that leukocytes and platelets indices may be used to predict the development of diabetic complications.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.04.046.

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