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Fetuin-A and Hemogram-Related Inflammatory Markers in type 2 Diabetic Retinopathy

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Abstract

Diabetic retinopathy (DR) is considered a microvascular complication of diabetes mellitus (DM) and is characterized clinically by progressive alterations in the microvasculature that result in retinal ischemia, neo-vascularization, altered retinal permeability and macular oedema. DR is currently considered a leading cause of blindness in the adult population. Fetuin-A is a multifunctional glycoprotein that could be an early initiator of DR pathogenesis due to its dual role in insulin resistance and angiogenesis. This study aimed to evaluate the level of Fetuin-A as well as CBC parameters and its related inflammatory markers in DR patients as markers that may help to predict the disease occurrence and may help to follow up and avoid complications along the disease course. A case-control study enrolled forty-five participants selected from Al-Zahraa University Hospital, between March 2021 and October 2021, they were divided into three groups; **group I**; control group (n=15) non-type 2 diabetic persons who appeared to be healthy, **group II**; type 2 diabetic patients without DR (n=15) and **group III**; type 2 diabetic patients with DR (n=15). All groups were age and sex-matched and were investigated by ELISA to evaluate the serum level of Fetuin-A, besides CBC parameters and its related inflammatory markers. Comparison study shows; a non-significant increase of Fetuin-A value (P=0.539), a significant increase of diabetic duration (P=0.041), a significant decrease of PLR (p=0.019), as well as significant decrease of NLR (P=0.041) in the group of diabetic retinopathies compared with that without retinopathy. Although there is non-significant increase of Fetuin-A value in the diabetics with and without retinopathy compared with that in the non-diabetics, there is a significant negative correlation between Fetuin-A value and RDW% in the apparently healthy group (0.03). There is highly significant negative correlation between FBG and HCT% (P=0.001), significant positive correlation between 2HPP and LMR (P=0.002), significant positive correlation between HbA1c and LMR (P=0.030), as well as significant positive correlation between 2HPP and diabetic duration(P=0.031) in the diabetic retinopathy group. There is a significant negative correlation between diabetic duration and HCT% (P=0.043) in the diabetics without retinopathy. Diabetes type 2 retinopathy is a complication of a sophisticated diabetic disorder associated with a state of immunodeficiency, systemic inflammation and anemia that need to be investigated beside the glycemic markers and diabetic duration to avoid serious complications. Fetuin-A has no significant role in the pathogenesis of diabetic retinopathy, yet it may have a role in RBCs development and maturation.

Keywords: DM, DR, T2DM, Fetuin-A.

1. Introduction

The DR is the most common and feared ophthalmic complication due to T2DM and signs of it are present in one-third of T2DM patients at the time of diagnosis. Over 60% of patients with DM type 2 will develop DR after 20 years of disease onset [1]. The DR is one of the leading causes of vision loss worldwide and preventable blindness in adults aged 20-74 years, particularly in higher-income countries [2]. According to the WHO report, DR accounts for 4.8% (~37 million cases) of blindness worldwide [3].

Fetuin-A is a member of a multifunctional glycoprotein exclusively secreted from the hepatocytes in human, it could be an early initiator of DR pathogenesis due to its dual role of insulin resistance as well as neovascularization and angiogenesis. Fetuin-A can stimulate several potent angiogenic factors, such as VEGF which is prominently involved in the pathogenesis of neo-vessels in DR. Fetuin-A is considered as an endogenous inhibitor of insulin receptor tyrosine kinase in the muscles and liver that results in decreased insulin signalling and insulin resistance [4].

Inflammatory abnormalities can be detected by an increase in WBCs consisting of several subtypes, including monocytes, lymphocytes, and granulocytes (neutrophils, eosinophil, and basophils). These cells and the NLR are crucial for innate and adaptive immune responses to attack organisms, and their numbers are influenced by infection, stress, and inflammation. Elevated WBCs and NLR were associated with increased severity of glycosylated hemoglobin. Also, WBCs and NLR were associated with increased insulin resistance. As such, both WBCs and NLR are key biological markers for evaluating T2DM [5].

The erythrocytes in diabetic patients face multiple risks, such as hyperglycemia, hyperosmolarity, oxidative stress, inflammation, and lipid metabolism disorder, which lead to increased aggregation, reduced cell deformability, and reduced membrane fluidity. These changes in erythrocytes eventually give rise to microcirculation disorder [6]. Evaluate the level of Fetuin-A as well as CBC parameters and its related inflammatory markers in DR patients as markers that may help to predict the disease occurrence and may help to follow up and avoid complications along the disease course.

2. Patients and Methods

The study design is a case-control study This study was conducted on 45 participants aged (25-60 years) of both sexes. Patients were recruited from the ophthalmology department at Al-Zahraa University Hospital during the period between March 2021 and October 2021. The T2DM were diagnosed according to the criteria established by the ADA.

2.1 Exclusion criteria

All patients known to have a history of micro and macrovascular complications of DM other than DR. Also, patients with cardiovascular disease, stroke or transient ischemic attacks, liver disease, evidence of sepsis and any autoimmune diseases such as systemic lupus erythematosus were excluded.

2.2 The matching criteria

The controls were age and sex-matched, free of personal history of diabetes disease.

2.3 All participants were subjected to the following

Complete medical history, clinical examination, complete ophthalmologic

examination and funds photography were done.

2.4 Laboratory investigations included

CBC, fetuin-A serum values evaluation and estimation of FBG, 2HPP and HbA1c.

2.5 Sample collection and preparation

Approximately 6 ml of peripheral venous blood were withdrawn aseptically from each participant in the morning after they had fasted for 8 hours and were divided into three tubes: 1) the first tube contained 2 ml of anticoagulated blood with EDTA for CBC and HbA1c, 2) the second plain tube contained 2 ml of blood left to clot and sera were separated for immediate measurements of FBG, 3) the third plain tube contained 2 ml of blood. The serum was separated and stored at −20°C till time of measurement of Fetuin-A in one assay to avoid repeated freezing and thawing process. Fetuin-A was analyzed according to the manufacturer's instructions by ELISA with a complete set of ELISA reader model Plate 1851, using Human Fetuin A ELISA Kit (Cat. NO. E1386Hu), supplied by Bioassay Technology Laboratory (China) and 4) after 2 hours the participants had a meal with at least 75grams of carbohydrates, 2 ml of venous blood were withdrawn aseptically in a plain tube, and sera were separated for immediate measurement of 2HPP blood glucose level.

2.6 Methods

CBC was performed using a fully automated hematology analyzer (Sysmex KX21N, Kobe, Japan), b. HbA1c was performed by fully automated chemistry analyzer cobas c 311 based on the turbidimetric inhibition immunoassay with reference range between 4% and 5.6%, c. fasting blood sugar and 2HPP were performed by fully automated chemistry analyzer cobas c 311(Germany). Serum FBG (with normal reference range 65-99 mg/dl) while serum 2HPP blood sugar with normal reference value less than140 mg⁄dl and d. enzyme-linked immunosorbent assay was used to quantify level of human Fatuin A. The Fatuin A in samples was captured by the purified specific antibody that was precoated into the microwells. Following washing, a specific monoclonal antibody conjugated with streptavidin-horseradish peroxidase was added to detect the captured Fatuin A. For signal development, substrate solution was added, and then sulfuric acid solution was used to stop color development and the color intensity, which is proportional to the quantity of bound protein was measured at 450 nm. The detection range of Fatuin A was 0.75–15 μmol/l.

2.7 Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data were summarized using mean and standard deviation for normally distributed quantitative variables or median and interquartile range for non-normally distributed quantitative variables. Correlations between quantitative variables were done using Spearman correlation coefficient. P-values $\leq$ 0.05 were considered as statistically significant and $\leq$ 0.001 were considered highly significant.

3. Results

This study included 45 participants (30 diabetic patients (T2DM) and 15 age and sex-matched healthy. There were 17 males and 28 females, ranging in age from 25 to 60 years, according to the clinical and laboratory data (FBG, 2HPP, and HbA1c), the T2DM patients of this study were divided according to fundus examination into; diabetic patients with retinopathy (n=15) and diabetic patients without retinopathy (n=15). All the participants were investigated for FBG, 2HPP, HbA1c,

and CBC parameters (RBCs, Hb, HCT, MCV, MCH, MCHC, RDW, PLT and MPV), that were presented as mean, standard deviation and range. While TLC, WBCs differential counts and ratios as well as Fetuin-A values were presented as median and interquartile range (IQR).

Statistically significant results are presented in tables (1 to 3), and figures (1 to 9). There is a highly significant increase in the diabetic patients without retinopathy group regarding FBG mg/dl ($p< 0.001$), 2HPP mg/dl ($p< 0.001$), and HbA1c% ($p< 0.001$) and a significant decrease regarding Plt × 10^3 /uL ($p=0.048$) compared with the healthy group. On the other hand, there are no significant differences between the two groups regarding RBCs 10^6/µL, Hb g/dl, HCT%, MCV fl, MCH pg, MCHC g/dl, RDW% and MPV fl (Table 1).

Table (1): Comparison between diabetic patients without retinopathy group and apparently healthy control group regarding blood glucose level, Hb, HCT, RBCs and PLT count and indices.

	Apparently healthy control (n=15)		Diabetic patients without retinopathy (n=15)		
	Mean	Standard Deviation	Mean	Standard Deviation	P value
FBG mg/dl	89.87	6.91	169.00	62.04	< 0.001
2HPP mg/dl	100.67	8.34	293.40	103.69	< 0.001
Glycated Hemoglobin A1c %	4.54	0.71	7.03	1.44	< 0.001
RBCs 10^6/µL	4.63	0.58	4.48	0.42	1.000
Hb g/dl	12.21	1.10	12.35	1.03	1.000
HCT%	41.07	3.51	38.33	4.28	0.242
MCV fl	84.06	6.06	82.46	5.37	1.000
MCH pg	26.32	1.82	26.09	2.49	1.000
MCHC g/dl	30.43	1.40	31.56	1.82	0.587
RDW%	14.40	3.44	13.52	3.03	1.000
Plt × 10^3 /uL	326.07	74.64	255.20	88.13	0.048
MPV fl	8.39	1.23	9.64	1.70	0.106

There is a highly significant decrease in the diabetic patients with retinopathy group regarding PLR ($p< 0.001$) and a significant decrease regarding NLR ($p=0.019$) compared with the apparently healthy group. The healthy control group showed NLR ranged 1.08-7.2, LMR ranged 1.3-7.2, PLR ranged 84.11-393.9 and Fetuin-A values ranged 130-1109 mg/ml (Table 2).

Table (2): Comparison between diabetic patients with retinopathy group and apparently healthy group regarding diabetic duration, serum Fetuin-A level, TLC, WBCs differential counts and ratios.

	Apparently healthy control (n=15)			Diabetic patients with retinopathy (n=15)			P value
	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	
Fetuin-A mg/ml	253.80	183.80	411.20	334.80	274.20	473.00	0.106
TLC × 10^3 /uL	7.00	5.10	8.51	6.23	5.40	8.44	0.935
Absolute Neutrophils count 10^3 /Ul	4.25	2.81	5.30	2.81	2.37	4.12	0.126
Absolute Lymphocyte count 10^3 /uL	1.80	1.24	2.36	2.13	1.84	2.60	0.126
Absolute Monocyte count 10^3 /uL	0.42	0.35	0.61	0.52	0.32	0.61	0.512
Neutrophils/ Lymphocytes ratio	1.90	1.29	3.20	1.39	1.11	1.70	**0.019**
Lymphocytes / Monocytes ratio	4.30	2.95	6.00	4.89	3.51	6.39	0.436
Platelets/ Lymphocytes ratio	198.20	122.60	231.40	86.10	70.40	145.40	**< 0.001**

There is a significant increase in the diabetic patients with retinopathy group regarding diabetic duration (years) (p=0.041), while there is significant decrease in the NLR (p=0.041) and PLR (p=0.019) compared with diabetic patients without retinopathy. On the other hand, there are no significant differences between the two groups regarding Fetuin-A mg/ml, TLC ×10³/uL, WBCs differential counts and LMR (Table 3).

Table (3): Comparison between diabetic patients without retinopathy group and diabetic patients with retinopathy group regarding diabetic duration, serum Fetuin-A level, TLC, WBCs differential counts and ratios.

	Diabetic patients without retinopathy (n=15)			Diabetic patients with retinopathy (n=15)			P value
	Median	1st quartile	3rd quartile	Median	1st quartile	3rd quartile	
Diabetic duration (years)	4.00	1.00	12.00	15.00	7.00	20.00	**0.041**
Fetuin-A mg/ml	310.40	238.30	431.30	334.80	274.20	473.00	0.539
TLC × 10^3 /uL	5.70	4.60	7.30	6.23	5.40	8.44	0.539
Absolute Neutrophils count 10^3 /uL	3.84	2.32	4.30	2.81	2.37	4.12	0.653
Absolute Lymphocyte count 10^3 /Ul	1.92	1.13	2.10	2.13	1.84	2.60	0.106
Absolute Monocyte count 10^3 /uL	0.42	0.31	0.63	0.52	0.32	0.61	0.539
Neutrophils/Lymphocytes ratio	1.76	1.20	2.67	1.39	1.11	1.70	**0.041**
Lymphocytes/Monocytes ratio	3.60	2.50	7.10	4.89	3.51	6.39	0.267
Platelets/ Lymphocytes ratio	142.00	94.00	228.90	86.10	70.40	145.40	**0.019**

There is a significant negative correlation between HbA1c % and PLR in the healthy group (P=0.042) (Fig. 1). There is a significant negative correlation between Fetuin-A mg/ml and RDW % in the apparently healthy group (P value=0.035) (Fig. 2). There is a significant increase in the diabetic duration in the diabetic patients with retinopathy group compared with the diabetic patients without retinopathy group (P value=0.041) (Fig. 3). There is significant negative correlation between diabetic duration (years) and HCT% in the diabetic patients without retinopathy group (P= 0.043) (Fig. 4). There is significant decrease in the PLR in the diabetic patients with retinopathy group compared with diabetic patients without retinopathy group (P value=0.019) (Fig. 5). There is a significant decrease in NLR in the diabetic patients with retinopathy group compared with the diabetic patients without retinopathy group (P value=0.041) (Fig. 6). There is a highly significant negative correlation between FBG mg/dl and HCT % in the diabetic patients with retinopathy group (P= 0.001) (Fig. 7). There is significant positive correlation between 2HPP mg/dl and LMR in the diabetic patients with retinopathy group (P= 0.002) (Fig. 8). There is a significant positive correlation between HbA1c % and the LMR in the diabetic patients with retinopathy group (P= 0.030) (Fig. 9).

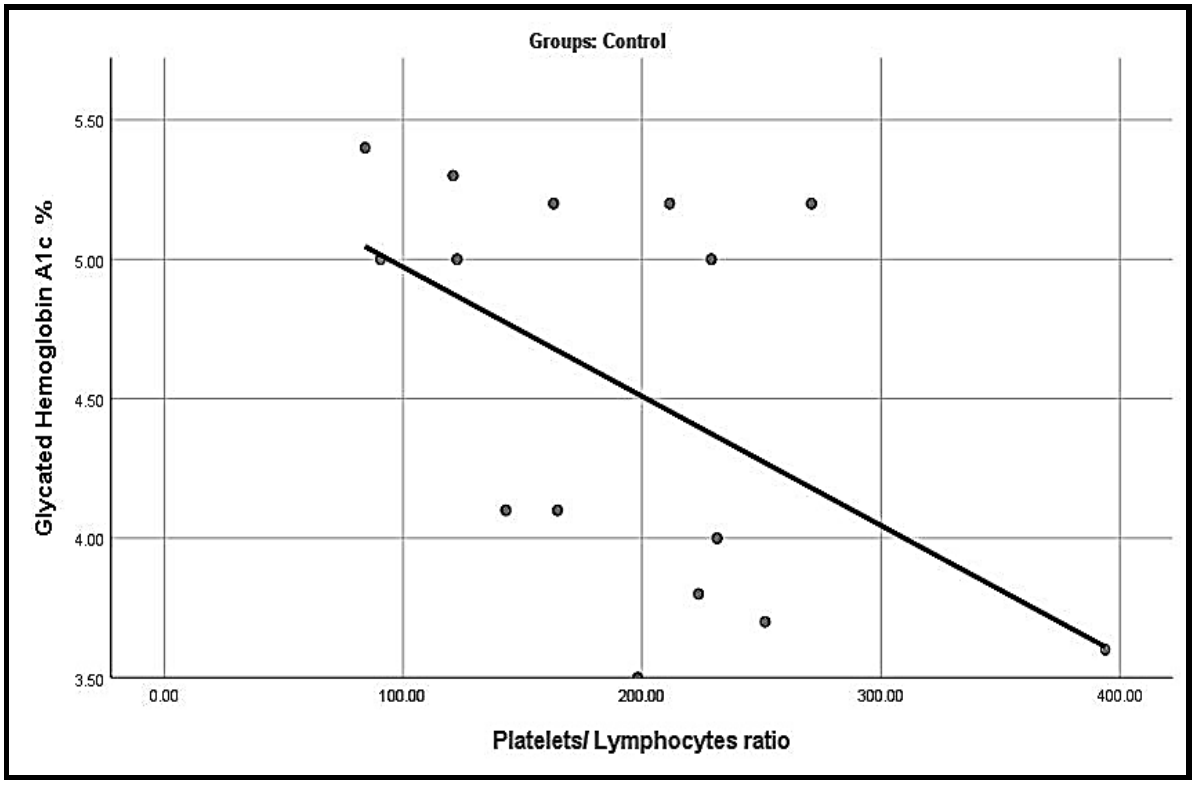

Figure (1): Correlation between HbA1c % and platelets/lymphocytes ratio in apparently healthy group.

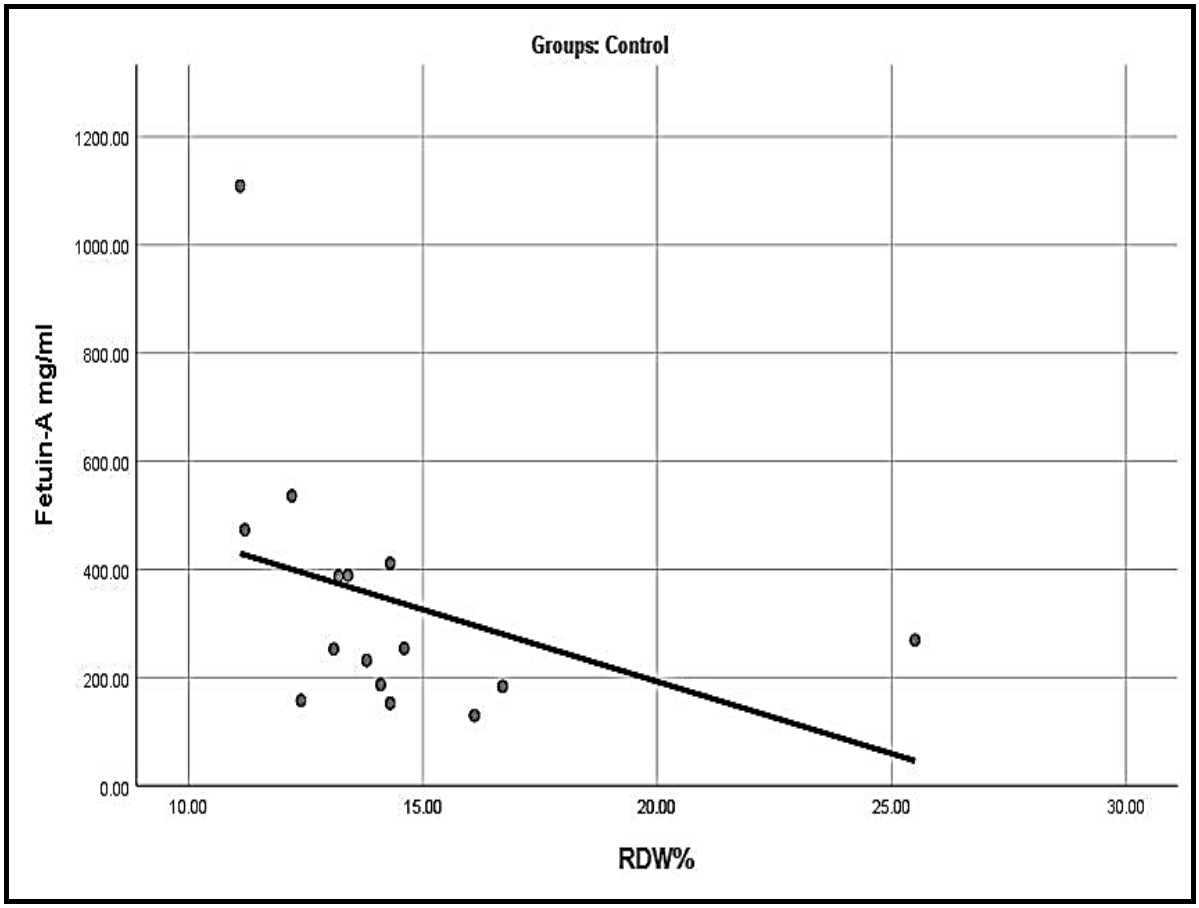

Figure (2): Correlation between Fetuin-A mg/ml and RDW % in the apparently healthy group.

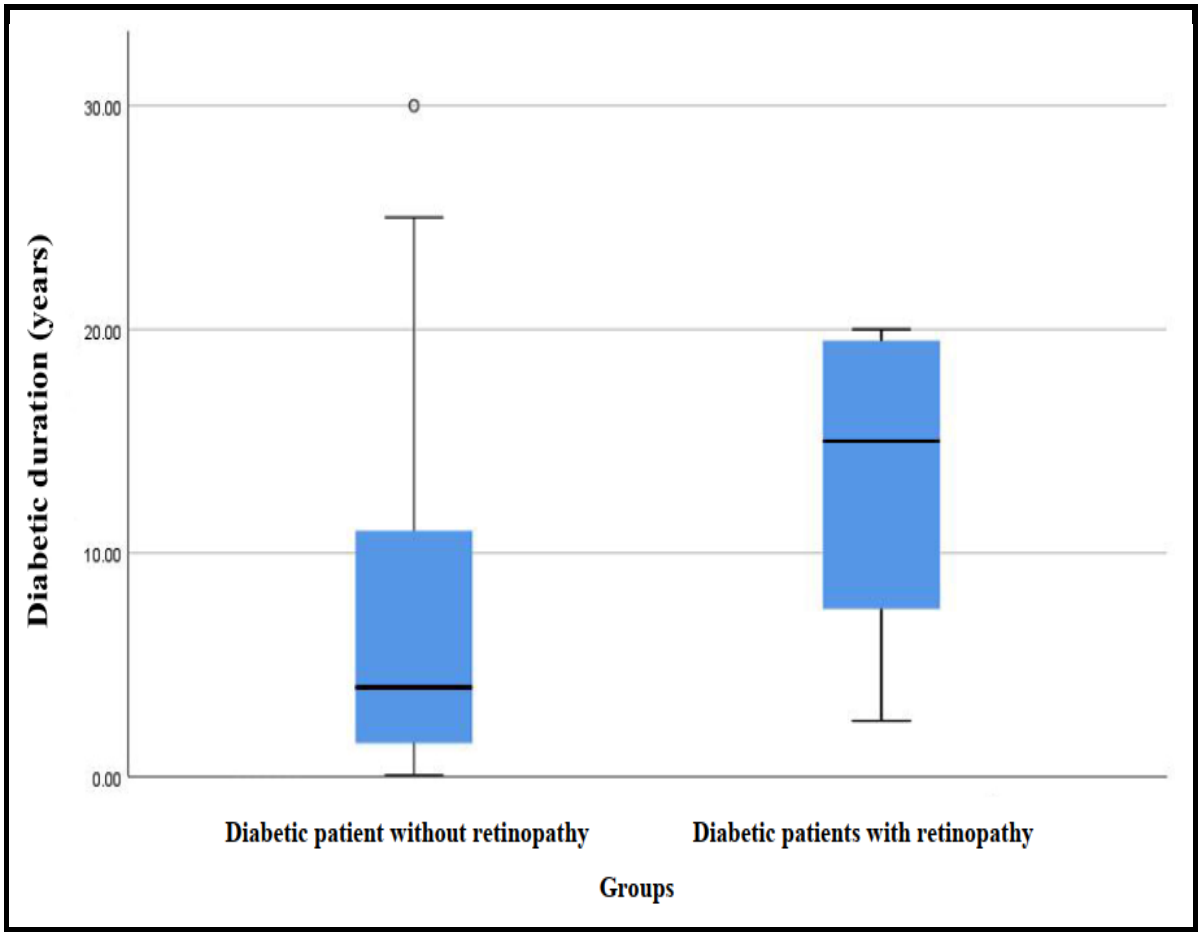

Figure (3): Comparison between diabetic patients with retinopathy group and diabetic patients without retinopathy group regarding diabetic duration (years).

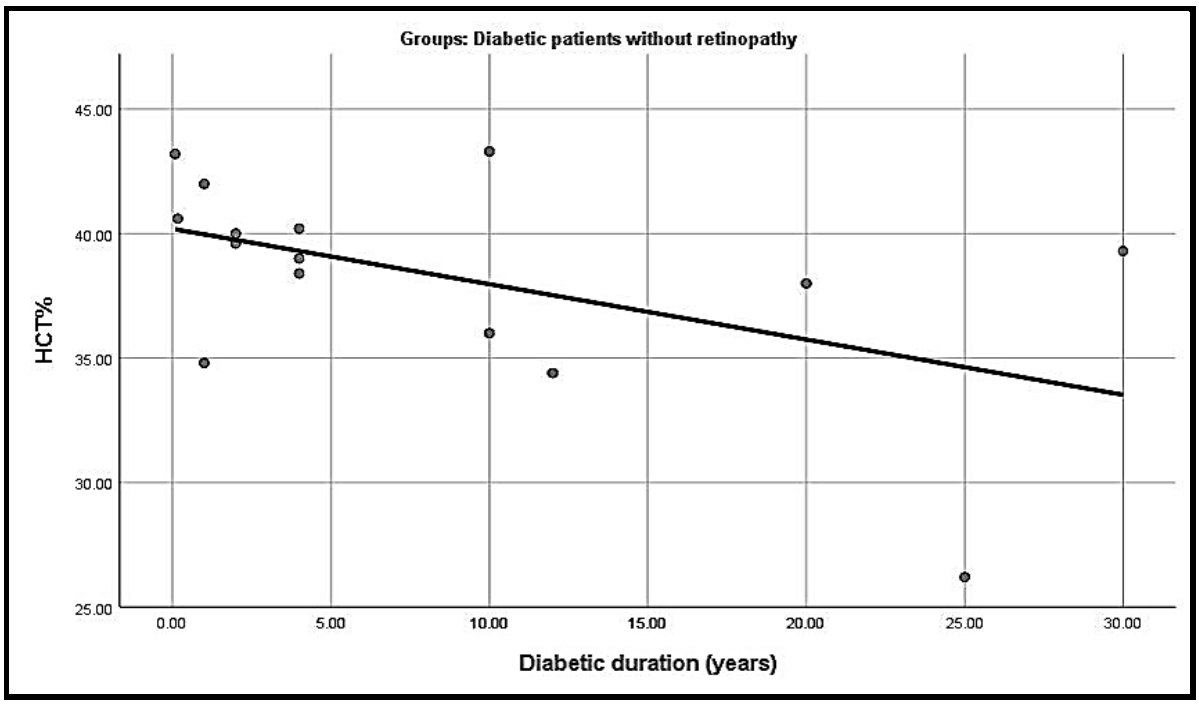

Figure (4): Correlation between diabetic duration (years) and HCT % in the diabetic patients without retinopathy group

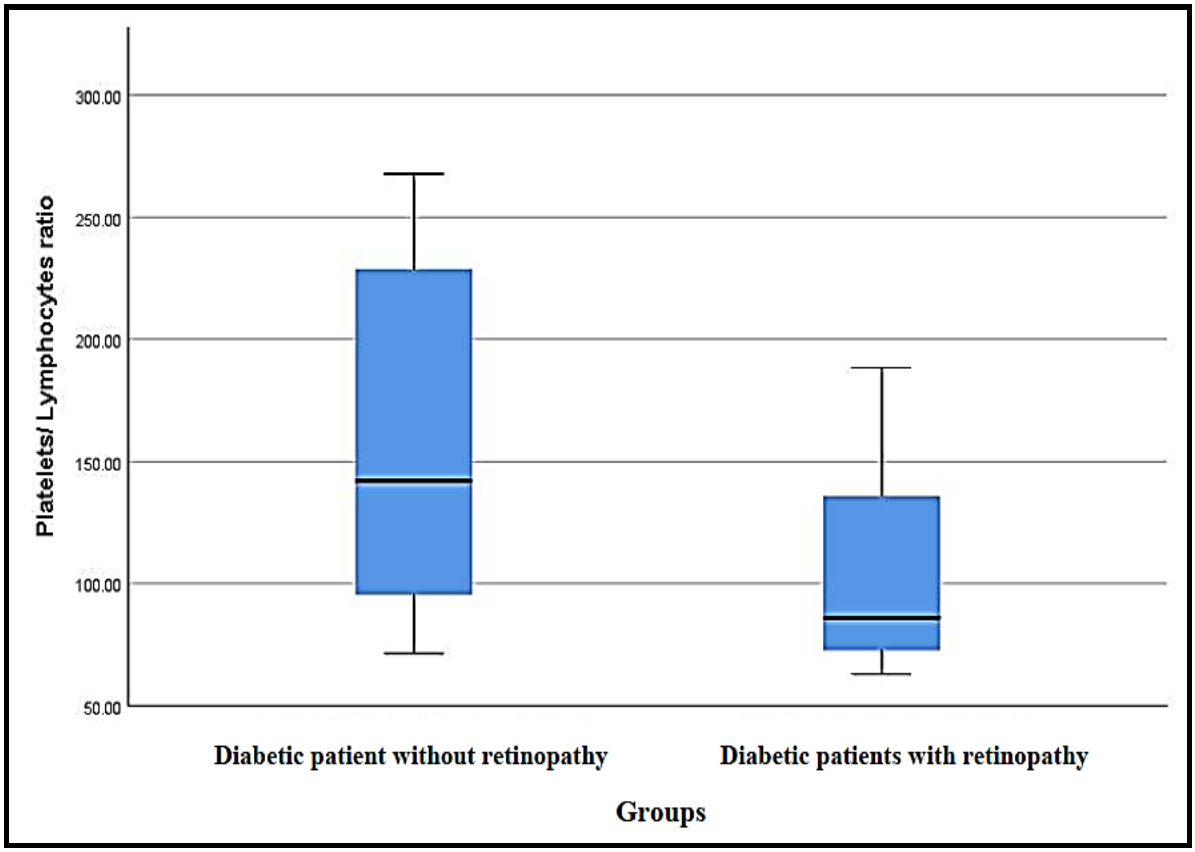

Figure (5): Comparison between diabetic patients with retinopathy group and diabetic patients without retinopathy group regarding PLR.

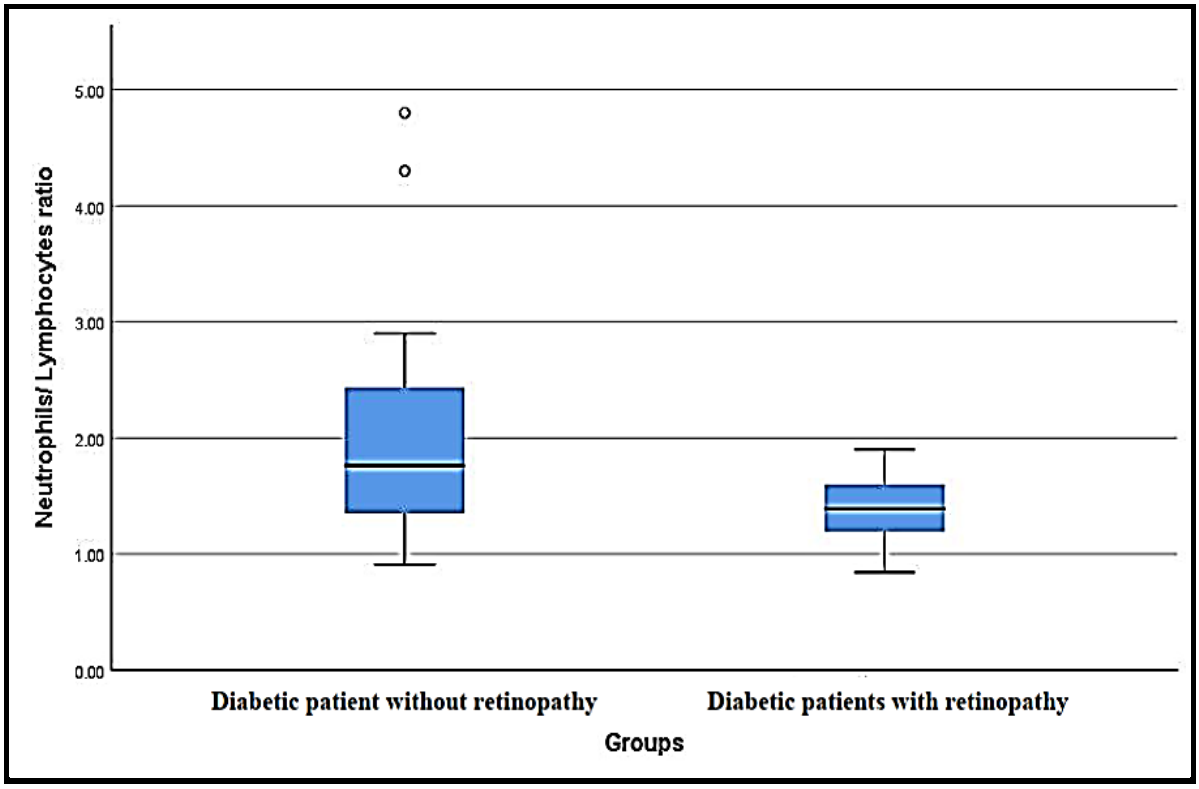

Figure (6): Comparison between diabetic patients with retinopathy group and diabetic patients without retinopathy group regarding the NLR.

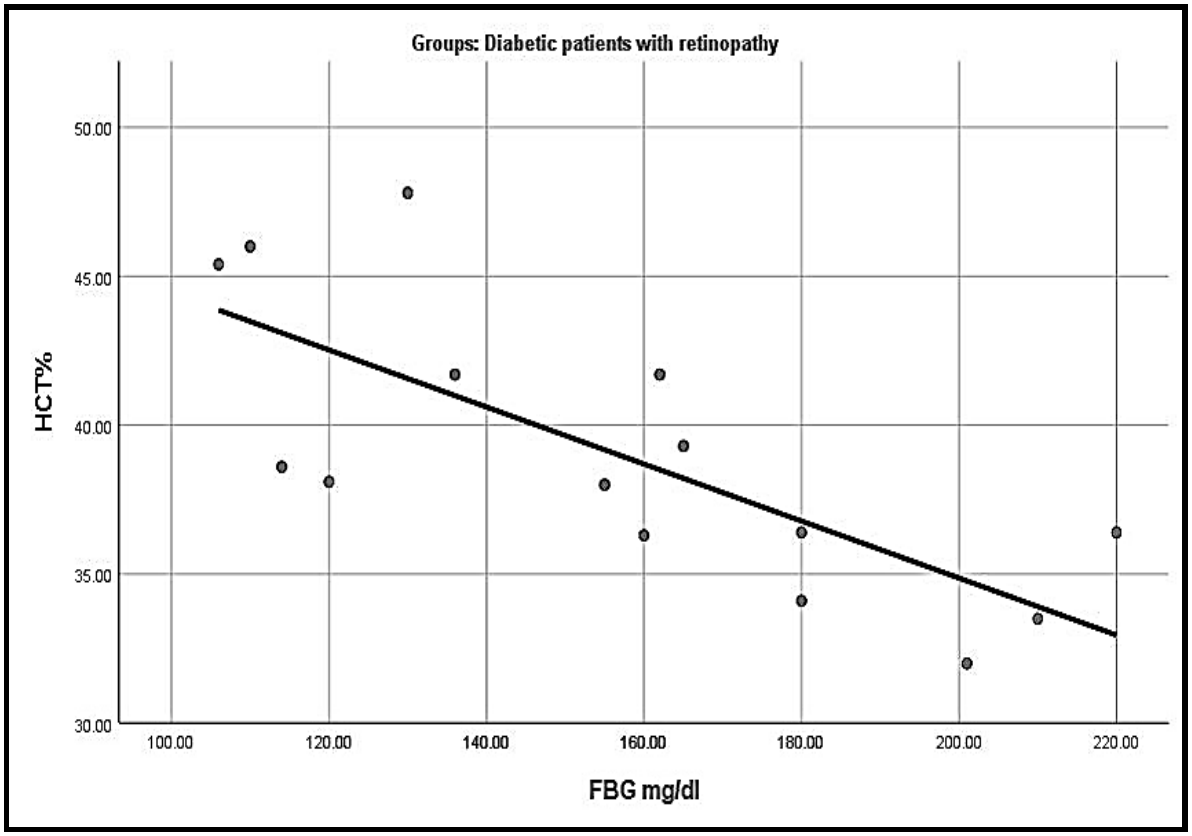

Figure (7): Correlation between FBG mg/dl and HCT % in the diabetic patients with retinopathy group.

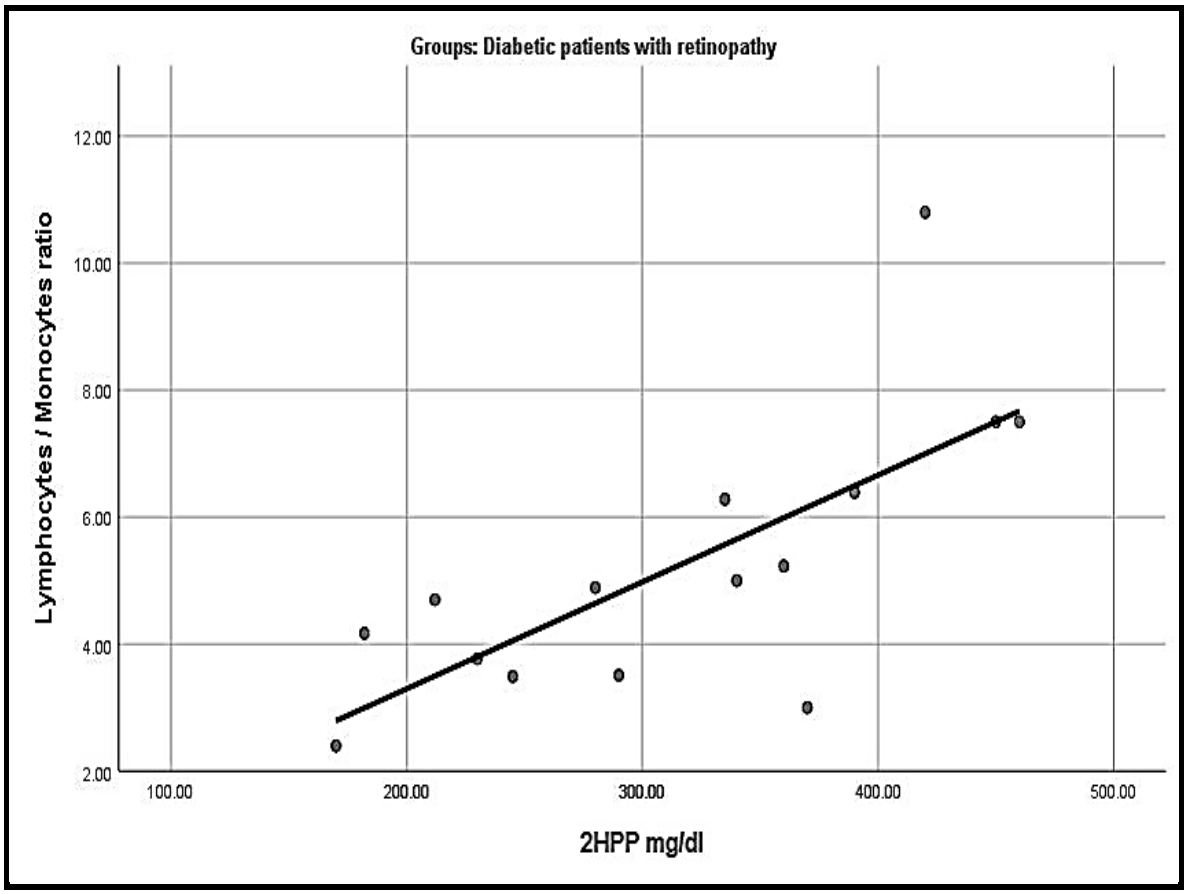

Figure (8): Correlation between 2HPP mg/dl and the LMR in the diabetic patients with retinopathy group.

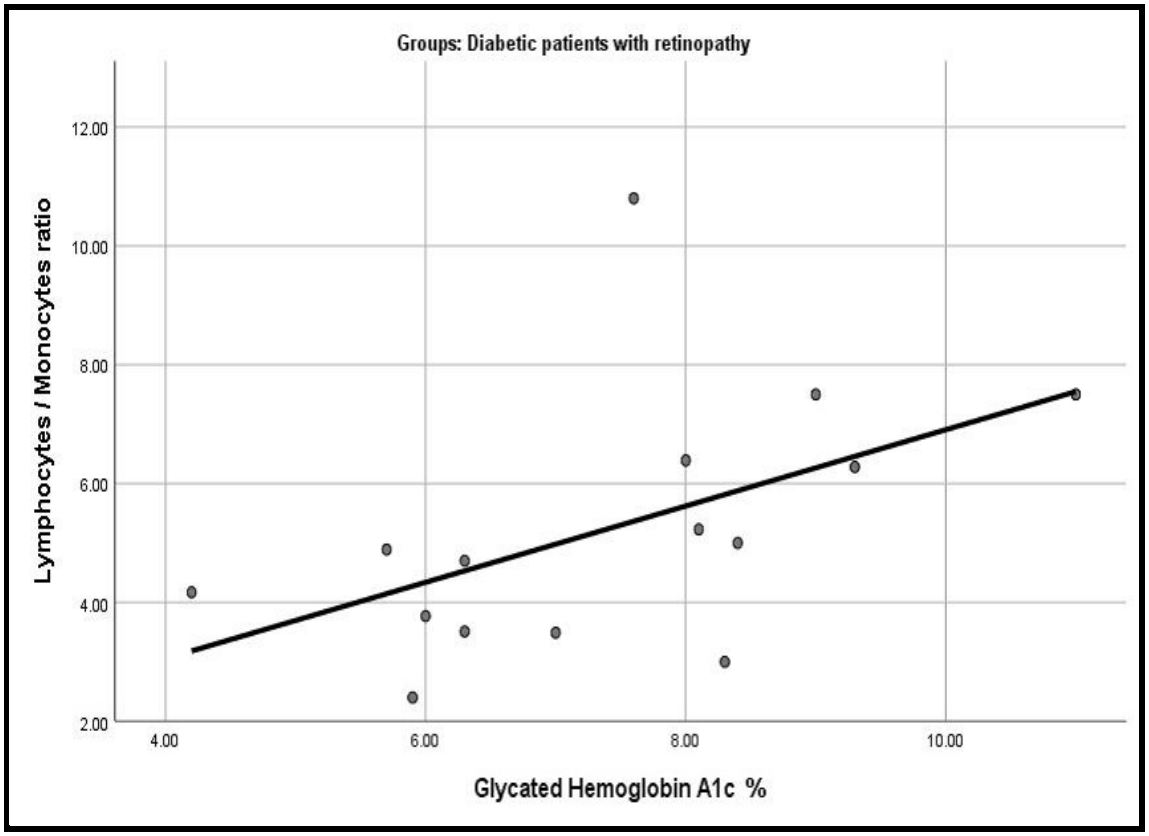

Figure (9): Correlation between HbA1c % and the LMR in the diabetic patients with retinopathy group.

4. Discussion

Diabetic retinopathy is the most common ophthalmic complication of DM and is one of the leading causes of vision loss, but preventable blindness in adults worldwide. Blindness due to DR is preventable with early detection and treatment [7]. However, examination of the retina is often delayed or missed until severe damage or even visual impairment occurs. Therefore, early detection of micro-vascular complications may give an opportunity to early identification of the pathological changes, stop, follow up or even delay the onset and improve T2DM prognosis [8]. Therefore, this study aimed to evaluate the level of Fetuin-A as well as CBC parameters and its related inflammatory markers in DR patients as markers that may help to predict the disease occurrence and may help to follow up and avoid complications along the disease course. Fetuin-A is known to act as a positive or negative acute phase protein (APP) in disease conditions [9]. Liu et al. [10] have reported that WBCs subtypes and platelets are closely associated with the inflammatory state of DR. That may indicate that peripheral blood parameters would be of great value for DR screening and follow up. There is highly significant increase of the three glycemic parameters (FBG, 2HPP and HbA1c) ($p<0.001$) and significant decrease of the platelet count in the diabetic group without retinopathy ($p=0.048$) compared with apparently healthy group (Table 1). The mean platelet count in the diabetic group is 255.2 ± 88.13 10^3/Ul. This result goes near to that reported by Akinsgum et al. [11] who published mean platelet count in diabetics as 235.29 ± 76.81 10^3/uL. They reported a significant increase in the mean platelet count of the diabetics compared with the control group, but that returned to lower value with treatment. In contrast, Ephraim et al. [12] found no significant difference in platelet count between diabetics and non-diabetic controls, while Arkew et al. [13] reported that platelet count was significantly higher in T2DM

compared to the controls. Tables (2, 3) and Fig (5, 6) show a significant decrease of both the NLR and the PLR in the diabetic patients with retinopathy group compared with diabetic patients without retinopathy and apparently healthy groups. Using IQR in statistics, the median values were as follow: -The apparently healthy group 1.9 and 198.2 respectively, -The diabetic patients without retinopathy group: 1.76 and 142.0 respectively and -The diabetic patients with retinopathy group: 1.39 and 86.1 respectively **.**

Being stable and efficient markers of inflammation, these results of the NLR and the PLR could be due to deficient immune responses in the diabetic patients that is manifested more in the diabetic retinopathy group. These results partially agree with Wan et al. [7] they concluded that higher NLR is associated with an increased prevalence of cardiovascular disease, cerebrovascular diseases and diabetic kidney diseases, other than DR in diabetic adult patients**.** Ilhan et al. [14] published that the NLR value of 2.11 or more predicts the DR with a sensitivity of 76% and a specificity of 80%. Again, Ilhan et al. [15] reported the NLR cut off value of ≥2.26 was identified as an indicator of the pathogenesis of the diabetic macular edema with high sensitively and specificity. Also, the NLR of the non-diabetic macular edema group was higher than that of the control group. In contrast to these results, compared with non-diabetic retinopathy patients, the NLR and the PLR values were significantly higher among DR patients, NLR=2.36 ± 1.16 in the DR group versus 1.97 ± 1.06 in the NDR group ($p \leq 0.001$), while the PLR = 11.62 ± 4.55 in the DR group versus (10.56 ± 4.45) in NDR ($p=0.012$) [6]. Again Ciray et al. [16] found that the NLR was not significantly different in diabetic patients with and without retinopathy and there was no correlation of NLR with the severity of the disease **.**

El Sayd and Araby [17] reported that patients with micro-vascular and macro-vascular complications of diabetes had significantly higher NLR and PLR than non-diabetic patients. Öztürk et al. [18] reported that diabetic patients with complications had higher NLR than diabetics without complications. This discrepancy may be due to differences in the immune system response as well as diabetic control**.**

Shiny et al. [19] reported that NLR is correlated with increasing severity of glucose intolerance and insulin resistance. Adnyani et al. [20] reported that there were significant differences between the NLR of the controlled and the uncontrolled T2DM groups. Higher HbA1c levels were associated with higher degree of inflammation. Increased NLR level is associated with elevated HBA1c and poor glycemic control in patients of T2DM**.[21]**

Also, Demirtas et al. [22] confirmed that PLR values were found to be independent predictors of impaired glucose regulation in diabetic patients**.**

Fig (8, 9) shows that there are significant positive correlation between both of 2HPP and HbA1c and the LMR in the diabetic patients with retinopathy group ($p=0.002$) and ($P= 0.030$) respectively, although tables (2 and 3) show no significant change regarding LMR in the comparison between DR group and both of apparently healthy and diabetics without retinopathy group. These results may suggest an inflammatory role of the glycemic markers and a predicative significance of the LMR in T2DR. This suggestion is nearer to that reported by Hunag et al. [23] who reported clinical and predictive significance of plasma fibrinogen concentration combined monocyte/ lymphocyte ratio (MLR) in the DR patients and Yue et al. [24] who reported that the use of the MLR to predict DR**.** The results of the present study concerning the DR group may suggest the fact that this group of diabetic patients seems to be associated with a degree of systemic inflammation masked by impaired immune response. The present study shows a non-significant increase in

Fetuin-A levels in DR group compared with NDR group and in the NDR group compared with the non-diabetic group (Tables 2, 3). These results are in agreement with Jung et al. [25] who reported that serum Fetuin-A levels did not differ among patients with and without retinopathy. These results may indicate that Fetuin-A does not play a role in the pathogenesis of DR. Again, Al-Saida et al. [26] reported that there was no significant increase in the mean value of Fetuin-A in patients with diabetic retinopathy compared with diabetic patients without micro-vascular complications**.**

On the line, serum Fetuin-A levels showed no significant difference between patients with T2DM and controls [27]. Also, Jung et al. [25] reported that mean serum Fetuin-A levels were not significantly different in T2DM patients with and without DR. Mori et al. [28] found no significant difference in serum Fetuin-A levels between type 2 diabetic and non-diabetic group. In contrast El-Deeb et al. [29] reported that T2DM patients had significantly higher level of Fetuin-A in comparison with non-diabetic control. In agreement with this study, Song et al. [30] reported that higher Fetuin-A concentrations were associated with type 2 diabetes and insulin resistance. Keskin et al. [31] reported a significant increase in Fetuin-A level in type 2 diabetes patients when compared to non-diabetics**.**

Also, data reported by Zhou et al. [4] showed that Fetuin-A was positively associated with insulin resistance in patients with newly diagnosed type 2 diabetes, but he demonstrated that serum Fetuin-A concentrations were positively associated with serum VEGF levels independent of other factors in type 2 diabetic patients**.**

Fig (1) shows a significant negative correlation between HbA1c and the PLR in the apparently healthy group (P= 0.042). That may indicate a role of blood glucose at normal level in healthy conditions in systemic inflammation control**.**

Fig (2) there is a significant negative correlation (p=0.035) between serum Fetuin-A levels and RDW in the apparently healthy group. This result may indicate that Fetuin-A may have a role in RBCs production, development and or life span. Fetuin-A was inversely correlated with RDW in the total study of metabolic syndrome patients [32]. No report could be detected concerning the correlation between Fetuin-A and RDW in apparently healthy subjects or T2DM**.**n Fig (3) comparison between diabetic patients with and without retinopathy regarding diabetic duration. There is a significant increase in the diabetic duration in the diabetic patients with retinopathy (p=0.041). On the other hand, comparison between the two groups as regards the three glycemic parameters (FBG, 2HPP and HbA1c) does not show significant difference**.**

These results indicate that the diabetic duration rather than the glycemic parameters may play a role in the pathogenesis of DR.

important role in the pathogenesis of systemic inflammation and anemia in DR. Diabetic duration is an important factor in the pathogenesis of DR. Glycemic markers, anaemia markers and inflammatory markers may be considered to predict or early detect DR.

5. Conclusion

Although Fetuin-A may not have direct effect in the pathogenesis of DR, it may have a role in hemopoiesis. T2DM should not be only considered as endocrinal disease, but it may also be considered as a sophisticated endocrinal, metabolic, immunological and inflammatory systemic disorder that may end by serious complications. The glycemic markers (FBG, 2HPP and HbA1c) may have an

6. Recommendations

Glycemic markers as well as diabetic duration should be considered through the continuous evaluation of T2DM to avoid

serious micro-vascular and macro-vascular complications that may end by DR or other diabetic complications. The role of Fetuin-A in hemopoiesis as well as association with DR needs further study. Hematological, immunological as well as inflammatory markers evaluation are worth considering and investigated as early markers to predict diabetic complications including DR.

Abbreviations

VEGF: Vascular Endothelial Growth Factor; NLR: neutrophil to lymphocyte ratio; ADA: American Diabetes Association; CBC: Complete blood count; FBG: fasting blood glucose; 2HPP: 2 hours postprandial; HbA1c: glycated haemoglobin.

Acknowledgements

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Authors' Contributions

EA is the corresponding author and has a major role in collecting the data and laboratory investigations of the patients in the study, BA made substantial contributions to the design of the work, the analysis, and interpretation of data and is a major contributor in revising the manuscript. SM made substantial contributions to the analysis, and interpretation of laboratory data and a major role in writing the manuscript, AM has a major role in doing the ophthalmologic examination and fundus photography of the patients in the study. All authors have read and approved the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate.

The study's protocol was approved by the Research Ethics Committee of the Faculty of Medicine for Girls Al-Azhar University (AFMG-201911270). Informed written consent was obtained from all participants.

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