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Evaluation of the Correlation of Trefoil Factor with Hemostatic Profile in Iraqi Diabetic Nephropathy Patients

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ABSTRACT

In the present study, the percentages of study variables including age, gender, residence, smoking habit, family history, treatment and duration of disease between study groups including patients with diabetic nephropathy, diabetic patients without diabetic nephropathy and control group were results showed presence of significant differences between study groups in age (P 0.007), family history (P<0.001), treatment (P 0.005), and duration of disease (P 0.044), while there were no significant differences between study groups in their gender (p 0.942), residence (p 0.848), and smoking (p 0.958). there were significant increase between means of biomarkers concentrations of those study groups in both smoker and nonsmoker which include trefoil (P 0.011, P 0.002), d-dimer (P 0.011, P 0.002), fibrinogen (P<0.001, P<0.001), According to smoking habit, there was no significant differences (P≤ 0.05) between smokers and non-smokers in all study groups there was significant increase (P 0.017) in trefoil concentration in diabetic without nephropathy group with increasing of duration of disease Furthermore, no significant between D-dimer (p 0.155, p 0.738) fibrinogen (p 0.935, p 0.797). There was significant increase in means of fibrinogen and d-dimer in DN groups with all duration of disease (P 0.013, P 0.002, P 0.021), (P 0.038, P 0.02, P<0.001) compared to DM group. In addition, the results showed presence of significant increase in means of trefoil concentration in DN and DM groups at age group 46-60 years (P<0.001), and there was significant increase of d-dimer concentration for DN and DM groups at age groups 30-45 years and 46-60 years (P 0.011, P 0.007) compared to control. While mean differences of fibrinogen concentration was significant increase in DN and DM groups at age groups 46-60 years and ≥ 60 years (P 0.001, P<0.001) respectively compared to control group. So, there were no significant difference between trefoil concentration in study groups including (diabetic with nephropathy, diabetic without nephropathy and control group) according to age (p 0.858, p 0.116, p 0.252), d-dimer (p 0.268, p 0.506, p 0.527), fibrinogen (p 0.307, p 0.978, p 0.703). There was significant difference between means of all biomarkers in this study for study groups with gender (male and female). These marker trefoil (P 0.001, P 0.018), d-dimer (P 0.002, P 0.005), fibrinogen (P<0.001, <0.001), Furthermore, no significant difference between males and females in all study groups (diabetic with nephropathy, diabetic without nephropathy and control group) including trefoil concentration (p 0.819, p 0.865, p 0.505), d-dimer (p 0.334, p 0.766, p 0.803), fibrinogen (p 0.207, p 0.595, p 0.15).

Objective: This study aimed to investigate the effect of diabetic nephropathy in thrombosis formation and hemostatic profile by study trefoil related with hemostasis

Keywords: Trefoil factor, Hemostatic profile, Diabetic Nephropathy, Fibrinogen, D-dimer.

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INTRODUCTION

Diabetic nephropathy (DN), also known as diabetic kidney disease, is the chronic loss of kidney function occurring in those with diabetes mellitus. Diabetic nephropathy is one of the leading causes of chronic kidney disease (CKD) and end-stage renal disease (ESRD) globally [1] .The risk factors of diabetic nephropathy are related either to chronic hyperglycemia (poor control of blood glucose), or a family history of diabetic nephropathy, long duration of diabetes, presence of other microvascular complication, pre-existing hypertension and genetic factors also play a role [2-4]. There is a paucity of sensitive and specific biomarkers for the early prediction of CKD progression [4]. Hemostatic factors are circulating proteins that are critical factors in, or indicators of the blood clotting/coagulation process. They include, but are not limited to, fibrinogen, factor VII (FVII), factor VIII (FVIII), von Willebrand factor (vWF), plasminogen activator-inhibitor 1 (PAI-1), and D-dimer [6]. The patients also may have an increased risk of thrombosis (particularly renal vein thrombosis, deep vein thrombosis) leading to an increased risk of PE as well as an increased rate of infectious complications due to a deficit in antibodies that are lost through the injured glomeruli [7; 8]. Fibrinogen is a glycoprotein complex that circulates in the blood of vertebrates. During tissue and vascular injury, it is converted enzymatically by thrombin to fibrin and then to a fibrin-based blood clot. Fibrin clots function primarily to occlude blood vessels to stop bleeding [9]. D-dimer is a marker for fibrinolytic/coagulation processes associated with disease conditions such as deep vein thrombosis and atherosclerosis. D-dimer testing was originally developed in the diagnosis of disseminated intravascular coagulation (DIC), deep venous thrombosis (DVT) or pulmonary embolism (PE), sepsis, cancer and other diseases as well as after major surgery [10]. Trefoil factors are peptides synthesized and secreted by the

mucin producing cells of epithelial surface .These peptides are thought to play an important role in maintenance and protection of mucosal surfaces in the gastrointestinal tract through an interaction with mucins, enhancement of "restitution" (i.e., rapid mucosal repair by cell migration), modulation of mucosal regeneration by differentiation from stem cells, and modulation of the mucosal immune response [11]. TFFs play a key role in the maintenance of the surface integrity of mucous epithelia in health and disease. Their multiple molecular functions include (1) formation of the mucus barrier, (2) enhancement of mucosal repair (restitution) on different levels, (3) modulation of mucosal differentiation processes, and (4) modulation of the immune response. Of special note, TFFs are also connected with oncogenic pathways [12].

MATERIALS AND METHOD

The present study is an observational case control design. The data of study were collected in the period from November 2019 to January 2020. The study was conducted in Marjan Teaching Hospital in Hilla City, Babylon province, Iraq. A total number of subjects involved in this study was 75 patients (50 patients suffering from diabetic nephropathy, 25 diabetic patients without nephropathy) and 25 as control healthy). All patients and control were from the same ethnic group (Arabic).

Research and sampling ethics

The project proposal and sampling method were approved by the Research Ethics Committee of Babylon Health Directorate according to the directorate administrative order No. 5393, date 12/11/2019. In addition, the project achieves the permission of research ethics in Marjan Medical City.

Estimation of D-dimer level: by using ELAZA kit from Boditch Biotechnology/ Korea.

Estimation of Hemostat Fibrinogen: by using ELAZA kit from Junior human/ Germany

Estimation of Human Trefoil Factor 1: by using ELAZA kit from Bioassay technology laboratory / China.

Statistical analysis

Statistical analysis was carried out using SPSS version 23. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups.

RESULTS AND DISCUSSION

Distribution of study groups by socio-demographic characteristics

In this study, the percentages of study variables including age, gender, residence, smoking habit, family history, treatment and duration of disease between study groups including patients with diabetic nephropathy, diabetic patients without diabetic nephropathy and control group were shown in Table (1). The results showed presence of significant differences between study groups in age (P 0.007), family history (P<0.001), treatment (P 0.005), and duration of disease (P 0.044), while there were no significant differences between study groups in their gender (p 0.942), residence (p 0.848), and smoking (p 0.958).

Table 41: Distribution of study groups by socio-demographic characteristics

Study variables	Study groups (%)			χ^2	P-value
	Diabetic nephropathy	Diabetic without nephropathy	Healthy control		
Age					
30-45 years	5 (10.0%)	4 (16.0%)	8 (32.0%)		
46-60 years	17 (34.0%)	13 (52.0%)	13 (52.0%)		**0.007***
≥ 61 years	28 (56.0%)	8 (32.0%)	4 (16.0%)		
Total	50 (100%)	25 (100.0%)	25 (100.0%)		
Gender					
Male	26 (52.0%)	13 (52.0%)	14 (56.0%)	0.12	0.942
Female	24 (48.0%)	12 (48.0%)	11 (44.0%)		
Total	50 (100.0%)	25 (100.0%)	25 (100.0%)		
Residence					
Urban	13 (26.0%)	5 (20.0%)	6 (24.0%)	0.329	0.848
Rural	37 (74.0%)	20 (80.0%)	19 (76.0%)		
Total	50 (100.0%)	25 (100.0%)	25 (100.0%)		
Smoking					
Yes	19 (38.0%)	10 (40.0%)	9 (36.0%)	0.085	0.958
No	31 (62.0%)	15 (60.0%)	16 (64.0%)		
Total	50 (100.0%)	25 (100.0%)	25 (100.0%)		
Family history					
Yes	27 (54.0%)	13 (52.0%)	0 (0.0%)	**22.25**	**<0.001***
No	23 (46.0%)	12 (48.0%)	25 (100.0%)		
Total	50 (100.0%)	25 (100.0%)	25 (100.0%)		
Treatment					
Insulin therapy	17(34.0%)	1 (4.0)	-		
Hypoglycemic agent	32(64.0%)	24 (96.0)			**0.005***
No treatment	1(2.0%)	0 (0.0)			
Total	50(100.0%)	25 (100.0)			

Duration of disease					
From (1-5) years	11 (22.0)	8 (32.0)	-	**6.24**	**0.044***
From (6-10) years	12 (24.0)	11 (44.0)			
≥ 11 years	27 (54.0)	6 (24.0)			
Total	50 (100.00	25 (100.0)			

* p value ≤ 0.05 was significant.

The figure (1) showed in significant increase (P=0.007) in age group ≥ 61 years in diabetic nephropathy group (56%) compared with diabetic without nephropathy and control group in which the age group 46-60 year composed the higher percentage (52%) than other age groups.

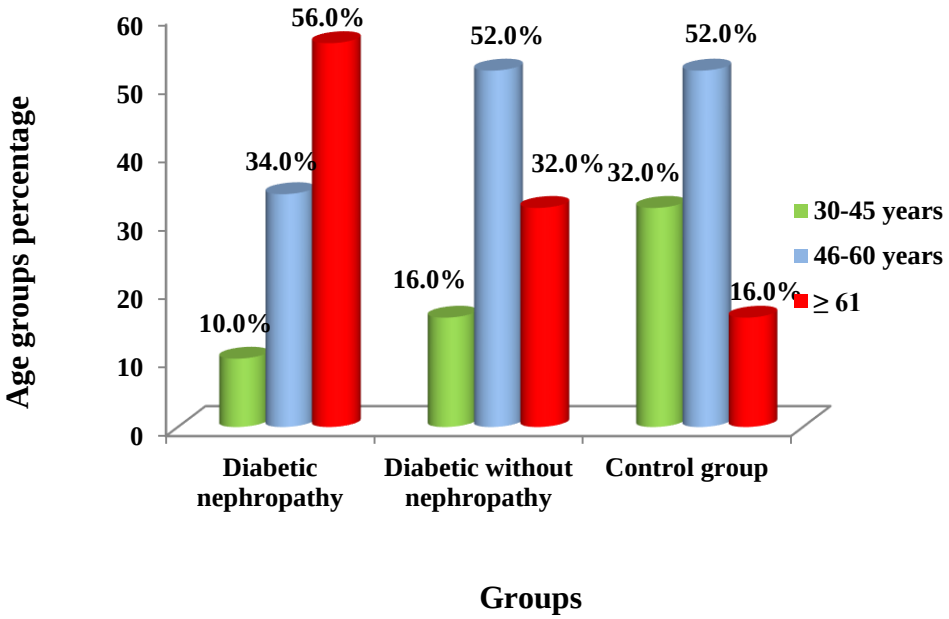

Figure 1: The age group percentages in study groups

However, the differences in the percentages of family history in study groups shown in figure (2) which showed that, there were significant increase in diabetic groups had family history (X2=22.25, P <0.001) compared with control group.

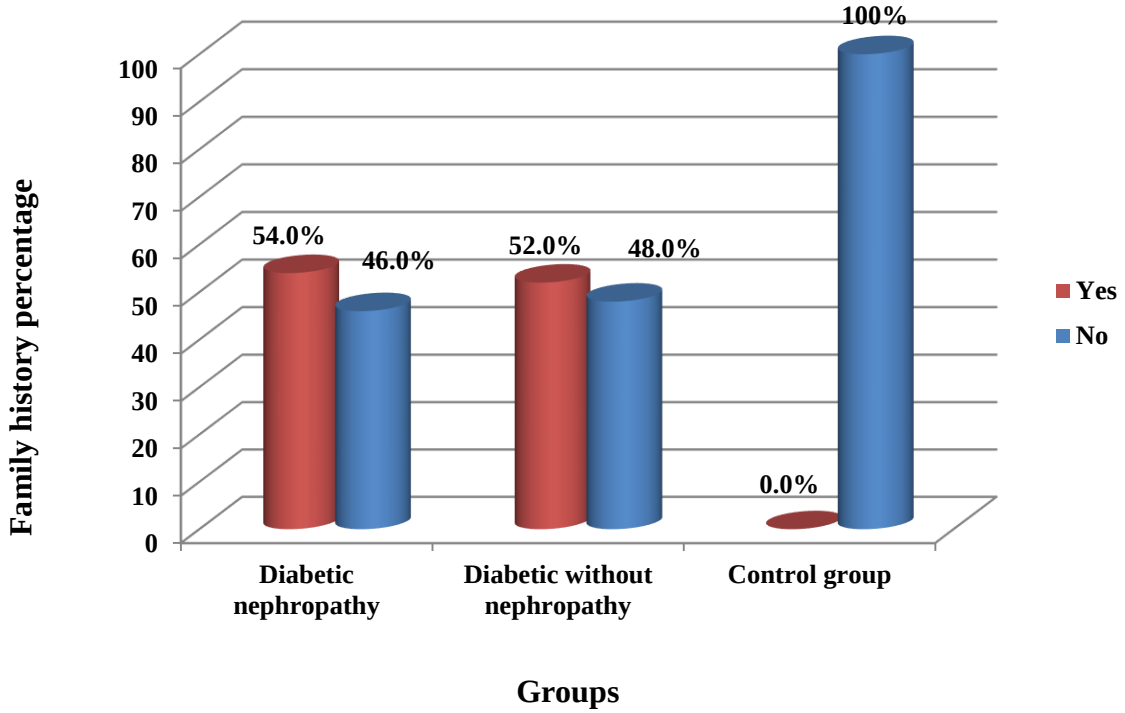

Figure 2: The percentages of family history in study groups

In figure (3), the results showed that, there were significant differences (P ≤ 0.05) between study groups (patients with diabetic nephropathy and diabetic patients without nephropathy) in duration of diabetes (P=0.044), where the higher percentage was in diabetic nephropathy patients with duration of disease ≥ 11 years.

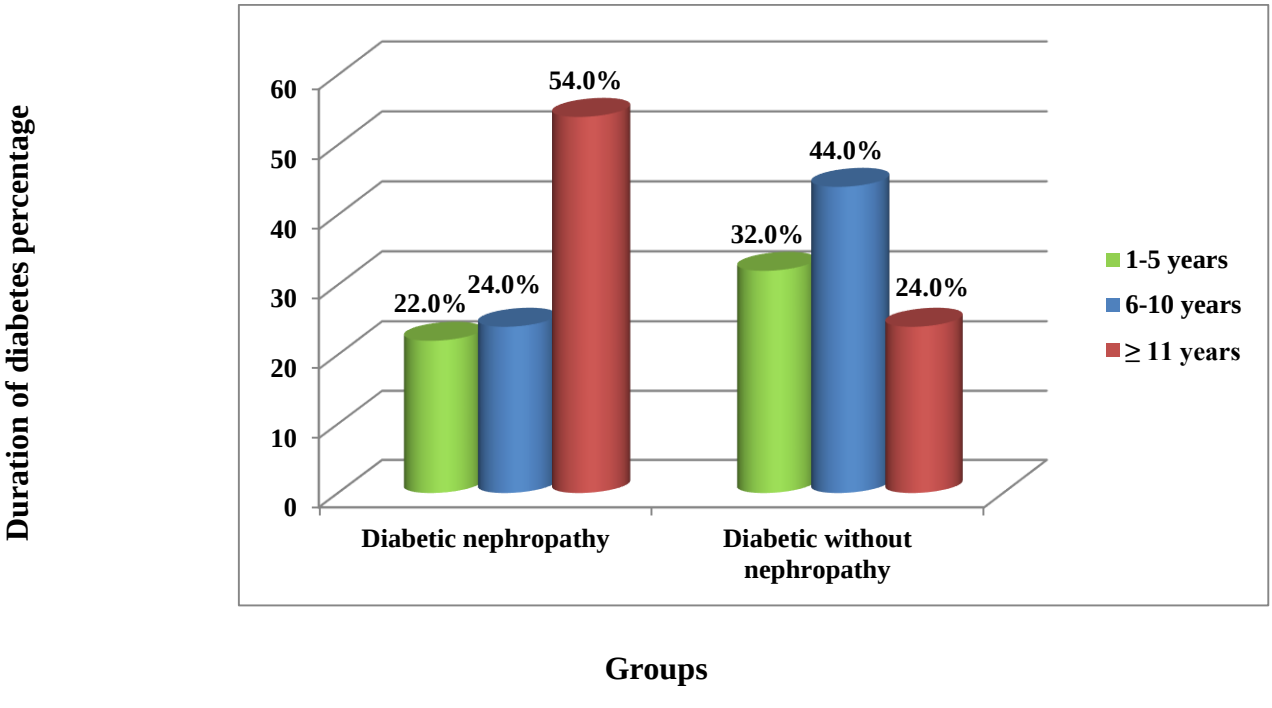

Figure 3: The percentages of diabetic patients according of duration of diabetes

In Figure (4), the results showed that, there were significant differences between study groups (patients with diabetic nephropathy and diabetic patients without nephropathy) and types of treatment of diabetes (P 0.005).

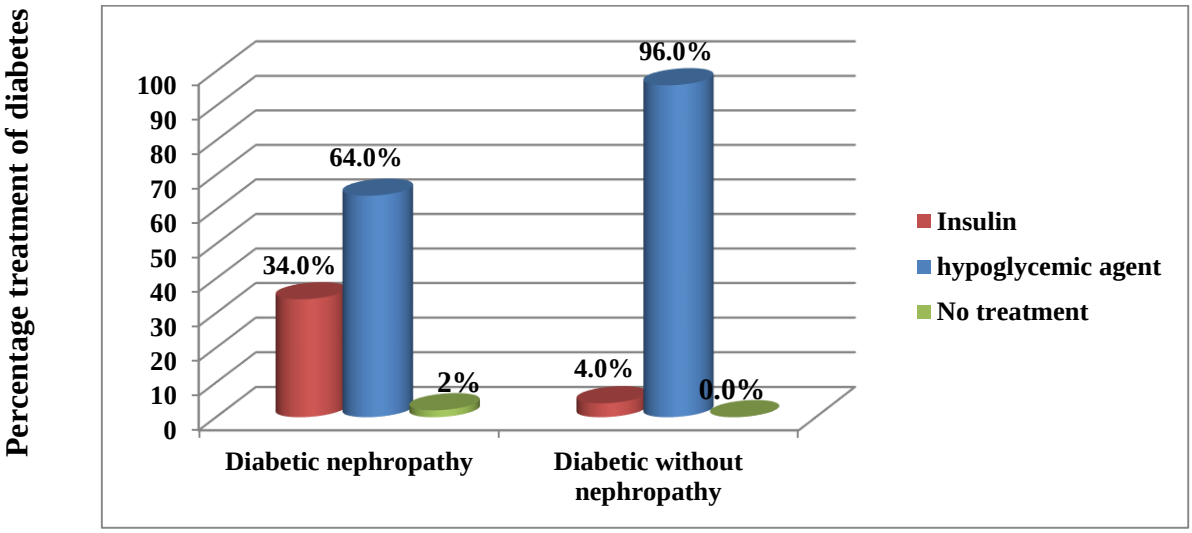

Figure 4: The mean differences between treatment of diabetes and study groups

These results were agreement with results obtained by [13] who found that the prevalence of diabetes and diabetic nephropathy markedly increased starting at age 30 years. A study of [14] found that patients with diabetic nephropathy was highest in patients aged 12 to <18 years. Type 2 diabetes is becoming more common with the increasing rate, and it is estimated that up to 45% of all new patients with diabetes in this age-group have type

2 diabetes [15]. With the rising prevalence of diabetes in children, a rise in diabetes-related complications, such as nephropathy, is anticipated. According to family history, the results in this study were agreement with results of [16] who found that there was significant increase in diabetic groups had family history. Family studies show that genetic factors are important in the pathogenesis of DN [17]. According to duration of disease, the results were agreement with results obtained by [18] who found that the higher percentage was in diabetic nephropathy patients with duration of disease ≥ 12 years. Many patients continue in this stage for life. However, stage III, represents the first clinically detectable sign of glomerular damage and microalbuminuria (albumin 30-300 mg/day). It usually occurs 5 to 10 years after the onset of the disease with or without hypertension [19]. There is no cure for diabetic nephropathy, but treatments can delay or stop the progression of the disease. According to treatment, the results were agreement with results obtained by [20] Treatments consist of keeping blood sugar levels under control and blood pressure levels within their target range through medications and lifestyle changes [21].

Mean differences of physio-biochemical and biomarkers concentrations according to smoking

Table (2), revealed that the mean differences of biomarkers according to smoking in study groups were studied. It was found that, there were significant increase between means of biomarkers concentrations of those study groups in both smoker and nonsmoker which include trefoil (P 0.011, P 0.002), d-dimer (P 0.011, P 0.002), fibrinogen (P<0.001, P<0.001), According to smoking habit, there was no significant differences (P≤ 0.05) between smokers and non-smokers in all study groups. These results were agreement with result of [22] who found that there was significant increase between trefoil and study groups in smoker and nonsmoker. Smoking is a preventable risk factor, identify as risk factor for induce complication of diabetic nephropathy [23]. The high percentage in diabetic nephropathy patient is related to fact that there is a relationship between smoking and biomarkers indicates direct or indirect renal damage induced by smoking. [24, 25] Fibrinogen may be indirectly associated with diabetic nephropathy as a marker of unstable lesions that are undergoing sub intimal hemorrhage or with potent risk factors such as smoking [26]. Diabetic smokers are usually associated with glomerular hypertrophy, glomerulosclerosis, tubulointerstitial fibrosis and mesangial cell expansion, followed by albuminuria and reduction in the glomerular filtration rate [27]. Thus, establishing the causes of smoking mediated progression of diabetic nephropathy remains the key step towards the prevention and amelioration of this disease [28].

Table 2: Physio-biochemical and biomarkers in study groups according to smoking

Biomarkers	Study groups	Smoking habit				P-value
		N	Present (Mean ± SD)	N	Absent (Mean ± SD)	
Trefoil (ng/ml)	DN (N=50)	19	12.07 ± 4.93	31	12.97 ± 6.82	0.621
	DM (N=25)	10	11.60 ± 3.21	15	11.78 ± 4.19	0.911
	Control group	9	6.96 ± 2.21	16	6.75 ± 2.75	0.847
P-value	**Total**	38	**0.011***	62	**0.002***	
D-dimer(ng/ml)	DN (N=50)	19	2488.37 ± 2873.71	31	2141.59 ±2602.22	0.662
	DM (N=25)	10	349.55 ± 133.92	15	346.63 ± 135.30	0.958
	Control group	9	323.85 ± 172.08	16	344.35 ± 227.22	0.816
P-value	**Total**	38	**0.011***	62	**0.002***	
fibrinogen(ml/dl)	DN (N=50)	19	493.78 ± 139.73	31	474.51 ± 168.55	0.678
	DM (N=25)	10	334.75 ± 60.08	15	304.53 ± 90.38	0.326
	Control group	9	204.94 ± 23.93	16	242.93 ± 65.30	0.108
P-value	**Total**	38	**<0.001***	62	**<0.001***	

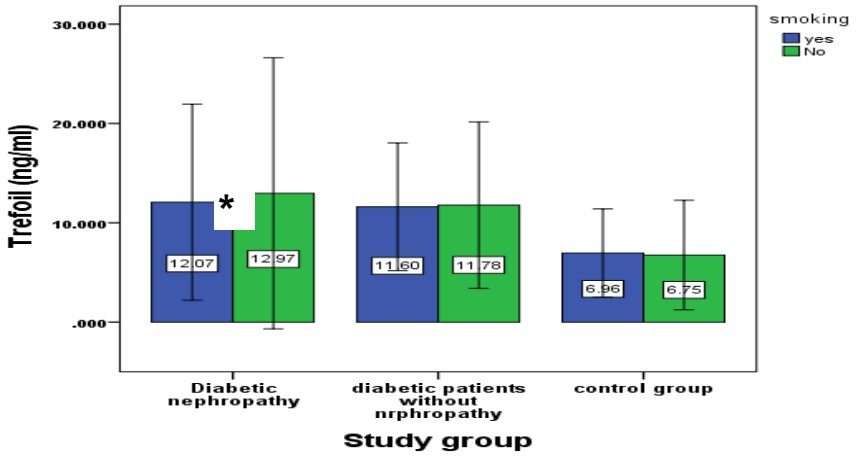

Figure 5: The mean differences of trefoil concentration according to smoking

Mean differences of physio-biochemical and biomarkers concentrations according to duration of disease:

In Table (3), the results showed that, there was significant increase (P 0.017) in trefoil concentration in diabetic without nephropathy group with increasing of duration of disease Furthermore, no significant between D-dimer (p 0.155, p 0.738) fibrinogen (p 0.935, p 0.797). There was significant increase in means of fibrinogen and d-dimer in DN groups with all duration of disease (P 0.013, P 0.002, P 0.021), (P 0.038, P 0.02, P<0.001) compared to DM group. These results were agreement with results obtained by [29] who found that trefoil concentration biomarker in diabetic without nephropathy with increasing of duration of disease, in addition, the same finding are reported by other studies [30], [31], [32], [33], in that increase duration of disease is a risk factor for development of diabetic nephropathy. Longer duration of diabetic is a risk factor for development and progression of Microvascular complication, and nearly universal finding in previous studies [34], [35].

Table 3: Mean differences of physio-biochemical and biomarkers in diabetic patients according to duration of disease

Study variables	Study groups	Duration of disease						P-value
		N	1-5 years	N	6-10 years	N	≥ 11 years	
Trefoil (ng/ml)	DN (N=50)	11	12.74 ± 5.86	12	10.77 ± 3.79	27	13.40 ± 7.02	0.476
	DM (N=25)	8	8.72 ± 4.00	11	13.21 ± 2.30	6	12.92 ± 3.70	**0.017***
P-value	Total	19	0.181	23	0.08	33	0.158	
D-dimer (ng/ml)	DN (N=50)	11	1443.29 ± 1535.41	12	3504.89 ± 3998.08	27	2064.21 ± 2208.77	0.155
	DM (N=25)	8	337.47 ± 136.23	11	370.70 ± 139.15	6	319.58 ± 128.71	0.738
P-value	Total	19	**0.038***	23	**0.02***	33	**<0.001***	
Fibrinogen (ml/dl)	DN (N=50)	11	468.18 ± 121.99	12	492.62 ± 152.20	27	482.61 ± 175.52	0.935
	DM (N=25)	8	331.68 ± 78.29	11	313.27 ± 82.49	6	302.66 ± 87.48	0.797
P-value	Total	19	**0.013***	23	**0.002***	33	**0.021***	

Mean differences of physio-biochemical and biomarkers in study groups according to age:

In table (4), the mean differences of physio-biochemical and biomarkers according to age were studied and the results showed presence of significant increase in means of trefoil concentration in DN and DM groups at age group 46-60 years (P<0.001), and there was significant increase of d-dimer concentration for DN and DM groups at age groups 30-45 years and 46-60 years (P 0.011, P 0.007) compared to control. While mean differences of fibrinogen concentration was significant increase in DN and DM groups at age groups 46-60 years and ≥ 60 years (P 0.001, P<0.001) respectively compared to control group. In addition, there were no significant difference between trefoil concentration in study groups including (diabetic with nephropathy, diabetic without nephropathy and control group) according to age (p 0.858, p 0.116, p 0.252), d-dimer (p 0.268, p 0.506, p 0.527), fibrinogen (p 0.307, p 0.978, p 0.703). These results were agreement with result obtained by [36] who found that of trefoil concentration was increase in DN and DM groups at age >40 years, while a study of [37] seams to decrease of trefoil concentration in DN and DM groups with age. Serum levels of trefoil were significantly higher in patients with diabetic nephropathy as compared to patients suffering from diabetic mellitus at age 50 [38].

Table 4: Mean differences of physio-biochemical and biomarkers in study groups according to age groups

Study variables	Study group	Age group						P-value
		N	30-45 years	N	46-60 years	N	≥ 60 years	
Trefoil (ng/ml)	DN (N=50)	5	12.76 ± 4.66	17	13.27 ± 5.95	28	12.21 ± 6.60	0.858
	DM (N=25)	4	8.31 ± 3.65	13	12.75 ± 3.38	8	11.70 ± 3.81	0.116
	Control group	8	8.02 ± 3.12	13	6.42 ± 2.26	4	5.76 ± 1.36	0.252
P-value	Total	17	0.10	43	**<0.001***	40	0.136	
D-dimer (ng/ml)	DN (N=50)	5	4129.06 ± 3632.45	17	2004.63 ± 2516.84	28	2105.16 ±2580.86	0.268
	DM (N=25)	4	411.57 ± 128.13	13	348.57 ± 141.73	8	314.65 ± 120.95	0.506
	Control group	8	402.94 ± 312.81	13	316.52 ± 143.58	4	271.51 ± 89.48	0.527
P-value	Total	17	**0.011***	43	**0.007***	40	0.073	
Fibrinogen (ml/dl)	DN (N=50)	5	399.10 ± 218.68	17	518.91 ± 152.95	28	474.107 ± 146.98	0.307
	DM (N=25)	4	316.00 ± 101.37	13	313.76 ± 78.16	8	321.56 ± 82.70	0.978
	Control group	8	241.75 ± 54.46	13	226.65 ± 62.25	4	212.75 ± 48.85	0.703
P-value	Total	17	0.146	43	**<0.001***	40	**<0.001***	

Mean differences of physio-biochemical biomarkers in study groups according to gender:

The mean differences of biomarkers according to gender were shown in Table (5), the results showed that, there was significant difference between means of all biomarkers in this study for study groups with gender (male and female). These marker trefoil (P 0.001, P 0.018), d-dimer (P 0.002, P 0.005), fibrinogen (P<0.001, <0.001), Furthermore, no significant difference between males and females in all study groups (diabetic with nephropathy, diabetic without nephropathy and control group) including trefoil concentration (p 0.819, p 0.865, p 0.505), d-dimer (p 0.334, p 0.766, p 0.803), fibrinogen (p 0.207, p 0.595, p 0.15). These results were agreement with results obtained by [39] who found that there was significant difference between trefoil with gender. A study of [40] found Fibrinogen is an independent risk factor for the development of renal disease. Factors influencing circulating levels of fibrinogen include age, smoking, gender, and genetic factors [41].

Table 5: Mean differences of physio-biochemical and epigenetic biomarkers in study groups according to the gender

Study variables	Study group	Gender				P-value
		N	Male	N	Female	
Trefoil (ng/ml)	DN (N=50)	26	12.82 ± 5.16	24	12.41 ± 7.14	0.819
	DM (N=25)	13	11.58 ± 3.49	12	11.84 ± 4.18	0.865
	Control group	14	7.13 ± 2.74	11	6.43 ± 2.30	0.505
P-value	Total	53	0.001*	47	0.018*	
D-dimer (ng/ml)	DN (N=50)	26	1917.52 ± 2151.76	24	2658.87 ± 3166.40	0.334
	DM (N=25)	13	355.59 ± 138.51	12	339.35 ± 130.00	0.766
	Control group	14	346.34 ± 225.41	11	325.05 ± 187.20	0.803
P-value	Total	53	0.002*	47	0.005*	
Fibrinogen (ml/dl)	DN (N=50)	26	508.96 ± 144.04	24	452.45 ± 168.05	0.207
	DM (N=25)	13	325.19 ± 59.07	12	307.33 ± 99.30	0.595
	Control group	14	212.82 ± 26.20	11	250.18 ± 77.03	0.15
P-value	Total	53	<0.001*	47	<0.001*	

The correlation between Trefoil and all study variables:

The correlation between trefoil and study variables were shown in Table (6), it was found the correlation was negative between trefoil and d-dimer in diabetic patients without nephropathy (r=-o.427,p=0.033) as shown in Figure (6), Furthermore, no correlation between trefoil and fibrinogen was found. These results were agreement with results of [42] who found that Serum fibrinogen is independently predictive of all-cause mortality in end-stage kidney disease [43]. Trefoil factor protein levels were markedly reduced in diabetic patients without nephropathy [42].

Table 6: The correlation between Trefoil and study variables

Study variables	Trefoil (ng/ml)					
	Diabetic nephropathy (N=50)		etic without nephropathy (N=2		Healthy control (N=25)	
	r	P-value	r	P-value	r	P-value
D-dimer (ng\ml)	-0.06	0.669	-0.427	0.033*	-0.343	0.093
Fibrinogen (ml\dl)	-0.182	0.205	-0.232	0.264	0.077	0.715

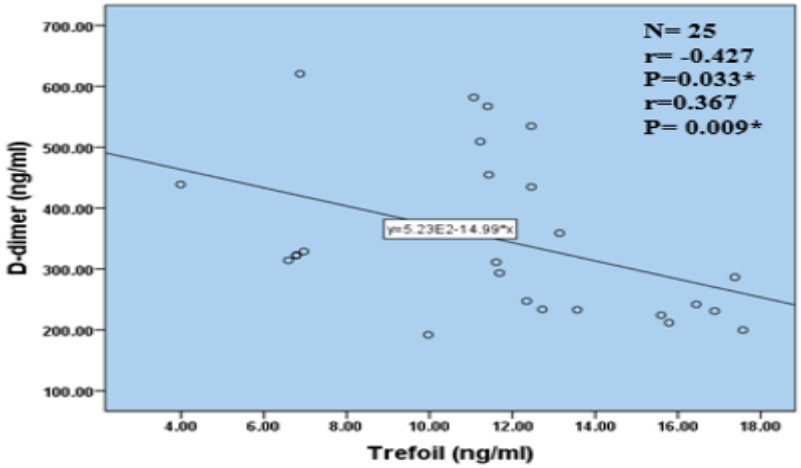

Figure 6: negative correlation between trefoil and d-dimer in diabetic patients without nephropathy

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