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Assessment of the autophagy biomarker level in Diabetic Kidney Disease Patients

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ABSTRACT

Introduction: The most frequent long-term consequence of diabetes mellitus is diabetic nephropathy (DN), which is also one of the main causes of end-stage renal disease (ESRD). Recent research indicates that impaired autophagy has a role in the development and course of diabetic kidney disease. So, we aimed in this study to assess the autophagy biomarker Beclin-1 level in diabetic kidney disease patients.

Methods: In this cross sectional study, we enrolled 100 patients with Diabetic Kidney Disease (DKD) referred to Ain-Shams University hospital in the period from January 2022 till September 2022. The patients were divided into 5 groups according to the estimated glomerular filtration rate (eGFR). Each group consisted of 20 patients to compare the level of serum Beclin-1 in different groups. Beclin 1 assay: Human Beclin 1 (BECN1) ELISA kit uses a double antibody sandwich enzyme linked immunosorbent assay (ELISA) to assay the level of Human Beclin 1 (BECN1) in samples.

Results: There was statistically significant difference between the studied groups ($p<0.001$) regarding hemoglobin, creatinine, hemoglobin A1c, calcium, phosphate, protein creatinine ratio, eGFR, and Beclin-1 levels with highest value of beclin among group (2, 5, 4, 2, 5, 5, 5 and 5) respectively and lowest value among group (5, 1, 5, 5, 2, 1, 5 and 5) respectively. There was statistically significant inverse correlation between Beclin- 1 and diabetes duration, creatinine, urea, CRP and protein creatinine ratio (r: -0.24; $p= 0.026$), (r: -0.44; $p< 0.001$), (r: -0.44; $p<0.001$), (r: 0.52; $p<0.001$), (r: -0.56; $p<0.001$) respectively. There was statistically significant positive correlation between Beclin- 1 and hemoglobin, calcium and eGFR (r: 0.242; $p= 0.015$), (r: 0.248; $p= 0.013$) and (r: 0.568; $p<0.001$) respectively. At cutoff value equal to (3.3, 1.8, 1.2 and 1.1) Beclin- 1 had sensitivity equal to (100%, 55%, 80% and 85%) respectively and specificity equal to (85%, 85%, 75% and 100%) respectively in prediction of CKD progression from (stage 1 to stage 2), (stage 2 to stage 3), (stage 3 to stage 4) and (stage 4 to stage 5) respectively.

Conclusion: In conclusion, and by using multivariate linear regression analysis we found that eGFR was the most influential parameter affecting Beclin-1 level. Beclin-1 can be used as a predictor for chronic kidney disease CKD progression among diabetic patients. The degree of Beclin-1 declining is correlated with hemoglobin drop and calcium level decrease with the CKD progression.

Keywords: Diabetic kidney disease, Beclin- 1, Autophagy biomarker.

INTRODUCTION

Patients with both type 1 and type 2 diabetes are more likely to have higher morbidity and mortality when they have diabetic nephropathy (DN), which is the most frequent cause of ESRD globally. Although microalbuminuria (MAU) is acknowledged as an early indicator of nephropathy, it has some limitations, including poor sensitivity and greater variability, as well as the inability to consistently predict a renal outcome or be specific to DN [(1)].

Thus, in addition to microalbuminuria, earlier, more precise, sensitive, and more predictable indicators are required for accurate early diagnosis and progression prediction in diabetic kidney disease [(2)]. The lysosomal pathway is used in the strictly controlled process of autophagy to break down damaged organelles and cellular protein aggregates. It has been proposed that dysregulated autophagy has significant pathogenic roles in a number of disease processes. A growing body of research links decreased autophagic function to the aetiology of diabetic kidney damage [(3)].

Autophagy appears to be crucial for maintaining renal function in both healthy and diseased settings, according to research on human kidneys, in vivo animal models, and in vitro cell cultures [(4)]. Role of Beclin- 1(autophagy biomarker) in diagnosis of diabetic kidney disease had been evaluated in previous studies on experimental levels only [(5, 6)].

METHODS

In this cross section study, we enrolled 100 patients with Diabetic Kidney Disease (DKD) referred to Ain-Shams University hospital at the period from January 2022 till September 2022. Based on the eGFR, which was determined using the modification of diet in renal disorders (MDRD) formula, the patients were split into five groups:

Group 1(CKD 1): Kidney damage with normal or increased (eGFR >90 mL/min/1.73 m^2).

Group 2(CKD 2): eGFR (60-89 mL/min/1.73 m^2).

Group 3(CKD 3): eGFR (30-59 mL/min/1.73 m^2).

Group 4(CKD 4): eGFR (15-29 mL/min/1.73 m^2).

Group 5(CKD 5): Kidney failure (predialysis) (eGFR < 15 mL/min/1.73 m^2).

Each group consisted of 20 patients to compare the level of serum Beclin-1 in different groups. All of the patients were older than eighteen years of age, with Type-2 diabetes mellitus and diabetic renal disease, and they all satisfied the inclusion criteria. Patients with infectious diseases, inflammatory disorders, immune disorders, heart failure, acute kidney injury and malignancy were excluded from our study.

All patients were undergone to: Complete history including, General Examination and The Following Investigations: CBC (complete blood picture), creatinine, urea, calcium (Ca), phosphorus (Po_4), Protein/ creatinine ratio, C- reactive protein (CRP).

Sample size calculation: Using pass 11 program for sample size calculation and according to Nagiub and Rashed, the expected mean Beclin -1 in the studied groups =

3.36±1.30,1.43±0.83 and 6.03±1.94.Sample size of 20 patients in each group achieves 100% power to detect difference between the study groups with α-error 0.09.

Statistical analysis:

After being gathered and edited, the data were imported into SPSS version 23.0. When it was determined that the quantitative data were not parametric, they were given as the median and inter-quartile range (IQR), and the mean±SD, and ranges for the parametric data. Furthermore, percentages and figures were used to represent qualitative factors. The Fisher exact test and the Chi-square test were used to compare groups using qualitative data. Four independent t-tests were used to compare the groups with quantitative data and parametric distribution. The One Way ANOVA test was used to compare more than two groups given quantitative data and a parametric distribution. The correlation between two quantitative characteristics was evaluated using Spearman correlation coefficients. The allowed error margin was set at 5%, and the confidence interval was set at 95%. $P < 0.05$ is therefore regarded as significant. The optimal cut off point was determined by utilising the ROC curve to evaluate the examined marker's (Beclin-1) AUC, sensitivity, specificity, positive predictive value, and negative predictive value.

RESULTS

Table (1): Demographic comparison between the studied groups:

Demographic data		**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Test value**	**P-value**	**Sig.**
		No. = 20	**No. = 20**	**No. = 20**	**No. = 20**	**No. = 20**			
Age (years)	Mean ± SD	40.95 ± 9.31	53.25±10.62	60.30 ± 6.87	66.75±10.59	55.60 ± 8.14	21.551•	***<0.001***	HS
	Range	30 – 59	31 – 72	49 – 71	36 – 82	40 – 68			
Sex	Female	14 (70.0%)	9 (45.0%)	13 (65.0%)	11 (55.0%)	5 (25.0%)	10.256*	***0.036***	S
	Male	6 (30.0%)	11 (55.0%)	7 (35.0%)	9 (45.0%)	15 (75.0%)			
diabetes duration (years)	Median (IQR)	3 (1 – 6)	9.5(5 – 12.5)	10 (6 – 17.5)	10 (5 – 25)	9 (4.5 – 15)	19.796‡	***0.001***	HS

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS);
*: Chi-square test; •: One Way ANOVA test; ‡: Kruskal Wallis test

There was statistically significant difference between the studied groups as regard age, sex and diabetes duration with p- value equal to ($p<0.001$), ($p=0.036$) and ($p=0.001$) respectively.

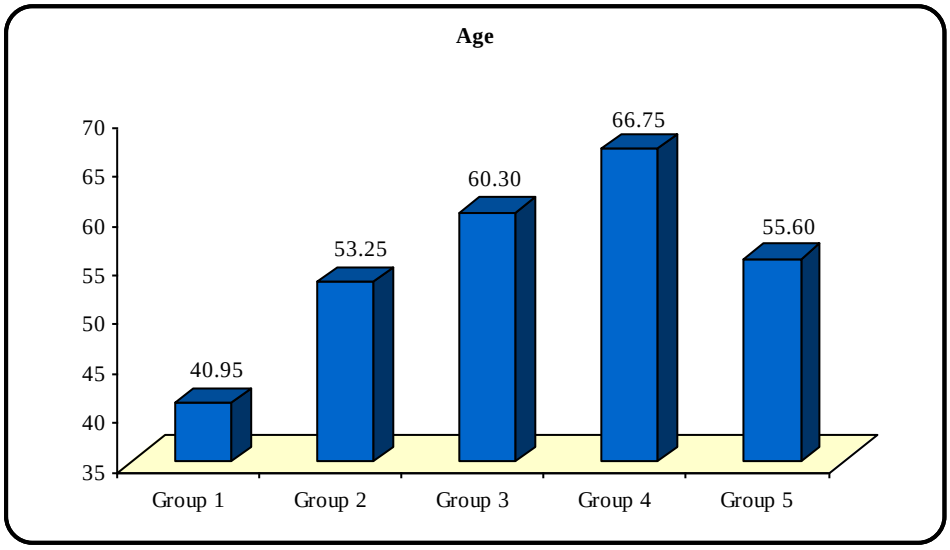

Figure (1): Age differences between the studied groups.

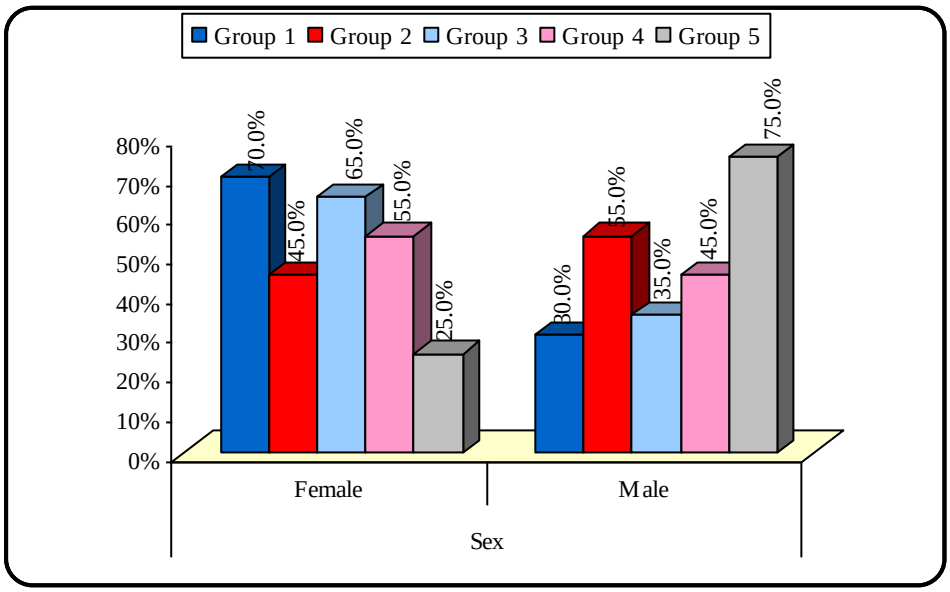

Figure (2): Sex distribution between the studied groups.

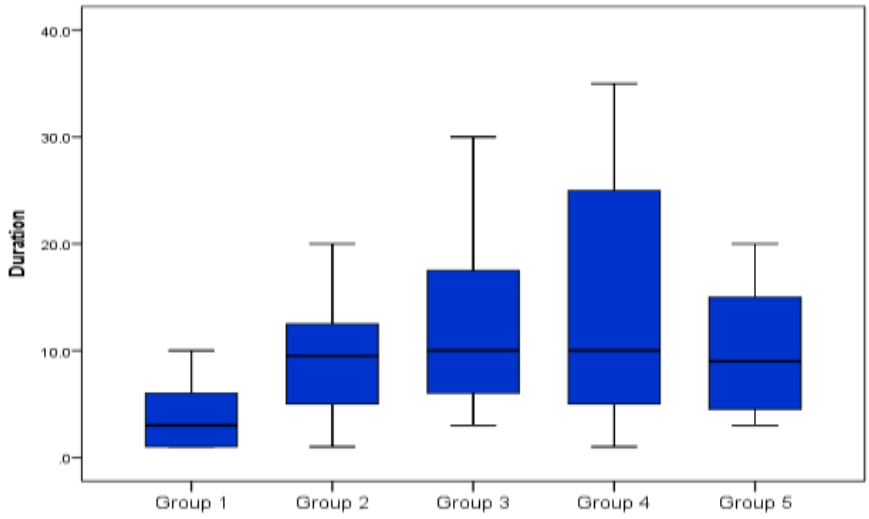

Figure (3): Diabetes duration differences between the studied groups.

Table (2a**):** Laboratory investigations differences:

Laboratory investigations		Group 1 No. = 20	Group 2 No. = 20	Group 3 No. = 20	Group 4 No. = 20	Group 5 No. = 20	Test value	P-value	Sig.
Hemoglobin (g/dL)	Mean ± SD	11.37 ± 1.00	12.63 ± 1.68	11.57 ± 1.52	11.44 ± 1.18	9.83 ± 1.06	11.626•	***<0.001***	HS
	Range	9.7 – 13.2	7.7 – 15.1	7 – 13.9	10 – 13.7	7.8 – 11.3			
Total leucocytic count ($*10^3/mm^3$)	Mean ± SD	6.06 ± 2.63	6.89 ± 1.74	7.42 ± 2.06	6.76 ± 1.90	5.76 ± 1.88	2.077•	0.090	NS
	Range	2.3 – 13.2	3.8 – 10.3	4.2 – 13	4.5 – 10.8	3.5 – 10			
Platelets ($*10^3/mm^3$)	Mean ± SD	200.40± 50.12	203.70± 49.09	227.00± 62.16	190.75± 59.68	176.85± 67.11	2.024•	0.097	NS
	Range	79 – 264	90 – 285	136 – 364	117 – 321	87 – 305			
S. creatinine (mg/ dL)	Median (IQR)	0.76(0.7 – 0.9)	1.1(0.92 – 1.2)	1.3 (1.1 – 1.6)	2.5 (2.1 – 2.8)	6.05 (5.6 – 7)	90.284	***<0.001***	HS
	Range	0.66 – 1	0.9 – 1.3	1 – 1.8	1.9 – 3.3	4.3 – 12.5			
Urea (mg/ dL)	Median (IQR)	24.5 (23 – 29)	35 (25.5 – 40)	37 (29.5– 42.5)	79 (50.5 – 90)	108(92– 132.5)	75.138	***<0.001***	HS
	Range	20 – 40	20 – 53	20 – 81	45 – 110	53 – 141			
C- reactive protein (mg/dL)	Median (IQR)	2.21 (1.41–7.92)	3.37 (2.39–4.84)	2.65 (1.82 – 4.13)	3.21 (1.99 – 12.5)	4.8 (2.3 – 36)	7.309 ‡	0.120	NS
	Range	0.95 – 27	0.84 – 13.25	1.23 – 16.45	1.05 – 28.4	0.54 – 96			
Hemoglobin A1c (%)	Mean ± SD	8.40 ± 1.76	8.59 ± 2.03	8.82 ± 2.12	7.46 ± 1.08	6.48 ± 1.15	6.658•	***<0.001***	HS
	Range	6 – 11.6	6.2 – 12.4	5.9 – 13.5	6.5 – 9.6	4.8 – 9			
Calcium (mg/ dL)	Mean ± SD	8.43 ± 0.72	8.86 ± 0.41	8.47 ± 0.71	8.20 ± 0.75	7.50 ± 1.16	8.165•	***<0.001***	HS
	Range	6.9 – 9.4	7.5 – 9.5	7.2 – 10.2	7 – 9.5	5.3 – 9.1			
Phosphate (mg/ dL)	Mean ± SD	3.27 ± 0.63	3.31 ± 0.74	3.27 ± 1.00	3.33 ± 0.69	4.57 ± 1.35	7.649•	***<0.001***	HS
	Range	2.1 – 4.3	1.8 – 4.6	1.6 – 5	2 – 4.4	2.9 – 7.9			
Protein/ creatinine ratio (gm/ mg)	Median (IQR)	0.2 (0.16 – 0.24)	0.26 (0.22 – 0.39)	0.31 (0.19 – 0.36)	0.36 (0.23 – 0.51)	2.4 (1.75 – 3.7)	56.156‡	***<0.001***	HS
	Range	0.05 – 0.29	0.06 – 0.52	0.09 – 0.52	0.11 – 1.36	1 – 6.2			
Glomerular filtration rate (mL/ min/ 1.37 m^2)	Median (IQR)	92.3 (91 – 100.6)	69.2 (62.8 – 72.95)	51.93 (42.65– 55.25)	25 (21.15– 27.79)	8.63 (7.3 – 11.47)	94.226	***<0.001***	HS
	Range	90 – 107	50 – 79	32.5 – 58.6	14 – 29	4.45 – 14			
BECLIN- 1 (ng/ mL)	Median (IQR)	6.35 (3.8 – 10.45)	1.96 (1.3 – 2.75)	1.2 (0.92 – 1.85)	2.85 (2 – 4.2)	0.42 (0.12 – 1)	57.370	***<0.001***	HS
	Range	2.2 – 21	0.9 – 3.3	0.66 – 12.2	1.2 – 6.4	0.02 – 10.3			

Table (2b): Post-hoc analysis of significant laboratory findings:

Laboratory investigations	Post Hoc analysis									
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**
Hemoglobin	0.003	0.623	0.867	<0.001	0.012	0.005	<0.001	0.746	<0.001	<0.001
Creatinine	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Urea	0.005	<0.001	<0.001	<0.001	0.255	<0.001	<0.001	<0.001	<0.001	<0.001
HbA1c	0.722	0.438	0.081	<0.001	0.674	0.036	<0.001	0.013	<0.001	0.067
Calcium	0.084	0.873	0.369	<0.001	0.116	0.009	<0.001	0.290	<0.001	0.006

Phosphate	0.893	0.999	0.847	<0.001	0.891	0.954	<0.001	0.846	<0.001	<0.001
Protein /creatinine ratio	0.003	0.008	0.003	<0.001	0.860	0.213	<0.001	0.208	<0.001	<0.001
eGFR	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Beclin- 1	<0.001	<0.001	<0.001	<0.001	0.044	0.019	<0.001	0.002	<0.001	<0.001

P1: Group 1 Vs Group 2 P2: Group 1 Vs Group 3
P3: Group 1 Vs Group 4 P4: Group 1 Vs Group 5
P5: Group 2 Vs Group 3 P6: Group 2 Vs Group 4
P7: Group 2 Vs Group 5 P8: Group 3 Vs Group 4
P9: Group 3 Vs Group 5 P10: Group 4 Vs Group 5

There was statistically significant difference between the studied groups **(p<0.001)** regarding hemoglobin, creatinine, hemoglobin A1c, calcium, phosphate, protein creatinine ratio, eGFR, and Beclin-1 levels. There were no statistically significant differences between the studied groups regarding total leucocytic count, platelets and CRP.

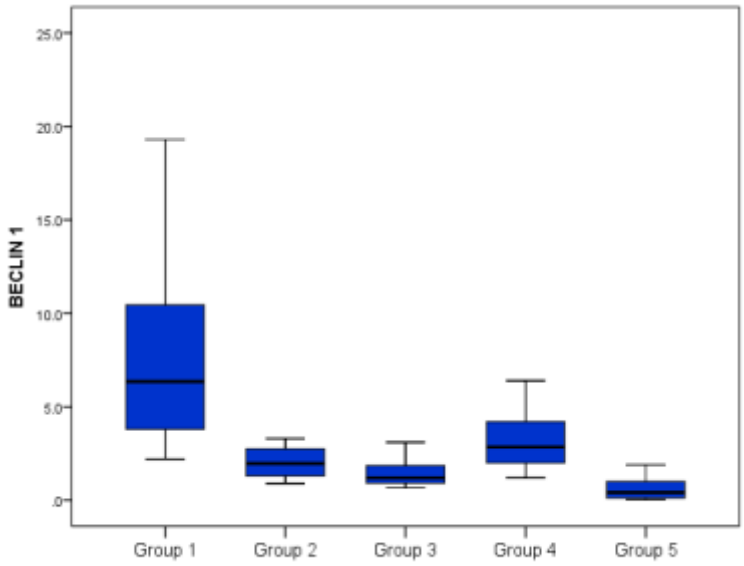

Figure (4): Beclin- 1 levels differences between the studied groups

Table (3): Correlation of Beclin- 1 to different parameters:

parameters	BECLIN 1	
	r	**P-value**
Age	-0.182	0.069
Diabetes duration	**-0.240***	**0.016**
Hemoglobin	**0.242***	**0.015**
Total leucocytic count	0.046	0.652
Platelets	0.061	0.546
Creatinine	**-0.521****	**<0.001**
Urea	**-0.443****	**<0.001**
C reactive protein	**-0.198***	**0.048**
Hemoglobin A1c	0.176	0.080
Calcium	**0.248***	**0.013**
Phosphate	-0.189	0.060
Protein /creatinine ratio	**-0.561****	**<0.001**

eGFR	**0.568****	**<0.001**

There was no significant correlation between Beclin- 1 and age, white blood cells, platelets, hemoglobin A1c, phosphate. There was statistically significant inverse correlation between Beclin- 1 and diabetes duration, creatinine, urea, CRP and protein creatinine ratio (r: -0.24; p= 0.026), (r: -0.44; p< 0.001), (r: -0.44; p<0.001), (r: -0.52; p<0.001), (r: -0.56; p<0.001) respectively. There was statistically significant positive correlation between Beclin-1 and hemoglobin, calcium and eGFR (r: 0.242; p= 0.015), (r: 0.248; p= 0.013) and (r: 0.568; p<0.001) respectively.

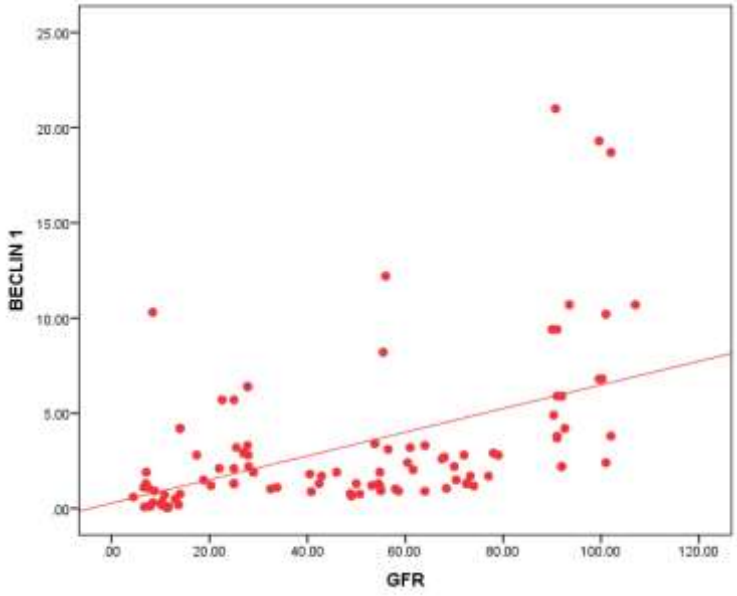

Figure (5**):** Significant positive correlation between Beclin- 1 and eGFR.

Table (4): Linear regression analysis of the factors affecting Beclin- 1level:

parameters	**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
	B	**SE**	**Beta**		
Duration	-0.015	0.051	-0.029	-0.287	0.774
Hemoglobin	-0.294	0.260	-0.116	-1.129	0.262
Creatinine	0.206	0.397	0.118	0.520	0.605
Urea	0.022	0.023	0.197	0.984	0.328
C reactive protein	-0.029	0.027	-0.115	-1.067	0.289
Protein /creatinine ratio	-0.152	0.450	-0.047	-0.338	0.736
eGFR	0.091	0.022	0.720	4.175	<0.001

The significant factors correlated with Beclin- 1 levels using univariate analysis were entered in multivariate model using linear regression analysis to adjust factors for confounders. eGFR was the only factor which kept his significant correlation with Beclin- 1.

Table (5): Accuracy of Beclin- 1 levels in prediction of CKD progression from one stage to another in diabetic kidney disease patients using ROC analysis

BECLIN	**AUC**	**Cut of Point**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Group 1 Vs Group 2	0.939	≤3.3	100.0	85.0	87.0	100.0

Group 2 Vs Group 3	0.686	≤1.8	55.0	85.0	78.6	65.4
Group 3 Vs Group 4	0.791	>1.2	80.0	75.0	76.2	78.9
Group 4 Vs Group 5	0.934	≤1.1	85.0	100.0	100.0	87.0

At cutoff value equal to (3.3, 1.8, 1.2 and 1.1) to differentiate between different stages of CKD.

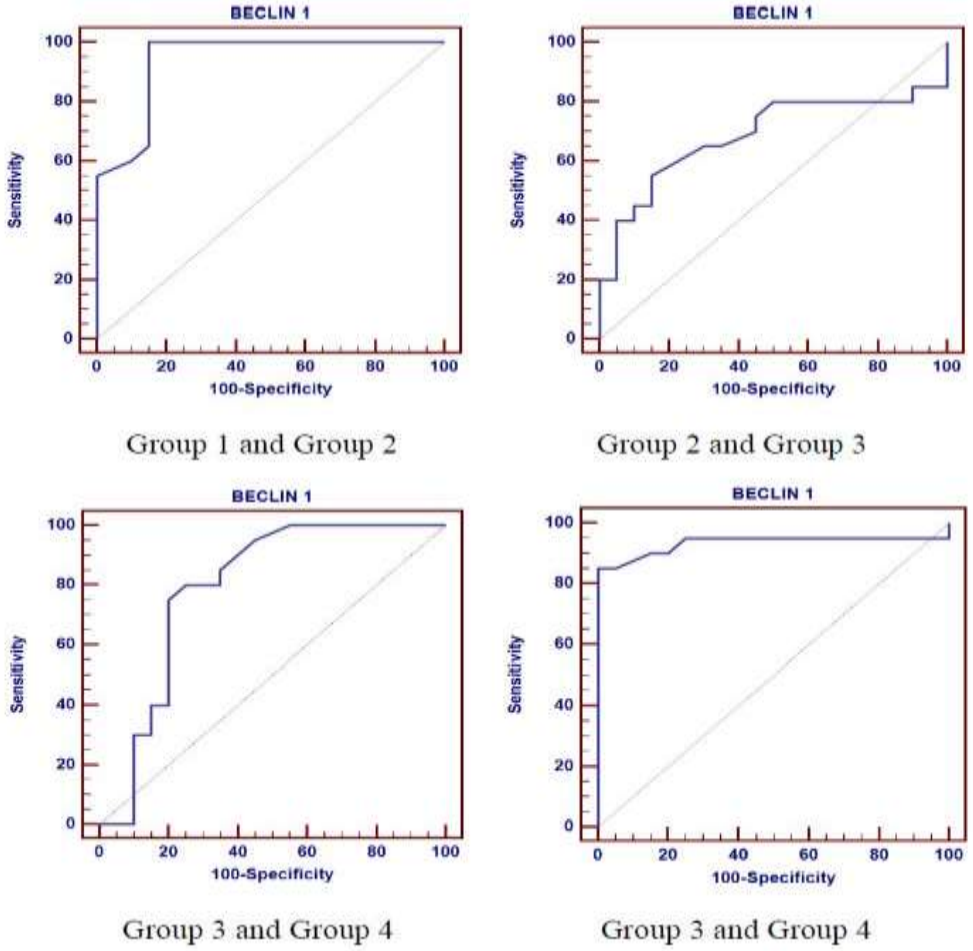

Figure (6): Accuracy of Beclin- 1 levels in prediction of CKD progression from one stage to another in diabetic kidney disease patients using ROC analysis**.**

DISCUSSION

Patients with both type 1 and type 2 diabetes are more likely to have higher morbidity and mortality when they have DN, which is the most frequent cause of ESRD globally. Although MAU is acknowledged as an early indicator of nephropathy, it has some limitations, including poor sensitivity and greater variability, as well as the inability to consistently predict a renal outcome or be specific to DN [(1)].

The aim of the current study was to evaluate the association between Beclin- 1 levels and diabetic kidney diseases. To achieve this aim, we conducted this study on 100 patients who were supposed to have diabetic kidney disease and were divided according to degree of

severity of renal disease into 5 groups. Beclin- 1 levels were assessed and analyzed in these different groups.

There was statistically significant difference between the studied groups patient age ($p< 0.001$). Regarding sex distribution, females had higher frequency among groups 1**&**3 and males had higher frequency in groups 4 & 5. In large annual survey study in United States, **Liu et al.** [7] reported that females had slightly higher prevalence of diabetic nephropathy in different age groups. They reported also increased prevalence of diabetic nephropathy with advanced age. Recent study by **Jiang et al.** [8] showed that female diabetic patients had higher incidence rate of CKD progression. There was statistically significant difference between the studied groups regard diabetes duration with longer duration in more advanced CKD groups ($p= 0.001$). Table (1)

There was statistically significant difference between the studied groups regarding patients' age as CKD stage progresses with age increases. This came in hand with **Jiang et al.** [8]**,** who reported that older age patients at higher risk for acute kidney injury due to decreased functional renal mass and increased sclerotic glomeruli and patients with mean age 61.4 ± 12.5 at higher risk for CKD than patients with mean age 54.7 ± 12.9 [8]**.**

Also, **Go et al.** [9]**,** reported significant difference between fast CKD progrressors and non-fast CKD progressors among diabetic kidney disease patients with older age among progressors**.** In hand with the current study, **Jiang et al.** [10]**,** showed statistically significant effect of disease duration of progression of diabetic nephropathy to advanced stages**.** Another recent study showed that diabetes duration is a significant predictor for development of renal impairment in diabetic patients beside age and female sex [8]**.**

Cheng et al. [9]**,** also showed statistically significant difference between CKD stages in diabetic patients regarding disease duration**. Naguib et al.** [11], showed significant longer diabetes duration in CKD group with eGFR less than 30 mL/ min**.** In the present study, we did not notice statistically significant difference between CKD stages in diabetic patients regarding C- reactive protein. In contrary to the current study, **Cheng et al.** [12]**,** reported that C- reactive protein increases with disease progression with higher level among hemodialysis patients reflecting the inflammatory state in hemodialysis**.**

In the current study, we reported significant increase of hemoglobin A1c mean values along with DKD stage advancement but not in group 5 as this group had the lowest mean values. Likely, **Jiang et al.** [10] reported statistically significant differences between the studied groups regarding hemoglobin A1C with higher levels among advanced stages**.**

Another recent study by **Jiang et al.** [8], showed statistically significant difference between patients who developed renal impairment and who did not regarding Hemoglobin A1c which was higher among patients with rise of serum creatinine**.** In our study there was a significant increase in proteinuria with advanced CKD progression. **Rodriguez et al.** [13] discovered that individuals with type 2 diabetes mellitus who had higher HbA1c variability had a considerably higher chance of increased albumin excretion, even after controlling for a wide variety of other clinical factors. **Naguib et al.** [11], also reported significant higher protein/ creatinine ratio in patients with eGFR less than 30 mL/ min**.**

In our study, there were statistically significant differences between different CKD stages in DKD patients regarding serum calcium and phosphorus as calcium decreased and phosphorus increased in concordance with CKD progression. This came in hand with the results by **Janmaat et al.** [14], who showed statistically significant differences between different stages regarding calcium and phosphorus**.**

Beclin- 1 levels were assessed in all groups in the current study and the results showed

statistically significant differences with higher median range among CKD stage 1 group (6.35; 3.8- 10.45) and lower median range in group 5 (0.42; 0.12- 1). In concordance with the current study, **Naguib et al. [11],** in his study which was conducted on 70 DKD patients (2 groups; group I: eGFR> 30 mL/ min & group II: eGFR< 30 mL/ min) and 20 healthy control revealed higher Beclin- 1 levels in normal healthy patients (6.03 ± 1.94) in comparison to other DKD groups. Also, Beclin- 1 levels were higher significantly among group I with eGFR> 30 mL/ min (3.36 ± 1.30) than group II with eGFR < 30 mL/ min (1.43 ± 0.83) ($p< 0.001$)**.** Table (2)

To the best of our knowledge, **Naguib et al. [11],** then our study are the only human studies evaluated Beclin- 1 levels in different CKD stages in DKD patients. **Fang et al. [5],** in his experimental study showed that autophagy protects against diabetic glomerular damage through inhibition of podocyte injury caused by the state of hyperglycemia. He reported increased Beclin- 1 levels, as a marker for autophagy, among healthy and early stages of renal affection mice than mice with advanced renal affection**.**

In fact, autophagy offers protection against diabetic nephropathy. It is unclear, therefore, if autophagy is active or inactive in DKD. It is well known that inflammation plays a key role in the pathophysiology of DKD. Crucially, autophagy may protect against renal inflammation in DKD, which might aid in our investigation of the disease's pathophysiology **[11].**

In the current study, we evaluated the correlation between Beclin- 1 as a marker for autophagy and multiple parameters. There was no statistically significant correlation between Beclin- 1 levels and patient age. In concordance with the current study, the previous study by **Naguib et al. [11]**, did not find significant correlation between Beclin- 1 and age in diabetic kidney disease patients**.** In contrary, experimental studies on mice showed statistically significant positive correlation between Beclin- 1 as autophagy marker and age **[15, 16].**

Also, in human study, **Xu et al. [17],** showed increased Beclin- 1 among healthy population with age progression and produced protective effect to the heart and kidney**.** These studies were not performed on diabetic cases. There was statistically significant inverse correlation between Beclin- 1 levels and diabetes duration and this result also was presented in the study by **Naguib et al. [11]**, However, both studies failed to demonstrate significant correlation between Beclin- 1 and Hemoglobin A1c**.** Also, **Fang et al. [5]**, showed that hyperglycemia could inhibit autophagy, thus with prolonged diabetes duration, Beclin- 1 would decline significantly**.** Table (3)

In the present study, there was statistically significant positive correlation between Beclin- 1 levels and hemoglobin levels however no correlations were reported between Beclin- 1 and white blood cells or platelets. **Okuyan et al. [18]**, showed statistically inverse correlation between hemoglobin levels and Beclin- 1 levels in another entity (COVID- 19) than diabetes or chronic kidney disease**.**

In our study, there was positive correlation between Beclin- 1 levels and serum calcium. This is in concordance with previous studies which reported that role of Beclin- 1 in autophagy is mediated mainly by calcium and sensitivity of Endoplasmic reticulum receptors to cellular calcium **[19, 20].** Also, **Vicencio et al. [21],** showed that Beclin- 1 had significant effect on calium levels**.** Table (3)

Multivariate linear regression analysis of the correlated parameters revealed that eGFR was the most influential parameters affecting the Beclin- 1 level. Table (4)

Regarding the diagnostic accuracy of serum Beclin- 1 as a predictor and a diagnostic marker for progression to different stages of CKD in diabetic kidney disease, we analyzed the ROC curve and showed that the cutoff value of serum Beclin- 1 to differentiate between different

stages of CKD were equal to (3.3, 1.8, 1.2 and 1.1) Beclin- 1 had sensitivity equal to (100%, 55%, 80% and 85%) respectively and specificity equal to (85%, 85%, 75% and 100%) respectively in prediction of CKD progression from (stage 1 to stage 2), (stage 2 to stage 3), (stage 3 to stage 4) and (stage 4 to stage 5) respectively. Table (5)

The study had some advantages as being conducted on sector of population with different CKD stages and wide variability in different parameters which allows generalizability of the results. Also, it is one of very limited studies evaluated the disease of interest on human patients. Also, we tested the accuracy of Beclin- 1 in prediction of disease progression.

CONCLUSION

In conclusion, and by using multivariate linear regression analysis we found that eGFR is the most influential parameter affecting Beclin-1 level. Beclin-1 can be used as a predictor for CKD progression among diabetic patients. The degree of Beclin-1 declining is correlated with hemoglobin drop and calcium level decrease with the CKD progression.

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