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Urinary Albumin Creatinine Ratio has no Significant Association with Retinopathy in Individuals with Type 1 Diabetes Mellitus

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The relationship between diabetic retinopathy (DR) and nephropathy in type 1 diabetes mellitus (T1DM) is controversial. This study assessed the utility of the spot urinary albumin creatinine ratio (UACR) as a parameter for correlating diabetic nephropathy with DR in individuals with T1DM in Basrah. The study was a cross-sectional observational study of 216 patients with T1DM (16–49 years old) with different DR types. We used demographic variables, different T1DM-related variables (onset and duration of T1DM, glycemic control, latency between T1DM and DR onset, age of onset of DR), and biochemical investigations, such as spot UACR, renal function tests, glycated hemoglobin (HbA1c), anti-glutamic acid decarboxylase-65 (anti-GAD-65) antibody, lipid profile, and thyrotropin. We used ordinal regression analysis to test for the possible primary covariates and adjusted the findings with an analysis of covariates (ANCOVA). The patient population showed a slight male preponderance, and uncontrolled T1DM with high HbA1c was found in 93% of the cohort. The mean UACR was 64.37 ± 8.99 mg/g. Normal UACR levels were seen in approximately 60% of the cohort (n=129). Sixty-five individuals were reported to have DR, with a median age of onset of 34 ± 8 years and a median latency period of 13 ± 7 years. UACR had no significant relationship with DR development at any association level, with or without adjustment for the composite regression factors. There was no significant association between UACR at any level and DR development before and after adjustment for all of the possible covariates in this study.

Keywords: Albuminuria; Basrah; diabetic nephropathy; diabetic retinopathy; type 1 diabetes mellitus; urinary albumin-creatinine ratio.

The mutual relationship between diabetic retinopathy (DR) and nephropathy is controversial[1]. Retinopathy may be proliferative (PDR) or nonproliferative (NPDR) and is considered the primary culprit for blindness in diabetes mellitus, with a prevalence of 17–97.5% [2].

Diabetes duration, proteinuria, blood pressure and glycemic control might be implicated in its pathogenesis[3] and may act synergistically with diabetic nephropathy in the development of DR[1]. Microalbuminuria may reflect the level of

retinal microvascular changes in different severities of PDR[3,5]

The urinary albumin creatinine ratio (UACR) is the best tool to assess the albuminuria level in patients with diabetes, and it has replaced the 24-hour collection of urine. Levels below 30 mg/g are acceptable[4].

This study aimed to assess the correlation between UACR and DR in individuals with type 1 diabetes mellitus (T1DM) in Basrah-Southern Iraq.

METHODS

This study is a cross-sectional observational study of 216 patients with T1DM who attended Faiha Specialized Diabetes Endocrine and Metabolism Center (FDEMC)-Basrah-Southern Iraq from March 2019 to October 2020. Every enrolled individual provided informed written consent for their data to be used. The ethical committee at FDEMC granted ethical approval.

After a full assessment of the patients with T1DM by the T1DM team, an expert ophthalmologist examined the patients for DR. The enrollment eligibility included individuals with T1DM older than 16 and less than 50 years old who had undergone UACR. We included patients with T1DM who were in G1 (eGFR = 90 mL/min/1.73 m^2 or higher) and G2 (eGFR =89–60 mL/min/1.73 m^2) only.

We excluded patients with any documented endocrine abnormalities of the thyroid, pituitary, adrenal, and gonads; any individual on hormonal or anti-hormonal medications including contraceptive medications; any patient with a previous diagnosis of hepatic, renal, cardiac, or bone diseases; any patient with malabsorption or celiac disease; any patient with hypertension and dyslipidemia whether primary or secondary; any patient with an active infection or inflammatory condition whatever the severity; patients with any type of malignancy whether on chemotherapy or not; pregnant; menopausal. Additionally, we excluded any patient with a previously diagnosed sight-related disease and any patient with a documented family history of eye-related genetic disorders.

A thorough ophthalmologic evaluation was performed for each patient to reveal the possibility of DR as no DR, nonproliferative DR, proliferative DR, or maculopathy. Patients with papilledema were excluded. Their worst eye was used to determine each patient's level of retinopathy.

Individuals who acquired T1DM before the age of 18 years were considered childhood-onset individuals, while individuals who acquired it after the age of 18 years were considered adult-onset T1DM individuals.

We evaluated the general characteristics of the enrolled cohort such as age, sex, body mass index (BMI), onset of T1DM, childhood or adult-onset, and latency duration between the diagnosis of T1DM and the onset of retinopathy.

Laboratory investigations

The enrolled patients were evaluated by a cascade of biochemical investigations that included blood urea and serum creatinine. The normal reference ranges for blood urea and serum creatinine in our laboratory were 15–45 mg/dl and 0.57–1.25 mg/dl, respectively.

The estimated glomerular filtration rate (eGFR) was calculated by the CKD-EPI equation and according to the KDIGO classification, with a reference level e" 90 mL/min/1.73 m^2.

The urinary albumin creatinine ratio (UACR) was determined at the enrollment visit with a spot urine sample, with a normal reference value below 30 mg/g. Levels between 30 and 300 mg/g indicate microalbuminuria, while levels greater than 300 mg/g indicate macroalbuminuria.

For UACR estimation, we used the immunoturbidimetry technique of COBAS (INTEGRA 400 PLUS, Roche, Switzerland). The first morning spot urine sample is preferred because more concentrated urine allows for better detection of analytes.

HbA1c measurements were assessed at the enrollment visit (reference normal value < 5.7%, and diabetes range > 6.5%). We used Bio-Rad Variant II Turbo HbA1c Kit–2.0 Quick Guide 270-2455EX.

We tested the patients for anti-glutamic acid decarboxylase-65 (anti-GAD-65) antibody (normal value < 5 U/L) using ELISA BioTek-USA (SN: 256905).

Lipid profiling and thyrotropin stimulation hormone (TSH) were performed at the enrollment visit and were used as a basis for enrollment.

We used Cobas e411-Roche Diagnostics (Germany) electrochemiluminescence technology

for immunoassay analysis and the different hormonal analyses.

Statistical analysis

Data were analyzed with IBM SPSS Statistics (Version 26). We used the mean and median to express the continuous data, with either standard deviation (SD) or standard error (SE). To express the categorical data, we used percentage (%).

We used ordinal regression analysis to analyze the relationship between independent variables and DR as a dependent variable.

To extract the net effect of UACR on retinopathy, we used principal component analysis

Table 1. General Characteristics of the Patients with T1DM in the Study (n=216)

Variables		Values
Male gender n (%)		123 (56.9)
Age years[a]	Mean ± SD (Median ± SE)	30 ± 8 (28 ± 1)
	Less than 28 years n (%)	93 (43.1)
	e”28 years n (%)	123 (56.9)
BMI kg/m2	Mean ± SD (Median ± SE)	22.45 ± 4.28 (22.08 ± .29)
	Underweight <18.5	27 (12.5)
	Normal weight 18.5-24.9	144 (66.7)
	Overweight 25-29.9	33 (15.3)
	Obese >30	12 (5.5)
Age onset of T1DM[a] Mean ± SD (Median ± SE) years		18 ± 8 (17 ± .5)
Duration of T1DM[a] Years Mean ± SD		13 ± 6
T1DM Onset n (%)	Childhood-onset	113 (52.3)
	Adulthood-onset	103 (47.7)
UACR	Median ± SE mg/g	22.05 ± 9.00
	<30 n (%)	129 (59.7)
	30-300 n (%)	73 (33.8)
	>300 n (%)	14 (6.5)
HbA1c	Mean ± SD (Median ± SE)	10.15 ± 2.50 (9.90 ± .17)
	Median (SE)	9.9 (.17)
	Less than 7	15 (6.9)
	7 to 10	100 (46.3)
	More than 10	101 (46.8)
Anti-GAD 65 antibody	Mean ± SE	50.32 ± 10.91
	Positive n (%)	117 (54.2)
	Negative n (%)	99 (45.8)
Serum Creatinine mean (SD) mg/dL		.71 ± .23
GFR mL/min/1.73 m^2	Mean (SD)	123.77 ±23.15 (125.5 ± 1.55)
	G1 n (%)	195 (90.3)
	G2 n (%)	21 (9.7)
DR categories (n=65) n (%)	PDR	36 (16.7)
	NPDR	22 (10.2)
	MP	7 (3.2)
Age at onset of DR[a]	Mean ± SD (Median ± SE)	33 ± 8 (34 ± 1)
	<34 years n (%)	30 (13.9)
	≥34 years n (%)	35 (16.2)
Latency duration till DR[a]	Mean ± SD (Median ± SE)	15 ± 7 (13 ± 1)
	<13 years n (%)	24 (11.1)
	≥13 years n (%)	41 (19.0)

[a] The values were approximated to the nearest integer.Abbreviations: BMI, body mass index; DR, diabetic retinopathy; GFR, glomerular filtration rate; HbA1c, glycated hemoglobin; MP, maculopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SE, standard error; SD, standard deviation; T1DM, type 1 diabetes mellitus; UACR, urinary albumin creatinine ratio.

and dimension reduction to create a composite regression factor. The composite factors included the effect of the independent factors (excluding UACR and the variables limited to individuals with DR), which might affect retinopathy with different component loadings.

Analysis of covariates (ANCOVA) provided the platform to assess the net relationship between UACR (as the independent variable) and retinopathy (as the dependent variable) before and after adjustment with the composite regression factor (as a covariate). The use of ANCOVA and ordinal regression analysis with adjustment could reduce the effect of a nonnormal distribution of the data.

Levene's test examines the null hypothesis in which the error variance of the dependent variable is equal across groups.

Partial eta squared is a measure of effect size. Values d" 0.02 represent a small effect size, d" 0.13 represent a medium effect size, and e" 0.26 represent a large effect size.

To examine the characteristics of cutoff values for the UACR for different DR categories, we used receiver operating characteristic (ROC) curves.

All of the data were examined with a two-tailed significance level d" 0.05 and 95% confidence interval (CI). Exp (B) represented the odds ratio.

Table 2. Ordinal Regression Analysis for Independent Variables for Different Types of Diabetic Retinopathy in T1DM

Independent Factors	SE	p	Exp (B) Odds Ratio	95% Confidence Interval Lower Bound	Upper Bound
Gender	.546	.986	0.99	-1.080-	1.060
Age of patients with T1DM	.850	.202	0.34	-2.749-	.581
BMI	.406	.986	1.01	-.789-	.803
Patterns of Onset of T1DM	.980	.384	0.43	-2.773-	1.068
Duration of T1DM	1.567	.509	2.82	-2.036-	4.107
HbA1c	.445	.457	1.39	-.541-	1.202
eGFR	.857	.968	0.97	-1.715-	1.646
Anti-GAD-65 antibody	.660	.533	1.51	-.882-	1.705
Latency between T1DM and DR onset	1.554	.728	0.58	-3.587-	2.506
Age onset of DR	.777	.623	1.47	-1.142-	1.905
UACR	.419	.722	0.86	-.971-	.672

Abbreviations: BMI, body mass index; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; GAD, glutamic acid decarboxylase; HbA1c, glycated hemoglobin; standard error; T1DM, type 1 diabetes mellitus; UACR, urinary albumin creatinine ratio.

Table 3. The Different Component Loading Which Was Obtained by Principal Component Analysis

Components	Component loadings
Gender	-.062-
Age of patients with T1DM	.907
Body mass index	.204
Patterns of Onset of T1DM	.771
Duration of T1DM	.339
Glycated hemoglobin	-.126-
eGFR	.369
Antiglutamic acid decarboxylase-65 antibody	.060

Abbreviations: eGFR, estimated glomerular filtration rate; T1DM, type 1 diabetes mellitus

RESULTS

Table 1 shows a slight male predominance of 56.9% (n=123) versus 43.1% females (n=93). This study included young normal-weight individuals with T1DM with an age range of 17–49 years and a BMI range of 14.53–39.45 kg/m^2. The percentage of individuals with childhood-

Table 4. Analysis of Covariance to Demonstrate the Net Effect of UACR on Different Types of DR before and after Adjustment for the Composite Regression Factor

Dependent Variables in ANCOVA	Levene's test				Test between subject							
	F		Sig		F		Sig		Partial Eta Squared		Observed power[b]	
	Before[a]	After	Before[a]	After	Before	After	Before	After	Before	After	Before	After
Retinopathy (overall)	1.84	1.38	.01	.06	.76	.88	.92	.75	.54	.58	.89	.95
PDR	3.74	1.71	<.001	.004	1.04	.99	.43	.54	.62	.61	.98	.97
NPDR	4.26	1.45	<.001	.034	.92	1.12	.67	.29	.59	.64	.96	.99
MP	8.62	3.25	<.001	<.001	1.77	1.75	.003	.003	.73	.73	1.00	1.00

[a] Using Trimmed mean[b] Calculated using alpha =0.05Abbreviations: DR, diabetic retinopathy; MP, maculopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

onset T1DM approximates that of adult-onset individuals, with an age range of 2–39 years.

The majority (93%) of the cohort showed uncontrolled T1DM with high HbA1c. More than 54% were anti-GAD65 positive. Their mean UACR was 64.37 ± 8.99 mg/g. Normal UACR levels were seen in approximately 60% of the cohort (n=129). However, all individuals in the study were either G1 or G2 regarding their eGFR. Sixty-five individuals were reported to have DR, with a median age onset of 34 ± 8 years and a median latency period of 13 ± 7 years.

Table 2 demonstrates the ordinal regression analysis of independent variables when we considered the DR presence as a dependent variable. No single independent variable had any significant relationship with DR development at any association level. The same independent variables in Table 2 were used to create a composite regression factor to adjust the UACR data for different individuals with DR. Table 3 demonstrates the different component loadings obtained by principal component analysis and factor rotation. Age had the highest component loading (.907) in a positive direction, while gender contributed with the lowest component loading (.062) in a negative direction.

In Table 4, we used ANCOVA to evaluate the effect size of the covariates (UACR readings) on different DR types before and after adjustment for the composite regression factor. The adjustment was useful to significantly decrease the (F) value to more than half of its original value, which is a measure of the heteroscedasticity of the UACR values in Levene's for different DR types. The adjustment of the UACR for different DR types did not alter the significance levels of the association or the relationship between the UACR and the DR types, with minimal changes of their F value during testing between subjects in both directions. Furthermore, the change was minimal and negligible for both the effect size and the power during the adjustment. Even after adjusting for the composite regression factor, the AUCR levels had no significant association with the overall DR incidence. The most powerful significant association with the changes in UACR was restricted to the seven individuals with MP.

For further analysis, we used ROC curves to define the UACR cutoff values after which DR

Table 5. Receiver Operating Characteristic (ROC) curves properties

Variables	Cut-Off	Sensitivity	1-Specificity	Area Under Curve	Asymptotic Significance	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
Retinopathy (overall)	33.5	.508	.318	.580	.062	.494	.666
PDR	33.5	.550	.335	.590	.055	.483	.698
NPDR	33.5	.552	.358	.610	.056	.511	.709
MP	33.5	.714	.364	.623	.270	.399	.847

Abbreviations: DR, diabetic retinopathy; MP, maculopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

might develop, and it was 33.5 mg/g for DR overall and all subtypes. (33.5 mg/g) represented a level that was slightly higher than the upper normal range. These cutoff values came with different sensitivities and specificities with asymptotic significance> 0.05. The findings from the ROC curves further confirmed the poor correlation between UACR and DR.

DISCUSSION

DR as an indicator for the state of generalized vascular damage progresses from mild nonproliferative abnormalities with increased vascular permeability to moderate and severe NPDR to PDR, characterized by the growth of new retinal vasculature and posterior surface of the vitreous. Macular edema, as part of MP, is characterized by retinal thickening from leaky blood vessels and can develop during any stage of retinopathy[5,6].

This study demonstrated a slight male predominance with a male to female ratio of 1.3:1, which was similar to that of Hietala et al. and with a similar age of onset of T1DM, i.e., 17 years in our study versus 15.3 years in Hietala's study[7].

Approximately 40% of the cohort had different severities of albuminuria, which was similar to other studies[8–10]. Glomerular diffusion hemodynamics, diffusion permeability, and renal functional reserve capacity are abnormal in T1DM and may predispose patients to overt diabetic glomerulopathy[8]. The prevalence of DR in this study was 30.09%, which was similar to the prevalence found in other studies[11,12] but lower than in other studies, which showed a higher prevalence (44.4% and 71.5%)[13,14]. The difference in the prevalence might be attributed to the different population characteristics and risk factors and could not be explained by the screening method alone but rather by the early age detection of diabetes.

There was no significant relationship between any independent variables (sex, age, BMI, patterns of onset of T1DM, HbA1c, anti-GAD-65 antibody, UACR, duration of T1DM, age onset of DR, latency between T1DM and DR onset, eGFR) and DR development in the ordinal regression analysis. In the ordinal regression analysis, we described the different variables that might affect the DR progression of different types. Although the different directions of association with different odds ratios were evident, it lacked significance, which merited further assessment by ANCOVA for the variable of interest, i.e., UACR. Similar findings regarding sex, age at T1DM diagnosis, latency between T1DM onset and DR and BMI were found by the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR)[3,15]. The statistically nonsignificant relationship between the patterns of onset of diabetes and the presence of retinopathy in this article is similar to the results of Esteves et al[13]. Hietala et al. and Hammes et al. found a significant relationship between the age of onset of T1DM and the presence of retinopathy[7,16].

There is a documented controversial relationship between the HbA1c level and DR. Our study found a nonsignificant association between HbA1c and DR, with an odds ratio of 1.39, similar to many previous studies[13,14,17–19]. Other studies

demonstrated a significant relationship between HbA1c and DR[1,7,10,12,16,20–25]. Fluctuations in HbA1c increase the risk for microvascular complications and alter the future progression of DR and possible treatment decisions[7,21,24]. However, in this study, we have only a single determination of the HbA1c level, which only measures blood glucose control during the preceding eight to 12 weeks.

Matuszewski et al. considered poor glycemic control to be the strongest modifiable factor for the development of DR through a cascade of oxidative stress and increased synthesis of prothrombotic factors, contributing to increased production of vascular endothelial growth factor (VEGF) [12]. VEGF is responsible for the development of pathological angiogenesis and PDR, leading to eyesight loss[26]. Data from DCCT show that intensive glycemic control in T1DM was associated with a significant reduction in the progression and a significant increase in the improvement of established DR independent of the duration of diabetes and level of baseline DR[27].

BMI failed to influence DR as an independent variable in this study, similar to some studies[12,13,17,20,21] but not others[7,19,28–31]. The relationship between BMI in T1DM and DR development is complex, and its pathophysiological basis remains unclear. This could be due to obesity-related risk factors such as hypertension and dyslipidemia or it may be directly related to obesity by increasing oxidative stress, inflammation, and macrovascular complications associated with obesity[12,29,30,32]. One of the pivotal inclusion criteria was the inclusion of patients with T1DM with normal renal function only. Normal glomerular filtration or hyperfiltration has been documented in patients with T1DM and G1 and G2 stages of CKD[20].

We did not find any significant association between the duration of T1DM and DR during ordinal regression analysis, yet the odds ratio was 2.82. A similar finding was found in the early phases of WESDR in patients with T1DM who had an onset of their diabetes at their thirties or later[3], but not among younger patients[2]. Many studies have shown such an association to be significant[2,3,6,7,10,12,33]. The latency between T1DM and DR development was not significantly associated with DR development in this study. In the WESDR, this association was significant during the initial assessment of patients with T1DM, but the significance was lost after controlling for or adjusting for other risk factors[15], which enforced our findings.

We could not find any significant association between eGFR and DR during ordinal analysis. It can be considered a covariate or confounding factor to be adjusted during further analysis. Changes in eGFR precede the UACR increase, with an initial increase in the early stages of DM, and then it later declines, mirroring the timely renal function decline[34].

Anti-GAD antibodies were positive in 54.2% (n=117) of patients, with no significant association with DR, although they had an odds ratio of 1.51. This finding was similar to other studies that showed a lack of a significant association between these antibodies and any T1DM microvascular complication, including retinopathy [35,36]. Mimura et al[37] suggested a protective mechanism of anti-GAD antibodies on the retinal tissues of individuals with T1DM. Due to these conflicting results between studies, we introduced the anti-GAD-65 antibody as a component in the composite regression factor to adjust for the UACR association with DR.

The ordinal regression analysis failed to identify any significant relationship between the different UACR categories and that of DR. ANCOVA with and without adjustment for the composite regression factor supported the original conclusion.

Although many studies found a significant relationship between the degree of albuminuria and different DR types[5,10,13,31,38,39], our study did not support that correlation, similar to other studies[1,14]. This controversy might be related to the study design, sample size, ethnic variation, and unmeasured residual confounding at the baseline retinopathy level.

ANCOVA with adjustment provided a unique opportunity to measure the net association between the DR and UACR. There was no association between UACR and DR, but it was significantly associated with MP in the subgroup analysis. This was verified previously by Astuti et al., who found that UACR in MP was higher than UACR without MP[40]. Earlier phases of WESDR provided robust statistical evidence about the correlation between UACR and MP and macular

edema after adjusting for possible covariates. They claimed a causal alteration in prorenin, renin, angiotensin, and fibrinogen, but they did not clarify the exact mechanism behind this association[21]. Benitez-Aguirre et al. proposed another theory about a possible association between high UACR and more narrowing and tortuosity of the retinal vessel caliber[41].

This study pointed to the importance of the standard baseline and frequent follow-up ophthalmological evaluations as a point of care in patients with T1DM.

The main limitation in this study was the noncompliance issue of the patients with the follow-up schedule and timing, which prevented the accumulation of long-term longitudinal follow-up data and reduced the total number of enrolled patients. In addition, the cross-sectional design of the study limited the generalizability of the results and did not provide a causal association of UACR with DR.

CONCLUSION

There was no significant association between UACR at any level with DR development before and after adjustment for all of the possible covariates in this study. The significant association between UACR and MP needs further clarification by a prospective longitudinal large sample size study.

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Conflict of interest

No potential conflicts of interest or financial sponsorship need to be declared.

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Authors Contribution

SAO, HAA and NTY contributed to the study design and execution, data analysis, manuscript drafting and critical discussion. MTA and OMK contributed to the study execution, data analysis, and manuscript drafting. AHA, ASB and AAM contributed to the critical discussion. All authors were involved in the preparation of the manuscript and approved the final manuscript.

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