

Original Article

Significance of microalbuminuria in predicting the risk of diabetic retinopathy in type 2 diabetes mellitus patients

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Abstract: To obtain the predictive value of microalbuminuria (UMA) for diabetic retinopathy (DR) by investigating the influence of UMA on the incidence of DR. The laboratory and clinical data of 512 type 2 diabetes mellitus (T2DM) patients were systematically collected and evaluated. Receiver operating characteristic (ROC) curve was used to determine the optimal cut-off value. The area under the ROC curve of UMA for incidence of DR was 0.730 [95% confidence interval (95% CI), 0.689 to 0.768], with a sensitivity of 67.8% and a specificity of 70.2%. The increase of UMA level was significantly correlated with duration of diabetes mellitus (DDM), fasting blood glucose (FBG), serum Hemoglobin A1c (HbA1c), triglycerides (TG), low-density lipoprotein (LDL) and DR in T2DM patients (all $P < 0.05$), bringing about that was a significantly risk factor for the development DR [Odds ratio (OR) 4.357, 95% CI 3.334 to 6.970, $P < 0.001$]. UMA was an important risk factor of DR, and it is recommended to add UMA as a follow-up project for T2DM patients.

Keywords: Diabetic retinopathy, diabetes mellitus, microalbuminuria, risk factor

Introduction

Diabetes mellitus (DM) developed rapidly throughout the world. At present, nearly 382 million people suffer from DM, of which 80% in developing countries, and this data is estimated to reach 529 million over the world in 2035 [1]. With the rapid economic development, life-style changes and the growth of life expectancy, DM prevalence is showing a rapid upward trend in china. China National Diabetes and Metabolic Disorders Study Group reported that the age standardized incidence of DM was 9.7%, accounting for 92.4 million adults with DM in 2009 [2]. DM has become a kind of important chronic non-communicable diseases which cause serious harm to public health following the cardio-cerebrovascular diseases and cancer. DR is the ocular manifestations of diabetic microvascular complications. The development of DR is a complex process involving many molecules and biochemical mechanisms, and interacts to influence the retinal

blood vessels and cell homeostasis, leading it to be the one of the main causes of irreversible blindness in the global adult population [3-6]. Some researchers such as Yau JW [6], SC Reddy [7], and Ponto KA [8] had reported that the incidence of DR was as a high morbidity rate in the DM population, respectively. Therefore, it can be considered that the number of DR is also very large based on such a large diabetic population. At present, the therapy methods of DR are invasive and aggressive, such as laser, intravitrea injection the drugs of anti-vascular endothelial growth factor (VEGF) and vitreoretinal operation, etc. Therefore, for the majority of clinicians and patients, a simple, inexpensive, and readily available predictive factor to early predict the incidence and development of DR is highly preferred and also has important and extensive clinical value. This study explored the related risk factors of DR through retrospective analysis of the clinical pathological data of 512 type 2DM (T2DM) patients and preliminary calculated the optimal cut-off for predicting the

Table 1. Clinical and biochemical data of examined patients (n = 512)

Parameter	Numerical value*
Age (years)	62.47±10.76
Gender: female/male (n)	254/258
Drinking: no/yes (n)	455/57
Smoking: no/yes (n)	430/82
DDM, years	9.71±7.28
BMI, kg/m ²	25.04±9.96
WBC, × 10 ⁹ /L	6.79±1.70
Platelets, × 10 ⁹ /L	210.91±59.67
FBG, mmol/L	8.77±3.26
PBG, mmol/L	16.22±4.67
HbA1c, %	8.92±2.27
TCH, mmol/L	4.78±1.12
TG, mmol/L	2.00±1.57
HDL, mmol/L	1.13±0.53
LDL, mmol/L	2.86±0.86
UMA, mg/L: median, range	16.22 (0.36-926.43)

*Data presented as mean ± SD or other.

incidence of DR in Chinese patients. Our study may provide reliable technical support and operating basis for finding the predictive and control index of DR in vast primary medical institutions.

Materials and methods

Study population

Between June 2012 to November 2014, 512 patients with T2DM hospitalized in the endocrinology department of the People's Hospital of Guilin (Guilin, Guangxi, P. R. China) were enrolled in this study, of which, 248 cases were DR patients and another 264 were NDR patients. The baseline information including gender, age, smoking, drinking, body mass index (BMI), duration of DM (DDM), microalbuminuria (UMA), fasting blood glucose (FBG), postprandial blood glucose (PBG), Hemoglobin A1c (HbA1c), lipid levels were collected. The following cases were excluded from this study, including: 1) Fever patient, 2) Malignant tumor patient, 3) Patient with coronary heart disease, 4) Uremic patient, 5) Pregnant women and lactating women.

Diagnosis of DM and inspection of DR

The diagnostic criterion for DM was based on the World Health Organization (WHO) criteria

which had been developed in 1999 [9]. All of the DR patients were diagnosed and assessed by the experienced ophthalmologists, who had received specialized DR-related disease training, according to international clinical DR disease severity scale [10]. All patients diagnosed with DM were treated with visual acuity, intraocular pressure, and slit lamp examination to exclude cataract and glaucoma. All patients received mydriasis by using Compound Tropic amide Eye Drops (Shenyang Xingqi Pharmaceutical Co., Ltd, China). Then the fundus oculi camera (Topcom TRC 50DX, Japan) was used to take the photos of fundus in darkroom through the enlarged pupil. The standard of the fundus images was that both macular and disc must be clear. All the inspection operations, diagnosis and classification of DR were completed by the experienced ophthalmologists with specialized DR-related disease training.

Assay method of UMA

UMA was measured by using the immune transmission turbidity, and the reagents used were from Beijing Leadman Biochemistry Co., Ltd. Detection principle: the albumin in the sample and the anti-human albumin antibody of the reagent rapidly form the antigen antibody complex in the buffer, so that the reaction liquor appear turbidity. When keeping antibody excess in the reaction solution, the complex formed increase with the increase of the antigen, and the turbidity of the reaction liquid also increase. The content of unknown albumin can be calculated by comparing with the calibration. The analytical sensitivity of the method is that the absorbance variation ranges from 0.005 to 0.12 on the concentration of 2.5 mg/L, with the linear correlation coefficient $r^2 \geq 0.995$ in regard to the linear ranging from 0 to 1000 mg/L). The kit test project precision is less than 10% and the value < 25 mg/L is treated as normal specimen. Urine samples were collected for 24 hours, of which 5-10 ml was taken to detect after the full mixing of the urine. The UMA was measured by Hitachi 7600.020 Full-automatic Biochemical Analyze (Hitachi High-Technologies Corporation Tokyo Japan). The Other biochemical items were measured by Cobas 8000 Full-automatic Biochemical Analyzer (Roche Diagnostics, Mannheim, Germany). All the blood samples were taken from the early morning fasting venous blood, except for 2 hour postprandial blood glucose.

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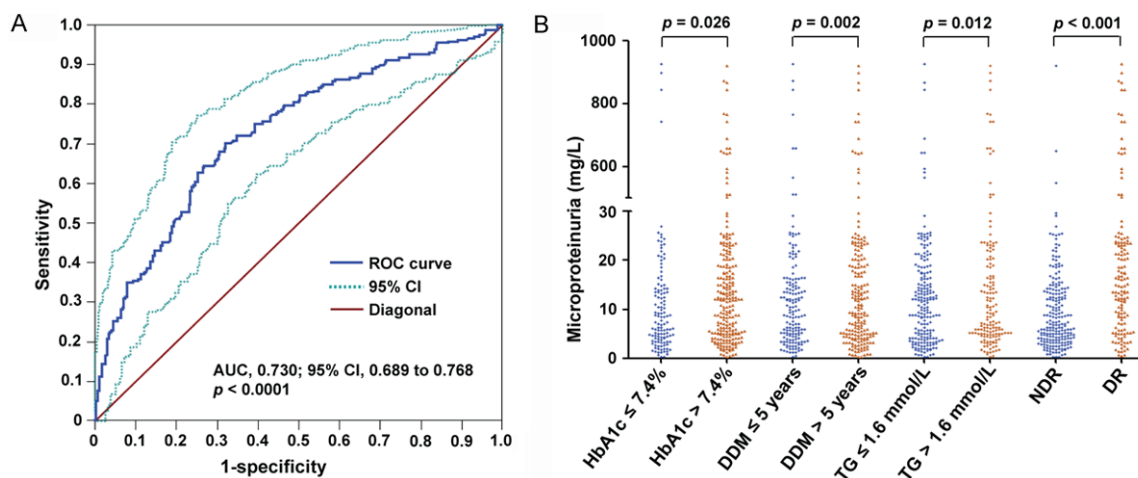


Figure 1. ROC curve and stratified analysis. A. ROC (Receiver operating characteristic) curve to assess the predictive cut off value of the UMA in patients with DR. For predicting the incidence of DR the optimal cut-off value of UMA was 15.6. B. All T2DM patients were stratified based on serum HbA1c, DDM, TG, and DR and compared based on UMA in T2DM subgroups. The patients with an elevated UMA along with an increased HbA1c level ($> 7.4\%$), DDM > 5 years, TG level (> 1.6 mmol/l), DR was much higher than those with HbA1c level ($\leq 7.4\%$), DDM ≤ 5 years, TG level (≤ 1.6 mmol/l), NDR ($P = 0.0026$, $P = 0.002$, $P = 0.012$, $P < 0.001$, respectively).

Before testing the sample of patients, all the test items should be monitored by the internal quality control (IQC), and then specimens can be measured only after the test items were qualified. All of the projects were participated in the Guangxi Zhuang Autonomous Region external quality control (EQC).

Statistical analysis

The statistical analyses were performed using SPSS 18.0 software. Variables were expressed in mean \pm SD unless otherwise stated. Receiver operating characteristic (ROC) curve was used to determine the optimal cut-off value and assess prediction accuracy. The correlations between clinical variables and UMA were calculated by Chi-Square Test. Multivariate logistic regression analysis was used to identify risk factors associated with DR. Odds ratios (OR) for individual risk factors were calculated using logistic regression analyses. OR > 1 was considered to be statistically significant. A $P < 0.05$ was defined as statistical significance.

Results

Basic clinical and laboratory data

Five hundred and twelve T2DM patients, with 248 DR patients and 264 NDR patients, were enrolled in the present study. The demographic, clinical, and laboratory data of the patients

are listed in **Table 1**. Of the 512 patients, 258 (50.39%) were male and 254 (49.61%) were female. Overall, the mean age was 62.47 ± 10.76 years (range, 26 to 92 years), and the mean DDM was 9.71 ± 7.28 years (range, 1 to 40 years). The median value of UMA was 16.22 mg/L (range, 0.36 to 926.43), the average values of HbA1c (%) and FBG (mmol/L) were 8.92 ± 2.27 , 8.77 ± 3.26 , respectively.

The best cut-off value of UMA for the prediction of DR

The UMA values of the T2DM patients ranged from 0.36 to 926.43. Optimal cut-off for the UMA of 15.6 to predict the incidence of DR was selected based on maximizing both of the sensitivity and specificity according to ROC curve. The area under ROC curve (AUC) was 0.730 [95% confidence interval (CI), 0.689-0.768], with a sensitivity of 67.8% and a specificity of 70.2% (**Figure 1A**).

Association of the clinical variables and DR

The relationship between clinical characteristics and DR was investigated and showed in **Table 2**. The data showed that data distribution was statistical significance with gender ($P = 0.003$), age ($P = 0.001$), DDM ($P < 0.001$), FBG ($P = 0.002$), HbA1c ($P < 0.001$), low-density lipoprotein (LDL) ($P = 0.019$), and UMA ($P < 0.001$) in NDR and DR groups. No obvious cor-

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Table 2. Correlation between the clinical variables of the diabetes patients with DR and NDR group

Clinical character	DM patients		P value
	NDR	DR	
	n = 264	n = 248	
Male sex, n (%)	116 (43.93)	142 (57.26)	0.003
Age, years	64.41±11.07	60.41±10.04	0.001
Drinking, n (%)	25 (9.47)	32 (12.90)	0.217
Smoking, n (%)	36 (13.64)	46 (18.55)	0.130
DDM, years	8.41±7.30	11.07±7.04	< 0.001
BMI, kg/m ²	24.76±3.21	25.35±13.93	0.500
FBG, mmol/L	8.07±3.02	9.51±3.35	0.002
HbA1c, %	8.48±2.11	9.38±2.36	< 0.001
TCH, mmol/L	4.83±1.20	4.73±1.02	0.319
TG, mmol/L	2.08±1.68	1.92±1.43	0.241
HDL, mmol/L	1.13±0.33	1.12±0.67	0.737
LDL, mmol/L	2.77±0.81	2.94±0.89	0.019
UMA, mg/L: median, range	13.3 (0.4-926.4)	20.4 (0.4-922.1)	< 0.001

Table 3. Risk factors according to presence or absence of DR as evaluated by multivariate logistic regression model in 512 diabetes patients

Clinical character	β	SE	Wald	P value	Odds ratio	95% CI
Age (years)	-0.541	0.243	4.963	0.026	0.582	0.361-0.937
FBG (mmol/L)	0.090	0.037	5.802	0.016	1.094	1.017-1.177
HbA1c (%)	0.130	0.053	6.040	0.014	1.139	1.027-1.264
TG (mmol/L)	-0.181	0.065	7.653	0.006	0.835	0.735-0.949
LDL (mmol/L)	-0.312	0.121	6.638	0.010	0.732	0.578-0.928
UMA (mg/L)	1.601	0.202	52.593	< 0.001	4.357	3.334-6.970
Constant	-1.172	0.561	4.361	0.037	0.320	

relations between DR and drinking, smoking, BMI, total cholesterol (TCH), triglycerides (TG), and high-density lipoprotein (HDL) were observed (all $P > 0.05$).

Risk factors for the development of DR

To further clarify the risk for the development of DR, the factors which were significantly correlated with DR were enrolled in logistic regression analysis. The OR of DR-related parameter was calculated. Statistically significant variables which were identified in univariate analysis were used as independent variables. Of note, the OR of DR for patients with UMA was 4.357 (95% CI, 3.334-6.970), which was significantly higher than that of FBG [OR = 1.094, 95% CI (1.017-1.177)], HbA1c [OR = 1.139, 95% CI (1.027-1.264)], indicating that UMA is

the most dangerous factor of DR and the increase of UMA is positively correlated with progress of DR. However, the OR of age, TG and LDL for the development of DR were less than 1 (**Table 3**).

Association between the clinical variables and UMA in the diabetes patients

The relationship between clinical characteristics and UMA was investigated and showed in **Table 4**. The data showed that UMA was significantly correlated with DDM ($P = 0.001$), FBG ($P = 0.002$), HbA1c ($P < 0.001$), TG ($P = 0.026$), LDL ($P = 0.035$), and DR ($P < 0.001$), suggesting that these factor might contribute to the increase of UMA and UMA could more comprehensively and effectively predict the development of DR.

Stratified analysis according to serum HbA1c level, TG level, DDM and DR

Patients were stratified according to serum HbA1c level, TG level, DDM and DR to compare the UMA level in two different T2DM subgroups (**Figure 1B**).

The results showed that the level of UMA in patients with serum HbA1c $> 7.4\%$ was significantly higher than those with serum HbA1c level $\leq 7.4\%$ ($P = 0.026$). Moreover, this trend was found in long DDM (> 5 years) compared to DDM (≤ 5 years) ($P = 0.002$). The level of UMA in patients with serum TG level > 1.6 mmol/L was also significantly higher than those with serum TG level ≤ 1.6 mmol/L ($P = 0.012$). Of note, the level of UMA in DR patients was much higher than that in NDR patients ($P < 0.001$).

Discussion

The most common complications of diabetes are microvascular disease, which mainly involve the kidney and eye, and the microangiopathy of the retina and the kidney has similar character-

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Table 4. Correlation between the clinical variables and UMA in the 512 diabetes patients

Clinical character	Clinical variable	No. of patients	UMA (mg/L)		P value
			≤ 15.6 (n)	> 15.6 (n)	
Gender	Female	254	135	119	0.078
	Male	258	117	141	
Age (years)	≤ 55	119	58	61	0.905
	> 55	393	194	199	
Drinking	No	455	226	229	0.577
	Yes	57	26	31	
Smoking	No	430	218	212	0.125
	Yes	82	34	48	
DDM (years)	≤ 5	207	120	87	0.001
	> 5	305	132	173	
BMI (kg/m ²)	≤ 25	286	145	141	0.451
	> 25	226	107	119	
FBG (mmol/L)	≤ 6.2	120	74	46	0.002
	> 6.2	392	178	214	
HbA1c (%)	≤ 7.4	159	98	61	< 0.001
	> 7.4	353	154	199	
TCH (mmol/L)	≤ 5.7	417	210	207	0.279
	> 5.7	95	42	53	
TG (mmol/L)	≤ 1.6	263	142	121	0.026
	> 1.6	249	110	139	
HDL (mmol/L)	≤ 1.0	222	104	118	0.348
	> 1.0	290	148	142	
LDL (mmol/L)	≤ 2.9	268	120	148	0.035
	> 2.9	244	132	112	
DR	No	264	178	86	< 0.001
	Yes	248	74	174	

istics. The increase of UMA excretion in the urine has been recognized as a clinical feature of diabetic nephropathy, and has also been recognized as a sensitive indicator of early diabetic kidney damage. In recent years, the close relationship between DR and UMA has been reported by scholars worldwide. The abnormal expression of UMA is considered to be the performance of microvascular endothelial cells dysfunction and will accelerate the microvascular disease. Scholars in many countries generally deem that the UMA is positive related to the incidence of DR [8, 11-14], and the elevation of UMA increased the risk of DR [8, 11, 13-18]. However, until now few scholars have used large sample data to determine the optimal cut-off of UMA with a relatively high sensitivity and specificity in the prediction and diagnosis of DR. Therefore, this study aimed to do some researches in this area.

Our current study demonstrated that UMA might be a potential predictive marker for the incidence of DR. According to the ROC curve, 15.6 appeared to be the most suitable cut-off value for UMA to predict the risk of DR with a sensitivity of 67.8% and a specificity of 70.2% in T2DM patients. This will provide a reliable technical support and operational basis for the majority of primary health care institutions in order to early diagnosis of DR and determine the appropriate intervention. To our knowledge, this is the first report to demonstrate the optimal cut-off of UMA for predicting the risk of DR in T2DM patients by using such a certain amount of sample data.

In this study, the associations between clinical variables and DR were further analyzed by stratified grouping. The results showed DR was significantly correlated with gender (male), age, DDM, FBG, HbA1c, LDL and UMA. Our findings are consistent with previous studies [19-22]. In addition, Elisa et al [18] and Benarous R et al [23] indicated that the incidence of DR was positively correlated with DDM, HbA1C and UMA,

yet negatively correlated with HDL. The UMA patients with a higher HbA1c and the older patients were more likely to concurrent DR [24]. Of note, our study showed that, when UMA > 15.6 mg/L, the incidence of DR was up to 70% (174/260), when UMA is less than or equal to 15.6 mg/L, the incidence of DR was 29% (74/252). Moreover, for most Chinese patients, elevated UMA increase the risk of DR with the highest OR (3.995). Abnormal UMA can at least show the presence of microvascular damage, such as microvascular leakage, increased brittleness and breakage hemorrhage etc. These manifestations may occur in the kidney, and also appear in the retina, which has been verified in clinical. Therefore, this study provides a powerful clinical basis for the application of UMA in the early diagnosis of DR.

Our study demonstrated that the level of UMA was significantly increased in patients with

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serum HbA1c, FBG, TG, DDM, LDL and DR. It can be speculated that the elevation of HbA1c, TG and DDM and so on was associated with the increase of UMA level and DR patients, and the common increase trend of these factors is closely related to the occurrence of DR in T2DM patients. It can be certain that risk factors associated with DR will increase with the increase of UMA for patients with T2DM, and these risk factors will work together to cause the occurrence of DR and further exacerbate the progression of DR. DR as a major global public health problem [25], early diagnosis and treatment of DR or delay this complication are of great help to maintain and improve the visual function of patients with diabetes. The American association of ophthalmology clinical guidelines about DR recommended blood pressure, blood lipids, renal function and blood glucose levels as follow-up projects. Through statistical analysis, this study confirmed that the value of UMA in predicting the occurrence of DR is also large, and this index is noninvasive, economic and practical. Accordingly, it is recommended to add UMA as a follow-up project for T2DM patients and it will be very helpful for the early the diagnosis of DR and monitoring the dynamic development of DR.

But there are still some limitations in this research. First, the patients selected in this study were mainly nonproliferative diabetic retinopathy (NPDR), and the patients with a more severity of proliferative diabetic retinopathy (PDR) were relatively small. Thus, it needs to be further explored in the future study. Second, the study patients were T2DM. The predictive value of UMA in patients with type 1 DM remains to be determined. Third, what extent of the UMA needed to control can effectively reduce the incidence and progression of retinopathy, and whether there is a correlation between the severity of the DR classification and the increase of UMA are needed to be further explored in large sample cases.

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Disclosure of conflict of interest

None.

Abbreviations

T2DM, type 2 diabetes mellitus; DM, diabetes mellitus; UMA, microalbuminuria; DR, diabetic retinopathy; NDR, non-diabetic retinopathy; n, number of patients; DDM, duration of diabetes mellitus; BMI, body mass index; WBC, white blood cell; FBG, fasting blood glucose; PBG, postprandial blood glucose; HbA1c, glycated haemoglobin; TCH, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; CI, confidence interval.

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