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ORIGINAL RESEARCH

Pentosidine levels in nonproteinuric diabetes associated with both low estimated glomerular filtration rate and cataract

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Xiu-Li Lim[1,*]
Boon-Wee Teo[2,*]
Bee-Choo Tai[1]
Tien Y Wong[3]
Daniel P-K Ng[1]

[1]Department of Epidemiology and Public Health, [2]Department of Medicine, Yong Loo Lin School of Medicine, National University Health System, [3]Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore

*Joint first authors

Background: The main objective of this study was to investigate whether plasma pentosidine levels were associated with cataract and low estimated glomerular filtration rate (eGFR) in nonproteinuric type 2 diabetic patients.

Methods: We characterized 888 nonproteinuric type 2 diabetic patients residing in Singapore according to their eGFR values. Proteinuria was excluded on the basis of multiple urinalyses. Patients with low renal function (cases, n = 125) and controls (n = 763) were defined as having eGFR $<$ and $\geq$60 mL/min/1.73 m^2, respectively. Pentosidine levels were measured by enzyme-linked immunosorbent assay. Multinomial logistic regression was used to test the association between plasma pentosidine levels and the joint phenotype of cataract and low eGFR.

Results: Cases had higher triacylglycerol values, higher systolic blood pressure, and were more likely to be treated with two or more antihypertensive medications. In univariate analysis, cases were potentially more than twice as likely to have had a history of cataract compared with controls. This association persisted in multivariate analyses after adjusting for the significant covariates, hypertension and triacylglycerol, but was attenuated when age was included in the model. Plasma pentosidine levels were significantly higher in cases with low eGFR who also had a history of cataract. This association persisted in multivariate analyses that included the covariates, glycosylated hemoglobin, hypertension, and diabetic retinopathy, as well as age.

Conclusion: Carbonyl stress, as reflected by pentosidine levels, is present in a subset of nonproteinuric diabetic patients. Clinically, this stress was associated with the joint presence of cataract and low eGFR.

Keywords: advanced glycation endproducts, Chinese, normoalbuminuria, renal function

Introduction

While the development of advanced diabetic nephropathy is marked clinically by proteinuria which subsequently leads to renal failure, it had been reported that low glomerular filtration rate is already present in a substantial proportion of type 2 diabetic patients even though they were microalbuminuric or even normoalbuminuric.[1–4] Fifty-one percent of patients in the UK Prospective Diabetes Study who developed chronic renal failure remained nonalbuminuric.[2] The prevalence of low estimated glomerular filtration rate (eGFR) in normoalbuminuric type 2 diabetic patients living in Brazil and Australia was 12.7% and 23%, respectively.[1,3] Low eGFR was present in 12.6% of East Asian normoalbuminuric type 2 diabetic patients in Korea.[5] In nonproteinuric type 2 diabetic Japanese and Chinese patients, the prevalence of low renal function had been reported as 11.4%[6] and 19.7%,[7] respectively.

Correspondence: Daniel PK Ng
Department of Epidemiology and Public Health, Yong Loo Lin School of Medicine, National University Health System, 16 Medical Drive MD3, Singapore 117597, Singapore
Tel +65 6516 4967
Fax +65 6779 1489
Email danielpkng@gmail.com

Aside from these reports, little else is currently known about low eGFR in nonproteinuric diabetes. As such, we sought to characterize 888 nonproteinuric Chinese patients with type 2 diabetes residing in Singapore according to their eGFR values. Specifically, the main objective of this study was to investigate whether there was an association between the presence of carbonyl stress as reflected by pentosidine levels, cataract, and low eGFR in these nonproteinuric patients. The underlying rationale for this hypothesis was that pentosidine, an advanced glycation endproduct, has been linked with chronic kidney disease and with cataract. However, it is not known if carbonyl stress was especially high in diabetic patients having both conditions in the absence of proteinuria.

Materials and methods

Patient population

All patients included in this cross sectional study were of Chinese ethnicity and from one large primary care clinic. These patients were all previously recruited into the Singapore Diabetes Cohort Study. Briefly, the recruitment process for the Singapore Diabetes Cohort Study was as follows. Since 2004, all patients previously diagnosed with type 2 diabetes and who were treated at primary care facilities of the National Healthcare Group Polyclinics in Singapore were invited to join the Singapore Diabetes Cohort Study. Patients with a history of mental illness were excluded. Of the patients approached, 91% agreed to participate in the study and formed part of the cohort. Consenting patients completed a questionnaire to elicit information on demographics, lifestyle factors, and medical family history, and also had their physical measurements taken. Blood and random spot urine specimens were obtained for laboratory analyses, and medical records were reviewed to obtain information on their metabolic control and the presence of comorbidities and complications. The research protocol was approved by both the National University of Singapore institutional review board and the National Healthcare Group domain-specific review board, and patients participating in this cohort gave informed consent.

Diagnosis of cataract

The presence of cataracts was screened for by funduscopy to look for a red reflex. Cataracts are also detected when the nurses perform diabetic retinal photography which reveals media opacity. Patients with visually significant cataracts (ie, visual acuity of 6/18 or more) were referred to the Eye Department for review in preparation for surgery. Patients who perform very fine work, such as microbiology and craftwork, were referred to the Eye Department even if the cataracts only caused very mild reduction in visual acuity that affected their work.

Definition of low renal function and absence of proteinuria

Renal function (expressed as glomerular filtration rate) was estimated using the simplified Modification of Diet in Renal Disease (MDRD) equation where eGFR (mL/min/1.73 m^2) = 186.3 × (plasma creatinine in mg/dL)$^{-1.154}$ × (age in years)$^{-0.203}$ × (0.742 for women) × (1.21 if subject is black).[8] Cases were defined as those with eGFR < 60 mL/min/1.73 m^2 (n = 125) and controls had eGFR values ≥60 mL/min/1.73 m^2 (n = 763).

A total of 888 patients were identified as being nonproteinuric based on multiple spot urine samples. To exclude the presence of proteinuria completely, urine samples were required to test negative on Labstix (Bayer, Leverkusen, Germany) or Micral-Test® (Boehringer Mannheim, Germany), or show an albumin-creatinine ratio <30 mg/g (Exocell, Philadelphia, PA) on at least two of the last three urinalyses. Most of the patients were therefore likely to be normoalbuminuric, although it is possible for some to have microalbuminuria, especially if this was transient. Accordingly, the patients included in this report were described as "nonproteinuric" rather than normoalbuminuric.

Measurement of plasma pentosidine

Plasma pentosidine was measured using a sandwich-based enzyme-linked immunosorbent assay kit which had intra-assay and interassay coefficients of variation <10% (USCN Life Science Inc, Wuhan, China). The kit was used according to the manufacturer's instructions. Standards were prepared by serial dilution to give a range of 0–100 ng/mL. The plasma samples stored at –80°C were first thawed at –20°C overnight and then on ice on the day of experiment. Each plasma sample was diluted 10-fold with the sample diluent that was provided. Next, 100 µL of the standard or sample was incubated in the respective wells for 2 hours. After aspiration, 100 µL of detection reagent A working solution was added per well and incubated for one hour. Each well was aspirated and washed three times with wash buffer before 100 µL of detection reagent B working solution was added. After one hour of incubation, the wash step was repeated. Then, 90 µL of substrate solution was added per well and incubated for half an hour. Next, 50 µL of stop solution was

added per well to stop development of color. Optical density was measured at 450 nm on a VERSAMax monochromatic spectrometer (Molecular Devices, Sunnyvale, CA).

Statistical analysis

All statistical analyses were carried out using STATA 11, assuming a two-sided test with a 5% level of significance. Differences in characteristics between groups of clinical interests were compared using the χ^2 test for categorical variables. For continuous variables which were normally distributed, the Student's *t*-test and the analysis of variance were used to compare differences in mean between two or more groups, respectively. Otherwise, the Wilcoxon rank sum test or the Kruskal-Wallis test was implemented, and the medians compared. Multivariate analysis of the association between history of cataract and low eGFR was performed by logistic regression. The presence of hypertension was defined as having a systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or being on antihypertensive medication. The following variables were considered for adjustment based on their significance at the univariate analysis: waist-hip ratio, triacylglycerol, hypertension, diabetic retinopathy, and cerebrovascular disease. In addition to these variables, we generated another model that considered age as a potential confounder, although it was used directly in the computation of eGFR.

The main objective of the study was to investigate whether pentosidine levels were associated with cataracts and low eGFR in patients without proteinuria. To test this association, multinomial logistic regression was performed in the multivariable analysis of the effect of plasma pentosidine levels on the joint presence of low eGFR and history of cataract. Pentosidine levels were defined based on a binary cutoff according to the median value of 188.74 ng/mL. In this model, the following variables were considered for adjustment based on their significance at the univariate analysis: waist-hip ratio, glycosylated hemoglobin, high-density lipoprotein cholesterol, hypertension and diabetic retinopathy. Again, in addition to these variables, we also considered age as a potential confounder. The above multinominal logistic regression was repeated with pentosidine levels treated as a continuous variable.

Results

Of 888 nonproteinuric type 2 diabetic patients, 125 (14.1%) had low eGFR values (<60 mL/min/1.73 m^2) and were considered as cases, while the remaining 763 (85.9%) patients were used as controls for comparison (Table 1). Cases were older and had higher serum creatinine values as compared with controls, as would be anticipated, since both parameters were used directly to compute eGFR values in the MDRD equation (both $P < 0.0001$). For other variables, cases had larger waist-hip ratio ($P = 0.017$), higher triacylglycerol values ($P = 0.004$), higher systolic blood pressure ($P = 0.007$), and were more likely to be treated with two or more antihypertensive medications ($P < 0.001$). There were no gender differences between cases and controls according to renal status (Table 1).

In total, 248 (28.0%) of the 888 patients had a positive history of cataract, while 128 (14.4%) had previous surgery for cataract removal. In univariate analysis, we observed a significant association between low eGFR and a positive history of cataract ($P < 0.001$, Table 1). Specifically, nearly half of cases (46.4%) had a history of this complication compared with a quarter (24.9%) among the controls (crude odds ratio [OR] 2.61, 95% confidence interval [CI] 1.77–3.85). There was a two-fold increase in risk for cataract surgery (crude OR 2.14, 95% CI 1.35–3.40) amongst cases (24.1%) as compared with controls (13.0%). The associations between low eGFR and other complications, including retinopathy ($P = 0.096$) and cerebrovascular disease ($P = 0.023$) were only of borderline significance (Table 1). In multivariate analysis, the association between low eGFR and cataract remained significant after adjusting for hypertension and triacylglycerol as significant covariates (OR 2.50, 95% CI 1.6–3.71, Table 2). With age included as a covariate, the association between low eGFR and cataract was reduced (OR 1.16, 95% CI 0.75–1.81). Similarly, the association between cataract surgery and low eGFR was reduced when adjusted for age (data not shown).

We next investigated if there was any biochemical evidence linking low eGFR and a history of cataract in our patients. As a candidate biomolecule, we considered pentosidine which had been extensively studied as an advanced glycation endproduct. Particularly, pentosidine had been implicated in human cataractogenesis.[9–14] At the same time, a number of reports had suggested that pentosidine levels were separately related to the presence of chronic kidney disease.[15–22]

Circulating pentosidine levels were measured in the plasma samples of 188 patients according to the following groups: group 1, controls with a positive history for cataract (n = 48); group 2, cases with a positive history for cataract (n = 44); group 3, controls with no history of cataract (n = 48); and group 4, cases with no history of cataract (n = 48). Univariate analyses revealed that plasma pentosidine levels

Table 1 Patient characteristics according to the presence or absence of low estimated glomerular filtration rate

	Case	Control	*P* value
n (%)	125 (14.1)	763 (85.9)	
Male, n (%)	55 (44.0)	341 (44.7)	0.885
Age (yrs)	70.8 (8.8)	62.1 (9.2)	<0.0001
Median diabetes duration (years)	6 (0–42)	7 (0–48)	0.653
BMI (kg/m^2)	25.1 (3.4)	24.8 (3.7)	0.372
WHR	0.90 (0.07)	0.89 (0.07)	0.017
HbA_{1c} (%)	7.3 (0.9)	7.3 (1.0)	0.909
HbA_{1c} (mmol/mol)	56.0 (9.9)	56.0 (11.2)	0.909
Systolic blood pressure (mmHg)	136 (14)	133 (13)	0.007
Diastolic blood pressure (mmHg)	76 (9)	77 (8)	0.076
Mean arterial pressure (mmHg)	96 (9)	96 (7)	0.758
Serum creatinine (μmol/L)	113.7 (20.5)	74.2 (16.5)	<0.0001
Triacylglycerol (mmol/L)	1.58 (0.70)	1.37 (0.74)	0.004
Total cholesterol (mmol/L)	4.70 (0.77)	4.72 (0.80)	0.890
LDL cholesterol (mmol/L)	2.72 (0.63)	2.81 (0.68)	0.201
HDL cholesterol (mmol/L)	1.28 (0.34)	1.29 (0.33)	0.802
Smoking, n (%)			
Current	10 (8.0)	66 (8.7)	0.607
Never	90 (72.0)	571 (74.9)	
Former	25 (20.0)	125 (16.4)	
Patients on antihypertensive drugs, n (%)			
1 drug	36 (29.5)	267 (35.9)	<0.001
2 drug	43 (35.3)	205 (27.6)	
3 or more drugs	36 (29.5)	111 (14.9)	
Not on medication	7 (5.7)	160 (21.5)	
Patients on lipid-lowering drugs, n (%)			
1 drug	86 (71.7)	596 (80.2)	0.177
2 drug	11 (9.2)	44 (5.9)	
3 or more drugs	0 (0.0)	1 (0.1)	
Not on medication	23 (19.2)	102 (13.7)	
Treatment for diabetes, n (%)			
Diet/exercise alone	33 (27.1)	179 (24.0)	0.556
Oral hypoglycemic drugs alone	78 (63.9)	520 (69.6)	
Oral hypoglycemic drugs with insulin	10 (8.2)	45 (6.0)	
Insulin	1 (0.8)	3 (0.4)	
History of diabetic retinopathy, n (%)			
None	87 (77.7)	589 (84.0)	0.096
Any retinopathy	25 (22.3)	112 (16.0)	
History of coronary artery disease, n (%)			
Yes	19 (15.2)	98 (12.8)	0.470
No	106 (84.8)	665 (87.2)	
History of cerebrovascular disease, n (%)			
Yes	14 (11.2)	44 (5.8)	0.023
No	111 (88.8)	719 (94.2)	
History of cataract, n (%)			
Yes	54 (43.2)	188 (24.6)	<0.001
No	71 (56.8)	575 (75.4)	

Note: Values are the mean (SD) unless stated otherwise.
Abbreviations: eGFR, estimated glomerular filtration rate; BMI, body mass index; WHR, waist-hip ratio; HbA_{1c}, Hemoglobin A_{1c}; LDL, low-density lipoprotein; HDL, high-density lipoprotein; SD, standard deviation.

were significantly higher in patients with both low eGFR and cataract, being approximately twice as high in group 2 than in the others ($P = 0.036$, Table 3).

In multivariate analyses, plasma pentosidine levels above the median was associated with a higher risk of the joint presence of low eGFR and cataract in group 2 (relative risk [RR] 4.43, 95% CI 1.69–11.66) after adjusting for other significant covariates, including hypertension and the presence of diabetic retinopathy (Table 4). When age was included in the multivariate analyses, the association between

Table 2 Multivariate association between cataract and low estimated glomerular filtration rate, adjusted for hypertension and triacylglycerol[a]

Variable	OR (95% CI)	*P* value
Model 1 (without age in the model)		
Cataract	2.50 (1.69–3.71)	<0.001
Hypertension	3.49 (1.59–7.70)	0.002
Triacylglycerol	1.36 (1.08–1.73)	0.010
Model 2 (with age in the model)		
Cataract	1.16 (0.75–1.81)	0.509
Hypertension	2.97 (1.32–6.72)	0.009
Triacylglycerol	1.46 (1.14–1.87)	0.003
Age	1.11 (1.08–1.14)	<0.001

Notes: [a]Apart from cataract, only covariates (hypertension and triacylglycerol) which were statistically significant in the final model are shown. Adjustment for age is included in Model 2.
Abbreviations: eGFR, estimated glomerular filtration rate; OR, odds ratio; CI, confidence interval.

raised pentosidine levels and the joint presence of low eGFR with cataract remained highly significant (RR 3.90, 95% CI 1.29–11.78, Table 4). In further multivariate analyses, with pentosidine levels being analyzed as a continuous rather than as a binary variable, plasma levels of this advanced glycation endproduct remained significantly associated with the joint presence of low eGFR and cataract after adjustment for various covariates including age (Supplementary Table S1). We also performed multivariate analyses considering the joint presence of low eGFR and cataract surgery and similarly observed a positive association with pentosidine levels (either as a binary or continuous variable) even after adjustment for age and other covariates (Supplementary Tables S2 and S3).

Discussion

Low renal function (as indicated by eGFR) appeared to be quite common (14.1%) in our Chinese nonproteinuric diabetic patient population. This estimate was comparable with that reported in studies from Japan (11.4%), Korea (12.6%), China (19.7%), and Brazil (12.7%), but seemed lower compared with that reported for patients in the UK (51%) and Australia (23%).[1–7] Strong associations were readily detected between certain patient variables, including age, systolic blood pressure, usage of antihypertensive drugs, and fasting triacylglycerol levels, while more moderate associations were seen for waist-hip ratio, diabetic retinopathy, and cerebrovascular disease. Thus, we were able to replicate some of the findings from earlier studies performed in patient collections from other human populations.

A finding from our study was the apparent association between low eGFR and a positive history of cataract. While clearly novel in the context of nonproteinuria, it is interesting to note that there may be some precedence in the literature linking cataract with low renal function. For instance, patients with cataracts have been reported to have higher blood levels of uric acid and creatinine and this may be consistent with the presence of low renal function.[23] Renal impairment has also been implicated as a possible factor for incident cataract surgery in a largely nondiabetic population-based cohort.[24] Consistent with this, the presence of low eGFR was strongly associated with a two-fold increased risk for cataract surgery in our patients. It should be noted that when adjusted for age, the above associations were attenuated. While this may certainly imply age as a confounder, an alternative explanation may also be considered. Specifically, it should be noted that age was a major factor used in the computation of eGFR (and therefore determining the presence of low renal function). Thus, the adjustment for age would be expected to nullify the association between low eGFR and cataract strongly, given the already well known relationship between age and cataract.

The most salient finding in our study was the evidence for carbonyl stress in a subset of our patients who had both low eGFR and cataract. This stress was alluded to by heightened circulating levels of the well known advanced glycation endproduct, pentosidine. Advanced glycation endproducts are formed from the nonenzymatic glycation of amino acids and other biomolecules. This process of nonenzymatic glycation is exacerbated in diabetes due to chronic hyperglycemia and accompanying reactive carbonyl stress. Of direct relevance to our study, pentosidine has been strongly implicated in human cataractogenesis.[9–14] At the same time, numerous reports have suggested that pentosidine levels are also separately related to chronic kidney disease.[15–22] Building on these prior reports, we have now demonstrated that the presence of low renal function and a history of cataract may be potentially linked through a systemic reactive carbonyl stress. This heightened carbonyl stress could have led to increased accumulation of advanced glycation endproducts in various body tissues, such as the lens and the kidney, thereby altering tissue structure and organ function. Consistent with this view, increased advanced glycation endproduct accumulation has been widely accepted to be a major cause of diabetic complications.[25]

Due to the cross-sectional nature of our study design, we are not able to establish causation, only associations. Thus, our observation of a significant association between raised levels of pentosidine and the joint presence of low eGFR and cataract remains open to various interpretations.

Table 3 Patient characteristics and plasma pentosidine levels according to the presence of low estimated glomerular filtration rate and history of cataract

	Group 1	Group 2	Group 3	Group 4	*P* value
Low eGFR	No	Yes	No	Yes	
History of cataract	Yes	Yes	No	No	
n (%)	48 (25.5)	44 (23.4)	48 (25.5)	48 (25.5)	
Male, n (%)	17 (35.4)	21 (47.7)	14 (29.2)	21 (43.8)	0.253
Age (years)	68.2 (7.1)	73.6 (6.7)	61.2 (9.4)	68.6 (8.9)	<0.0001
Median diabetes duration (years)	8 (0–48)	6 (0–27)	7 (0–32)	5 (0–42)	0.882
BMI (kg/m^2)	24.6 (3.7)	25.1 (3.5)	24.8 (3.7)	25.0 (3.6)	0.907
WHR	0.90 (0.08)	0.91 (0.08)	0.87 (0.06)	0.90 (0.06)	0.060
HbA_{1c} (%)	7.5 (1.3)	7.1 (0.7)	7.1 (0.7)	7.4 (1.0)	0.051
HbA_{1c} (mmol/mol)	58.3 (13.8)	54.1 (8.4)	53.8 (7.3)	57.4 (10.5)	0.051
Systolic blood pressure (mmHg)	133 (9.0)	137 (15)	133 (12)	137 (15)	0.249
Diastolic blood pressure (mmHg)	77 (6)	77 (9)	76 (7)	75 (9)	0.554
Mean arterial pressure (mmHg)	96 (6)	97 (9)	95 (6)	96 (9)	0.863
Serum creatinine (μmol/L)	74.9 (15.8)	110.7 (15.9)	74.6 (16.4)	115.1 (23.0)	<0.0001
Triacylglycerols (mmol/L)	1.52 (1.19)	1.53 (0.64)	1.40 (0.51)	1.64 (0.78)	0.570
Total cholesterol (mmol/L)	4.87 (0.83)	4.73 (0.78)	4.69 (0.88)	4.69 (0.84)	0.656
LDL cholesterol (mmol/L)	2.87 (0.73)	2.79 (0.63)	2.77 (0.76)	2.68 (0.69)	0.632
HDL cholesterol (mmol/L)	1.40 (0.36)	1.26 (0.29)	1.28 (0.30)	1.26 (0.29)	0.099
Smoking, n (%)					
Current	2 (4.2)	4 (9.1)	3 (6.3)	6 (12.5)	0.699
Never	36 (75.0)	30 (68.2)	38 (79.2)	34 (70.8)	
Former	10 (20.8)	10 (22.7)	7 (14.6)	8 (16.7)	
Patients on antihypertensive drugs, n (%)					
1 drug	10 (21.7)	12 (27.3)	15 (34.9)	18 (38.3)	0.045
2 drug	19 (41.3)	15 (34.1)	10 (23.3)	15 (31.9)	
3 or more drugs	9 (19.6)	14 (31.8)	8 (18.6)	13 (27.7)	
Not on medication	8 (17.4)	3 (6.8)	10 (23.3)	1 (2.1)	
Patients on lipid-lowering drugs, n (%)					
1 drug	34 (73.9)	32 (72.7)	36 (83.7)	32 (71.1)	0.518
2 drug	4 (8.7)	2 (4.6)	3 (7.0)	6 (13.3)	
3 or more drugs	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Not on medication	8 (17.4)	10 (22.7)	4 (9.3)	7 (15.6)	
Treatment for diabetes, n (%)					
Diet/exercise alone	14 (29.8)	12 (27.3)	9 (20.9)	13 (27.7)	0.477
Oral hypoglycemic drugs alone	29 (61.7)	30 (68.2)	33 (76.7)	28 (59.6)	
Oral hypoglycemic drugs with insulin	2 (4.3)	2 (4.6)	1 (2.3)	5 (10.6)	
Insulin	2 (4.3)	0 (0.0)	0 (0.0)	1 (2.1)	
Diabetic retinopathy, n (%)					
None	39 (83.0)	30 (69.8)	39 (90.7)	31 (75.6)	0.085
Any retinopathy	8 (17.0)	13 (30.2)	4 (9.3)	10 (24.4)	
History of coronary artery disease, n (%)					
Yes	40 (83.3)	35 (79.6)	44 (91.7)	42 (87.5)	0.378
No	8 (16.7)	9 (20.5)	4 (8.3)	6 (12.5)	
History of cerebrovascular disease, n (%)					
Yes	6 (12.5)	8 (18.2)	4 (8.3)	3 (6.3)	0.281
No	42 (87.5)	36 (81.8)	44 (91.7)	45 (93.8)	
eGFR, mL/min/1.73 m^2	82.7 (16.9)	52.3 (4.8)	83.3 (15.8)	51.0 (7.5)	<0.001
Pentosidine, ng/mL	173 (22–1147)	313 (2–1342)	166 (16–1846)	150 (28–1416)	0.036

Note: Values are the mean (SD) unless stated otherwise.

Abbreviations: eGFR, estimated glomerular filtration rate; BMI, body mass index; WHR, waist-hip ratio; HbA_{1c}, Hemoglobin A_{1c}; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

For instance, one might consider whether a greater exposure to hyperglycemia may have led to higher levels of advanced glycation endproducts in these patients. However, this notion was not supported by the observation that these patients were at least comparable with the others in terms of glycosylated hemoglobin values, diabetes duration, and diabetes treatment modalities. Another possibility was that higher plasma pentosidine might have accumulated due to inefficient removal of advanced glycation endproducts or their precursor molecules when eGFR was low. While this might

Table 4 Multivariate analysis of the joint phenotype of low estimated glomerular filtration rate and history of cataract with plasma pentosidine levels as a binary variable[a]

	Group 1[b]	Group 2[b]	Group 4[b]
Low eGFR	No	Yes	Yes
History of cataract	Yes	Yes	No
	RR (95% CI)	**RR (95% CI)**	**RR (95% CI)**
Model 1 (without age in the model)			
Pentosidine	0.98 (0.40–2.36)	4.43 (1.69–11.66)	1.03 (0.40–2.62)
HbA_{1c}	1.53 (0.93–2.52)	0.67 (0.37–1.24)	1.26 (0.74–2.13)
Hypertension	1.33 (0.44–4.04)	10.27 (1.82–58.05)	12.33 (1.41–107.54)
Diabetic retinopathy	1.77 (0.47–6.61)	6.73 (1.76–25.71)	3.55 (0.94–13.47)
Model 2 (with age in the model)			
Pentosidine	0.85 (0.33–2.15)	3.90 (1.29–11.78)	0.89 (0.33–2.34)
Age	1.12 (1.06–1.19)	1.25 (1.15–1.36)	1.09 (1.02–1.16)
HbA_{1c}	1.84 (1.06–3.18)	0.76 (0.37–1.55)	1.44 (0.82–2.54)
Hypertension	1.01 (0.31–3.28)	4.97 (0.80–30.85)	10.44 (1.17–93.12)
Diabetic retinopathy	2.05 (0.49–8.59)	7.85 (1.70–36.27)	4.03 (0.95–17.13)

Notes: [a]Apart from pentosidine, only covariates (HbA_{1c}, history of hypertension and diabetic retinopathy) which were statistically significant in the final model are shown; [b]group 3 is the referent category.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; RR, relative risk.

be true when comparing cases with controls as a whole, it did not adequately explain why pentosidine levels were different between the two subgroups of cases, namely, those with and without cataract, especially given that we did not observe any difference in eGFR values between the groups. It also failed to explain why cases with low eGFR who did not have cataracts had similar levels of pentosidine compared with controls. Additional environmental and possibly genetic factors may therefore need to be invoked to clarify this phenomenon.

Notwithstanding our positive findings, a few limitations of our study should be highlighted apart from that naturally endowed by the cross-sectional study design. Firstly, we had limited information on the type of cataracts present, ie, nuclear, cortical, or posterior subcapsular. This was because in the setting of our primary care clinic, patient management was oriented towards diagnosis and treatment (by surgery) of visually significant cataracts, regardless of their type or etiology. Secondly, our measurement of pentosidine was not performed using liquid chromatography. Although liquid chromatography has been historically used due possibly to the lack of alternative methods, newer reagents have recently become available for the measurement of advanced glycation endproducts, including pentosidine. Thirdly, glomerular filtration rate was not measured directly using invasive techniques which would have been difficult to carry out in our large patient collection. Instead, an estimation of glomerular filtration rate was obtained using the MDRD formula. This may have inadvertently led to some degree of case misclassification. Also, as elaborated above, this use of eGFR (rather than actual measures of renal function) may have inadvertently raised some difficulty in interpreting the effect of age on the association between low eGFR and cataract. However, this limitation did not extend to the novel association between pentosidine levels and the joint presence of low eGFR and cataract which was statistically robust after adjusting for age and other covariates.

Finally, we did not have biopsy information on the patients in our study. In the absence of this information, it was conceivable that the mechanism responsible for low eGFR in our mostly elderly patients might not be entirely attributed to diabetic nephropathy. With aging, there is an increased risk of other conditions such as arteriosclerosis, atherosclerosis (microvascular and macrovascular), and hypertension. These conditions also cause renal damage and reduced GFR over time, and these can compound injury from poorly controlled diabetes. The functional renal reserve is also reduced or absent in elderly patients, suggesting that reduced GFR was truly lower. Nonetheless, the evidence for a carbonyl stress in our group of diabetic patients was novel and would merit further investigation.

Conclusion

Carbonyl stress, as reflected by pentosidine levels, was present in a subset of nonproteinuric diabetic patients. Clinically, this stress was associated with the joint presence of cataract and low eGFR.

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Disclosure

The authors report no conflict of interests in this work.

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Supplementary tables

Table S1 Multivariate analysis of the joint phenotype of low eGFR and history of cataract with plasma pentosidine levels analyzed as a continuous variable

	Group 1[c]	Group 2[c]	Group 4[c]
Low eGFR	No	Yes	Yes
History of cataract	Yes	Yes	No
	RR (95% CI)	**RR (95% CI)**	**RR (95% CI)**
Model 1 (without age in the model)[a]			
Pentosidine	0.999 (0.998–1.002)	1.002 (1.001–1.004)	0.999 (0.998–1.001)
HbA_{1c} (%)	1.52 (0.93–2.48)	0.70 (0.38–1.29)	1.26 (0.75–2.12)
Hypertension	1.32 (0.44–3.96)	9.04 (1.60–50.93)	12.39 (1.43–107.41)
Diabetic retinopathy	1.78 (0.48–6.64)	6.89 (1.82–26.04)	3.59 (0.95–13.55)
Model 2 (with age in the model)[b]			
Pentosidine	0.999 (0.998–1.001)	1.002 (1.0002–1.003)	0.999 (0.998–1.001)
Age	1.12 (1.06–1.20)	1.25 (1.15–1.36)	1.09 (1.02–1.16)
HbA_{1c} (%)	1.80 (1.05–3.10)	0.80 (0.39–1.63)	1.42 (0.81–2.49)
Hypertension	0.99 (0.31–3.20)	5.02 (0.79–31.94)	10.56 (1.19–93.61)
Diabetic retinopathy	2.09 (0.50–8.71)	8.25 (1.78–38.17)	4.13 (0.98–17.50)

Notes: [a]Apart from pentosidine, only covariates (HbA_{1c}, history of hypertension and diabetic retinopathy) which were statistically significant in the final model are shown; [b]apart from pentosidine, only covariates (HbA_{1c}, history of hypertension [$P = 0.058$], diabetic retinopathy) which were statistically significant in the final model are shown; [c]group 3 is the referent category.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; HbA_{1c}, hemoglobin A_{1c}; RR, relative risk.

Table S2 Multivariate analysis of the joint phenotype of low estimated glomerular filtration rate and history of cataract surgery with plasma pentosidine levels as a binary variable

	Group 1[c]	Group 2[c]	Group 4[c]
Low eGFR	No	Yes	Yes
History of cataract surgery	Yes	Yes	No
	RR (95% CI)	**RR (95% CI)**	**RR (95% CI)**
Model 1 (without age in the model)[a]			
Pentosidine	0.89 (0.30–2.62)	3.48 (1.24–9.76)	1.59 (0.76–3.32)
Hypertension	0.44 (0.13–1.49)	3.39 (0.63–18.40)	13.57 (1.64–111.93)
Diabetic retinopathy	2.84 (0.77–10.52)	6.45 (1.96–21.16)	3.21 (1.15–8.97)
Model 2 (with age in the model)[b]			
Pentosidine	0.76 (0.25–2.33)	2.81 (0.91–8.71)	1.33 (0.61–2.89)
Age	1.14 (1.06–1.24)	1.21 (1.12–1.31)	1.08 (1.03–1.14)
Hypertension	0.26 (0.07–0.996)	1.29 (0.21–8.06)	9.97 (1.19–83.58)
Diabetic retinopathy	3.80 (0.95–15.17)	8.81 (2.33–33.35)	3.60 (1.20–10.74)

Notes: [a]Apart from pentosidine, only covariates (history of hypertension and diabetic retinopathy) which were statistically significant in the final model are shown; [b]apart from pentosidine, only covariates (history of hypertension and diabetic retinopathy) which were statistically significant in the final model are shown; [c]group 3 is the referent category.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; RR, relative risk.

Table S3 Multivariate analysis of the joint phenotype of low estimated glomerular filtration rate and history of cataract surgery with plasma pentosidine levels analyzed as a continuous variable

	Group 1[c]	Group 2[c]	Group 4[c]
Low eGFR	No	Yes	Yes
History of cataract surgery	Yes	Yes	No
	RR (95% CI)	**RR (95% CI)**	**RR (95% CI)**
Model 1 (without age in the model)[a]			
Pentosidine	1.001 (0.998–1.002)	1.002 (1.001–1.004)	1.001 (0.999–1.002)
Hypertension	0.47 (0.14–1.58)	3.25 (0.58–18.15)	13.42 (1.63–110.77)
Diabetic retinopathy	2.95 (0.80–10.93)	7.10 (2.13–23.69)	3.26 (1.17–9.12)
Model 2 (with age in the model)[b]			
Pentosidine	1.000 (0.998–1.002)	1.002 (1.001–1.004)	1.001 (0.999–1.002)
Age	1.14 (1.06–1.23)	1.21 (1.12–1.32)	1.08 (1.03–1.14)
Hypertension	0.28 (0.07–1.05)	1.38 (0.21–9.6)	9.94 (1.18–83.49)
Diabetic retinopathy	3.86 (0.96–15.47)	9.79 (2.53–37.84)	3.67 (1.23–10.98)

Notes: [a]apart from pentosidine, only covariates (history of hypertension and diabetic retinopathy) which were statistically significant in the final model are shown; [b]apart from pentosidine, only covariates (history of hypertension and diabetic retinopathy) which were statistically significant in the final model are shown; [c]group 3 is the referent category.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; RR, relative risk.