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Elevated Level of Glycated KQTALVELVK Peptide of Albumin Is Associated with the Risk of Diabetic Nephropathy

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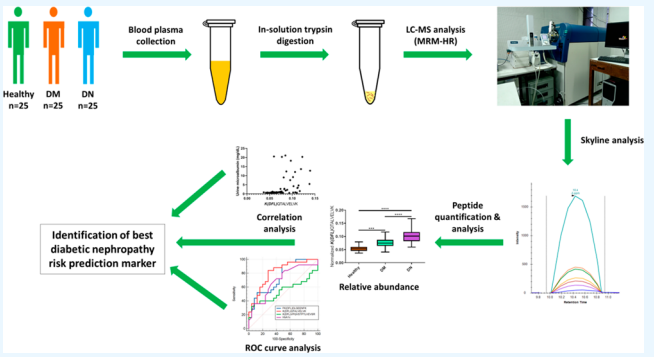
ABSTRACT: Diabetic nephropathy is a leading cause of end-stage renal disease. Hence, early detection of diabetic nephropathy is essential to mitigate the disease burden. Microalbuminuria, the currently used diagnostic marker of diabetic nephropathy, is not efficient in detecting it at an early stage. Therefore, we explored the utility of glycated human serum albumin (HSA) peptides for risk prediction of diabetic nephropathy. Three glycation-sensitive HSA peptides, namely, FKDLGEENFK, KQTALVELVK, and KVPQVSTPTLVEVSR, with deoxyfructosyllysine (DFL) modification were quantified by targeted mass spectrometry (MS) in a study population comprising healthy and type II diabetes subjects with and without nephropathy. Mass spectrometry, receiver operating characteristic (ROC) curve, and correlation analysis revealed that the DFL-modified KQTALVELVK peptide was better than other glycated HSA peptides and HbA_{1c} for identifying diabetic nephropathy. DFL-modified KQTALVELVK could be a potential marker for risk prediction of diabetic nephropathy.



INTRODUCTION

Diabetic nephropathy is one of the major complications that affects about 20−40% of patients with diabetes. Due to glomerular damage, diabetic nephropathy patients have a progressive decrease in the glomerular filtration rate.[1] In diabetic nephropathy, there is an accumulation of nitrogenous waste in the blood, such as urea nitrogen and creatinine,[1,2] and increased excretion of blood proteins in the urine.[3] The diagnosis of diabetic nephropathy is typically based on the measurement of albumin concentration in the urine. The first clinical sign of diabetic nephropathy is microalbuminuria, which is detected by urinary albumin excretion of 30−299 mg in 24 h urine collection or 30−299 μg of albumin/mg creatinine in a spot urine sample.[4] The development and progression of diabetic nephropathy involve different stages.[5,6] Since the initial stages of diabetic nephropathy, before the development of microalbuminuria, are subclinical, there is a risk of developing diabetic nephropathy even when the urinary albumin concentration is within the normal range. Thus, it is crucial to detect diabetic nephropathy early to take timely preventive measures, but the currently used diagnostic markers may not be sensitive enough to detect diabetic nephropathy at an early stage. Therefore, there is an urgent need of a marker to predict the risk of diabetic nephropathy development.

Increased glycation is known to promote the development of several complications in patients with diabetes, including diabetic nephropathy. Several studies have evaluated the ability of well-established glycemic markers like glycated hemoglobin (HbA_{1c}) and other markers, such as glycated albumin, to predict diabetic nephropathy.[1,7−10] Due to the causal link of glycation with diabetic complications,[1] glycated proteins could be useful markers for risk prediction of diabetic nephropathy. HbA_{1c} and glycated albumin have been evaluated for their association with the progression of diabetic nephropathy.[7,11] In a longitudinal study involving 449 patients with type II diabetes, glycated albumin levels were found to be better predictors of diabetic nephropathy development than HbA_{1c} levels.[8] In another longitudinal study conducted on 154 participants, glycated albumin could predict the progression of diabetic nephropathy, but HbA_{1c} could not.[12] Similarly, another study reported superior performance of glycated albumin over HbA_{1c} for the prediction of diabetic nephropathy.[9] Therefore, it is worth evaluating the diabetic nephropathy risk prediction performance of glycated albumin.

Human serum albumin (HSA) has a half-life of 19 days;[13] hence, it represents a short-term glycemic status. HSA is directly exposed to reducing sugars in plasma, and it is the

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most abundant plasma protein containing 59 lysine and 24 arginine residues, many of which can be glycated.[14] Therefore, glycated albumin represents a large share of glycated plasma proteins, and measuring glycated albumin could be more sensitive than other glycated plasma proteins.

Glycated albumin has been increasingly investigated in recent years for its role in the pathogenesis of diabetic complications, as well as for its prediction.[1,11,15] Several reports have found an association between glycated albumin and diabetic nephropathy.[1,9,11,12] However, most of the reports on the association of albumin glycation and diabetic nephropathy have focused on the quantification of total glycated albumin, but it has been reported previously that the lysine residues of albumin vary in their glycation sensitivity.[16−18] Therefore, quantifying glycation-sensitive peptides rather than overall glycated HSA can make the analysis more sensitive. Mass spectrometry is being increasingly used for the discovery and quantification of disease biomarkers.[19] Mass spectrometry is the best tool to characterize and quantify glycated peptides by bottom-up proteomics. It offers great sensitivity and resolution to measure the level of peptides and proteins accurately. In this context, targeted mass spectrometry-based approaches, such as high-resolution multiple reaction monitoring (MRM-HR), offer accurate and sensitive quantification of target peptides with the ease of the mass spectrometry acquisition method setup. Herein, we selected three glycation-sensitive HSA peptides, namely FKDLGEENFK, KQTALVELVK, and KVPQVSTPTLVEVSR, corresponding to sites K36, K549, and K438, respectively, based on previous literatures;[16,18,20−23] quantified these peptides in unmodified and DFL-modified form by MRM-HR approach in a study group comprising healthy and type II diabetes subjects with and without nephropathy; and evaluated if these glycated peptides can be good predictors for diabetic nephropathy.

MATERIALS AND METHODS

Materials. All chemicals, including ammonium bicarbonate, dithiothreitol, iodoacetamide, formic acid, and proteomics grade trypsin, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bradford reagent was purchased from Bio-Rad Laboratories Inc. (Hercules, CA, USA). LC-MS grade water and acetonitrile (ACN) were purchased from JT Baker (PA, USA). Sep-Pak C18 cartridges were purchased from Waters (Waters Corporation, MA, USA). Eksigent micro-LC column (ChromXP C18, 100 × 0.3 mm, 3 μM, 120 Å) was procured from Sciex (Framingham, MA, USA).

Study Design. This cross-sectional study was designed to assess three glycated HSA peptides as a marker for the risk prediction of diabetic nephropathy. Peripheral blood samples from healthy, type II diabetes subjects without nephropathy (DM) and type II diabetes subjects with nephropathy (DN) were collected, and the plasma was separated. Plasma samples were digested by trypsin, and the tryptic peptides were analyzed by targeted mass spectrometry for the quantification of deoxyfructosyllysine (DFL)-modified HSA peptides, namely, FKDLGEENFK, KQTALVELVK, and KVPQVSTPTLVEVSR. Statistical analysis was performed to check if these glycated peptides were significantly elevated in DM and DN groups. Also, ROC curve analysis was performed to compare the accuracy of these glycated peptides in differentiating between healthy, DM, and DN groups.

Subjects. Five mL blood samples were collected from 25 healthy, 25 DM, and 25 DN subjects in EDTA-coated vacutainers. Subjects without a history of known diabetes having HbA_{1c} < 5.7% were categorized as healthy, while those having HbA_{1c} ≥ 6.5% and spot urine microalbumin <2 mg/dl were categorized as type II diabetes subjects without nephropathy. Subjects with HbA_{1c} ≥ 6.5% and spot urine microalbumin ≥2 mg/dl were grouped as subjects with diabetic nephropathy. Subjects with chronic disease, inflammation, thyroid disorder, cardiovascular disease, and pregnancy were excluded from the study. Written informed consent was obtained from all the study participants before blood sample collection, and the study was performed in accordance with the principles of the Declaration of Helsinki. The study was approved by the institute ethics committee of Chellaram Diabetes Institute, Pune. Clinical characteristics of the subjects, such as fasting blood glucose (FBG) level, 2 h postprandial blood glucose (PPBG) level, HbA_{1c}, total cholesterol, triglycerides, HDL, LDL, VLDL, serum creatinine, serum albumin, and urine microalbumin were measured. Blood samples were centrifuged at 1000 rpm at 4 °C for 15 min to separate plasma from erythrocytes. Plasma in the supernatant was collected in a fresh collection tube and centrifuged again at 14000 rpm at 4 °C for 20 min to separate out cellular contaminants and debris. The clear plasma was collected and stored at minus 80 °C until further analysis.

Sample Preparation for MS Analysis. Plasma protein concentration was estimated by Bradford's method. 50 μg of the protein from each sample was diluted with 50 mM ammonium bicarbonate buffer (pH 8.0) to get the final protein concentration of 1 mg/mL in 50 μL reaction volume and was heated at 80 °C for 15 min to denature proteins. 2.5 μL of 100 mM dithiothreitol was added to the solution and heated at 60 °C for 15 min for disulfide bond reduction. Alkylation of the sulfhydryl group was performed by adding 2.5 μL of 200 mM iodoacetamide and incubating the solution in the dark at room temperature for 30 min. Trypsin was added at a 1:25 (w/w) trypsin to protein ratio, and the digestion was performed for 16 h at 37 °C. Trypsin action was stopped by adding 1 μL of formic acid and incubating at 37 °C for 20 min. The tryptic peptides were desalted using Sep-Pak C18 cartridges. The desalted tryptic peptide solution was dried by vacuum concentrator and stored at minus 80 °C until reconstitution for MS analysis.

Liquid Chromatography−Mass Spectrometry (LC-MS) Analysis. HSA peptides were reconstituted in 2% ACN with 0.1% formic acid. The peptide concentration was estimated by NanoDrop 2000 spectrophotometer (Thermo Scientific) at 205 nm, and 0.6 μg of peptides were loaded onto Eksigent C18-RP HPLC column (length 100 mm, ID 0.3 mm, particle size 3 μm, pore size 120 Å) using an Eksigent Ekspert MicroLC 200 system (Sciex, Framingham, MA, USA) which was connected to Triple TOF 5600 mass spectrometer (Sciex). The column temperature was maintained at 40 °C. Peptides were separated using a mixture of mobile phase A (water with 0.1% formic acid) and mobile phase B (ACN with 0.1% formic acid) at a flow rate of 7 μL/min and two linear LC gradient segments, i.e., 2 to 20% mobile phase B for 20 min followed by 20 to 40% mobile phase B for 14 min, were used. MRM-HR approach was used for the data acquisition. The m/z values of the unmodified and DFL-modified HSA peptides FKDLGEENFK, KQTALVELVK, and KVPQVSTPTLVEVSR and their corresponding collision energies were specified in the acquisition method (Table S1) with a collision energy spread as 10 eV. The ESI source was maintained at a temperature of

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Table 1. Details of Precursor Ions and Their Corresponding Fragment Ions Used for Quantification

Modification site	Peptide	Precursor m/z	Precursor charge state	Fragment ions used for precursor quantification (m/z)
K36	FKDLGEENFK	613.8062	2	$y6^+$ (723.3308), $y7^+$ (836.4149), $y8^+$ (951.4418), $y9^{+2}$ (540.272), $b2^+$ (276.1707), $b3^+$ (391.1976), $b8^+$ (933.4312)
K36	F***K(DFL)***DLGEENFK	694.8326	2	$y2^+$ (294.1812), $y6^+$ (723.3308), $y7^+$ (836.4149), $y8^+$ (951.4418), $b3^+$ (553.2504), $b4^+$ (666.3345), $b5^+$ (723.3559)
K549	KQTALVELVK	564.853	2	$y4^+$ (488.3079), $y7^+$ (771.4975), $y8^+$ (872.5451), $y9^+$ (1000.6037), $b7^+$ (770.4407), $b8^+$ (883.5247), $b9^+$ (982.5932)
K549	***K(DFL)***QTALVELVK	645.8794	2	$y2^+$ (246.1812), $y7^+$ (771.4975), $y8^+$ (872.5451), $y9^+$ (1000.6037), $b6^+$ (803.4509), $b7^+$ (932.4935), $b8^{+2}$ (523.2924)
K438	KVPQVSTPTLVEVSR	547.3174	3	$y4^+$ (490.262), $y5^+$ (589.3304), $y6^+$ (702.4145), $y8^+$ (900.5149), $b7^+$ (740.4301), $b9^+$ (938.5306), $b10^+$ (1051.6146)
K438	***K(DFL)***VPQVSTPTLVEVSR	601.335	3	$y5^+$ (589.3304), $y6^+$ (702.4145), $y7^+$ (803.4621), $y8^+$ (900.5149), $b4^+$ (615.3348), $b5^+$ (714.4032), $b7^+$ (902.4829)

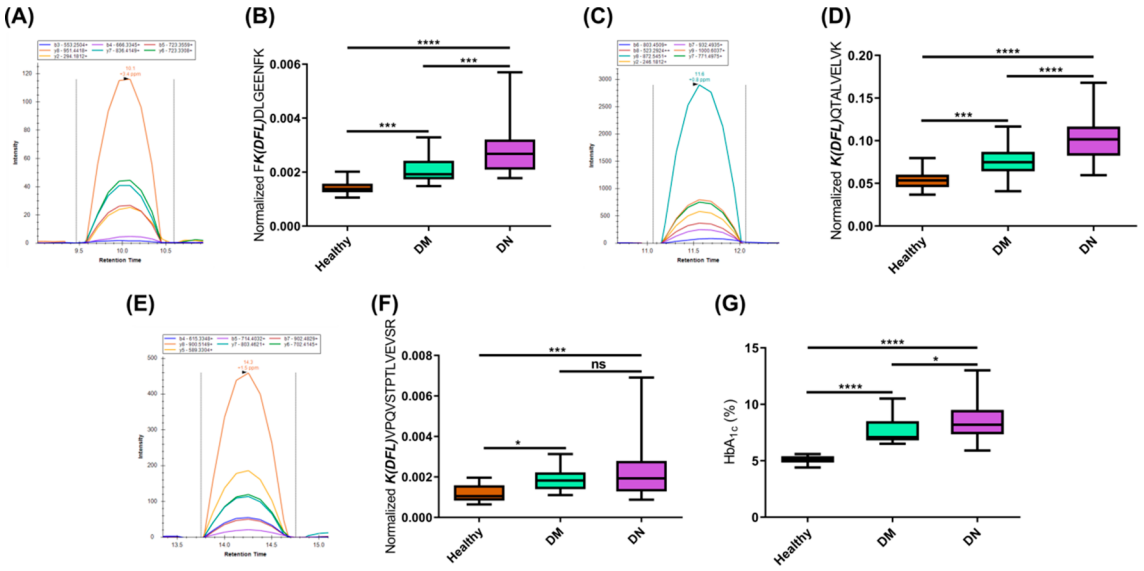

Figure 1. Representative DFL-modified peptide chromatograms and their quantification in healthy, type II diabetes without nephropathy (DM) and diabetic nephropathy (DN) groups. Chromatogram showing coeluting fragment ion peaks of F***K(DFL)***DLGEENFK (A); DM group showed an increased normalized AUC of F***K(DFL)***DLGEENFK than the healthy group. DN group had significantly elevated normalized AUC of F***K(DFL)*** DLGEENFK compared to the healthy and DM group (B); Chromatogram showing coeluting fragment ion peaks of ***K(DFL)***QTALVELVK (C); DM group had an increased normalized AUC of ***K(DFL)***QTALVELVK than the healthy group. DN group had significantly elevated normalized AUC of ***K(DFL)***QTALVELVK compared to the healthy and DM group (D); Chromatogram showing coeluting fragment ion peaks of ***K(DFL)*** VPQVSTPTLVEVSR (E); DM and DN groups had significantly elevated normalized ***K(DFL)***VPQVSTPTLVEVSR compared to the healthy group, but there was no difference in its level between DM and DN group (F); DM group had significantly elevated HbA_{1c} compared to the healthy group, whereas the DN group had elevated HbA_{1c} compared to both healthy and DM groups (G). ns: nonsignificant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

200 °C and a voltage of 5500 V. Nebulizer gas, heater gas, and curtain gas were set at 25 psi each. Declustering potential was set to 80 V. Accumulation time was set to 0.25 s for the TOF MS scan over the mass range of 350−1250 Da and 0.6 s for the fragment ion scan over the mass range of 100−1200 Da.

Data Analysis. The MS data generated were analyzed using Skyline version 22.2.0.255 (MacCoss Lab, University of Washington, WA, USA). The proteolytic enzyme was specified as trypsin, with one missed cleavage. Carbamidomethylation at cysteine (57.021464 Da) was specified as fixed modification, whereas DFL modification at lysine (162.052795 Da) was specified as variable modification. Target HSA peptides for quantification were specified as FKDLGEENFK, KQTALVELVK, and KVPQVSTPTVEVSR. The resolution was specified as 30000. Skyline-generated theoretical precursor and fragment ion masses based on the specified parameters served as the spectral library for precursor quantification. The retention times of the peptides were manually corrected wherever necessary. High-intensity b and y fragment ions of each precursor detected in all samples were selected, and their areas under curve (AUC) were integrated to calculate the AUC of the respective precursor. The AUC of DFL-modified peptides was normalized with the AUC of corresponding unmodified peptides to quantify the respective glycated peptides in each sample.

Statistical Analysis. One-way analysis of variance (ANOVA) followed by a *post hoc* Tukey's test was performed to compare the glycated HSA peptide level among different subject groups. Correlation analysis of DFL-modified peptides with all the recorded clinical parameters was performed using Pearson's correlation method. The ROC curve analysis was performed using MedCalc version 20.0.1 (MedCalc Software Ltd., Ostend, Belgium) to assess and compare the accuracy of glycated HSA peptides as a marker to detect diabetic nephropathy. *p*-values less than 0.05 were considered statistically significant.

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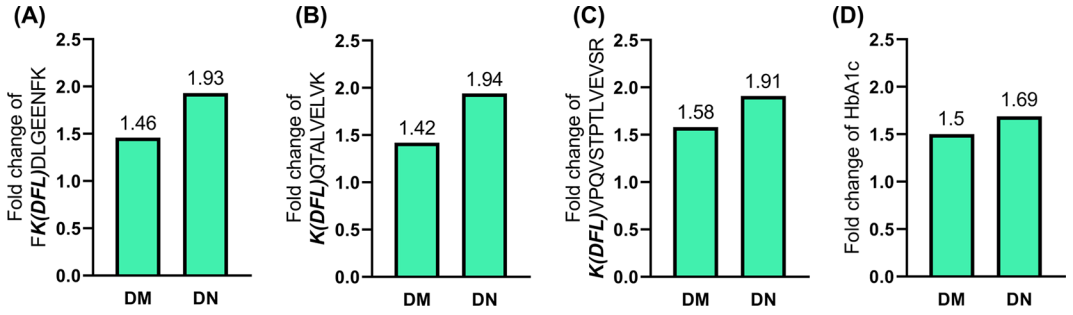

Figure 2. Fold-change in DFL-modified HSA peptide levels and HbA_{1c} in different subject groups. Compared to the healthy group, an increase in the level of F***K(DFL)***DLGEENFK (A), ***K(DFL)***QTALVELVK (B), ***K(DFL)***VPQVSTPTLVEVSR (C), and HbA_{1c} (D) was observed in both DM and DN groups.

RESULT AND DISCUSSION

Diabetic nephropathy is a major complication of diabetes. With the increasing prevalence of diabetes, the burden of diabetic nephropathy will also increase in parallel. At the last stage of diabetic nephropathy, known as end-stage renal disease, patients must undergo dialysis or renal transplant.[24] Therefore, it is vital to predict and detect diabetic nephropathy early to resort to early preventive and therapeutic measures to delay or stop the progression to end-stage renal disease. However, it has been reported that the currently used markers, based on urinary albumin concentration measurement, are not efficient in detecting diabetic nephropathy at early stages; and it can be detected only when there is increased albumin excretion in urine, a condition called microalbuminuria. At this stage, the kidneys are already damaged.[5] Hence, there is an urgent need of a marker for predicting diabetic nephropathy.

Plasma proteins with different half-lives can reflect the glycemic status of varying periods.[16] Among these, there has been a growing interest in glycated albumin as a marker for monitoring glycemic status and predicting diabetic complications.[1,11,15] Glycated albumin has several advantages as a marker. Being the most abundant plasma protein with many glycation-sensitive lysine and arginine residues directly exposed to plasma, HSA is prone to extensive glycation.[14] It has a half-life of 19 days, which is sufficient for glycation reactions. Therefore, glycated albumin could be a potential candidate marker for the risk prediction of diabetic nephropathy. Also, since different lysine and arginine residues of albumin have different propensities for glycation,[18,25] measuring the glycation on glycation-sensitive residues can be more sensitive for diabetic nephropathy prediction instead of measuring total glycation on albumin. In this context, we evaluated the level of glycation of the three glycation-sensitive HSA peptides, namely, FKDLGEENFK, KQTALVELVK, and KVPQVSTPTLVEVSR, in healthy, type II diabetes and diabetic nephropathy subjects using a targeted mass spectrometry approach, MRM-HR, to assess the utility of glycated HSA peptides as marker for risk prediction of diabetic nephropathy.

The site of glycation-sensitive lysine residues in HSA focused in this study, m/z values of glycated HSA peptides, and their corresponding unmodified peptides are shown in Table 1. The DFL-modified peptides are characterized by a mass increase of 162 Da. The MS output data were analyzed using Skyline. Representative chromatograms depicting coeluting fragment ions of DFL-modified HSA peptides are shown in Figure 1. Fragment ions present in all samples with good intensity were used for precursor quantification (Table 1). The b ions bearing DFL modification were considered as qualifier ions for glycation. The precursor AUC was derived by integrating the AUC of selected fragment ions, and the DFL-modified precursor AUC was normalized with the respective unglycated precursor AUC. The normalized AUC of DFL-modified HSA peptides and HbA_{1c} were compared between healthy, DM, and DN groups (Figure 1).

An increase in DFL modification of HSA peptides was observed from healthy to type II diabetes and further to diabetic nephropathy condition. DM group had significantly elevated levels of F***K(DFL)***DLGEENFK, ***K(DFL)***QTALVELVK, and ***K(DFL)***VPQVSTPTLVEVSR compared to the healthy group. Similarly, the level of F***K(DFL)***DLGEENFK, and ***K(DFL)***QTALVELVK was elevated in the DN group compared to the healthy and DM group. However, the DN group showed a significantly elevated level of ***K(DFL)***VPQVSTPTLVEVSR compared to the healthy group but not when compared to the DM group. Thus, all three DFL-modified HSA peptides, namely, F***K(DFL)***DLGEENFK, ***K(DFL)***QTALVELVK, and ***K(DFL)***VPQVSTPTLVEVSR, were able to differentiate between healthy and type II diabetes subjects. However, for diabetic nephropathy detection, only F***K(DFL)***DLGEENFK and ***K(DFL)***QTALVELVK were useful. ***K(DFL)***QTALVELVK levels showed a relatively higher statistically significant difference ($p < 0.0001$) between DM and DN groups than F***K(DFL)***DLGEENFK ($p = 0.0006$). Although HbA_{1c} also could differentiate between DM and DN groups, it had a lesser statistically significant difference ($p = 0.0163$) than that observed with F***K(DFL)***DLGEENFK and ***K(DFL)***QTALVELVK for detecting diabetic nephropathy. This was also evident when the increase in DFL modification of HSA peptides in DM and DN groups compared to the healthy group was represented in terms of fold change and compared to that of HbA_{1c} (Figure 2). While HbA_{1c} showed only a 1.69 fold increase in the DN group, F***K(DFL)***DLGEENFK and ***K(DFL)***QTALVELVK were increased by 1.93 and 1.94 fold, respectively, in the DN group compared to healthy subjects. This finding indicates that F***K(DFL)***DLGEENFK and ***K(DFL)***QTALVELVK are more sensitive than HbA_{1c} and may better predict diabetic nephropathy risk. The higher DFL modification of HSA peptides in the DN group could be attributed to the faster DFL modification of albumin than hemoglobin.[1] Also, since albumin has a short half-life than hemoglobin, glycated albumin can reflect glycemic changes faster than HbA_{1c}.[9] Therefore, DFL-modified HSA peptides may detect diabetic nephropathy sensitively and earlier than HbA_{1c}. Thus, the faster increase in DFL modification of albumin compared to hemoglobin translates into higher

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glycated albumin to HbA_{1c} ratio observed in diabetic nephropathy.[9]

Glycated albumin has previously been assessed for the risk prediction of diabetic nephropathy and has been compared with HbA_{1c}.[1,11,15] Our results are in agreement with the earlier reports that glycated albumin predicts early diabetic nephropathy development than HbA_{1c}.[8,9] This can be explained by the fact that the glycation of albumin changes faster than glycated hemoglobin in response to the serum glycemic index, i.e., albumin is more sensitive to glycation than hemoglobin.[26,27] Different plasma proteins get glycated to different extents,[16,17] and the degree of glycation increases with the increase in glycemia.[28] Glycated albumin level also increases with the severity of diabetes.[10] Thus, being the most abundant plasma protein with a large number of lysine and arginine residues, the DFL-albumin accumulates in patients with diabetes and later promotes the development of diabetic complications, such as diabetic nephropathy.[1] While previous studies mostly quantified total albumin glycation using an enzymatic assay, quantification of glycation-sensitive HSA peptides by mass spectrometry could be more sensitive.

ROC curve analysis was performed to assess the diabetic nephropathy detection accuracy of glycated HSA peptides F*K(DFL)*DLGEENFK, *K(DFL)*QTALVELVK, and *K(DFL)*VPQVSTPTLVEVSR, and compared to that of HbA_{1c} (Figure 3). The sensitivity, specificity, and AUC of these peptides and HbA_{1c} are summarized in Table 2. The ROC plot for *K(DFL)*VPQVSTPTLVEVSR indicated that it was not useful as a marker for diabetic nephropathy detection (p = 0.6256). Among the other two DFL-modified peptides, the area under the ROC curve of *K(DFL)*QTALVELVK was more (AUC: 0.81, 95%CI: 0.674−0.907) than that of F*K(DFL)*DLGEENFK (AUC: 0.752, 95%CI: 0.610−0.863) for diabetic nephropathy detection. However, the difference between the AUCs of *K(DFL)*QTALVELVK and F*K(DFL)*DLGEENFK was insignificant (Table S2). Similarly, the AUC of the ROC curve for both F*K(DFL)*DLGEENFK and *K(DFL)*QTALVELVK was more than that of HbA_{1c}, though not statistically significant. Thus, among the three DFL-modified HSA peptides, *K(DFL)*QTALVELVK and F*K(DFL)*DLGEENFK could be useful markers for predicting diabetic nephropathy.

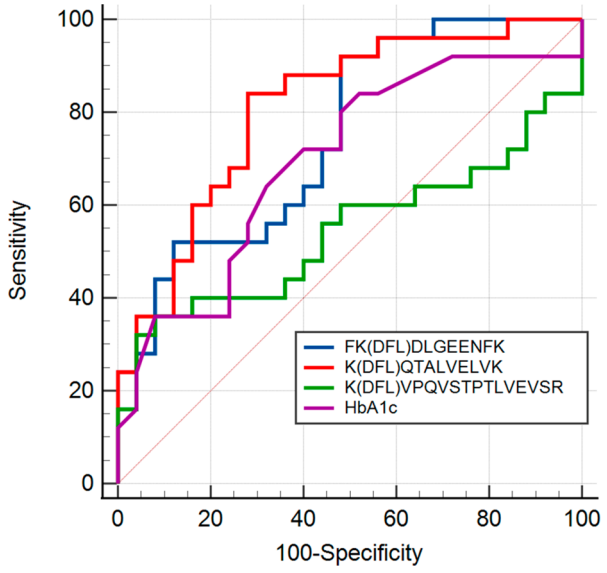

Figure 3. ROC curve analysis for DFL-modified HSA peptides and HbA_{1c} in the detection of diabetic nephropathy.

Pearson's correlation analysis was performed to check for the association of DFL-modified HSA peptides and HbA_{1c} with clinical parameters, especially with diabetic nephropathy-associated parameters, i.e., urine microalbumin and serum creatinine (Table 3). F*K(DFL)*DLGEENFK, *K(DFL)*QTALVELVK, and *K(DFL)*VPQVSTPTLVEVSR showed a strong positive correlation with fasting and postprandial blood glucose level and HbA_{1c}. Urine microalbumin represents the extent of urinary albumin excretion, a parameter used in diabetic nephropathy diagnosis. *K(DFL)*QTALVELVK showed a stronger positive correlation with urine microalbumin (r = 0.429***) than F*K(DFL)*DLGEENFK (r = 0.333**) and HbA_{1c} (r = 0.351**), while all these three markers showed a negative correlation with serum albumin. Only *K(DFL)*QTALVELVK showed a significant positive correlation with serum creatinine (r = 0.326**), a nitrogenous waste elevated in diabetic nephropathy. *K(DFL)*VPQVSTPTLVEVSR was not associated with urine microalbumin or serum creatinine levels. Therefore, *K(DFL)*QTALVELVK showed the best association with diabetic nephropathy-associated markers.

Thus, based on mass spectrometry, ROC curve, and correlation analysis, it can be concluded that *K(DFL)*QTALVELVK could be a better risk prediction marker for diabetic nephropathy, followed by F*K(DFL)*DLGEENFK and HbA_{1c}.

CONCLUSION

Currently, there are no markers in clinical practice that can detect diabetic nephropathy at very early stages. Therefore, in the search for a potential marker, we evaluated the glycated HSA peptides for the risk prediction of diabetic nephropathy. *K(DFL)*QTALVELVK and F*K(DFL)*DLGEENFK could detect diabetic nephropathy better than HbA_{1c}. *K(DFL)*QTALVELVK was better correlated with diabetic nephrop-

Table 2. Summary of ROC Curve Analysis for Assessing the Accuracy of DFL-Modified HSA Peptides and HbA_{1c} for Detecting Diabetic Nephropathy

	DM vs DN			
Peptide	Sensitivity	Specificity	p-value	AUC (95% CI)
F*K(DFL)*DLGEENFK	92	52	0.0002**	0.752 (0.610−0.863)
*K(DFL)*QTALVELVK	84	72	<0.0001***	0.81 (0.674−0.907)
*K(DFL)*VPQVSTPTLVEVSR	36	92	0.6256	0.542 (0.396−0.684)
HbA_{1c}	82	52	0.0101*	0.696 (0.550−0.818)

*p < 0.05. **p < 0.001. ***p < 0.0001

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Table 3. Correlation of DFL-Modified HSA Peptides and HbA_{1c} with Clinical Parameters

Clinical parameters	Correlation coefficient (r)			
	F*K(DFL)*DLGEENFK	*K(DFL)*QTALVELVK	*K(DFL)*VPQVSTPTLVEVSR	HbA_{1c}
FBG (n = 75)	0.606****	0.635****	0.574***	0.788****
PPBG (n = 74)	0.7****	0.656****	0.578***	0.783****
HbA_{1c} (n = 75)	0.727****	0.787****	0.558***	1****
Total cholesterol (n = 75)	−0.107	−0.127	−0.098	−0.026
Triglycerides (n = 75)	0.06	0.088	0.046	0.189
HDL (n = 75)	−0.116	−0.09	−0.027	−0.157
LDL (n = 75)	−0.158	−0.193	−0.111	−0.105
VLDL (n = 75)	0.061	0.098	0.043	0.194
Serum creatinine (n = 74)	0.161	0.326**	−0.091	0.221
Serum albumin (n = 74)	−0.28*	−0.356**	−0.107	−0.449****
Urine microalbumin (n = 63)	0.333**	0.429***	0.152	0.351**

*p < 0.05. **p < 0.01. ***p < 0.001. ****p < 0.0001.

athy-associated clinical parameters, i.e., serum creatinine and urine microalbumin, than F***K(DFL)***DLGEENFK. Absolute quantification and determining the range and cutoff value in a large cohort would help in developing ***K(DFL)***QTALVELVK peptide as a risk prediction marker for diabetic nephropathy.

■ ASSOCIATED CONTENT

Data Availability Statement

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (https://www.ebi.ac.uk/pride) with the data set identifier PXD040028.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01219.

Precursor masses and collision energies specified in the MRM-HR acquisition method (Table S1); Comparison of potential markers for diabetic nephropathy risk prediction based on ROC curve (Table S2) (PDF)

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Notes

The authors declare no competing financial interest.

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