Original Article

Circulating 18-Glycosyl Hydrolase Protein Chitiotriosidase-1 is Associated with Renal Dysfunction and Systemic Inflammation in Diabetic Kidney Disease

Abstract

Introduction: Chitotriosidase-1 (CHIT-1) is a marker of macrophage activation and recently attributed to type 2 diabetes mellitus (T2DM). However, its role in the development and progression of diabetic kidney disease (DKD) has been sparsely discussed in the recent literature. **Materials and Methods:** In this cross-sectional exploratory study, 81 participants with T2DM were classified into two groups based on the presence of DKD. Their anthropometric, biochemical, and pathological profiles were estimated. Circulatory CHIT-1 concentration was determined using the enzyme-linked immuno-sorbent assay (ELISA) in plasma. **Results:** CHIT-1 was significantly elevated in diabetic nephropathy, independent of age and gender. It is associated with severity of kidney disease, as assessed using urinary protein-creatinine ratio (uPCR) in a multiple linear regression model, independent of age, gender, diabetes duration, and insulin resistance. CHIT-1 positively predicted the likelihood of DKD in the study population (area under the curve = 0.724, $P < 0.05$). The duration of diabetes correlated positively with uPCR and negatively with estimated glomerular-filtration rate. Neutrophil-Lymphocyte ratio was elevated in participants with DKD. This well-established marker of systemic inflammation exhibited significant positive association with CHIT-1. **Conclusion:** Plasma CHIT-1 protein is elevated in DKD and associated with disease progression. It is capable of reflecting disease severity and is closely related to systemic inflammation possibly caused by pro-inflammatory circulatory immune cells.

Keywords: *Chronic low-grade inflammation, diabetic kidney disease, neutrophil-lymphocyte ratio, systemic inflammation, type 2 diabetes mellitus*

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Introduction

Chitotriosidase-1 (CHIT-1), belongs to the 18 glycosyl hydrolase (18 GH) group of enzymes, and is the first discovered chitinase in humans. It is mainly expressed by activated macrophages and neutrophils.[1,2] The elevation of CHIT-1 in human diseases was identified through several biomarker studies. It is involved in several infectious, allergic, and auto-immune conditions. Later, it was also found elevated in lifestyle-associated chronic low-grade inflammation (CLGI) conditions.[3] In atherosclerosis, CHIT-1 is associated with severity of the sclerotic lesions, and plaque development, and with increased pro-inflammatory status of circulatory innate immune cells in atherosclerosis.[4] In acute coronary syndrome, there was an association between plasma CHIT-1 activity and inflammatory proteins, i.e., high sensitivity C-reactive protein.[5]

In type 2 diabetes mellitus (T2DM), very few recent studies have discussed the significance of CHIT-1. There are especially sparse studies delineating its role in inflammatory processes occurring in T2DM. In 2010, a study was published, where it was found that CHIT-1 activity in plasma is increased in uncomplicated and untreated type 2 diabetes, compared to healthy controls. Plasma CHIT-1 activity exhibited differences when compared between albuminuric and nonalbuminuric T2DM.[6] Several studies have been done in the following years, nearly establishing that plasma CHIT-1 activity is related to T2DM and development of associated angiopathies. A recent study on CHIT-1 in T2DM showed that the 24-bp duplication mutation in the exon 10 of CHIT-1 gene served a protective role against the

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development of nephropathy.[7] It is also implicated in endothelial dysfunction in both type 1 and type 2 DM.[6,8] Mechanistic role of CHIT-1 and the significance of its expression in diabetic kidney disease (DKD) is unknown, although the aforementioned studies have established its implication in diabetic complications and endothelial dysfunction.

CHIT-1 is a well-established marker of macrophage activation and reflects the systemic immune activation status. However, according to Kumar and Zhang the exact molecular involvement of CHIT-1 in immunity and inflammation is yet to be clearly understood.[9] So far, it is understood that inflammatory stimuli attract innate immune cells to the site of inflammation, and induce their pro-inflammatory status. The consequent expression of inflammatory cytokines has been found to induce the production of CHIT-1.[10]

Elevated CHIT-1 could be associated with tissue remodeling, and therefore involved in fibrosis-linked molecular mechanisms in inflammatory conditions.[11] A few studies have reported the involvement of CHIT-1 in inflammation-associated events, including the recent work of Tans *et al*.[12] However, the mechanistic insights into CHIT-1 function need further research. Although in recent years, the role of CHIT-1 in T2DM has garnered much attention, evidence regarding its significance in inflammation has largely been derived from infective and autoimmune conditions.

Essentially, T2DM is a metabolic condition characterized by chronic systemic low-grade inflammation, where immune response is likely influenced by metabolic alterations. In addition, DM is a key contributor to end stage renal disease (ESRD), accounting for about 50% of all ESRD cases in the entire world. According to the 2017 Global Disease Burden Study, an estimated 219,451 deaths were attributed to DKD.[13] Hence, the relationship between CHIT-1 in T2DM and its role in development of diabetic complications warrants significant research. Studies so far have discussed the role of CHIT-1 activity in T2DM, but the circulating concentration and its significance has not been explored. This is to be investigated to gain the understanding of the molecular pathways involved in CHIT-1 expression, as this might help understand disease progression further in DKD. In order to explore the evidence in this regard, this study aims to study the role of CHIT-1 in DKD, and its possible role in systemic inflammation and disease progression in DKD.

Materials and Methods

Ethics approval

Prior to the beginning of participant enrolment, this study was approved by the Institutional Ethics Committee (JIP/IEC/2019/046, Dated May 21, 2019) and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

All the study participants were explained in detail about the study and written informed consent of voluntary participation was obtained from all the participants enrolled into the study. Written informed consent was also obtained from all the participants before enrolment, to publish their data in journals, in a de-identified format.

Study setting and participant enrolment

For the current study, type 2 diabetic patients aging between 35 and 65 years, were recruited from the Medicine and Nephrology outpatient clinics at a Tertiary Care Hospital located in Puducherry, India. Enrollment of patients took place over the period from January 2020 to June 2022.

This study was designed as a cross-sectional observational study. Participants were recruited following consecutive sampling and enrolled into two groups, namely, T2DM patients without diabetic nephropathy (n = 41) and T2DM patients with diabetic nephropathy presenting with either microalbuminuria or macroalbuminuria (n = 41), as evidenced by the reports of renal function parameters including urinary protein-creatinine ratio (uPCR). Patients presenting with non-DKDs, cardiovascular and hepatic diseases, known endocrinological conditions, recent infections, neoplasia, auto-immune conditions, and other inflammatory conditions, were excluded from the study. Alcoholics, individuals with smoking history, and pregnant or lactating women were also excluded.

Sample size estimation

The primary hypothesis we tested in this study was that plasma CHIT-1 protein levels are significantly elevated in T2DM patients with nephropathy, when compared to T2DM patients without nephropathy. Therefore, an *a priori* power analysis was conducted using G * Power (Version 3.1.9.6, Heinrich Heine University, Düsseldorf, Germany) to determine the minimum sample size required to test this hypothesis.

Although a few previous reports of circulating CHIT-1 levels in diabetic nephropathy were available, recent reports and papers related to our study population were not available. Hence, we calculated sample size in GPower software, choosing *t*-test under Test family and "Means: Difference between two independent means (two groups)" under Statistical Tests and proceeded with the following assumptions for the sample size calculation: For n1 not equal to n2 and effect size of 0.67, power of 90%, alpha of 5% and allocation ratio of 1, GPower 3.1.9.6 gave the sample size of 40 in each arm, coming to a total sample size of 80.[14]

Data collection

Participant anthropometric characteristics, disease history, and blood pressure data were collected. The presence of other microvascular complications such as retinopathy and/or neuropathy was determined based on the reports of standard fundoscopy and neurological examinations, respectively. Fasting venous blood samples of the participants were obtained, and the serum and plasma were collected. Serum was used for the estimation of glucose, lipid profile, and renal function markers, using the Beckman Coulter AU680 and AU5800 Biochemistry Autoanalyzer. Plasma was used for the estimation of complete blood count using Sysmex 4000i Automated Blood Cell Counter. Plasma was also used for the estimation of CHIT-1 using enzyme-linked immune-sorbent assay (Finetest Human Plasma CHIT-1 ELISA kit, Catalog number: EH1551, manufactured by Wuhan Fine Biotech Co., Ltd., China) as per the manufacturer's instructions. The plasma aliquots for ELISA were stored at − 40°C after collection, and analyzed at the end of the data collection. Urine samples were collected to estimate uPCR, and the corresponding urinary protein and creatinine values were measured in the AU 680 Biochemistry Autoanalyzer. Albuminuria was estimated by immuno-nephelometry.

Estimated glomerular filtration rate (eGFR) was calculated based on the CKD-EPI (2009) equation.[15]

The CKD-EPI equation, expressed as a single equation, is GFR = 141 × min (Scr/κ, 1)α × max (Scr/κ, 1) - 1.209 × 0.993 Age × 1.018 (if female) ×1.159 (if black), where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is − 0.329 for females and − 0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

Triglyceride-to-Glucose (TyG) index = ln ([Serum Triglycerides (mg/dL) * (Serum Fasting Glucose (mg/dL)]/2).

Triglyceride-to-high density lipoprotein cholesterol (TG-HDL) ratio = Serum Triglycerides (mg/dL)/(serum high-density lipoprotein-cholesterol (mg/dL).

Plasma atherogenic index = log10 (TG/HDL-C).

Neutrophil-lymphocyte ratio (NLR) = Ratio of neutrophil cell count to lymphocyte count.

Platelet-lymphocyte ratio (PLR) = Ratio of platelet count to lymphocyte count.

Statistical analysis

SPSS version 20.0 (IBM Corporation, New York, USA) was used for conducting all the statistical tests in this study.[16] All continuous data are presented as mean ± standard deviation (SD) or median with interquartile range, depending on their distribution, which we assessed using Shapiro–Wilk test of normality. The categorical data are depicted as percentages and frequencies. Cases and controls were compared for differences between the continuous variables using Independent *t*-test or Mann–Whitney *U*-test, as appropriate. We used Pearson and spearman rank correlation test to assess the relationship between the study parameters. Receiver operator characteristic (ROC) analysis was done to measure the ability of the study parameters to accurately classify the cases and control groups. Binomial logistic regression analysis was performed to test the association of CHIT-1 with the likelihood of presence of kidney disease. Linearity assumption of the regression model was tested following the Box-Tidwell procedure. Visual analysis using box plot of the observations in both groups was used to remove extreme values, i.e. values present outside ± 3 SD.

Multivariate linear regression was performed for estimating the strength of association between the continuous variables. Linearity assumption was assessed by the visual inspection of the LOESS scatterplot with unstandardized residuals at Y-axis and unstandardized predicted value at the X-axis and comparison of the LOESS line with the zero values of y-axis. The confirmatory test was also carried out by the calculation of the mean of residuals.

Independence of residuals was confirmed using the Durbin-Watson statistic, and accepted when the statistic was close to 2 and statistically significant. According to the test, for an α of 0.05, and depending on the number of independent variables and observations, the lower (dL) and upper (dU) limit of the test statistic was assessed and if greater than the dU value, the null hypothesis, i.e., independence of residuals was not rejected.

Homoscedasticity was assessed by visual inspection of a plot of standardized residuals against standardized predicted values. A confirmation of homoscedascity was additionally done using the Bruesch–Pagan test. The absence of multicollinearity was confirmed if the VIF was <3.[17]

One-way analysis of covariance (ANCOVA) was done to assess the difference in CHIT-1 levels between cases and controls, after adjusting for age and gender.

$P < 0.05$ was considered statistically significant in all the statistical procedures.

Results

In this cross-sectional study, type 2 diabetic subjects with nephropathy ($n = 41$) and without nephropathy ($n = 41$), were compared. The aim was to determine the levels of plasma CHIT-1 protein in DKD and T2DM, in order to elucidate possible relationship with DKD disease progression and pathogenesis. The study flow diagram [Figure 1] depicts the process of participant recruitment. Our study did not contain any missing values. Hence, corresponding missing data analysis was not required.

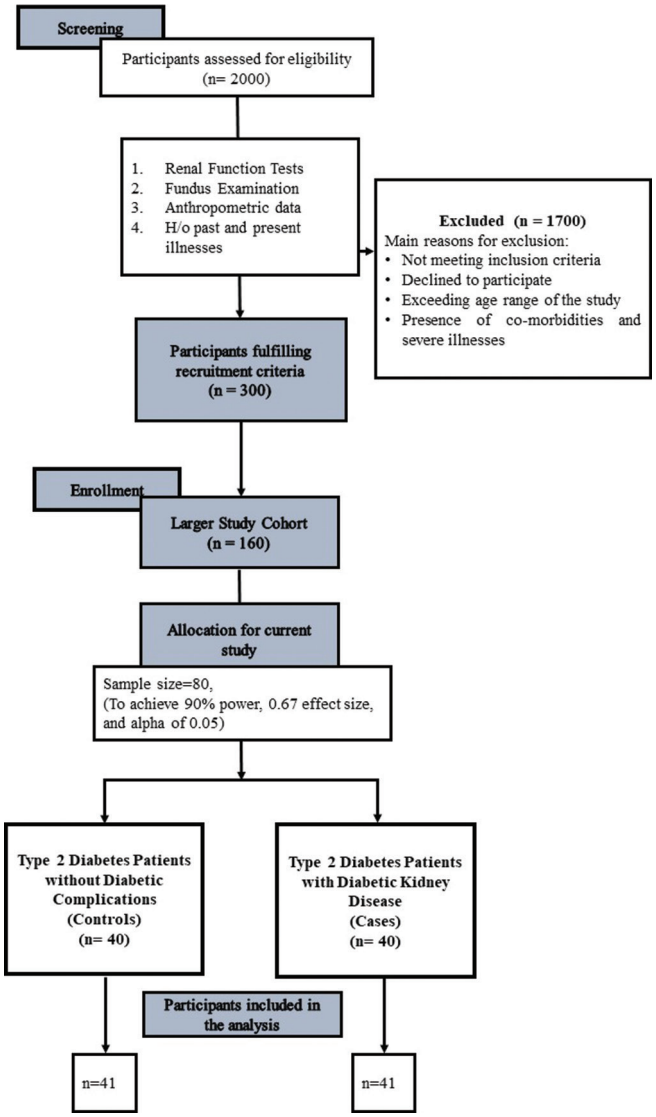

Figure 1: Study flow diagram

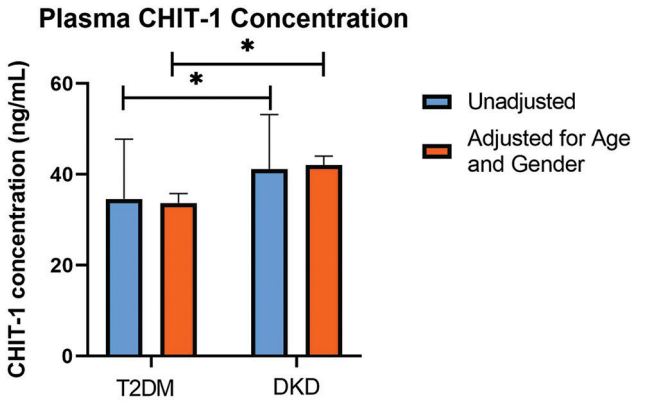

Figure 2: CHIT-1 levels compared between T2DM and DKD, showing comparison for both unadjusted and adjusted data. *P less than 0.05 considered significant. CHIT 1: Chitiotriosidase-1, T2DM: Type 2 diabetes mellitus, DKD: Diabetic kidney disease

Comparison of patient characteristics between controls and cases

The results of comparison carried out for the anthropometric parameters, biochemical profile, pathological indices, and plasma CHIT-1 levels are listed in Table 1. As expected, eGFR, hemoglobin were decreased in the cases. Urinary PCR, neutrophil-to-lymphocyte count, and platelet-to-lymphocyte count were higher in cases than controls. These parameters are known to be positively reflective of disease severity in earlier literature. There was no difference in the values observed for glucose and lipid profile parameters. Among the calculated lipid indices, TyG index has been described as a reliable surrogate measure of insulin resistance. There was no difference observed for this parameter, suggesting insulin resistance to be of similar level in both study groups. This was, however not confirmed by actual estimation of plasma insulin levels.

CHIT-1 was significantly increased in diabetic nephropathy compared to the T2DM group [Figure 2]. To account for the possible confounding effect of age and gender between the two groups, we performed ANCOVA, and observed that CHIT-1 levels exhibited a statistically significant elevation in individuals with DKD. There was a linear relationship between the covariates and CHIT-1, as assessed by visual inspection of a scatterplot. Homogeneity of regression slopes was not violated as the interaction term was not statistically significant, F (1,76) =0.464, P = 0.498. Standardized residuals for the interventions and for the overall model were normally distributed, as assessed by the Shapiro–Wilk's test ($P > .05$). There was homoscedasticity and homogeneity of variances, as assessed by visual inspection of a scatterplot and Levene's test of homogeneity of variance ($P = 0.804$), respectively. There were no outliers in the data, as assessed by no cases with standardized residuals present outside ± 3 SDs. After adjustment for age and gender, there was a statistically significant difference in plasma CHIT-1 levels between the two study groups, F (1, 77) =7.961, P = 0.006.(adjusted and unadjusted differences between the two groups are given in Table 1.

Correlation of study variables

Plasma CHIT-1 levels were correlated significantly with various estimates of kidney disease progression in our study population and the major correlation results are shown in Table 2. Correlation analysis was performed by treating the entire sample population as a single group. We observed that CHIT-1 correlated positively with NLR, a marker of systemic inflammation and with uPCR, the established indicator of kidney disease severity in T2DM.

We also observed significant correlations of other parameters with uPCR and eGFR, which are in agreement with the expected clinical reports of DKD. Red blood cell count was negatively correlated with uPCR and positively with eGFR. The same was observed with hemoglobin, serum albumin, and lymphocyte percentage. However,

Table 1: Comparison of anthropometric and laboratory parameters between study groups

Parameters	Mean±SD		*P*
	Control group (T2DM) (*n*=41)	Case group (DKD) (*n*=41)	
Anthropometric parameters			
Age (years)	51.47±9.3	57.26±7.91	0.007*
SBP (mm/Hg)	134.92±16.64	145.71±18.72	0.048*
DBP (mm/Hg)	81.35±11.63	86.31±14.41	0.22
BMI (kg/m^2)	25.24±3.16	24.91±2.96	0.62
DM duration (months)	90.05±79.80	150.14±56.73	<0.001*
eGFR (mL/min/1.73 m^2)	114.15±55.53	36.82±26.29	0.001*
Biochemical and hematological parameters			
Glucose (mg/dL)	211.05±91.61	240.33±111.03	0.17
Total cholesterol (mg/dL)	165.66±56.38	166.74±42.70	0.94
TGs (mg/dL)	194.97±153.56	175.02±90.07	0.3
HDL (mg/dL)	40.89±8.96	41.41±7.98	0.57
LDL (mg/dL)	110.25±42.23	114.86±33.52	0.81
VLDL (mg/dL)	36.13±18.58	36.14±17.81	0.62
Urea (mg/dL)	28.3±13.86	66.78±43.59	<0.001*
Creatinine (mg/dL)	0.99±0.75	3.23±3.72	<0.001*
Uric acid (mg/dL)	5.36±2.19	7.21±2.29	0.001*
TyG index	9.6±0.58	9.7±0.7	0.48
TG/HDL-C ratio	5.05±4.03	4.52±2.45	0.40
Atherogenic index of plasma	0.61±0.25	0.58±0.25	0.26
uPCR	0.21±0.19	2.96±2.52	<0.001*
Hemoglobin (g/dL)	11.92±1.94	10.90±2.43	0.016*
RBC count (×10^6 µL)	4.46±0.49	3.95±0.84	<0.001*
WBC count (×10^3/µL)	8.98±2.16	9.26±1.97	0.54
Absolute neutrophil count (×10^3/µL)	4.93±1.32	6.04±1.71	0.003*
Absolute lymphocyte count (×10^3/µL)	2.79±0.57	2.38±0.79	<0.001*
Neutrophil-to-lymphocyte ratio	1.79±0.53	3.04±1.61	<0.001*
Platelet-to-lymphocyte ratio	104.12±30.44	123.16±42.4	0.003*
Plasma CHIT-1			
Plasma CHIT-1 (ng/mL) (unadjusted)	34.57±13.2	41.19±12.01	0.012*
Plasma CHIT-1 (ng/mL) (adjusted)	33.69±2.06$^{\#}$	42.04±2.00$^{\#}$	0.006*

*P<0.05; $^{\#}$SE measured for adjusted means in ANCOVA for CHIT-1. ANCOVA: Analysis of covariance; CHIT-1: Chitotriosidase-1; SE: Standard error; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; eGFR: Estimated glomerular filtration rate; HDL: High density lipoprotein; TG: Triglyceride; TyG index: TG-glucose index; HDL-C: HDL-cholesterol; BMI: Body mass index; DM: Diabetes mellitus; LDL: Low density lipoprotein; VLDL: Very LDL; RBC: Red blood cell; WBC: White blood cell; DKD: Diabetic kidney disease; T2DM: Type 2 DM; SD: Standard deviation; uPCR: Urinary protein-creatinine ratio

urea positively correlated with uPCR. Serum urea, uric acid as well as uPCR, exhibit negative linear correlation with eGFR. These relationships are consistent with disease characteristics in DKD. NLR and PLR, which are known inflammatory markers, exhibited significant positive correlation with uPCR and negative correlation with eGFR. Moreover, NLR was positively correlated with PLR (r = 0.623; P = .001).

Association of chitotriosidase-1 with the presence of diabetic kidney disease

To ascertain the association of CHIT1 with DKD, binomial logistic regression was performed on the entire sample population and the results are tabulated in Table 3. Elevation of CHIT-1 in circulation is significantly associated with increase in the likelihood of DKD. After inclusion of NLR as an additional independent variable, statistical significance of the predictive association was not retained. We therefore observe that NLR had a significant confounding role on the relationship between chitoriosidase-1 levels and occurrence of DKD. In addition, our analysis showed that NLR exhibited statistically significant positive association with the presence of DKD. The results of this regression model were independent of the confounding effects of age and gender of the study population.

Association of chitotriosidase-1 with urinary protein-creatinine ratio and neutrophil-lymphocyte ratio-multiple linear regression analysis

In order to elucidate any relationship between CHIT-1 and DKD severity, we needed to determine their linear association. Therefore, to quantify the linear relationship

Table 2: Correlation of study parameters with (A) Chitiotriosidase-1, (B) Urinary Protein Creatinine Ratio, and (C) Estimated Glomerular Filtration Rate in total study population

	R	*P*
CHIT-1 (ng/mL)		
RBC count (×10⁶ µL)	−0.256	0.021
Urine total protein (mg/day)	0.4	<0.001
uPCR	0.361	0.001
Serum creatinine (mg/dL)	0.244	0.029
NLR	0.287	0.015
uPCR (mg/g)		
Serum uric acid (mg/dL)	0.386	<0.001
Hemoglobin (g/dL)	−0.426	<0.001
RBC count (×10⁶ µL)	−0.463	<0.001
Hematocrit (%)	−0.456	<0.001
Neutrophils (%)	0.385	0.001
Lymphocytes (%)	−0.44	<0.001
Duration of DM (months)	0.464	<0.001
NLR	0.551	<0.001
PLR	0.338	0.003
eGFR (mL/min/1.73 m²)		
Duration of DM (months)	−0.291	0.009
Serum urea (mg/dL)	−0.871	<0.001
Serum uric acid (mg/dL)	−0.643	<0.001
Hemoglobin (g/dL)	0.285	0.010
Serum albumin (g/dL)	0.295	0.024
Urine total protein (mg/dL)	−0.697	<0.001
uPCR	−0.692	<0.001
NLR	−0.389	0.001
PLR	−0.239	0.037

P<0.05 was considered statistically significant. CHIT-1: Chitotriosidase-1; RBC: Red blood cell; uPCR: Urinary protein-creatinine ratio; NLR: Neutrophil-lymphocyte ratio; PLR: Platelet lymphocyte ratio; eGFR: Estimated glomerular filtration rate; DM: Diabetes mellitus

between CHIT-1 and disease severity markers, we conducted a hierarchical multiple linear regression analysis with CHIT-1 as the outcome variable. CHIT-1 was associated with indices of both disease severity as well as systemic inflammation. It exhibited positive association with NLR. Regression coefficients and standard errors can be found in Table 4. This predictive positive association between NLR and CHIT-1 were confounded with statistical significance by urinary PCR. We observed from the model that, uPCR exhibited an independent positive association with the elevation of CHIT-1 levels in plasma. This significant predictive association was not confounded by age, gender, duration of diabetes, and TyG index, which were adjusted for, as covariates in the hierarchical regression model.

Receiver operator characteristic analysis of discriminatory potential of chitotriosidase-1

This study aimed to investigate the significance of circulating CHIT-1 protein levels in DKD. Hence, the discriminatory capability of CHIT-1 was analyzed using the ROC curve. When compared to other known and well-established parameters of DKD, CHIT-1 exhibited decent discriminatory ability in classifying DKD in T2DM. CHIT-1 was acceptably closer in area under the curve (AUC) values to NLR and serum uric acid. This signifies the possibility of an important pathological mechanism that may be underway in the increased expression of CHIT-1 in patients with DKD. The ROC analysis results are presented in Table 5.

Discussion

CHIT-1 or Chitinase-1 is a protein of the human chitinase family, called 18 GHs. It is one among the only two functional chitinases found in humans: CHIT-1 and Acid Mammalian Chitinase. All other members of the 18 GH family do not have the chitinolytic function. CHIT-1 is suggested to be involved in several innate immune processes associated with chronic low-grade inflammatory conditions. However, very few studies have assessed plasma CHIT-1 in diabetes mellitus and its significance in DKD. Therefore, the objective of this study was to measure circulating protein levels of CHIT-1 and to determine its association with systemic markers of disease progression, and thus elucidate its possible involvement in the pathogenesis of DKD.

The study population comprised of type 2 diabetes patients without and with DKD, and their plasma was assessed for circulatory CHIT-1 concentration. An age-and gender-independent elevation was observed for CHIT-1 in DKD group. It was accompanied by decrease in hemoglobin, albumin and eGFR, and elevated levels of uPCR, neutrophils and lymphocytes. These were in line with the expected laboratory observations in DKD. CHIT-1 is known to be largely produced and secreted by neutrophils, monocytes, natural killer cells and lymphocytes, generally upon immune stimulation.

Elevated CHIT-1 activity was first observed in untreated, newly diagnosed T2DM individuals by Sonmez *et al*. in 2010.[6] This was the earliest report finding CHIT-1 to be linked to T2DM pathogenesis. Later, few further studies also found CHIT-1 activity in plasma to be elevated in T2DM patients presenting with diabetic complications such as, DKD and neuropathy.[18] Early evidence of differential concentration of CHIT-1 in T2DM was presented in the work of Żurawska-Płaksej *et al*. Their study found that the concentration of CHIT-1 significantly increased in plasma of T2DM patients with proteinuria (both micro and macro-albuminuria).[14,19] These studies strongly suggest that CHIT-1 could be involved in the pathogenesis of DKD. Our findings corroborate with these previous findings on the levels of CHIT-1 activity. Protein concentrations of CHIT-1 are independently elevated in DKD.

In our study, CHIT-1 was positively associated with disease progression in DKD. There was significant positive

Table 3: Logistic regression model for predicting the likelihood of diabetic kidney disease

Model (n=82)	χ^2 statistics	P	Nagelkerke R^2	Model accuracy	B	SE	Significant	OR	95% CI for OR	
									Lower	Upper
1										
CHIT-1	5.114	0.024	9.3	60.6	0.043	0.020	0.029*	1.044	1.004	1.086
2										
CHIT-1	15.43	<0.001	26.1	73.2	0.057	0.022	0.010*	1.059	1.014	1.106
Age					0.095	0.033	0.004	1.099	1.032	1.172
3										
CHIT-1	22.437	<0.001	36.2	74.6	0.064	0.023	0.006*	1.066	1.019	1.116
Age					0.080	0.034	0.018	1.084	1.014	1.158
Gender					1.603	0.639	0.012	4.970	1.421	17.385
4										
CHIT-1	39.33	<0.001	56.8	85.9	0.044	0.026	0.084	1.045	0.994	1.099
Age					0.102	0.038	0.008	1.108	1.027	1.194
Gender					1.026	0.729	0.159	2.790	0.669	11.644
NLR					1.655	0.527	0.002*	5.232	1.861	14.708

*P<0.05 is statistically significant. CHIT-1: Chitotriosidase-1; NLR: Neutrophil-lymphocyte ratio; SE: Standard error; CI: Confidence interval; OR: Odds ratio

Table 4: Association of markers of inflammation and diabetic kidney disease severity with plasma chitotriosidase-1 concentration

Model (n=82)	F	P	Adjusted R^2	Model parameters	B	β	P
1	5.905	0.018	0.068	NLR	40.737	0.287	0.018*
2	14.152	0.000	0.282	NLR	3.179	0.022	0.851
				uPCR	2.991	0.539	0.000*
3	6.030	0.000	0.311	NLR	7.037	0.049	0.680
				uPCR	3.395	0.612	0.000*
				Gender	−5.651	−0.210	0.063
				Age	−0.119	−0.084	0.432
				Duration of DM	−0.024	−0.144	0.172
				TyG index	0.546	0.029	0.783

*P<0.05 is statistically significant. NLR: Neutrophil-lymphocyte ratio; uPCR: Urinary protein-creatinine ratio; DM: Diabetes mellitus; TyG index: Triglyceride glucose index

Table 5: Receiver operator characteristic curve analysis of chitotriosidase-1 and other major markers of kidney disease in diabetes mellitus

	Area under the curve	P	Asymptotic 95% CI	
			Lower bound	Upper bound
eGFR (mL/min/1.73 m^2)	0.897	<0.001*	0.827	0.967
uPCR	0.979	<0.001*	0.950	1.0
NLR	0.781	<0.001*	0.665	0.897
Serum uric acid (mg/dL)	0.745	0.001*	0.619	0.872
CHIT-1 (ng/mL)	0.724	0.003*	0.592	0.856
PLR	0.724	0.003*	0.596	0.853
Hemoglobin (g/dL)	0.644	0.027*	0.521	0.767
TG/HDL-C ratio	0.481	0.795	0.332	0.629

*P<0.05 is statistically significant. CHIT-1: Chitotriosidase-1; eGFR: Estimated glomerular filtration rate; uPCR: Urinary protein-creatinine ratio; NLR: Neutrophil-lymphocyte ratio; PLR: Platelet lymphocyte ratio; TG: Triglyceride; HDL-C: High-density lipoprotein-cholesterol; CI: Confidence interval

association between CHIT-1 and uPCR, independent of age, gender, duration of diabetes and insulin resistance. This was further strengthened by the logistic regression that showed an age- and gender-independent ability for CHIT-1 to discriminate the likelihood of DKD in the study population. This is corroborated by similar findings that were observed in a study which reported that CHIT-1 activity could discriminate between normal and abnormal

albumin excretion in T2DM individuals, suggesting its link to disease progression in DKD.[20]

CHIT-1 levels increasing with increase in disease severity could underlie an associated increase in induction and transcription of CHIT-1 gene. This is evident from some recent studies that have observed tissue-level expression of CHIT-1 to be strongly associated with disease progression and inflammation in type 2 diabetes. In adipose tissue of obese and T2DM patients, CHIT-1 mRNA levels were found elevated and associated with inflammatory and oxidative stress levels (Tans *et al*. 2019).[12] This study also reported that adipose tissue expression of CHIT-1 could be considered as a tissue biomarker of inflammation. Similarly, in Kupffer cells of patients with nonalcoholic steatohepatitis, CHIT-1 mRNA was increased, and is proposed to be elevated due to immune cell activation as a response to lipid peroxidation and free radical generation.[21,22] Such mechanistic evidences are lacking in DKD, regarding its involvement in inflammation.

In our work, we tested the relationship between CHIT-1 concentration and systemic inflammatory status. The relationship between NLR and CHIT-1 has not yet been reported in literature. Neutrophil-to-lymphocyte ratio is a well-known indicator of systemic inflammation in DKD. It is significantly increased in DKD compared to uncomplicated diabetes or early DKD.[23] NLR exhibits a significant positive correlation with CHIT-1 in our study. Importantly, our study has observed that systemic inflammatory status is strongly associated with circulatory changes of CHIT-1 protein levels in DKD. In addition, this positive association was independent of age, gender, and duration of diabetes. Thus, a strong agreement between the macrophage activation status and systemic inflammation exists in DKD, evidenced through CHIT-1. This association was, however, confounded only by urinary PCR. These evidences show that expression of CHIT-1 could be influenced by molecular pathways likely attributed in severity of DKD and associated inflammatory events.

We further observed that NLR is significantly elevated in DKD compared to T2DM. Additionally, NLR is also significantly associated with the presence of DKD in our study population as observed in the logistic regression model. This association of NLR with microvascular diabetic complications has been consistently reported by several works, suggesting its potential as a marker of endothelial dysfunction, systemic inflammation, and early renal complications in T2DM.[24,25] There is a possibility of disease severity being closely related to the immune activation status at a systemic level, and might be well reflected through CHIT-1 protein levels in plasma in DKD. Since CHIT-1 is highly expressed by active immune cells, the protein expression in plasma could also be an indicator of indicate the effect of infiltrated immune cells resident in the diseased kidney of DKD individuals. The ROC analysis made it evident that, the difference in CHIT-1 levels could decently discriminate T2DM with and without Nephropathy, and its comparability to AUC exhibited by known markers involved in pathogenesis (including uric acid and NLR) therefore suggests that, the marked elevation of CHIT-1 expression in diabetic nephropathy could carry biological significance relating to the pathological changes occurring due to DKD in T2DM.

Taken together with our study inferences, these evidences suggest the possible occurrence of a scenario in diabetic nephropathy, whereby, glycemic and inflammatory insults to the kidney tissue could induce the expression of CHIT-1 by resident and infiltrating immune cells. CHIT-1 expression is closely associated with disease progression. But this is not reflected by CHIT-1 activity estimated in plasma. This observation was suggested to be attributed to loss of enzyme activity as a result of the 24-bp duplication mutation in the exon 10 of CHIT-1 gene, a common occurrence in all populations. However, the findings of our study have shown that estimation of CHIT-1 protein levels in plasma is equally informative of disease status, and inflammation. Although the significance of CHIT-1 enzyme is not yet clearly known in T2DM, understanding the upstream pathways of the CHIT-1 protein transcription and secretion in immune cells might serve in understanding CLGI, a major cause of diabetic complications, and the conclusions of our study are in support of this perspective.

Our study also has some limitations. We could not conduct tissue level expression studies in human kidney samples to test the association between plasma CHIT-1 and its gene expression. This could have helped understand how strongly plasma protein concentrations reflect tissue level changes and their association with disease progression. Since DKD and T2DM patients do not commonly receive kidney biopsies, we decided to avoid tissue collection with regard to ethics. We could not assess CHIT-1 activity levels and compare with CHIT-1 concentration. This could have helped comparatively evaluate their relationship with disease parameters.

Conclusion

Our study has shown that CHIT-1 protein concentration is associated with DKD disease severity and might therefore be essential to the understanding of DKD development and progression. We have also shown that CHIT-1 levels are independent of age and gender. Based on evidence from literature on diabetes and other diseases, we investigated the relationship between CHIT-1 and systemic inflammation. Our analysis proved that circulatory CHIT-1 protein is strongly associated with NLR. In total, the evidence from our observational study suggests that there is a link between CLGI pathways and DKD progression, reflected by CHIT-1 expression in plasma. Further research on CHIT-1 expression could potentially inform us regarding the pathways involved in immune cell activation

and infiltration in the diabetic kidney and the possible molecular mechanisms associated with kidney disease in diabetes.

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Conflicts of interest

There are no conflicts of interest.

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