TISSUE BARRIERS
2022, VOL. 10, NO. 3, e1994823 (11 pages)
https://doi.org/10.1080/21688370.2021.1994823

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Urinary IgG, serum CX3CL1 and miRNA-152-3p: as predictors of nephropathy in Egyptian type 2 diabetic patients

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ABSTRACT

The purpose of this study was to assess the role of urinary IgG, serum CX3CL1 and miRNA 152–3p levels as predictors of nephropathy in type 2 Egyptian diabetic patients. Sixty type 2 diabetic patients and twenty healthy controls were enrolled in a cross-sectional study. Then they were grouped into: three groups based upon urine albumin excretion (UAE). The expression of miRNA 152–3p in serum was measured using quantitative polymerase chain reaction (RTq-PCR). Serum CX3CL1 and urinary IgG concentrations were measured by ELISA. RTq-PCR revealed that serum miRNA-152-3p levels in patients were significantly higher than in controls. There was significant differences between group with normoalbuminuria and groups with diabetic nephropathy DN as regard to age, duration of nephropathy, Albumin/Creatinine ratio (A/C ratio), creatinine, urine IgG, CX3CL1 and HbA1c. In diabetic patients, there was a significant positive correlation between miRNA-152-3p levels and disease duration only as well as significant positive correlations between urinary IgG levels and age, disease duration, serum creatinine, A/C ratio, and urea. Positive correlation between serum fractalkine CX3CL1 level and age, duration of disease, urea, creatinine, A/C ratio, HbA1C and IgG in patient with DN. Serum CX3CL1 level, urinary IgG were significantly increased with the progress of nephropathy so these integrated biomarkers could be used as good predictors for early identification of nephropathy. But miRNA- 152-3p has inadequate prognostic indicator for ESRD progression.

ARTICLE HISTORY
Received 10 September 2021
Revised 2 October 2021
Accepted 10 October 2021

KEYWORDS
Diabetic nephropathy; miRNA 152-3p; CX3CL1; Urinary IgG; Fractalkine; Albuminuria

Introduction

Type 2 diabetes play a vital role in the global problem due to obvious effect of related complications.[1] Diabetic nephropathy (DN) is increasingly to become a main reason of cardiovascular diseases and end-stage renal disease), which can develop after long period of diabetes.[2] Inflammatory cells, adhesion molecules, cytokines, and all chemokines are implicated in the development of DN, verifying that it is a chronic inflammatory and immunological disease.[3]

Albuminuria, identified by the urinary albumin creatinine ratio (UACR), also has limitations although being an initial predictor of DKD.[4] Patients with diabetes may have reduced renal function but no significant increases in albuminuria. The risk of diabetic nephropathy development starts even when urinary excretion of albumin is within the normoalbuminuric range, and the progression from normo-albuminuria into micro/macroalbuminuria occurs more frequently in type 2 DM patients with baseline urinary albumin > 2.5 mg/24 hour.[5] Accordingly, there is a necessity to explore a biomarker that can aid in early detection and serve as a prognostic indicator for disease development.[6]

Micro-RNAs are short noncoding RNAs that play a role in many of pathophysiological processes including the development of chronic renal illness such as DN.[7] Many human miRNAs involved in the pathogenesis of renal disease have been discovered

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in recent years, among these, miRNA-152-3p may considered an important role in the progression of type 2 diabetes mellitus (T2DM), as well as in the development of new mechanisms in the pathophysiology of DN.

C-X3-C motif chemokine 2 (CX3CL1) upregulated in diabetes produced mostly by glomerular endothelial cells and the tubular epithelium and as well as in many other cells, such as podocytes, stromal cells and renal tumor cells.[8] CX3CL1, also known as fractalkine. CX3CL1 has two types (membrane and soluble). Membrane CX3CL1 is an adhesion molecule, but it is a chemoattractant for Chemokine (C-X3-C motif) Receptor 1 (CX3CR1 + cells) in the soluble form.[9] The most of leukocytes that invade the kidney during nephropathies were shown to express CX3CR1. Chemokine (C-X3-C motif) receptor 1 (CX3CR1) is frequently expressed on monocytes and T cells in most organs. CX3CL1 is the only ligand for CX3CR1, a single chemokine acts as a chemoattractant as well as helps CX3CR1+ cells bind together. As a result, the CX3CL1/CX3CR1 axis represents a novel type of leukocyte-migration regulator.[10] Furthermore, it has a role in the enhancement of chronic renal disorders like DN.[11] High glucose levels, AGE formation, and cytokine activation in diabetes may induce fractalkine upregulation in the kidneys and lead to progression of diabetic nephropathy[12] (McDermott et al. 2003).

Many urine markers have been proposed for detecting early DN and one of them has been implemented. Urine IgG is an anionic plasma protein that has a difficult time passing the glomerular barrier.[13] Urine IgG can be released prior to the onset of microalbuminuria, along with elevated urinary transferrin, urinary ceruloplasmin, and urinary orosoplasmin levels. Increased urine IgG elimination could thus be used to predict microalbuminuria in diabetic patients. So accessibility of novel biomarkers that are sensitive, specific besides able to detect kidney injury and expect clinically significant outcomes would be widely beneficial in diabetic.[14]

Subjects and methods

This is a cross-sectional study. Sixty adult patients with type 2 diabetes enrolled in the study. Patients were recruited from Internal medicine department, Cairo University's as well as Internal medicine department and Endocrinology outpatient clinic of Al-Zahraa University Hospital, Cairo, Egypt from August 2020 till April 2021. All patients were clinically studied and selected according to the criteria for T2DM patients was set by the American Diabetes Association (2017).[15] Patients were classified into three groups based upon urine albumin excretion (UAE). Group I: included 20 diabetic patients with normolbuminuria and without nephropathy, ACR less than 30 mg/g. Group II: included 20 diabetic patients with micro albuminuria, ACR ranged from 30 to 300 mg/g. Group III: included 20 diabetic patients with macro albuminuria, ACR more than 300 mg/g. Twenty age and sex matched healthy participants served as a control group. Patients with end-stage renal illness, urinary tract infection, obstructive uropathy, diabetic foot infection, autoimmune disorders or heart failure as well as pregnant women were excluded from the study. An informed written consent was acquired from each patient and control subject. This study was conducted in accordance with the Declaration of Helsinki principles.

Sample size calculation

The calculated sample size of the study will be 16 participants for each group at 5% level of significance and 95% power of the study, using G*Power 3 sample size calculator.

Mean (SD) Micro RNA 152–3p in diabetic neuropathy group = 2.52 (1.52)

Mean (SD) Micro RNA 152–3p in control group = .93 (0.42).

The sample size will be increased to be 20 participants for each group to compensate for incomplete data and to increase the study power.

Sample collection

Five milliliters of venous blood were drawn without anticoagulant from each patient and healthy subjects, sera were separated and divided into three portions: one portion was stored at −20°C until use in measurement of CX3CL1 levels by ELISA, second portion stored at −20°C until use for miRNA extraction and the third portion was used for biochemical tests. Five ml urine samples

were collected from each patient and control subjects for urine albumin creatinine ratio and the rest was kept at −20°C until use in measurement of IgG level by ELISA

Methods

Measurement of HbA1c by D-10 BIO-RAD.; urea and creatinine by BIOLIS 24i.

Quantification of miRNA 152 by real time PCR (RT-qPCR)

To identify the expression levels of miRNA-152-3p of patients and healthy subjects, Quantitative Real Time Polymerase chain reaction (RT-q-PCR) was used by AB Applied Biosystems, Thermo, Inc., Foster City, CA (USA).

Extraction of RNA

Total RNA was extracted from serum samples using the miRNeasy Mini Kit (Qiagen Hilden, Germany, Cat. No. 217,004) according to manufacturer's protocol. In summary, 200 µl of serum sample was mixed with 1 ml QIAzol lysis reagent (Lot No. 557,010,548) and incubated for 5 minutes at room temperature. The tube was then vortexed for 15 seconds with 200 µl of chloroform (Sigma). A new collecting tube was used to transfer 600 of upper aqueous phase. 900 µl of 99.9% ethanol was added and carefully mixed by pipetting. In a 2 ml collection tube, 700 µl of sample was transferred to an RNeasy min column. To elute RNA, the RNeasy Min column was washed once with buffer RWT and then with buffer RPE, subsequently, 30–50 µl of RNase-free water was pipetted directly onto the RNeasy membrane. The RNA purity of the extract was determined using a Thermo Fisher Nano drop spectrophotometer.

Reverse transcription

Purified RNA was then utilized for one-step reverse transcription using the TaqMan Micro-RNA Reverse Transcription kit (Applied Biosystems, Cat. No. 4,366,596, USA), as directed by the manufacturer recommendations. Briefly, a total reaction volume 15 µl/reaction. Consisted of 5 µl RNA sample, 7 µl master mix and 3 µl of 5x RT primer. After a gentle mix, the tubes were set in a thermal cycler (Bio-Rad, T100, Singapore) with the following run conditions: incubation for 30 minutes at 16°C, 30 minutes at 42°C, followed by 5 minutes at 85°C.

Quantification was used to perform with real time PCR using the TaqMan® miRNA Assays (Cat. No. (4,427,975) according to manufacturer's methodology. In Brief, 20 ul total reaction volume were prepared consisted of 10 ul of TaqMan® universal master mix II with no UNG 2X, 1ul of 20x TaqMan® Assay, 7ul of cDNA template and 2 ul of RNase free water. hsa miRNA let7i-5p (assay ID 002221) were used as endogenous control in normalizing micro-RNA expression. The reaction tubes were heated by initial activation step at 95°C for 10 minutes before being exposed to 40 cycles of denaturation at 94°C for 15 seconds, annealing, and extension at 60°C for 60 seconds. After each cycle, fluorescence readings were taken. Relative quantification with hsa micro-RNA let7i-5p as an endogenous control was done using the 2-ΔΔ Ct equation.

Measurement of serum CX3CL1 and urine IgG by ELISA

Serum CX3CL1 level was measured using ELISA Basic kit provided by Elabscience (Cat. No E-EL-H0044) USA. Procedure was performed according to operational guidelines. Serum CX3CL1 levels were calculated using a standard curve. IgG level in urine was measured using ELISA kit provided by Elabscience (Cat No. E-EL-H0169) USA.

Statistical analysis

SPSS for Windows was used to conduct all of the comparisons (SPSS version 21 Armonk, NY: IBM. Corp, released 2012, USA). The chi-square test was used to compare the differences between the controls and patients. Descriptive analyses were performed for age, gender and laboratory investigation with data shown as mean ± SD (range). Statistical comparisons between cases and controls were carried out using the χ^2 test to assess the Mann–Whitney U-test was used to compare between two groups. *P* values less than 0.05 were regarded as

statistically significant. For data correlation, Pearson's correlation coefficient (r) test was employed. The optimal cutoff values, overall predictive values, and calculation of sensitivity and specificity of parameters were demonstrated using receiver operating characteristic (ROC) curve analysis.

Results

Demographic and laboratory data of patients and healthy subjects

Table 1 summarizes the demographic and laboratory characteristics of diabetic patients and controls. Significant difference was observed between group I and controls in the A/C ratio, HbA1c, urine IgG, serum CX3CL1 ($p \leq 0.001$) and miRNA152 = 3p ($p = 0.029$). There were significant difference between group II and controls in urea, A/C ratio, HbA1c, urine IgG, serum CX3CL1 ($p \leq 0.001$) and miRNA152 ($p = 0.007$). A significant difference was observed between group III and controls in urea, creatinine, A/C ratio, HBA1c, urine IgG, serum CX3CL1 and miRNA152-3p ($P \leq 0.001$) (Table 1).

Furthermore, a significance difference between group I and group II regarding to age, duration of disease, urea, creatinine, A/C ratio, IgG levels $P \leq 0.001$. Also a significance difference between group II and III regarding to urea, creatinine, A/C ratio and IgG levels ($P \leq 0.001$) (Table 1).

Correlation study

Correlation coefficient study revealed positive correlation between serum fractalkine CX3CL1 level and age, duration of disease, urea, creatinine, A/C ratio, HbA1C and IgG in diabetic nephropathy patients groups (II & III) ($P = 0.001$) (Table 2). Positive correlation between urinary IgG level and age, duration of disease, serum creatinine, Albumin /Creatinine ratio (A/C) ($P = 0.001$) and urea ($P = 0.006$) in the diabetic patients with nephropathy (groups II & III) (Table 3). A significant positive correlation was observed between the expression of miRNA-152 and duration of disease among patient groups with T2 diabetes $r = 0.394$, $P = 0.002$.

Expression of serum miRNA 152-3p

The expression of miRNA-152-3p was significantly upregulated in patients with DN (macroalbuminuria and microalbuminuria) followed by diabetic patients with normoalbuminuria compared with healthy controls (Figure 1).

Table 1. Demographic and laboratory data of diabetic patients and controls.

					Test of significance				
	Group I ($n = 20$)	Group II ($n = 20$)	Group III ($n = 20$)	Control ($n = 20$)	GI Vs. control	GII Vs. control	GIII Vs. control	GI Vs. GII	GII Vs. GIII
Age (years) Mean ± SD	48.95 ± 7.9	56.10 ± 3.67	62.75 ± 8.82	56.60 ± 7.7	-	-	-	$t = 5.65$ $P \leq 0.001$*	$t = 0.26$ $P = .796$
Sex									
Male	10 (50%)	10 (50%)	9 (45%)	11 (55%)	-	-	-	$\chi^2 = 0$	$\chi^2 = 0.1$
Female	10 (50%)	10 (50%)	11 (55%)	9 (45%)				$P = 1$	P = .752
Duration Median (Min–Max)	4 (1–8)	9 (4–15)	11.5 (5–20)	-	-	-	-	$Z = 4.44$ $P \leq 0.001$*	$Z = 1.92$ $P = .055$
Urea (mg/dl) Mean ± SD	23.65 ± 5.01	16.75 ± 4.35	32.25 ± 8.65	25.7 ± 8.57	$t = 0.92$ $p = .36$	$t = 4.2$ $p \leq 0.001$*	$t = 2.4$ $p = .021$*	$t = 4.65$ $P \leq 0.001$*	$t = 7.16$ $P \leq 0.001$*
Crea (mg/dl) Mean ± SD	0.83 ± 0.12	0.99 ± 0.21	1.49 ± 0.31	0.89 ± 0.16	$t = 1.43$ $p = .16$	$t = 1.9$ $p = 0.07$	$t = 7.6$ $p \leq 0.001$*	$t = 3.23$ $P = .003$*	$t = 5.83$ $P \leq 0.001$*
A/C Median (Min–Max)	14.5 (8–30)	126 (58–280)	924 (395–2640)	7 (4–9)	$Z = 5.21$ $p \leq 0.001$*	$Z = 5.4$ $p \leq 0.001$*	$Z = 5.44$ $p \leq 0.001$*	$Z = 3.62$ $P \leq 0.001$*	$Z = 5.41$ $P \leq 0.001$*
HbA1c (%) Mean ± SD	10.38 ± 1.73	10.94 ± 1.41	11.64 ± 1.70	5.71 ± 0.49	$t = 11.6$ $p \leq 0.001$*	$t = 15.6$ $p \leq 0.001$*	$t = 14.9$ $p \leq 0.001$*	$t = 1.13$ $P = .266$	$t = 1.41$ $P = .166$
IgG/C Mean ± SD	7.41 ± 1.75	15.81 ± 2.51	25.21 ± 1.88	0.78 ± 0.42	$t = 16.4$ $p \leq 0.001$*	$t = 26.3$ $p \leq 0.001$*	$t = 56.5$ $p \leq 0.001$*	$t = 5.33$ $P \leq 0.001$*	$t = 5.78$ $P \leq 0.001$*
CX3CL1 (ng/ml)	2.89 ± 1.19	5.61 ± 1.35	9.02 ± 1.03	0.65 ± 0.25	$t = 8.2$ $p \leq 0.001$*	$t = 16.1$ $p \leq 0.001$*	$t = 35.1$ $p \leq 0.001$*	$t = 6.70$ $P \leq 0.001$*	$t = 8.96$ $P \leq 0.001$*
miR-152 Median (Min–Max)	1.95 (0.4–7)	2.1 (0.3–5.3)	2.6 (0.5–9.3)	0.9 (0.34–2)	$Z = 2.18$ $p = .029$*	$Z = 2.68$ $p = 0.007$*	$Z = 4.05$ $p \leq 0.001$*	$Z = 0.379$ $P = .705$	$Z = 1.71$ $P = .088$

A/C: albumin creatinine ratio, HbA1c: glycated hemoglobin, IgG: immunoglobulin G. *: means significant.

Table 2. Correlation between serum CX3CL1 (ng/ml) and other variables among diabetic nephropathy patients (groups II & III).

	CX3CL1 (ng/ml)	
Variable	*r*	*P* value
Age	0.648	≤0.001*
Duration of DM	0.606	≤0.001*
Urea (mg/dl)	0.412	0.001*
Creatinine (mg/dl)	0.711	≤0.001*
A/C	0.842	≤0.001*
HA1c (%)	0.549	≤0.001*
IgG (ng/ml)	0.855	≤0.001*
miR-152	0.182	0.164

*: means significant.

Table 3. Correlation between IgG and other variables among diabetic nephropathy patients (group II and III).

	IgG	
Variable	*r*	*P* value
Age	0.507	0.001*
Duration	0.722	≤0.001*
Urea (mg/dl)	0.349	0.006*
Creatinine (mg/dl)	0.763	≤0.001*
A/C	0.883	≤0.001*
HA1c (%)	0.229	0.078

*: means significant.

Serum concentrations of CX3CL1 (ng/ml) and urine IgG

There was a significant increase in Urine IgG and CX3CL1 among the studied groups. In patients with DN (macroalbuminuria and microalbuminuria) followed by diabetic patients with normal albuminuria compared with healthy controls (Figures 2 and 3).

Logistic regression analysis for diabetic nephropathy DN (groups II and III)

A significant difference was found in HbA1c with odd ratio 1.8, CX3CL1 with odd ratio 6.8 and urinary IgG with odd ratio 4.7 among diabetic nephropathy group II and group III (Table 4).

Diagnostic performance of serum Cx3CL1, serum miRNA, urine IgG and albumin/creatinine ratio in discrimination of the patients

The receiver operating (ROC) curve was used to establish the best cutoff value for serum CX3CL1, serum miRNA-152-3p, urine IgG and albumin / creatinine ratio which were (>3.85, >1.21 > 12.35, >44.00) respectively, with sensitivity of 97.5%, 80%, 92.5%, 90%, respectively, specificity of 82.5%, 55%, 95%, 87.5%, respectively, of the patients with diabetic nephropathy. AUC values (0.9) of CX3CL1, IgG and A/c ratio provide good predictors role in DN (Table 5; Figures 4 and 5).

Discussion

Microalbuminuria is the first sign to predict the progression of renal failure. However, albuminuria has many limitations. As a result, more sensitive and

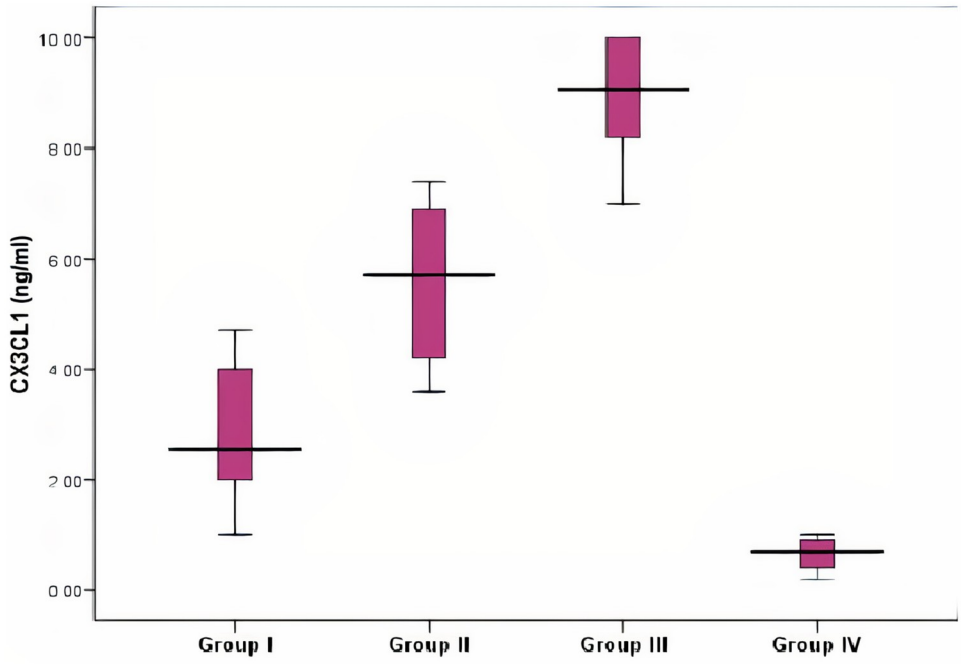

Figure 1. Box plot for median serum CX3CL1 (ng/ml) among the studied groups.

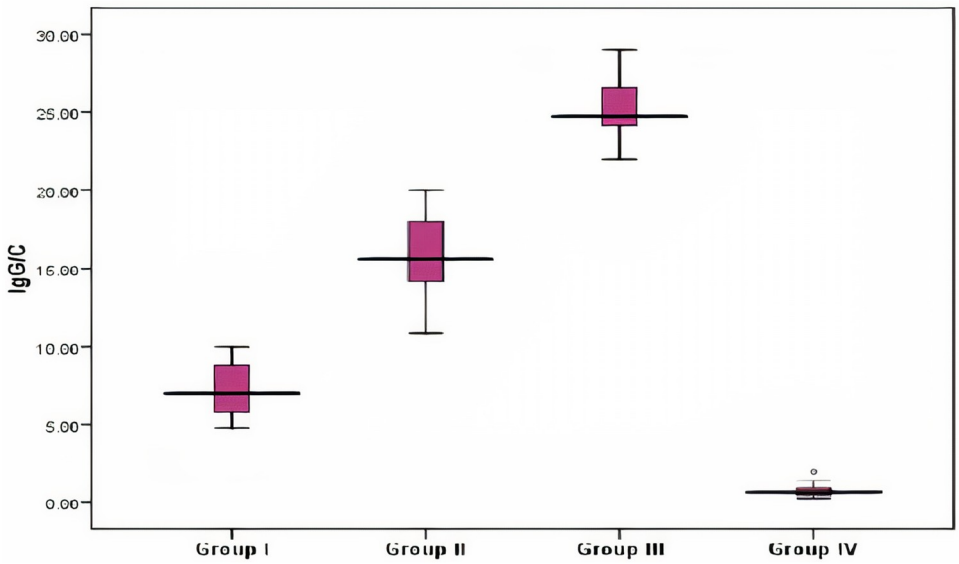

Figure 2. Box plot for median urine IgG/C among the tested groups.

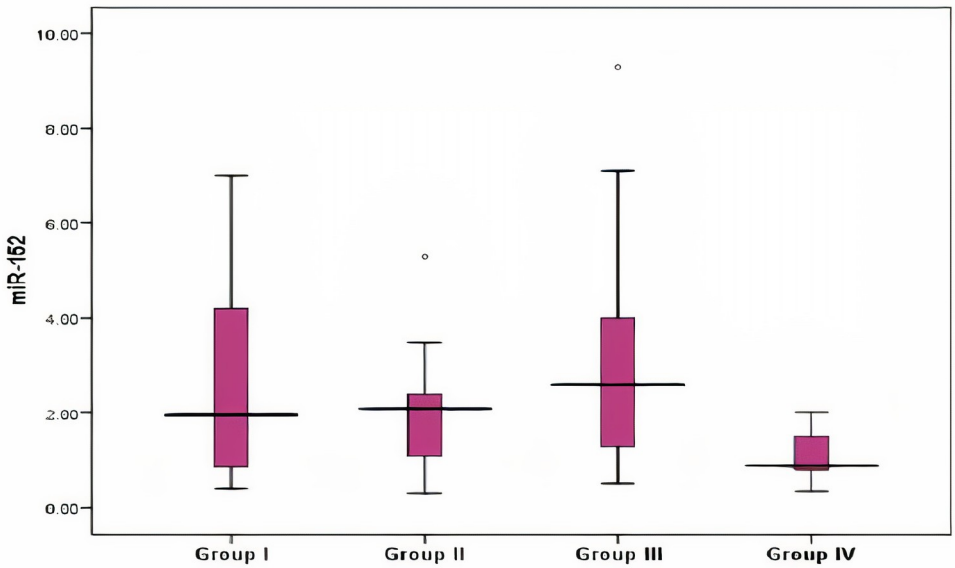

Figure 3. Box plot representation of miRNA-152-3p expression levels in diabetic cases and controls among the tested groups.

Table 4. Multivariate logistic regression analysis for independent predictors of Groups II & III.

	β	SE	*P* value	OR	95% CI
HbA1c	0.607	0.135	≤0.001*	1.83	1.4–2.4
Serum CX3CL1 (ng/ml)	1.917	0.656	0.003*	6.8	1.9–24
Urine IgG	1.540	0.577	0.008*	4.7	1.5–14.5

OR: odds ratio; CI: Confidence interval; HbA1c: glycated hemoglobin; IgG: immunoglobulin G

specific biomarkers have been developed to predict the renal dysfunction before the detection of microalbuminuria. CX3CR1 are expressed by the most of leukocytes invading the kidney in human renal disorders demonstrating its clinical relevance. CX3CL1 is a chemoattractant and adhesion molecule that plays an important role in glomerulonephritis.[16]

This study found that serum fractalkine concentrations were significantly higher in diabetic patients with nephropathy than in diabetic patients without nephropathy as well as in the control group $p \leq 0.001$. These findings are also in concordance with other finding observed by Zakaria et al. (2013)

Table 5. Diagnostic relevance of serum CX3CL1, serum mRNA, urinary IgG and albumin/creatinine ratio in discrimination of the patients.

	AUC	95% CI	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum CX3CL1 (ng/ml)	0.979	0.96– 1.0	>3.85	97.5%	82.5	84.8	97.1	90
Urine IgG (ng/ml)	0.961	0.91–1.0	>12.35	92.5%	95%	94.5%	92.6%	93.7%
A/C ratio	0.946	0.89–0.99	>44.00	90%	87.5%	87.8%	89.7%	88.7%
miRNA-152-3p	0.672	0.55–0.79	>1.21	80%	55%	64%	73.3%	67.5%

AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value

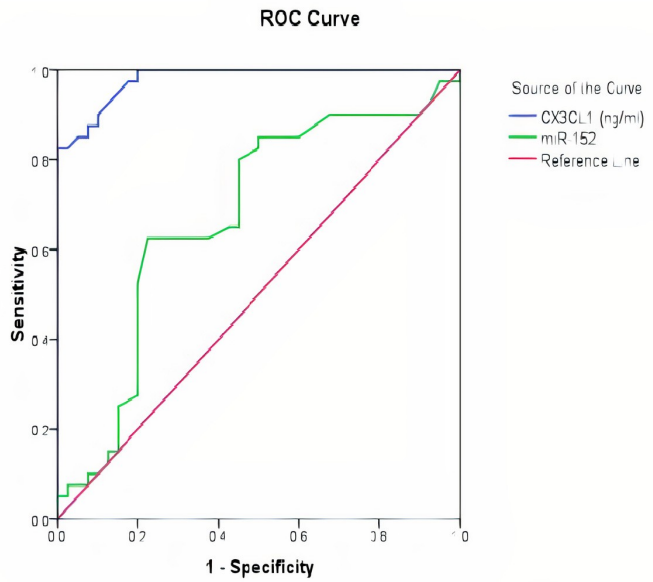

Figure 4. Receiver operating curve (ROC) to define the best cut off values of serum CX3CL1, serum miRNA 152–3p concentrations.

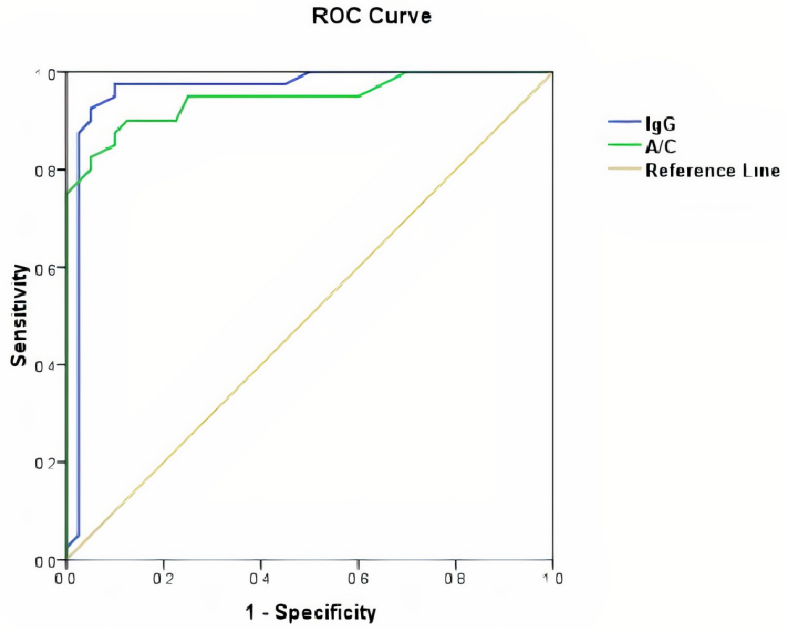

Figure 5. Receiver operating curve (ROC) to define the best cut off values of urine IgG concentration and albumin /creatinine ratio.

and Kikuchi et al. (2008) who demonstrated that fractalkine and CX3CL1 were increased in an early phases of diabetic kidney.[17,18]

These findings recommend that fractalkine expression and CX3CR1-positive cell infiltration in diabetic kidneys might play risk factors in deteriorating of diabetic nephropathy. In addition, it was demonstrated that CX3CL1 level was higher than a six-fold increased odds ratio for diabetic nephropathy. The current findings are consistent with previous study that showed plasma CX3CL1 levels were considerably greater in diabetic patients than non-diabetics, with a more than two fold increased odds ratio of diabetes.[19]

Without early detection, 50% of microalbuminuria patients would progress to macroalbuminuria. The presence of microalbuminuria in T2DM patients is determined by a high A/C ratio and a high HbA1c level.[20] In this study, a correlation was found between serum fractalkine level and the other parameters including age, duration of disease, urea, serum creatinine, A/C ratio, HbA1c and IgG (P = .001) in the patient groups studied with nephropathy. Our results are in agreement to those of Zakaria et al. who noticed correlations between serum fractalkine level and the other variables including microalbuminuria, HA1c, and serum creatinine. In addition, they were demonstrated that HbA1c level was more than one fold increased odds ratio (1.8) for diabetic nephropathy.[17]

In the present work, urine IgG excretion levels were found to be significantly higher in diabetic patients (groups I, II and III) compared to a control group ($P < 0.001$) as well as significantly elevated in patients with diabetic nephropathy than patients without diabetic nephropathy ($p \leq 0.001$).

The level of urine IgG, which is more sensitive than albuminuria as a marker of diabetic nephropathy in the current work, shows the extent of significant lesions in the kidney and may be useful for identifying patients who have a higher risk of DN. These findings were in agreement with those of Narita et al., who reported that increased urine IgG, ceruloplasmin, and transferrin but does not cause increase in albuminuria throughout the early stages of diabetic nephropathy.[21]

Furthermore, Mistry and Kalia found a 156% rise in urine IgG in diabetic nephropathy patients compared to non-diabetic nephropathy patients, as well as a 75% increase in IgG excretion in diabetic nephropathy patients.[22]

In the present work, there was a significantly positive correlation between urine IgG level and other studied parameters, such as age, duration of disease, urea, creatinine, A/C ratio (P = 0.001) and HbA1c (P = 0.05) in studied diabetic patients with nephropathy. The result is also in concordance with other findings reporting positive correlation between IgG creatinine ratio and ACR and serum creatinine and blood urea.[23] Also Yadav et al., who found that urine IgG levels are correlated to urine albumin excretion. In comparison to diabetic patients with normoalbuminuria, there was a significant rise in IgG creatinine ratio and ACR with increase in patients' age and duration of diabetic nephropathy.[24] This could be explained by longer time of diabetes has been considered a strong risk factor in the development of DN.

The same finding was reported by Assal et al., who realized a significant difference in diabetes duration between diabetic patients with normoalbuminuria and diabetic nephropathy with microalbuminuria.[25]

In the present work, also reported that the urine IgG significantly increased in DN with 4.7 fold increased risk to develop end stage renal disease. Zhang et al. also found the glomerular IgG an independent risk factor with odds ratio 1.2 fold for the renal clinical outcomes suggest that increased urinary excretion of IgG might have a role imply a worse kidney outcome.[26]

In the current work, the expression level of serum miRNA- 152-3P was remarkable elevated in both diabetic patients with or without nephropathy compared to control group. In agreement with our results Nasser et al. in Egypt reported that the miRNA 152–3p levels were overexpressed in diabetes mellitus and DN compared to the controls.[27]

There was a statistically insignificant difference detected between serum level of miRNA- 152-3P in the diabetic group with microalbuminuria versus DN group macroalbuminuria. Our results agreed with Bijkerk et al., who reported statistically

insignificant difference between level of serum miRNA 152–3P in type 1 diabetes with good renal function and DN.[28] In addition, Nasser et al., who found insignificant difference in the serum miRNA level which could be detected between the diabetics with nephropathy and without nephropathy.[27] In contrast to our findings, Roux et al. (2018) found that plasma expression of miRNA-152-3p was significantly different in T2DM patients without nephropathy compared to diabetic nephropathy patients, and that miRNA is correlated with the risk of DN in patients with T2DM.[29] This result is in disagreement with the Chen et al., who showed the expression of miR-152 was significantly decreased in patients with T2D compared with the control group. This could be explained by high frequency of patients with macroalbuminuria. As a result, it could be argued that miRNA 152–3p was not a promising marker for DN as its expression insignificant different between the DN and DM groups although the level increased in the both diabetes groups compared to controls.

Our results confirmed the association of miRNA-152-3p with diabetic nephropathy in two features; first, the significant difference in the expression of micro-RNA 152–3p between DN and control group (P <0.001). Second, a positive correlation between the miRNA 152–3p and duration of disease was detected ($P < 0.002$). The same finding was reported by Nasser et al. (2020) who observed a positive correlation between miRNA 152–3p and duration of diabetes was observed ($P < 0.001$).[27] In the current work, there are no correlation between miRNA 152–3p with HbA1c ($p = 0.781$). Conversely, Nasser et al. (2020) reported that a positive correlation was observed between serum micro-RNA 152–3p with level of HbA1c levels ($p < .001$). The analysis of discrepancy between these studies supported the linkage between T2DM and micro-RNA.[27]

ROC curve analysis in the present study revealed that CX3CL1 could be an excellent predictor of DN risk with 97.0% sensitivity and 82.5% specificity, AUC = 0.9, (cutoff 3.8 ng/ml) compared to urine IgG level and A/C ratio with sensitivity (92.5%, 90%), respectively, (AUC 0.9) and specificity (95%, 87.5%, respectively). These results are also consistent with other findings reported that the ROC curve analysis of plasma CX3CL1 which CX3CL1 cutoff (0.511 ng/ml), AUC = 0.780, sensitivity = 0.818, specificity = 0.693).[30,31]

Limitation of the study

The current study has some limitation related to correlation of biomarkers with glomerular filtration rate in both groups studied that might provide insight to possible involvement in progress of DN.

Conclusion

Serum CX3CL1 level, urinary IgG were significantly increased with the progress of nephropathy so the results of the present work suggest that these integrated biomarkers could be used as good predictors for early identification of nephropathy. In addition, miRNA- 152-3p has inadequate prognostic indicator for ESRD progression.

Author Contributions

Conceptualization, A.E A, H.M.A, E.E.E, S.I, Data collection, H.M.A, H.M.M, E.K.A, S.I, E.K.A, methodology H.AA, H.F.I, E.E.E, N.S, E.A.Y, N.M.G, A.S.H, M.A.R, A.E.A, writing N.S, S. I, writing-original draft preparation, HA.A, A.E.A, H.M.A writing review and editing, H.M.M, H.A.A, project administration, H.M.A, H.F.I, E.E.E, N.M.G, funding acquisition, all authors

All authors have read and agreed to the published version of the manuscript.

Disclosure statement

No conflict of interest was reported by the author(s).

Funding

There is no external funding to report.

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