Oxidized-LDL, Toll-like Receptor 4, Lipocalin 2 and Omentin1: Role in Diabetic Nephropathy

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Authors' contributions

This work was carried out in collaboration between both authors. Author WAK reached literature and conceived the study, contributed to biochemical/molecular assays, performed data analysis and wrote the first draft of the paper. Author NSE was involved in protocol development, gaining ethical approval, patient recruitment and data analysis. Both authors reviewed, edited the manuscript and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Diabetic nephropathy is the major micro-vascular complication of type 2 diabetes mellitus (T2DM) and is the main cause for end-stage kidney disease. In view of metabolic derangements of T2DM, we went further to investigate the role played by oxidized low density lipoprotein (ox-LDL), toll like receptor 4 (TLR4), lipocalin-2 (LCN2), and omentin-1 in DN.
Patients and Methods: 15 normo-albuminuria T2DM, 15 micro-albuminuria T2DM and 15 macro-albuminuria T2DM in addition to 15 apparently healthy volunteer who served as control group were enrolled in this study. Demographic and clinical data were recorded. Plasma Ox-LDL, omentin-1 and urinary LCN2 levels by immunoassy and TLR4 mRNA level with real time PCR were assessed.
Results: *TLR4* gene expression, Plasma ox-LDL, urinary LCN2 levels were increased in T2DM cases as compared to their allied control group with the higher values were for macro-albuminuria

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T2DM cases. Meanwhile Plasma omentin-1 level was decreased in T2DM cases when compared to their allied control group with least values were for macro-albuminuria T2DM cases. Also there were positive correlations between TLR4 mRNA, ox-LDL, urinary LCN2 levels and serum creatinine, fasting blood glucose, urinary albumin/creatinine ratio. Meanwhile, omentin 1 showed negative correlations with serum creatinine, fasting blood glucose, urinary albumin/creatinine ratio. **Conclusions:** Ox-LDL, TLR4, LCN2 and omentin 1 may confer a relevant role in diabetic nephropathy development and progression.

1. INTRODUCTION

Diabetes mellitus (DM) is the third serious chronic metabolic disease after cardiovascular disease and cancer which is characterized by hyperglycemia, the main precipitating factor for micro and macro-vascular complications [1]. Diabetic nephropathy (DN) is one of the micro-vascular diabetic complications and is the main cause of end-stage renal disease (ESRD). Metabolic abnormalities, systemic inflammation and oxidative stress associated with type 2 DM (T2DM) are the main players in the pathophysiological processes of DN and are facilitated by innate immune responses [2]. Renal infiltration with monocyte, macrophages and T lymphocytes in addition to intrinsic renal cells (endothelial, mesangial, glomerular, and tubular epithelial cells) are the main sources of cytokines mediating inflammation and renal injury in DM [3]. Diagnostic marker to detect DN at early stage is important as early intervention can slow the loss of kidney function and reduce adverse outcomes [4].

Toll-like receptors (TLRs) are trans-membrane pattern recognition receptors that recognize conserved pathogen-associated molecular patterns to initiate an inflammatory response essential for host defense. TLRs also interact with endogenous ligands, such as oxidized- low density lipoprotein (ox-LDL), heat-shock proteins [5]. Lipocalin 2 (LCN2) is a member of the lipocalin family covalently associated with matrix metalloproteinase 9 (MMP-9) and is expressed by renal tubular cells, hepatocytes and cells of the innate immune system such as polymorphonuclear neutrophils (PMN) and macrophages. LCN2 has many roles as MMP-9 transport to renal tubular cells, activation, induction of apoptosis, and regulation of the immune response [6]. Omentin-1 is an anti-inflammatory adipokine primarily expressed in stromal vascular cells and adipocytes of visceral adipose tissue. Omentin-1 has important roles in cellular energy homoeostasis, increases insulin signal transduction, regulation of lipid metabolism, involved in stress responses, expression of heat shock proteins, T-cell differentiation and apoptosis [7,8]. As DN represents one of the most devastating outcomes during the progression of T2DM, early detection strategies to diagnose loss of renal function would be of essential to improve quality of life. Therefore, we investigated molecular mechanism through ox-LDL, TLR4 and LCN2 axis in addition to omentin1 level that may have role in pathogenesis and diagnosis of DN. Also we went further to determine urinary LCN2 to evaluate its role as an early noninvasive biomarker of renal damage during the course of DM.

2. PATIENTS AND METHODS

2.1 Participants and Study Design

All patients have given their informed consent, and the study was institutionally approved by the Research Ethical Committee of Faculty of Medicine, Tanta University, Egypt (Approval code: 30745). This current study included forty five type 2 diabetic patients who were selected from the outpatient and inpatient Clinic, Internal Medicine Department, Tanta University Hospitals and were diagnosed according to the 1999 World Health Organization (WHO) criteria [9]; 35 of them were receiving oral hypoglycaemic agents in the form of Biguanides and Sulfonylureas and 10 were receiving insulin. Diabetic cases were subdivided into three groups according to urinary albumin/creatinine ratio (UACR) which was measured by early morning spot urine sample into 3 groups [10]: normo-albuminuria T2DM cases (n=15; 10 female and 5 male) with urinary albumin to creatinine ratio (UACR) < 30 mg albumin/g creatinine; micro-albuminuria T2DM cases (n=15; 11 females and 4 males) with UACR = 30-299 mg/g creatinine and macro-albuminuria T2DM cases (n=15; 10 females and 5 males) with UACR more than 299 mg/g creatinine in addition to 15 age and sex matched

apparently healthy individuals without any systemic diseases as diabetes, hypertension, cardiovascular disease, or renal insufficiency and they will be served as healthy controls (group I). Subjects with acute illness, liver dysfunction, heart failure, malignancy, history of cerebrovascular or cardiovascular disorders and other renal disorders were excluded from the study. All subjects enrolled in this study were subjected to full clinical history taking, clinical examination and calculation of BMI (weight in kilograms divided by the square of the height in meters (kg/m2).

2.2 Sampling

Overnight fasting blood samples were obtained from each participant on sterile EDTA treated and plain tubes for serum and plasma separation. Serum, plasma and EDTA treated blood were stored at -80℃ till analysis. First morning mid-stream urine samples were collected centrifuged and the supernatant was stored at –80℃ for further analysis.

2.3 Biochemical and Immunoassays

Serum lipid profile including triacylglycerol (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assayed by enzymatic-colorimetric method using commercial assay kits (Biodiagnostic., Egypt). Fasting blood glucose (FBS) was detected by oxidase method using commercial assay kit (Biodiagnostic., Egypt). Serum/urinary creatinine and urea levels were estimated using commercial kits (Diamond Diagnostic, Egypt). Glycated haemoglobin (HbA1c) was measured in whole EDTA blood using quantitative colorimetric measurement of glycohemoglobin as percent of total hemoglobin using commercial kits (STANBIO, San Antonio, Texas, USA). Urinary albumin was measured using commercial kit (BioSystems Company, Spain). Enzyme-linked immunosorbent assay (ELISA) was used to detect plasma ox-LDL level (OxiSelectTM, Cell Biolab Inc., STA-369, CA, USA), Urinary LCN2 level (R&D systems, Minneapolis, MN, USA) and plasma omentin 1 level (Avisera Bioscience, Inc., USA) using ELISA Reader (Star fax 2001).

2.4 Molecular Assessement

2.4.1 RNA extraction, cDNA synthesis and real-time PCR for TLR4 gene expression

Total RNA was extracted from EDTA peripheral blood using QIAamp RNA mini kit (Qiagen, Hiden, Germany) according to manufacturer's instructions. Total RNA was treated with DNase I to eliminate genomic DNA contamination, followed by synthesis of the first strand cDNA using SuperScript ® III First-Strand Synthesis System for RT-PCR kit (Life Technologies) according to the manufacturer's instructions. Real-time PCR was carried out with cDNAs. PCR reactions were performed using Power SYBR Green PCR Master Mix (Life Technologies) following the manufacturer's instructions. TLR mRNA transcripts were quantified, relative to the housekeeping gene, glyceraldehyde-3-phosphatedehydrogenase (GAPDH) which was used as an internal control. Sequence specific primers were designed by Primer3 software: (http://bioinfo.ut.ee/primer3/) as follows: *TLR4 (NM_138557.2) forward, (5'-TGTCCTCCCACTCCAGGTAAGT-3'), reverse, (*5'-GATTGCTCAGACCTGGCAGTT-3'); GAPDH (No: NM_001289746.1): forward primer (5'-AGTGCCAGCCTCGTCTCATAG -3'), and reverse primer (5'-CGTTGAACTTGCCGTGGGTAG -3'). PCR cycling was set as follows: a single cycle of DNA polymerase activation for a 10 min hold at 95℃ followed by 40 cycles of 95℃ for 15 s for denaturation, 60℃ for 1 min for annealing and 60℃ for 1 min for extension . Amplification and data analysis were conducted on a Rotor-Gene Q 6plex and its specific software (Qiagen, Valencia, CA, USA). Relative gene expression was automatically calculated from the cycle threshold (ct) values of the target and the reference gene.

2.5 Statistical Analysis

Statistical analysis was conducted as mean and standard deviation using Statistical Package for Social Sciences (SPSS), version 16.0 for Windows (SPSS, Chicago, IL). One-way analysis of variance (ANOVA) was used for multiple comparisons to evaluate the statistical significance between experimental groups followed by post hoc test. The correlation study was calculated using Pearson's correlation. *P* value < 0.05 was considered significant.

3. RESULTS

3.1 Clinical and Metabolic Characteristics

Demographic variables and clinical characteristics of the studied groups are shown in Table 1. There was no significant difference in age, body mass index between the studied

groups. Meanwhile, there were statistically significant increase in disease duration, TG, TC, FBG levels and HbA1C percentage in diabetic cases when compared to their allied control group with higher values were for macro-albuminuria T2DM group. LDL-C and HDL-C levels showed statistically significant difference in diabetic cases when compared to their allied control group but with no difference between micro and macro albuminuria T2DM groups. Serum urea, creatinine and UACR were statistically significantly higher in T2DM cases when compared to their allied control group and normo-albuminuria T2DM groups with higher values were for macro-albuminuria T2DM group (Table 1).

3.2 Immune/Inflammatory Status and Tubular Damage

This current study revealed statistically significant increase in plasma ox-LDL level and up-regulation of TLR4 expression in T2DM cases when compared to their allied control group with higher values were for macro-albuminuria T2DM group. Meanwhile, plasma omentin1 level was statistically significantly decreased in T2DM cases than their allied controls with the lowest values were for macro-albuminuria T2DM group. This current study revealed statistically significant increase in urinary LCN2 level in T2DM cases than their allied control group with higher values were observed in macro-albuminuria T2DM group (Table 2).

3.3 Correlation Matrix

All correlations are summarized in Tables 3. In T2DM cases, FBG, serum creatinine, disease duration, UACR were correlated positively with ox-LDL, TLR4 mRNA, LCN2 levels but negatively with plasma omentin-1 level. LCN2 level showed positive correlation with TLR4 mRNA, ox-LDL levels, meanwhile, it showed a negative correlation with omentin-1 level (Table 3).

3.4 Results from ROC Curve

Area under the ROC curve for LCN2 was 0.966, with optimal cutoff value of >37 pg/ml. Using this cutoff value, LCN2 showed a sensitivity of 97% and a specificity of 67% for early diagnosis of DN. Area under the ROC curve for ox-LDL was 0.981, with optimal cutoff value of >39 ng/ml. Using this cutoff value, ox-LDL showed a sensitivity of 97% and a specificity of 13% for early diagnosing DN. Area under the ROC curve

for TLR4 mRNA expression was 0.924, with optimal cutoff value of >0.45. Using this cutoff value, TLR4 showed a sensitivity of 97% and a specificity of 63% for early diagnosis of DN. Area under the ROC curve for omentin1 was 0.031, with optimal cutoff value of >20.1 ng/ml. Using this cutoff value, omentin1 showed a sensitivity of 1% and a specificity of 87% for early diagnosis of DN (Table 4; Fig.1).
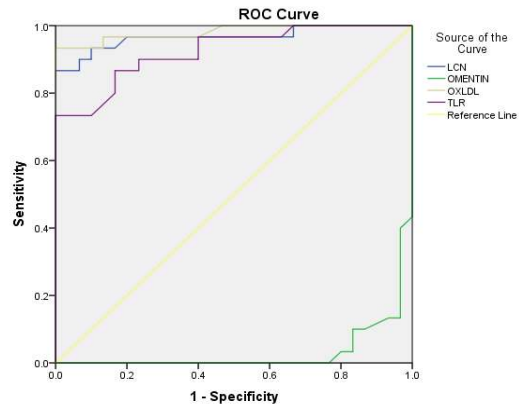
Fig. 1. Receiver Operating Characteristics (ROC) curve for lipocalin2 (LCN2), ox-low density lipoprotein (ox-LDL), omentin1 and toll like receptor4 (TLR4) gene expression

4. DISCUSSION

Diabetic nephropathy (DN) is one of the leading causes of chronic kidney diseases worldwide and most of the affected patients have T2DM. A considerable number of patients newly diagnosed with T2DM may already have developed nephropathy due to a preceding period of undiagnosed diabetes and impaired glucose tolerance [11]. According to the classification of American Diabetes Association, DN is divided into three stages, incipient nephropathy (micro-albuminuria), clinical diabetic nephropathy (macro-albuminuria) and ESRD. To diagnose those DN patients in its early stage can effectively prevent or delay the progression to ESRD [12]. Renal biopsy is a useful way to diagnose DN but is an invasive method so we are in need to simple, accurate and non-invasive test for early diagnosis and/or monitoring DN progression [13]. Several mechanisms, including hyperglycemia, advanced glycation end products (AGEs), oxidative stress, and inflammation are the main contributors to DN pathogenesis through activation of nuclear factor κB (NF-κB) signaling which is the key regulator of inflammation and apoptosis [14].

Table 1. Demographic characteristics and routine biochemical findings in studied groups

Parameters/Groups	Control group (n=15)[a]	Normo-albuminuria T2DM (n=15)[b]	Micro- albuminuria T2DM (n=15)[c]	Macro- albuminuria T2DM (n=15)[d]	P-value
Age(years)	53.75±5.58	52.35±3.88	51.9±2.9	51.73±3.78	0.464
Sex Male (%)	6(40%)	5 (33.3%)	6(40%)	4(26.66%)	-
Female (%)	9(60%)	10(66.6%)	9(60%)	11(73.3%)	-
Duration (years)	-	7.25±1.77[cd]	13.07±2.3[bd]	16.03±2.08[bc]	0.001*
BMI (kg/m2)	25.3±1.18	25.73±1.16	24.6±1.88	25.26±1.43	0.21*
FBS level(mg/dL)	88.15±4.17[bcd]	149.15±9.6[acd]	174.19±17.25[abd]	283.15±32.3[abc]	0.002*
Hb A1c%	4.4±0.54 [bcd]	6.6±0.51 [acd]	7.8±0.37 [abd]	8.7±0.41 [abc]	0.001*
Systolic blood pressure (mmHg)	110.6±7. 5 [bcd]	125.3±4.4 [acd]	128.6±4.1 [abd]	136.6±4.8 [abc]	0.004*
Diastolic blood pressure (mmHg)	71.7±3.07 [bcd]	75.45±1.3 [ad]	81.55±3.1 [abd]	87.65±2.4 [abc]	0.003*
Serum urea level (mg/dL)	23.35±2.08 [bcd]	25.1±2.38 [acd]	45.25±11.68 [abd]	63.3±8.2 [abc]	0.002*
Serum creatinine level (mg/dL)	0.71±0.12[cd]	0.77±0.1 [cd]	1.01±0.17 [abd]	1.28±0.4 [abc]	0.005*
UACR (mg/g)	18.75±2.9 [cd]	19.1±2.09 [cd]	133.63+31.1 [abd]	380.33±45.6 [abc]	0.01*
TC level (mg/dL)	142.73±6.7 [bcd]	192.6±15.9 [acd]	277.93±15.48 [abd]	303.4±17.8 [abc]	0.003*
TG level (mg/dL)	136.75±9.67 [bcd]	163.3±16.24 [acd]	196.46±13.52 [abd]	223.37±31.1 [abc]	0.0021*
LDL-C level (mg/dL)	98.4±7.1 [bcd]	111.1±20.5 [acd]	162.76±7.58[ab]	164.5±11.58 [ab]	0.0032*
HDL-C level (mg/dL)	66.8±1.3 [bcd]	62.1±5.8 [acd]	42.7±4.7[ab]	40.58±4.1 [ab]	0.0024*

*Data presented as mean ± SD; n number of cases; BMI Body mass index; FBS fasting blood sugar; HbA1c: Glycated hemoglobin; UACR urinary albumin/creatinine ratio ;TC total cholesterol; TG triacylglycerol; LDL-C low-density lipoprotein cholesterol; HDL-C high-density lipoprotein cholesterol;. * Significant difference. P Value was calculated by one-way ANOVA test followed by Tukey's post hoc test. Identical superscript letters indicate nonsignificant differences while different superscript ones show statistically significant results. P Value was considered significant at 0.05*

Table 2. Biochemical and molecular findings of the studied groups

Parameters/Groups	Control group (n=15)[a]	Diabetic cases with normo-albuminuria (n=15)[b]	DN with micro-albuminuria (n=15)[c]	DN with macro-albuminuria (n=15)[d]	P-value
Urinary LCN2 level (pg/ml)	34.35+4.2 [bcd]	46.5+5.9 [acd]	59.76+9.43 [abd]	73.8+5.1 [abc]	0.0032*
Plasma omentin-1 level (ng/ml)	26.08+1.8 [bcd]	22.4+3.1 [acd]	18.7+1.6 [abd]	14.37+1.53 [abc]	0.014*
Plasma ox-LDL level (ng/ml)	19.65+2.2 [bcd]	33.9+6.5 [acd]	64.25+13.18 [abd]	117.78+21.8 [abc]	0.0031*
Relative TLR4 mRNA expression	0.37+0.02 [bcd]	0.8+0.3 [acd]	1.08+0.3 [abd]	1.34+0.37 [abc]	0.002*

*Data presented as means ± SD; n number of cases; Ox-LDL oxidized low-density lipoprotein; TLR4 toll like receptor4; LCN2 lipocalin 2. * Significant difference. P Value was calculated by one-way ANOVA test followed by Tukey's post hoc test. Identical superscript letters indicate nonsignificant differences while different superscript ones show statistically significant results. P Value was considered significant at 0.05*

Table 3. Correlations matrix of glycemic status, creatinine, urinary albumin creatinin ratio, lipocalin 2 in relation to plasma ox-LDL, peripheral bloodTLR4 mRNA and plasma omentin-1 levels

		Fasting blood sugar (mg/dl)	Serum creatinine (mg/dl)	Urinary albumin/ creatinine ratio	Disease duration (years)	Urinary LCN2 level (pg/ml)
Plasma Ox-LDL level (ng/ml)	r	0.454**	0.491**	0.932**	0.806**	0.844**
	p	**0.004**	**0.003**	**0.0032**	**0.0051**	**0.0041**
Plasma omentin1 level (ng/ml)	r	-0.699**	-0.515**	-0.861**	-0.927**	-0.848**
	p	**0.0041**	**0.0002**	**0.0001**	**0.0003**	**0.0004**
Relative TLR4 mRNA expression	r	0.647**	0.519**	0.890**	0.900**	0.851**
	p	**0.0042**	**0.0034**	**0.0025**	**0.0044**	**0.00016**
Urinary LCN2 (pg/ml)	r	0.482**	0.423**	0.864**	0.833**	-
	p	**0.0002**	**0.00012**	**0.0003**	**0.00024**	-

Values are Pearson correlation coefficients. Bold font marks significance at P< 0.05

Table 4. Receiver Operating Characteristics (ROC) curve for lipocalin2 (LCN2), ox-low density lipoprotein (ox-LDL), omentin1 and toll like receptor4 (TLR4) gene expression

Parameters	Cut-off value	Sensitivity (%)	Specificity (%)	Area under the curve (AUC)	P-value
LCN2 (pg/ml)	>37	97%	67%	0.966	<0.001*
Ox-LDL(ng/ml)	>39	97%	13%	0.981	<0.001*
Omentin1(ng/ml)	>20.1	1%	87%	0.031	<0.001*
Relative TLR4 mRNA expression	>0.45	97%	63%	0.924	<0.001*

TLRs have been implicated in diabetes-induced inflammation and vascular complications. TLRs recognize various damage-associated molecular patterns such as heat-shock proteins, fatty acids, ox-LDLs and AGEs. Activation of the immuno-inflammatory reactions through TLR4 was supposed to play a role in the pathogenesis of DN [15]. This current study revealed up-regulation of TLR4 in diabetic patients when compared to control with higher expression was in macro-albuminuria diabetic cases; also it was correlated positively to serum creatinin, disease duration, UACR. These results indicate that up-regulation of TLR4 expression might contribute to the pathogenesis, diagnosis and progression of DN. Lin et al. [16] found that silencing of TLR4 gene expression with small interfering RNAs (siRNA) or anti-TLR4 antibodies attenuated high-glucose-induced NF-κB activation. Devaraj et al. [17] reported up-regulation in TLR4 expression in type 1 diabetes mellitus patients (T1DM) with micro-vascular complications compared without micro-vascular complications T1DM and may contribute to increased inflammation and subsequent T1DM complications.

Ox-LDL causes endothelial injury, atherosclerosis, enhancing albumin excretion and can induce ischemia, stress and damage to cultured renal tubular cells with further development of advanced nephropathy [18]. The present study revealed increased s ox-LDL level in micro and macro albuminuria diabetic cases than control and normo-albuminuria diabetic cases with higher value were for macro-albuminuria cases. These findings in addition to the up-regulation of TLR4 expression may suggest a role played by ox-LDL in pathogenesis, progression of DN through activation TLR4. Moreover, ox-LDL was correlated positively with serum creatinine, UACR and disease duration revealing its role not only in pathogenesis but also in diagnosis of DN and its progression. Geng et al. [19] reported up-regulation of TLR4 expression in response to ox-LDL and the inhibition of TLR4 expression by small interference RNA(siRNA) caused decrease in NF-κB activity and monocyte chemoatractant protein 1 (MCP-1) and interleukin 8 (IL-8) secretion in response to ox-LDL so atherogenic effects of ox-LDL could be mediated in part via the TLR4 pathway [19]. There are other more adequate methods to study lipidic oxidative stress, but this was not the aim of the present study due to its limitations.

Proximal tubules are particularly susceptible to injury associated with diabetes and tubular damage may occur before glomerular damage causing appearance of several tubular proteins in

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urine which may occurs before the appearance of microalbuminuria [20]. So that it is important to find new predictive biomarkers of tubular damage. LCN2 is one of proteins that expressed abundantly in ischemic or nephrotoxic kidney injury and serum lipocalin-2 has been described as a sensitive and specific biomarker for early identification of acute kidney injury as a result of cardiac surgery [21]. LCN2 binds to its receptor and transports small lipophilic substances, such as retinoids, arachidonic acid, steroids, iron, and fatty acids in addition it has roles in induction of apoptosis, the inhibition of bacterial growth, regulation of iron metabolism, and insulin resistance. LCN2 is induced by inflammatory stimuli through the activation of the NF-kB pathway [22]. This current study revealed increased urinary LCN2 level in T2DM cases than controls with more increase were observed in macro-albuminuria T2DM. Increased levels of urinary LCN2 were already found in diabetic patients without early signs of glomerular damage in the form of normo-albuminuria, demonstrating usefulness of urinary LCN2 as a marker of early DN. LCN 2 was correlated positively to serum creatinine, disease duration, UACR. The significant increase in macro-albuminuria T2DM cases may indicate that urinary LCN2 level may be a useful biomarker for reflecting the severity of renal damage caused by diabetic disease. These results may be due to impaired tubular reabsorption as a result of specific saturation of the endocytic pathway durring heavy proteinuria or proximal tubules dysfunction [23].

These results indicate that LCN2 might contribute to early diagnosis of DN and its progression. These results were in agreements with those obtained previously [24,25]. Wu et al. 2015 reported that high glucose caused activation of TLR4/NF-κB p65/ LCN2 signaling pathways and promoted the downstream fibrosis and inflammatory reaction in cultured mesangial cells (MCs) [26]. Flo et al. [27] demonstrate that injection of mice with the TLR4 ligand lipopolysaccharide induces increased expression of LCN2 in a TLR4-dependent manner. So that from the results obtained from the present study; oxidized lipids through acting as ligand and up-regulator for TLR4 expression with subsequent increased LCN2 production can cause renal damage in diabetic patients.

Omentin-1 is an anti-inflammatory adipokine with important role in cellular energy homoeostasis, vascular tone regulation, stress responses, T-cell differentiation and apoptosis [28]. Tan et al. [29] reported that omentin-1 can attenuates C-reactive protein (CRP) and TNF-α-induced NF-κB activation in human endothelial cells suggesting that omentin-1 might be an anti-inflammatory adipokine in humans. However, little information is available with respect to the associations of serum omentin levels and diabetic microvascular complications. This current study showed decreased omentin 1 level in diabetic cases than their allied control group with lowest levels were observed in macro-albuminuria T2DM cases, also it was correlated negatively with serum creatinine, disease duration, UACR. These results indicate that omentin 1 might contribute to the pathogenesis, diagnosis and progression of DN. Tekce et al. [30] suggested that DM is associated with low omentin 1 levels in chronic kidney disease population. Zhang et al. [31] suggested that decreased omentin-1 level may contribute to the development of insulin resistance and T2DM. Furthermore, ROC curve results indicated that urinary LCN2 and TLR4 had the best specificity and sensitivity for early diagnosis of DN. In view of correlation study so it can be concluded that LCN2 is a non-invasive, easily assessed with the best specificity and sensitivity. Major limitations of our study remain the small number of cases included.

5. CONCLUSION

The synergetic increase of ox-LDL, TLR4, LCN2 and decreased omentin-1 level may mediate DN pathogenesis and progression in addition to the novel proposed role of LCN2 as a non-invasive biomarker for early detection of DN. The results of this study may support further diagnostic and therapeutic approaches that ensure modification of metabolic and immune-modulatory status to prevent premature micro-vascular complications in diabetic patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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