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Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	Ms_IJBCRR_25286
Title of the Manuscript:	Oxidized-LDL, Toll-like receptor 4, Lipocalin2 and Omentin1: Role in diabetic nephropathy
Type of the Article	Original Research Article

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Major comments 1. Although authors measured plasma ox-LDL, TLR4 mRNA in whole blood, urinary LCN2 and plasma omentin-1 levels, the authors failed to demonstrate these are the early markers for diabetic nephropathy. To emphasize the importance for diagnosis, authors should set the cut-off points and perform the ROC analyses. 2. Authors failed to reveal the functional link among the ox-LDL, TLR4, urinary LCN2 and omentin-1 in the diabetic nephropathy. Are there tight correlations among these factors? In table 2, urinary LCN2 levels demonstrate tight correlations with plasma omentin-1, plasma ox-LDL and blood TLR4 mRNA. 3. Authors may want to step-wise regression analysis in addition to the simple correlation appeared in table 3. Authors should carefully select the dependent and independent variables to provide the clinically relevant results. 4. In line 177, how did the authors conclude ox-LDL increased the expression of TLR4. 5. In line 178, it is not clear why ox-LDL can be used for the diagnosis of diabetic nephropathy. 6. In lines 183-186, the relation between ox-LDL and proximal tubular injuries is not described. 7. In lines 206-208, the evidence is lacking in the statement that TLR4 increased LCN2 production.	We appreciated the constructive criticisms of the reviewer and the valuable comments that have helped us greatly to improve our manuscript. I have addressed each of their concerns as outlined below. 1 ROCcurve was performed 2. 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., 2004 demonstrate that injection of mice with the TLR4 ligand lipopolysaccharide induces increased expression of LCN2 in a TLR4-dependent manner [27]. 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 can attenuates C-reactive protein (CRP) and TNF-α-induced NF-κB



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		activation in human endothelial cells suggesting that omentin-1 might be an anti-inflammatory adipokine in humans [29]. However, little information is available with respect to the associations of serum omentin levels and diabetic microvascular complications. 3. Performing regression analysis is a good idea and we already perform it (alb/ceat. Ratio was used as dependent variable but LCN2 .TLR4,oxLDL and omentin1 were independent variable) but the results obtained were the same as correlation matrix and we found that the statistics had too much tables so include only the matrix 4. as it was reported previously that ox-LDL is an inducer to TLR4 GENE EXPRESSION Geng et al, 2010 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 chemoattractant 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]. 5. Moreover, ox-LDL was correlated positively with serum creatinine, UACR
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		and disease duration revealing its role not only in pathogenesis but also in diagnosis of DN and its progression. 6. 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]. AND ALSO THROUGH NUCLEAR FACTOR KAPP B ACTIVATION (OXIDATIVE STRESS)
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Minor REVISION comments	Minor comments 1. In line 16, omentin-1 levels negatively correlated with various clinical parameters. 2. In line 26, 'main player' should be 'main players'. 3. In line 38, please specify 'MMP-9 transport'. Transport to renal tubular cells? 4. In line 51, please describe the numbers for approval of local ethic committee. 5. In line 54, authors should describe the oral agents for the treatment of diabetes. 6. In line 86, did authors isolate total RNA from whole blood or isolated leukocytes? 7. In table 1, authors should use SI units for the clinical data. 8. In table 2, urinary LCN2 levels should be standardized by urinary creatinine levels. How did authors calculate the relative TRL4 mRNA in blood samples? 9. In lines 194-196, please refer the article. 10. In line 22, what did authors mean in 'synergistic increase'?	7. LCN2 is induced by inflammatory stimuli through the activation of the NF-kB pathway [22] WHICH WAS ACTIVATED BY TLR4 MINOR COMMENTS 1.It was corrected 2. corrected 3.corrected 4. Added 5.added 6. from whole blood 8. - From ct of target and housekeeping gene and it is automatically calculated by soft ware 11. They are increased with each other
Optional/General comments	The authors investigated the plasma ox-LDL, TLR4 mRNA in whole blood, urinary LCN2 and plasma omentin-1 levels in control, normo-, micro- and macroalbuminuria T2DM patients. TLR4 mRNA, plasma ox-LDL, and urinary LCN2 levels were higher in T2DM cases and further higher in macroalbuminuria DM patients. Omentin-1 levels were lower in T2DM cases and further lower in macroalbuminuria DM patients. These parameters correlated with serum creatinine, fasting blood glucose, urinary albumin/creatinine ratio. Although plasma ox-LDL, TLR4 mRNA in whole blood, urinary LCN2 and plasma omentin-1 levels were measured, the significance of these parameters in the diagnosis of diabetic nephropathy is not clear and functional link among these factors are not demonstrated in the study.	