ORIGINAL ARTICLE

Retinol-binding protein 4 versus albuminuria as predictors of estimated glomerular filtration rate decline in patients with type 2 diabetes

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Background: Since the increase in some tubular damage biomarkers can be observed at the early stage of diabetic nephropathy, even in the absence of albuminuria, we aimed to investigate if urinary albumin is superior than tubular damage marker, such as serum retinol-binding protein 4 (RBP4), in predicting renal function decline (defined as estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m^2) in the cohort of patients with type 2 diabetes mellitus (T2D). **Materials and Methods:** A total of 106 sedentary T2D patients (mean [± standard deviation] age 64.9 [±6.6] years) were included in this cross-sectional study. Anthropometric and biochemical parameters (fasting glucose, glycated hemoglobin [HbA1c], lipid parameters, creatinine, RBP4, high sensitivity C-reactive protein [hsCRP], urinary albumin excretion [UAE]), as well as blood pressure were obtained. **Results:** HsCRP (odds ratio [OR] =0.754, 95% confidence interval [CI] (0.603–0.942), P = 0.013) and RBP4 (OR = 0.873, 95% CI [0.824–0.926], P < 0.001) were independent predictors of eGFR decline. Moreover, although RBP4 and UAE as single diagnostic parameters of renal impairment showed excellent clinical accuracy (area under the curve [AUC] = 0.900 and AUC = 0.940, respectively), the Model which included body mass index, HbA1c, triglycerides, hsCRP, and RBP4 showed statistically same accuracy as UAE, when UAE was used as a single parameter (AUC = 0.932 vs. AUC = 0.940, respectively; P for AUC difference = 0.759). As well, the Model had higher sensitivity and specificity (92% and 90%, respectively) than single predictors, RBP4, and UAE. **Conclusion:** Although serum RBP4 showed excellent clinical accuracy, just like UAE, a combination of markers of tubular damage, inflammation, and traditional markers has the higher sensitivity and specificity than UAE alone for prediction renal impairment in patients with T2D.

Key words: Albuminuria, diabetic nephropathy, inflammation, retinol-binding protein 4

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INTRODUCTION

Diabetic nephropathy (DN) has been widely recognized as a common complication of type 2 diabetes mellitus (T2D), which may further progress into end-stage renal disease and premature mortality.[1]

Oxidative stress and increased inflammation are considered as key determinants of DN.[2-4] Due to increased reactive oxygen species (ROS) and inflammatory cytokines production, glomerular filtration membrane becomes permeable for plasma proteins, resulting in albuminuria, a hallmark of early loss of renal function.[1,5] However, not always renal impairment is accompanied with albuminuria since it is known that DN is observed among normoalbuminuric patients with T2D, as well.[1,6] In addition, in some cases with T2D, microalbuminuria (30–300 mg/24 h) can have transient character, especially along with improvement of glycemic or blood pressure control.[7] All of this may in part explain why changes in albuminuria are now considered as complementary rather than obligatory manifestations of DN.[1] In addition, not only the dysfunction of glomeruli but also the impairment of renal tubules also plays a significant role in the pathogenesis of DN.[1] As well, the increase in some of

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the tubular damage biomarkers has been observed at the early stage of DN, even in the absence of albuminuria, thus making them specific and sensitive markers of DN.[1]

Retinol-binding protein 4 (RBP4) has been widely explored as adipokine, closely related to cardiometabolic indices.[8-11] Furthermore, due to its low molecular weight (21 kDa), it is freely filtered through the glomeruli and then almost completely reabsorbed in the proximal tubuls, which makes this protein as useful biomarker of tubular renal impairment. Namely, a significant rise of this biomarker has been observed in the end-stage renal disease,[12] which was decreased after kidney transplantation.[13] As well, serum RBP4 levels were associated with estimated glomerular filtration rate (eGFR), as well as positively correlated with changes in serum creatinine, confirming its association with renal function.[13]

In order to get better insight into the pathophysiological mechanisms of renal function decline, we aimed to examine markers of glomerular damage (i.e., urinary albumin), markers of tubular damage (i.e., serum RBP4), and inflammation markers (i.e., serum high sensitivity C-reactive protein level [hsCRP]) in patients with T2D. Furthermore, we aimed to investigate if urinary albumin is superior than tubular damage, inflammation, and some traditional markers in predicting renal function impairment in the cohort of patients with T2D.

MATERIALS AND METHODS

Study population

The current cross-sectional study derived from our previous works investigating the utility of cardiometabolic, inflammation, and oxidative stress markers in individuals with T2D.[14-17]

The study enrolled a total of 106 patients with T2D (mean age 64.9 ± 6.6 years, of them 61.3% females). All patients with T2D were consecutively recruited by the endocrinologist in the Center for Laboratory Diagnostics of the Primary Health Care Center in Podgorica, Montenegro, for their regular checkup in a period from October 2012 to May 2016.

Participants that were included in the study were patients with T2D without acute inflammatory disease, or urinary infection and/or hematuria. Diabetes cases were defined as described in our previous reports.[14-17]

Exclusion criteria from the current investigation were participants with diabetes mellitus type 1, with eGFR <15 mL/min/1.73 m^2, patients on chronic dialysis, with kidney transplantation, renal disease other than DN, diseases other than diabetes which induce proteinuria (e.g., vasculitis and amyloidosis), hsCRP >10 mg/L, those with a recent (6 months) history of acute myocardial infarction or stroke, carcinoma, pregnancy, and with history of alcohol abuse (i.e., ethanol consumption >20 g/day). All the examinees signed informed consent. Ethical Committee of Primary Health Care Center in Podgorica, Montenegro (number 317/2) approved the study protocol, and the investigation was carried out in compliance with the Declaration of Helsinki.

Anthropometric measurements

Basic anthropometric measurements were obtained, as described previously.[18]

Biochemical analyses

After at least 8 h of an overnight fasting, cubital venous sample blood (10 mL) was collected from each participant for biochemical analyses (fasting glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides [TG], creatinine, glycated hemoglobin [HbA1c], hsCRP, and RBP4 levels), as described elsewhere.[14,18] Examinees were requested to provide two blood samples, one for whole blood in K_2EDTA for HbA1c determination and the other for serum extraction. Patients were also asked to provide 24 h urine sample. Rate of urinary albumin excretion (UAE) <30 mg/24 h was considered as normoalbuminuria; UAE within the range 30–300 mg/24 h was considered as microalbuminuria, while UAE rate ≥300 mg/24 h was regarded as macroalbuminuria. All the examinees were instructed on how to collect 24 h urine and asked to store the urine on cold (4°C).

Blood pressure was measured as described previously.[18]

Glomerular filtration rate was estimated by using creatinine in the Modification of Diet in Renal Disease Study equation ($eGFR_{MDRD}$).[14] Renal function decline is defined as $eGFR_{MDRD}$ <60 mL/min/1.73 m^2.

Statistical analysis

Statistical analysis was performed with MedCalc Version 12.5 (Mariakerke, Belgium) and SPSS® Statistics version 22 (Chicago, Illinois, USA) statistical softwares for Windows.

The distributions of variables were checked by Kolmogorov–Smirnov test. Differences in clinical parameters between individuals were analyzed by Student's *t*-test for normally and log-normally distributed variables and by Mann–Whitney U-test for skewed distribution. Bivariate correlation between $eGFR_{MDRD}$ and other clinical parameters were analyzed by nonparametric Spearman correlation analysis.

Logistic regression analysis was used to elucidate the association between $eGFR_{MDRD}$ and other clinical

parameters. The dependent variable was $eGFR_{MDRD}$ coded as 0 for $eGFR_{MDRD}$ <60 mL/min/1.73 m² and coded as 1 for $eGFR_{MDRD}$ ≥60 mL/min/1.73 m².

Since we aimed to get better insight into the pathophysiological mechanisms of renal function decline in patients with T2D, we included marker of glomerular damage (i.e., UAE), marker of tubular damage (i.e., serum RBP4), and inflammation marker (i.e., serum hsCRP). In addition, we included traditional risk factors such as body mass index (BMI), HbA1c, and TG. Therefore, independent variables were BMI, HbA1c, TG, hsCRP, RBP4, and UAE (all continuous). Those continuous variables which had $P < 0.05$ when testing bivariate correlations with $eGFR_{MDRD}$ were included in univariate and further multivariate logistic regression analysis. Because they entered the equation for GFR calculation, age and creatinine were excluded from logistic regression analysis. To examine tested independent variables, independent predictions on $eGFR_{MDRD}$ multivariate logistic regression analysis were employed. The explained variation in $eGFR_{MDRD}$ was given by Nagelkerke R^2 value. Receiver operating characteristic (ROC) curve analysis was used to test the diagnostic performance of each independent variable and the Model to discriminate patients that suffered from renal function decline from those that did not have it. Differences between curve areas for UAE and the Model were also tested.

Data are shown as mean ± standard deviation for normally distributed continuous variables, as geometrical mean (95% confidence interval [CI]) for log-normally distributed variables, median (interquartile range), and as absolute frequencies for categorical variables.[19] All tests were considered significant at the probability level $P < 0.05$.

RESULTS

Table 1 shows the biochemical parameters in diabetic patients with renal decline ($eGFR_{MDRD}$ <60 mL/min/1.73 m²) and those that did not have it ($eGFR_{MDRD}$ ≥60 mL/min/1.73 m²). Unequal distribution of patients taking antihyperglycemic or insulin therapies was established among groups. Patients with $eGFR_{MDRD}$ ≥60 mL/min/1.73 m² were older and had higher BMI than those with $eGFR_{MDRD}$ <60 mL/min/1.73 m². Furthermore, HbA1c, TG, creatinine, RBP4, and UEA concentrations were significantly higher among patients with $eGFR_{MDRD}$ <60 mL/min/1.73 m². No other significant differences in clinical parameters were present between these two groups [Table 1].

Spearman's correlation analyses were performed to test the associations between $eGFR_{MDRD}$ and other clinical parameters. Estimated GFR_{MDRD} was significantly negatively correlated with age, TG, hsCRP, creatinine, RBP4, UAE, and positively correlated with BMI [Table 2].

Table 3 summarizes results of logistic regression analysis applied to examine the associations of parameters significantly correlated with $eGFR_{MDRD}$ such as BMI, HBA1c, TG, hsCRP, RBP4, and UAE as independent variables (predictors) on $eGFR_{MDRD}$ as dependent variable. Age and creatinine were excluded from further analysis because they were used for $eGFR_{MDRD}$ calculation. Predictors were unadjusted and adjusted for other parameters and tested by univariate and multivariate analysis, respectively. In order to test if RBP4 together with other routinely determined parameters could be as good indicator of renal function as UAE, the latter was tested only in univariate analysis and it did not enter the Model like all the other parameters. HsCRP, RBP4, and UAE showed significant odds ratio (OR) in univariate logistic regression [Table 3]. As hsCRP rose for 1 mg/L, RBP for 1 mg/L, and UAE for 1 mg/24 h, probability for $eGFR_{MDRD}$ ≥60 mL/min/1.73 m² decreased for 14.3%, 11.2%, and 0.4%, respectively. Nagelkerke R^2 showed that each predictor in univariate analysis such as hsCRP, RBP4, and UAE could explain the variation in $eGFR_{MDRD}$ by 5.4%, 58.4%, and 55.9%, respectively. Multivariate logistic regression analysis showed that only hsCRP and RBP4 kept independent prediction on $eGFR_{MDRD}$ [Model, Table 3]. As hsCRP rose for 1 mg/L and RBP4 for 1 mg/L, the probability for $eGFR_{MDRD}$ ≥60 mL/min/1.73 m² decreased for 24.6% and 12.7%, respectively. Adjusted R^2 for the Model was 0.733, which means that even 73.3% of variation in $eGFR_{MDRD}$ could be explained with this Model [Table 3].

ROC analysis was used to discriminate patients with renal function decline from those who did not have it [Table 4]. The calculated AUC for BMI, HbA1c, TG, and hsCRP were ranking from 0.600 to 0.700 indicated that the clinical accuracy of each diagnostic parameter was low according to Swets.[20] On the contrary to these single predictors, RBP4 and UAE as single diagnostic parameters of renal impairment showed excellent clinical accuracy (AUC = 0.900 and AUC = 0.940, respectively) [Table 4]. Furthermore, the same was established for the Model which included BMI, HbA1c, TG, hsCRP, and RBP4 (continuous variables). The calculated AUC for the Model was 0.932 which suggested statistically same accuracy as UAE, when UAE was used as a single parameter [Figure 1]. Accordingly, the difference between areas was 0.008, SE = 0.026, 95% CI (−0.043–0.059) and $P = 0.759$. As well, the Model had higher sensitivity and specificity (92% and 90%, respectively) than single predictors (i.e., RBP4 and UAE) [Table 4 and Figure 1].

DISCUSSION

The main finding of the current study is that tubular damage marker such as serum RBP4 as single diagnostic parameter of renal impairment showed excellent clinical accuracy, just like UAE (AUC = 0.900 and AUC = 0.940,

Table 1: Clinical characteristics of patients with diabetes according to estimated glomerular filtration rate

	$eGFR_{MDRD}$ <60 mL/min/1.73 m²	$eGFR_{MDRD}$ ≥60 mL/min/1.73 m²	*P*
n (male/female)	40 (16/24)	66 (25/41)	0.840
Age (years)	62.72±8.31	63.88±5.13	0.031
BMI (kg/m²)	26.44±2.44	27.55±2.33	0.021
Glucose (mmol/L)*	8.10 (6.15-10.95)	7.80 (6.90-9.70)	0.964
HbA1c (%)	8.34±2.09	7.57±1.21	0.017
TC (mmol/L)	5.97±1.24	5.71±1.20	0.289
HDL-c (mmol/L)	1.21±0.32	1.31±0.38	0.162
LDL-c (mmol/L)	3.82±1.06	3.56±1.00	0.204
TG (mmol/L)**	2.17 (1.82-2.59)	1.63 (1.50-1.78)	0.001
hsCRP (mg/L)*	3.13 (1.53-5.65)	2.41 (0.94-3.81)	0.085
Creatinine (μmol/L)**	144 (107-202)	63 (52-74)	<0.001
RBP4 (mg/L)**	74.43 (68.17-81.27)	46.89 (8.58-23.06)	<0.001
UAE (mg/24 h)**	825.00 (88.99-2094.25)	12.46 (44.08-79.90)	<0.001
$eGFR_{MDRD}$ (mL/min/1.73 m²)	39.86±13.26	100.28±25.50	<0.001
Antihyperglycemics (no/yes)	19/21	2/64	<0.001
Insulin (no/yes)	11/29	60/6	<0.001

Data are presented as arithmetic mean±SD and compared by Student's *t*-test. *Skewed distributed data are presented as median (interquartile range) and compared by Mann–Whitney test, **Log-normal distributed data are presented as geometric mean (95% CI) compared by Student's *t*-test. Antihyperglycemic and insulin therapies are given as absolute frequencies and compared by Chi-square test. BMI=Body mass index; HbA1c=Glycated hemoglobin; TC=Total cholesterol; HDL-c=High-density lipoprotein cholesterol; LDL-c=Low-density lipoprotein cholesterol; TG=Triglycerides; hsCRP=High-sensitivity C-reactive protein; RBP4=Retinol-binding protein 4; UAE=Urinary albumin excretion rate; $eGFR_{MDRD}$=Estimated glomerular filtration rate in the modification of diet in renal disease study equation; SD=Standard deviation; CI: Confidence interval

Table 2: Spearman's correlation analysis between $eGFR_{MDRD}$ and clinical parameters in patients with diabetes

	Rho (ρ)	*P*
Age (years)	−0.343	<0.001
BMI (kg/m²)	0.234	0.016
Glucose (mmol/L)	0.005	0.963
HbA1c (%)	−0.220	0.030
TC (mmol/L)	−0.080	0.414
HDL-c (mmol/L)	0.174	0.075
LDL-c (mmol/L)	−0.135	0.168
TG (mmol/L)	−0.294	0.002
hsCRP (mg/L)	−0.214	0.048
Creatinine (μmol/L)	−0.959	<0.001
RBP4 (mg/L)	−0.754	<0.001
UAE (mg/24 h)	−0.635	<0.001

Data are presented as correlation coefficient Rho (ρ). BMI=Body mass index; HbA1c=Glycated hemoglobin; TC=Total cholesterol; HDL-c=High-density lipoprotein cholesterol; LDL-c=Low-density lipoprotein cholesterol; TG=Triglycerides; hsCRP=High-sensitivity C-reactive protein; RBP4=Retinol-binding protein 4; UAE=Urinary albumin excretion rate; $eGFR_{MDRD}$=Estimated glomerular filtration rate in the modification of diet in renal disease study equation

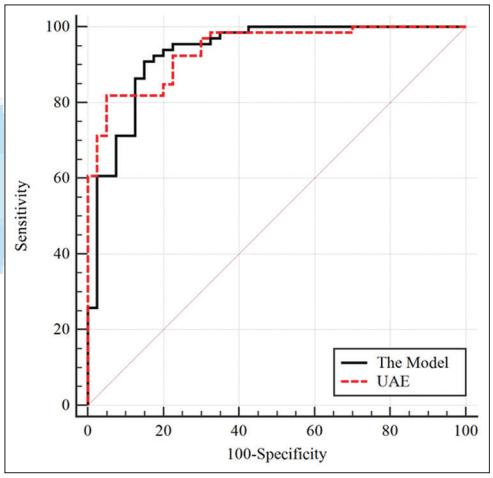

Figure 1: Discriminatory abilities of UAE as a single parameter and the Model regarding renal function decline. Model: BMI, HbA1c, TG, hsCRP, and RBP4 (all continuous variables). BMI = Body mass index; HbA1c = Glycated hemoglobin; TG = Triglycerides; hsCRP = High-sensitivity C-reactive protein; RBP4 = Retinol-binding protein 4; UAE = Urinary albumin excretion rate

respectively) [Table 4]. Furthermore, we have shown that serum RBP4, hsCRP and some routinely determined parameters, could be as good indicators of renal function decline (defined as eGFR <60 mL/min/1.73 m²) as UAE.

Even though albuminuria has been considered as the gold standard biomarker for DN onset and progression, it lacks specificity for diagnosing disease progression (i.e., when UAE is 30–300 mg/24 h), as well as sensitivity, since DN can often progress even without albuminuria.[7,21] Hence, the quest for a better biomarkers with high sensitivity and specificity for early detection of DN is needed.

Since renal proximal tubular injury may occur before a reduction of GFR, we examined the utility of tubular biomarker, such as serum RBP4, in comparison with glomerular biomarkers, such as urinary albumin. Previous study by Mahfouz *et al*.[22] showed that RBP4 was more specific (90% specificity) than albumin-to-creatinine ratio for discriminating DN onset (72% specificity), suggesting that RBP4 may serve as an efficient diagnostic tool for clinical monitoring of kidney disease progression. However, our study reported that both of those biomarkers had excellent clinical accuracy for eGFR decline prediction. Several previous studies also reported elevated serum RBP4 levels in kidney disease[12,13,23,24]

but did not make a comparison between those two biomarkers.

Oxidative stress and increased inflammation play a key role in DN development.[1,2] Chronic hyperglycemia enhances ROS production which causes the damage of the glomerular filtration barrier integrity, leading to albumin leakage, which can with ROS in the tubular ultrafiltrate further activate a variety of aberrant signaling pathways to cause overall renal function deterioration.[1] Increased activation of different signaling mediators such as transcription factors, inflammatory agents, and cytokines can compromise renal hemodynamics and increase glomerular extracellular matrix accumulation, thus further leading to interstitial fibrosis and glomerulosclerosis to eventual end-stage renal disease.[25]

Indeed, individuals with DN have increased low-grade inflammation for years before renal impairment can become clinically detectable.[21]

Multivariate logistic regression analysis in the current study showed that both hsCRP and RBP4 kept independent prediction on $eGFR_{MDRD}$ [Model, Table 3]. As hsCRP rose for 1 mg/L and RBP4 for 1 mg/L, probability for $eGFR_{MDRD}$ ≥60 mL/min/1.73 m² decreased for 24.6% and 12.7%, respectively.

Table 3: Univariate and multivariate logistic regression analysis for clinical parameters predicting estimated glomerular filtration rate in patients with diabetes

Predictors	Unadjusted OR (95% CI)	*P*	Nagelkerke R^2
BMI (kg/m²)	1.224 (1.025-1.461)	0.913	0.067
HbA1c (%)	0.740 (0.571-0.958)	0.313	0.071
TG (mmol/L)	0.408 (0.241-0.688)	0.212	0.179
hsCRP (mg/L)	0.857 (0.738-0.995)	0.042	0.054
RBP4 (mg/L)	0.888 (0.847-0.930)	<0.001	0.584
UAE (mg/24 h)	0.996 (0.993-0.998)	0.010	0.559
Model	**Adjusted OR (95% CI)**	***P***	**Nagelkerke R^2**
hsCRP (mg/L)	0.754 (0.603-0.942)	0.013	0.733 (for Model)
RBP4 (mg/L)	0.873 (0.824-0.926)	<0.001	

Model: BMI, HbA1c, TG, hsCRP, and RBP4 (all continuous variables). BMI=Body mass index; HbA1c=Glycated hemoglobin; TG=Triglycerides; hsCRP=High-sensitivity C-reactive protein; RBP4=Retinol-binding protein 4; UAE=Urinary albumin excretion rate; OR=Odds ratio; CI=Confidence interval

Pro-inflammatory cytokines are considered as determining factors in the development of microvascular diabetic complications, acting through nuclear transcription factor-kappa B (NF-κB) signaling hsCRP pathway.[26]

In line with our results, previous studies also reported high hsCRP in patients with DN.[26,27] Furthermore, earlier studies reported the utility of some other parameters such as cystatin C, for estimation of eGFR decline, suggesting its high diagnostic accuracy for screening of DN.[28]

In our study, to seek for the panel of parameters that might display the best specificity and sensitivity for discrimination of patients with renal function decline from those who did not have it, ROC analysis was used [Table 4]. Model which included RBP4, hsCRP, gender, BMI, HbA1c and TG, suggested statistically same accuracy as UAE, when UAE was used as a single parameter (AUC = 0.932 vs. AUC = 940, respectively; p for AUC diff erence = 0.759) [Table 4 and Figure 1]. Of note, the Model had higher sensitivity and specificity (92% and 90%, respectively) than single predictors RBP4 and UAE [Table 4], suggesting that other traditional markers should not be underestimated when examining diabetic kidney disease.[29,30]

The limitations of our study are cross-sectional design and small sample size. However, in addition to urinary albumin we examined a broad panel of biomarkers, such as marker of tubular damage but also inflammation and several well-known traditional markers.

CONCLUSION

The novel finding of the current study is that even though that tubular damage marker such as serum RBP4 as single diagnostic parameter of renal impairment showed excellent clinical accuracy, just like UAE, a combination of markers of tubular damage, inflammation markers, and traditional markers has the higher sensitivity and specificity than

Table 4: Receiver operating characteristic analysis for single parameters and the Model discriminatory abilities regarding renal function decline in patients with diabetes

Predictors	AUC (95% CI)	SE	Sensitivity (%)	Specificity (%)	*P*
BMI (kg/m²)	0.638 (0.539-0.729)	0.057	55	70	<0.001
HbA1c (%)	0.598 (0.498-0.692)	0.061	89	37	0.106
TG (mmol/L)	0.700 (0.602-0.784)	0.057	85	57	0.005
hsCRP (mg/L)	0.600 (0.501-0.694)	0.058	91	30	0.087
RBP4 (mg/L)	0.900 (0.816-0.948)	0.034	82	85	<0.001
UAE (mg/24 h)	0.940 (0.876-0.977)	0.021	84	95	<0.001
Model	0.932 (0.881-0.983)	0.026	92	90	<0.001

Model: BMI, HbA1c, TG, hsCRP, and RBP4 (all continuous variables). BMI=Body mass index; HbA1c=Glycated hemoglobin; TG=Triglycerides; hsCRP=High-sensitivity C-reactive protein; RBP4=Retinol-binding protein 4; UAE=Urinary albumin excretion rate; AUC=Area under the curve; SE=Standard error; CI=Confidence interval

urinary albumin alone. Given that the early prediction of the onset of renal function decline is of urgent need to prevent further possible complications, the quest for more biomarkers with higher sensitivity and specificity is of great clinical importance.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Fiseha T, Tamir Z. Urinary markers of tubular injury in early diabetic nephropathy. Int J Nephrol 2016;2016:4647685.
2. Miranda-Díaz AG, Pazarín-Villaseñor L, Yanowsky-Escatell FG, Andrade-Sierra J. Oxidative stress in diabetic nephropathy with early chronic kidney disease. J Diabetes Res 2016;2016:7047238.
3. Saif-Elnasr M, Ibrahim IM, Alkady MM. Role of Vitamin D on glycemic control and oxidative stress in type 2 diabetes mellitus. J Res Med Sci 2017;22:22.
4. Papaetis GS, Papakyriakou P, Panagiotou TN. Central obesity, type 2 diabetes and insulin: Exploring a pathway full of thorns. Arch Med Sci 2015;11:463-82.
5. Nasri H, Rafieian-Kopaei M. Diabetes mellitus and renal failure: Prevention and management. J Res Med Sci 2015;20:1112-20.
6. Porrini E, Ruggenenti P, Mogensen CE, Barlovic DP, Praga M, Cruzado JM, *et al.* Non-proteinuric pathways in loss of renal function in patients with type 2 diabetes. Lancet Diabetes Endocrinol 2015;3:382-91.
7. Glassock RJ. Control of albuminuria in overt diabetic nephropathy: Durability counts. Nephrol Dial Transplant 2016;31:1371-3.
8. Tabesh M, Noroozi A, Amini M, Feizi A, Saraf-Bank S, Zare M, *et al.* Association of retinol-binding protein 4 with metabolic syndrome in first-degree relatives of type 2 diabetic patients. J Res Med Sci 2017;22:28.
9. Klisić A, Kavarić N, Bjelaković B, Soldatović I, Martinović M, Kotur-Stevuljević J, *et al.* The association between retinol-binding protein 4 and cardiovascular risk score is mediated by waist circumference in overweight/Obese adolescent girls Acta Clin Croat 2017;56:92-8.
10. Chen D, Huang X, Lu S, Deng H, Gan H, Du X, *et al.* RBP4/Lp-PLA2/Netrin-1 signaling regulation of cognitive dysfunction in diabetic nephropathy complicated with silent cerebral infarction. Exp Clin Endocrinol Diabetes 2017;125:547-53.
11. Klisic A, Kotur-Stevuljevic J, Kavaric N, Matic M. Relationship between cystatin C, retinol-binding protein 4 and Framingham risk score in healthy postmenopausal women. Arch Iran Med 2016;19:845-51.
12. Rubinow KB, Henderson CM, Robinson-Cohen C, Himmelfarb J, de Boer IH, Vaisar T, *et al.* Kidney function is associated with an altered protein composition of high-density lipoprotein. Kidney Int 2017;92:1526-35.
13. Zhang WX, Zhou W, Zhang ZM, Zhang ZQ, He JF, Shi BY, *et al.* Decreased retinol-binding protein 4 in the sera of patients with end-stage renal disease after kidney transplantation. Genet Mol Res 2014;13:8126-34.
14. Klisic A, Kavaric N, Jovanovic M, Zvrko E, Skerovic V, Scepanovic A, *et al.* Association between unfavorable lipid profile and glycemic control in patients with type 2 diabetes mellitus. J Res Med Sci 2017;22:122.
15. Klisic A, Isakovic A, Kocic G, Kavaric N, Jovanovic M, Zvrko E, *et al.* Relationship between oxidative stress, inflammation and dyslipidemia with fatty liver index in patients with type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2017;125:1-8.
16. Kavaric N, Klisic A, Ninic A. Are visceral adiposity index and lipid accumulation product reliable indices for metabolic disturbances in patients with type 2 diabetes mellitus? J Clin Lab Anal 2018;32:e22283.
17. Klisic A, Kocic G, Kavaric N, Jovanovic M, Stanisic V, Ninic A, *et al.* Xanthine oxidase and uric acid as independent predictors of albuminuria in patients with diabetes mellitus type 2. Clin Exp Med 2017. DOI: 10.1007/s10238-017-0483-0.
18. Klisic A, Kavaric N, Jovanovic M, Soldatovic I, Gligorovic-Barhanovic N, Kotur-Stevuljevic J, *et al.* Bioavailable testosterone is independently associated with fatty liver index in postmenopausal women. Arch Med Sci 2017;13:1188-96.
19. Bland JM, Altman DG. Transformations, means, and confidence intervals. BMJ 1996;312:1079.
20. Swets JA. Measuring the accuracy of diagnostic systems. Science 1988;240:1285-93.
21. Al-Rubeaan K, Siddiqui K, Al-Ghonaim MA, Youssef AM, Al-Sharqawi AH, AlNaqeb D, *et al.* Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. Sci Rep 2017;7:2684.
22. Mahfouz MH, Assiri AM, Mukhtar MH. Assessment of neutrophil gelatinase-associated lipocalin (NGAL) and retinol-binding protein 4 (RBP4) in type 2 diabetic patients with nephropathy. Biomark Insights 2016;11:31-40.
23. Domingos MA, Queiroz M, Lotufo PA, Benseñor IJ, Titan SM. Serum RBP4 and CKD: Association with insulin resistance and lipids. J Diabetes Complications 2017;31:1132-8.
24. Majerczyk M, Choręza P, Bożentowicz-Wikarek M, Brzozowska A, Arabzada H, Owczarek A, *et al.* Increased plasma RBP4 concentration in older hypertensives is related to the decreased kidney function and the number of antihypertensive drugs-results from the PolSenior substudy. J Am Soc Hypertens 2017;11:71-80.
25. Fakhruddin S, Alanazi W, Jackson KE. Diabetes-induced reactive oxygen species: Mechanism of their generation and role in renal injury. J Diabetes Res 2017;2017:8379327.
26. Liu Q, Jiang CY, Chen BX, Zhao W, Meng D. The association between high-sensitivity C-reactive protein concentration and diabetic nephropathy: A meta-analysis. Eur Rev Med Pharmacol Sci 2015;19:4558-68.
27. Varma V, Varma M, Varma A, Kumar R, Bharosay A, Vyas S, *et al.* Serum total sialic acid and highly sensitive C-reactive protein: Prognostic markers for the diabetic nephropathy. J Lab Physicians 2016;8:25-9.
28. Javanmardi M, Azadi NA, Amini S, Abdi M. Diagnostic value of cystatin C for diagnosis of early renal damages in type 2 diabetic mellitus patients: The first experience in Iran. J Res Med Sci 2015;20:571-6.
29. Al-Rubeaan K, Youssef AM, Subhani SN, Ahmad NA, Al-Sharqawi AH, Al-Mutlaq HM, *et al.* Diabetic nephropathy and its risk factors in a society with a type 2 diabetes epidemic: A Saudi National Diabetes Registry-based study. PLoS One 2014;9:e88956.
30. Svensson MK, Tyrberg M, Nyström L, Arnqvist HJ, Bolinder J, Östman J, *et al.* The risk for diabetic nephropathy is low in young adults in a 17-year follow-up from the Diabetes Incidence Study in Sweden (DISS). Older age and higher BMI at diabetes onset can be important risk factors. Diabetes Metab Res Rev 2015;31:138-46.