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http://rjdnmd.org
Rom J Diabetes Nutr Metab Dis. **26**(2):169-175
doi: 10.2478/rjdnmd-2019-0018

TGF-β1 AND VCAM-1 SERUM CONCENTRATIONS AS DIAGNOSTIC BIOMARKERS OF DIABETIC KIDNEY DISEASE PROGRESSION

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received: May 06, 2019 *accepted*: June 10, 2019
available online: June 30, 2019

Abstract

Background and aims: *Transforming growth factor-beta 1 (TGF-β1) and vascular adhesion molecule 1 (VCAM-1) have been proposed as promising biomarkers for multiple diseases. TGF-β1 and VCAM-1 are reported to be associated with diabetic kidney disease (DKD) and end stage renal disease in patients with type 1 and type 2 diabetes mellitus (T1DM and T2DM).* ***Material and methods:*** *The aim of this study was to investigate the expression of circulating TGF-β1 and VCAM-1 and to assess their potential as a blood-based biomarker for DKD in T1DM and T2DM patients.* ***Results:****. The study included 124 participants: 66 patients with T1DM, 58 with T2DM and 20 healthy controls. The diabetic patients were classified according to the estimated glomerular filtration rate (eGFR). First group - eGFR ≥90ml/min/1.73 m² (n=39), second group eGFR 89-60 ml/min/1.73m² (n=45), and third group eGFR 59-45 ml/min/1.73m² (n=40). Enzyme-linked immunosorbent assay for the quantitative detection of was used to evaluate blood TGF- β1 and VCAM-1 expression. It was found that there were higher TGF- β1 and VCAM-1 in all diabetic patients compared with healthy controls (P<0.05). TGF- β1 and VCAM-1 were higher in group with eGFR ≥90ml/min/1.73 m² and gradually increased in the groups with eGFR89-60 ml/min/1.73m² and eGFR 59-45 ml/min/1.73m². TGF- β1 and VCAM-1 were less in T1DM, than T2DM in all study groups. Regression analysis revealed reverse associations between TGF- β1, VCAM-1 and eGFR (P<0.05). TGF- β1 and VCAM-1 correlated positively with albuminuria and negatively with renal function.* ***Conclusion:*** *In discriminating overall patients from healthy subjects, ROC analysis revealed areas under the curve (AUCs) of 1,0 for TGF- β1 for T1DM and T2DM, VCAM-1 0,866 for T1DM, 0,923 for T2DM (P<0.001). The results suggested that blood-based TGF- β1 and VCAM-1 may serve as potential biomarkers for early detection of DKD.*

key words: *VCAM-1; TGF- β1; endothelial dysfunction; diabetes mellitus; diabetic kidney disease progression*

Background and aim

The prevalence of diabetes mellitus (DM) has tendency to increase worldwide. It is about 425 million people with diabetes now and it may rise to 693 million by 2045 [1]. Epidemiological studies in Ukraine also indicate ongoing increase the number of patients with DM.

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Chronic hyperglycemia is accompanied by lesion, dysfunction or insufficiency of various organs and systems. Eye retinal blood vessels and the kidney are primary affected by increased glucose level. Damage of kidney leads to diabetic kidney disease (DKD), which often leads to disability. The increasing number of patients with end stage renal disease (ESRD) cause significant social and economic losses around the world.

Five-year survival rate of patients with T1DM and T2DM after DKD manifestation is about 77 and 70%, respectively [2]. These and other facts encourage researchers to seek new approaches to determining the early stages of DKD, for slowing the disease progression. Preclinical diagnostics of DKD may enable to prescribe therapy earlier and slow down the kidney disease progression. Thus, scientists are continuously looking for markers to detect DKD at the earliest stages, when changes in kidneys are reversible.

Endothelial cells intensively regulate basal vascular tone tension and vascular reactivity under physiological and pathological conditions, by responding to mechanical forces and neurohumoral mediators with release of variety relaxing and contracting factors. Loss of the endothelium modulatory role may be a critical and initiation factor in the development of diabetic vascular disease. Now it is possible to select several variants of changes in the endothelium functional activity. Endothelial dysfunction as a reduction of factors that are constantly formed and excreted from endothelial cells like nitric oxide or prostacyclin. Stimulation of endothelium cause an increase in blood factors such as Willibrand, R-selectin and tissue plasminogen activator. Activation of endothelium include strong increase in blood concentrations of endothelin-1, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, plasminogen activator inhibitor-1 (PAI-1). Notably, the concentrations of these factors are very low under heath conditions [3].

Studies of the factors released during endothelial activation suggest their important role in the development of DKD [4,5]. Multiply studies showed that VCAM-1 is associated with DKD development in patients with T2DM [6,7]. Dong-Jin Kim and coauthors found that increased levels of transforming growth factor beta 1 (TGF-β1) were associated only with increased kidney fibrosis in mice with diabetes induced by ischemic reperfusion injury in kidney [8]. TGF-β1, a key profibrotic cytokine, is involved in renal fibrosis processes, dysfunction and renal tubular atrophy and reduces small vessels number causing chronic hypoxia. The studying of endothelial dysfunction factors is an actual now, therefore the diagnostic and prognostic value of these markers needs to be clarified and should be made statistically-mathematical substantiation for detection of DKD progression.

To evaluate the significance of using transforming growth factor-beta 1 and vascular adhesion molecule-1 as prognostic markers of diabetic kidney disease progression in patients with diabetes mellitus type 1 and 2.

Material and methods

The study involved 124 patients with diabetes, 66 with T1DM and 58 with T2DM. Among the patients - 68 women and 56 men aged from 18 to 64 (33 ± 5.6). Duration of DM was 3.5-25 (12 ± 6.3) years. Patients were divided into three groups depending on the eGFR. Patients of first group had eGFR ≥90ml/min/1.73 m²; of the second group - 89-60 ml/min/1.73m², and third group - 59-45 ml/min/1.73m^2. Next patients were excluded from the study: patients with T1DM, T2DM and

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eGFR ≤45 ml/min/1.73 m^2, secondary diabetes, gestational diabetes, diabetic ketoacidosis, severe hypoglycemia, severe concomitant pathology. The control group consisted of 20 healthy volunteers. The study was performed at endocrinology department of the "Clinic of the Medical Academy", Dnipro, 2016-2017.

Creatinine levels were measured using the test kit «SpinLab» (Ukraine). eGFR was calculated by CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). The endothelial function was determined by measurements of concentration of TGF-β1 and VCAM-1 using the «Bender Medsystems» (Austria) and «BCM Diagnostics» (Ukraine). Measurements were conducted in Diagnostic Center of «Pharmacy of Medical Academy», Dnipro.

The study was conducted according to the ethical principles of the Helsinki Declaration with the permission of the Bioethical Commission of the SE "Dnipropetrovsk Medical Academy of the Ministry of Health of Ukraine".

Data were analyzed using Microsoft Excel add-on program AtteStat, software STATISTICA 6.1, and MedCalc Version 18.2.1 (https://www.medcalc.org/download.php).

ROC (Recognitive Operating Characteristic) was used to assess the discrimination ability (diagnostic test value) of the TGF-β1 and VCAM-1 as predictors of DKD progression. The result was represented by the area under the ROC curve (AUC) based on test sensitivity (Se) and specificity (Sp) and with a 95% confidence interval (95% CI). Calculations of the AUC and the difference between two AUCs were performed using DeLong et al. [9]. ROC-analysis considered adequate if statistically significant differences in AUC value was more than 0.7. The values of the area under the ROC curve were interpreted as diagnostic accuracy: 0.9-1.0 – excellent method operating characteristic; 0,8-0,9 - very good; 0,7-0,8 - good; 0.6-0.7 - average; 0.5-0.6 - unsatisfactory; the value less than 0.5 mean that the method is non-effective [10]. The threshold predictive values of the diagnostic tests were determined by the optimal cut-off point of the ROC curve calculated using the J-Youden index (the maximum vertical distance between the ROC curve and the diagonal referential line) by the ratio (J = Se + Sp-1) [11]. The critical level of statistical significance (p) was taken <5% (p<0,05).

Results and Discussion

Patients of all studied groups had significantly higher levels of TGF-β1 in the circulation (Fig. 1). This clearly demonstrate that diabetes on both types induce endothelial dysfunction. Shortly, patients of first group with T2DM had 1.14 fold higher level of TGF-β1. TGF-β1 levels were 3.85 and 4.68 fold increased in T1DM and T2DM patients of second eGFR group. Patients of third group with diabetes had 8.0 and 8.2 fold higher levels of TGF-β1 compared to control. Interestingly, the difference in TGF-β1 levels were also different in patients with T1DM and T2DM related to eGFR groups one and two (Fig. 1). TGF-β1 was increased with decreasing of eGFR regardless of type of diabetes. The levels of TGF-β1 were 57 and 78% higher in patients of second and third groups in comparison with those of first group.

It was shown that differences between levels of TGF-β1 (p1-2 = 0.001; p1-3 <0.001) and VCAM-1 (p1-2 = 0.001; p1-3 <0.001) in the study groups were the statistically significant.

It was determined that levels of TGF-β1 and VCAM-1 have by reverse strong correlation with eGFR (ρ = -0.56, p <0.001) and (ρ = -0.51, p<0.001) respectively. The less eGFR, the higher rates of TGF-β1 and VCAM-1, which indicates the progression of endothelial dysfunction.

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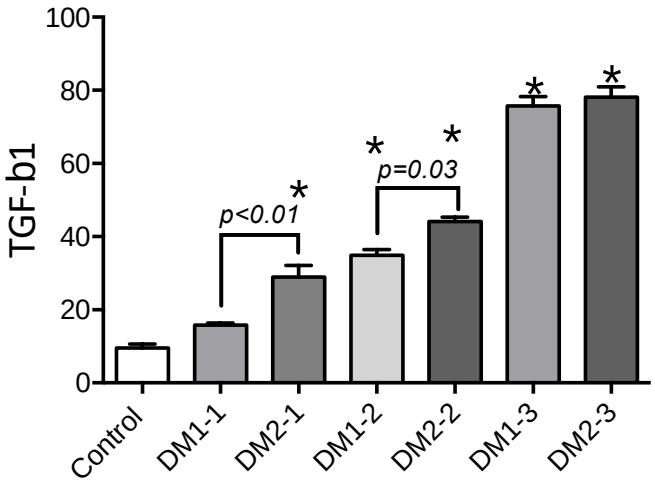

Fig. 1. *The TGF- β1 levels in healthy and individuals T1DM and T2DM ranged into three types of eGFR (1, 2 and 3).*

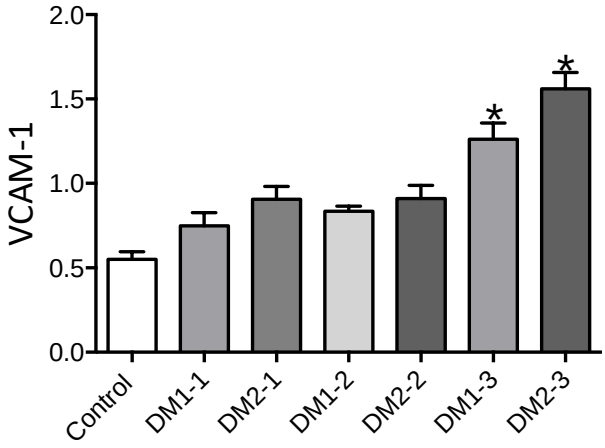

Fig. 2. *The VCAM-1 levels in study groups.*

ROC analysis was conducted to evaluate the possibility of using endothelial function proteins TGF-β1 and VCAM-1 as prognostic markers of DKD progression. For such an analysis the patients were separated into two subgroups based on eGFR. Subgroup A included 39 patients without nephropathy and eGFR 90 ml/min/1.73 m^2. Patients of subgroup B (40 patients) had clinically proven DKD and eGFR 59-45 90 ml/min/1.73 m^2. The analysis was conducted among all patients in general and taking into account the DM type. Since albuminuria (AU) is a one of the main diagnostic criteria for DKD [12] the diagnostic values of TGF-β1 and VCAM-1 were compared with the value of AU.

Test sensitivity it's a probability of positive test result in the presence of the disease, and test specificity it's a probability of negative test result in the absence of the disease. We compared the predictive power of the studied tests on DKD progression using ROC-analysis. The area under the ROC curve of all investigated laboratory tests is more than 0.7 ($p < 0.001$), so they all have a predictive value for detection of DKD progression.

We have done a pairwise comparison of the tests operating characteristics and found that TGF-b1 had a statistically significant difference (Fig. 3) with AU (difference between AUCs 0.204 at 95% CI 0.067-0.340; p = 0.004) and withVCAM-1 (difference between AUCs 0.134

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at 95% CI 0.002 - 0.268; p = 0.049) in patients with T1DM. While there was no statistically significant difference between the ROC curves for predicting DKD progression of AU and VCAM-1 (p> 0.05).

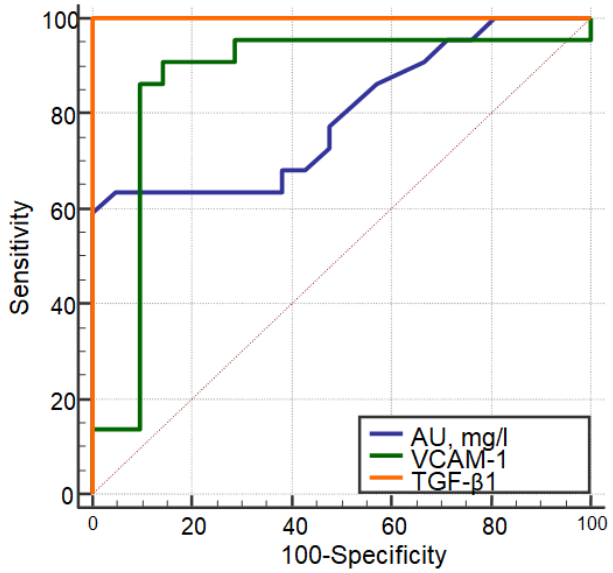

Fig. 3. *Prognostic ROC curves of diabetic kidney disease based on the levels of albuminuria, VCAM-1 and TGF-b1 in patients with diabetes mellitus type1.*

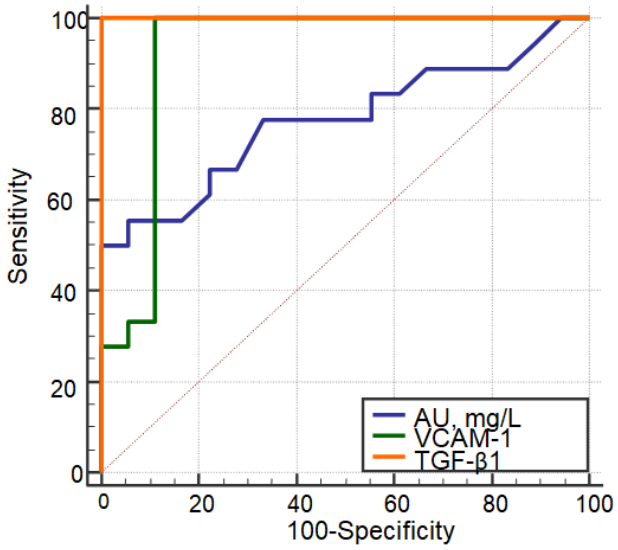

Fig. 4. *Prognostic ROC curves of diabetic kidney disease based on the levels of albuminuria, VCAM-1 and TGF-b1 in patients with diabetes mellitus type2.*

The difference between the areas under ROC curves of AU and VCAM-1 was also not statistically significant (p> 0.05) in patients with T2DM. We also didn't find statistically significant differences between ROC curves of VCAM-1 and TGF-β1 in patients with T2DM (difference between AUCs was 0.077 at 95% CI -0.029-0.184; p = 0.156). The difference between areas under ROC curves of AU and TGF-b1 was 0.225 (95% CI 0.065 - 0.386, p = 0.006).

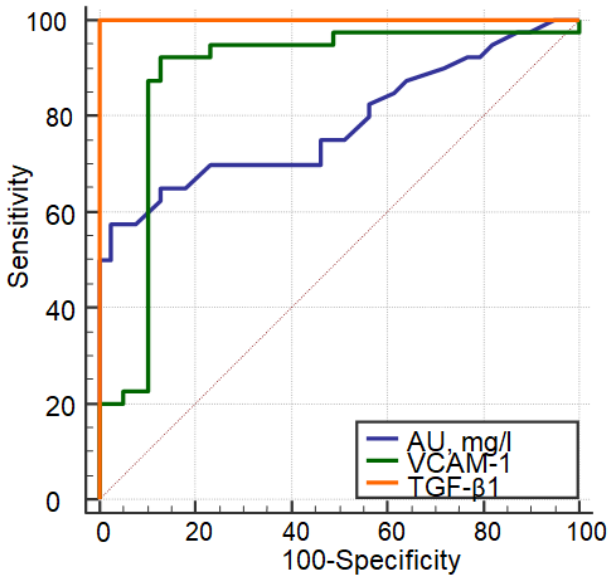

Fig. 5. *Prognostic ROC curves of diabetic kidney disease based on the levels of albuminuria, VCAM-1 and TGF-b1 in patients with diabetes mellitus types1 and 2.*

Thus, selected endothelial function markers - VCAM-1 and TGF-β1 can be used for predicting DKD progression in patients with diabetes both types. It is important to emphasize that the diagnostic capabilities of VCAM-1 and TGF-β1 in predicting DKD progression exceed the diagnostic capabilities of AU.

The optimal cut-off point used as the critical level for deciding DKD is progressing. According to our research for the AU the critical value corresponds to well-known data and is> 35.2 mg / l. For the VCAM-1 in patients with T1DM the cut-off point level is higher than 901.92 pg / ml; in patients with T2DM - more than 964.44 pg / ml, for both types of diabetes - more than 922.5 pg / ml. For the TGF-β1 the

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optimal cut-off point level for deciding that DKD is going to progress in patients with diabetes 1 and 2 types is more than 52281.6 ng/ml.

In our study, it was determined that albuminuria has “good” operating characteristics concerning DKD progression: Se = 57,50; Sd = 97.44; AUC = 0.785; p <0,001 for type1 and 2 DM. However, the evaluation of albuminuria in diabetes has a high specificity, but low sensitivity of the prognosis of DKD progression. We traced this pattern for both types of diabetes.

VCAM-1 has “excellent” operating characteristics for predicting DKD progression in patients with T2DM (Se = 100,0; SP = 88,89; AUC = 0,923; p <0.001) and “very good” in patients with T1DM (Se = 86,36; Sp = 90.48; AUC = 0.866; p <0.001).

The best operating characteristics (the highest sensitivity and specificity, AUC) has TGF-β1, its predictive ability concerning DKD progression can be assessed as “exellent” for both types of diabetes (Se = 100.0; Sr = 100.0; AUC = 1.000; p < 0.001).

Conclusions

In our study it has been determined that endothelial function markers TGF-β1 and VCAM-1 can be used to predicting diabetic kidney disease progression in patients with type 1 and type 2 diabetes.

Diagnostic capabilities VCAM-1 (the difference between AUCs of 0.134 at 95% CI 0.002 - 0.268; p = 0.049) and TGF-β1 (difference between AUCs 0.225 at 95% CI 0.066 - 0.386; p = 0.006) for predicting DKD progression significantly exceed the diagnostic capabilities of albuminuria, which determines VCAM-1 and TGF-β1 as early markers.

According to the results of the pairwise comparison of predictive capabilities between TGF-β1 and VCAM-1, it was determined that the best operating characteristics for the prognosis of DKD progression has TGF-β1, in patients with both types of diabetes. That is why TGF-β1 can be recommended for using as an early diagnostic marker of diabetic kidney disease progression.

We defined the optimal cut-off point levels for predicting DKD progression: for the VCAM-1 in patients with T1DM – more than 901.92 pg/ml, in patients with T2DM – more than 964.44 pg/ml, for both types of diabetes – more than 922.5 pg/ml. For the TGF-b1 the optimal cut-off point level for predicting DKD progression is more than 52281.6 ng/ml in patients with T1DM and T2DM separately and in general.

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