IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 18, Issue 8 Ser. 6 (August. 2019), PP 16-20
www.iosrjournals.org

Dynamics ofEndothelialDesquamationinPatientswithDiabeticKidneyDisease

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Abstract:The objective of our study*– to determine the effect of glucose-lowering therapy on blood glucose indices, vessels' structure and markers of functional conditions of kidneys in patients with diabetes mellitus.* ***Methods:*** *11 type-1 and 39 type-2 diabetic patients were studied with assessment of vascular endothelium state and renal function parametersfor a period of 3 months. Endotheliocytemia level was estimated using Hladovec method.*

Results and discussion*. 3-month course of insulin glargine in combination with short-acting insulin in type-1 diabetic patients and the administration of oral hypoglycemic therapy in combination with insulin glargine in type 2 diabetic patients has been associated with clinically significant improvement in glycemic control. Thenumberofcirculating endothelial cellswasestablishedtobe 738 ± 67.8 cells/ml, which is 29,1 % lowerafter 3-months oftreatment (p < 0,001).Thedecreaseinthelevelofendothelialdesquamationwasobservedinallgroupsofpatientswithdiabetes, regardlessoftheinitial functionalstateofthekidneys.*

Conclusion: *Improvement of glycemic control and complex therapy of existing complications of diabetes leads to a significant reduction in endothelial damage in patients with diabetes, and, therefore, contributes to the protection of blood vessels (p < 0.001). The analysis of the functional state of the kidneys for a period of 3 months indicates that it is possible to prevent or slow down the progression of diabetic kidney disease using the prescribed treatment scheme.*

Key words: *diabetes mellitus; endothelial dysfunction; diabetic kidney disease, circulating endothelial cells, vascular endothelium, vascular complications*

Date of Submission: 29-07-2019 Date of Acceptance: 14-08-2019

I. Introduction

This work is a fragment of the research and development work 'Investigation of endothelial dysfunction in diabetes mellitus and the possibility and effectiveness of the use of a new method for treating patients with diabetes mellitus'. № of state registration 0118U001852.

The global prevalence of diabetes mellitus among the adult population reached a worldwide epidemic. A steady increase in the incidence of diabetes mellitus (DM)in all age and ethnic groups has been observed. About 5% of the world's population already suffers from this disease, and the number of patients, despite the active efforts of modern medicine, is increasing annually by 5-7% [1].

DM is accompanied by the lesion of almost all organs and systems. It should be noted that the main mechanism of the pathogenesis of all vascular diabetic complications is endothelial damage with endothelial dysfunction (ED). One of the most serious vascular complications of diabetes is diabetic kidney disease (DKD), which is one of the main causes of chronic kidney disease (CKD) with an outcome in chronic renal failure[2],[3].

DKD is an integral component of the cardiorenal continuum, which determines the relationship between the pathological processes of the cardiovascular system and the kidneys.The development of DKD is associated with structural and functional changes caused by metabolic and hemodynamic factors that can lead to irreversible life-threatening outcomes[3].

Different pathogenesis links are closely related due to hyperglycemia, so we cannot highlight any specific mechanism for the development of ED. Under condition of hyperglycemia, the following pathological processes are developed: the polyol pathway ofglucose metabolism, glycosylation of proteins with increased formation of glycation end products, hyperactivation of protein kinase C and renin-angiotensin-aldosterone

system,the oxidative stress, mitochondrial and endoplasmic reticulum stress, low-grade inflammation, desquamation of endotheliocytes, etc. [[4]-[6].

The increased endothelium desquamation intensity is regarded as the first stage of endothelial dysfunction [3],[7].Therefore, the determination of the plasma level of circulating endothelial cells (CECs), a recognized morphological marker of structural damage of the endothelium, makes it possible to reveal the presence and degree of endothelial damage both in the early stages of ED and in the development and progression of vascular complications, including DKD[8].

Aim of the study is todeterminethepossibilityofreductionofendothelialdamageduetothenormalizationofglycemiainpatientswithtype 1 (T1DM) and type 2 diabetes(T2DM) withnormal and depressed glomerular filtration rate(GFR)within 3 monthsoftreatment.

Dataanalysis.Statistical analysis and the visualization of the obtained data carried out using Plotus software [9]. Data were presented as mean (M) and the standard error of mean (SEM) in comparison of group means. Levene's test was used to assess the homogeneity of variances; the analysis of normal distribution was performed using the D'agostino-Pearson test. The relationships and the correlations between the studied parameters were determined using Pearson's rank correlation coefficient (r). The difference between the groups was determined using one-way analysis of variances (ANOVA). The difference was considered statistically significant at $p < 0.05$.

II. Methodology

50 patientswithT1DMand T2DM, whomettheeligibilitycriteriaandagreedtoenrollintothestudy, wereinvolved. Patientswerehospitalizedin ME 'OdessaRegionalClinicalMedicalCenter'intheperiodfromAprilto December 2018.

The study included an assessment of vascular endothelium state and renal function parameters in examined patients.Patients with T1DM received combination of insulin glargineIlar, a long acting human insulin analogue,and short-acting insulin as hypoglycemic therapy. For type 2 Patients with type 2 DM received metformin, glimepiride ± voglibose in combination with insulin glargine. The correction of a concomitant pathology and progression rates of micro- and macrovascular diabetic complications were carried out with the agreed decision of the physician and the patient and in accordance with the instructions of the drugs' application.

Diabetes has been diagnosed within at least a year since the initial diagnosis. DKD was exposed on the basis of either of the following criteria: abnormalities of kidneystructure or function presented for > 3 months[[9]]:

- Urine sediment abnormalities, albuminuria or albumin-to-creatinine ratio (ACR) ≥ 30 mg/g
- Electrolyte and other abnormalities due to tubular disorders
- Abnormalitiesdetectedbyhistology
- Structuralabnormalitiesdetectedbyimaging
- Historyofkidneytransplantation
- Decreased GFR < 60 ml/min/1.73 m^2

The National Kidney Foundation guidelines recommend using the CKD-EPI Creatinine Equation (2009) to estimate GFR as thebestoverallindexofkidneyfunction[9]. EndothelialdesquamationintensitywasdeterminedusingHladovec J. methodwith the estimation of both the total number of CECs and the determination of CECs at different stages of decay in blood plasma[11]. DependingonthemorphologicalchangesofCECs, 3 stagesofdecayareconditionallydistinguished: theinitial (S1), thestageoftheexpressedchanges (S2), andthefinalstage (S3) [10].

III. ResultsAndDiscussion

Amongdiabeticpatients, 25 men (50 %) and 25 women (50%)wereincludedintothestudy. Theaverageageofthepatientswas 56.32 ± 12.4 years. DurationofDM wasonanaverageof10.3 ± 7.3 years. T1DM patients(n = 11) sufferedfromdiabetesonanaverageof 6.81 ± 5.11 years; T2DM patients(n = 39) – 11.28 ± 7.6 years, respectively.

Theinitiallevelofglycosylatedhemoglobin (HbA_{1c}) was 8.42 ± 1.2 %. Theinitiallevelofalbuminuriawasonanaverageof-0.06 ± 0.1 g/ l, serumcreatinineconcentration– 91.46 ± 4.3 μmol/l withhighervalueingroupof T2DM patients. Concentrationofcreatinineexceededtheupperlimitofthereferencevalueby 28 % (n = 14). Themeanvalueof GFR was 73.17 ± 18.7 ml/min/1.73 m^2.

Patientsweredividedinto4groups: group1– patientswithnormal GFR (n = 7); mean GFR was 104.68 ± 10.27 ml/min/1.73 m^2. 2ndgroup –GFR tendstodecline(n = 31)andthemean GFR was75 ± 8.9 ml/min/1.73 m^2.The3rd groupconsistedofpatientswith DKD stage 3 (n = 9); meanlevelofGFR was 53.79 ± 4 ml/min/1.73 m^2.Group 4 includedpatientswithDKD stage 4 (n = 3) withaveragemeanof 38.83 ± 5.6 ml/min/1.73 m^2.

Correlationanalysisrevealedsignificantrelationshipsbetweenrenalfunctionparameters. Thehighestvaluesofthelinearcorrelationcoefficientswereobservedinthefollowingpairs: ureaandcreatinineconcentrations (r = 0.6; p < 0.001), GFR andureaconcentration (r = -0.57; p< 0.001), GFR andserumcreatinineconcentration (r = -0.76; p < 0.001).

Afterthehospitaltreatment, theaveragefastingplasmaglucosedecreasedfrom 13,72 ± 0,5 to 6,53 ± 0,2 mmol/l (p < 0.001). Thepercentage ofpatientswithplasmaglucoseupto ≤ 7.0 mmol/l increasedto 84 %. The postprandial glycemia level decreased from 14.62 ± 1.1 to 6.67 ± 0.3 mmol/l (p< 0.001). The average level of fructosamine in the period of hospital treatment decreased by0.75 mmol/l, with a statistically significant difference of 16.7 % (p < 0.001).

Attheendofthestudy, themeanlevelof HbA_{1c}decreasedfrom 8.42 ± 0.2 % to 7.36 ± 0.3 %, with a differenceof -1.05 ± 0.2 % (p< 0.001). 3-month courseofinsulinglargineincombinationwithshort-actinginsulininpatientswith T1DM andtheadministrationoforalhypoglycemictherapyincombinationwithinsulinglarginein T2DM hasbeenassociatedwithclinicallysignificantimprovementinglycemiccontrol.

Table 1: The functional state of the kidneys during the study period

Indicators	**main group** n = 50	**T1DM** n = 11	**T2DM** n = 39
Initialserum creatinineconcentration, µmol /l	91.46 ± 4.3	81.81 ± 4.9	94.17 ± 3.8
Initial GFR, ml/min/1.73 m^2	73.17 ± 7.1	89.85 ± 6.6*	68.46 ± 2.7*
Initial level of albumin in urine, g/l	0.06 ± 0.01	0.03 ± 0.01	0.07 ± 0.01
Serum creatinineconcentration after hospital treatment, µmol /l	86.16 ± 4.4	77.72 ± 5.1	88.53 ± 2.7
GFR after hospital treatment, ml/min/1.73 m^2	77.52 ± 8.1	95.02 ± 5.3*	72.58 ± 2.4*
Finalserum creatinineconcentration, µmol /l	85.78 ± 4.5	81.09 ± 3	87.1 ± 3.7
Final GFR, ml/min/1,73 m^2	78.58 ± 7.3	89.89 ± 3.9	75.39 ± 3.4
Final level of albumin in urine, g/l	0.04 ± 0.007	0.02 ± 0.009	0.04 ± 0.009
Final creatinineconcentration in urine, µmol /l	7.53 ± 0.7	8.27 ± 2	7.32 ± 0.8
ACR, mg/mmol	*10.9 ± 2.69*	*9.54 ± 7.7*	*11.28 ± 2.7*

*Notes: *p ≤ 0,05 difference between indicators of T1DM and T2DM groups; data are presented as M ± SEM.*

Dependingonthetypeofdiabetes,GFR levelsincreasedafterhospitaltreatment(p < 0.001). The level of GFR at different stages of the study isshowninFigure 1.

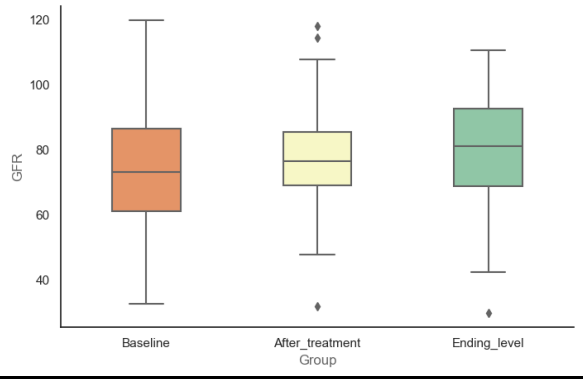

Figure 1: Comparativecharacteristicsof GFR levels amongpatientswithdiabetesduringthestudyperiod.
Notes: data are presented asmedians and interquartile ranges.

Theanalysisofbaselineindicatorsofkidneys' functionalstatebeforetreatment, andtheindicatorsafterhospitaltreatmentandonthefinalvisit, hasshownthatprescribedtreatmentofpatientswith T1DM and T2DM couldpreventorslowdowntheprogressionofkidneydiseaseindiabeticpatients.

Theexpressivenessofendotheliocytemiawasrevealedinthebloodofallpatients. TheconcentrationofCECsvariedfrom 1200 to 4600 cells/ml. ThemeanlevelofCECsinpatientswith DM was 2530

± 694 cells/ml.ThenumberofCECswasestablishedtobe738 ± 67.8 cells/ml, whichis29,1 % lowerafter 3-months oftreatment(p < 0,001).Thereasonforthedecreasedendotheliumdesquamationintensityisprobablyduetonormalizationofglycemiccontrolandcomplextherapyofexistingcomplicationsof DM.

Thelevelofendotheliocytemiawasanalyzedaccordingtothe GFR ofdiabeticpatients. Thedynamicsofthedegreeofendothelialdesquamationandthedistributionof CECsfor GFR aregiveninTable. 2

Table 2: Levels of endothelial desquamation during the study in dependence of GFR

Indicators	**Stagesofthestudy**	**GFR ≥ 90 мl/min/1.73² m²**	**GFR 60-89 ml/min/1.73 m²**	**GFR 59-45 ml/min/1.73m²**	**GFR 35-44 ml/min/1.73m²**
		n = 7	n = 30	n = 9	n = 3
GenerallevelofCECs, cells/ml	Baseline stage	2828.57 ± 2000	2366.66 ± 346.9	2711.11 ± 394.49	2825 ± 523.8
	Stage after hospital treatment	1900 ± 450	1733.33 ± 199.8	1844.44 ± 119	1925 ± 321.4
	Output stage	1771.42 ± 180	1635.48 ± 76.2	1844.44 ± 133.5	1866.66 ± 150
S1,cells/ml	Baseline stage	400 ± 100	310 ± 89.7	322.22 ± 125.8	350 ± 88.1
	Stage after hospital treatment	214.28 ± 50	243.3 ± 47.1	266.66 ± 100	200 ± 100
	Output stage	214.28 ± 44.7	183.87 ± 21.1	155.55 ± 36.8	300 ± 8150
S2, cells/ml	Baseline stage	2014,28 ± 200	1700 ± 246.3	1866.66 ± 150	1875 ± 392.9
	Stage after hospital treatment	1442.85 ± 500	1220 ± 158.1	1322.22 ± 85.3	1200 ± 145.2
	Output stage	1285.71 ± 168.5	1135.48 ± 72.7	1300 ± 110	1133.3 ± 250
S3, cells/ml	Baseline stage	428.57 ± 100	356.66 ± 40.8	522.22 ± 179.6	600 ± 66.6
	Stage after hospital treatment	242.8 ± 100	273.33 ± 37.2	255.55 ± 75	525 ± 176.3
	Output stage	271.42 ± 50.9	270.42 ± 26.2	388.88 ± 57.14	433.33 ± 100

Notes:data are presented as M ± SEM

Thedecreaseinthelevelofendothelialdesquamationwasobservedinallgroupsofpatientswithdiabetes, regardlessofthefunctionalstateofthekidneys, which is showninFigure 2.

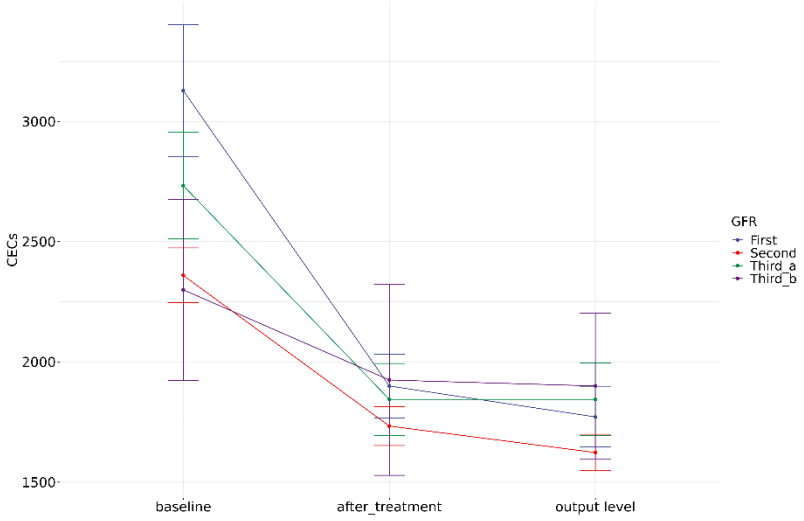

Figure2: Dynamicsofthelevelofendotheliocytemiain 3 monthsperiodindiabeticpatientswithdifferentinitialGFR.

Laboratorydatacharacterizingendothelialdysfunctiondidnotdiffersignificantlybetweengroupsofpatientswithdifferent GFR. Despitedifferentinitialnephropathicchangesinthesepatients, statisticallysignificantdecreaseinthelevelofendothelialdesquamationwasobservedinallgroupsofpatientswithdiabetes (p < 0.001).

Inconclusion, thekeyroleinthevascularwalllesionspathogenesisinpatientswith DM belongstohyperglycemia and to the hypoinsulinemia. The correction of this disturbancesleadstotheimprovementof the structural and functional state of the kidneys and decrease of the degree of endothelial desquamation.

IV. Conclusion

1. Improvementofglycemiccontrolandcomplextherapyofexistingcomplicationsofdiabetesleadsto a significantreductioninendothelialdamageinpatientswith DM, and, therefore,contributestotheprotectionofbloodvessels ($p < 0.001$).
2. A persistentdecreaseinendothelialdesquamationdue totreatmentisobservedinalldiabeticpatients, regardlessoftheinitial GFR.
3. Theanalysisofthefunctionalstateofthekidneysforperiodof 3 monthsindicatesthatitispossibletopreventorslowdowntheprogressionofdiabetickidneydiseaseusingtheprescribedtreatmentscheme.
4. Determinationofthedegreeoftheendothelialdamageisaninformativeresearchthatallows, byanalyzingboththegenerallevelofCECsandthelevelofendothelialcellsatdifferentstagesofdecay, topredicttheformationandprogressionofvascularcomplicationsofdiabetes, timelydetectandcorrectprescribedtherapyandpreventfurtherdevelopmentofcardiovascularcomplications.
5. Basedonourfindings, itisproposedtodeterminetheconcentrationofCECsasanindependentmarkerof ED inpatientswithT1DM andT2DMindynamics.

PROSPECTS FOR FURTHER RESEARCH

The study results indicate the necessity of effective measures for endothelial dysfunction correction development in patients with DM

Acknowledgements

This research has been supported by JSC 'Farmak'

Abbreviations:ACR: albumin-to-creatinine ratio,CECs: circulating endothelial cells, DKD: diabetic kidney disease, DM: diabetes mellitus, ED:endothelial dysfunction, GFR: glomerular filtration rate, M: mean, S1: theinitial stage of endothelial cell decay, S2: thestageoftheexpressedchanges of endothelial cell decay S3: finalstage of endothelial cell decay, SD: standard deviations, SEM: standard error of mean, T1DM: Type-1 diabetes mellitus, T2DM: Type-2 diabetes mellitus

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GozhenkoAnatoliy. "Dynamics of Endothelial Desquamation in Patients with Diabetic Kidney Disease." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 8, 2019, pp 16-20.