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Blood selectin levels as a predictive factor for diabetic retinopathy and diabetic macular edema in type 2 diabetes

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Background: *Diabetes mellitus (DM) is still a noninfectious global pandemic. Diabetic retinopathy (DR) is one of the most common and socially significant complications of both type 1 and type 2 diabetes. Predicting the probability of DR progression and potential diabetic macular edema (DME) development is still important. Currently available predictive models include a wide number of predictors, and such predictors as blood selectin levels seem to be promising.*
Purpose: *To determine relationships between blood levels of selectins and DR progression and DME development in patients with T2DM, and to develop particular predictive models.*
Material and Methods: *Of the 124 patients (124 eyes) involved into this study, 95 (95 eyes) had T2DM and retinopathy (group 1 of 29 eyes with mild non-proliferative DR (NPDR); group 2 of 35 eyes with moderate or severe non-proliferative NPDR; and group 3 of 31 eyes with proliferative DR (PDR)), and 29 (29 eyes) had no diabetes (controls). Patients underwent a routine eye examination and spectral domain optical coherence tomography (SD-OCT) to determine central retinal thickness (CRT). The presence of DME was based on an increased macular thickness in the Early Treatment Diabetic Retinopathy Study (ETDRS) subfields compared to the upper limit of normal for patient's age and gender. Enzyme-linked immunosorbent assay (ELISA) kits from Invitrogen Thermo Fisher Scientific (USA) were used to determine selectin levels in blood.*
Results: *There was a statistically significant tendency for an increase in progression of DR to be accompanied by an increase in blood selectin levels. In the presence of DME, blood LS levels were statistically significantly increased in mild NPDR; blood PS levels, in PDR; and blood ES levels, in all stages of DR. A regression model for predicting the progression of DR was built. In the model developed, diabetes duration and blood selectin levels were positively correlated, while HbA1c level was inversely correlated with DR progression. The model was found to be adequate R2adjust = 0.84; F = 97.9, p < 0.001), demonstrating high correlation of the selected independent variables with the stage of DR. The calculated Y index may be considered a quantitative reflection of the severity of DR. A patient is predicted to have mild NPDR (with a prediction accuracy of 86.2%) if Y < 1.5, moderate of severe NPDR (with a prediction accuracy of 88.6%) if 1.5 ≤ Y < 2.35, and PDR (with a prediction accuracy of 100%) if Y ≥ 2.35. A regression model for predicting the development of DME was built, with CRT and blood ES level found to increase the risk of the development of DME. A high AUC value for the ROC curve (AUC = 0.97; 95% CI, 0.92-0.99) indicated a very strong correlation of the risk of DME development with the levels of selected independent variables. In addition, sensitivity and specificity values at the optimal cut-off point were 93.2% (95% CI, 83.5%-98.1%) and 92.3% (95% CI, 83.0%-97.5%), respectively.*
Conclusion*: Our findings confirmed the current view that high blood selectin levels are important in the presence of T2DM and related to major pathogenetic mechanisms of microvascular complications, and blood selectin levels can be considered predictors of the progression of DR as well as the development of DME.*

Keywords:
diabetic retinopathy, diabetic macular edema, type 2 diabetes mellitus, soluble selectins, regression predictive model

Introduction

Diabetes mellitus (DM) is still the largest noninfectious global pandemic [1-3]. Diabetic retinopathy (DR) is one of the most common and socially significant complications of both type 1 and type 2 diabetes. Among individuals with diabetes, global prevalence was 22.27% (95% confidence interval [CI], 19.73%-25.03%) for DR and 4.07% (95% CI, 3.42%-4.82%) for clinically significant macular edema (CSME). In 2020, the number of adults worldwide with DR and CSME was estimated to be 103.12 million and 18.83 million, respectively. DR and diabetic macular edema (DME) are common ocular complications of DM and the leading causes of severe vision loss and blindness in adults of working age [1, 3]. Predicting the probability of DR progression and potential diabetic macular edema (DME) development is still important [2].

Several models with easily measurable predictors are available for predicting the development of DR in type 2

diabetes mellitus (T2DM); using these models improves the medical efficacy of treatment and reduces the financial burden of the patients [4]. Poor glycemic control, systemic hypertension, diabetes duration, dyslipidemia, microalbuminuria and increased aortic stiffness have been confirmed as risk factors for the development and progression of DR.

In addition to general demographic factors, clinical and biochemical factors, the important value of anti-inflammatory, angiogenic and growth factors, as well as oxidative stress markers, has been established. It has been demonstrated that platelet-derived growth factor (PDGF), tumor necrosis factor alpha (TNFα), and Endothelin-1 (ET1) are involved in the development of DR and diabetic maculopathy (DMP) and are associated with outcomes of surgical treatment of these disorders [5].

Furthermore, it has been established that the inflammatory and prothrombogenic changes affecting the blood retinal barrier (BRB) are of key value in DR. The key inflammatory events involved in BRB alteration appear to be: (1) Increased expression of endothelial adhesion molecules (e.g., selectins), (2) adhesion of leukocytes to the endothelium, (3) release of inflammatory chemokines, cytokines, and vascular permeability factors, (4) alteration of adherens and tight junctional proteins between the endothelial cells, and (5) infiltration of leukocytes into the neuro-retina [6].

Selectins are a family of adhesion molecules comprised of L-, E-, and P-selectin (LS, ES and PS, respectively). They are important factors of vascular regulation and can mediate leukocyte rolling on the endothelial surface [7]. Soluble LS (sCD62L) is a glycoprotein expressed on lymphocytes, neutrophils, monocytes and other myeloid cells, and mediates their rolling along the vascular wall [8]. In addition, LS promotes transendothelial migration of neutrophils through TNF-activated endothelial monolayers [8]. Serum level of sL-selectin was found to be decreased in patients with T2DM. Serum level of sL-selectin did not significantly vary between diabetic groups. In patients with diabetic microangiopathy and macroangiopathy leukocyte expression of L-selectin was significantly lower in comparison with the healthy control and patients without vascular complications [9].

The soluble E-selectin level was significantly higher in patients with T2DM compared to controls [10]. When Kasza et al [10] examined diabetic patients by the severity of retinopathy, they did not find any significant difference in soluble ES levels. On the other hand, increased blood levels of ES and other vascular factors in patients with DR allowed demonstrating a role of endothelial dysfunction in the development of DR, whereas a gradual increase in blood ES levels with an increase in the stage of DR demonstrated the role of ES in DR progression [11].

Soluble PS (CD62, PADGEM, GMP-140) is a glycoprotein located in the dense storage granules of platelets and in the Weibel-Palade body storage granules of the endothelial cells [12]. The prompt translocation of PS from the storage granules within platelets to the cell surface is induced by endothelial stimulation with inflammatory factors, and enables neutrophil recruitment through carbohydrate residues of leukocyte glycoproteins [13]. Plasma levels of PS increased with the progression of DR, which was associated with monocyte activation and retinal capillary occlusion [14].

Therefore, the role of selectins in the development of DR and DME in DM remains unclear, given contradictory data in the literature. In addition, our current understanding of the pathogenetic role of selectins as important factors of microvascular complications prompts the need to study their role in the prediction of progression of DR and DME.

The purpose of the study was to determine relationships between blood levels of selectins and DR progression and DME development in patients with T2DM, and to develop particular predictive models.

Material and Methods

One hundred and twenty-four patients (124 eyes) were involved into this prospective cohort case-control study. Of these, 95 (95 eyes) had type 2 diabetes and retinopathy, and 29 (29 eyes) had no diabetes (controls). Patient age ranged from 43 to 85 years (mean ± standard deviation, 66.8 ± 0.75 years). The control group consisted of 16 (55.2%) women and 13 (44.8%) men, and the DR group, of 65 (68.4%) women and 30 (31.6%) men, with no significant difference by the Fisher exact test in gender distribution between groups ($p = 0.164$).

The study followed the ethical standards stated in the Declaration of Helsinki, the European Convention on Human Rights and Biomedicine, relevant provisions of the WHO, Council for International Organizations of Medical Sciences, International Code of Medical Ethics (1983), as well as the Ministry of Health of Ukraine Order No. 690 dated 23.09.2009.

Informed consent was obtained from all participants.

Patients underwent an eye examination which included visual acuity assessment, static Humphrey perimetry, refractometry, intraocular pressure measurement, slit lamp biomicroscopy, gonioscopy, ophthalmoscopy with Volk Super Field lens and Goldmann three-mirror lens (Volk Optical, Mentor, OH) and fundus photography (the Early Treatment Diabetic Retinopathy Study (ETDRS) seven standard fields). In addition, they underwent spectral domain optical coherence tomography (SD-OCT; 3D OCT-1000 (Topcon Corporation, Tokyo, Japan); scan programs, Macula 3D and RetinaRaster). The presence of DME was based on (a) an increased macular thickness in the ETDRS subfields compared to the upper limit of normal for patient's age and gender and (b) the presence of intraretinal fluid on OCT scans. Central retinal thickness (CRT) was measured and expressed in millimeters.

DR severity was graded as per the 2002 guidelines of the American Academy of Ophthalmology. DME severity was graded as per the 2003 guidelines of the American Academy of Ophthalmology.

Patients were divided into three groups based on examination results and as per the International clinical DR

Severity Scale: group 1 of 29 patients with mild non-proliferative DR (NPDR); group 2 of 35 patients with moderate or severe non-proliferative NPDR; and group 3 of 31 patients with proliferative DR (PDR). The presence of DME was based on an increased macular thickness in the ETDRS subfields compared to the upper limit of normal for patient's age and gender as assessed by SD OCT scans, and marked by a color scale, with yellow and red colors used to denote a significance of $p<0.05$ and $p<0.01$, respectively.

Carbohydrate metabolism was assessed based on blood glucose and glycosylated hemoglobin A1c (HbA1c) levels.

Enzyme-linked immunosorbent assay (ELISA) kits from Invitrogen Thermo Fisher Scientific (USA) were used to determine selectin levels in blood.

Statistical analyses were conducted using MedStat and MedCalc v.15.1 (MedCalc Software bvba) software [15]. Odds ratio (OR) and 95% confidence interval (CI) values were computed to determine significant differences in regression analysis.

Results

Patients with DR (n = 95) had hyperglycemia (mean ± SD blood glucose level, 8.51 ± 0.82 mmol/l), increased blood HbA1c (7.36 ± 0.15%), and worse visual acuity (0.60 ± 0.03) and thicker CRT (318 ± 11.2 μm) compared to controls ($p < 0.001$ for all comparisons). This was confirmed by the presence of durable carbohydrate metabolism abnormalities, increased non-enzymatic protein glycosylation and specific diabetogenic damage to the retina.

The mean incidence of DME in total patients with DR was 62.1%, while in groups 1, 2 and 3, it was 48.3%, 77.1% and 58.1%, respectively, but no significant difference in DME incidence between the groups was detected ($p > 0.2$).

The blood levels of LS in groups 1, 2 and 3 were 2.0 times, 2.3 times and 3.2 times, respectively, and significantly higher than that in controls ($p < 0.05$ for all comparisons). Blood LS levels were significantly higher in the presence than in the absence of DME for DR group 1 ($p = 0.006$; a 1.4-times increase in blood level of LS), but not for DR groups 2 and 3 ($p > 0.1$ for both comparisons). Therefore, it could be hypothesized that increased blood LS levels may be important for the development of DME only in early DR stages.

The blood level of ES increased definitely and significantly with an increase in the severity of DR ($p < 0.05$ for all comparisons). Blood ES levels were significantly higher in the presence than in the absence of DME for each of the DR groups ($p < 0.01$; a 1.2 to 1.3-times increase in blood level of ES).

The blood level of PS was significantly higher in each of the DR groups than in controls, with a 2.3-times increase for group 3 ($p < 0.001$). Blood PS levels were significantly higher in the presence than in the absence of DME for DR group 3 ($p < 0.001$; a 1.2-times increase in blood level of PS), but not for DR groups 2 and 3 ($p > 0.05$ for both comparisons).

Therefore, there was a significant tendency for an increase in progression of DR to be accompanied by an increase in blood selectin levels. In addition, blood selectin levels were generally (but not universally) increased in the presence of DME. Thus, blood LS levels were statistically significantly increased in mild NPDR; blood PS levels, in PDR; and blood ES levels, in all stages of DR.

A method for the development and analysis of multivariate linear regression models [15] was applied to identify the relationships of the independent variables with the stage of DR. Age, diabetes duration, glucose level, HbA1c

Table 1. Selectin levels in the serum of patients of groups 1, 2 and 3 in the presence versus in the absence of diabetic macular edema (DME) (mean values ± standard deviation)

Marker	Presence of DME	Controls and diabetic retinopathy groups, ng/ml			
		Controls	1-a	2-a	3-я
LS	Both	12.5±3.5	24.7±6.4*	29.3±5.4*	39.5±6.5*
	DME-	-	21.9±5.7	31.1±5.8	39.9±6.7
	DME+		31.2±3.8	38.6±10.9	44.4±6.6
$p_{DME- vs DME+}$		-	0.006	0.146	0.392
ES	Both	26.3±5.5	34.9±5.4*	45.9±8.8*	52.4±8.4*
	DME-	-	31.2±3.8	38.6±10.9	44.4±6.6
	DME+		38.7±3.9	48.1±7.0	55.9±5.8
$p_{DME- vs DME+}$		-	<0.001	0.003	<0.001
PS	Both	59.9±7.5	68.5±9.7*	72.3±7.6*	134.9±17.0*
	DME-	-	65.8±10.0	75.1±7.6	121.2±13.1
	DME+		71.4±8.7	71.5±7.6	141.5±14.3
$p_{DME- vs DME+}$		-	0.062	0.122	<0.001

level, visual acuity, CRT, and blood LS, ES and PS levels were used as independent variables, and the stage of DR, as a dependent variable.

A stepwise approach was used to select and retain variables, using the forward selection criterion of $p < 0.1$, and backward selection criterion of $p > 0.2$. Five risk factors (diabetes duration, HbA1c level, and blood LS, ES and PS levels) were selected based on this approach. The model built on the selected variables was found to be adequate, and the adjusted coefficient of determination (R2adjust) was 0.84 (Fisher test: $F = 97.9$, $p < 0.001$), demonstrating high correlation of the selected independent variables with the stage of DR.

The coefficients of the model developed are presented in Table 2. A low value of the variance inflationary factor (VIF < 3 for all the coefficients) indicates the absence of an interrelationship among the selected variables (multicollinearity) and, consequently, the presence of independent contributions of each of the selected covariates to the prognosis of the stage of DR. In the model developed, diabetes duration and blood LS, ES and PS levels were positively correlated, while HbA1c level was negatively correlated with DR progression.

The obtained relationship can be expressed by the following equation:

$$Y = 0.58 + 0{,}022\times DD - 0.21 \times HbA1c + 0.019\times LS + 0{,}022\times ES + 0.015\times PS \quad (1),$$

where Y is DR severity index;

DD is diabetes duration expressed in years;

HbA1c is blood glycosylated hemoglobin level expressed as a percentage;

and LS, ES, and PS are blood levels of L-selectin, E-selectin, and P-selectin, respectively, expressed in ng/ml.

The calculated Y index may be considered a quantitative reflection of the severity of DR. Figure 1 shows Y index values calculated for each of the patients in groups.

With the patient's Y value calculated as per formula (1), a patient is predicted to have mild NPDR if $Y < 1.5$, moderate of severe NPDR if $1.5 \le Y < 2.35$, and PDR if $Y \ge 2.35$. The accuracy of predicting mild NPDR was 86.2%, moderate of severe NPDR, 88.6%, and PDR, 100%.

A method for the development and analysis of univariate and multivariate linear regression models [15] was applied to identify the relationships of the independent variables with the risk of DME development. Age, gender, diabetes duration, blood glucose level, HbA1c level, best-corrected visual acuity (BCVA), CRT, and blood LS, ES and PS levels were used as independent variables, and the risk of DME development, as a dependent variable (Table 3).

The risk of DME development increased with an increase in diabetes duration, CRT, and blood selectin levels. The risk of DR progression was inversely associated with an increase in age and BCVA. Gender and blood glucose level had no significant impact on the risk of DME development. Based on the receiver operating characteristic (ROC) analysis, the risk of DME development correlated most closely with CRT (area under curve (AUC) = 0.89; 95% CI, 0.82-0.94) and ES (AUC = 0.89; 95% CI, 0.83-0.94).

Figure 2 shows the ROC curve for predicting the risk of DME development in DR patients based on CRT values. The AUC for this curve was 0.89 (95% CI, 0.82-0.94), and sensitivity and specificity at optimal cut-off point were 98.3% (95% CI, 90.9%-100%) and 73.9% (95% CI, 61.5%-84.0%), respectively.

Figure 3 shows the ROC curve for predicting the risk of DME development in DR patients based on ES values. The AUC for this curve was 0.89 (95% CI, 0.83-0.94), and

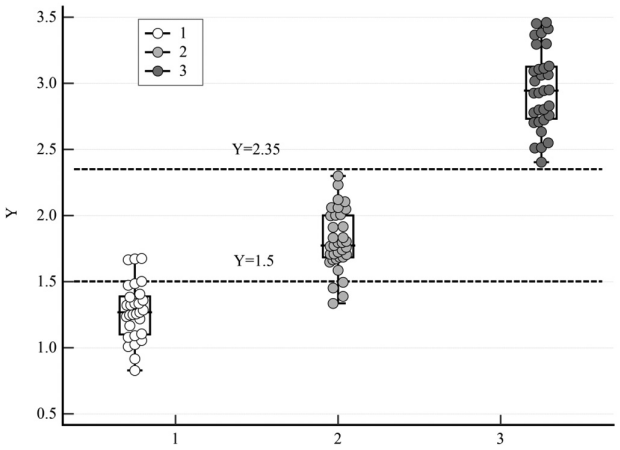

Fig. 1. Values of the calculated Y index for each of the patients. Note: 1, group 1; 2, group 2, 3, group 3.

Table 2. Coefficients of the multivariate linear regression model for predicting progression in the stage of DR

Chatacteristic	Value of model coefficient, b±m	Significance of difference	Partial correlation coefficient	Variance inflationary factor (VIF)
Const	0.58	-	-	-
Diabetes duration	0.022±0.006	0.001	0.354	1.2
HbA1c	-0.21±0.03	<0.001	-0.631	1.3
LS	0.019±0.006	0.003	0.313	2.4
ES	0.022±0.004	<0.001	0.477	1.7
PS	0.015±0.002	<0.001	0.699	2.4

sensitivity and specificity at optimal ES cut-off point (37.8 ng/ml) were 89.8% (95% CI, 72.9%-96.2%) and 80.0% (95% CI, 68.2%-88.9%), respectively.

A stepwise approach was used to select and retain variables for a multivariate model, using the forward selection criterion of $p < 0.1$, and backward selection criterion of $p > 0.2$. Four risk factors (diabetes duration, CRT, and blood ES and PS levels) were selected based on this approach. The model built on the selected variables was found to be adequate ($\chi 2=108.5$ for 4 degrees of freedom; $p < 0.001$).

The results of multivariate analysis for predicting the risk of DME development are presented in Table 4. The risk of DME development increased with an increase in CRT and blood ES level, and was inversely associated with diabetes duration and blood PS level.

Figure 4 shows the ROC curve for predicting the risk of DME development in DR patients based on the four-variate regression model. A high AUC value for this curve (AUC = 0.97; 95% CI, 0.92-0.99) indicated a very strong correlation of the risk of DME development with the levels of selected independent variables. In addition, sensitivity and specificity values at the optimal cut-off point were 93.2% (95% CI, 83.5%-98.1%) and 92.3% (95% CI, 83.0%-97.5%), respectively.

Table 3. Coefficients of the univariate linear regression model for predicting DME

Independent variable		Value of model coefficient, b±m	Significance of difference	OR (95% CI)	AUC (95% CI)
Sex	Fem	Reference			0,56 (0,46-0,65)
	Masc	–0.50±0.38	0.193	–	
Age		–0.055±0.023	0.017	0.95 (0.90-0.99)	0.63 (0.54-0.71)
Diabetes duration		0.04±0.031	0.007	1.09 (1.02-1.15)	0.70 (0.61-0.78)
Glucose		–0.003±0.020	0.881	–	0.66 (0.57-0.75)
HbA1c		0.56±0.14	<0.001	1.75 (1.33-2.31)	0.71 (0.63-0.79)
BCVA		–3.87±0.69	<0.001	0.02 (0.01-0.08)	0.83 (0.75-0.89)
CRT		0.023±0.004	<0.001	1.02 (1.02-1.03)	0.89 (0.82-0.94)
LS		0.089±0.020	<0.001	1.09 (1.05-1.14)	0.74 (0.66-0.82)
ES		0.17±0.03	<0.001	1.19 (1.12-1.26)	0.89 (0.83-0.94)
PS		0.020±0.007	0.003	1.02 (1.01-1.03)	0.70 (0.61-0.78)

Note: OR, odds ratio; CI, confidence interval; AUC, area under curve

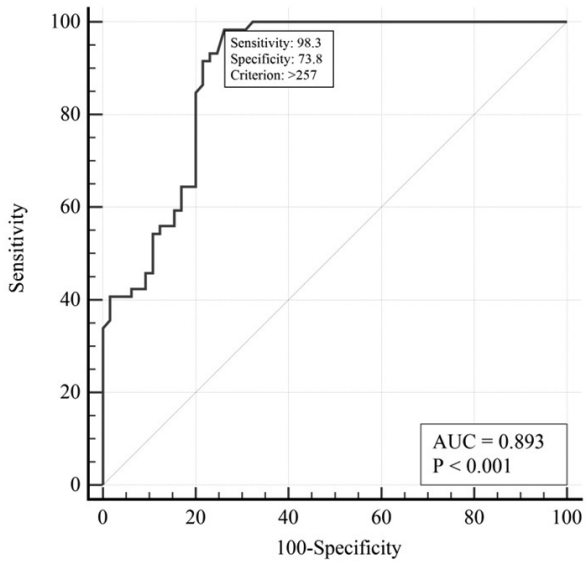

Fig. 2. Receiver operating characteristic (ROC) curve for predicting the risk of diabetic macular edema (DME) based on the central retinal thickness (CRT)

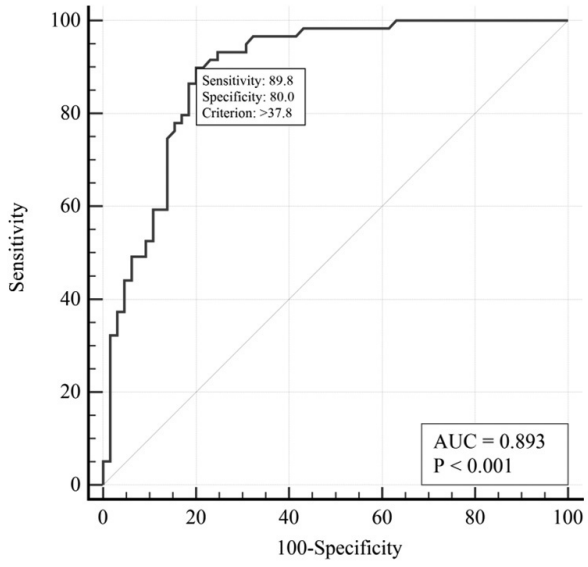

Fig. 3. Receiver operating characteristic (ROC) curve for predicting the risk of diabetic macular edema (DME) based on the blood E-selectin (ES) level

Table 4. Coefficients of the four-variate linear regression model for predicting DME

Variate	Value of model coefficient, b±m	Significance of difference	OR (95% CI)
Diabetes duration	–0.16±0.07	0.022	0.85 (0.74 – 0.98)
CRT	0.048±0.013	<0.001	1.05 (1.02 – 1.08)
ES	0.25±0.06	<0.001	1.29 (1.1 – 1.43)
PS	–0.09±0.024	<0.001	0.91 (0.87 – 0.95)

Note: OR, odds ratio; CI, confidence interval

Discussion

We found blood LS levels to be significantly increased in all patients with DR. In addition, blood LS levels were significantly higher in the presence than in the absence of DME for DR group 1, but not for DR groups 2 and 3. Karadayi and colleagues [16] observed significantly higher serum concentrations of sL-selectin in Type 2 diabetic patients with retinopathy than in healthy subjects (36.5 ± 18.1 vs. 11.4 ± 7.5 ng/ml, $p < 0.001$). They also found a significant difference between diabetic patients with DR and those without DR (36.5 ± 18.1 vs. 24.2 ± 13.5 ng/ml, $p < 0.05$). Thus, their findings are in agreement with ours and confirmed the value of LS in DR. This was confirmed in our regression analysis, with an increased blood LS level resulting in an increased risk of DR as well as DME (OR = 1.09; 95% CI, 1.05-1.14). These high blood LS levels reflected the involvement of polymorphonuclear neutrophils which play an important role in the pathogenesis of diabetic vascular complications [16].

In the current study, blood ES levels were found to be substantially and progressively increased in patients with DR. Similar findings (a substantial increase in blood ES levels with an increase in the stage of DR, with the largest increase in blood ES levels seen in PDR) have been reported in studies by other researchers [10, 17]. An increase in blood ES levels was closely and directly associated with the progression DR and development of DME. The latter was confirmed by our univariate and multivariate analyses, with blood ES level having a larger effect on the risk of the development of DME (OR =1.19) than blood levels of other selectins in the univariate. In this prognostic model, the sensitivity and specificity at the optimal ES cut-off point (37.8 ng/ml) were satisfactory (89.8% and 80.0%, respectively).

An important role of ES as a biomarker of vascular damage was confirmed by the Treatment Options for type 2 Diabetes in Adolescent and Youth study [18]. They found that, over 1 to 3 years, for every 1% increase in HbA1c, ES increased by 6.8% ($P < 0.0001$). In addition, ES increased by 3.7% and 4.2% for every 10 mm Hg increase in systolic and diastolic blood pressure, respectively (both $P < 0.0001$). Moreover, ES was 15.5% higher in participants with microalbuminuria ($P < 0.01$).

Therefore, no doubt there is a close association of the progression of DR and development of DME with a high blood ES level, an informative prognostic biomarker of vascular complications in T2DM.

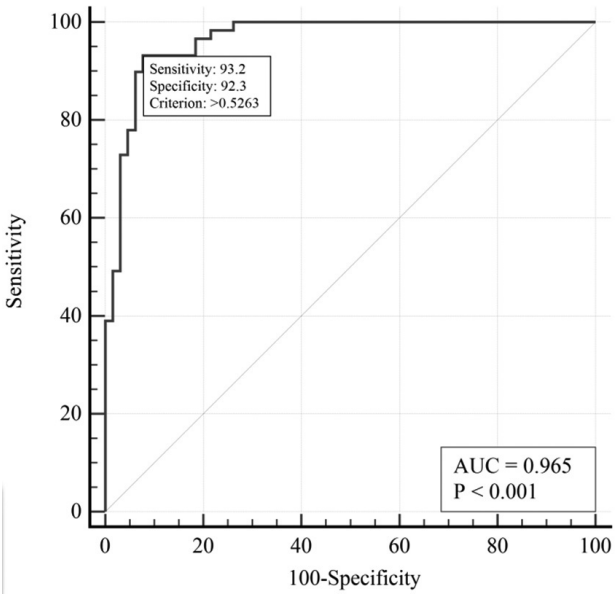

Fig. 4. Receiver operating characteristic (ROC) curve for predicting the risk of diabetic macular edema (DME) in a four-variate model

With regard to the discussion on the mechanisms of the association of an increased blood ES level with DR, it may be noted that serum obtained from T2DM patients with DR induced a significantly higher expression of ES than serum from non-diabetic healthy individuals, in association with an enhanced production of reactive oxygen species in in vitro-grown human coronary artery endothelial cells [19]. Given the established association of ES with protein glycosylation, it may be hypothesized that diabetic metabolic factors are capable of increasing endothelial ES expression.

In the current study, we found substantially increased blood PS levels in patients with PDR. Other researchers also reported on the association of PS with platelet activation and the development of vasculopathies and complications in T2DM [20].

We found that blood PS level has a value as an independent predictor of the progression of DR and the development of DME. Penman and colleagues [21] reported that, among African Americans with T2DM, in multivariate models adjusted for age, sex, and other traditional risk factors, higher PS levels were associated with any DR (OR

= 1.11, 95% CI = 1.02-1.21, p = 0.02) and proliferative DR (OR = 1.23, 95% CI = 1.03-1.46, p = 0.02). This is in agreement with our finding that there is (1) an association of high blood PS levels with any DR as well as (2) a substantially increased risk of PDR in patients with significantly elevated blood PS levels. We agree with the opinion of Penman and colleagues [21] in that this association may provide insight into the pathogenesis of retinopathy.

Wang and colleagues [22] aimed to develop a DR hazard nomogram for a Chinese population of patients with T2DM and used the least absolute shrinkage and selection operator (LASSO) to identify the following eight predictive variables: disease duration, body mass index, fasting blood glucose, HbA1c level, homeostatic model assessment-insulin resistance (HOMA-IR), triglyceride, total cholesterol, and vitamin D. We found that longer diabetes duration was the most important clinical characteristic for the progression of DR (p = 0.001). HbA1c level had a negative beta coefficient (-0.214 ± 0.006), which likely reflected the feature of this cohort of patients – they had compensated T2DM, were regularly seen by an endocrinologist for diabetes management, and were treated with hypoglycemic medications. However, such factors glucosemia, age and male gender were noted in the predictive models of DR progression described by others, but were not included as independent predictors in our model (p>0.2).

While not denying the importance of general clinical prognostic factors, we argue that the use of the biomarkers contributing to the pathogenesis of vascular complications is not less efficient. Thus, our multivariate linear regression model for predicting the risk of a particular stage of DR included not only the duration of diabetes and blood HbA1c level but also blood selectin levels, and showed a high predictive ability: the accuracy of predicting mild NPDR was 86.2%, moderate of severe NPDR, 88.6%, and PDR, 100%.

Our study demonstrated the importance of longer diabetes duration, CRT and blood HbA1c and selectin levels in predicting the risk of DME. The risk of DME development was inversely associated with age and visual acuity. Gender and blood glucose level had no significant effect on the risk of DME development. In addition, based on the ROC analysis, the risk of DME development correlated most closely with CRT (AUC = 0.89; 95% CI, 0.82-0.94) and ES (AUC = 0.89; 95% CI, 0.83-0.94). An important role of selectins in the development of DME and the potential for their use as prognostic biomarkers is an interesting finding of the current study. This seems very promising since our multivariate linear regression model built for predicting the risk of DME development had high performance characteristics (AUC = 0.97; sensitivity, 93.2%; and specificity, 92.3%).

Given that (a) current methods of diagnosing and predicting the outcomes of treatment for DME include mostly ophthalmological diagnostic procedures (first and foremost, optical coherence tomography) and (b) some selectins are directly related to the mechanisms of DME, incorporating these selectins in the diagnostic panel might improve the efficacy of the prediction, whereas blood selectin levels might be considered as a criterion of efficacy of treatment for DME.

Therefore, our findings confirmed the current view that high blood selectin levels are important in the presence of T2DM [24] and related to major pathogenetic mechanisms of microvascular complications, and blood selectin levels can be considered predictors of the progression of DR as well as the development of DME.

Conclusion

First, a relationship was established between blood selectin levels and the progression in DR stage in T2DM. In the presence of DME, blood LS levels were statistically significantly increased in mild NPDR; blood PS levels, in PDR; and blood ES levels, in all stages of DR.

Second, a regression model for predicting the progression of DR was built. In the model developed, diabetes duration and blood selectin levels were positively correlated, while HbA1c level was inversely correlated with DR progression. The model built on the selected variables was found to be adequate R2adjust = 0.84; F = 97.9, p < 0.001), demonstrating high correlation of the selected independent variables with the stage of DR.

Third, the calculated Y index may be considered a quantitative reflection of the severity of DR. A patient is predicted to have mild NPDR (with a prediction accuracy of 86.2%) if Y < 1.5, moderate of severe NPDR (with a prediction accuracy of 88.6%) if 1.5 ≤ Y < 2.35, and PDR (with a prediction accuracy of 100%) if Y ≥ 2.35.

Finally, a regression model for predicting the development of DME was built, with CRT and blood ES level found to increase the risk of the development of DME. A high AUC value for the ROC curve (AUC = 0.97; 95% CI, 0.92-0.99) indicated a very strong correlation of the risk of DME development with the levels of selected independent variables. In addition, sensitivity and specificity values at the optimal cut-off point were 93.2% (95% CI, 83.5%-98.1%) and 92.3% (95% CI, 83.0%-97.5%), respectively.

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