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Tear and serum levels of the neuromarker BDNF in type 2 diabetes mellitus patients with and without diabetic retinopathy

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Background: *Numerous studies have been conducted to examine the growth factors capable of exerting simultaneous effects on the vascular and metabolic components of the pathogenesis of diabetic retinopathy (DR).*
Purpose: *To examine tear and serum brain-derived neurotrophic factor (BDNF) levels in type 2 diabetes mellitus (DM2) patients with and without DR.*
Material and Methods: *One hundred and seventy patients with DM2 and 84 practically healthy individuals without any eye disease (totally, 254 individuals and 504 eyes) were included in the study. Of the total individuals included in the study, 49.2% were males and 50.8% were females. The mean age plus or minus standard deviation was 67 ± 4.2 years for men and 64 ± 5.6 years for women.*
Results: *It was found that tear BDNF levels lower than 97.5 pg/ml and serum BDNF levels lower than 23.8 ng/ml indicate the transition to DR, whereas tear BDNF levels lower than 62.0 pg/ml and serum BDNF levels lower than 12.0 ng/ml indicate the development of proliferative DR.*
Conclusion: *Mean serum BDNF levels as low as below 23.8 ± 1.33 ng/ml and mean tear BDNF levels as low as below 97.5 ± 5.57 pg/ml are an early sign of DR.*

Keywords:
retina, diabetic retinopathy, BDNF, diabetic retinopathy biomarkers

Introduction. In recent decades, numerous studies have been conducted to examine the growth factors capable of exerting simultaneous effects on the vascular and metabolic components of the pathogenesis of diabetic retinopathy (DR). [1, 2]. These factors include brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF). BDNF and VEGF have been found to be increased in early diabetic polyneuropathy, which is believed to be a manifestation of compensatory mechanisms. In addition, they have been found to be significantly decreased in decompensated diabetic polyneuropathy. Moreover, in these processes, neurotrophic factor deficiency becomes a stand-alone pathogenetic component.

Filimonova reported on the role of BDNF in the development of diabetic foot syndrome (DFS) and obtained a patent for the method of diagnostic assessment of subclinical diabetic neuropathy through determining BDNF levels in patients with DFS in 2018. [4]. To the best of our knowledge, however, the literature of the countries of the former Soviet Union is scant on the topic. [3-7].

The available methods of DR treatment are commonly used in late disease, which underlines the need for informative biomarkers for early identification and adequate treatment of the disease. Further research should be aimed at the investigation of novel DR biomarkers, and the latter should be easily available, non-invasive, economic and accurate, and capable of assessing the presence and progression of DR. [8-12].

The purpose of the study was to examine tear and serum BDNF levels in type 2 diabetes patients with and without DR.

Material and Methods

One hundred and seventy patients with type 2 diabetes mellitus (DM2) and 84 practically healthy individuals without any eye disease (totally, 254 individuals and 504 eyes) were included in the study. Group 1 was composed of 89 patients (174 eyes) with DR and was divided into the following subgroups: mild non-proliferative DR (NPDR) (24 patients; 47 eyes); moderate NPDR (22 patients; 43 eyes); severe NPDR (23 patients; 45 eyes); and proliferative DR or PDR (20 patients; 39 eyes). Group 2 (DR0) was composed of 81 patients (162 eyes) without ocular signs of DR, and group 3 (controls), of 84 practically healthy individuals without any eye disease. Of the total individuals included in the study, 49.2% were males and 50.8% were females. The mean age plus or minus standard deviation (SD) was 67±4.2 years for men and 64±5.6 years for women.

Subjects underwent biomicroscopy using the Goldmann lens, B scan ocular ultrasound using the VuMAX HD A/B/UBM Ophthalmic Ultrasound System (Sonomed Escalon, New Hyde Park, NY), and macular optical coherence tomography using Spectralis HRA-OCT (Heidelberg Engineering, Heidelberg, Germany). The BDNF-IFA-BEST enzyme-linked immunosorbent assay (ELISA) kit (Vektor-Best, Novosibirsk, Russia) was used to measure serum and tear BDNF levels according to the manufacturer's instructions.

After an overnight fast, 7-ml blood samples were collected from the antecubital vein into blood collection tubes under sterile conditions. Blood sera were separated by centrifugation at 3000 rpm for 15 mins, aliquoted and stored at -20°C for 1 to 3 months. For running ELISAs, serum samples were thawed at room temperature. Serum BDNF concentration was calculated from the standard curve.

A 100-ml tear sample was collected with a sterile pipette from the lower conjunctival fornix in the subject in the sitting position and placed in an Eppendorf tube (Eppendorf International, Hamburg, Germany). Tear samples were frozen at -20°C and stored for 1 to 3 months. They were thawed at room temperature immediately before analysis and centrifuged at 4000 rpm for 10 mins to measure tear BDNF levels in pg/ml.

DM2 was diagnosed according to American Diabetes Association (ADA) criteria: fasting plasma glucose (FPG) ≥ 7.0 mmol/L or 2 hour plasma glucose ≥ 11.1 mmol/L. Demographic data recorded included patient age, sex and disease duration.

Exclusion criteria included ocular disorders capable of impacting retinal vascular pathology; history of intravitreal injections, surgery or laser treatment; systemic vascular disorders impacting BDNF levels; cardiac ischemic disease; current or planned dialysis; or malignant tumors.

Informed consent was obtained from all study subjects. The study was approved by the Ethics Committee of the Ministry of Health of Republic Uzbekistan (Committee Meeting Minutes No.3 of April 11, 2023) and followed the ethical standards stated in the Declaration of Helsinki.

Microsoft Excel 2010 software was used for statistical analysis. Data are presented as mean±standard error of mean. The level of significance $p \leq 0.05$ was assumed.

Results

Serum BDNF levels were significantly lower in patients in group 1 and group 2 compared with controls (25.6±2.64 ng/ml; $p < 0.05$) (Fig. 1).

Particularly, serum BDNF levels were 23.8±1.33 ng/ml in patients without ocular signs of DR; 21.5±0.6 ng/ml in patients with mild NPDR; 19.7±1.04 ng/ml in patients with moderate NPDR; 13.5±1.22 ng/ml in patients with severe NPDR; and 11.2±1.21 ng/ml in patients with PDR. There was a 2.2-times reduction in serum BDNF levels with an increase in DR severity (Fig. 1).

Tear BDNF levels were significantly lower in patients without ocular signs of DR compared with controls (97.5±5.57 pg/ml; $p < 0.05$). Tear BDNF levels were 82.7±7.69 pg/ml in patients with mild NPDR; 73.4±4.39 pg/ml in patients with moderate NPDR; 65.6±4.66 pg/ml in patients with severe NPDR; and 46.2±3.43 pg/ml in patients with PDR. There was a 2.4-times reduction in tear BDNF levels with an increase in DR severity (Fig. 2). There was a significant difference among all groups ($p<0.05$).

Discussion

It was found that tear BDNF levels lower than 97.5 pg/ml and serum BDNF levels lower than 23.8 ng/ml indicate the transition to DR, whereas tear BDNF levels lower than 62.0 pg/ml and serum BDNF levels lower than 12.0 ng/ml indicate the development of PDR. We suppose that early retinal neurodegeneration in patients with DR is associated with reduced BDNF expression. Our findings are in agreement with those of others [4, 6], who demonstrated that significantly decreased serum BDNF levels in patients with NPDR as well as those with PDR. It should be noted that a reduction in BDNF levels is also observed with an increase in the severity of diabetic neuropathy [9]. The results of our study demonstrated that tear BDNF levels as well as serum BDNF levels decreased with DR progression. Tear BDNF levels and serum BDNF levels may be used as biomarkers of disease severity in patients with DR and as prognostic biomarkers of DR onset. We have previously studied correlations of ocular hemodynamics parameters and tear antioxidant protection with the stage

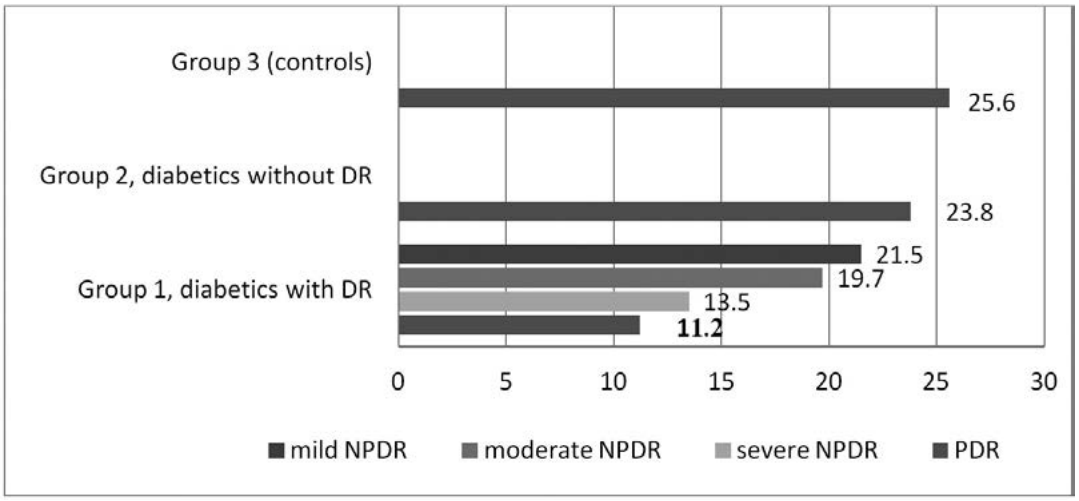

Fig. 1. Serum levels of the neurotrophic factor BDNF (ng/ml) in type 2 diabetes patients with DR, type 2 diabetes patients without DR, and controls

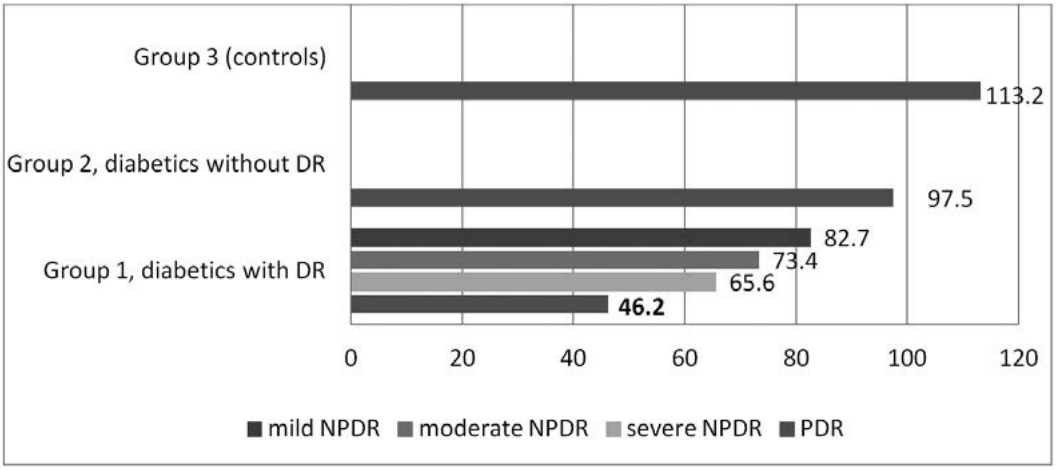

Fig. 2. Tear levels of the neurotrophic factor BDNF (pg/ml) in type 2 diabetes patients with DR, type 2 diabetes patients without DR, and controls

of DR [13, 14]. Our further research will study correlations of serum and tear levels of BDNF with the severity of DR.

Conclusion

First, mean serum BDNF levels as low as below 23.8±1.33 ng/ml and mean tear BDNF levels as low 97.5±5.57 pg/ml as below are an early sign of DR.

Second, in patients with DR, retinal neurodegeneration is supposed to be associated with a progressive decrease in BDNF expression, which characterizes the clinical course of DR.

Finally, tear BDNF levels and serum BDNF levels may be used as biomarkers of disease severity in patients with DR and as prognostic biomarkers of DR onset.

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Рівні нейромаркера BDNF у сльозі та сироватці крові хворих на цукровий діабет 2 типу з діабетичною ретинопатією та без неї

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Вступ. *Проведено значну кількість досліджень з метою вивчення факторів росту, які мають можливість одночасного впливу на судинний та метаболічний компоненти патогенезу діабетичної ретинопатії.*

Мета дослідження*: вивчити вміст нейротрофічного фактора головного мозку (brain-derived neurotrophic factor – BDNF) у слізній рідині та сироватці крові у пацієнтів з цукровим діабетом (ЦД) 2 типу без та при розвитку діабетичної ретинопатії (ДР).*

Матеріал та методи. *Клінічне дослідження проведено серед 254 осіб (n=504), у тому числі у 170 хворих на цукровий діабет 2 типу та у 84 здорових осіб (контроль). Усього чоловіків було 49,2%, жінок 50,8%. Середній вік чоловіків склав (67,0±4,2) років, середній вік жінок – (64,0±5,6) років.*

Результати. *Встановлено, що дефіцит BDNF у слізній рідині < 97,5 пг/мл та у сироватці крові менше <23,8 нг/мл свідчить про перехід до діабетичної ретинопатії, а рівень BDNF у слізній рідині <62,0 пг/мл та у сироватці крові <12,0 нг/мл свідчить про розвиток проліферативної діабетичної ретинопатії.*

Висновок. *Найбільш ранньою ознакою діабетичної ретинопатії є зниження середнього рівня нейротрофічного фактора головного мозку BDNF у слізній рідині нижче 97,5±5,57 пг/мл та сироватці крові пацієнтів нижче 23,8±1,08 нг/мл.*

Ключові слова: *діабетична ретинопатія, нейротрофічний фактор мозку BDNF, біомаркери розвитку діабетичної ретинопатії, сітківка.*