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# THE CONTENT OF NEURON-SPECIFIC ENOLASE IN AQUEOUS HUMOR MAY BE A PREDICTOR OF DIABETIC RETINOPATHY IN TYPE 2 DIABETES

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**Resume**. The study investigated the potential value of measuring neuron-specific enolase (NSE) as a marker of neuronal-glial damage in diabetic retinopathy. Its local measurement in intraocular fluid (IOP) may reflect the severity gradient of diabetic retinopathy (DR) and enhance risk stratification.

**Keywords**: diabetic retinopathy; type 2 diabetes; neuron-specific enolase; biomarkers; IOP; disease progression.

**Background**. Diabetic retinopathy (DR) is one of the leading causes of blindness and moderate/severe visual impairment worldwide [1]. According to the GBD/VLEG consortium, in 2020 it accounted for a significant share of vision loss among people ≥50 years of age, which has been increasing over the past 30 years [1]. Against the background of the global epidemic of diabetes mellitus (DM) (≈537 million patients as of 2021 with a prospect of ≈783 million by 2045 [2]), the number of patients at risk of DR is expected to continue to increase. This highlights the need for better risk stratification and personalized care for such patients.

Neuron-specific enolase (NSE) is a marker of neuronal-glial injury. Its local measurement in aqueous humor (AH) may reflect the severity gradient of diabetic retinopathy (DR) and enhance risk stratification. In ophthalmology, increased NSE has been described in retinal detachment (serologically and in intraocular media) [3], as well as in retinoblastoma, where NSE levels in the intraocular fluid (IOL) and vitreous humor reach high concentrations and are of diagnostic significance [4, 5]. Regarding diabetic pathology, systemic studies have shown increased serum NSE in patients with

DR and diabetic macular edema (DMO) [14] and increased serum and vitreous humor NSE in PD [6]. Together, this forms a biologically motivated hypothesis regarding NSE as a marker of retinal neuronal glial stress in DR.

**Aim** of the study was to determine AH-NSE levels and their association with DR severity.

**Material and methods.** We examined 110 patients with type 2 diabetes mellitus (DM) and classified eyes by the International Clinical Diabetic Retinopathy (ICDR, 2003) scale into five groups: DR0 (no retinopathy; n=15), mild non-proliferative DR (NPDR) (n=40), moderate NPDR (n=25), severe NPDR (n=12), and proliferative DR (PDR) (n=18). A control group included 25 age- and sex-matched individuals without DM/DR. AH was obtained during cataract phacoemulsification; NSE concentration (mg/mL) was measured. Statistical analysis was performed with EZR v1.54 (Austria).

**Results and Discussion.** Age was comparable across groups (p=0,108), while DM duration increased with DR stage (p<0,001). AH-NSE rose from control to PDR (p<0,001); medians (mg/mL): control 4,07 (3,42-4,70), DR0 4,66 (4,28-6,23), NPDR1 12,28 (10,2-15,4), NPDR2 19,20 (16,4-26,9), NPDR3 47,63 (42,3-51,6), PDR 59,19 (45,6-65,1). Multiclass cut-offs (mg/mL): <8,63 (DR0); 8,63-20,0 (NPDR1); 20,1-40,4 (NPDR2); 40,5-64,0 (NPDR3); >64,0 (PDR). For PDR, sensitivity 27,8%, specificity 100%; overall accuracy 0,64 (95% CI: 0,56-0,73). Binary classification (mild/moderate = NPDR1+NPDR2; severe = NPDR3+PDR) with thresholds 8,27-40,45 and >40,45 mg/mL yielded sensitivity/specificity of 90,8%/94,3% (mild/moderate) and 86,7%/98,1% (severe); overall accuracy 0,844 (95% CI: 0,772-0,901).

In our cohort, the content of NSE in the AH showed a monotonic increase with the progression of DR, which allowed us to define class-specific intervals for multiclass grading and working thresholds for practical binary stratification (mild/moderate vs severe DR). Although multiclass delineation provides moderate accuracy, combining adjacent nonproliferative stages into two clinically relevant groups significantly improves the metrics, forming a convenient “rule out/rule in” logic: values in the intermediate range with high sensitivity delineate mild/moderate forms, while high concentrations allow us to confirm severe phenotypes with very high specificity.

Such results are expected for a continuous pathobiological process that we are forced to discretize with a clinical scale. Binarization allows us to turn some of the “interclass noise” into a source for decision-making in clinical practice. Our One vs All approach for estimating multiclass ROC is fully consistent with accepted practices and theoretical generalizations of ROC/AUC to multimodal problems [7]. In systemic samples (serum), findings on DR and related complications (particularly neuropathy) also suggest an association of elevated NSE with neurological dysfunction in diabetes, although effect sizes and specificity vary due to the dilution of information about ocular tissue status in the large volumes of circulating blood and extracellular fluid [8]. Recent clinical studies confirm that higher serum NSE levels are associated with peripheral neuropathy and a worse metabolic profile in patients with type 2 diabetes [9]. These observations are

consistent with our local data from ocular tissue, but also highlight the advantage of analyzing the composition of the AH for more accurate assessment of retinal status.

The strengths of the study include the local definition of a biomarker (in the AH) with direct grading according to the DR phenotype, the construction of class-specific intervals and practical binarization with increased accuracy of the research method, and the consistency with current AH/vitreous proteomics data on neuro- and angioactive biomolecules in DR [10, 11].

Limitations include the single-center and cross-sectional nature of the study, which requires the creation of prospective trajectory designs with independent validation cohorts in the future; possible class imbalance between stages, which is typical for clinical samples and can underestimate the sensitivity at the poles in multiclass models and improve with binarization; possible influence of preanalytical factors on the NSE level (time to freezing, lack of control/documentation of hemolysis in the AH [12], use of local laboratory analysis protocols and units of measurement).

**Conclusions.** AH-NSE reflects the DR severity gradient and provides stage-specific and binary thresholds for practical stratification (a rule-out/rule-in logic for severe forms). External validation is required for broader implementation.

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# MicroRNA-146A-5p IN DIFFERENT STAGES OF DIABETIC RETINOPATHY

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**Resume**. The expression of microRNA-146a-5p in a cohort of patients with type 2 diabetes clearly corresponded to the stages of diabetic retinopathy. In addition, its diagnostic and prognostic significance as a marker of type 2 diabetes, diabetic retinopathy and its severe stages in patients from the Ukrainian population was shown.

**Keywords:** Diabetic Retinopathy; Diabetes Mellitus, Type 2; MicroRNAs; MicroRNA-146a; Gene Expression Regulation; Biomarkers; Disease Progression.

**Background**. MicroRNAs (miRs) are a class of evolutionarily conserved short non-coding RNAs, containing about 19-22 nucleotides, that regulate the expression of messenger RNA [1]. By participating in the regulation of mRNA target expression, miRs play important roles in a wide variety of biological systems and processes, such as cell differentiation, proliferation, apoptosis, and metabolism, making them promising