Modern Biomarkers for Early Diagnosis of Ocular Surface Disease in Type 1 Diabetes. A Pilot Study

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SUMMARY

Patients with dry eye syndrome form a significant proportion of those treated in everyday ophthalmology practice. Diabetes mellitus is a major risk factor for the development of dry eye syndrome. Changes in tear film homeostasis, chronic inflammation and subsequent corneal nerve fiber pathology play a key role in its progression.

The aim of this study was to describe the status of modern biomarkers of ocular surface damage in patients with type 1 diabetes and asses their utility in early diagnosis of dry eye syndrome.

Material and methods: In total the pilot study included 19 patients with type 1 diabetes (T1D) and 15 patients in the control group. All patients underwent a detailed ocular surface examination, sample collection for matrix metalloproteinase-9 (MMP-9) laboratory analysis and epithelial HLA-DR expression evaluation, and in-vivo corneal confocal microscopy.

Results: T1D patients showed statistically significantly reduced corneal nerve fiber length ($p = 0.0482$). The differences between the groups in terms of osmolarity, corneal sensitivity, Oxford score, tear break-up time and MMP-9 level were not statistically significant ($p = 0.8272$, $p = 0.6029$, $p = 0.3507$, $p = 0.7561$ and $p = 0.0826$ respectively). HLA-DR expression was examined in 10 T1D patients and 8 patients in the control group. Both groups showed minimal or no expression ($p > 0.9999$).

Conclusion: The previously published literature supports our finding of corneal nerve fiber length reduction in T1D patients compared to controls. However, we did not find any significant changes in standard or modern ocular surface markers (MMP-9 levels, HLA-DR expression) measured in patients with dry eye syndrome.

Key words: diabetes mellitus, dry eye disease, biomarker, MMP-9, HLA-DR, confocal microscopy

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INTRODUCTION

Diabetes mellitus (DM) is considered a risk factor for the development of ocular surface pathology, including dry eye disease (DED) [1].

DED is a multifactorial disorder associated with increased tear film osmolarity and inflammation, which is considered one of the key factors in its pathogenesis [2]. The diagnosis of DED is often difficult. At present, efforts are under way to introduce new, objectively measurable biomarkers that would assess DED more precisely and potentially describe its etiopathogenesis. The expression of human leukocyte antigen (HLA-DR) by ocular surface tissues is one of such markers. HLA-DR expression plays a significant role in the process of immune response initialization. In healthy eyes Langerhans cells present this glycoprotein on the ocular surface. It is uncommon for it to be presented by any other cell of the conjunctival epithelium. However, in patients with ocular surface pathology, aberrant overexpression by epithelial cells has been described [3–5].

Another notable surface marker associated with DED is the expression of matrix metalloproteinases (MMP), specifically MMP-9. In response to injury MMP-9 expression increases in corneal tissues as a component of

the immune response with subsequent elevation of MMP-9 in the lacrimal film [5,6].

The precise mechanism of DED development in patients with DM is unknown. Hyperglycemia has a fundamental influence on changes in ocular surface tissues, but it is assumed that multiple factors play a role, including corneal nerve fiber damage, decrease of fiber density and reduction of corneal sensitivity, associated with subsequent dysregulation and instability of the lacrimal film. Studies on animal models have also indicated a possible alternative mechanism of DED pathogenesis in patients with autoimmune type 1 diabetes (T1D) and type 2 diabetes. Symptoms of DED appear significantly earlier in pediatric patients with T1D rather than alongside with later metabolic complications [7]. It is assumed that lymphocyte mediated autoimmune reaction within the lacrimal gland disrupts its normal function [8].

The aim of the study was to describe ocular surface and tear film changes in patients with T1D. Concurrently with standardized ocular surface parameters, HLD-DR expression by the conjunctival epithelium and MMP-9 tear levels were assessed for their utility in early diagnosis of DED.

MATERIALS AND METHODS

The study included patients aged between 20 and 50 years with T1D, who were examined at the Department of Ophthalmology, 2nd Faculty of Medicine and Motol University Hospital in Prague during the course of 2022, as a part of their regular screening for diabetic retinopathy, as well as consenting patients without DM who were capable of undergoing the relevant examinations and could therefore be included in the control group. All participants in the study signed an informed consent form approved by the Ethical Committee of Motol University Hospital. Detailed medical history was recorded for all the participants. The exclusion criteria were systemic disorders, other than DM1, which may cause ocular surface changes, chronic ocular disease, previous eye operation, chronic ocular medication, and previously established diagnosis of DED.

A total of 19 patients with a diagnosis of T1D were included in the study, with an average age of 32.9 ±6.4 years, as well as 15 control subjects aged 29.7 ±6.5 years, so as to ensure that the two groups did not differ statistically significantly in terms of age ($p = 0.1636$). In the group of patients with T1D there were 6 patients without diabetic retinopathy, 9 patients with non-proliferative diabetic retinopathy and 4 patients with proliferative diabetic retinopathy. The average time since T1D diagnosis was 20.7 ±5.3 years.

All patients underwent a standard ophthalmological examination, including detailed examination of the ocular surface, tear osmolarity (TearLab), corneal sensitivity testing (Cochet-Bonnet esthesiometer, Luneau) and tear film stability evaluation using the tear break-up time test (TBUT). To measure tear osmolarity the TearLab Osmolarity System (TearLab Corp, San Diego, CA, USA) was used according to the manufacturer's instructions. For the TBUT and corneal sensitivity testing, the measurement was performed three times and the mean value was recorded.

The Oxford schema for epitheliopathy was used to assess the degree of vital ocular surface staining. The schema contains 5 panels graded A-E, in each of which 3 zones are displayed: temporal bulbar conjunctiva – cornea – nasal bulbar conjunctiva. The degree of epitheliopathy is evaluated for each zone from 0 to 5, the maximum total value being 15 (corresponding to severe epitheliopathy in all three zones) [5].

A tear sample was collected from the lower lacrimal meniscus using a micropipette and immediately stored at a temperature of -20˚C as previously described [9]. MMP-9 levels were evaluated using the Human MMP-9 ELISA (ThermoFisher Scientific, Human MMP-9 Platinum ELISA) commercial kit according to the manufacturer's instructions.

Impression cytology as per Jirsová et al. [10] was used to collect superficial conjunctival cells from the temporal region of the left eye using a Biopore membrane (Millicell-CM, PICM01250, Millipore). The samples were then frozen at a temperature of -80˚C until processing. A mouse monoclonal primary antibody (anti HLA-DR antibody, SAB4700731, Sigma-Aldrich) was used for HLA-DR detection, followed by a goat secondary antibody against mouse IgG marked with Alexa Fluor 488 (Alexa Fluor® 488 AffiniPure™ Goat Anti-Mouse IgG (H+L), 115-545-003, Jackson Laboratories) fluorescein stain. The nuclei were further stained with propidium iodide. The imprints were subsequently scanned with an epifluorescein microscope BX50 (Olympus) equipped with a DFK 23UX174 (Imaging Source) (JU) camera and NIS Elements AR (Laboratory Imaging) image analysis software. The scans were subsequently evaluated by two examinators (JŠ, GM), who judged the intensity of the staining as either unequivocal positivity, slight positivity or no staining (Figure 1).

In-vivo corneal confocal microscopy (IVCM) was performed using the HRTIII RCM (Heidelberg Engineering,

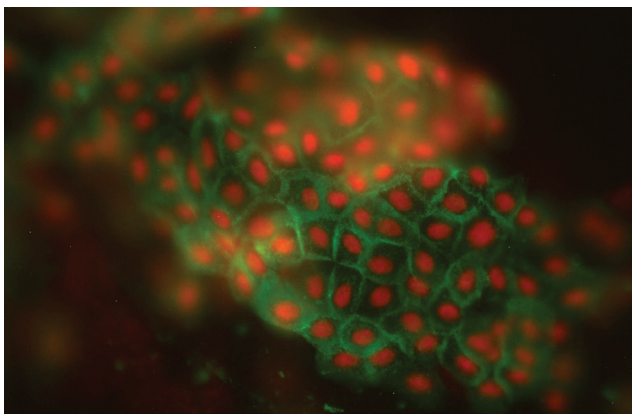
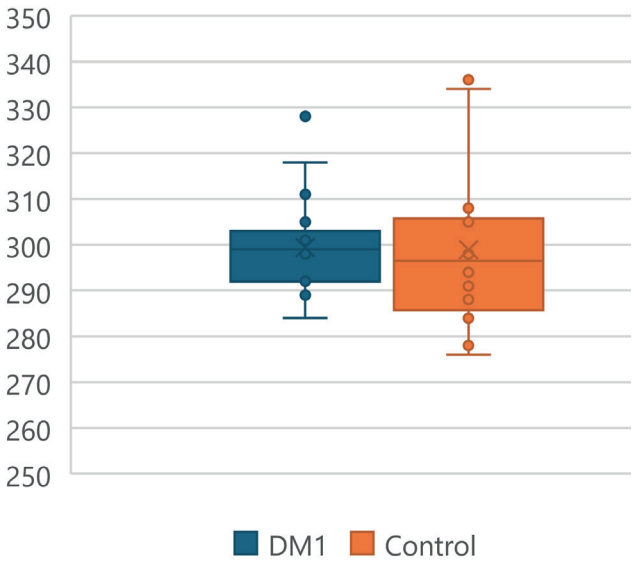


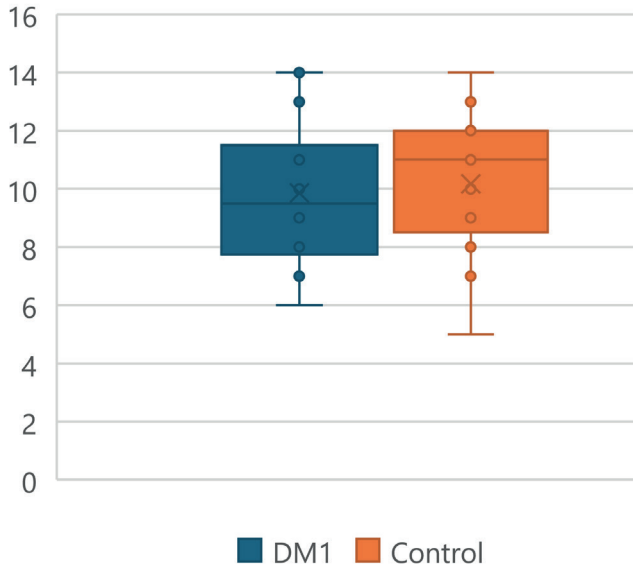
Figure 1. HLA-DR positivity
HLA-DR – Human leukocyte antigen

Germany) corneal confocal microscope. Local anesthesia was induced by two drops of oxybuprocaine-hydrochloride 4mg/ml and immersion was attained with gel (Recugel,Bausch & Lomb) and a sterile TomoCap (Heidelberg Engineering, Germany). Six corneal regions were subsequently scanned (superior, superior temporal, temporal, central, inferior temporal and inferior), and 5 scans optimally capturing the subbasal corneal nerve plexus were selected for measuring the length of the corneal nerve fiber layer (CNFL) for each eye. The automated ACCMetrics v0.3 software was used for their analysis [11,12].

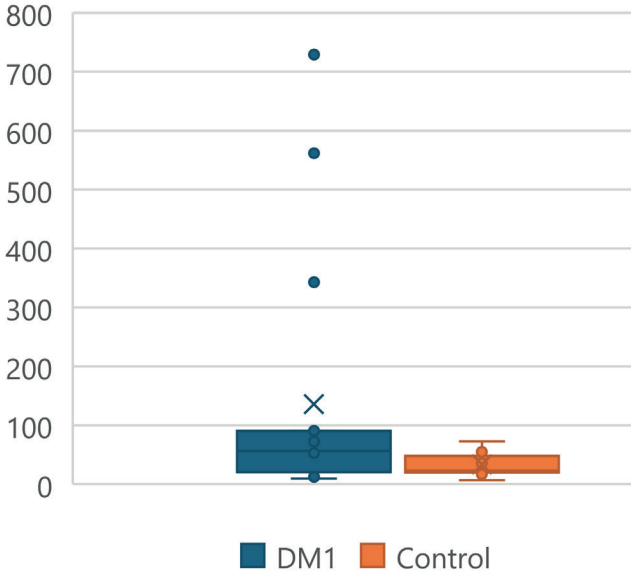
The results are presented as the mean ± standard deviation. Only measurements from the left eye were used in the analysis. The data was statistically processed using a non-paired t-test for parametrically distributed data (age, tear osmolarity, TBUT) and a Mann-Whitney test for non-parametric data (corneal sensitivity, Oxford score, MMP-9). The degree of HLA-DR antigen positivity in conjunctival epithelium was evaluated with contingency



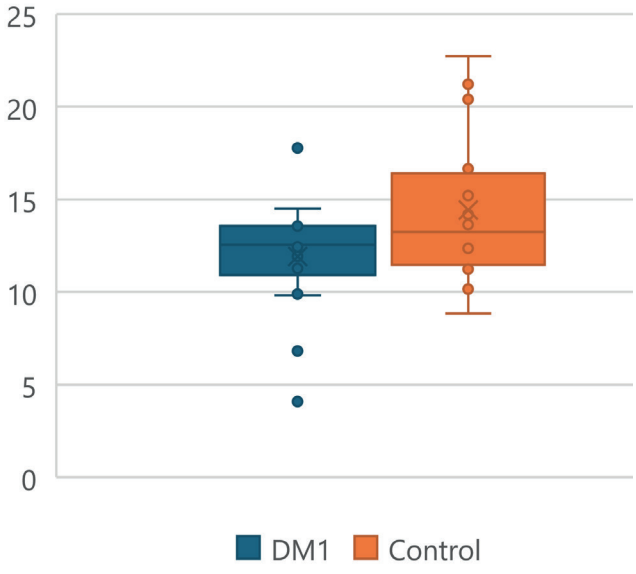
Graph1. Osmolarity
DM1 – diabetes mellitus type 1



Graph 2. TBUT
DM1 – diabetes mellitus type 1, TBUT – tear break-up time



Graph 3. MMP-9 levels
DM1 – diabetes mellitus type 1, MMP-9 – matrix metalloproteinase-9



Graph 4. Average corneal nerve fiber length in scanned sections
DM1 – diabetes mellitus 1. typu, CNFL – corneal nerve fiber length

tables and a Fisher exact test (software for statistical data processing StatView 5.0; SAS Institute Inc, Cary, NC, USA). The value of $p \leq 0.05$ was considered the limit of statistical significance.

RESULTS

We did not demonstrate any statistically significant difference between the group of patients with T1D and the control group in terms of tear osmolarity ($p = 0.8272$, Graph 1), corneal sensitivity ($p = 0.6029$), Oxford score ($p = 0.3507$) or TBUT ($p = 0.7561$, Graph 2). We did not demonstrate a statistically significant difference in MMP-9 tear levels between the groups either ($p = 0.0826$, Graph 3).

Expression of HLA DR antigen through the conjunctival surface cells was evaluated in a group of 10 patients with T1D and with varying degrees of diabetic retinopathy, and in 8 control group patients.

Positivity of the conjunctival epithelium for HLA-DR did not differ between the groups, in both groups it was mild or absent ($p > 0.9999$).

The mean total corneal nerve fiber length (CNFL) was statistically significantly lower in the group of patients with T1D than in the control group ($p = 0.0482$, Graph 4).

The results are summarized in Table 1.

DISCUSSION

DED is a chronic disease that often requires a demanding diagnostic process, a multimodal approach on the part of the doctor, and patient motivation to share in the treatment. The correlation of subjective and objective symptoms is not generally unequivocal, and there is pronounced variability depending on the cause of DED and between specific individuals.

Diabetes mellitus is considered one of the risk factors for developing ocular surface pathology and DED. It is assumed that an accumulation of advanced glycation end products (AGE) within the complex of epithelial basal membranes activates a proapoptotic and antiproliferative cascade, which leads to epithelial injury, subsequent epitheliopathy, and resulting in an inflammatory response and the release of pro-inflammatory cytokines [1,13]. Complications of hyperglycemia (formation of AGE, microvascular changes) also directly damage the lacrimal gland, which may lead to reduction of tear production and changes of tear composition [14]. In the case of T1D, a direct association between dysfunction of the lacrimal film, autoimmune nature of the disease and damage to the lacrimal gland has been previously described in a mouse model as a consequence of infiltration by lymphocytes, preceding the development of organ complications [8].

Insulin deficiency and hyperglycemia cause damage and dysfunction of the Meibomian glands [15]. Nerve fiber damage, a sequela of diabetic neuropathy, and the resulting disruption of nerve regulation further increases the risk of inflammation and DED [5].

HLA-DR expression by corneal epithelial cells is considered as a possible biomarker of ocular surface inflammation and DED [16]. It is assumed that aberrant epithelial expression plays a key role in activating the immune system, inflammation, and in autoimmunity [4,16]. Aberrant expression of HLA-DR has been described in a number of chronic and autoimmune diseases, e.g. Crohn's disease (esophageal epithelium) or primary biliary cirrhosis (bile duct epithelium). Aberrant expression of HLA-DR antigen has also been described in patients with T1D (in pancreatic β-cells) [3].

Within our study we focused on aberrant expression of HLA-DR as a factor in the pathological cascade of DED in patients with T1D, however, we did not demonstrate any difference in expression of this antigen by the conjunctival epithelium in comparison with a group of healthy individuals.

Evaluation of MMP-9 levels in tears is considered as another potential biomarker for monitoring the development of ocular surface inflammation and DED. Increased systemic production of MMP-9 has been described in immune and chronic diseases, including diabetes [17].

In our study we did not demonstrate any statistically significant difference in MMP-9 tear levels between the two groups. It was possible to observe a certain trend to-

Table 1. Results overview

	DM1	control	p
osmolarity (mOsmol/l)	299.37 ±10.63	300.40 ±16.60	0.8272
sensitivity (mm)	5.87 ±0.28	5.83 ±0.24	0.6029
TBUT (s)	9.86 ±2.60	10.13 ±2.13	0.7561
Oxford score	0.27 ±0.46	0.07 ±0.26	0.3507
MMP-9 (ng/ml)	455.31 ±1371.52	34.74 ±19.92	0.0826
OSDI	6.55 ±5.17	10.51 ±13.41	0.8555
CNFL (mm/mm2)	11.93 ±2.96	14.23 ±3.34	0.0482

DM1 – diabetes mellitus type 1, TBUT – tear break-up time, MMP-9 – matrix metalloproteinase-9, OSDI – Ocular Surface Disease Index, CNFL – corneal nerve fiber length

wards higher values in the group with T1D, though this was accompanied by a pronounced dispersion of results in both groups. A problem with the use of this marker is the fact that its levels fluctuate significantly depending on the time of day [18].

In accordance with previous studies, we demonstrated a statistically significant reduction in corneal nerve fiber length (CNFL) in patients with DM in comparison with the control group. However, we did not demonstrate a difference between the groups in standardized parameters of tear film disruption and ocular surface disease (tear osmolarity, TBUT or Oxford score).

A limitation of our study is the relatively small number of patients, as well as the fact that we included individuals with varying degrees of diabetic retinopathy in the tested group, though primarily with non-proliferative diabetic retinopathy. It is possible to assume that the risk of ocular surface damage in patients with T1D will increase with the development of further complications of DM. As a result, in the future it would be appropriate to conduct a more detailed observation of the incidence of ocular surface changes depending on the degree of diabetic retinopathy, or other diabetic complications (diabetic neuropathy, nephropathy).

CONCLUSION

In accordance with previously published studies we confirmed a reduction of the overall corneal nerve fiber length of the subbasal plexus in patients with T1D in comparison with control group patients. However, we did not demonstrate ocular surface changes (signs of DED) with either standard or modern biomarkers of DED (MMP-9 tear levels, HLA-DR expression on the ocular surface) in patients with T1D in comparison with the control group. A more detailed description ocular surface changes in patients with T1D, and defining the utility of modern DED biomarkers for early diagnosis of at risk patients and complications prevention would require further extensive studies as well testing the potential relationship of ocular surface damage to the compensation diabetes and the presence of its complications.

REFERENCES

1. Shih KC, Lam KSL, Tong L. A systematic review on the impact of diabetes mellitus on the ocular surface. Nutr Diabetes. 2017;7(3):e251–e251.
2. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK, et al. TFOS DEWS II Definition and Classification Report. Ocul Surf. 2017;15(3):276-283.
3. Jirsova K, Stangova PS, Palos M, et al. Aberrant HLA-DR expression in the conjunctival epithelium after autologous serum treatment in patients with graft-versus-host disease or Sjögren's syndrome. Plos One. 2020;15(4):e0231473.
4. Brignole-Baudouin F, Riancho L, Ismail D, Deniaud M, Amrane M, Baudouin C. Correlation Between the Inflammatory Marker HLA-DR and Signs and Symptoms in Moderate to Severe Dry Eye Disease. Inv Opth Vis Sci. 2017;58(4):2438.
5. Bron AJ, Paiva CS de, Chauhan SK, et al. TFOS DEWS II pathophysiology report. Ocul Surf. 2017;15(3):438-510.
6. Smith VA, Rishmawi H, Hussein H, Easty DL. Tear film MMP accumulation and corneal disease. Br J Ophthalmol. 2001;85(2):147.
7. Akinci A, Cetinkaya E, Aycan Z. Dry Eye Syndrome in Diabetic Children. Eur J Ophthalmol 2007;17(6):873-878.
8. Humphreys-Beher MG, Hu Y, Nakagawa Y, Wang PL, Purushotham KR. Lacrimal Gland, Tear Film, and Dry Eye Syndromes, Basic Science and Clinical Relevance. Adv Exp Med Biol. 1994;350:631-636.
9. Minaříková M, Fík Z, Štorm J, Helisová K, Ferrová K, Mahelková G. Tear matrix metalloproteinase-9 levels may help to follow a ocular surface injury in lagophthalmic eyes. Plos One .2022;17(9):e0274173.
10. Jirsova K, Juklova K, Vesela V, Filipec M. Morphological and immunocytochemical characterization of snake-like chromatin cells. Histol Histopathol. 2006;21(4):355-360.
11. Petropoulos IN, Alam U, Fadavi H, et al. Rapid Automated Diagnosis of Diabetic Peripheral Neuropathy With In Vivo Corneal Confocal MicroscopyAutomated Detection of Diabetic Neuropathy. Inv Oph Vis Sci. 2014;55(4):2071-2078.
12. Tavakoli M, Ferdousi M, Petropoulos IN, et al. Normative Values for Corneal Nerve Morphology Assessed Using Corneal Confocal Microscopy: A Multinational Normative Data Set. Diabetes Care. 2015;38(5):838-843.
13. Kim J, Kim CS, Sohn E, Jeong IH, Kim H, Kim JS. Involvement of advanced glycation end products, oxidative stress and nuclear factor-kappaB in the development of diabetic keratopathy. Graefe's Arch Clin Exp Ophthalmol. 2011;249(4):529-536.
14. Markoulli M, Flanagan J, Tummanapalli SS, Wu J, Willcox M. The impact of diabetes on corneal nerve morphology and ocular surface integrity. Ocul Surf. 2018;16(1):45-57.
15. Ding J, Liu Y, Sullivan DA. Effects of Insulin and High Glucose on Human Meibomian Gland Epithelial Cells. Inv Opth Vis Sci. 2015;56(13):7814.
16. Epstein SP, Gadaria-Rathod N, Wei Y, Maguire MG, Asbell PA. HLA-DR expression as a biomarker of inflammation for multicenter clinical trials of ocular surface disease. Exp Eye Res. 2013;111:95-104.
17. Cabral-Pacheco GA, Garza-Veloz I, Rosa CCD, et al. The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases. Int J Mol Sci. 2020;21(24):9739.
18. Markoulli M, Papas E, Cole N, Holden BA. The Diurnal Variation of Matrix Metalloproteinase-9 and Its Associated Factors in Human Tears. Inv Opth Vis Sci. 2012;53(3):1479.