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Effect of a combination of 0.001% hydrocortisone and 0.2% hyaluronic acid on conjunctival cytology in patients with dysregulatory inflammatory changes of the conjunctiva in type 2 diabetes

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Purpose. *To assess and evaluate cytological changes of conjunctival epithelial cells collected by CIC and relative expression of CD15 before and after 2-month treatment with sodium hyaluronate 0.2% and 0.001% hydrocortisone among patients with dysregulatory inflammatory changes of the conjunctiva in type 2 diabetes mellitus.*
Methods. *We prospectively enrolled 38 T2DM patients (76 eyes). All patients were further divided into two groups: Group I - study group (n=23; 46 eyes); Group II - controls (n=15; 30 eyes). Group I received one drop of sodium hyaluronate 0.2% and 0.001% hydrocortisone (HA/Hydrocortisone); group II – one drop of sodium hyaluronate two times daily for two months, respectively.*
Results. *Relative expression of epithelial CD15 was significantly reduced after the treatment in Group I (p<0,001) in contrast to Group II (p=0,8726). Absence of CD 15 expression was found in five cases (11%) before treatment and seven cases (15%) after HA/Hydrocortisone administration (p=0,7582). Macrophage infiltration was observed in 28% of participants upon baseline examination and it completely disappeared after treatment with HA/Hydrocortisone. These findings show that HA/hydrocortisone drops should be considered as an effective treatment for patients with dysregulated parainflammation of the conjunctiva.*
Conclusions. *Treatment with hyaluronate/hydrocortisone eye drops showed a statistically significant improvement of conjunctival cytology and functional tests: lipid layer thickness (LLT), tear breakup time (TBUT), meibomian gland loss (MGL) along with reduction of relative expression of epithelial CD15 among T2DM patients.*

Key words:
hydrocortisone 0.001%, hyaluronic acid, type 2 diabetes, dry eye disease, impression cytology, inflammatory infiltration, ocular surface

Background. Conjunctival epithelial cells provide a strong barrier of the anterior ocular surface against aggressive environmental factors [1]. Chronic hyperglycemia results in a significant damage of these cells identified by CIC (conjunctival impression cytology), namely squamous metaplasia, loss of tight junctions, dysplastic cells and increased relative expression of proinflammatory marker CD15 [1,2]. The latter may serve as a biomarker to measure targeted clinical therapies along with the morphological changes among T2DM patients [3, 4].

Recent data suggest that inflammation, mediated through the expression of IL-1, TNF-α, and IL-6, activate adaptive immunity and lead to chronic ocular surface damage [5]. Taking into account multifactorial impairment of ocular surface among T2DM patients, this group of patients requires not only moisturizing but also an anti-inflammatory agent; hence, an efficient combination is required. Hyaluronic acid-Trehalose has been considered a natural preservative-free bioprotector and was primarily used among patients with moderate and severe DED (dry eye disease) [6]. However, recent research anticipates that hydrocortisone allows controlling macrophage infiltration and may prevent the sub-clinical inflammatory component from progression to the evident chronic ocular surface damage [7]. Moreover, low concentration of hydrocortisone are adequate and safe to prolonged use in DED, chronic ocular surface inflammation etc [8]. Thus, it may be a beneficial pathogenetic treatment of ocular surface damage for T2DM patients.

Currently available eye drops formulation contains 0.2% hyaluronic acid and a low concentration of hydrocortisone (0.001%) (HA/hydrocortisone). It has been developed to manage the dysregulated parainflammation in patients with DED and reduce subsequent macrophage infiltration [9]. To our knowledge, there are no studies published regarding effectiveness of HA/hydrocortisone eye drops on the ocular surface damage and macrophage

infiltration, and expression of proinflammatory markers in the population of T2DM patients.

Hence, we **aimed** to assess and evaluate cytological changes of conjunctival epithelial cells collected by CIC and relative expression of CD15 before and after 2-month treatment with HA/Hydrocortisone among patients with dysregulatory inflammatory changes of the conjunctiva in type 2 diabetes mellitus.

Methods

The study was conducted from December 1, 2023 to January 31, 2024 in the setting of Vinnytsia Clinical Hospital Named after Pirogov. We prospectively enrolled 38 T2DM patients (76 eyes). Exclusion criteria included ocular surgical procedures in the past, use of contact lenses, arterial hypertension, and endocrine disorders affecting a lacrimal function unit. Subjects with rheumatic health conditions and poor control of T2DM (HbAc1 > 9) were also excluded from further research. Ocular surface assessments were measured at the baseline and after two months of treatment with HA/Hydrocortisone. All patients were further divided into two groups: Group I - study group (n=23; 46 eyes); Group II - controls (n=15; 30 eyes). Group I received one drop of sodium hyaluronate 0.2% and 0.001% hydrocortisone (combined eye drops); group II – one drop of sodium hyaluronate two times daily for two months, respectively.

All participants had an Ocular Surface Disease Index (OSDI) score of 13-32.

All procedures were performed following the Declaration of Helsinki and approved by the local institutional Committee of Bioethics. All participants had provided written informed consent prior to enrolment.

All patients underwent comprehensive ophthalmological examination using the expert class stationary equipment (SLM Ophthalmic Slit Lamp; SLM-6E): tear meniscus height test (TMH), noninvasive tear break-up time (NIBUT), lipid layer thickness (LLT), Meibomian gland loss (MGL). Conjunctival impression cytology (CIC) was performed according to our standard patent technique. Grades 0 and 1 characterized intact conjunctival structure whilst grades 2 and 3 were considered pathological. Expression of CD15 was detected via a multi-step immunocytochemical staining process with Clone Carb-3 and further evaluated using light microscopy.

Lower tear meniscus height was automatically measured in tear meniscus mode utilizing the mean height of three measurements after fluorescein staining. TMH <0.2 mm was considered abnormal.

Automated measurement of NIBUT was based on two measurements and the average value was recorded.

A set of standard templates was used to evaluate LLT automatically. LLT was measured and interpreted according to the following standards: Level 1:<15 nm; Level 2:=15 nm; Level 3:=30 nm; Level 4:[30-80 nm]; Level 5:=80 nm; Level 6:[80-120 nm]; Level 7:[120-160 nm].

Meibomian gland loss of the upper and lower eyelid were evaluated utilizing infrared imaging technology with high-definition digital shooting to generate the meiboscore: score 0 for no MGL, score 1 for less/equal to one-third MGL, score 2 for one-third to two-thirds MGL, and score 3 for more than two-thirds MGL.

Statistical analysis was performed with statistical package STATISTICA v.10.0 (StatSoft, USA). Descriptive analysis was performed with the mean ± standard deviation (SD). The normality distribution of the data was assessed with the Kolmogorov-Smirnov test. Differences in qualitative variables were assessed with the exact Fisher's test and Wilcoxon matched pairs test. The differences between the first and second measurements were performed with the two-tailed T-test for dependent groups. The correlation study was evaluated with the Spearman's rank test. P-value < 0.05 was considered significant.

Results

Thirty-three diabetic patients (46 eyes) with mean age of 61,6±4,6 years and good-to-fair glycemic control underwent objective tests including measurement of TMH, NIBUT, LLT, MGL (Group I). Fifteen age- and sex-matched patients were enrolled as controls (Group II). Table 1,2 represents the demographic and objective characteristics of the patients before and after treatment with HA/hydrocortisone drops (Group I) and HA (Group II).

The results show significant increase in NIBUT (p<0,001) and level of LLT (p<0,001) along with improvement of meiboscore and subsequent reduction of MGL (p<0,001) after treatment with HA/Hydrocortisone. Although, the average TMH was within a reference range at the baseline, it significantly improved after treatment (p=0,005). It may be clinically beneficial for patients with TMH lower or at the point of 0,2 mm in this population. Similar trends were observed in Group II (Table 1).

According to CIC, the vast majority of patients show Nelson's grade III both before and after treatment (Table 3).

Table 1. Demographic characteristics of the patients of both groups with HA/hydrocortisone drops (Group I) and HA (Group II)

Demographic characteristic of the groups		Group I	Group II	p-value
Age (m±SD); years		61,6±4,6	62,1±3,8	0,5936
Sex	M	12	8	1,000
	F	11	7	1,000
Duration of T2DM; years		8,1±3,5	8,9±3,7	0,2830

Group I – patients with received combined eye drops Hyalruonic acid/hydrocortisone; Group II - patients with received Hyaluronic acid.

Table 2. Objective characteristics of the patients before and after treatment with HA/hydrocortisone drops (Group I) and HA (Group II).

Objective tests		Before treatment	After treatment	p-value
TMH (m±SD); mm	Group I	0,25±0,08	0,27±0,07	0,005*
	Group II	0,22±0,07	0,25±0,05	0,0004*
NIBUT (m±SD); sec	Group I	5,81±2,05	7,72±2,23	<0,001*
	Group II	6,01±1,92	7,67±1,91	<0,001*
Lipid layer (m±SD); level	Group I	4,1±1,48	4,7±1,28	<0,001*
	Group II	4,10±1,39	5,13±1,00	0,0001*
MGL (upper) (m±SD) (score)	Group I	1,46±0,67	1,21±0,6	<0,001*
	Group II	1,62±0,61	1,2±0,55	0,0006*
MGL (lower) (m±SD) (score)	Group I	2,01±0,85	1,57±0,77	<0,001*
	Group II	1,85±0,70	1,32±0,57	<0,001*

* statistically significant at <0,05 before and after treatment within the group; TMH – Lower tear meniscus height, NIBUT – noninvasive tear break-up time, LLT – lipid layer thickness, MGL (upper) – meibomian gland loss (upper eyelids), MGL (lower) – meibomian gland loss (lower eyelids).

Table 3. Treatment outcomes based on CIC and relative epithelial expression of CD15+

Test title			Before treatment, persons (n)	After treatment persons (n)	p-value
CIC	Nelson's grade 0-I	Group I, n	1	5	0,2031
		Group II, n	2	4	0,6707
	Nelson's grade II	Group I, n	21	20	1,000
		Group II, n	18	19	1,000
	Nelson's grade III	Group I, n	24	21	0,6768
		Group II, n	10	7	0,5675
	Improved Nelson's grade	Group I, n	n/a	6	0,3507**
		Group II, n	n/a	7	
Relative expression of CD 15+ (%) (M±SD)		Group I	5,78±2,83	4,36±2,66	<0,001*
		Group II	5,56±3,20	5,6±2,91	0,8726

*statistically significant at <0,05 within the group; ** difference between the groups; Group I – patients with received combined eye drops Hyalruonic acid/hydrocortisone; Group II – patients with received Hyaluronic acid; CIC – conjunctival impression cytology.

However, the number of participants with Nelson's grade I increased from 2% to 11% (p=0,2031) in Group I and similar trends were observed with decrease in Nelson's grade III. This fact may suggest longer treatment required to detect significant difference. Overall, the number of patients improved their grades after treatment reached 13% and 15% providing evidence of positive effect of HA/Hydrocortisone and HA on epithelial cytology among T2DM patients, respectively.

Relative expression of epithelial CD15 was significantly reduced after the treatment in Group I (p<0,001) in contrast to Group II (p=0,8726). Absence of CD 15 expression was found in five cases (11%) before treatment and seven cases (15%) after HA/Hydrocortisone administration (p=0,7582). It means that even after 2 months of using this combination in 5% of cases, the disappearance of CD 15 expression was observed, which indicates the anti-inflammatory effect (Figure 1).

Furthermore, conjunctival cytology of patients with Nelson's grade III has been shown potential to improve based on restoration of intercellular connections, narrowing of intercellular space in epithelial blocks, reduction of dystrophic changes and following reappearance of Goblet cells.

Macrophage infiltration was observed in 28% of participants upon baseline examination and it completely disappeared after treatment with HA/Hydrocortisone.

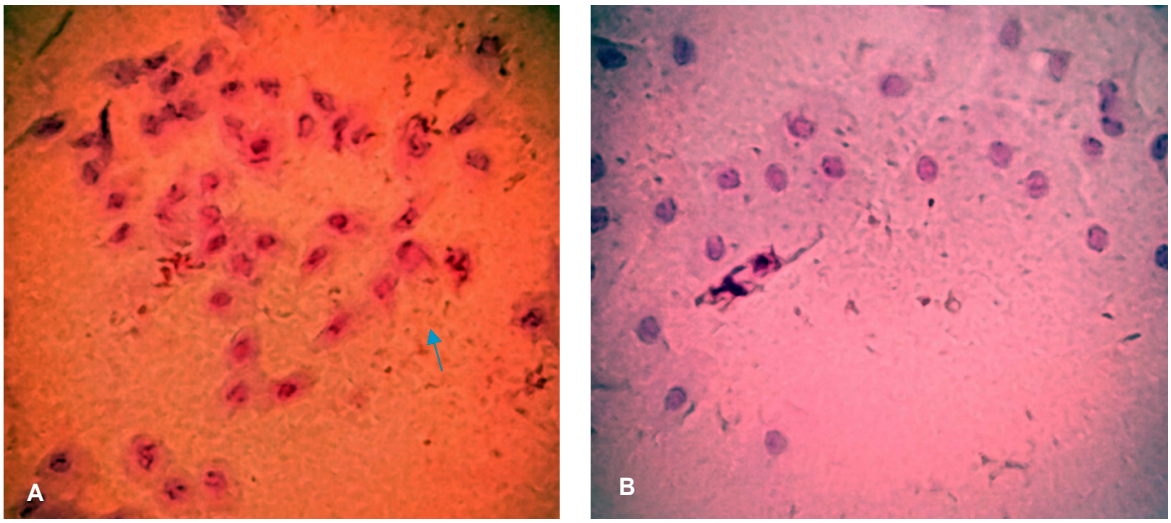

Figure 1. CIC – conjunctival impression cytology (A – before treatment, expression of CD15 – 3%; B – after treatment, absence of expression of CD15). Original magnification *400.

Discussion

Ocular surface damage and subsequent DED pathogenesis trigger the vicious circle of tear instability, ocular surface inflammation, epithelial damage etc. Para-inflammatory phase precedes these detrimental changes and aims to protect ocular surface homeostasis. Endogenous glucocorticoids, namely cortisol may play a crucial role in this protective mechanism and prevent its progression to the chronic ocular surface damage [5]. Therefore, we considered the treatment with 0.001% Hydrocortisone/HA as a promising approach to reduce structural damage and improve functional tests among patients with T2DM.

Fogagnolo P. at al. states that this formulation of eye drops has a positive effect on dysregulated parainflammation [9], which has been confirmed in our study. We have noticed that macrophage infiltration was completely resolved after two-month treatment with HA/Hydrocortisone and conjunctival cytology was improved significantly in contrast to baseline findings.

Another study conducted by Maurizio Rolando et. al. (2023) observed a significant improvement of the TBUT ($p \leq 0.05$) and infiltrated macrophages ($p < 0.05$) and a reduction in fluorescein and Lissamine staining ($p < 0.05$), suggesting damage reduction at both corneal and conjunctival levels whilst receiving treatment with hydrocortisone [7]. Borroni D. et al. measured similar outcomes of common functional tests, namely NIBUT, LLT, TBUT. Furthermore, vision and chronic fatigue syndrome improved significantly after the therapy with low-dose hydrocortisone ($p < 0.05$). Average increase of 7.8 s in NIBUT scores was observed after 3 months of treatment. In contrast, neither TMH nor meiboscore showed significant changes during the treatment period ($p > 0.05$) [5]. Similarly, to the study mentioned above, we have observed improvement of objective ocular tests; however, we also noticed significant reduction of meiboscore suggesting positive effect of HA/Hydrocortisone on the function of Meibomian glands ($p<0,001$).

A recent comparative study conducted by G. R. Tedesco et. al. has shown a significant increase in LLT and NIKBUT using hyaluronate/hydrocortisone eye drops at all visits (15d, 45d, 90d) in comparison to the treatment with hyaluronate/trehalose [10]. We observed similar patterns in our study before and after treatment.

Limitations of this study include different duration of T2DM among the patients with may contribute to pathogenesis and severity of ocular surface damage and interactions with eye drops; automated scoring of LLT levels with secondary data recorded, and a single evaluation of outcomes at the endpoint. We will address these issues in our further research to evaluate clinical response during the treatment.

Borroni D. et al. demonstrated that early treatment of mild/moderate DED with low doses of cortisol may be a valuable strategy to prevent progression from subclinical parainflammation to overt chronic disease [5]. Also our findings show that HA/hydrocortisone drops should be considered as an effective treatment for patients with type 2 diabetes mellitus and dysregulated parainflammation of the conjunctiva and ocular surface.

Conclusions

Treatment with hyaluronate 0,2 % and hydrocortisone 0,001 % eye drops showed a significant improvement of conjunctival cytology in patients with type 2 diabetes mellitus.

The statistically significant improvement of functional tests (LLT, TBUT, MGL ($p<0.001$)) was observed after treatment with the combination of hyaluronate 0,2 % and hydrocortisone 0,001 %. Thus, it may be the basis for stabilization of the functional and cytological state of the eye surface. Moreover, it might prevent the progression of eye surface damage in patients with type 2 diabetes.

Reduction of relative expression of epithelial CD15 ($p<0.001$) after treatment with a combination of low-dose hydrocortisone and hyaluronic acid may be effective in controlling parainflammation in patients with type 2 diabetes.

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Abbreviation. *T2DM – Diabetes mellitus type 2, DED – dry eye disease, CIC – conjunctival impression cytology, LLT – lipid layer thickness , TBUT – tear breakup time, MGL – meibomian gland loss, OSDI – Ocular Surface Disease Index, NIBUT – noninvasive tear break-up time, SLM – Ophthalmic Slit Lamp, TMH – Lower tear meniscus height, HA – hyaluronic acid.*